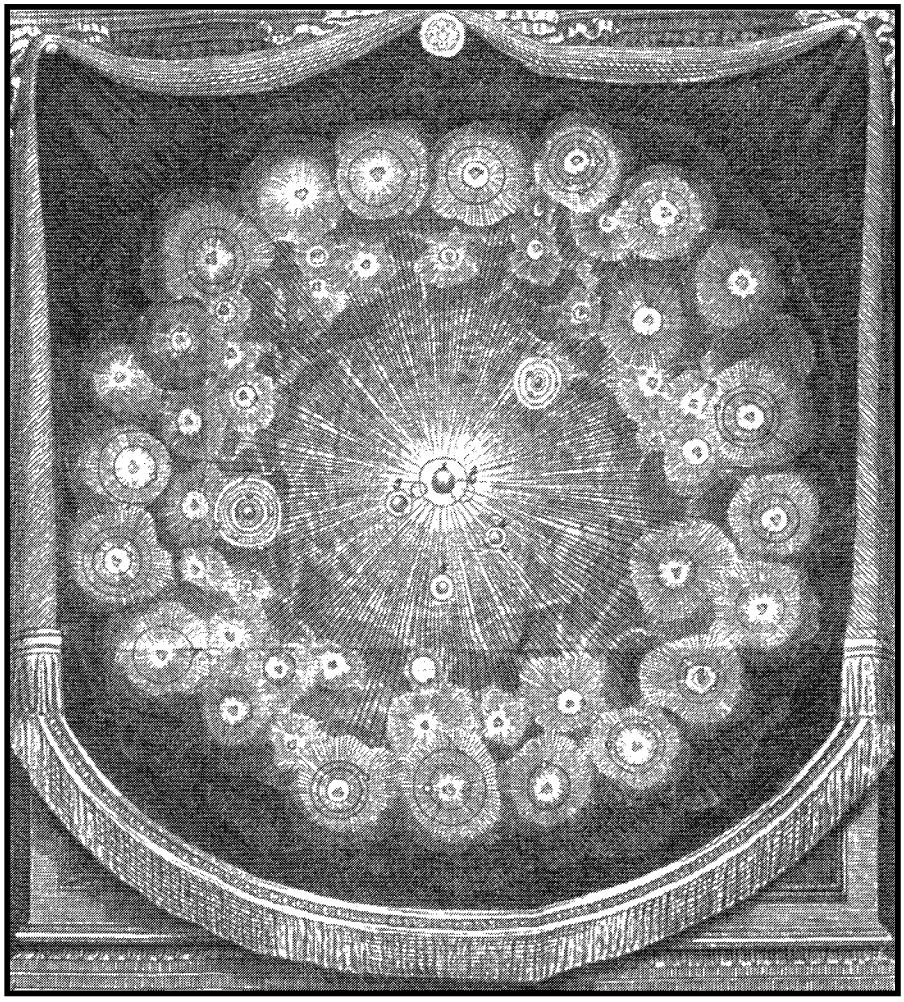
The NASA Astrobiology Institute

The Research Proposals of its New Lead Teams: Year 2001

August 2001



Cover Illustration:

This illustration is the frontispiece from *Fontenelle's Entretriens sur la pluralite des mondes* (Paris, 1766). Fontenelle was a Cartesian, but has now clearly placed planets around the other stars. Note our solar system is still central. Courtesy, Dibner Library, Smithsonian Institution Libraries.

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The Research Proposals of its New Lead Teams: Year 2001

August 2001

National Aeronautics and
Space Administration

Ames Research Center
Moffett Field, CA 94035-1000

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in memoriam

Dr. Gerald A. Soffen

Scientist, Pioneer, Viking, Mentor, Friend

1926 – 2000

"Gerald came to visit me shortly after I was appointed Director of the NASA Astrobiology Institute (NAI). He brought with him pictures related to the Viking and other NASA missions and spoke of the spirit of discovery and adventure that is the central spirit of the NASA experience. Gerald was dedicated to the furtherance of biology. The establishment of the NAI is a consequence of his enthusiasm and determination; we are grateful to him for his vision."

Baruch S. Blumberg
Director, NASA Astrobiology Institute

"[Soffen] was a guiding light in the formulation of astrobiology and critical in the creation of the NASA Astrobiology Institute . . . Gerry's talent for knowing the best [served the NAI] review process by bringing in many independent premier scientists able to judge the excellent and broad science base represented in the proposals."

Michael Meyer, Astrobiology Senior Scientist, NASA HQ
from *Astrobiology*: Volume 1, Number 1

A close advisor to NASA Administrator Daniel S. Goldin, Dr. Soffen helped shape NASA's Astrobiology program, the study of life in the Universe. Dr. Soffen also was instrumental in the establishment of the NASA Astrobiology Institute (NAI), a unique organization of NASA Centers, universities and research organizations dedicated to studying the origin, evolution, distribution and future of life in the universe. In recognition of his many contributions, the NAI has established the Gerald Soffen Student Award in Astrobiology, for research conducted through one of the Institute's Lead Teams, and presented at its General Meeting. Dr. Soffen was also responsible for the first volume of this series, documenting the science being conducted by the initial Lead Teams of the NAI.

Dr. Soffen was born in Cleveland, Ohio on Feb. 7, 1926. He received his Ph.D. in Biology in 1961 from Princeton University. He earned his master's of science degree from the University of Southern California and bachelor's degree from the University of California, Los Angeles.

Preface

A Cooperative Agreement Notice (CAN Cycle-2) was issued June 23, 2000 with the intent of adding new Lead Teams to the NASA Astrobiology Institute (NAI). Four new Lead Teams were selected from this competition: Michigan State University, the University of Rhode Island, the University of Washington, and a second team at the Jet Propulsion Laboratory. This document is a compilation of the proposed research plans from these four teams, and serves as a supplement to the compendium of research proposals produced for the initial 11 Lead Teams of the Institute[1]. All four new Principal Investigators agreed that the scientific sections of their proposals be assembled into a single document to assist in the coordination of research among the individual members of the NAI.

Wording within the CAN Cycle-2 announcement stated that proposals addressing any one or more of the research goals of Astrobiology would be considered for selection. However, in order to fill some perceived gaps in the current NAI research program, strong preference was to be given to proposals that:

> "**(a)** address the general issue of the nature and/or search for habitable worlds outside the solar system, as well as methods for detecting the presence of life thereon; **(b)** explore the role of ecosystem processes (e.g., large-scale episodic disturbance, nutrient dynamics and trace gas production, biogeographic change) in the global carbon cycle and other biogeochemical cycling processes on centennial to millennial time scales with a goal of contributing understanding that could advance our ability to predict future conditions; and **(c)** provide understanding of the response of life to the space environment, from gene expression to microbial evolution and ecological interactions."

Fifty-five Notices of Intent were received, and twenty-eight complete proposals were submitted before the closing date of September 22, 2000. These submissions represented 411 individual researchers and 132 research institutions in the US and abroad. Three of the 28 PIs, and nine of the proposing institutions, also submitted to the NAI CAN Cycle 1 solicitation.

A Review Panel of 28 members was assembled to evaluate the proposals, nearly all of whom did **not** serve on the review panel for the CAN Cycle 1 (24 of the 28). This approach assured the consideration of new views and insights into the make-up of the Institute. The presence of four repeat members of the panel provided continuity and perspective, as did the three panel members who are current Lead Team PIs. Following a mail review period in October and November, a Review Panel Meeting was held December 6-7, 2000 in Monterey, California to evaluate the proposals and make recommendations to the Institute. The Review Panel Meeting concluded with the convening of the Advisory Committee, which was composed of the Chairs and Co-chairs of the three Subpanels (Chairs Alan Boss, Robert Dorit, Richard Gammon; and Co-chairs David McKay, Patrick Crill, Steve Moorman).

The Institute's Director recommended the four Teams identified above to the NASA Selecting Official, Dr. Edward Weiler (Associate Administrator for Space Science). These selections were based on the evaluations of the Advisory Committee for scientific merit, together with consideration for a comprehensive research approach within the Institute, the development of strong academic training programs, and the coalescence of the Astrobiology community.

[1]The NASA Astrobiology Institute: A Compendium of the Science of its Initial Set of Members. Compiled by NASA Headquarters, Washington D.C.

Michigan State University

East Lansing, Michigan

Center for Genomic and Evolutionary Studies on Microbial Life at Low Temperature

Principal Investigator:

Michael Thomashow, Michigan State University

Co-Investigators/Collaborators:

Lycely Sepulveda, University of Puerto Rico, Mayaguez
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David Lubman, University of Michigan
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Executive Summary

Low temperature is a predominant environmental characteristic of interstellar space, our solar system, including most of the planets and their satellites, and asteroids and meteors. An understanding of the impact of low temperatures on the responses and evolution of biological organisms is, thus, integral to our knowledge of Astrobiology. The research that we propose will explore multiple aspects of microbial adaptation to low temperatures. One major line of investigation will be to conduct structural and functional genomic and proteomic analyses of bacteria that have been isolated from the Arctic and Antarctic permafrost. What genes and proteins enable the permafrost bacteria to inhabit these subfreezing environments? Do they have specific "freezing tolerance" genes and proteins, or "specialized alleles" of commonly found bacterial genes, or both? How is expression of the bacterial genome affected by low temperatures and other conditions that "hitchhiker" bacteria might encounter during travel through space on natural objects or spacecraft? In a second line of investigation, we will directly examine, through "test-tube evolution" experiments, bacterial adaptation to low temperatures. The studies will provide insight into how an organism, with a given complement of genes, can cross niche barriers that are defined by decreasing temperatures. And finally, we will use the information gained to explore the potential development of "signatures" for the presence of life in cold environments including Earth and other bodies such as Mars and Europa.

The proposed studies will provide significant new information relating to multiple goals outlined in the "Astrobiology Roadmap" including: Explore how life evolves on the molecular, organism and ecosystem levels (goal 3); Determine how the terrestrial biosphere has co-evolved with the Earth (goal 4); Establish limits for life in environments that provide analogues for condition on other worlds (goal 5); Determine how to recognize the signature of life on other worlds (goal 7); and Understand the response of terrestrial life to conditions in space or on other planets (goal 10). Moreover, the proposed studies address one of the perceived gaps in the current NAI research program, namely, "**provide understanding of the response of life to the space environment, from gene expression to microbial evolution.**" Finally, there are significant potential practical applications of the work ranging from the identification of genes that may be used to confer improved environmental stress tolerance in crop plants to the discovery of enzymes uniquely suited to catalysis at low temperature, a characteristic of importance in numerous biotechnology applications.

The proposed lines of interrelated investigations call for a broad range of expertise. Consequently, we have assembled a group of investigators with diverse backgrounds, training and research interests. The areas of expertise include microbial ecology (Tiedje, Sepulveda), the isolation and characterization of permafrost bacteria (Tiedje), molecular genetics and gene regulation (Kathariou, Thomashow), evolution and population genetics (Lenski, Bennett), cryobiology and mechanisms of freezing tolerance (McGrath, Thomashow), proteomics and protein evolution (Lubman, Goldstein), and structural genomics (Branscomb, Hawkins, Predki). As the proposed lines of research develop, we anticipate that the Center will evolve, bringing in new investigators to provide expertise to attack the next generation of questions. For instance, the structural genomic and gene expression profiling studies will lead to lines of investigation focused on specific genes with the objectives of establishing their roles in cold tolerance; determining their modes of action; and developing hypotheses as to how the genes evolved.

While the current Center investigators are well qualified to conduct these lines of research, it may, perhaps, become evident that adding an investigator with expertise in using X-ray crystallography and/or NMR-spectroscopy to determine protein structure-function relationships would strengthen the research efforts.

We also envision that additional lines of investigation will evolve out of our interactions with researchers in other Centers of the Astrobiology Institute. For instance, among our objectives are to conduct experiments to determine how bacterial gene expression and evolution are affected by conditions that relate to the space environment. Our major focus will be on cold temperatures. However, it seems likely that it would be of interest and importance to other Center members to understand how microorganisms react, in terms of gene expression and evolution, to other environmental conditions of space such as the Martian atmosphere and regolith or perhaps microgravity and solar radiation. We view the defining of mutual research interests and development of collaborative Center research efforts to address fundamental questions in astrobiology to be an underlying goal of our research and educational programs.

Our educational objective is to provide in-depth training in structural and functional genomics, but to encompass that training in a context relevant to astrobiology. In this way our students will have cutting-edge knowledge for the genomics era, but will be able to use that knowledge to explore questions important to understanding how biological organisms may adapt to and evolve in response to extreme environmental conditions present in the universe. We will implement this educational objective by having students major in their existing degree-granting programs, but establish an "area of specialization" in astrobiology. In this way students will not be diluted in their training in their basic discipline but will gain the perspective and knowledge needed to make significant contributions to the field of astrobiology. The specialization approach will also make it possible for students from a variety of departments to enter the program and gain an astrobiology emphasis. The astrobiology specialization will comprise coursework, including a new graduate level course in "Genomics and Evolution of Environmental Stress Tolerance" and short-courses to augment keys areas of student training. One such course that we have in mind initially would focus on the cryosoil environment and history of the Arctic and Antarctic, drawing similarities and dissimilarities to Europa and Mars. Other components of the astrobiology specialization will include an internal seminar series; research exchanges in which graduate students or postdocs go to another institution either within our Center or within the NAI to conduct a collaborative experiment that draws on the unique resources of the partners; and a symposium on "Genomics and Evolution of Extremophiles" (to be held in year three or four) where the research results from our Center will be presented along with findings by prominent invited speakers in related fields. The objective will be to bring together the cutting-edge body of knowledge on the symposium topic for Center members, NAI members and interested peers.

At present, we are not proposing a separate E/PO program. However, what we would like to suggest to the NAI, given our theme of genomics, is our developing a high school level genomics module that incorporates concepts central to astrobiology. Ideally, students would be able to explore actual extremophile genomic data using well constructed queries. The information they learn could be put in the context of "the limits of life." In addition, for the K-8 group, we suggest an update and take-off on the "Microbial Zoo" unit which the CME developed

with MSU's Communications Technology Laboratory. The module, which is available on the Web (http://commtechlab.msu.edu/sites/dlc-me/zoo/) and on CD-ROM (with sound) allows young people to explore environmentally important microbes as if they were in a zoo. Already included in the module is "Space Adventure" (http://commtechlab.msu.edu/sites/dlc-me/zoo/zsmain.html) which includes Martian meteorite images as a center-piece. This is an existing example of our contribution to astrobiology and what we have done for the K-12 sector. The resources necessary for development a full K-14 astrobiology Web site (including the professional educator involvement and design testing on target groups) is beyond the resources available for an individual Center activity, but should not be beyond what the NAI could do. We would have the scientists, communication technologist and educator experience to share if NAI undertook such an effort.

Professor Michael Thomashow will be the PI and Director of the Center and Professor James Tiedje will serve as Associate Director to aid the Director in duties determined by the Director. We have found such a management scheme to work well in our NSF-STC Center for Microbial Ecology; one person has point responsibility, but has a second person fully cognizant of the breadth of Center activities with whom to discuss pertinent issues and implement actions. In addition, there will be an Executive Committee consisting of three co-investigators (membership will rotate). The Executive Committee will establish operating policy, aid in making major decisions, make sure that all perspectives are heard and help ensure that the research being conducted is of the highest possible quality. In addition, we will name a small group of external advisors for the purpose of providing Center management with critique and suggestions on Center activities, progress and direction. After year two, we would specifically ask the group for advice on mid-course adjustments so that these could be implemented before the year three NASA review.

Our proposed Center is strongly supported by the institutions involved and includes cost sharing that will significantly enhance the proposed research effort. The institutional financial commitments include: 1) three graduate student lines in years 1 and 2, and four in years 3-5 plus all faculty and administrative salaries at Michigan State University to be provided by MSU, together totaling approximately $900k; 2) some 30 million base-pairs of DNA sequence provided by the Joint Genome Institute, a value of approximately $100k; and 3) virtually all faculty salary provided by the partner universities at an estimated value of $300k. **By not burdening the Center with faculty salaries, most NASA funds will go directly to researchers in the laboratory, at their computers, or in the field.**

Research/Training/Management Section

I. Research

The overarching research theme of our Center is understanding biological adaptation to environmental conditions that are common in space. Our focus will be on microbial adaptation to low temperature. Cold temperature is a predominant environmental characteristic of interstellar space, our solar system, including most of the planets and their satellites, as well as asteroids, comets and other objects. An understanding of the impact of cold temperatures on the responses and evolution of biological organisms is thus an integral component of an overall knowledge of astrobiology.

In one line of investigation, we will focus on structural and functional genomic analyses of bacteria that are present in permafrost environments on Earth. As discussed below, the permafrost environment provides an excellent model "system" to study microbial adaptation to cold temperatures and offers the unique opportunity to study organisms that have survived freezing for more than a million years. Fundamental objectives of this work include determining how expression of the genome is affected by low temperature and dehydration, two cardinal characteristics of subfreezing environments, and identifying genes and proteins that enable bacteria to inhabit cold environments. As a part of this investigation, we will include a targeted search for, and basic characterization of, two classes of proteins associated with freezing tolerance: antifreeze proteins and cryoprotective polypeptides. In addition, we will conduct "Field Truth" experiments to probe for the presence of hypothesized key cold tolerance genes and proteins *in situ* with an associated objective of developing potential "signatures" indicative of present or past life. Finally, we will conduct "test-tube" evolution experiments with *Escherichia coli* to identify genes that, through natural mutation, can impart increased "fitness" to cold temperatures. Through these studies, we will explore how an organism, with a given complement of genes, may cross niche barriers that are defined by low temperature.

The proposed studies will provide significant new information relating to multiple goals outlined in the "Astrobiology Roadmap." The include: Explore how life evolves on the molecular, organism and ecosystem levels (goal 3); Determine how the terrestrial biosphere has co-evolved with the Earth (goal 4); Establish limits for life in environments that provide analogues for condition on other worlds (goal 5); Determine how to recognize the signature of life on other worlds (goal 7); and Understand the response of terrestrial life to conditions in space or on other planets (goal 10). Moreover, the proposed studies address one of the perceived gaps in the current NAI research program, namely, "provide understanding of the response of life to the space environment, from gene expression to microbial evolution." Finally, there are significant potential practical applications of the work ranging from the identification of genes that may be used to confer improved environmental stress tolerance in crop plants to the discovery of enzymes uniquely suited to catalysis at low temperature, a characteristic of importance in various biotechnology applications.

A. Background Information

1. The permafrost environment and microbial community: a model for astrobiology

The ability of microorganisms to live in deep cold environments continually forces us to redefine the spatial and temporal limits of life in our biosphere and other bodies in space. From an astrobiological point of view, seven of the nine planets of our Solar System, as well as their satellites, comets and asteroids have a cryogenic nature, i.e. the permafrost is a common phenomenon in space. Here on Earth, permafrost is also an important environmental niche. Permafrost, defined as ground (including bedrock, soil and sands) that remains below 0°C for more than two years (Muller, 1943), makes up more than 20% of the land surface of the Earth, including 82% of Alaska, 50% of Russia and Canada, 20% of China, and most of the surface of Antarctica (Harris, 1986; Williams and Smith, 1989; Storad, 1990). Permafrost poses unique challenges to its resident biota:

- Temperatures that have remained below 0°C over geologically significant periods of time.
- Presence of only minute amounts of water in the liquid form, 93-98% of water being in solid (ice) form.
- Inaccessibility of organic matter to the resident microbes, due to the frozen state.
- Minimal diffusion and infiltration of water, organic compounds and minerals.
- Long-term influence of gamma radiation from soil minerals.

These challenges, considered alone and in combination, appear severe. Permafrost, however, is also a stable environment, without sharp fluctuations. A unique outcome of this specialized ecosystem is the preservation of life for the longest documented periods on Earth. Viable bacteria in permafrost soils were first discovered in Russia in connection with investigations of mammoths in Siberia, and reported sporadically thereafter (Becker and Volkmann, 1961; Cameron and Morelli, 1974). Most recently, and almost unnoticed by the western scientific community, Russian scientists under the leadership of D. Gilichinsky reported the existence of high numbers of viable microorganisms (up to 10^5-10^7 cells/g) in buried Siberian permafrost soils of depths varying from a few meters below surface to 400 m (Zvyagintsev et al., 1985; Zvyagintsev et al., 1990; Khlebnikova et al., 1990, Gilichinsky et al., 1988; Gilichinsky et al., 1992). This was surprising, not only because of the constant subzero temperature of the soils, averaging -10°C to -12°C, but also because of the length of time over which the soils were frozen, from a few thousand years up to 2-3 million years. These organisms may well be the only living cells that have survived for a geologically significant period of time. Although viable eukaryotes (yeasts, fungi, mosses) have been isolated from the permafrost, their numbers seem to be low in comparison to those of prokaryotes, especially in samples older than 10,000 years (Gilichinsky et al., 1992).

The permafrost microbial community has been described as "a community of survivors" (Friedman, 1994). Viable bacterial populations in the permafrost may be viewed as the result of a continuous process of selection for those capable of withstanding prolonged exposure to subzero temperature, accompanying low water activity, and other special challenges posed by the permafrost. Furthermore, they are also the outcome of tens of thousands of years of cyclic freeze-thaw selection in the original "active" tundra layers where they were exposed to warmer growth periods in summer and colder surface temperatures in the winter. Enumeration of culturable bacteria has yielded minimal estimates of the number of viable bacteria in the buried

permafrost, and some data suggest that the ratio of readily culturable (hypometabolic) bacteria to those that are in the viable but nonculturable state (deep resting cells) is determined by the length of time for which the buried soils have been frozen (for review, see Vorobyova et al., 1997). Several lines of evidence indicate that ***the microorganisms that have survived in the buried soils are not simply representatives of cold-region surface soil communities.*** The oldest samples have relatively low numbers of culturable bacteria, 10^2-10^4 cells per gram in Pliocene soil of 1 million years or older. Bacteria surviving in these ancient samples were frequently Coryneforms, whereas actinomycetes were rarely isolated from samples below the Holocene stratum (10^4 years). Importantly, non-spore forming bacteria always dominated, independent of depth or age (Gilichinsky et al., 1992). The prevalence of non-spore formers was also found in Antarctic soil (Horowitz et al., 1972), but not in core samples of the Antarctic ice sheet; Abyzov et al. (1987) reported increasing numbers of spore-forming bacteria with increasing depth (down to 320 m) and age of the ice cores (up to 12,000 years). The glacial ice, therefore, appears to provide an environment that supports a fundamentally different, much less dense, microbial community than that supported by the permafrost environment.

If non-spore-forming bacteria are dominant in ancient frozen soil samples, they have either survived in a state of suspended animation (anabiosis) or are metabolically active. There are several indications of metabolic activities *in situ*. Examples include the simultaneous presence of nitrifying bacteria and nitrites, of methanogens and methane, and the presence of fresh ferrous sulfite (Gilichinsky and Wagener, 1994). In addition, it has been estimated that in permafrost, background radiation (about 0.03 rad/yr taking into account the protective effect of frozen soil) would have lethal cumulative effects on the genomes of the endemic bacteria in about 200,000 years. Thus, survival of cells in frozen soils as old as 2-3 million years would seem highly unlikely without the implementation of DNA damage repair processes *in situ* (Friedman, 1994). Indeed, metabolic activity at temperatures of -7 to -10°C has been reported both in lichens (Friedman, 1982; Kappen 1989) and bacteria (Straka and Stokes, 1960; Larkin and Stokes, 1968).

Microbiological studies of permafrost have predominantly involved Arctic samples. Antarctic permafrost imposes even more stringent requirements for microbial survival: longer time periods (up to ~ 10^7 years); lower temperatures (average -20 to -25°C); and lower water activity (1% soil water content with a computed a_w~ 0.82 to 0.78). These are much more challenging conditions than certain privileged Antarctic microenvironments such as the recently described "oases" harboring microbial consortia under the ice cover of Antarctic lakes (Priscu et al., 1998). The survey and characterization of the Antarctic permafrost bacterial community will be of special interest in the context of a growing body of data on the bacterial communities of several other Antarctic ecosystems (e.g. Horowitz et al., 1972; Franzmann et al., 1990; Delille, 1992, 1993; Palmisano and Garrison, 1993; Grossmann and Dieckmann, 1994; Helmke and Weyland, 1995; Bowmann et al., 1997) as well as for comparisons with the data available on the microbiota of Arctic permafrost. Comparisons between Arctic and Antarctic permafrost bacteria should help reveal the adaptive strategies used for survival of the resident microbes in these extreme environments.

The existing bacterial permafrost communities are the result of a long-term natural experiment in cell preservation under specialized, stringent conditions. A pivotal condition,

time, cannot be readily simulated. Nonetheless, ***valuable information on microbial life's potential for long-term preservation and persistence can be obtained from the characterization of these communities in situ, and from the study of the adaptive physiology of representative community members.***

2. Bacterial cold shock and cold acclimation

When bacteria are exposed to sudden temperature downshifts, two major responses are commonly observed, changes in membrane lipid composition and production of novel proteins Jones et al., 1987; Gounot, 1991). Increases in lipid unsaturation and decreases in the length of the fatty acyl chains result in maintaining the membrane in a fluid state (Russell, 1990). Although bacteria that can grow at low temperature appear to have enhanced potential to maintain membrane lipid fluidity (Gounot, 1991), the molecular and physiologic mechanisms underlying the differences in growth temperature minima among microbes remain largely unclear. In discussions of bacterial low temperature physiology, the terms cold shock and cold acclimation refer to the transitory period following sudden temperature downshift and to steady-state growth at low temperature, respectively.

a. Studies on *Escherichia coli*

When *E. coli* cells are subjected to a rapid downshift in temperature from about 37 to 15°C, the synthesis of most proteins stop and growth ceases. A number of "cold shock" proteins, however, are synthesized and gradually (within a few hours) the bacteria resume growth. Significantly, many of the cold shock proteins have roles in gene expression including protein synthesis. The four most abundant cold shock proteins, CspA, CspB, CspG and CspI (Goldstein et al., 1990; Lee et al., 1994; Nakashima et al., 1996; Wang et al., 1999), are members of a gene family comprising nine closely related homologs. Remarkably, CspA constitutes more than 10% of protein synthesized within an hour of cells being shifted to low temperature (Goldstein et al., 1990). Intriguingly, CspA, CspB and CspG are synthesized in large amounts upon cold shock under conditions that normally inhibit protein synthesis, such as amino acid starvation and treatment by inhibitory antibiotics (Etchegaray and Inoye, 1999). Each of the CspA homologs, referred to as "RNA chaperones," bind to RNA without apparent sequence specificity. Recent evidence indicates that CspA and its homologs act as transcription antiterminators. Moreover, they appear to act to induce cold-regulated expression of four other cold-shock proteins, RbfA, IF2, NusA and PNP. Both RbfA and IF2 are involved in the initiation of translation; NusA associates with RNA core polymerase and is involved in both transcriptional termination and antitermination; and PNP (polynucleotide phosphorylase) is a 3' to 5' exonuclease that interacts with Ribonulease E to degrade mRNA.

Other cold shock proteins that have roles in *E. coli* acclimating to low temperature include CsdA, H-NS, trigger factor and Hsc66. CsdA, which is associated with 70S ribosomes at 15°C, has helix-destabilizing activity which is proposed to be important in removing secondary structures in mRNAs at low temperature. While a *csdA* knockout mutant shows no difference in growth from its parent strain at 37°C, it has an increased generation time of 16 hours at 15°C (compared to an 8 hour generation time of the wild type strain). H-NS is an abundant DNA-binding protein that affects DNA supercoiling and impacts the expression of several unrelated

genes, including the *rrn* operons (reviewed in Atlung and Ingmer, 1997). Binding of H-NS to the *rrn* promoter region specifically counteracts activation by FIS protein (Afflerbach et al., 1998). In an *hns* insertional mutant expressing no detectable H-NS protein, growth was significantly inhibited at 12°C and 25°C but only slightly affected at 37°C (Dersch et al., 1994). Finally, trigger factor and Hsc66 appear to be molecular chaperones with roles in acclimation to low temperature. Trigger factor, which has peptidyl prolyl isomerase activity, is thought to promote folding of nascent polypeptides (Kandror and Goldberg, 1997). Following incubation for one week at 4°C, a non-permissive growth temperature for *E. coli*, overexpression of trigger factor increased survival from 15% to 40%. Hsc66 has 42% similarity to the heat shock protein DnaK and can prevent aggregation of proteins *in vitro* (Silberg et al., 1998). An insertion mutation in the gene encoding Hsc66, *hscA*, was not greatly altered in growth following a cold shock although the expression of 5 proteins was altered at low temperature. The characterization of trigger factor and Hsc66 as cold shock proteins suggests that multiple steps in protein folding are negatively affected by low temperature in *E. coli*.

b. Cold shock proteins in other bacteria

The cold shock response has been investigated in several gram-negative and gram-positive bacteria. The most highly conserved feature of the response is the expression of CspA homologs, having been documented in hyperthermophiles, thermophiles, mesophiles and psychrotrophs. (We recognize that there is difference of opinion in the microbiological community concerning the terms most suitable to describe microbes in regard to their ability to grow at low temperature. In our proposal, we use the term **psychrophiles** to refer to microbes that grow at 4°C or lower, but not above 15°C; **psychrotrophs** are microbes which grow at 4°C or lower, but which have optimal temperature of growth above 15°C; and **mesophiles** are microbes which do not grow at 4°C or lower, and have optimal temperature for growth at 35-38°C.) In several species, multigene families have been identified that contain from three to nine CspA homologs. In each case, at least one of these proteins is increased in expression upon temperature downshift (Graumann et al., 1996; Mayr et al., 1996; Michel et al., 1997; Wouters et al., 1998). Interestingly, in the case of the psychrotrophic bacterium *Arhrobacter globiformis* SI55, production of the protein is not limited to the cold shock period immediately following the temperature downshift but continues during growth at low temperature (Berger et al., 1997) suggesting that the protein functions in cold acclimation (in addition to its function during cold shock). Investigation of CspA expression in additional psychrotrophs will reveal whether in these bacteria, unlike the situation in mesophiles, the major cold shock proteins continue to be expressed during steady-state growth in the cold.

Cold shock and cold acclimation proteins other than the Csp family have also been studied, albeit to a much lesser extent. In *B. subtilis*, the peptidylprolyl isomerase cyclophilin, like *E. coli* trigger factor (which has peptidylprolyl isomerase activity) is a cold shock protein. In *Anabaena* sp. strain PCC 7120, two RNA helicases, CrhC and CrhB, show increased expression following a temperature downshift of 10°C (Chamot et al., 1999). Both of these proteins belong to the DEAD-box family of RNA helicases as does the *E. coli* cold shock gene, *csdA*. Studies with psychrotrophic and psychrophilic bacteria are beginning to provide insight on the involvement of cold-shock and cold acclimation proteins in the ability of the organisms to grow at low temperatures. Most of these studies have involved the psychrotrophic food-borne

pathogens *Yersinia enterocolitica* and *Listeria monocytogenes*. In *Y. enterocolitica*, polynucleotide phosphorylase (involved in mRNA degradation) is a cold shock protein, as it is in *E. coli*. Moreover, PNP is required for growth at 5°C in *Y. enterocolitica* (Goverde et al., 1998); strains carrying transposon insertions in this gene were unable to form colonies at 5°C yet were indistinguishable from the wild type strain at 30°C. In *L. monocytogenes*, twelve proteins are induced following temperature downshift from 37 to 5°C, and four of these proteins continued to be expressed during cold growth, thus being cold acclimation proteins (Bayles et al., 1996). Transposon mutations in several chromosomal loci render *L. monocytogenes* cold-sensitive (Zheng and Kathariou, 1994). The Kathariou lab found that in one such mutant, the insertion was in a locus highly conserved between *L. monocytogenes* and *B. subtilis* and harbored genes involved in DNA repair and cell division. A gene encoding a polypeptide with a RNA-binding domain typical of PNP was also identified at this locus. Other investigators have identified cold-sensitive mutants of *L. monocytogenes* in which membrane fatty acid composition was markedly altered (Annous et al., 1997).

Although no fatty acid desaturase has been identified as a cold shock protein in *E. coli*, cold induced desaturases have been identified in *B. subtilis* and at least two cyanobacteria. In *B. subtilis*, a *des-lacZ* fusion was induced 10 to 15-fold following a temperature shift from 37 to 20°C (Aguilar et al., 1998). Levels of unsaturated fatty acids increased at 20°C, but were not required for viability as a *des* null mutant showed growth similar to that of the parent strain at both 37°C and 20°C. Of the four desaturases identified in the cyanobacterium *Synechocystis* sp. PCC 6803, three are clearly induced following a temperature downshift albeit at different rates (Los et al., 1997). In *Synechococcus* sp. strain PCC 7002, three desaturases, DesA, DesB, and DesC, have been identified (Sakamoto and Bryant, 1997). The expression of all three genes was induced within five minutes of a shift from 38 to 22°C. The three genes were also expressed more highly in cells grown continuously at 22°C. Insertion mutants of *desA* and *desB* were unable to grow at 15°C (Sakamoto et al., 1998).

3. Current understanding of freezing tolerance

Poikilothermic organisms (those having body temperatures that fluctuate with the environment) vary markedly in their ability to cope with freezing temperatures. For instance, plants of tropical origin are typically killed by the slightest freeze, while plants from temperate and polar environments can survive freezing at high subzero temperatures (see Thomashow, 1999). Moreover, most temperate and polar plants increase in freezing tolerance in response to low nonfreezing temperatures, a process referred to as "cold acclimation" or "cold hardening." Nonacclimated rye plants are killed upon freezing at about -5°C, but after cold acclimation, can survive freezing down to about -30°C. The ability to cold harden is not limited to plants, but includes other organisms including certain bacteria (Pannoff et al., 1998; Thammavongs et al., 1996). A fundamental goal in low temperature biology research is to determine the mechanisms of freezing tolerance and to identify the genes and proteins that enable poikilothermic organisms to contend with freezing temperatures.

a. Bacterial tolerance to freezing and thawing

Bacteria differ greatly in their ability to tolerate freezing and thawing, for reasons that are poorly understood. Exposure of mesophilic bacteria to low temperatures has been shown to enhance their ability to withstand repeated freeze-thawing ("cryotolerance") (Panoff et al., 1995; Thamavongs et al., 1996). In addition, exposure of lactic acid bacteria to osmotic stress has been recently shown to confer tolerance to freeze-thawing (Panoff et al., 2000). The molecular basis of cryotolerance, conferred either by cold pre-exposure or by osmotic stress, remains to be determined. In contrast to the situation in plants and animals, antifreeze proteins have not been frequently identified in prokaryotes. Bacteria have unique adaptations to low temperature and may have unique and currently unknown mechanisms not only to withstand prolonged exposure to freezing, but also to actually grow at sub-zero temperatures. The remarkable ability of several bacterial species to grow at temperatures as low as -8°C has been described as early as the 1930s (Haines, 1931; Tschistajakow and Botescharowa, 1938a,b).

In *Bacillus subtilis*, where synthesis of three Csp proteins is induced upon cold shock, a minimum of one *csp* gene is required for viability at all temperatures (Graumann et al., 1997). Interestingly, the CspB protein of this organism has been shown to be required for viability of the cells following freezing and thawing (Willimsky et al., 1992), but the role of CspB in freezing tolerance is unknown.

b. Freezing injury and tolerance mechanisms: insights from studies on plants

Many of the basic insights into freezing injury have come from studies with plants (Hughs and Dunn, 1996; Thomashow, 1999). These investigations have revealed that the membrane systems of the cell are the primary site of freezing injury and that freeze-induced membrane damage results primarily from the severe dehydration associated with freezing (Steponkus et al., 1993). As temperatures drop below zero, ice formation is generally initiated in the extracellular spaces due largely to the extracellular fluid having a higher freezing point (lower solute concentration) than the intracellular fluid. Because the chemical potential of ice is less than that of liquid water at a given temperature, the formation of extracellular ice results in a drop in water potential outside the cell. Consequently, there is movement of water down the chemical potential gradient from inside the cell to the extracellular spaces where it freezes. This process continues until an equilibrium in chemical potential is achieved. If the freezing temperature is –10°C, the unfrozen liquid will have an osmolarity in excess of 5 and typically, greater than 90% of the osmotically active water will have moved out of the cell. Formation of intracellular ice is generally thought to be a fatal event.

Although freezing injury is thought to result primarily from membrane lesions caused by cellular dehydration, additional factors may also contribute to freezing-induced cellular damage. There is evidence that freeze-induced production of reactive oxygen species contributes to membrane damage (McKersie and Bowley, 1997) and that extracellular ice can form adhesions with cell walls and membranes and cause cell rupture (Olein and Smith, 1977). In addition, there is evidence that protein denaturation occurs in plants at low temperature (Guy et al., 1998), which could potentially result in cellular damage.

Most of what is know about freezing tolerance mechanisms has come from the study of the cold acclimation response. These studies have shown that key function of cold acclimation is

to stabilize membranes against freezing-injury. Multiple mechanisms appear to be involved in this stabilization. The best documented are changes in lipid composition (Uemura and Steponkus, 1997). However, the accumulation of "compatible solutes," such as proline and simple sugars, that typically occurs with cold acclimation is likely to contribute to the stabilization of membranes as these molecules can protect membranes (as well as proteins) against freeze-induced damage *in vitro* (Strauss and Hauser, 1986; Anchordoguy et al., 1987) and *in vivo* (Nanjo et al., 1999). There is also emerging evidence that certain cold-inducible hydrophilic novel and LEA (late embryogenesis abundant) polypeptides also participate in the stabilization of membranes against freeze-induced injury.

Role of cold-regulated genes encoding unusually hydrophilic polypeptides: COR and LEA proteins. Cold acclimation in plants and other organisms is associated with changes in gene expression (see Thomashow, 1998, 1999). In plants, where the phenomenon has been best studied, it has been shown that the most highly expressed cold-regulated genes encode either newly discovered proteins, such as the Arabidopsis COR6.6, COR15a and COR78 polypeptides, or homologs of LEA proteins, such as Arabidopsis COR47 (Arabidopsis is a small flowering plant that has become an important "model" organism to study basic plant processes) (see Hughs and Dunn, 1996; Thomashow, 1999). Moreover, recent evidence from the Thomashow and Steponkus laboratories (Artus et al., 1996; Steponkus et al., 1998) indicates that at least one of these genes, *COR15a*, encodes a cryoprotective polypeptide.

COR15a encodes a 15 kDa polypeptide that is targeted to the chloroplasts. Upon import into the organelle, COR15a is processed to a 9.4 kDa polypeptide designated COR15am. Constitutive expression of *COR15a* gene in nonacclimated transgenic Arabidopsis plants increases the freezing tolerance of chloroplasts by 1 to 2°C (Artus et al., 1996). This results from the COR15am polypeptide decreasing the propensity of membranes to enter into the hexagonal II phase, a deleterious non-bilayer membrane structure that can form upon freezing-induced dehydration (Steponkus et al., 1998). Moreover, this effect appears to be due to the COR15am polypeptide altering the intrinsic curvature of the membrane monolayers. Studies in mammalian systems have shown that certain polypeptides that form amphipathic α-helices can stabilize membranes against the formation of hexagonal II phase lipids and that this is accomplished by the polypeptides altering the intrinsic curvature of membrane monolayers. A sensitive test of whether a polypeptide has an effect on monolayer curvature is to determine whether the polypeptide causes a shift in the lamellar-to-hexagonal II phase transition temperature. Indeed, Steponkus et al. (1998) have found that the COR15am polypeptide increases the lamellar-to-hexagonal II phase transition temperature of dioleoylphosphatidylethanolamine and promotes formation of the lamellar phase in a lipid mixture composed of the major lipid species that comprise the chloroplast envelope. Whether this effect is dependent on COR15am forming an amphipathic α-helix is not known, but the COR15am is indeed predicted to form an amphipathic α-helix.

It remains to be established whether other COR and LEA genes encode cryoprotective polypeptides that stabilize membranes. In this regard, however, it is significant to note that most of the COR and LEA proteins have a set of distinctive properties in common: they are unusually hydrophilic; many have been shown to remain soluble upon boiling in dilute aqueous buffer; many have relatively simple amino acid compositions, being composed largely of a few amino

acids; many are composed largely of repeated amino acid sequence motifs; and many are predicted to contain regions capable of forming amphipathic α-helices. For example:

- *Arabidopsis COR15a* (Lin and Thomashow, 1992), as mentioned above, encodes a novel 15 kDa polypeptide that is targeted to the chloroplasts. The mature 9.4 kD polypeptide COR15am (Fig. 1) is highly hydrophilic; remains soluble upon boiling; is rich is alanine, lysine, glutamic acid and aspartic acid (they account for 64% of the amino acid residues); and is composed largely of a 13 amino acid sequence that is repeated (imperfectly) four times. Regions of the polypeptide that include the repeated sequences are predicted to form amphipathic α-helices.

- Alfalfa *cas15* (Monroy et al., 1993) encodes a novel 15 kDa polypeptide CAS15 (Fig. 1) that is highly hydrophilic; is rich is glutamate, glycine, histidine and lysine (they account for 68% of the amino acid residues); and nearly a third of the protein is composed of a 10 amino acid sequence that is repeated (imperfectly) four times. Regions of the polypeptide that include the repeated sequences are predicted to form amphipathic α-helices.

- Wheat *wcs120* (Houde et al., 1992) encodes a 39 kDa polypeptide WCS120 (Fig. 1) that is a member of the LEA II group of polypeptides (Close, 1997). It is highly hydrophilic; remains soluble upon boiling; is rich in glycine, histidine and threonine residues (they account for 54% of the amino acid residues); and is composed largely of a lysine-rich sequence, referred to as a K-segment, that is repeated (imperfectly) six times and a glycine-rich sequence, referred to as a ϕ-segment, that is repeated (imperfectly) 11 times. The K-segments are present in all LEA II proteins and are predicted to form amphipathic α-helices (Close, 1997).

- Barley *HVA1* (Hong et al., 1992) encodes a 22 kDa polypeptide HVA1 (Fig. 1) that is member of the LEA III group of proteins (Dure, 1993). It is highly hydrophilic; rich in alanine, lysine and threonine (they account for 53% of the amino acid residues); and is composed largely of an 11 amino acid sequence that is repeated (imperfectly) nine times. The repeated sequences are present in all LEA III proteins and are predicted to form amphipathic α-helices (Dure, 1993).

The intriguing possibility raised is that the similar biochemical properties shared by the novel hydrophilic and LEA polypeptides may reflect a common function of the proteins, namely to stabilize membranes against freezing damage. In this regard, it will be interesting to determine whether any of these proteins effect on the intrinsic curvature of membranes and if so, whether this is due to the regions of the proteins predicted to form amphipathic α-helices.

Cold-regulated genes impart both freezing and dehydration tolerance. As discussed above, freezing tolerance must include tolerance to severe dehydration stress. Thus, one might expect that some of the genes that are important for freezing tolerance would also be important in dehydration tolerance. Indeed, many of the COR, LEA and other cold-responsive genes discovered in plants are also induced upon dehydration stress. Moreover, recent results indicate that the cold-responsive genes impart both freezing and drought tolerance. In particular, the Thomashow (Stockinger et al., 1996; Jaglo-Ottosen et al., 1998; Gilmour et al., 1998) and Shinozaki (Liu et al., 1998; Kasuga et al., 1999) laboratories have identified a family of

Arabidopsis COR15am (novel hydrophilic)

AKGDGNILDDLNEATK **KASDFVTDKTKEA** LADGE
KAKDYVVEKNSET ADTLGKEAE
KAAAYVEEKGKEA AN
KAAEFAEGKAGEA KDATK

Alfalfa CAS15 (novel hydrophilic)

MAGIMNKIGDALHGGGDKKEGEH **KGEQHGHVGG** EHHGEY
KGEQHGFVGG HAGDH
KGEQHGFVGG HGGDY
KGEQHGFGHG DHKEGYHGEEHKEGFADKIKDKIHGEGADGEK
KKKKEKKKHGEGHEHGHDSSSSDSD

Wheat WCS120 (LEA II)

MENQAHIA **GEKKGIMEKIKEKLPGGHGDHKE** TAGTHGHPGTATHGAPA **TGGAYGQQGHAGTT** GTGLHGAHA
GEKKGVMENIKDKLPGGHQDHQQ **TGGTYGQQGHTGTA** THGTPA
TGGTYGQQGHTGTA THGTPA
TGGTYGEQGHTGVT GTGTHGT
GEKKGVMENIKEKLPGGHGDHQQ **TGGTYGQQGHTGTA** THGTPA
GGGTYEQHGHTGMT GTGTHGT
GEKKGVMENIKDKLPGGHGDHQQ **TGGTYGQQGHTGTA** TQGTPA
GGGTYEQHGHTGMT GAGTHST
GEKKGVMENIKEKLPGGHSDHQQ **TGGAYGQQGHTGTR** HMAPL
PAGTYGQHGHAGVI GTETHGTTA
TGGTHGQHGHTGTT GTGTHGSDGI
GEKKSLMDKIKDKLPGQH

Barley HVA1 (LEA III)

MASNQNQGSYHAGETKARTEEKTGQM **MGATKQKAGQT**
TEATKQKAGET
AEATKQKTGET
AEAAKQKAAEA KDKTAQT
AQAAKDKTYET
AQAAKERAAQG KDQTGSALGEK
TEAAKQKAAET
TEAAKQKAAEA
TEAAKQKASDT AQYTKESAVAGKDKTGSVLQQAGE
TVVNAVVGAKDAVANTLGMGGDNT
SATKDATTGATVKDTTTTTRNH

Figure 1. Examples of novel hydrophilic and LEA proteins encoded by genes induced during cold acclimation in higher plants. The amino acid sequences are presented with the repeated motifs aligned (in bold) to highlight this attribute of each protein. Additional details about the proteins are presented in the text.

transcriptional activator proteins, known as CBF1, CBF2 and CBF3 (or DREB1b, DREB1c and DREB1a, respectively), that control the expression of a battery of cold-responsive genes in Arabidopsis including the *COR6.6*, *COR15a*, *COR47* and *COR78* genes mentioned above. Constitutive overexpression of the *CBF* genes in transgenic Arabidopsis plants induces the expression of the *COR* and other cold-responsive genes without a low temperature stimulus and results in increased freezing tolerance at the whole plant level (in contrast, expression of *COR15a* alone has no apparent effect on the freezing survival of whole plants) (Jaglo-Ottosen et al., 1998; Liu et al., 1998). In addition, it results in increased tolerance to stresses that involve dehydration including drought and high salinity (Kasuga et al., 1999).

c. Role of antifreeze proteins in freezing tolerance

Antifreeze proteins (AFPs) have been identified in distantly related organisms (see Ewart et al., 1999). The best studied are from fish and insects, but recently, they have also been described in plants and bacteria. A hallmark characteristic of these proteins is "thermal hysteresis" activity: the proteins decrease the temperature at which ice is formed, but do not affect the melting point of the solution. This effect results from the AFPs binding to the surface of ice nuclei and inhibiting ice crystal growth. In addition, AFPs affect the shape of the ice crystals that form when temperatures drop below the freezing point of the solution and are potent inhibitors of ice recrystallization (they inhibit the coalescing of small ice crystals into large ice crystals).

Significantly, AFPs are highly diverse in amino acid sequence and structure (Ewart et al., 1999). In fish, there are four classes of AFPs: type I are alanine-rich α-helixes (M*r* ca. 3300-5000): type II are cystine-rich globular proteins (M*r* ca. 14000); type III proteins form a compact β-sheet structure (M*r* ca. 6500); and type IV proteins are though to form a four-helix bundle. Two AFPs from insects, spruce budworm and mealworm beetle, are rich in threonine and cytosine and have been shown to form β-helixes. In winter rye plants, six AFPs ranging in molecular mass from 16 to 35 kD have been shown to accumulate in the extracellular fluids of cold acclimated plants (Antikainen and Griffith, 1997). Surprisingly, these AFPs are related to pathogenesis-related (PR) proteins: two of the AFPs are endochitinase-like proteins; two are β-1,3-glucanase-like proteins; and two are thaumatin-like proteins (Antikainen and Griffith, 1997). One of the rye AFPs has been purified and shown to have both endochitinase and antifreeze activity (the protein alters the shape of ice crystals and has a low level of thermal hysteresis activity). In contrast, a purified endochitinase from tobacco, a freezing sensitive plant, was devoid of antifreeze activity. In addition, endochitinase-, glucanase- and thaumatin-like proteins are present in cell extracts of nonacclimated rye plants, but no antifreeze activity can be detected. Thus, antifreeze activity is not an inherent property of these proteins. Taken together, these results indicate that antifreeze activity has evolved independently multiple times.

It is important to note that the different activities of the APFs do not appear to strictly correlate. An AFP from perennial ryegrass has been described that has very low thermal hysteresis activity, but is a very potent inhibitor of ice recrystallization (Sidebottom et al., 2000). In addition, the functions of the AFPs appear to differ among organisms (Antikainen and Griffith, 1997; Ewart et al., 1999). In some cases, the AFPs have a role in frost avoidance. In

polar fish living in waters that are about two degrees below freezing, the AFPs, through their thermal hysteresis activity, contribute to keeping the blood serum in the liquid state. However, in organisms that do not avoid freezing, like cold-acclimated rye plants, AFP function is presumably different. In the case of extracellular AFPs, it is possible that the inhibition of ice recrystallization, or the effects that AFPs have on ice crystal shape, might mitigate against physical damage caused by ice and thereby enhance freezing tolerance. Intracellular AFPs, which do not come into direct contact with ice, might have fundamentally different roles. Indeed, it has been shown that certain AFPs (antifreeze gluycoproteins) can inhibit leakage of ions from liposomes during thermotropic phase transitions (Hays et al., 1996). Thus, some AFPs might have important roles in stabilization membranes at low and freezing temperatures. In this regard, it is interesting that certain of the AFPs, like the COR and LEA proteins described above, are hydrophilic; remain soluble upon boiling; and are composed in large part of simple repeated amino acidic sequences.

B. Preliminary Results

1. Sites of isolating permafrost bacteria

Our Arctic study site is the Kolyma-Indigirka Lowland (152-162° E and 68-72° N), which is a tundra ecosystem in Northeastern Siberia adjacent to the East Siberia Sea. Permafrost occurs throughout the area to depths of ~600-800m. These layers were syngenetically frozen (i.e., sedimentation occurred concurrent with freezing) and thus, the age of the permafrost is equal to the age of the sediment. Evidence suggests that buried permafrost in this region has remained continuously frozen since its period of deposition (Gilichinsky et al., 1995). Permafrost cores of Arctic and Antarctic sediments have been collected by slow rotary drilling, so as not to melt them, and without the use of any muds or solutions. The temperature of the extracted core was never higher than -7°C. Surfaces of the extracted core were trimmed away with a sterile knife and the remaining core was immediately placed in pre-sterilized tins, sealed and kept frozen during storage and transport. When ready to use in the laboratory, the frozen core was placed in a sterile hood and fractured with a sterile knife. ***Only internal segments of the core were used for microbiological studies.*** Previously Khlebnikova et al. (1990) had shown that *Serratia marcescens* could not be detected in internal segments of cores that had purposely received surface contamination with this organism in the field.

Our Antarctic permafrost samples were collected in January 1999 during the Beringia expedition to the Antarctica Dry Valleys (Beacon Valley) by the Gilichinsky team. The same coring methods described above were used. The Antarctic sediments are colder (-18 to -25°C), have less organic content (down to 0.1%), and have been frozen longer than those from the Arctic. Our bore hole went through an ash layer that had been dated at 8.1 million years, so the deeper samples must be at least this old.

2. rDNA analysis of prokaryotic permafrost communities

We have sequenced the small subunit (SSU) rRNA genes of dominant bacteria and Archaea clones amplified from Arctic community DNA extracted from permafrost sediments ranging in (frozen) age from 25,000 years to 2-3 million years. Nearly complete SSU rRNA

gene sequences from bacterial clone libraries revealed two major classes of organisms: (i) clones with sequences very similar to *Arthrobacter* and (ii) clones representing a novel gamma Proteobacteria group. The latter bears resemblance to recently reported Fe(II) oxidizing chemolithotrophs that grow at neutral pH (Emerson and Moyer, 1997). Numerous isolates of *Arthrobacter*-like organisms have been obtained from the permafrost, but no isolates of the latter group have yet been recovered. The Archaea clone libraries are even more interesting (Moyer, Tiedje and Petrova, unpublished). Nearly full-length sequences of the majority of the clones reveal a novel cluster within the *Crenarchaeota* which is clearly distinguished from the other *Crenarchaeota* groups, most significantly from the soil and low temperature marine clusters.

Having completed the SSU rDNA analysis of several bore holes and age layers provides us with considerable information for comparison to proposed Antarctic data. This also provides sequence information for design of probes and primers to quantify the major groups, and to aid in cultivation and perhaps isolation of the novel groups.

3. Bacteriologic and molecular characterization of culturable bacteria from Arctic permafrost

Several types of growth media, differing both in nutrient content and in the presence or absence of osmoprotectants, were used to successfully isolate aerobic bacteria, yeasts and fungi from permafrost samples collected in the period 1991-1997. Isolations were all done at 4°C immediately following thawing of the samples, as well as following natural growth of strains in the permafrost soil held at 4°C for 2-18 weeks. Strains from selected samples were also isolated at -2.5 and -4.5°C. Direct bacterial counts in the samples were monitored by staining with epifluorescence using DTAF (Bloem et al., 1995). When surveyed immediately upon thawing, all samples had comparable numbers of total bacterial counts as detected by DTAF staining (average, 10^8 bacteria/g), whereas the number of culturable aerobic bacteria ranged from 0 to 10^2-10^3 CFU/g in samples dating from middle Pleistocene to Pliocene (100,000 years up to 2-3 million years), to ca. 10^5-10^6 in samples dating 25,000-40,000 years. Similarly processed modern tundra soil had 10^5-10^6 CFU/g. Although most isolated cultures were bacterial, several strains of yeasts and fungi were also recovered (Vishnivetskaya et al., 2000).

Over 400 strains, representing as many diverse cultural phenotypes as possible, were isolated and characterized further. With very few exceptions, the isolated bacterial cultures were non-spore forming and grew well in the -2.5 to 20°C range, suggesting that they were psychrotrophic (growing at low temperature but also above 15°C). This was in contrast to the high frequency of psychrophiles (growing at low temperature but not above 15°C) documented in Antarctic sea ice (Bowmann et al., 1997) and in Burton Lake, Antarctica (Franzmann et al., 1990). Growth in the low temperature range, however, was prolific for all strains that we isolated. The bacteria grew readily at -2.5°C, both in liquid and on solid media, and screening of 6 randomly selected strains at -4.5°C revealed that within 7-8 weeks all formed colonies on agar plates. In contrast, an *Arthrobacter* strain isolated from Michigan soil (LTER3) grew at 4°C but was unable to grow below 0°C.

Selected gram-positive and gram-negative strains isolated from different samples, and from different depths of one core sample, were typed by repetitive element Polymerase Chain

Reaction fingerprinting (rep-PCR) (Versalovic et al., 1991). The rep-PCR fingerprints suggested several clusters within both the gram-positive and gram-negative lineages, which were analyzed further by restriction fragment length polymorphisms within PCR-amplified portions of the gene encoding 16S RNA (rDNA). Forty four strains representing distinct molecular fingerprints were characterized by partial 16S rDNA sequencing. These preliminary molecular taxonomy data revealed that the strains were predominantly of 4 groups: Gram-negative strains were in *Psychrobacter* sp. (gamma division of proteobacteria) and in the *Flavobacterium-Bacteroides - Cytophaga* group); Gram-positive bacteria were mostly in the high GC *Arthrobacter* group and in the low GC *Planococcus* group. Gram-positive bacteria tended to predominate (58-85% in the different sediments) and a large proportion were of the high GC groups, especially *Arthrobacter*. About 50% of the strains closely matched (98-100% identity) other lineages in the ribosomal database. Of the 44 strains that were analyzed, 6 were found to have 92-96% identity to their closest relatives in the database, suggesting that they represent novel taxonomic units.

Several themes emerged from the phylogenetic analysis of the bacteria which were isolated from arctic permafrost. It was noted that certain lineages were more frequently retrieved from relatively young (20,000-40,000 yrs) sediments than from the more ancient (200,000 to 2-3 million yrs) permafrost. Thus, *Planococcus* (Fig. 2: Low G+C gram-positives) and *Psychrobacter* (Fig. 2: Proteobacteria) were repeatedly isolated, but only from relatively young sediments. Strains with highest similarity at the level of rDNA sequence (97-99%) to *Arthrobacter psychrolactophilus* were isolated on multiple occasions from different sediments in the relatively young age range, whereas *Arthrobacter* from the most ancient sediments had highest similarity (98-99%) to *A. citreus* and *A. agilis* (Fig. 2: High C+G gram-positives). Certain lineages, such as those with highest similarity (98-99%) to *Rathayibacter, Clavibacter* and *Curtobacterium* (Fig. 2: High G+C gram-positives) and to the gram-negative *Sphingomonas* sp. Ant20 (99-100% identity) (Fig. 2: Proteobacteria) were only isolated from the very ancient (200,000 to 2-3 million yr) sediments. It is worthy of note, although not readily interpretable with currently available data, that the *Rathayibacter-Clavibacter-Curtobacterium* lineages (Fig. 2: High G+C gram-positives) from the ancient sediments were closest to modern bacteria which are associated with plants and agricultural soils. The overall findings, nonetheless, suggest that certain lineages have adaptive features which allow them to compete effectively and predominate in relatively young sediments that contain high numbers of other culturable bacteria but become eliminated as the permafrost ages, whereas others seem to survive preferentially in the very ancient sediments where most other microbes have been eliminated.

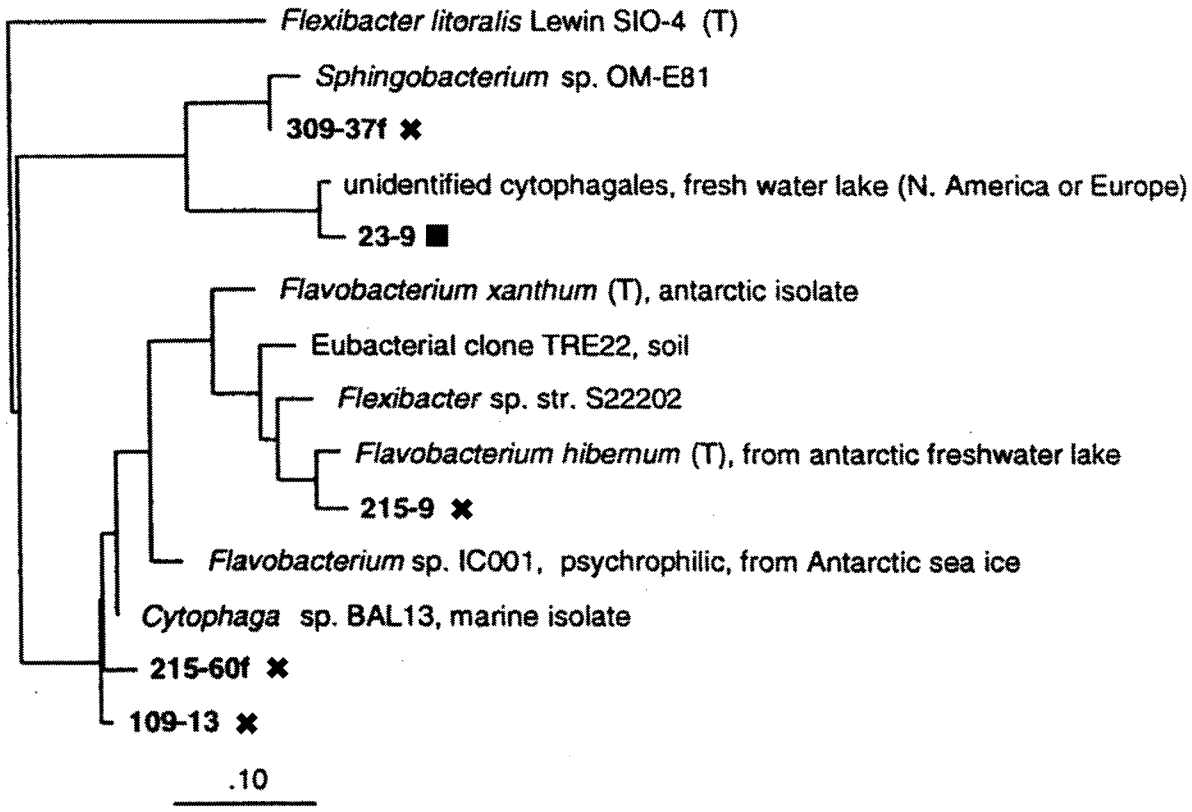
Analysis of the taxonomic data identified one lineage, represented by *Exiguobacterium* strains, which was retrieved from sediments of variable ages (20,000 to 2-3 million yrs) as well as from modern tundra soil (Fig. 2: Low G+C gram-positives). The *Exiguobacterium* lineage appears to represent microbes which can effectively survive under the distinct conditions (including the key impact of time) that characterize permafrost of widely variable ages.

Another interesting finding that emerged from the phylogenetic analysis of the lineages was that 16S rDNA sequences of *Psychrobacter*, *Flavobacterium-Bacteroides-Cytophaga, Sphingomonas* and *Planococcus* strains most closely matched (94-99% identity) with the sequences of strains of the same genera isolated from Antarctic sea ice (Bowmann et al., 1997), Antactic sea brine (Junge et al., 1998), Antarctic soil, and from a freshwater Antarctic lake

Figure 2. Phylogenetic relationships among permafrost isolates.

16S rRNA sequences were compared to public databases (Ribosome Database Project, GenBank) to identify nearest relatives. The phylogenetic trees, generated by maximum likelihood using 337 to 395 homologous nucleotide positions, illustrate permafrost isolates and nearest relatives. Additionally, related type strains (designated "(T)") were included to place relationships within the context of the global bacterial phylogeny. When available, sources of isolation of all relatives are illustrated on the trees. Isolates and clones are color coded based upon the age of their sediments of origin (see code on each tree). Isolates from the current study are indicated by bold type face.

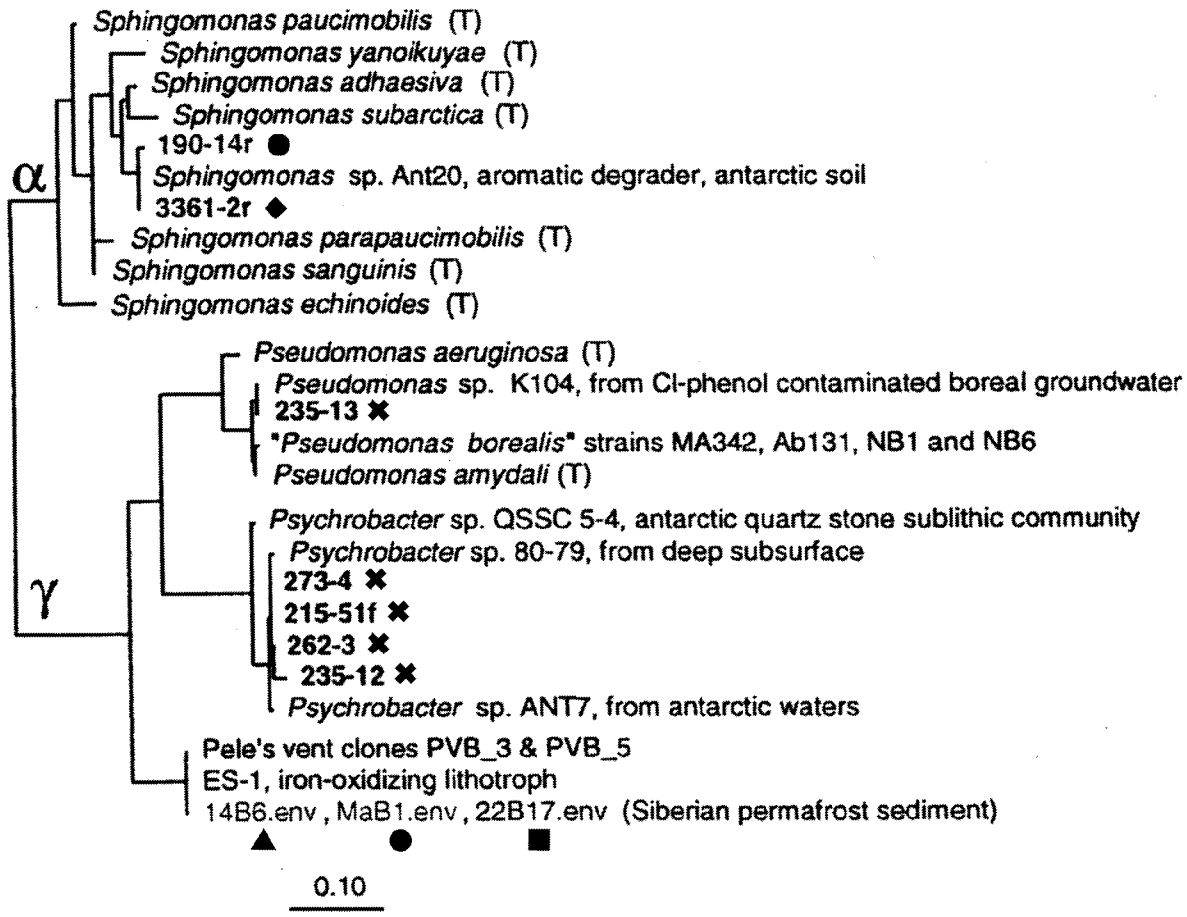
Flavobacteria/Cytophaga



Key:

- **Modern day sample or reference sequence**
✖ **20-30K years**
▲ 60-100K years
● 200-600K years
■ **0.6-2 million years**
◆ **2-3 million years**

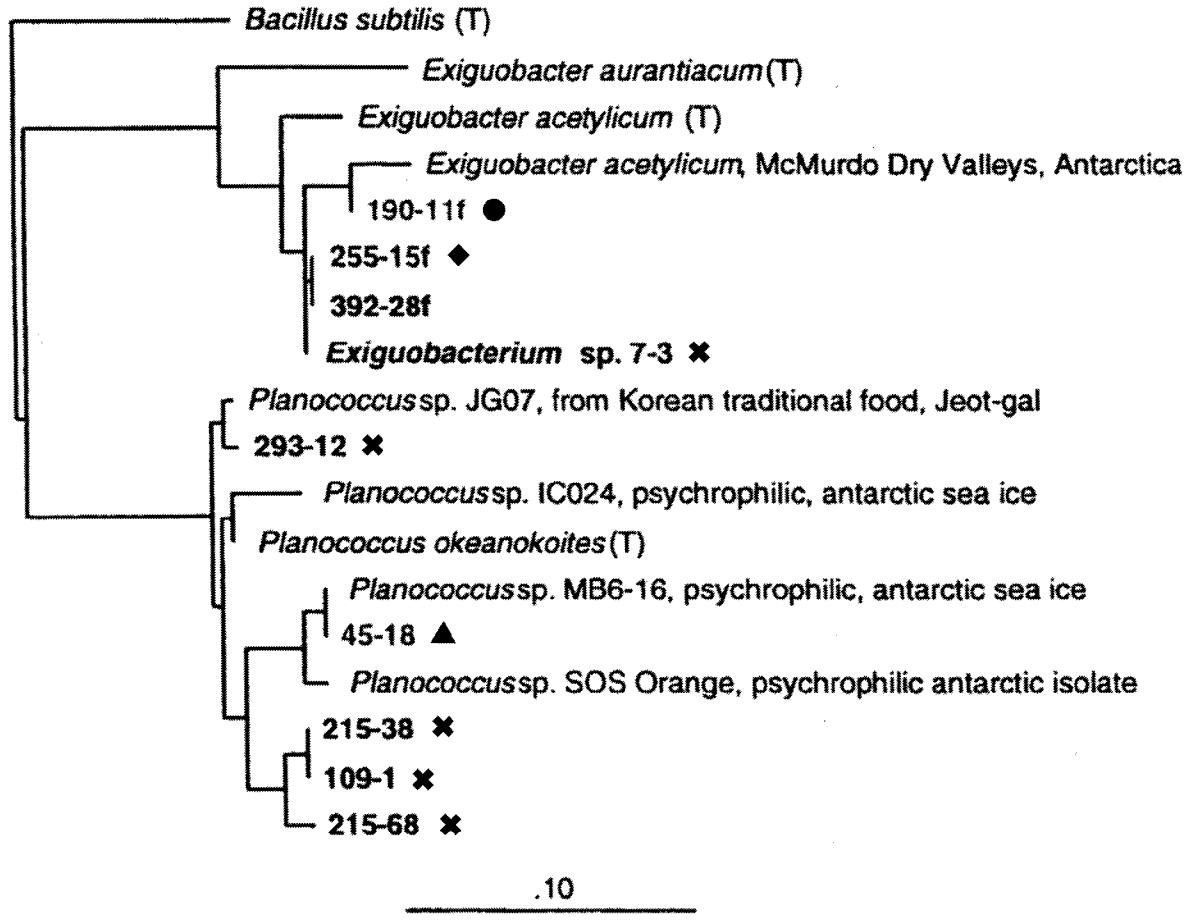
Figure 2. (cont.): Proteobacteria (alpha and gamma subdivisions)



Key:

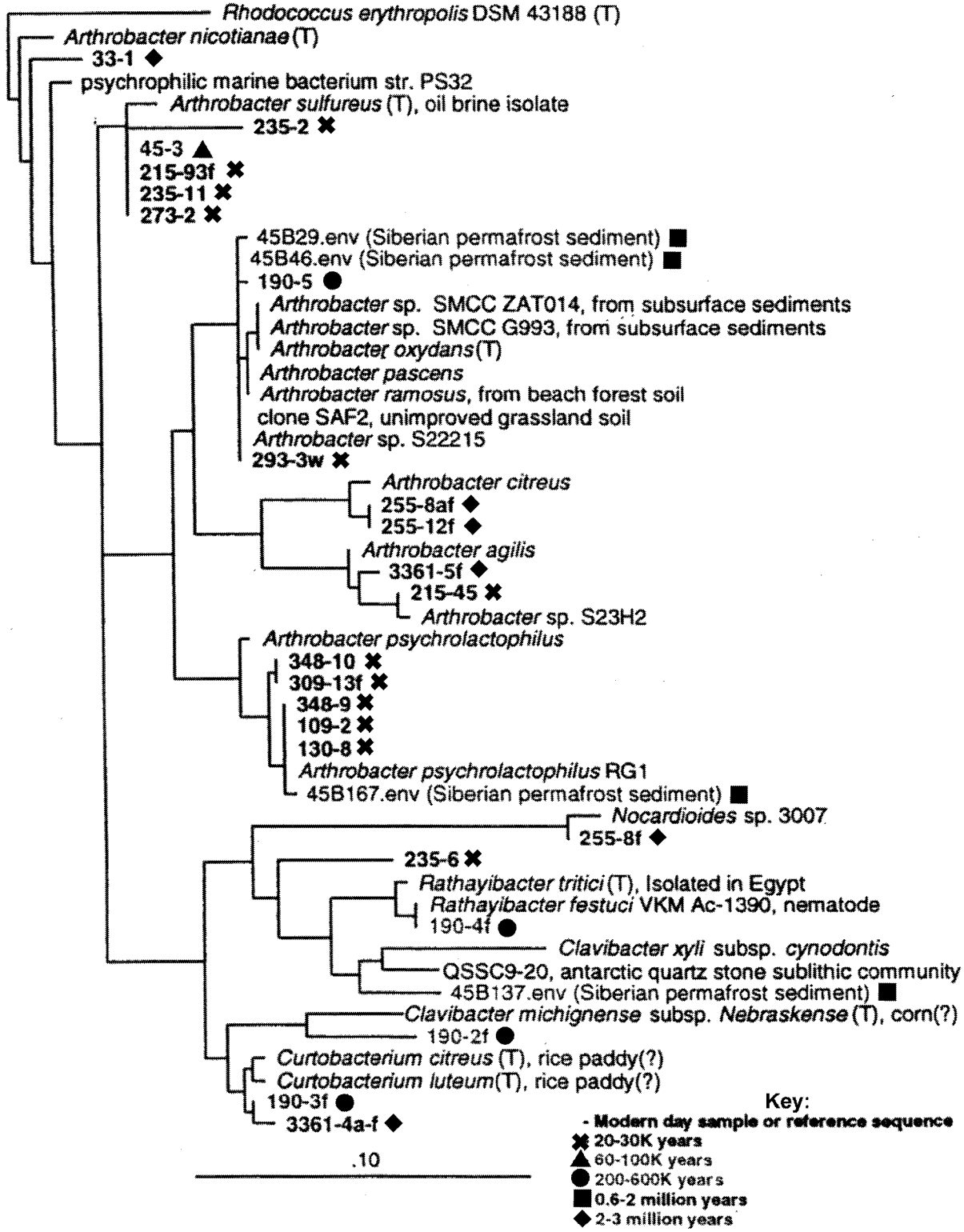
- **Modern day sample or reference sequence**
✖ **20-30K years**
▲ 60-100K years
● 200-600K years
■ **0.6-2 million years**
◆ **2-3 million years**

Figure 2. (cont.): Low G+C gram-positives



Key:
- **Modern day sample or reference sequence**
✖**20-30K years**
▲60-100K years
●200-600K years
■**0.6-2 million years**
◆**2-3 million years**

Figure 2. (cont.): High G+C Gram-positives



(McCammon et al., 1998) (Fig. 2). In addition, the closest matches for several of the *Arthrobacter* strains were *Arthrobacter* from Antarctic sea ice brine (Junge et al., 1998) and from deep subsurface sediments (Boivin-Jahns et al., 1995).

These findings suggest that there may be an ecological and/or adaptive link between bacteria from the Arctic permafrost and those from Antarctic habitats. The fact that the Arctic permafrost bacteria were proficient in low temperature growth, but were not limited to it, may suggest that they represent globally distributed, ancient lineages of highly cold-tolerant microorganisms that specialized in the Antarctic sea ice and lakes to become more cold-adapted, and more limited in their temperature range. This has been suggested by other studies of the comparative distribution of psychrophilic bacteria and their non-psychrophilic relatives (Franzmann, 1996).

4. Responses of permafrost bacteria to low temperature

a. Compatible solutes and lipids

We have used HPLC to show that several of our permafrost strains produce compatible solutes when they are grown at low temperature (4°C) compared to growth at 20°C (McGrath, unpublished). In three of the four strains tested estimated intracellular concentrations of solutes increased significantly. In one case, glutamate + proline increased from 200 mM (at 20°C) to 1200 mM (at 4°C). However, in another, the metabolite pool (per unit quantity of cells) decreased in total metabolites when cells were grown at 4°C.

In agreement with findings from numerous other bacterial systems (Russell, 1990), examination of the cell lipids of seven permafrost strains using the MIDI system and FAME analysis showed that the lipids were altered when the permafrost microbes were grown at low temperature (4°C) compared to those grown at 20°C (Wagener et al., 1993). The general trend was increased fatty acid unsaturation when grown at low temperature, and no significant change in average fatty acid chain length.

b. Freezing tolerance

Freeze tolerance of bacteria has mostly been studied using rapid, serial freeze-thawing treatments (Pannoff et al., 1998; Thammavongs et al., 1996). The ecology of the permafrost, however, suggested that it would be more relevant to determine tolerance of the bacteria to prolonged exposure to subzero temperatures, or to intermittent thawing followed by prolonged freeze periods. Experiments with several permafrost strains showed marked impact of low temperature growth on freeze tolerance. Bacteria grown at 20°C and 4°C were frozen at -20°C and survival was monitored over 5 months. It was found that during this time survival of 20°C-grown bacteria decreased markedly (in strain ED 23-9 survival dropped from 0.8×10^8 to $<10^4$ CFU/ml in a representative experiment), whereas survival of 4°C-grown bacteria was much less affected (a drop from 0.9×10^8 to 0.5×10^8). Interestingly, growth of the bacteria at 10°C did not confer any protection to prolonged freezing, and growth at temperatures below 4°C (2.5, 0 and -2.5°C) was as protective as growth at 4°C. We found that 4°C growth also conferred significant protection to cells subjected to intermittent thawing followed by prolonged (1 week)

periods of freezing. In contrast, 4°C or 2.5°C growth failed to confer marked protection to long-term freezing and freeze-thawing in a psychrotrophic strain from Michigan soil, LTER3 or another psychrotrophic bacterium, *Listeria monocytogenes*.

c. Detection of respiratory activity in permafrost bacteria

The dye CTC (5-cyano-2,3-ditolyl tetrazolium chloride) can be used as a fluorescent redox probe for the direct visualization of actively respiring bacteria. CTC is a nearly colorless artificial electron acceptor that is readily reduced to insoluble, highly fluorescent, and intracellularly accumulated CTC-formazan by bacterial respiration. Since the fluorescence emission of CTC-formazan is primarily in the red region, actively respiring bacteria can be distinguished from nonrespiring bacteria and nonbiological material that typically emits in the blue to blue-green regions (Rodriguez *et al.*, 1992).

We have assessed the use of CTC to determine metabolic activity of bacterial cells from permafrost soils using both laboratory strains and the permafrost samples themselves. We have found that that the detection of metabolic activity using CTC reduction is possible under all of the conditions of interest to us. These include: both gram- positive and gram-negative permafrost strains grown at both low and high temperatures, in the frozen state, with and without high salt concentrations, in cells grown in liquid or on solid medium, in assays done at low or high temperatures, within soil slurries with and without the addition of an additional carbon source. In addition, activity was also detected in the permafrost samples (which were thawed and kept at 4°C during the assay) by direct epifluorescence. Control experiments show that only intact, living, and respiring cells seem to exhibit reducing activity on the CTC dye. Sensitivity and kinetic details remain to be established. We believe that it will be possible to use laser confocal microscopy to determine metabolic activity *in situ* in permafrost soil samples in their original state, i.e. to study a freshly thawed permafrost sample, rather than samples incubated as a soil slurry in a buffered aqueous solution with CTC.

5. Development of methods for proteomic analysis of permafrost bacteria

The Lubman laboratory has been developing methodologies for rapid screening of the protein content of bacterial cells using fast nonporous reversed-phase (NP-RP) HPLC separation of proteins (Kalghatgi and Horvath, 1990; Jenke, 1996; Barder et al., 1997). As each protein fraction elutes, it is collected in the liquid state from the HPLC separation for analysis by mass spectrometry (MS). The use of NP C18 coated silica based packing materials, as compared to the conventional porous materials, is the key issue in this work. The separation of complex protein mixtures from cells can be accomplished in 1/3-1/4× the time required for porous packed column separations with much enhanced protein recovery and resolution. The use of NP packing materials prevents proteins from sticking inside the pores of porous materials, thus allowing much greater speed of separation, a decrease in chemical noise background, and enhanced protein recovery.

In complex protein mixtures, a single NP-RP-HPLC separation may not be sufficient to resolve all the proteins. In present work, Lubman and coworkers have been using two NP columns on-line to separate out complex mixture of proteins. By using a 4.6×33 mm column at

60°C followed by a second shorter column, 4.6 × 14 mm, held at 25°C, over a hundred proteins from a Siberian Permafrost bacterial whole cell lysate could readily be separated. One important advantage of the method is that excellent separations can be obtained using two columns held under different conditions and this can be preformed on-line in 15-30 minutes. A second important advantage is that a substantial amount of material can be loaded on the column and high efficiency in recovery achieved. In recent experiments, it has been shown that over 350 μg can be loaded onto the system and the final recovery after separation by the second column is nearly 90 % for water-soluble proteins under 30 kDa. The recovery of protein will be critical for detailed sequencing experiments. The key here is that although some overloading with loss of resolution may occur on the first column, this is compensated for by separation of the eluting peaks from column 1 to column 2. Even though not a genuine 2-D LC separation, the results are very encouraging for the proposed work. In addition, the HPLC separation is highly reproducible on each different run and we are able to quantitate changes in peak profile for expressed proteins with ± 10 %.

The tandem-column NP-RP-HPLC has been used as a rapid method for separation and isolation of proteins in the liquid phase from bacterial whole cell lysates for further analysis by MALDI-TOF MS and CE-ESI-MS. Bacterial strain ED 7-3, isolated from the Siberian Permafrost, was studied in order to examine cold shock response and resulting induction of cold shock proteins (CSPs) at 4°C and room temperature (25°C). The whole cell lysates are rapidly profiled and separated using a tandem-column NP-RP-HPLC method where distinct protein profiles were obtained from each culture. Proteins that were differentially expressed at 4°C were collected and sized for molecular weight by MALDI-TOF MS. The proteins were digested with trypsin to generate peptide maps for identification by MALDI-TOF MS analysis and further confirmed by CE-ESI-MS using database searching procedures (Mortz et al., 1994; Ducret et al., 1998; Chen et al., 1999). As a result, several CSPs and other cold-shock related proteins could be identified.

As an example, Figures 3a and 3b show the protein profiles of the whole cell lysates of ED 7-3 using the tandem-column NP-RP-HPLC method. Employing a relatively short gradient program (about 25 minutes), distinct profiles were obtained for each growth temperature. For the cold (4°C) culture, 7-3-4 (Fig. 3a), the majority of proteins elute between 3 and 17 minutes, while for warm (25°C) culture, 7-3-25 (Fig. 3b), most of the proteins elute after 15 minutes into the gradient. The profiles show that in the culture grown at 4°C, a large number of new proteins were expressed relative to the 25° culture. Some of these protein peaks have been identified (Table 1) including several CSPs such as CSP C, CSI14B, CSI15, and CSI5. Other proteins that are highly expressed belong to the general stress family (GSP170), translational and transcriptional family (IF-1, SENS, and ABRB), degradation regulation enzyme family (DEGQ), and the ribosomal family (S6). Trisephosphate isomerase (TIM) and RNA polymerase sigma-B factor (Sigma-37) are also highly expressed. This RNA-polymerase is also known as general stress protein 84 or GSP84. Many of these proteins are housekeeping proteins and have been previously identified in *B. subtilis* and other bacteria (Jones and Inouye, 1994; Bayles et al., 1996; Berger et al., 1996; Bae et al., 1997; Panoff et al., 1997). They are essential for the proper function of the bacterial cell during the cold shock process (Graumann et al., 1996, 1997, 1999; Kaan et al., 1999).

Figure 3a. NP-RP-HPLC protein profile of the whole cell lysate of 7-3-4 culture (4°C).

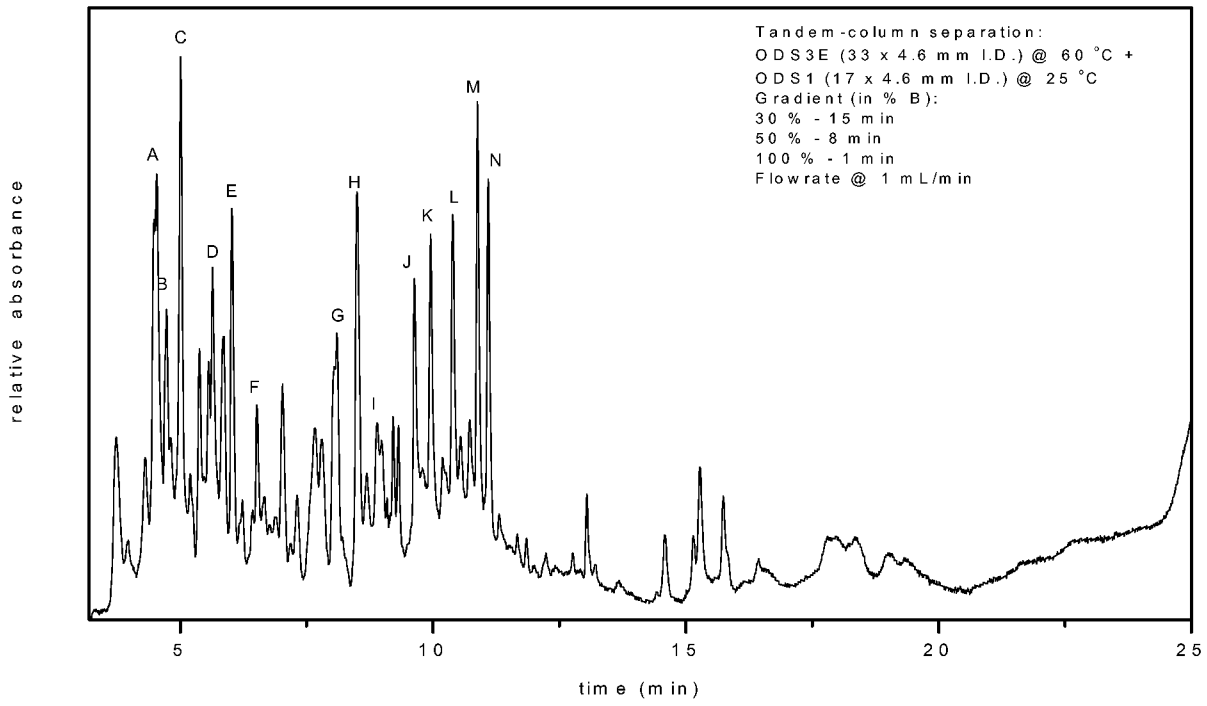
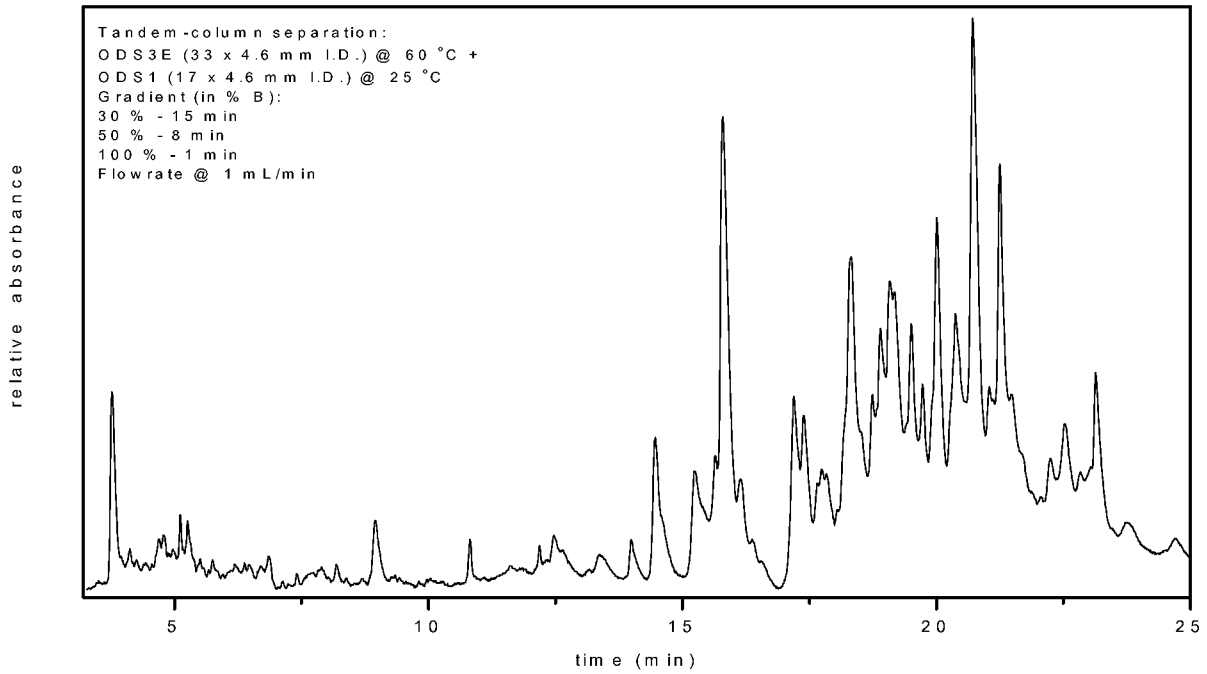


Figure 3b. NP-RP-HPLC protein profile of the whole cell lysate of 7-3-25 culture (25°C).



This tandem-column NP-RP-HPLC method has several advantages for separation of proteins from whole cell lysates in these studies compared to more traditional 1-D or 2-D polyacrylamide gel electrophoresis methods. A major advantage of the NP-RP-HPLC method is the separation and isolation of proteins in the liquid phase. The purified proteins in the liquid phase are readily amenable to analysis by MALDI-TOF MS or CE-ESI-MS as compared to gel methods, which require time consuming extraction, destaining, and purification procedures for analysis of the proteins embedded in the gels. In addition, even after these purification procedures the percent coverage of the protein digests of proteins isolated by NP-RP-HPLC is generally much improved compared to that observed for proteins excised from gels. This will be important for identification and detailed sequencing by MS analysis.

Table 1. Proteins induced during cold shock response in ED 7-3 at 4 °C . Tryptic peptide maps used for MS-Fit database-search were obtained from both CE-ESI-MS and MALDI-TOF MS

Peak	Protein Name	**kDa / *pI***	**AC**
A	[still to be determined]	–	–
B	[still to be determined]	–	–
C	DNA-binding protein II (HB)(HU)	9884.3 / 8.96	P08821
C	Stage V Sporulation protein M	3017.8 / 10.57	P37817
D	Cold shock protein CSI4B (9 KD Cold shock protein)	1924.3 / 4.52	P81094
D	General stress protein 170 (GSP170)	2158.4 / 8.80	P80242
E	Cold-shock induced protein 15 (CSI15)	2376.8 / 10.83	P54616
E	Transcriptional regulatory protein SENS	7912.5 / 10.00	P21344
F	Translation initiation factor IF-1	8213.6 / 6.83	P20458
G	[still to be determined]	–	–
H	Cold shock proteinC (CSPC)	7255.0 / 4.72	P39158
H	Cold shock protein CSI5 (11 KD Cold shock protein)	1359.7 / 11.17	P81095
I	Transcription state regulatory protein ABRB	10772.7 / 6.29	P08874
J	Degradation enzyme regulation protein DEGQ	5546.5 / 6.16	Q99039
K	30S Ribosomal protein S6 (BS9)	11124.6 / 5.2	P21468
L	[still to be determined] (General stress protein 84) (GSP84)	–	–
M	Trisephosphate isomerase (TIM)	27029.8 / 5.00	P27876
N	RNA polymerase sigma-B factor (Sigma-37) (General stress protein 84) (GSP84)	29901.4 / 5.55	P06574

A second advantage of the NP-RP-HPLC method in these studies is the speed of analysis, whereby a typical tandem-column separation of cell lysate may only take 30 minutes. In comparison, an equivalent 1-D gel separation might require at least 6 hours to obtain reasonable separation, whereas 2-D gel separation would require up to 24 hours. Further, the liquid separation can be totally automated and the data is obtained in digitized form. The gel methods are not readily automated and the gel image must be digitized. Moreover, the NP-RP-HPLC method provides improved separations over 1-D gels. Although 2-D gels provide higher resolution than the NP-RP-HPLC method, the resolution obtained is sufficient for these studies when combined with MS capabilities.

The use of MALDI-TOF MS can provide molecular weight information of the proteins separated in HPLC, while MALDI-TOF MS and CE-ESI-MS can provide mass analysis of peptides generated by enzymatic mapping. By using this information with a database search, we were able to identify many of the bacterial proteins separated by NP-RP-HPLC. Only proteins that appeared in both search results were used as possible candidates for identification. It should be noted that many of the collected fractions from NP-RP-HPLC were shown to contain two or more proteins when sized by MALDI-TOF MS. For these fractions, the MS-Fit database search on the tryptic peptides was performed twice (or more) using the corresponding mass range for each protein. When digesting these fractions, a blank tryptic digest was always performed and then analyzed on MALDI-TOF MS for trypsin autolysis peaks. A continuation of this work will involve performing another digest, possibly CNBr followed by trypsin, to further ascertain the identity of these proteins as well as tandem MS analysis on peptides of interest.

Both MALDI-TOF MS and CE-ESI-MS were used together to confirm the validity of protein identification. CE-ESI-MS detects mainly the lower mass range (up to 1500 Da) of the tryptic peptide fragments, whereas MALDI-TOF MS is more efficient in detecting peptides between 1000 and 3000 Da. Due to interference from MALDI matrix, peptides of 500 Da and under are generally suppressed. The two methods complement each other in terms of total peptide coverage of the total protein map. By combining the peptide maps from both techniques, the total peptide coverage for CSP C, for example, was increased to 85 % (55/66 amino acids matched). Similar results were obtained for other proteins in Table 1.

It should be noted that a database for ED 7-3 is currently not available for MS-Fit searches. In these studies, *B. subtilis* was used to conduct the MS-Fit searches. *B. subtilis* is the species phylogenetically closest to ED 7-3, for which the genome has been completely sequenced and annotated. Even though strain ED 7-3 (*Exiguobacterium sp*) and *B. subtilis* are distinct phylogenetic units, and may not be closely related, the mass spectral tryptic mapping procedure from CE-ESI-MS and MALDI-TOF MS provided reasonable close matches to known proteins of *B. subtilis*. However, the unavailability of an appropriate existing database for other Permafrost bacteria under study in this work, would limit the usefulness of these methods. The ability to sequence these organisms and generate such database is an important part of this study and makes our MS methods powerful tools for identification and sequence verification.

C. Experimental Plan

Objective 1. Permafrost Structural Genomics: determine the complete genome sequence of one permafrost bacterium and obtain substantial coverage of nine additional isolates.

The permafrost bacterial isolates that we will be examining are ones that had been frozen for a minimum of 20 to 30k years up to more than 1 million years. A fundamental question raised is whether these bacteria have unique genes that have enabled them to remain viable in this environment for such long periods of time. As a first step to address this question, we will determine the complete genome sequence of one permafrost organism and obtain substantial sequence coverage of nine additional isolates. These results will provide a broad view of the "informational content" of the genomes such as knowledge of the basic housekeeping genes that are carried by the permafrost bacteria; insights into the metabolic pathways used by the bacteria, some of which might be involved in the synthesis of compatible solutes or other low molecular weight cryoprotectants; and may provide signs of peculiar genome features (sequence or organization) that may be a consequence of the bacteria having been exposed to low background radiation for long periods of time while confined to an environment capable of supporting only low metabolic rates. Of paramount interest, however, will be a gaining of insight into the novel informational content of the genomes. How much of the genome of each isolate is composed of novel genes? Are any of these novel genes highly conserved among the permafrost bacteria even though they represent distantly related species (potentially signaling significant lateral gene transfer)? Are such novel genes present in closely related bacteria that reside in warm environments that have experienced little or no freezing for long periods of time? What is the nature of the polypeptides encoded by the novel genes? Do they have structural and biochemical features that resemble the highly expressed COR and LEA-like polypeptides associated with freezing tolerance in plants? The results from the sequencing efforts will provide fundamental information needed to address these questions. In addition, they will provide the basic resources required for the expression profiling experiments that will be conducted as part of our Functional Genomic analyses, Objective II of our Experimental Plan.

a. Choice of permafrost bacteria to be sequenced.

Table 2 describes the characteristics of a selected group of 10 permafrost strains which appear especially promising candidates for genomic studies. These strains were chosen following a careful consideration of their potential to serve as model systems for special adaptive features of relevance to survival in the permafrost. The selection includes representatives of all lineages that have been isolated from the very ancient sediments as well as lineages predominant or exclusively isolated from relatively young sediments (e.g. *Planococcus, Psychrobacter*). The high-GC gram-positives, which predominate in permafrost, are well-represented. In the case of *Exiguobacterium*, the two listed strains represent isolates from sediments of markedly different age, thus providing special opportunities for comparative genomic analyses. In addition, the selection includes bacteriologic features which are predominant among permafrost strains, and which may have special adaptive physiology roles, such as pigmentation and motility. It is of interest that both of these features have been noted in cultures grown at -2.5°C. It would be of interest to pursue the complete genome sequencing of

strain ED7-3, since this strain has been already included in the proteomic studies, and pursue partial genomic sequencing (80% coverage) of the other nine strains.

Table 2. Characteristics of selected strains from Siberian permafrost of special interest for genomic studies.

Strain	Age of sediment (years)	Group	Bacteriologic features	Closest relative in database
255-12f[1]	2-3 million	High-GC gram-positive	Pigmented (yellow), rods, motile, growth at –2.5°C	*Arthrobacter citreus*
215-93f[1]	20-30K	High-GC gram-positive	Pigmented (yellow), rods, motile, growth at –2.5°C	*Arthrobacter sulfureus*
190-4f[1]	200-600K	High-GC gram-positive	Pigmented (orange), oval rods, growth at –2.5°C	*Rathayibacter tritici*
3361-4a-f[1]	2-3 million	High-GC gram-positive	Pigmented (orange), cocci, motile, growth at –2.5°C	*Curtobacterium* sp.
ED7-3[2]	20-30K	Low-GC gram-positive	Pigmented (orange), rods, motile, growth at –2.5°C	*Exiguobacterium sp.*
255-15f[2]	2-3 million	Low-GC gram-positive	Pigmented (orange), oval rods, growth at –2.5°C	*Exiguobacterium sp.*
45-18[2]	20-30K	Low-GC gram-positive	Pigmented (orange), cocci, growth at –2.5°C	*Planococcus sp.*
3361-2r[3]	2-3 million	Gram negative α proteobacteria	Pigmented (orange), rods, motile, growth at –2.5°C	*Sphingomonas sp.* Ant20
23-9	20-30K	Gram negative Favobacterium-Cytophaga	Pigmented (yellow), rods, growth at –2.5°C	*Flavobacterium sp.*
262-3[3]	20-30K	Gram negative γ proteobacteria	Rods, growth at –2.5°C	*Psychrobacter sp.*

[1]Phylogenetic placement shown in Fig. 2: High GC gram-positives
[2]Phylogenetic placement shown in Fig. 2: Low GC gram-positives
[3]Phylogenetic placement shown in Fig. 2: Proteobacteria

Further characterization will determine whether these strains satisfy additional criteria of relevance for genomic and genetic studies including: 1) the genome should be 2-3 Mb or less (i..e., an average size for bacteria, or smaller; in this regard it is important to note that we have recently determined that the *Psychrobacter* isolate 262-3 listed in Table 2 has a genome size of 2 Mb); 2) the isolate should be more freezing tolerant that typical mesophiles such as *E. coli*, and preferably, would increase in freezing tolerance upon low temperature treatment (as noted above, this characteristic has been demonstrated for several of the permafrost strains); 3) there should be the potential for developing a genetic system for the organism (e.g., it should be sensitive to standard antibiotics used in transformation selection); and 4) for at least some isolates, we will want to have available closely related bacteria from an environment that has not been exposed to

freezing to conduct comparative genomic and adaptive physiology studies. We anticipate that the availability of the *Exiguobacterium* isolates will be of substantial value in this regard. Currently our collection includes a "temporal freeze series" of closely related *Exiguobacterium* strains derived from permafrost layers that have been frozen for 20-30K, 200-600K, and 2-3 million years, as well as from modern tundra soil.

2. Genome sequencing

DNA sequencing will be carried out at the DOE Joint Genome Institute using a whole-genome shotgun approach. For complete genome sequencing, the majority of sequence will be generated from ~2 kb and ~10 kb insert libraries (6x and 3x sequence coverage respectively), along with a small amount of fosmid end-sequence (5x genome coverage). The JGI sequencing operation is a high-throughput facility employing 84 capillary sequencers (MegaBACE 1000). At the current pass rate (~85%), readlength (550bp) and throughput (>1 million lanes per month), it is possible to draft sequence a 3 Mb genome in approximately 1 day of sequencer time (1-2 weeks prep time). Sequencing from both ends of plasmid DNA generates paired-end sequences critical for providing order and orientation information for the contigs resulting from sequence assembly. This, combined with a new whole-genome assembly program currently in development at the JGI, is expected to produce near-finished quality sequence from the shotgun data. The few remaining gaps and low quality sequence regions can be addressed manually.

The resulting genome sequence will be run through our semi-automated microbial annotation pipeline. Gene models are selected using a combination of 3 gene-finding programs (Generation, Glimmer, Critica), and annotated based on BLAST match, as well as CoGs and metabolic pathway assignments. The various RNA genes as well as sequence repeats are identified separately. Final assignments and genome annotation will be done by manual curation by both JGI personnel and outside experts. Of particular interest will be predicted ORFs encoding polypeptides similar to described antifreeze proteins, as well as ORFs similar to the freezing tolerance associated COR and LEA-like proteins of plants.

DNA sequencing of 9 additional microbes will be performed at 2-3x coverage using ~2kb insert libraries; a process estimated to require ~2-3 days of sequence capacity. This scanning should be sufficient to identify the majority of genes in these microbes. Plasmid DNA from all ~2 kb libraries will be made available for ORF amplification using a common set of primers.

3. Gene conservation.

Computational comparisons of DNA sequences obtained for the permafrost bacteria will enable us to determine whether there are genes, other than ones involved in "housekeeping" functions, that are conserved between the permafrost bacteria. Such genes would be of great interest, especially if found among distantly related isolates (as determined from the 16S rRNA gene sequences) as they might have roles in freezing tolerance and might be examples of lateral gene transfer. We will also, however, determine whether the genes from a given "sequenced" permafrost isolate are present in closely related isolates obtained from either the permafrost or "warm" environments. Genes present in the permafrost isolates and missing from the "warm" isolates would again be candidate "freezing tolerance" genes.

We will conduct this comparative analysis by "DNA-DNA" microarray analysis. Microarray slides will be prepared from PCR products generated from the genomes of the sequenced permafrost isolates. With the organism for which we have determined the entire genome sequence, we will design oligonucleotides to amplify PCR products for all of the open reading frames that are predicted to encode proteins. Given what has been found in other bacteria for which there is complete genome sequence, we anticipate there being about 3000 open reading frames (assuming a 2.5 Mb genome). For the permafrost genomes that have not been completely sequenced, we will choose about 2000 plasmids carrying open reading frames (we will include all "novel" sequences) and amplify PCR products using a set of primers that surround the cloning site in the plasmid (the same set of primers will be able to be used for all of the cloned inserts). Because the yield of PCR product can vary quite a bit for a given reaction (more than 10 fold), we will quantify the PCR products and adjust all DNA concentrations to a fixed amount before preparation of arrays. Preparing thousands of plasmids, PCR reactions and adjusting DNA solutions will be a major time and resource investment. Fortunately, the MSU Genomics Technology Support Facility has BioMek 2000 and Quiagen 3000 robots which can be programmed to carry out many of the repetitive manipulations. Moreover, Dr. Tom Newman, who is the director of the facility, has extensive experience in handling, cataloging and storing more than 35,000 clones and DNA preparations from previous projects, including a pioneering Arabidopsis EST project conducted at MSU (Newman et al., 1994).

The microarray slides will be hybridized with Cy-labeled genomic DNA isolated from the "reference" (i.e. organism from which the PCR products were produced) and "test" (other isolates from permafrost and warm environments) organisms. Standard protocols will be used (e.g., http://cmgm.stanford.edu/pbrown/protocols/4_genomic.html). In brief, total DNA from the homologous and test organisms will be labeled with either FoluroLink Cy5-dCTP or Cy3-dCTP (Amersham Pharmacia) by random-priming (e.g., using the BioPrime labeling kit of Gibco/BRL). The probes will be purified, combined, denatured and then hybridize to the microarray slides as described (http://cmgm.stanford.edu/pbrown/protocols/5_hyb_yeast.html). Hybridized arrays will be scanned with a GenePix 4000 laser scanner (Axon) using laser lights of wavelength at 532 and 635 nm to excite the Cy3 dye and Cy5 dye, respectively. Fluorescent images will be captured as multi-image tagged image file format (TIFF) and analyzed with GenePix Pro 3.0 software (Axon) and software obtained from the National Human Genome Research Institute (NHGRI). This publicly available software consists of a suite of programs that performs spot location; background calculation; signal intensity measurements for each channel; and calculations of the ratio of intensities. The software normalizes the signals and provides a statistical estimate of the significance of the ratio values. This data will be entered into a database constructed using the schema provided by the NHGRI. The NHGRI has also developed tools that allow sophisticated analysis of large data sets. Using Websql from Sybase and Java applets from NHGRI, the database containing the accumulated array data can be queried using a web browser.

Although microarray analysis is becoming incorporated into more and more research programs, it is not a trivial undertaking. However, the Tiedje, Lenski and Bennett laboratories have experience conducting such analyses and our colleagues at MSU have been at the forefront of structural and function genomic analyses. Researchers at MSU, including Thomashow, were

among the first to conduct a large scale Arabidopsis EST project (Newman et al., 1994). Moreover, faculty at MSU are investigators in the Arabidopsis Functional Genome Consortium (AFGC), an NSF-funded plant genome project (http://afgc.stanford.edu/). The specific task that MSU has in this multi-institutional research effort is to conduct microarray analysis experiments in collaboration of individual labs in the Arabidopsis research community. In short, we will have access to requisite equipment (robotics, slide printers, array readers) and technical expertise to carry out the comparative DNA-DNA experiments described here and the RNA profiling experiments described below.

Objective 2. Permafrost Functional Genomics: gene expression profiling, genetic analysis and targeted protein exploration to identify genes and proteins that enable bacteria to inhabit the permafrost environment.

As in space, low temperature and low water activity are cardinal characteristics of the permafrost environments. Thus, a fundamental "first stage" goal of our research program will be to detail how the genomes of the permafrost bacteria respond to these conditions at a gene expression level. How much of each permafrost bacterial genome is devoted to encoding cold- and dehydration-responsive genes? What is the nature of the genes that are induced in response to low temperature and dehydration stress? Are bacteria in a frozen environment capable of altering gene expression? These experiments will provide a broad picture about the numbers and types of genes that are specifically induced at low temperature and low water activities. In addition, they will set the stage for detailed genetic analysis of genes and proteins that enable bacteria to inhabit the permafrost environment. And finally, we will conduct a targeted search for candidate proteins with potential roles in freezing tolerance. In particular, we will explore whether the permafrost bacteria synthesize antifreeze proteins and potential cryoprotective COR and LEA-like hydrophilic polypeptides.

1. RNA microarray profiling

RNA microarray analysis is one approach that we will used to determine how gene expression in the permafrost bacteria is effected by temperature and water availability. DNA microarrays will be prepared as described above for the "DNA-DNA" hybridization experiments. RNA will be isolated from bacterial samples, Cy-3- and Cy5-labeled probes prepared and microarrays hybridized using protocols that have been proven effective with other bacteria (Richmond et al., 1999; Talaat et al., 2000; de Saizieu et al., 2000; http://cmgm.stanford.edu/pbrown/protocols), though it is likely that modifications will be needed to optimized the procedures for our specific isolates. The bacterial isolates will be grown in liquid culture at temperatures ranging from the maximum growth rate of the isolate down to 0°C (e.g. 20, 10, and 0°C). In addition, we will expose cells to subzero temperatures. High subzero temperatures, such as -2°C, will not result in freezing due to the solute concentration of the medium, and thus, will simply represent a continuum of temperature range. However, at lower subzero temperatures, freezing will occur which, as described above, will add a second environmental "stress," namely cellular dehydration. At -5°C, for instance, the nonfrozen liquid will be at approximately 2.5 osmolar (the amount of unfrozen water at -5°C will depend on the starting osmolarity of the medium.)

Our primary interest is to determine what genes are continually expressed during active growth at a given temperature. In these experiments, bacterial cultures will be maintained at the temperature of interest for many generations and gene expression will be determined in cells from exponential phase. This strategy will hold for all temperatures where the cultures remain liquid. However, the situation becomes more problematic with the cultures are in the frozen state. In this case, we will take cells that have been grown at relatively high (e.g. 20°C) and low (e.g. 0°C) temperatures and transfer the cultures to subfreezing temperature of -5, -10 and -20°C. RNA samples will be prepared from the starting cultures before freezing and harvested from the frozen cultures after 1 week, 1 month, 1 year and then once a year thereafter. The major question will be whether the bacteria are capable of altering gene expression in a frozen environment and if so, to determine what portions of the genome are affected. In our experiments, we will also expose bacteria to osmotic "stress" to determine how they respond to decreased water activity at a given temperature. The osmolarity of the cultures will be adjusted by the addition of molecules that the organisms cannot metabolize such as sodium chloride.

Managing the data associated with the proposed microarray experiments will require a well-planned bioinformatics and laboratory information management (LIMS) support system. This is due, in part, to the fact that the volume of data produced by these projects is very large. As the research community gains more experience with microarray technology, the preferred algorithms for analysis of microarray data continue to evolve. In addition, our data management solution needs to be able to serve geographically distributed collaborating researchers (web based). These features are not specific to microbial microarray projects. However, microbial projects present some specific additional challenges. For example, unlike EST-based efforts, we are not guaranteed that each probe targets a single gene, or even a single transcription unit, and our concept of what each probe targets will likely change over the course of the project. It will be important for the information management system to automatically link all array hybridization results to our most current target picture. Other unique features include the project's multi-organism focus and resulting need to track potential for cross-hybridization.

Tiedje and co-workers are currently developing the data management system for another microbial microarray project and intend to use this system with modification on this project. The system is being built on top of the Oracle RDBMS and uses a layered architecture conforming to the Sun Enterprise Java specification. It will consist of one unit that will provide tracking of the probes and other reagents used to construct the microarrays and perform the hybridizations, including organism, gene target, and probe synthesis aspects. A second unit will store post-hybridization scan and analysis data in a software package neutral format. The system will accept and provide these data through a web interface to support remote collaborators. We have completed design and implementation of the 20 relational tables making up the probe tracking unit. We are currently implementing the Java - web layers to handle the requests (queries) required for this unit. In addition, we have developed detailed designs (schemas) for the second unit and expect to begin implementation as soon as the first unit is operational. We believe that this information system can be easily modified for the work proposed here. We anticipate that most of the work will involve gathering and implementation of project-specific use cases and presentation logic, with only relatively minor changes necessary in the lower levels of our layered architecture. We will need to implement an additional unit to periodically search for and store updates to our understanding of our probes' targets.

2. Proteomic profiling

As described above, we will use 2-column nonporous (NP) RP-HPLC to profile the highly expressed protein from selected Permafrost bacteria. This profiling will be performed on bacteria cultured at various temperatures used in the RNA profiling experiments. The major proteins that show differential expression between the temperatures will be collected in the liquid phase and sized for molecular weight (MW) by MALDI-TOF MS. The proteins will then be digested by trypsin and CNBr to obtain peptide maps, which will be used to identify the proteins. An important aspect of this work is that the database will now become available for these bacteria due to DNA sequencing efforts of the group. MALDI-TOF MS and CE-ESI-MS can then be used to perform detailed sequencing of the proteins from multiple digests. In addition, MS/MS is available in our CE-ESI-MS experiments so that even small changes in protein structure can be detected and post-translational modifications (PTM) detected against the database.

Once the expressed proteins that change as a function of temperature are identified, gene knockout experiments can be performed in collaboration with the Kathariou laboratory. These gene knockout experiments can be used to determine which of the differentially expressed proteins at low temperature are significant in terms of cold tolerance. Changes in the expressed of the protein profile as genes are knocked out will be monitored using 2-column NP-RP-HPLC. In addition to knocking out individual proteins, one might expect changes in protein-protein interactions and the resulting cascades downstream. These changes can be monitored using our liquid separation techniques. The proteins that change in the subsequent protein profile can be collected and identified using MALDI-TOF MS. Furthermore, these proteins in the altered profiles can be sized by MALDI-TOF MS for MW and detailed sequencing performed by CE-ESI-MS/MS. One might expect that as protein interactions are altered, one would observe various modifications on the proteins expressed.

An important aspect of these studies will involve studying the evolution of the proteins that impart cold tolerance. Once key proteins are identified, they can be collected and isolated from permafrost bacterial strains ranging from 10^6 years to 6×10^4 years. CE-ESI-MS/MS can be used to perform detailed sequence analysis on these proteins to identify changes in sequence or PTM that have occurred through evolution. These analysis will be combined with functional analyses with a view to understand the evolution of specific activities. Integral to these studies will be application of the computer modeling methodologies developed in the Goldstein laboratory for studying evolutionary changes in protein sequence and function (see below).

An important feature of the liquid separation methodologies being developed in the Lubman laboratory is that they can be used to prepare significant amounts of protein for further analysis. Once proteins are identified as being significant to life at low temperature, then relatively large quantities can be collected using the nonporous separations. Using 8 mm diameter columns specially prepared for us by Eichrom Technologies, we can load > 1 mg of sample on the 2-column separation. Considering the number of highly expressed proteins observed, as much as 30-50 μg of each major protein can be collected. This can be performed in 10-20 minutes. Further isolation of the proteins can be performed on an additional NP column

with different properties. The isolated proteins can be studied for a number of properties including hydrophobicity/hydrophilicity, structure, effects of proteins on membrane layers, effects on dehydration properties of cells, antifreeze activity, etc., by McGrath, Thomashow and collaborators.

In initial work, the 1-D/2-column experiment will be sufficient for identifying the highly expressed proteins related to cold tolerance. However, as the bacterial systems and key proteins of interest are identified, more detail on the total protein expression might be required. This will be performed using a preparative 2-D liquid separation that has been used to successfully map large numbers of protein cells in our lab (Wall et al., 2000). The method uses a liquid IEF-based separation in a Rotofor device, which produces 20 pH fractions. The loadability of this device can be in the tens of mg range so that large amounts of protein can be prepared. In addition, relatively low abundance proteins can be detected using this method. Each *pI* fraction is then separated by the NP-RP-HPLC to produce a total 2-D map of the protein content. This map covers a pH range from pH 2.5 to 11, therefore, it is more comprehensive than most 2-D gels. It can resolve the lower MW proteins (< 15 kDa) not readily resolved by gels, which are so important in the proposed work. Furthermore, by the use of solubilization methods including 9 M urea and n-octyl (sugar)pyranoside (a nonionic detergent), large proteins > 100 kDa including protein complexes, can often be separated and detected. The method still uses a total liquid format and is readily interfaced to our MS analysis. It will be used to obtain more detailed information on the less highly expressed proteins during the cold shock processes and the various protein cascades that result. Although these detailed 2-D separations require more effort than the 1-D liquid separation, they still can be prepared in 5-7 hours, provide pure proteins in the liquid phase for future study and provide a 2-D map for study of the less abundant protein especially in the lower mass range required in this work.

3. Genetic analysis

The overall aim of the genetic analyses is to identify genes that have key roles in cold tolerance. We will address this aim using three experimental approaches.

a. Experimental approach 1: mutagenesis of candidate genes identified from the RNA and proteomic profiling studies

The experiments described above will point to candidate cold tolerance genes. These would include genes that are induced in response to low temperature and/or dehydration as well as genes that encode potential antifreeze proteins or polypeptides that resemble the COR and LEA hydrophilic polypeptides of plants. To evaluate the functional role of these genes, mutants will be constructed in which expression of the gene is inactivated. The choice of the mutagenesis scheme to be used will depend on the strain. In the case of low-GC gram-positive bacteria (e.g. strain ED7-3 and Planococcus strains), suicide vectors utilized for insertional inactivation of genes in *B. subtilis* will be employed. For instance, the temperature-sensitive shuttle vector pKSV7 (Smith and Youngman, 1992), and its conjugative derivative, pCON-1, can be introduced to the permafrost strain by electroporation and conjugation, respectively. In the latter case, conjugation will be between an antibiotic-resistant variant of the permafrost strain (we have successfully generated streptomycin-resistant derivatives of numerous permafrost

strains in the laboratory) and *E. coli* S17-1, which can serve as conjugative donor of the plasmid pCON-1. The selection marker on the vector (chloramphenicol) can be used with several of the permafrost strains, which are chloramphenicol-sensitive. These integration mutagenesis schemes will require the cloning of an internal portion of the gene into the vector, and selection for integrants under conditions which prevent replication of the plasmid in the permafrost bacteria. The available temperature-sensitive plasmids such as pKSV7 and pCON-1 will be utilized first. If these replicons prove suboptimal (e.g. because the restrictive temperature for replication is lethal for the permafrost bacteria) alternative suicide plasmids which can be efficiently introduced into the permafrost strain but cannot replicate will be utilized.

Our intent is to start the genetic analysis studies with strain ED7-3, since proteomic analysis of this organism is already under way and at least partial genomic sequences will be available. Another pre-disposing factor for the choice of this strain is the potential to utilize genetic approaches already established for several other low-GC gram-positive bacteria (e.g. *B. subtilis*, *Listeria*, lactic acid bacteria). In addition, strain ED7-3 is a member of a cluster of genetically closely related strains which have been isolated from permafrost sediments of variable ages (20,000-40,000 years, 200,000-600,000 years and 2-3 million years) as well as from modern tundra soil. Depending on genomic conservation among the strains, it may be possible to utilize PCR (polymerase chain reaction) to amplify the homologous genes from the different members of the cluster, thus identifying possible changes in nucleotide sequences that may be of relevance to the age of the bacteria in the sediments. The role of genes of special interest in ED7-3 can be investigated in similar strains from variable ages, utilizing mutagenesis approaches used for ED7-3.

Once the mutants are constructed, they will be used in functional studies to determine the impact of the mutation in phenotypes of interest, primarily low temperature growth and survival upon exposure to sub-zero temperature and freeze-thawing. In addition, the mutant strains will be characterized at the proteomic level in the Lubman laboratory to determine the impact of the mutation on the levels of expression of different proteins. Mutants of special interest will also be characterized at the level of RNA expression utilizing microarrays as described above. Such analyses will determine whether a single protein and/or RNA species is impacted by the mutation, or whether a number of proteins and RNAs are affected. The latter, for instance, may be expected if the inactivated gene encodes a regulatory protein essential for transcription of several others. An additional reason concerns the possibility of polar mutations. Depending on the genomic organization of the gene of interest, the insertional mutation may have polar effects on downstream genes that may be co-transcribed with the gene that harbors the insertion. In such cases, and if the mutant appears to have interesting phenotypes, additional work will be done to construct mutants bearing an internal gene cassette that does not have polar effects or in-frame deletions of the gene of interest. The genetic tools for such constructs are available for *B. subtilis* and in-frame deletion constructions have been used extensively in the Kathariou laboratory for mutant constructions in *L. monocytogenes*. Since cassette constructs and deletions are substantially more labor-intensive than insertional inactivations, they will be pursued only if (1) the gene is indeed a member of an operon, and is followed by additional downstream genes and (2) the insertionally inactivated mutant does indeed have a phenotype of interest. To determine whether the gene is a member of an operon, we will rely on both the analysis of genomic organization and sequence features in the region (e.g. transcriptional orientation,

presence of terminators, length of intergenic regions) and on experimental data utilizing Reverse-Transcription PCR (RT-PCR).

An important factor in these experiments is the accessibility of nucleotide sequences for site-specific construction of mutants. It is anticipated that the complete genome of one permafrost strain and partial (at least 80% coverage) genome sequences of nine additional strains will be available during this project. Although the relevant genes are expected to be readily identified among the sequences of the completely-sequenced genome, in the case of other strains it is conceivable that the genes of interest may be absent from the sequences (i.e. may be included in the ca. 20% of the genome which will not be covered by the sequencing project), for reasons having to do with peculiarities in nucleotide sequence and under-representation of the corresponding sequences within the cloned libraries. If an especially interesting gene appears to be lacking from the known sequences, special effort will be made to clone the gene by PCR using degenerate primers constructed on the basis of the available protein sequence data. Otherwise, our attention will be focused on those genes the sequences of which have been revealed in the course of the genome sequencing projects.

Another situation to consider is the potential inability to obtain mutations is specific genes. Certain genes may have functions essential for bacterial survival, and their inactivation is expected to be lethal. Thus, inability to obtain a specific mutant (when the mutagenesis scheme is operational for the organism of interest) suggests that the protein has an essential function. The mutagenesis schemes will be pursued with a number of genes of the genome of ED7-3 early in the course of the project, to ascertain the feasibility of the insertional inactivation and/or deletion protocols. If a specific mutant cannot be obtained, additional evidence for its essential role can be obtained utilizing a recombinant strain which harbors the gene of interest cloned on a plasmid, or integrated elsewhere in the chromosome, as has been described in other systems (Graumann et al., 1997).

b. Experimental approach 2: transposon mutagenesis of permafrost bacteria

In addition to site-specific mutagenesis, transposon mutagenesis of selected strains will be done to generate mutants in selected phenotypes of interest. Preliminary data of ours suggest that the conjugative transposon Tn916 (tetracycline resistance) (Clewell et al., 1995) and its erythromycin-resistance derivative, Tn916 E can be used to generate mutants of strain ED7-3 and a similar strain, ED5138, from Siberian permafrost (20,000-40,000 yrs). Southern blot data suggested that single copies of these transposons were inserted in various locations on the chromosome of different mutants, indicating that such mutagenesis will be suitable for genetic analysis. Alternative transposon systems, such as Tn917 (Tomich et al., 1980), can be used to enhance the range of target sites on the genome. The Kathariou and Thomashow laboratories have had experience with transposon mutagenesis systems of a number of different bacteria.

The transposon mutants will be screened for several phenotypes of interest, including ability to grow at different low temperatures (e.g. 4, 0, and -2.5°C) and to survive prolonged freezing and repeated freeze-thawing. In addition, mutants deficient in pigmentation will be isolated to evaluate the impact of pigmentation on low temperature survival and freeze tolerance. Mutants which are non-motile will be isolated as the screening for such mutants is easy, and can

provide information on the efficiency of the mutagenesis scheme. In addition, the possible impact of motility on low temperature survival will be evaluated. It is of interest that in several permafrost bacteria appear to be motile when grown at low temperatures. Transposon mutants with phenotypes of interest (e.g. cold-sensitive, freeze-sensitive etc) will be evaluated at the proteomic level in the Lubman laboratory as well as in terms of RNA expression profiles using microarrays, to evaluate the impact of the mutation on expression of distinct protein and RNAs, as described above. Transposon mutations are expected to have polar effects on transcription of downstream genes in the same operon, and mutants of interest may have to be characterized further with additional mutagenesis schemes, if the insertion is indeed found to be in a location that would allow such polar effects.

To identify the inactivated gene in a transposon mutant, genomic DNA of the mutants will be digested with an enzyme that does not cut into the transposon, ligated to a vector of known sequence (e.g. pUC18) and used as template in single-specific primer PCR (Shyamala and Ferro-Luzzi Ames, 1989) employing a primer at the terminus of the transposon and a vector primer. Such PCR products will contain one of the transposon-flanking fragments. The sequence of this fragment will be analyzed against the available genomic sequences of the bacterium. Homologies with other genes and gene products in the databases may provide clues as to the function of the gene. If the gene is not present in the available genomic sequences of the permafrost bacterium, the entire gene can be obtained by inverse-PCR. The utilization of SSP-PCR and additional approaches to obtain the sequences of transposon-targeted genes has been used extensively in the Kathariou laboratory.

To confirm that the mutant phenotype is due to the transposon insertion, genetic complementation will be employed, introducing the wild type gene cloned into a plasmid that can replicate in the permafrost bacteria. Our intent is to pursue transposon mutagenesis of ED7-3 first, and follow with similar work of high GC- gram-positive bacteria from the permafrost. Of special interest would be some of the *Arthrobacter* strains, which are prevalent in permafrost sediments of various ages.

On the basis of available preliminary data we anticipate that mutagenesis of ED7-3 and other low GC-gram-positive bacteria will be accomplished without undue difficulty. If transposons other than Tn916 and Tn917 are desired, transposon systems based on Insertion sequences (IS) identified during the genome sequencing of the bacteria, as constructed for other low GC-bacteria (Maguin et al., 1996). Transposon mutagenesis of Arthrobacter may prove more challenging, as transposon delivery systems for this class of high GC-gram-positives have not been established yet. We will examine the suitability of Tn916 and Tn917 for mutagenesis of these organisms, and will include a number of different permafrost strains, to maximize the likelihood of at least some them serving as transposon recipients. Tn*5564*, successfully used in *Corynebacterium glutamicum* (Tauch et al., 1998), and Tn*5367*, utilized with *Mycobacterium paratuberculosis* will also be employed. It is expected that novel genetic tools suitable for use in the high GC-gram-positive bacteria will be also becoming available, and these will be included in the investigations as well.

Genetic complementation in trans, with the wild type gene on a plasmid, may be difficult under the low-temperature conditions of the assays. It is possible, for instance, that replication of

the plasmids may be sub-optimal at 4°C or other low temperatures (the replicons of available plasmids are derived from mesophilic bacteria). Proper complementation under such conditions may require integration of the gene in a chromosomal location. Such integration constructs can be made for mutants of special phenotypic interest.

c. Experimental approach 3: isolation and characterization of genetic variants in phenotypes of interest

In the course of our work with several permafrost strains, including ED 7-3 we encountered genetic variants in certain phenotypes, such as pigmentation, filamentation of cells, and production of polysaccharide. The variants were identified on the basis of their distinctive phenotypes and were stable in the laboratory. Such variants likely represent spontaneous mutants of the progenitor culture, and can be used to evaluate the impact of the mutation on additional phenotypes of interest, such as low temperature growth and freeze tolerance. Variants with especially interesting phenotypes in these regards will be analyzed at the proteomic level to determine the impact of the mutation(s) on expression of different proteins. Such analysis will reveal possible changes in the size of the protein conferred by the mutation. In addition, the mutant protein is likely to yield an altered peptide map.

It is recognized that the variants may harbor mutations in more than one locus, thus hindering the association between the original phenotype (e.g. pigmentation) and the functional effects (e.g. freeze tolerance). If the proteomic analysis shows that a specific protein is affected, the corresponding gene can be mutated separately to confirm the function of the protein and create isogenic mutants. This additional confirmatory work will be done only if the variant appears to have unusually interesting phenotypes in terms of cold and freeze tolerance.

4. Targeted exploration for candidate freezing tolerance proteins

a. Antifreeze proteins

As described earlier, antifreeze proteins have been described in diverse organisms including fish, insects, plants and bacteria. These studies have revealed that proteins with very different amino acid sequences and structure (secondary and tertiary) are capable of displaying the activities associated with antifreeze proteins—namely thermal hysteresis, inhibition of ice recrystallization and altering the shape of ice crystals—indicating that antifreeze activity has evolved multiple independent times. Many fundamental issues, however, remain to be addressed regarding these fascinating proteins. The precise structural features of antifreeze proteins that impart each of the associated activities is not clear; the roles that the proteins play in organisms that tolerate freezing, as opposed to avoid freezing, remains to be determined; and the diversity and evolution of proteins with antifreeze proteins has only begun to be explored. Indeed, to our knowledge, only one antifreeze protein from a bacterium has been identified, and in that case, the protein appears to have a novel activity, that of ice-nucleation (Xu et al., 1998).). Although the *B. subtilis* CspB has been shown to be essential for freeze tolerance in this organism (Willimsky et al., 1992) it has not yet been shown that the protein actually acts in an anti-freeze capacity.

Given that the permafrost bacteria have been frozen from tens of thousands to millions of years, it does not seem unreasonable to think that they are potentially a good source to "mine" for proteins with antifreeze activity. Thus, we (McGrath and Thomashow laboratories) plan to conduct a survey of all the permafrost isolates that we have obtained for antifreeze activity. The isolates will be grown at low temperature and the cells and supernatant fluids will be collected and assayed for antifreeze activities. Supernatant fluids will be tested directly and after being concentrated by lyophilization and suspension in buffer. Cells will be disrupted, fractionated into soluble and insoluble fractions and then tested for activity. The activities to be examined are the three that are best characterized: thermal hysteresis, inhibition of ice recrystallization, and effect on ice crystal shape. Thermal hysteresis activity will be measured by the capillary freezing-melting point techniques as described by DeVries (1986) and the nanoliter osmometer method described by Chakrabartty and Hew (1991). Inhibition of ice recrystallization will be measured using cryomicroscopy as described by Chao et al. (1996) and effect on ice crystal growth will be monitored as described by Griffith et al. (1997) and Sidebottom et al (2000).

If antifreeze activities are observed, we will purify the proteins, obtain a partial amino acid sequences and isolate and sequence the corresponding genes. From the sequence information, we will be able to determine whether the proteins are related in amino acid sequence or share common structural features that have been observed in other antifreeze proteins such as repeated amino acid motifs. This information will provide the foundation for future detailed analysis to understand better structure-function relationships among antifreeze proteins and the evolution of antifreeze activities (see section on computational analysis of protein evolution below). As a part of this analysis, we will test for two additional activities that have been observed with antifreeze proteins including the ability to nucleate ice formation (Xu et al., 1998) and to stabilize membranes (specifically inhibit the leakage of small molecules from liposomes during thermotropic phase transitions) (Hays et al., 1996). Of course, we would also want to make mutants of the isolates that are no longer able to produce the antifreeze protein and asses what effects this has on growth at low temperature and the ability of the isolates to tolerate freezing. Whether this can be done in the short run will depend on how easily the specific isolates can be genetically manipulated (see section on genetic analysis above). Other issues will also be of interest to address. Do increased levels of thermal hysteresis activity and ice recrystallization inhibition correlate with enhanced resistance to freeze/thaw damage of these microbes, suggesting that they are important determinants of freeze protection? Since there are lower water activities and lower temperatures in the Antarctic compared to the Arctic, are the thermal hysteresis activities and ice recrystallization inhibition capacities more pronounced in the former? What are the limits of low water activity and low temperature for Arctic and/or Antarctic microbes that express AFPs- i.e. can they survive more extreme conditions that may exist at extraterrestial sites. Are the thermal hysteresis activities or ice recrystallization inhibition capacities of AFPs produced by Arctic or Antarctic microbes unusual in any way compared to known behavior? For example, do they produce unusually potent activity or inhibition? What environmental conditions (e.g. low temperature) trigger the appearance of thermal hysteresis activity and ice recrystallization inhibition?

b. COR and LEA-like hydrophilic polypeptides

As noted earlier, it is widely observed in plants that during cold acclimation, the most highly expressed cold-inducible genes encode extremely hydrophilic polypeptides that are thought to be involved in freezing tolerance, though their specific roles remain largely unknown. Our goal here is to determine whether the synthesis of extremely hydrophilic polypeptides occurs upon exposure to low temperature in the permafrost bacteria and if so, to explore their roles in life at low temperature. In short, is the production of such polypeptides a conserved feature of organisms that must contend with freezing temperatures?

One way in which we will address this issue is to analysis the genomic sequence that we obtain for ORFs that encode cold-regulated hydrophilic polypeptides that resemble those found in plants and, in the microarray analysis experiments, determine whether they are induced in response to low temperature. In addition, we will screen directly for the synthesis of "boiling-soluble" polypeptides in cultures of permafrost bacteria that are grown at low temperature. In these cases, culture supernatants and cell extracts will be boiled, the coagulated proteins will be removed by centrafugation, and the soluble polypeptides will be fractionated in the Lubman laboratory using the NP columns described above.

If our results indicate that the synthesis of novel COR and LEA-like hydrophilic polypeptides occurs in the permafrost bacteria, we will want to determine whether they have importance roles in life at low temperature. An important approach will be to make mutants that do not express the proteins. As with the antifreeze protein experiments, however, our ability to perform these experiments will be subject to how easily we can genetically manipulate the given permafrost isolate. We are also aware that if a given isolate produces multiple COR and LEA-like polypeptides in response to low temperature, that we may not detect any effect on cold tolerance by "knocking out" one gene as the proteins may have redundant functions (a similar situation exists with the *Csp* genes in *E. coli*). In this case, we would have to construct strains with multiple mutations to asses their importance to life at low temperature. As a part of our initial analyses, the McGrath and Thomashow laboratories will also conduct *in vitro* experiments to see if the proteins have activities that have been associated with other cyroprotective molecules such as sucrose and proline, namely the stabilization of proteins, membranes and cells against freezing and desiccation damage (Csonka, 1981; Anchordoguy et al., 1987; Carpenter and Crowe, 1988; Artus et al., 1996; Hayes et al., 1996; Steponkus et al., 1998). Given some of the overt similarities between many of the novel, COR and LEA-like hydrophilic polypeptides and certain antifreeze proteins, we include in our analyses whether the proteins have activities displayed by antifreeze proteins, namely thermal hysteresis, inhibition of ice recrystallization and effect on ice crystal shape.

Objective 3. Evolutionary Adaptation to Low Temperature: experimental evolution studies on improving "cold fitness" and modeling evolutionary relationships of "cold tolerance" proteins.

1. Experimental evolution studies on cold fitness

Historically, most studies of organic evolution have been phylogenetic and comparative in their approach (Harvey and Pagel 1991; Avise 1994; Lenski 1995a; Larson and Losos 1996; Bennett and Lenski 1999). In recent years, experimental studies of evolutionary change using

bacteria and other microorganisms have flourished while addressing a wide range of questions from dynamics of adaptation and bacteria-virus interactions to molecular evolution and social behaviors (Chao et al. 1977, 1983; Dykhuizen 1978, 1990; Hartl and Dykhuizen 1979; Hall 1983; Lenski and Levin 1985; Adams and Oeller 1986; Bouma and Lenski 1988; Lenski 1988, 1995a, 1995b, 2000; Chao 1990; Lenski et al. 1991; Bull and Molineux 1992; Hillis et al. 1992; Cohan et al. 1994; Lenski and Travisano 1994; Rosenzweig et al. 1994; Elena et al. 1996; Bull et al. 1997; 2000; Schrag et al. 1997; Sniegowski et al. 1997; Nakatsu et al. 1998; Rainey and Travisano 1998; Turner at al. 1998; Velicer et al. 1998, 2000; Appenzeller 1999; Burch and Chao 1999, 2000; De Visser et al. 1999; Papadopoulos et al. 1999; Turner and Chao 1999; Vulic et al. 1999; Wichman et al. 1999; Bohannan and Lenski 2000; Cooper and Lenski 2000; Schneider et al. 2000). Bacteria have many features that facilitate an experimental approach to evolution, including their rapid generation times, large population sizes and ease of culture. Albert Bennett and Richard Lenski have been among the pioneers in using this approach, including their collaborative work on thermal adaptation in *E. coli* (Bennett et al. 1990, 1992; Bennett and Lenski 1993, 1996, 1997, 1999; Lenski and Bennett 1993; Leroi et al. 1994a, 1994b; Travisano et al. 1995; Mongold et al. 1996, 1999).

Experiments to study evolutionary (genetic) adaptation to very low temperature pose a particular challenge, namely that the absolute rate of adaptation by natural selection depends on genotypic differences in the net rate of cell growth (cell division minus cell death), and these rates are extremely slow at very low temperature. Nonetheless, we believe that we can make significant progress toward understanding the process and mechanisms of evolutionary adaptation to low temperatures by a series of four experimental studies, which are described below. The first of these concerns a further analysis of an already existing evolutionary lineage, which demonstrates the feasibility of this approach. The next three studies are proposed new experiments in which new lineages of bacteria will be evolved and subsequently analyzed. Following a description of these four evolution experiments, we then describe two types of genomic analyses that we will perform to understand the genetic bases of adaptation in all four of these experimental systems. Related studies exploring the evolution of cold fitness traits in permafrost isolates would be considered once they are more thoroughly characterized at a genetic and physiological level.

a. Experiment 1: evolutionary adaptation to 20°C, a moderately low temperature

This experiment started with a clone from a population of *E. coli* that had previously evolved in a minimal glucose-supplemented medium at 37°C for 2,000 generations (Lenski et al. 1991). From this founding clone, six new populations were propagated in the same medium at 20°C for 2,000 more generations (Mongold et al. 1996). 20°C was chosen as the selective temperature because it was within 1°C of the minimum temperature at which the ancestral population could replicate itself under the defined culture conditions. After 2,000 generations at 20°C, new clones were sampled from each of the populations and placed in competition with their 37°C-selected ancestor across a wide range of temperatures from 20-40°C. Details of competition experiments can be found elsewhere (Lenski et al. 1991; Mongold et al. 1996), but the important points are these: (1) The ancestral strain was stored as a clone in glycerol at -80°C during the time that the 20°C-selected populations were evolving. (2) All evolved clones were

also subsequently stored at -80°C. (3) Prior to competitions at each assay temperature, the evolved and ancestral clones were separately acclimated for several generations to the assay temperature; hence, any difference between them has a heritable genetic basis. (4) During the competition between the evolved 20°C clones and their ancestor, the competitors were distinguished on the basis of a selectively neutral genetic marker.

Figure 4 shows the average fitness of the 20°C-selected clones relative to their 37°C-selected ancestor across a range of temperatures from 20 to 40°C. Fitness is calculated here simply as the ratio of the number of doublings achieved by the two competitors during their competition for limiting glucose at that temperature (Lenski et al. 1991; Mongold et al. 1996). Evidently, there has been significant genetic adaptation to the lower temperature, such that the 20°C-selected clones now grow about 10% faster than their ancestor at 20°C. The 20°C-selected clones retain their competitive advantage at 27 and 32°C, whereas they are marginally less fit than their ancestor at 37°C and much less fit at 40°C.

In this experiment, populations were propagated by serial transfer with daily dilution of 1:100 into fresh medium. In order to persist against this rate of dilution, a population must achieve 6.64 (= $\log_2$ 100) doublings per day. The 37°C-selected ancestral strain is just able to persist at 19.5°C, whereas it is progressively diluted to extinction at 18°C (Bennett and Lenski 1993; Mongold et al. 1996). As a consequence of their genetic adaptation to 20°C, all six of the 20°C-selected lines can persist indefinitely at 18°C against a 100-fold daily dilution, but they cannot persist at 17°C with this same dilution factor (Mongold et al. 1996). In essence, the 2,000 generations of evolution at 20°C (which required 300 days in real time) allowed us to push downward by 1.5°C the thermal limit for persistence against a 1:100 daily dilution. In the next experiment, we will use the 20°C-selected clones as starting material for continued selection at progressively lowered temperatures. Also, we will use these 20°C-selected lines as the first material for genomic analysis of the genetic basis of adaptation to low temperature. These genetic analyses can be undertaken while the three additional evolution experiments are in progress.

b. Experiment 2: evolutionary adaptation to progressively lower temperatures

In this proposed experiment, we will take the six 20°C-selected lines and use of each of them to found four new populations (24 populations in all). The new populations will then be propagated under identical conditions, including the 100-fold daily dilution into fresh medium, except they will be maintained at 18°C, where they just barely persist. At 500-generation intervals, we will assay the lower thermal limit for persistence (as defined in the preceding section) of each 18°C- evolved line, including as controls their proximate 20°C-selected progenitors and their common 37°C-selected ancestor. At such time as 50% or more of the 18°C-selected lines have evolved the capacity to persist (against a 100-fold daily dilution) at a temperature at 17°C or lower, we will then shift the incubation temperature to that new lower limit. In the event that some lines have not evolved this capacity, we will replicate other lines that did so in order to maintain a total of 24 populations at the new temperature. We will then propagate the 24 lines at this new lower temperature, assay their lower limits for persistence at 500-generation intervals, and shift them to a still lower temperature once a majority have evolved the capacity to persist at their former low temperature.

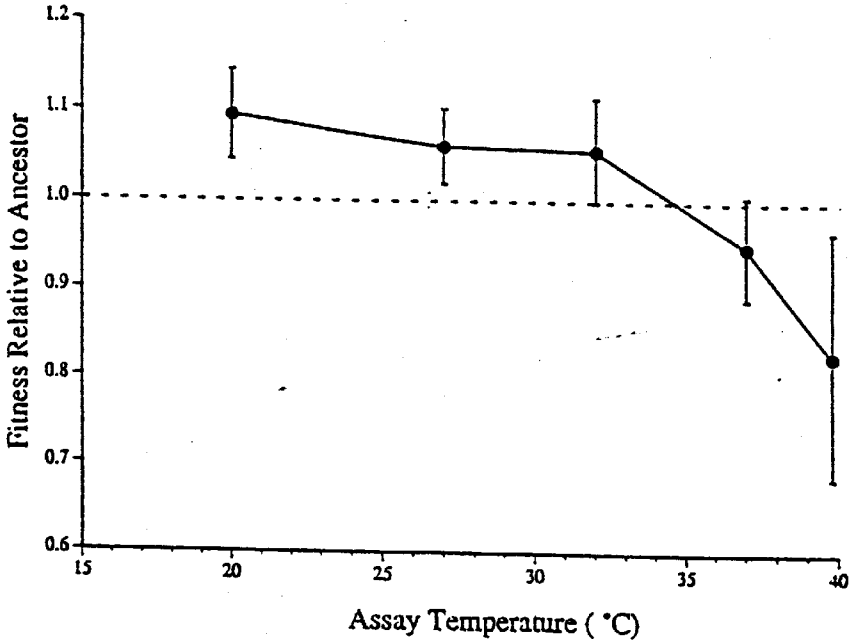


Figure 4. Average fitness of 20°C-selected lines in competition with their 37°C-selected ancestor across a range of assay temperatures. Error bars indicate the 95% confidence intervals. A value >1 indicates the 20°C-selected lines are more fit; a value <1 indicates the 37°C-selected ancestor is more fit. (From Mongold et al., 1996.)

We will continue this selection scheme indefinitely through the first 4 years of this proposal. This should allow more than 10,000 generations of evolutionary adaptation to progressively lower temperatures (given 6.6 doublings per day to offset the 100-fold dilution). Note that the Lenski lab has successfully maintained *E. coli* lines for more than a decade – more than 25,000 generations – as part of another long running experiment (Lenski et al. 1991; Lenski and Travisano 1994; Papadopoulos et al. 1999; Cooper et al. 2000; see also feature article in *Science* by Appenzeller, 1999, posted at http://www.msu.edu/user/lenski/).

All the evolved clones with progressively reduced thermal limits for persistence will be stored in glycerol at -80°C, where they and their progenitors (which serve as controls) will be available for the genomic analyses described later.

c. Experiment 3: evolutionary adaptation following a sudden shift to 4°C

Experiment 2 is predicated on the assumption that evolutionary adaptation to low temperatures is most likely to be achieved in a "stepping-stone" fashion, that is by a long series of incremental reductions in temperature. This approach is demonstrably successful (see results of Experiment 1) but it is also time consuming; for example, the first 1.5°C shift required 2,000 generations.

Experiment 3 will be run concurrently with Experiment 2. In Experiment 3, however, we will use a strategy in which we look for adaptation to a sudden and more extreme shift in incubation temperature. We have previously used this "sudden shift" approach to find several mutant clones that can grow at temperatures 1-2°C above those that allowed growth by their progenitor, which we did not obtain by more gradual changes in temperature (Bennett and Lenski 1993; Mongold et al. 1999). Specifically, for the present project, we will initiate 72 experimental populations, including six started from each of the six 20°C-selected lines and 36 started from their 37°C-selected ancestor. These 72 populations will be grown to stationary phase at 20°C, and then inoculated into fresh medium at 4°C, which is several degrees below where "wildtype" *E. coli* is reported to sustain growth in minimal medium (Ng et al. 1962; Shaw et al. 1971; Ingraham and Marr 1996). Nonetheless, there may be some mutations that permit sustained growth at this temperature, even if the resulting growth is very slow. By incubating these populations for weeks and even months at 4°C, any such mutant should increase in the population, eventually resulting in a dense, turbid culture. We will monitor these populations indefinitely, isolating clones from populations that achieve increased numbers of cells over time.

Assuming that one or more populations respond, we must confirm that: (a) the clone is not a contaminant; and (b) the difference between the 4°C-selected clone and its ancestor is a heritable, genetic difference, as opposed to some very slow and strictly phenotypic acclimation response. The former is readily determined by a series of phenotypic and genetic markers that uniquely characterize the strains used in all the Lenski and Bennett evolution experiments (Lenski et al. 1991; Bennett et al. 1992; Papadopoulos et al. 1999). The latter is best achieved by performing a series of paired assays in which the putative 4°C-selected mutant and its immediate progenitor are removed from storage at -80°C, re-acclimated to 20°C, then re-transferred to 4°C. If the difference is inherited, then the mutant should consistently achieve high density at 4°C,

whereas the progenitor should not. Alternatively, no consistent difference between the putative mutant and its progenitor is predicted if the original growth at 4°C represented a slow acclimation response.

If 4°C-adapted mutants are found and confirmed, they will be subjected to the genetic analyses described below. And if we find multiple 4°C-adapted mutants, then the above experimental design with 72 populations will also allow us to test the hypothesis that prior adaptation to 20°C (as opposed to 37°C) genetically predisposed the populations to be able to make the shift more often and/or faster at 4°C. Similarly, among the six independently evolved 20°C-selected lines, we can assess whether certain ones were better able than others to make the evolutionary transition to 4°C. Thus, we have an experimental system that allows us to examine not only the process of evolutionary adaptation, but also the potential role of historical contingency in promoting or constraining adaptation (Gould 1989; Travisano et al. 1995; Huey et al. 2000).

d. Experiment 4: evolutionary adaptation to freeze/thaw cycles

As discussed elsewhere in this proposal, the capacity to survive and even grow at a sub-zero temperature is likely to present a bacterium with a different set of physiological challenges from growing in liquid medium at a low temperature, including hyperosmotic stress. Therefore, as a bridge between the two realms, we propose in Experiment 4 to examine the evolutionary adaptation of *E. coli* to repeated cycles of freezing and thawing.

At first glance, it might seem improbable that a mesophile like *E. coli* could evolve – over an experimental time scale – new mechanisms to withstand freezing, and we recognize that this proposed experiment is a long-shot in that regard. On the other hand, many compatible solutes that allow an organism to withstand freezing (such as proline, trehalose, and glycerol) are in fact produced by *E. coli* as routine parts of its existing metabolism. Indeed, production of trehalose is induced in wild-type *E. coli* by high external osmolality, and some mutants of *Salmonella* over-produce proline and can thus grow at higher osmolality than their wild-type counterparts (Csonka 1981; Strom et al. 1986; Ingraham and Marr 1996). It is quite possible, therefore, that existing metabolic pathways might be co-opted to allow cells to accumulate an adequate quantity of a cryoprotective metabolite.

We will pursue two experimental approaches to evolve populations under a freeze-thaw regime. In the first one, we will allow populations to grow to stationary phase (where cells are generally more robust to stress than during exponential growth), then subject them to varying duration of freezing without providing any extrinsic cryoprotective substance. Survivors will then be transferred to fresh medium, allowed to grow to stationary phase, and again subjected to sudden freezing. If this protocol is unsuccessful – for example, there may be few or no survivors even for the briefest periods of freezing – then we will repeat the experiment, except by varying the concentration of glycerol added to the medium while keeping the duration of freezing constant at 24 h. We know, from past experience, that our *E. coli* strains survive in high numbers for more than a decade at -80°C in medium containing 15% glycerol. In essence, we will identify the glycerol concentration that just permits survival and then gradually reduce the

concentration below that limit to see whether we can identify mutant genotypes with reduced requirements.

As with Experiment 3, we can run this experiment with various 20°C- and 37°C-selected lines, to determine whether they are differentially predisposed to adapt to the freeze/thaw challenge. As with all of the experiments, any cryotolerant mutants that are isolated will then be subjected to the genomic analyses described below.

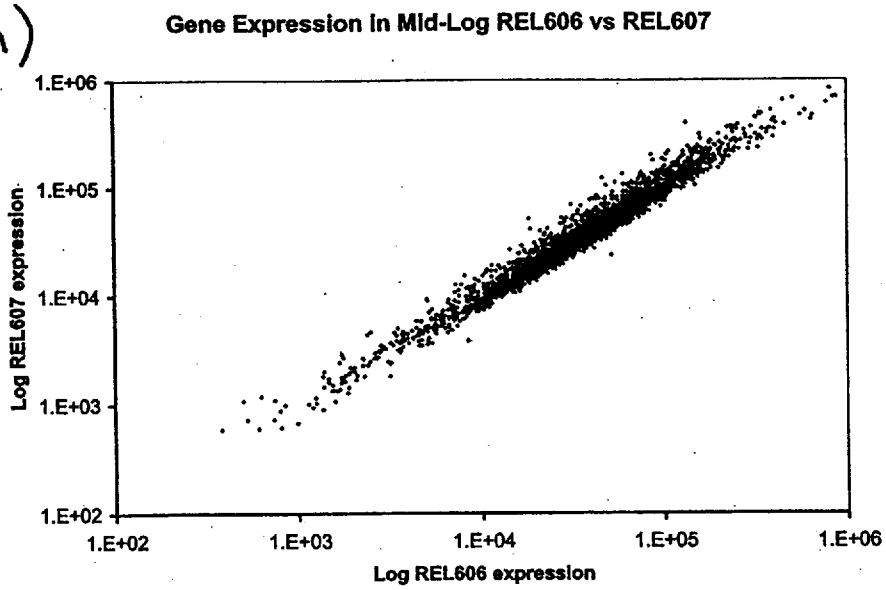
e. Genetic analysis

Genomic mRNA expression profiles. We will examine the mRNA expression profiles of selected lines from each of the four evolution experiments described above, along with the profiles of their progenitors as paired controls. To do so, we will use the commercially available Panorama™ *E. coli* gene expression arrays from Sigma-Genosys Biotechnologies. Each expression analysis module will ideally consist of four arrays, with mRNA isolated from the following cell populations: (i) the ancestral strain grown under the conditions used during its own evolution; (ii) the selected mutant grown under the ancestral conditions; (iii) the selected mutant grown under conditions corresponding to its own evolutionary derivation; and (iv) the ancestral strain grown under the conditions used to obtain the derived mutant. (In practice, the fourth category may sometimes be impossible to include because ancestral cells will be nonviable under the corresponding selective regime.) Standard protocols will be used to isolate the mRNA; synthesize ^{33}P-labeled cDNA using primers that exclude rRNA and tRNA; hybridize the labeled cDNA probes to the array; and scan the arrays using a PhosphoImager to record the intensity of hybridization (Tao et al. 1999).

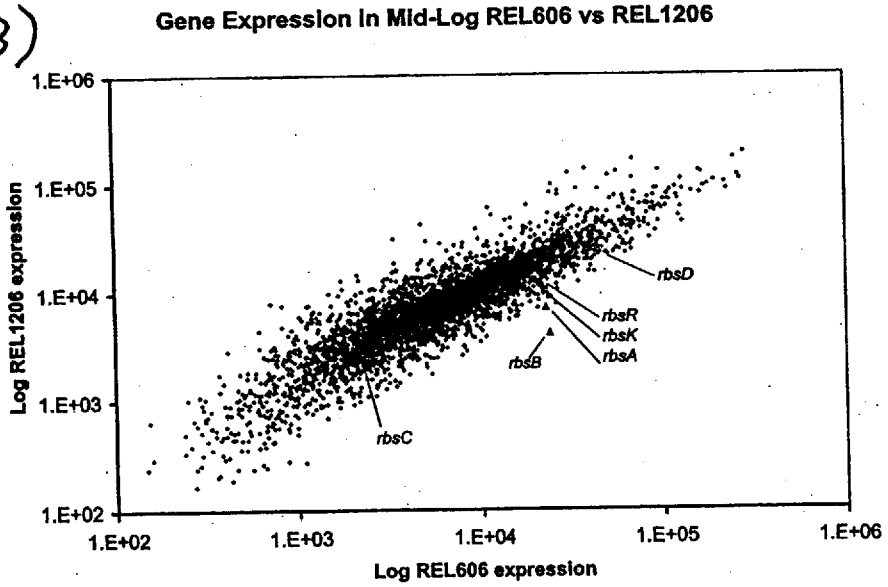
Both the Bennett and Lenski labs have successfully employed these gene expression arrays. Figure 5 shows the results of a preliminary microarray experiment using three clones from the long-term evolution experiment from the Lenski lab (Lenski et al. 1991; Lenski and Travisano 1994; Cooper and Lenski 2000). In Panel A, the relative intensities of all mRNA are contrasted for the ancestral clone (REL606) and another clone that differs only by a single neutral mutation that is used as a marker in competition experiments (REL607). Note the extremely tight correspondence of the expression profiles for these two genotypes, which show the high reproducibility of these profiles. Panel B shows the expression profile of a selected genotype (REL1206) isolated after 2,000 generations of evolution at 37C against its ancestor (REL606). Notice the greater variation between these two strains in their expression profiles; more than 100 genes differ in their level of expression by at least 3-fold, whereas fewer than 10 did when the two ancestral variants were compared. Among the genes that show much lower level of hybridization in the evolved mutant are those in the *rbs* operon, which encodes the ability to grow on ribose. This catabolic function has in fact been lost by this evolved genotype; the underlying mutational event has been characterized; and subsequent genetic manipulation and competitions have demonstrated the selective benefit of this mutation (Cooper 2000; Cooper and Lenski 2000).

Transposon tagging of selected mutations. A second approach that we can use to identify the mutations that were selected under any of the four selective regimes is transposon tagging. This approach is considerably more labor-intensive than genomic expression profiles,

(A)


Gene Expression In Mid-Log REL606 vs REL607
Log REL607 expression
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+02
1.E+03
1.E+04
1.E+05
1.E+06
Log REL606 expression

(B)


Gene Expression In Mid-Log REL606 vs REL1206
Log REL1206 expression
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+02
1.E+03
1.E+04
1.E+05
1.E+06
Log REL606 expression
rbsD
rbsR
rbsK
rbsA
rbsB
rbsC

and hence can be applied to only the most interesting evolved lines. But it allows the unambiguous identification of the exact mutational event responsible for a given improvement under one of the selective regimes.

To identify a beneficial mutation, we apply the familiar genetic approach of transposon-tagging in a novel way to what seems like a daunting problem — how to find one or a few beneficial mutations, among 5 x 10^6 bp and in nearly isogenic lines, whose only known phenotypic effect is improvement in growth rate or survival under a particular thermal regime. Using a mini-Tn*10* transposon that carries an antibiotic-resistance marker and is engineered to insert randomly into the genome (Kleckner et al. 1991), we create ~1500 sub-clones from an evolved line of interest, each one carrying an inserted marker. We mix these sub-clones, infect them with transducing phage P1, use the lysate to infect the ancestor, and select for resistance. Most ancestral cells that acquire the resistance marker will not carry a beneficial mutation, but among the 1500 marker insertions, a few will be near a beneficial mutation; when these markers are transduced into the ancestor, the beneficial mutation will often be co-transduced. We then screen the transduced ancestors for those that have a fitness advantage under the relevant thermal regime (corresponding to their selective history). In those cases where the transductant has improved fitness, we now have a well-defined marker that is linked to the trait of interest. We can then quantify the linkage by measuring the co-transduction frequency between the marker and improved fitness; we use these data to estimate the physical distance between the marker and the beneficial mutation; and we sequence regions on either side of the marker at the appropriate distance, thereby finding the beneficial mutation of interest.

The Lenski lab has successfully applied this approach to one of the 37°C-selected long-term lines, which allowed them to: (i) identify a 1-bp insertion in an upstream regulatory region of two co-transcribed genes involved in cell wall synthesis; (ii) demonstrate that this mutation confers a fitness advantage of 8% or so on the ancestral background at 37°C; and (iii) show that this mutation swept through that population between generation 500 (at which time 0 of 50 clones sequenced carry the mutation) and 1500 (when 29 of 30 clones carry it). Moreover, after having transduced a beneficial mutation of interest into the ancestral background, one can use genomic mRNA expression profiles of the ancestor, the evolved line, and the transductant bearing a known beneficial mutation to determine quantitatively which aspects of the resulting phenotypic expression profile can be attributed to that one mutation as opposed to others that might have been fixed in the population during the evolution experiment.

2. Modeling evolution of cold tolerance proteins

Central goals of the experiments described in Objectives 1 and 2 above are to identify proteins with roles in cold tolerance and to determine their modes of action. As these proteins are defined, we will also want to understand and model their evolution. How did the proteins evolve their capacities? What are the intermediary steps in the evolutionary process? How do we define the evolutionary changes in terms of the physical chemical characteristics of the proteins and their constituent amino acids? How do we identify when, in the evolutionary process, changes in selective pressure occurred? And can we use these models to provide important information about how the proteins function and to identify other putative "cold tolerance" proteins?

Every evolutionary analysis starts with a model of how sequences change. The standard approach towards modeling natural site substitutions in proteins is with a substitution matrix, a 20 X 20 array that represents the probability of any given amino acid changing to any other in a given length of evolutionary time. These substitution models directly encode the selective pressure acting on the proteins by representing which substitutions are likely or unlikely. Most methods for deriving these matrices use the approach developed by Dayhoff, based on an analysis of corresponding amino acids in pairs of closely-related homologous proteins (Dayhoff and Eck, 1968). Given such an evolutionary model and a set of putative homologs, it is possible to recreate the most probable phylogenetic relationship of the proteins using standard computer software. However, these standard substitution models are based on a number of highly problematic assumptions particularly poorly suited for the work described in this proposal. Generally researchers use matrices derived on a broad range of proteins, the vast majority of which are from mesophilic organisms. It is not clear how well suited these models are for representing the evolutionary changes of proteins from cold adapted bacteria. It is difficult to prepare more specialized models for the permafrost bacteria due to the large number of adjustable parameters and the limited amount of available data. Most importantly, the models assume that the rates of substitutions are the same in all locations in all proteins for all time, effectively assuming that all regions of the proteins are under constant homogeneous selective pressure. Obviously this assumption is inconsistent with exploring variations and changes in the selective pressure resulting from adaptation to cold environments.

For the past few years, the Goldstein lab has been working to create models of the evolutionary process that overcome the limitations of the current model. Initial work involved constructing substitution matrices specific for different types of local protein structure (Koshi and Goldstein, 1995). While this approach includes some aspects of structural heterogeneity in the evolutionary model, it is still based on the assumption that *all* positions in a particular local environment have the same fitness requirements and thus similar substitution rates. More recently, a set of more general probabilistic models have been developed (Koshi et al., 1997; Koshi and Goldstein, 1998) and used in phylogenetic analyses (Koshi et al., 1999). In this approach, rather than creating one "one-size-fits-all" model for all locations in the protein for all time, site heterogeneity is explicitly included in the model. Different *types* of locations, termed "*site classes*," are considered, each with its distinct fitness function and corresponding substitution matrix. While in principle these different site classes might correspond to locations with different secondary structures or functional significance, the nature of these site classes do not need to be define *a priori*. Nor does one need to assign different locations in the protein to specific site classes. Rather, there are adjustable probabilities that any location can be described by each site class. These probabilities are optimized simultaneously with the adjustment of the underlying fitness functions corresponding to the different site classes in order to best fit the observed sequences. Thus, one can *a posteriori* assign locations, at least probabilistically, to the various site classes and interpret the corresponding substitution matrices in terms of the selective pressure acting on each of those locations (Koshi and Goldstein, 1997). This approach achieved impressive gains in the ability to model the evolutionary process, compared with the more traditional single-model method.

In order to use this approach with limited data-sets (such as proteins from specific sets of organisms) we must greatly reduce the number of adjustable parameters. Rather than express the substitution rate as a function of the identity of the amino acids (*e.g.* the rate of alanine being substituted for glycine), the rates are expressed as functions of the corresponding physical-chemical properties of these residues (*e.g.* the rate of an amino acid with a given hydrophobicity, size, and charge being substituted for another amino acid of different hydrophobicity, size, and charge). Besides making the models more computationally tractable, it also allows one to "*read off*" the selective pressure at each location in the protein (Koshi and Goldstein, in press). It has been demonstrated how these methods can be applied to specific groups of proteins, such as hypervariable and constant regions of antibodies (Koshi and Goldstein, 1998) and to thermophilic proteins (manuscript in preparation).

A straightforward use of these models is in phylogenetic analysis. Analysis of protein sequences (in comparison with DNA sequences) allows us to "see" further back in evolutionary time, due to the higher degree of conservation at the amino acid level. In addition, the ability to model protein evolution on the amino acid level allows us to more accurately compute protein phylogenetics, in order that the resulting evolutionary trees can be better compared with the organism phylogenetics. In this way, we hope to determine the origin of the cold tolerance proteins that we identify in our structural and functional genomic studies, regardless of whether they arose through simple adaptation of pre-existing proteins, through gene-duplication, or through horizontal transfer. In addition, we will use the models to recreate ancestral protein sequences and examine specific changes in the sequences during particular periods of the evolutionary history of the protein (Koshi and Goldstein, 1996). This analysis will include expressing the proposed ancestral protein sequences in the laboratory and testing their specific biochemical properties. For instance, if we find that there are antifreeze proteins in the permafrost bacteria, we would model their evolution and test the intermediates for the activities associated with these proteins. Such experiments not only have the potential to reveal how a given antifreeze activity evolved, but has the potential to shed new light on the interrelationships between protein structure and the various activities observed in antifreeze proteins, a fundamental issue regarding these proteins. In these experiments, the "ancestral genes" will be created using standard molecular genetic/biology approaches, cloned into appropriate expression vectors and expressed at high levels in the host organism. The Thomashow lab has extensive experience making recombinant gene constructs and conducting protein overexpression experiments (e.g., Thomashow et al, 1986; Gilmour et al., 1996; Stockinger et al., 1997; Jaglo-Ottosen et al., 1998).

Most current evolutionary models assume constant selective pressure during the entire modeled evolutionary process. If we are interested in studying how proteins respond to *changes* in selective pressure resulting from the need to adapt to cold conditions, we need to have evolutionary models that include the possibility of such changes, especially if we want to explicitly include both psychrotrophs and non-psychrotrophs in the analysis. In this way we can directly separate sequence modifications due to cold adaptation from other random changes, as well as identify when in the evolutionary process the cold adaptation occurred. The models currently being used in the Goldstein laboratory assume that the selective pressure is constant during the evolutionary process. Our "site class" approach is, however, ideally suited to relaxing this constraint. We are now making the simple modifications necessary in order to explicitly

consider different substitution models at different periods of evolutionary time, providing information regarding these changes in selective pressure. Comparisons of protein and DNA evolution can also allow us to identify adaptive evolution from the background of random substitutions, using such conventional methods as comparing synonymous and non-synonymous DNA substitution rates.

There is already a set of proteins that have been identified to have antifreeze activity. Insight into how these proteins function can be gained by comparing their sequences and evolutionary patterns with related proteins (homologs) in other organisms. We will initially use standard methods of homology detection such as FASTA (Pearson, 1991) and PSI-BLAST (Altschul et al., 1997). Additional homologs may be detected by creating more specific statistical models for these particular proteins, including developing substitution models optimized for homolog detection for this particular set (Kann and Goldstein, in press) and by using more advanced methods such as Hidden Markov Models (HMMs) (Krogh et al., 1994). It may be possible to utilize the evolutionary approaches described above for homology detection; for instance, we can possibly improve our sensitivity by searching for homologs to our reconstructed ancestral sequences. As described in the background section of the proposal, proteins having very different amino acid sequence and structures can have similar antifreeze activity. In addition to finding homologous proteins, we are also interested in searching the psychrotrophic bacteria sequences for *analogous* proteins, proteins with similar antifreeze activity but with no evolutionary, sequence, or structural relationship. While these proteins may have no evolutionary relationship with the known proteins, it is likely that there would be similarities and regularities in the selective pressure acting on all of these various proteins. Using the methods described above, it may be possible to characterize the types of selective pressure acting on antifreeze proteins, and to use these characterizations to identify putative members of this set.

Finally, the Goldstein laboratory has developed a number of different approaches towards incorporating evolutionary information in the prediction of protein properties (Thompson and Goldstein, 1996a; Thompson and Goldstein, 1996b; Koshi and Goldstein, 1997; Thompson and Goldstein, 1997). While up until now they have concentrated on the prediction of local structure, the methods are robust and can be extended to prediction of other local attributes.

Objective IV. "Field Truth" Studies: assessing the importance of identified cold adaptive traits to success in the field and development of potential signatures for extraterrestrial life.

The first goal of these studies will be to bring the laboratory findings from above full circle, namely to test whether the cold adaptive traits that we identify have significant roles in the field. The second goal is to determine whether these traits can be converted to signatures of life useful for detecting extraterrestrial life. Toward these ends, we propose to address the following five questions.

1. Are cold adaptive physiological traits present in permafrost bacteria found in related bacteria from warm environments?

Our rationale is that the organisms we have isolated carry traits that have been selected for growth and survival in the permafrost habitat, and that these traits will not be as frequent in isolates from constantly warm soil environments. To test this hypothesis, we propose to compare various physiological traits of the permafrost strains with nearest relatives isolated from Puerto Rico. Puerto Rico occupies a tropical oceanic position at latitude 23.5°N, just south of the Tropic of Cancer. Persistent northeast trade winds maintain a uniform climate during the year with average temperatures ranging from 24 to 27°C. The island was formed by volcanic activity during the Lowest Cretaceous, approximately 120 million years ago, although the oldest rocks located in Southwestern Puerto Rico were created during the Jurassic era, approximately 195 million years ago (Jolly et al., 1998). **We have chosen to use tropical isolates for comparisons rather than temperate isolates to avoid any seasonal selection that would occur in temperate winters.**

The environmental temperatures prevailing in Puerto Rico and the permafrost are radically different. However, there are potential parallels regarding water availability. Precipitation is heavy in eastern Puerto Rico because the trade winds arrive at this side of the island first, leaving behind an average rainfall of more than 510 cm per year. Puerto Rico has an inner mountain range, the Cordillera Central, which covers the island from east to west, and determines the rain pattern observed in different parts of the island. Most of the residual rain remains in northern Puerto Rico with an average of 150 cm rain per year. Leeward of the Cordillera, rainfall is much less, averaging 0.9 cm per annum on the south coast and less than 0.8 cm for southwestern Puerto Rico (htpp://www.fs.fed.us/colorimagemap/images/ m411.html). Southwestern Puerto Rico has an average temperature of 30°C and the evaporation rate is 2.5 times faster than the annual precipitation rate, making this side of the island one of the most arid ecosystems observed in the tropics (Jolly et al., 1988). Thus, it is possible that genetic mechanisms important for dehydration tolerance in permafrost bacteria may have counterparts in bacterial isolates from southwestern Puerto Rico.

Our tropical sampling sites will be located in different sites in Puerto Rico. Some samples will be taken from the hot and moist soils that the northern side of Puerto Rico (no cold or desiccation stress) while other samples will be taken from the southern part of the island where hot and dry conditions prevail (no cold stress). In order to avoid biases introduced by sample-specific conditions, three samples of each soil type will be analyzed, each consisting of a composite of several representative samples. Bacteria will be isolated from these soil composites following the protocols used to isolate bacteria from the permafrost sites (Vishnivetskaya et al., 2000). Two incubation temperatures will be used, 27 and 4°C. The 16S rDNA gene of these organisms, as well as from DNA isolated directly from the soil samples, will be amplified by PCR using primers designed to amplify the 16S rDNA gene of bacteria isolated from Siberian and Antarctic sites. A phylogenetic tree will be constructed between the Puerto Rican and the polar isolates in order to determine the relatedness of these bacteria. Phylogenetically close bacteria isolated from Puerto Rican and polar soils will be compared in terms of their metabolic capabilities relating to cold and dehydration tolerance including: growth rates at 20, 10, 0 and -2.5°C; long-term freeze survival and freeze-thaw survival; synthesis of compatible solutes and alterations in membrane composition in response to low temperature and low water activity.

2. Are the taxa that appear uniquely cold distributed in fact found principally in polar habitats?

It is striking that four of the six major taxa isolated from the Siberian permafrost have strains from the Antarctic or other cold habitats as their closest known phylogenetic relatives. This suggests the selection for more than a few traits to realize this pattern. This correlation has not been thoroughly tested however since the postulated polar-unique taxa have not been directly searched for in warm environments. We will attempt to isolate from Puerto Rican soils taxa that are postulated as uniquely polar by using the enrichment conditions that were used for obtaining the permafrost isolates. Since this method may not be highly selective, we will also develop primers for the 16S rDNA of the permafrost clades and use these to for a more sensitive detection of these taxa in tropical soil DNA. Since several of the polar groups form a monophyletic lineage, it should be possible to identify clade specific primers. Any PCR products recovered will be cloned and sequenced to determine whether the clone belongs to the clade. If two primers can be successfully identified, we will try to identify a third probe/primer region so that we can quantify rDNA for these clades using quantitative (realtime PCR). We have successfully developed, verified and used this method for quantifying another (*Rhodococcus)* clade (Rodrigues, Aiello and Tiedje, unpublished). With this method we can also quantify how dominant the Siberian clades are in permafrost DNA, and thus have a better indication of the success of these groups in polar regions.

3. Are the genetic traits identified in the above objectives as likely important for cold adaptation found in the polar isolates but absent or rare in tropical strains?

If the traits are uniquely selected during the permafrost organisms' long term polar life, then these traits would not be expected to be maintained in the tropical bacteria. These traits will be screened for by using genes identified from the above studies to probe the DNA of the tropical isolates. If hybridization is found, we will identify primers from the genome sequence to attempt to amplify segments for sequencing to see if the nucleotide sequence is in fact similar. We can also screen for the particular expressed protein using the HPLC MALDI-TOF mass spectrometry protocal that will have been worked out under Objective 2 for these proteins. We will initially focus this test on the Arthrobacter group since we expect to be able to isolate tropical relatives of the Siberian strains. If question #2 above does not yield any tropical relatives of the postulated polar-unique taxa, then we will complete this test with other tropical isolates we recover.

4. Do the genetic traits identified in the above objective actually confer improved survival or growth under polar conditions?

This is an important test of the information gleaned from the genomic and proteomic studies. We will determine whether mutants with the target traits deleted have reduced survival or growth in simulated polar conditions, in other space-like conditions, and after several years when returned to their Siberian permafrost site. Similarly, we will add the trait to wild-type strains that lack the trait to see if their survival or growth is improved under the polar conditions. Under this objective we hope to cooperate with other NAI members who have facilities to simulate other planetary or space environments. We will return the mutant strains to the site in

microcosms perpared with sterile soil or in microcosms with genetically marked strains. Survival and growth will be measured by plant counts.

5. Can the cryo-adaptive traits be detected in permafrost samples?

If the above tests have identified traits important to microbial survival in permafrost, then we should be able to directly test for those traits in polar samples. We would initially attempt to devise specific primers for conserved regions of such genes since that is currently the easiest, most sensitive and specific means to detect particular genetic traits in environmental samples. Furthermore, the trait can be quantified, in principle, to one gene copy per DNA sample. But, the test does not have to be limited to PCR and DNA. Other chemical biomarkers that could be assayed are specific proteins, membrane components or electron carriers. If some taxa are proven polar-unique, there are likely a variety of chemical differences distinctive for these groups that could become potential assays. We will initially compare the permafrost and tropical sites to determine the presence (and perhaps quantity) of these cryo-traits in the two environments.

If cryo-markers (or, at a later stage, other markers for extraterrestrial features), are identified and validated as above, we will explore whether they can be automated and miniaturized for extraterrestrial life detection. We would intend to do this in cooperation with another MAI group who has expertise in approaches to reducing the idea to practice. We would bring to the team the biological ideas and approaches but the planetary chemistry, experience with feasible approaches for non-earth measurements, device engineering would need to be provided by others. Dr. McGrath, a member of our Center, is a mechanical engineer so he can at least provide us with beginning considerations on what might be feasible.

Our ultimate goal is to mine genomic and proteomic information to better understand how microbial life on earth adapts to cold temperatures using the ancient permafrost microbes as the model, and to mine that information for approaches to search for and help characterize any life in extraterrestrial environments.

D. Expertise and responsibilities of Center Co-investigators

1. Michigan State University

Michael Thomashow will be responsible for integrating the structural and function genomics studies and will directly supervise the gene conservation and gene expression microarray analyses. He will also assist McGrath is the proteomic studies targeted at identifying candidate freezing tolerance proteins (antifreeze proteins and potential cryoprotective polypeptides). Dr. Thomashow is molecular geneticist who has worked on both plant and bacterial systems and has extensive experience working on the cold acclimation response in plants with emphasis on the regulation and function of genes induced in response to low temperature.

James Tiedje will be responsible for the permafrost culture collection, the physiological characterization of these strains, development and use of probes to detect cryo-adapted strains

and genes, and for coordination of the work on Field Truth. Tiedje will also assist Thomashow in array expression studies. Tiedje has expertise in microbial ecology, including use of molecular and array technologies for understanding microbial communities and their activities.

Richard Lenski will supervise one of the three new evolution experiments described in this proposal (adaptation following a sudden shift to 4°C). He will also supervise some of the genetic analyses of the derived lines from the evolution experiments, including transposon tagging of selected mutations and genomic mRNA expression profiles. All of these techniques are currently being performed successfully in his laboratory. Lenski will collaborate closely with Bennett (University of California, Irvine) in all aspects of the design and analysis of the evolution experiments. Lenski is a leader in the field of evolutionary biology and is a pioneer of experimental evolution studies.

John McGrath will supervise, in close collaboration with Thomashow, the proteomic studies targeted at identifying and determining the specific activities of antifreeze proteins and potential cryoprotective polypeptides. In addition, he will lead the efforts in applying HPLC methods to analyze compatible solutes. McGrath is recognized for his published work on thermodynamic and mathematical modeling of the responses of cells to freezing and thawing which provides a means of predicting the state of cells in the frozen state. He has been a leader in the development and application of instrumentation aimed at exposing cells to controlled low water activity and low temperature states and is currently President of the International Society for Cryobiology.

2. University of Michigan

David Lubman will lead the proteomic profiling studies and closely collaborate with Thomashow, McGrath and Kathariou on the purification of specific cold tolerance proteins. Lubman has developed a number of separation methods for screening proteins that include liquid phase separations of proteins, chromatographic separation, isoelectric focussing of proteins and various ion-exchange methods for separating proteins. In addition, he is involved in developing sequencing methodologies using a variety of capillary separation methods including capillary electrophoresis, capillary chromatography and capillary electrochromatography for separation of protein digests. He has also been involved in the development of new mass spec techniques for the characterization of proteins including ESI and MALDI and the development of new hybrid mass spectrometers for on-line sequencing.

Richard Goldstein will develop and implement the bioinformatics techniques for analyzing the protein sequence information and model the evolution of cold tolerance proteins. This work will involve delineating the phylogenetic relationships between the sequenced psychrotrophic proteins as well as those of related species, and using this information to understand the origin and evolution of the proteins responsible for cold adaptation. He will also use standard and novel statistical approaches to identify proteins that are homologous or analogous to "antifreeze" proteins. Goldstein has developed a number of novel methods for understanding proteins in their evolutionary context, as well as using evolutionary information to understand the properties of specific proteins. He also has experience in developing models of evolution that explicitly consider protein structure and amino acid properties.

3. University of California, Irvine

Albert Bennett will supervise two of the three newly proposed evolution experiments with laboratory populations of the bacterium *E. coli* (adaptation to progressively lower temperatures, and adaptation to freeze/thaw cycles). He will also supervise the mapping of the thermal niches of these derived lineages and their fitness changes. He will be responsible for conducting the genomic analyses of these and previously derived lineages using high-density arrays. All of these techniques are currently being undertaken successfully in his laboratory. Bennett will work closely with Lenski (Michigan State University) in all aspects of the design and analysis of the evolution experiments. Bennett is a leader in the field of ecology and evolutionary biology.

4. North Carolina State University

Sophia Kathariou will supervise the genetic experiments directed at identifying genes with key roles in cold adaptation. Karhariou is an expert in microbial genetics and for a number of years has been studying low temperature gene expression and cold-essential genes in the food-associated psychrotropic bacterium (and pathogen) Listeria monocytogenes. Kathariou spent a sabbatical at MSU in 1997-1998 and worked with McGrath, Tiedje and Gilichinsky on the isolation and characterization of cold tolerance of the Arctic permafrost isolates.

5. University of Puerto Rico-Mayaguez

Lycely Sepulveda is the new microbial genetics faculty member at UPR-Mayaguez. She has expertise in cloning sequencing and characterizing functional genes in environmentally important organisms, and is well trained in microbial ecology. She will be responsible for isolating tropical organisms for comparison to the permafrost strains, their 16S rDNA sequencing, measurement of some of the cryo adaptive traits, and to coordinate with Tiedje the DNA-based methods for organism and trait detection.

6. Joint Genome Institute (JGI)

The JGI is one of the five major international high-throughput genome centers that participated in completing the human genome. They have also completed or are currently completing several microbial genomes, e.g. *Nitrosomonas, Prochlorococcus, Rhodobacter.* The JGI has high capacity and high quality product at cost-effective rates. In this project they are responsible for delivering approximately 90 million base pairs of sequence, assembly as appropriate for the level of coverage proposed, and the standard annotation. We are pleased that their science leaders (Brauscomb, Hawkins and Predki) have joined the team since their interest in these novel organisms, advances in technology expected during this project, and suggestions and interpretations for the array and proteomics phase, will be important to the research group.

II. Training

Our educational objective is to provide in-depth training in structural and functional genomics, including both an overall understanding and knowledge of experimental approaches, but to encompass that training in a context relevant to astrobiology. In this way our students will have cutting-edge knowledge for the genomics era, but will be able to use that knowledge to explore questions important for understanding the possible strategies of extraterrestrial life. We will implement this strategy by having students major in their existing (strong) degree-granting programs, but to establish an "area of specialization" in astrobiology. In this way students will not be diluted in their training in their basic discipline but will gain the perspective and knowledge needed to make significant contributions to the field of astrobiology. The specialization approaches also makes it possible for students from a variety of departments to enter the program and gain an astrobiology emphasis.

A. Educational program

The educational program for the "astrobiology specialization" will consist of the following:

1. A new graduate level course at MSU in "Genomics of Environmental Stress Tolerance." The course will cover the latest developments in our understanding of structural and functional genomics in prokaryotes, eukaryotes and archea; the experimental approaches and methodologies used in such studies including fundamental bioinformatics; and applications of the science to understanding the systems organisms have evolved to tolerate environmental stresses such as extremes in temperature, dehydration and desiccation, and UV-light and other forms of radiation. The course will be taught by Thomashow and other faculty at MSU and will include guest lectures from other Center investigators. The lecture outlines, references and other materials related to the course will be made available on the Web for students at the other Centers, members of the Astrobiology Institute and interested public

2. Short-courses to augment keys areas of student training. We have two courses in mind initially. One by Dr. Gilichinsky during his visits to MSU that is focused on the cryosoil environment and history of the Arctic and Antarctic and drawing similarities and dissimilarities to Europa and Mars. The second would be in informatics and data analysis relevant to genomic studies. This would help our students and post-docs get an early start on more in-depth research.

3. Access NAI internet courses of relevance to our theme. There are many institutions in NAI that have existing excellence in relevant planetary, space and geochemical sciences. It makes sense for our students to access that excellence instead of trying to offer this portion of the cross-training at our Center. In return, our Center will offer its expertise in genomics and evolution to other Centers that lack this expertise. We would expect our students with this specialization to take one such course provided that an appropriate quality course exists

4. Other relevant courses. To provide the breadth of knowledge for the specialization, MSU students would be required to take at least 2 of the following courses: Diversity of Prokaryotes (MIC 827); Advanced Microbial Ecology (MIC 829); Molecular Evolution: Principles and Techniques (ZOL 855); Quantitative Methods in Ecology and Evolution (ZOL

851); Evolution (ZOL 445); and Environmental Geochemistry (GLG 421). Students not at MSU would take courses of similar subject matter. These courses provide knowledge of the extant microbial physiologies, microbial interactions, molecular and deduced evolution and the geochemical environment of the lithosphere, all topics important to astrobiology.

Because our Center is distributed among institutions, we will use the following operating principles for students outside MSU. Those students and postdocs will participate in the Center's annual science retreat. Second, they will enroll in courses with content similar to the above at their institutions. Students at RAS and at UPR will spend one summer or similar time at MSU for a phase of their research. Students at UM, because of proximity, will participate in some of the MSU components depending on their relevance to their program.

B. Internal seminars

Three times each semester all Center researchers will hear half-hour seminar presentations on the progress and plans of several of the graduate students and post-docs. With this schedule, each researcher would present once per year. This mechanisms keeps everyone informed of the entire research spectrum, and provides both an incentive and check-point for progress and quality. One session per year would be at UM. Since MSU and UM are 1 hour drive apart, this activity is feasible for members at both institutions.

C. Research exchanges

We have budgeted funds for graduate students or postdocs to go to another institution either within our Center or within the NAI to conduct a collaborative experiment that draws on the unique resources of the partners. We can envision that some of our mutant strains with postulated special survival properties could be tested in space/planetary-like conditions available at other NAI Centers.

D. Symposium on "Genomics and Evolution of Extremophiles"

In year three or four, we would host an open symposium in which our results are summarized along with presentations by relevant invited speakers. The purpose is to bring together the cutting-edge body of knowledge for Center members, NAI members and interested peers. Our intent is to stimulate new ideas for the next phase our experimentation both with the Center and with other NAI members. At this stage in our research we should have several genomes well analyzed, a good idea of at least postulated evolutionary strategies, and hopefully some genes and their proteins identified that have key roles in life at low temperature.

The above program we believe provides for the appropriate cross-training and provides the astrobiology context so that the power of genomics, proteomics and evolutionary mechanisms can be brought to bear on astrobiology.

III. Management

Professor Michael Thomashow will be the PI and Director of our Center. Professor James Tiedje will serve as Associate Director and aid the Director in duties determined by the Director. We have found such a management scheme to work well in our NSF-STC Center for Microbial Ecology; one person has point responsibility, but has a second person fully cognizant of the breadth of Center activities with whom to discuss pertinent issues and implement actions.

An Executive Committee of three will be named from among the Center co-investigators that will establish operating policy and aid in making major decisions. The membership of the Executive Committee will rotate. The Executive Committee will make sure that all perspectives are heard and help ensure that the science conducted is cutting-edge in nature.

A significant challenge for any large integrated research effort is to ensure that members know each other, their expertise and their project goals, and to achieve this without burdensome requirements that sap enthusiasm. We propose to achieve this by our educational and training program (above) and the following activities.

A. Center Programs

1. Annual Retreat. We will have an annual retreat for all Center members, i.e. graduate students, post-docs and faculty, in which all work is presented as posters or oral talks and working groups meet to discuss plans in subtopics areas. Especially in the first years members need to get to know others on a personal basis so that anyone, even new students, know who has what expertise and feel comfortable in directly accessing that expertise. The Center for Microbial Ecology was able to achieve this level of familiarity and much success and efficiency derived when students perceived opportunities or solutions via others and acted on them directly. In a dispersed Center, it is money well spent to bring all members together once per year (at least in initial years) to review and plan the science.

2. External Seminar Program. We will have a seminar program coordinated and co-sponsored with the Center for Microbial Ecology and relevant departments. The speakers will be asked to stay long enough to meet with students and post-docs and interested faculty. In the first year we will emphasize inviting key scientists at other NAI institutions so that we can gain a better idea of possible research or educational collaborations.

3. Web-based genomics database. As detailed above, we propose to enhance our Web-based microbial informatics database to store and make accessible to center scientists the genome, proteome and expression data that is the core of this project. Our design serves researchers well at any partner institution, and the information is immediately available to all other members. We envision this tool to be at the heart of our research interactions.

4. External Advisors. We will name a small group of external advisors for the purpose of providing Center management with critique and suggestions on Center activities, progress and direction. We would likely invite these advisors to our annual retreat. After year two, we would

particularly ask them for advice on mid-course adjustments so that these could be implemented before the third year NASA review.

Our operating philosophy, which is consistent with the cooperative agreement mode of funding, will be to adjust our project funding in subsequent years based on our recent discoveries, relevant external advice, effectiveness of members and the needs of the NAI. We would use information from the retreat and planning sessions, the external advisors and the NAI management in making these decisions. Because of this operating principle the subsequent year budgets in this proposal are subject to change.

B. Minority program

The Center includes the University of Puerto Rico (UPR), Mayaguez, as one of the partner institutions for three reasons. First, one important test of genes hypothesized as important to cold adaptation is whether they are uniquely selected in polar regions or whether they also exist in ancient tropical environments. Hence the Center research need matches the involvement of UPR. Secondly, UPR students, because of their tropical ecosystem awareness, are attracted to other environmental research problems. Hence, our UPR collaborator believes that recruiting minority students to astrobiology research should be successful. Finally CME has built a strong relationship over a 15 year period with the three UPR campuses that teach microbiology. We draw on our linkages and mutual program experience in what is proposed here. We believe our past program has been successful. More the 30 UPR undergrads have done summer research internships at the CME and most have gone on to science careers. Four have received PhDs (one NIH and one NSF Fellow) under the CME program and four more are at various stages of their PhD program. We believe the key to minority progress, at least our sciences, lies in establishing and maintaining relationships with minority institutions; building a reputation where the minority students knows previous students have been successful; and listening to the minority institutions programmatic ideas since they know their students well. We, therefore, propose the following:

a) Summer internships at the majority institutions for undergraduate and graduate students to experience research in the former case and in the latter to learn particular skills for the thesis research.
b) Focus on a genomics/astrobiology theme for the molecular microbial ecology workshops offered annually by CME and UPR faculty to UPR microbiology majors on the three campuses. This is a two day intensive hands on laboratory during their spring vacation and rotated among the campuses. These have been oversubscribed and extremely popular. We propose that our Astrobiology Center takes over organizing two of these in the five year period.
c) Develop an astrobiology module for undergraduate microbial diversity course at UPR. In this manner advanced students who already have a budding interest in the diversity (including extremophiles) of microbes would be introduced to the special conditions and opportunities of astrobiology. This same module could also be used in MSU senior level microbial ecology course to interest students in the field. Since this course is spring term, students could also be attracted to joining Center research projects in the summer.

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Jet Propulsion Laboratory (2)

Pasadena, California

Astronomical Detection of Biosignatures from Extrasolar Planets

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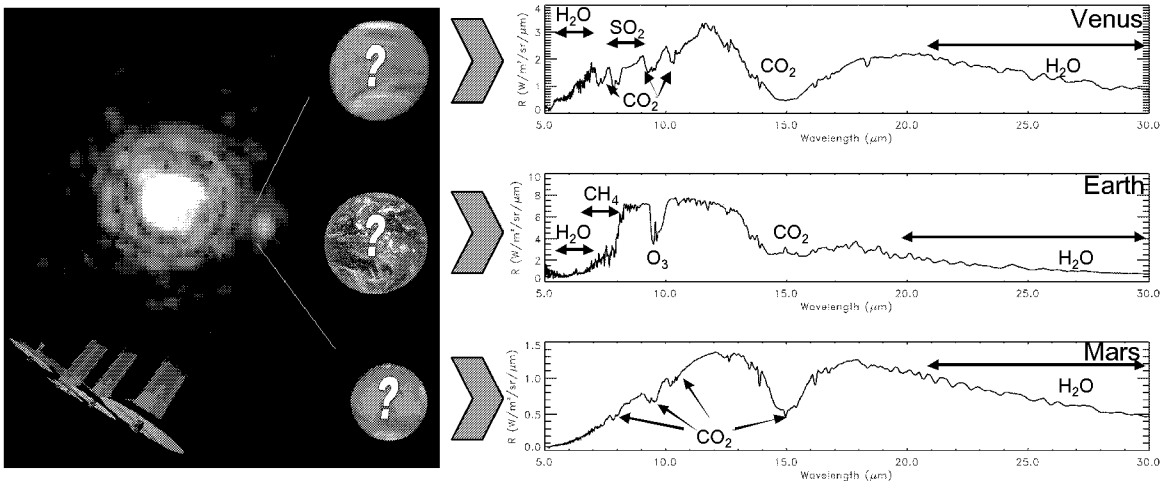


Are we alone? The goals of the proposed research are to understand the plausible range of atmospheric and surface compositions for terrestrial planets, and to learn how to use spectra to discriminate between extrasolar planets with and without life. The results of this research will provide essential material to drive the instrument design and search strategies for future NASA extrasolar planet detection and characterization missions.

A.1 PROPOSED PROGRAM

A.1.1 RATIONALE

Motivated by the recent discoveries of a multitude of extrasolar planets, NASA has initiated a series of studies for space-based observatories that will be able to search for life on these worlds. To optimize the designs of these NASA missions and to ultimately interpret the data that they return we must have the capability to recognize habitable worlds and to discriminate between planets with and without life. The intent of the proposed modeling work is to learn how to recognize the presence of life on extrasolar terrestrial planets by identifying the signatures of life in their spectra.

A.1.2 INNOVATIONS

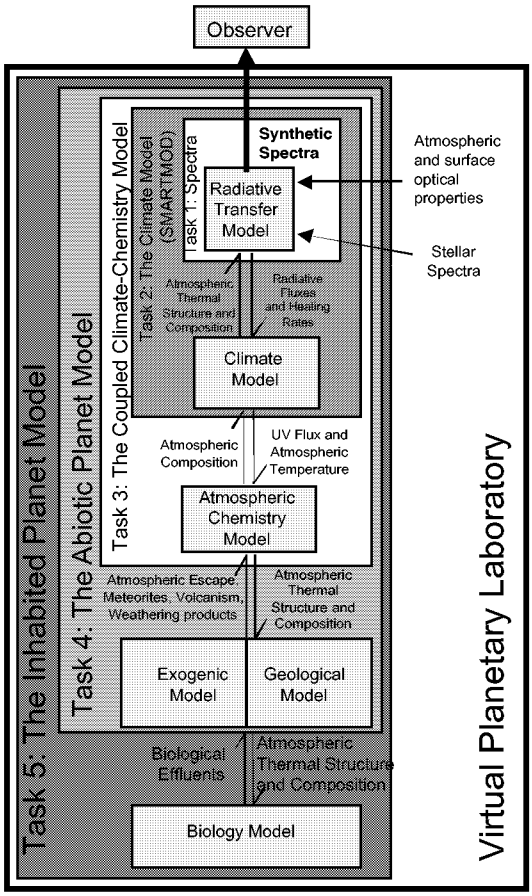
To achieve our goals we will develop a suite of innovative modeling tools to simulate the environments and spectra of extrasolar planets. These modeling tools will constitute a Virtual Planetary Laboratory and will provide the first models to couple the radiative fluxes, climate, chemistry, geology and biology of a terrestrial planet, to produce a self-consistent planetary state. This powerful new facility will generate of a wide range of plausible atmospheres for extrasolar planets, and for the atmospheres of early Earth.

A.1.3 DISTINGUISHING FEATURES

The proposed research is a significant multidisciplinary effort that combines existing models from five major research fields to develop a theoretical framework which parameterizes all the major processes of a terrestrial planet. The comprehensiveness and flexibility of the modeling tools enables a non-Earth-centric study of terrestrial planet atmospheres and the signs of life to extend our search for the signs of life to non-oxygen producing life, around stars very different to our own.

A.1.4 UNIFYING INTELLECTUAL FOCUS

Our objectives are to understand the characteristics and environments of plausible extrasolar planets both with and without life, and to use that understanding to drive the design and search strategies for future planet detecting and characterizing observatories.



The Virtual Planetary Laboratory

A.1.5 Proposed Research

The proposed research will provide improved understanding of the nature and characteristics of extrasolar planets, and the early Earth, and allow us to recognize the signs of life in these environments. This research will specifically address the question of false positive detections of life, and also help to quantify the effect of life on the atmospheric composition and spectrum of a terrestrial planet. The research tasks will ultimately provide a comprehensive spectral catalog, a "menu" of biosignatures, which will be used to determine the optimum wavelength range, spectral resolution and sensitivity required to remotely sense the signs of life in the atmosphere or on the surface of another world.

A.1.6 Training Plans

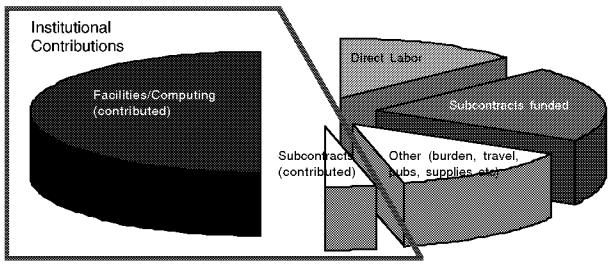
Training is an important part of this effort. Training is already in place for undergraduate and graduate students through the current education projects of its members. We will support 6 postdoctoral scholars with this effort and will train them in astrobiology and multidisciplinary research.

A.1.7 Management Approach of Personnel and Institution

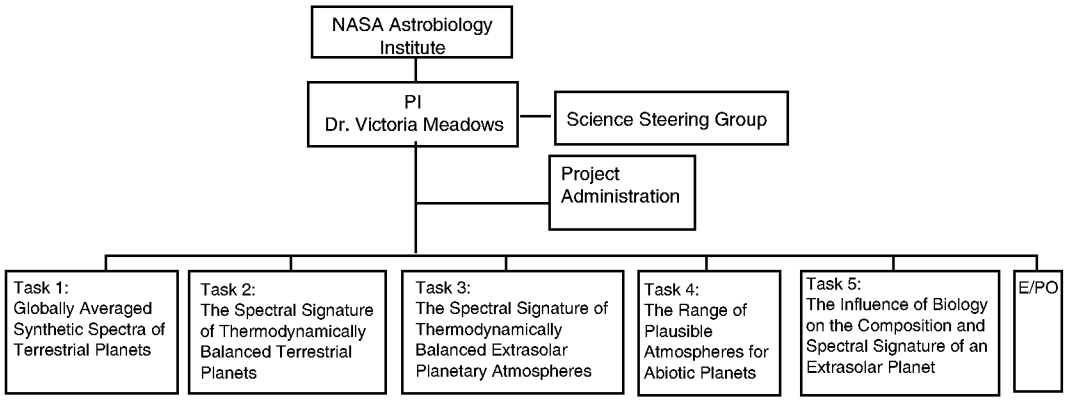
This research program is a cooperative effort consisting of a 17 member multidisciplinary team that leverages the resources and knowledge from 7 academic and research institutions. The majority of the computational research will be accomplished at JPL/Caltech, although all institutions will provide investigators' scientific expertise. Coordination and integration of the efforts of the research team members, in a timely manner, is the focus of this management activity.

The PI, Dr. Victoria Meadows, is accountable to NAI for the implementation, outcome, and scientific integrity of the research and the resulting products. The research efforts of the program will be accomplished within five major task groups, each with a Co-I task team and a Co-I assigned as Task Lead. A Science Steering Group, consisting of lead CoIs from five of the institutions, will assist the PI. The organizational structure also shows the EPO effort and the administrative support. The EPO effort will be managed in manner similar to the research tasks.

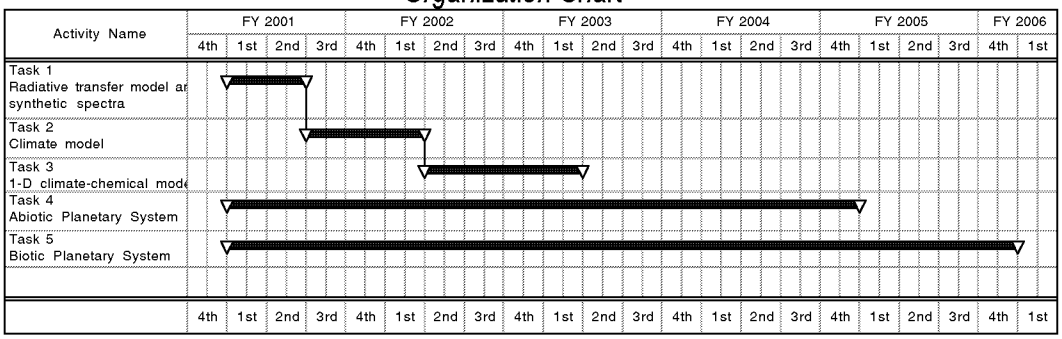
Tasks 1, 4 and 5 start in the first year of this effort. Task 1 starts the radiative-climate-chemical model development and Tasks 4 and 5 start by defining geological, exospheric and biological modules required for the planet models. Task 2 and 3 integrate climate and chemistry components to complete the mechanistic core of the planet models to be developed in Tasks 4 and 5. Work in the final two years of this effort will concentrate on integration of the geological, exospheric and biological modules with the mechanistic core to produce the planet models.



Funding Profile showing Institutional Commitments



Organization Chart



Schedule of Tasks

A.1.8 EPO Activities

This proposal contains an Education/Public Outreach proposal. The EPO proposal uses the research proposal focus of developing methods to characterize and search for life on other worlds to offer a compelling context for learning fundamental concepts in Earth and space science, physical science and life science. This EPO plan will partner proposal scientists with education specialists to implement educator workshops and public symposia. We will also produce web resources that will prepare and follow up with workshop and symposia participants. These will include a low-resolution interactive version of the Virtual Planetary Laboratory being designed by the research team, and a "Family Guide" addressing the search for life on other worlds.

A.1.9 Institutional Commitments

This proposal has a strong institutional commitment. JPL will provide a significant commitment in the form of $5.2M of supercomputing facilities, as well as office space and administrative and budgetary support for the project. Five of our other team institutions will provide commitments in the form of salaries, office space and computational facilities for five of the Co-Is.

A.1.10 Implementation of Collaborative and Networking Concepts of the NAI

Our research program will also support NAI s annual integration workshop and we will participate in the preparation of workshop reports. All research products will be documented in the open literature and available for the NAI/scientific community for review. The Virtual Planetary Laboratory will be a valuable tool for enhancing our understanding in a broad range of astrobiology fields. The VPL will be developed in collaboration with three other NAI member institutions, and will ultimately be made available for collaborative use with other members of the NAI.

The Next Generation Internet (NGI) will be used heavily to manage the research activities outlined in this proposal. Since all CoIs are at or near institutions already having a NAI-supplied videoconferencing area, a large fraction of the Science Steering Group meetings and the Research Program Team Progress meetings will be held as videoconferences.

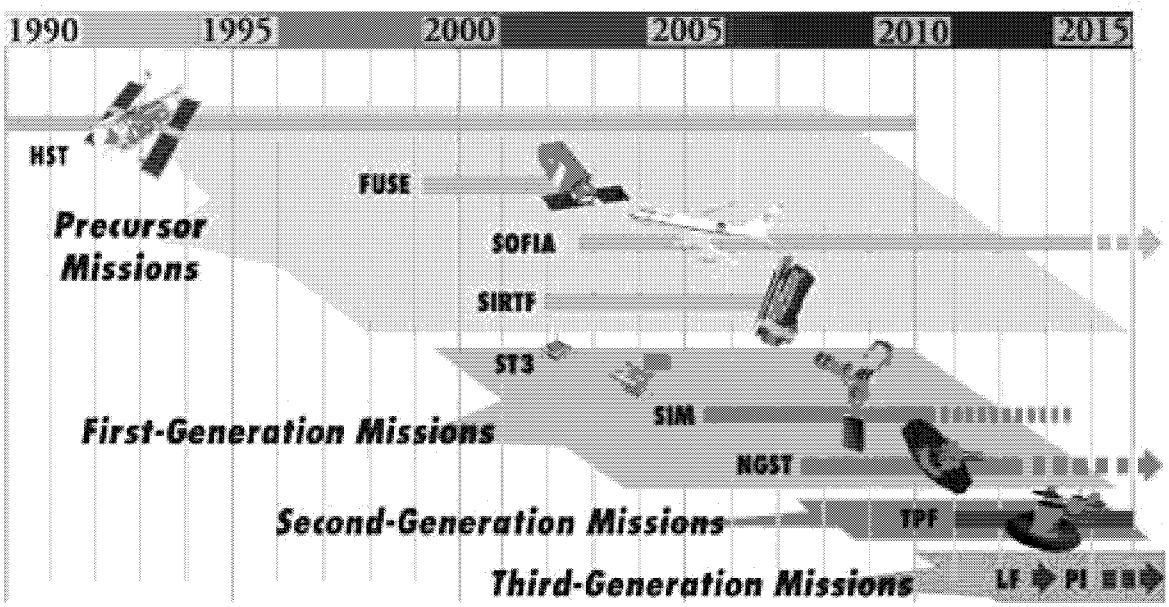


Figure C.1.1 Timeline of origins astronomical observing platforms (Origins Web site)

C.1 Objectives and Expected Significance of Research

The principal goal of this proposal is to learn to recognize biospheres on extrasolar planets by identifying the signatures of life that could be detected in their spectra (Goal 7 of the Astrobiology Roadmap, a preferred, currently underrepresented Roadmap goal).

Spurred by the recent discoveries of a multitude of extrasolar planetary systems, studies are already underway for instrumentation that can search for life on extrasolar planets, such as the space-based Terrestrial Planet Finder (TPF) mission, to be launched in 2011, and the next generation follow-on, the Life Finder mission. With these observatories currently in the early planning stages, our proposed work will provide timely resources and improved guidelines to better define the required capabilities and optimum survey strategies for these missions (Figure C.1.1).

To support these future missions, we will develop the ability to recognize habitable worlds and to discriminate between the spectra of planets with and without life.

We will also determine the optimum wavelength range, spectral resolution, and signal-to-noise ratio required to detect the signs of life on other planets.

C.1.1 What is an Astronomical Biosignature?

Some of the most overt astronomical biosignatures (signs of life visible from space) on our own planet are spectral features indicating the presence of liquid H_2O and chlorophyll on the surface, and abundant oxygen in the atmosphere (Figure C.1.2).

However, some of these astronomical biosignatures have only been characteristics of the Earth's spectrum for about half the time that the Earth has supported life (Holland, 1994). The Earth's spectrum and any astronomical biosignatures detectable *before* the advent of oxygen-producing life are currently not well-understood.

The simultaneous presence of strongly oxidized and reduced gases that are not in chemical equilibrium (e.g. O_2 and CH_4 in the Earth's atmosphere, Lovelock and Margulis, 1974) is thought to be a robust biosignature for many different kinds of planetary atmospheres. However, these robust indicators are generally much harder to detect via astronomical (remote-sensing) techniques.

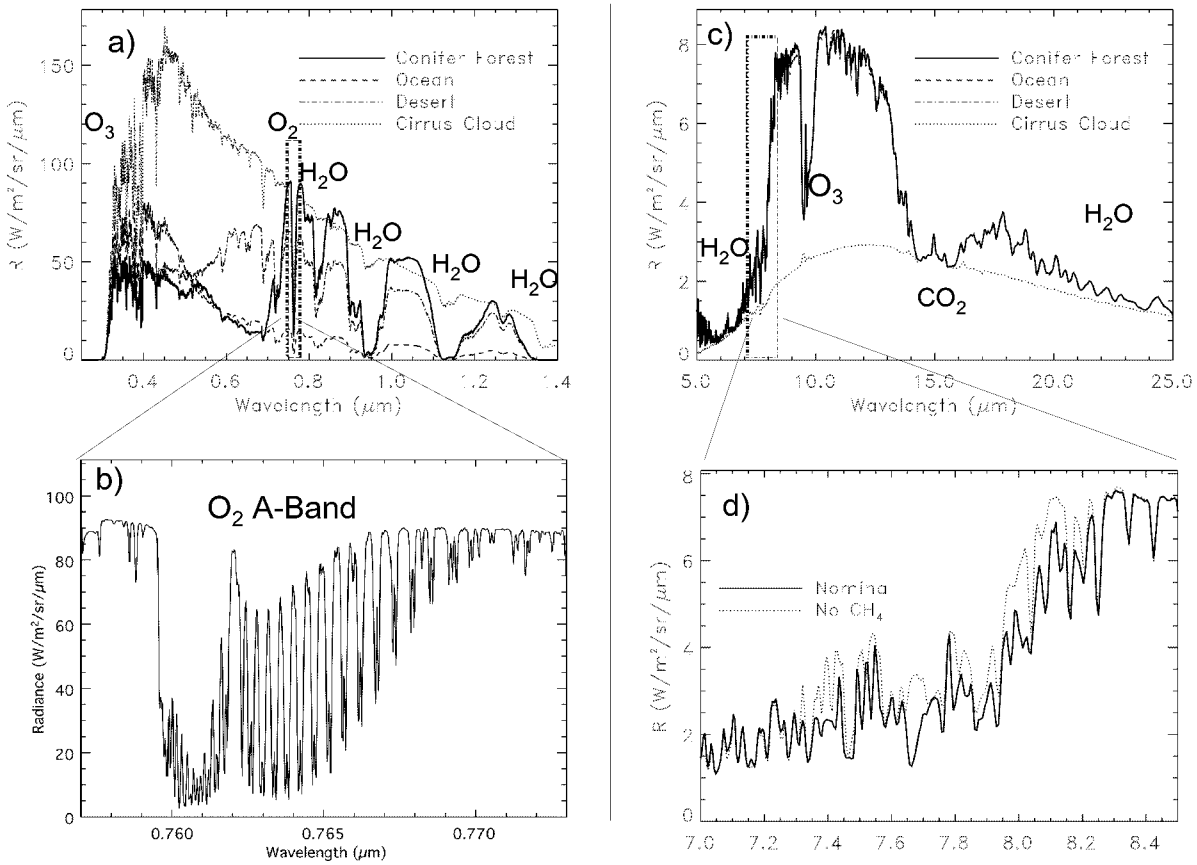


Figure C.1.2 The Earth's Biosignatures. Synthetic spectra of clear and cloudy terrestrial environments at solar (left) and thermal (right) wavelengths generated with the spectral mapping atmospheric radiative transfer (SMART) model. a) Synthetic solar reflection spectra of clear atmospheres over a conifer forest (solid line), a clear atmosphere over ocean (short dash line), clear sky over desert (dash-dot line), and for a sounding that includes a moderately thick (optical depth = 10) cirrus ice cloud over an ocean surface (dotted line). In each case, the sun is 60° from the zenith and the atmospheric thermal structure and the abundance profiles for H_2O, CO_2, O_3, N_2O, CO, CH_4, and O_2 are from the McClatchy et al. (1972) mid latitude summer atmosphere. Chlorophyll, a potentially important biosignature, has strong absorption in the UV and blue (<0.5μm) and in the red (0.6-0.7 μm), slightly less absorption in the green (0.55 μm). It is also sunlight highly reflective just beyond the visible range (>0.7 μm). Ozone (<0.3 μm), water vapor, and the O_2 A-Band (panel c) are also prominent in the Earth's solar spectrum. However, high clouds can hide most of these features. c) Synthetic thermal spectra of the environments described above. At these wavelengths, the spectrum is dominated by water vapor, ozone and CO_2 absorption, but is much less affected by surface cover. Reduced gases, such as CH_4 also absorb at these wavelengths (panel d), but their spectral signatures are far more subtle than the biosignatures at solar wavelengths.

C.1.2 Research Objectives

To reach our goal of being able to recognize the signatures of life on extrasolar planets, we have developed a series of research objectives, which are described below. These research objectives will be achieved with our research tasks, which will provide the theoretical framework required to recognize biosignatures in the spectra of extrasolar planets and to provide recommendations for instrument capabilities and search strategies required to detect astronomical biosignatures on extrasolar planets.

Objective 1: Requirements for Detecting Atmospheric and Surface Characteristics on an Earth-like Planet

We will explore the sensitivity of astronomical instrumentation to globally-averaged

surface and atmospheric properties, and astronomical biosignatures for modern-day Earth.

Objective 2: Extrasolar Planet Atmospheres and the Detectability of Biosignatures

We will move beyond the Earth-centric study of biosignatures to determine plausible atmospheric and surface compositions for a range of different terrestrial planet types around stars other than our own Sun. The detectability of biosignatures from these extrasolar atmospheres and surfaces will also be studied. This study for detectability, and subsequent studies throughout the proposed work will focus on detectability of biosignatures over a broad spectral range from the UV to the far-IR, and for different types of astronomical instrumentation.

Objective 3: Early Earth Atmospheres and the Detectability of Biosignatures

We will determine plausible environments for various stages of the Earth s abiotic and inhabited early history. We will understand the spectroscopic appearance of these early Earths, and how to identify biosignatures in the inhabited environment using astronomical instrumentation.

Objective 4: Abiotic Planet Atmospheres and the Determination of False Positives

We will understand the range of planetary atmospheres possible on a terrestrial planet without life. This study will allow us to identify possible false positives , planets without life that display spectral features that would otherwise be considered a biosignature. We will identify the circumstances under which a false positive can occur, and make recommendations on how to discriminate between a false positive signature and a true biosignature.

Objective 5: Inhabited Planet Atmospheres and the Definition of New Biosignatures

We will explore the effects of life on a planetary atmosphere and determine new biosignatures for a range of different terrestrial planet atmospheres.

C.1.3 The Virtual Planetary Laboratory

To achieve our research objectives we will develop a Virtual Planetary Laboratory (VPL), a suite of computer models that will allow us to simulate a broad range of planetary environments both with and without life, and to determine the spectral signature of these environments. These tools provide a significant innovation, because they will contribute completely new capabilities for understanding extrasolar planetary atmospheres and for astronomical biosignatures. These tools will be validated using observations of the current Earth's environment, with other terrestrial planets within our own solar system as controls. We will then realistically simulate the range of environments of the Early Earth and their spectra. To further expand our ability to recognize life on other planets we will ultimately generalize this effort to simulate biosignatures in terrestrial planetary environments very different from Earth around stars other than our own Sun.

Results generated throughout this multidisciplinary investigation will improve our understanding of the habitable zone (HZ) around other stars (Astrobiology Roadmap Objective 12) and will allow us to identify new potential biosignatures for a range of planetary environments other than that of present day Earth (Astrobiology Roadmap Goal 7 and Objective 13). The vast compendium of realistic synthetic spectra generated by this study will be analyzed to yield recommendations for required capabilities and optimum search strategies for future extrasolar planet detecting and characterizing instrumentation.

C.1.4 Simulating Planets and Their Spectra With the Virtual Planetary Laboratory

The spectrum of a planet is the product of the complex interplay of a broad range of environmental components and processes (Figure C.1.3). Hence, to generate realistic spectra of a range of plausible extrasolar terrestrial planets we must simulate planetary environments that include all the basic environmental components. The models used to generate these conditions must be consistent with known physical, chemical, and biological processes. The basic components include:

- incident solar/stellar flux
- thermal structure and composition of the atmosphere including gases, clouds and aerosols
- surface properties (land/ocean/ice/biology)

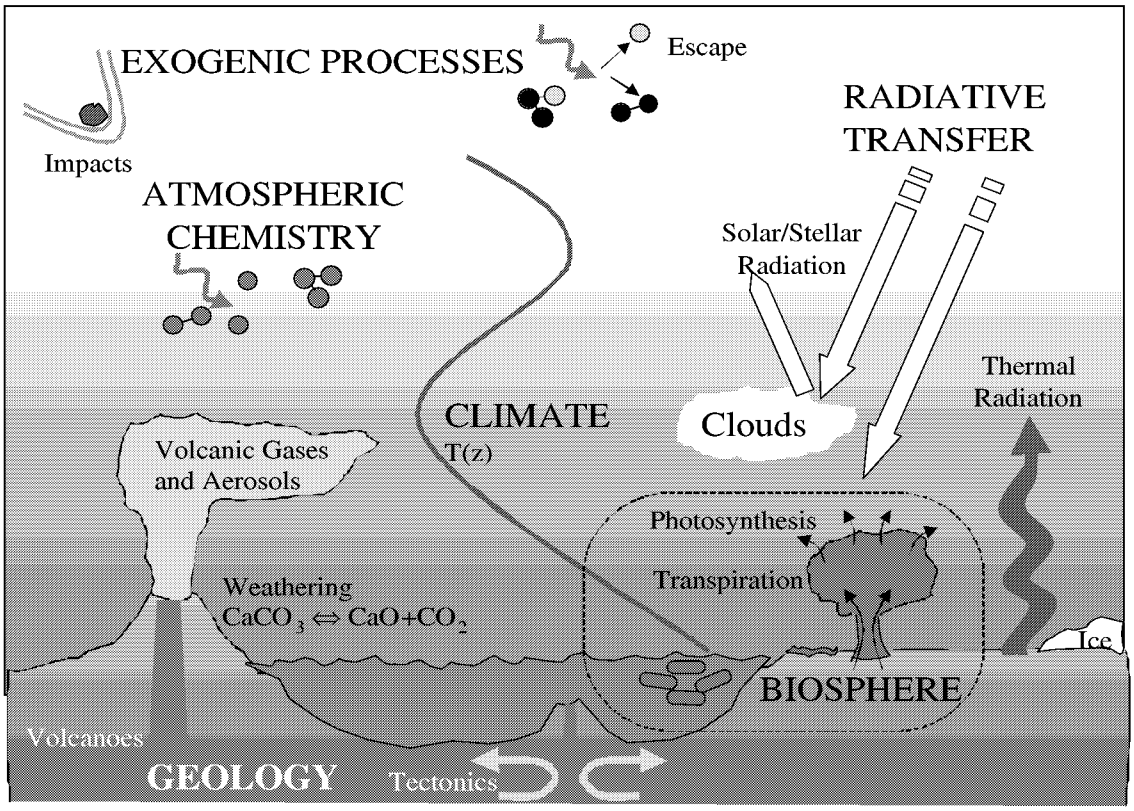


Figure C.1.3 Diagram demonstrating the basic components and planetary processes that ultimately contribute to the spectrum of a planet

The evolution and equilibrium state of a planet's environment are governed by a series of coupled physical, chemical, and biological processes. These processes are:

- Atmospheric and surface radiative heating and cooling rates
- atmospheric thermal and photochemistry
- impacts and atmospheric escape
- the hydrological cycle (oceans, clouds)
- geological processes such as volcanism, tectonics, weathering
- life processes such as respiration and photosynthesis

The Virtual Planetary Laboratory will simulate these planetary components and processes by integrating a series of existing, rigorous, radiative-transfer, climate, and chemical models that are currently used for studies of planets in our solar system, with geological and biospheric models that will be developed during the course of this activity.

The synthetic spectra generated by these models will be analyzed using a suite of statistical tools and astronomical instrument simulators to provide recommendations for the required spectral resolution, wavelength range, and signal to noise ratio needed to detect life on extrasolar planets. Once validated and documented, the VPL will be made available to the Astrobiology community through a Web-based interface or collaboration with our team. This powerful and versatile tool has many potential applications beyond the scope of this current proposal, and we have already identified a number of potential uses by other NAI members. These include:

- derivation of plausible extrasolar atmospheric compositions that can be generated for laboratory tests on microbial evolution and adaptation, and
- providing a theoretical framework in which to model and visualize the NAI's ongoing work on geological and biological processes and their effects on plausible atmospheres for early Earth and extrasolar planets.

Construction of the VPL will be achieved through five tasks, where each successive task builds substantially on the work of previous tasks. This hierarchical implementation plan

allows us to validate the model and produce interim results at each stage of added complexity and versatility. The major components of the VPL and their interrelationships are shown in Figure C.1.4. Note that this proposal is principally a computer modeling task. No new instruments or hardware are needed to achieve our stated objectives.

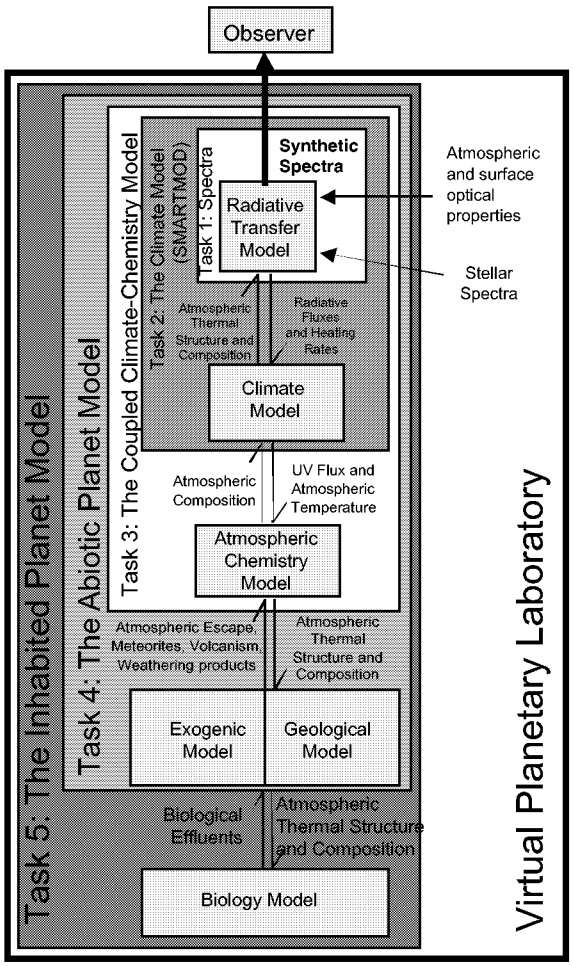


Figure C.1.4 This diagram shows the structure of the Virtual Planetary Laboratory. The suite of radiative transfer, climate, chemical, geological, and biological component models are shown as boxes, and their interactions with each other are shown by the arrows. The information transferred between these component models is labeled at each interface. The order in which these component models are coupled to each other during the course of this proposed work is specified by the Task number. The principal product at each Task development stage is a suite of synthetic spectra which can be used to identify potential biosignatures and derive required capabilities for astronomical instrumentation.

C.1.5 Research Tasks

To address our goals and objectives, the proposed research is divided into five tasks. With our final task, we will have developed a suite of tools for modeling biosignatures that will provide a significant improvement over currently available models. We will also provide improved understanding of plausible extrasolar and paleoatmospheres, and biosignatures and their detectability over a broad wavelength range applicable to many different astronomical technologies. The five tasks and the research objectives they address are:

- Task 1: Globally averaged synthetic spectra of terrestrial planets (Objective 1)
- Task 2: The spectral signature of thermodynamically balanced terrestrial planets (Objective 2)
- Task 3: The spectral signature of chemically and thermodynamically balanced extrasolar planetary atmospheres (Objectives 2 and 3)
- Task 4: The range of plausible atmospheres for abiotic planets (Objectives 2, 3 and 4)
- Task 5: The Influence of Biology on the Composition and Spectral Signature of an Extrasolar Planet (Research Objectives 2, 3, 4 and 5)

The first three tasks will also indicate the best wavelength range and spectral resolution for detecting the bulk atmospheric composition, physical properties, and trace gas abundances for a range of plausible atmospheres and for a number of different parent stars. The final two tasks will culminate in the development of coupled radiative and chemical models, with geological and biological processes, to determine the chemical equilibrium state for terrestrial planets with and without life. The atmospheres generated in this task will be visualized by tools developed in the second and third tasks, and the synthetic spectra of inhabited planets generated by this task will be used to identify new potential biosignatures.

To execute these tasks and develop the Virtual Planetary Laboratory, we require expertise in atmospheric photochemistry, atmospheric radiative transfer, molecular spectroscopy, climate modeling, paleoatmospheres, geochemistry, oceanography, and stellar spectral energy distributions. We also require plau-

sible constraints on the sources and sinks of abiotic volatiles (impacts, volcanism, tectonics), as well as the sources and sinks of biogenic gases and biogenic surface signatures. Finally, the sophisticated modeling tools required to simulate these processes also necessitate high-speed computing.

To provide this expertise, we have assembled a multidisciplinary team of 17 experts from 7 institutions. This team and their roles are described in detail in the management plan.

C.1.6 Significance of The Proposed Work and Anticipated Products

Much of the existing research in astrobiology focuses on the detection and recognition of the signs of life using *in situ* techniques to search for life on the surface of bodies in our own solar system.

This research will take the search for life beyond the confines of our own solar system and will provide a solid foundation for a new research field which has been identified as an underrepresented Astrobiology Roadmap Goal.

This work will provide the first systematic exploration of plausible atmospheres and conditions on extrasolar planets. In doing so, we will provide additional insight into the extent of the habitable zones around a range of stellar types. It will also elucidate the range of equilibrium atmospheric compositions that are associated solely with abiotic processes. Comparisons between an abiotic planetary model and a planetary model with life will allow us to assess the effects that life has on a planetary atmosphere, and to identify potential new biosignatures for a range of plausible extrasolar planet atmospheres and surfaces.

The principal product of the proposed work will be an improved understanding of the detectability of biosignatures in the a broad range of planetary environments. This effort will also produce a suite of powerful and versatile computer models that can be accessed by the Astrobiology community to run a wide range of experiments. The results of this research will provide a comprehensive spectral catalog, a menu of biosignatures, and recommendations for instrument capabilities for the development of missions that seek to detect life on other planets using astronomical techniques.

C.2 How This Work Will Build on and Extend the State of Knowledge

With this proposed work, we will provide a solid theoretical foundation for a field that is currently in its infancy. We will address the shortcomings that exist in the current models being used to study the detectability of biosignatures, and we will provide improvements in modeling methods, inputs and computational efficiency. We will also expand the existing modeling studies to cover a broad range of wavelengths, and include non-oxygen producing life, on planets other than Earth, around stars other than our Sun. In so doing we will improve our understanding of extrasolar planets, and provide the rigorous and comprehensive modeling work required to support and guide every step of the observational process for detecting biosignatures in extrasolar planets.

C.2.1 Improvements in Modeling

Existing studies of the detectability of astronomical biosignatures are extremely limited. Preliminary work on this topic can be found in the many feasibility studies for the Terrestrial Planet Finder mission (TPF book), and in conference proceedings for the NAI sponsored Pale Blue Dot Workshops (e.g. Traub and Jucks, 2000).

However, the most comprehensive paper on this topic published in a refereed journal is that of Schindler and Kasting (2000). To support the design of TPF-like instruments, these authors calculated synthetic thermal-IR spectra of oxidizing atmospheres containing various amounts of O_2 (and hence, O_3) and of reducing atmospheres containing various amounts of CH_4. They found that atmosphere containing more than about 1% of the present atmospheric level (PAL) of O_2 should have a detectable ozone signal for a high-resolution TPF, while atmospheres containing more than about 100 ppm of methane should have a detectable CH_4 signal.

These calculations were not, however, performed in a fully self-consistent manner. In the O_2/O_3 calculations, the temperature bulge in the stratosphere caused by heating by O_3 was simply removed for calculations at lower O_2 levels. In the CH_4-rich, primitive atmosphere calculations, stratospheric heating by CH_4

(which absorbs visible and near-IR solar radiation) was neglected. Incorrect estimates of the resultant temperature structure can affect the detectability of trace gases. Thus, these calculations are suggestive of what atmospheric biosignatures might be present, but they need to be redone with more self- consistent models.

Previous models used for these studies also have other shortcomings, which include:

- Limited spectral range (solar and near infrared wavelengths neglected)
- Multiple scattering by clouds and aerosols is not treated realistically, or neglected entirely
- Except for the present-day Earth cases, the chemical composition of the atmospheres are not self-consistent
- Spectra have been generated for isolated atmospheric profiles, neglecting spatial gradients in temperature or optical properties that might affect disk-averaged measurements.
- The effects of attenuation by zodiacal dust and limitations in instrument performance (spectral resolution, spectral range, and signal to noise) are not considered.

Models developed by this research effort as part of the Virtual Planetary Laboratory will overcome all the above shortcomings to derive plausible extrasolar atmospheres that are radiatively, physically, and chemically self-consistent.

C.2.2 Expanding The Wavelength Coverage

Existing work on the detectability of astronomical biosignatures has also focused principally on a narrow region of the mid-infrared. This focus on mid-infrared wavelengths was driven by initial designs for missions like Terrestrial Planet Finder, which are constrained by astronomical and technical implementation considerations to favor observations in the mid-infrared. At these wavelengths, the contrast between the radiation from the planet and its parent star is down by a factor of 1000 compared with the contrast in the optical, thereby providing higher signal to noise (S/N) information about the planet (Beichman, et al 1999). However, new studies indicate that coronagraphic techniques operating in the visible and near-IR may provide sufficient suppression of light from the parent star that they could also be feasible within the TPF time frame-generation. Coronagraphs could therefore provide a means to search for biosignatures over a promising spectral range for which there are few if any focused studies on their detectability.

To improve our understanding of potential biosignatures and their detectability throughout the spectrum, we therefore need to attack on two fronts. First, we need to better understand the features available in the mid-infrared region for a range of plausible extrasolar planets, not just for the commonly-modeled clear-sky Earth. Secondly, we need to comprehensively explore biosignatures available at other wavelengths that may be accessible to technology different than that currently planned for TPF.

C.2.2.1 Further Work Required in the Mid-IR

If nulling-interferometery is chosen as the observing technique for TPF, then clearly we need to rigorously model the mid-IR spectral region for a range of plausible extrasolar planet atmospheres to determine:

- Sensitivity to known mid-IR biosignatures for a range of different trace gas abundances and bulk atmospheric composition (Figure C.1.2d)
- Sensitivity (if any) to surface biosignatures (Figure C.1.2c)
- Potential new biosignatures for a range of paleo-Earth atmospheres and sensitivity to these biosignatures
- Potential new biosignatures for a range of atmospheric types other than that of present day or paleo-Earth and sensitivity to these biosignatures
- Potential sources of spectral confusion due to overlapping features from other molecules
- The effect of clouds on the detectability of biosignatures (Figure C.1.2c)
- Potential false positives in this wavelength range. Spectral signatures that mimic those produced by life, but are instead derived from an abiotic source in the planet s atmosphere or on its surface
- Improved estimates for S/N, spectral resolution, and optimum wavelength range for robust detection of biosignatures

All this information will be provided by the Virtual Planetary Laboratory over the course of our proposed work.

Why We Should Explore Wavelengths Other Than the Mid-IR

In addition to exploring the 5-30μm region in more depth than existing studies have done, there are many reasons to explore wavelength regimes beyond this region.

Enhanced Detection of Disequilibrium Biosignatures: The mid-IR region is not optimal for detection of classic disequilibrium biosignatures (like CH_4 or N_2O in the presence of O_2) These features are much more obvious at optical and near-infrared wavelengths, as already demonstrated during the Galileo flyby of Earth (Sagan, et al 1993). These disequilibrium biosignatures are likely to be more robust indicators of life, and desirable targets for the next generation of planet detection and characterization missions. These classic biosignatures are difficult to detect at mid-IR wavelengths for 2 reasons:

1) O_2 has no prominent spectral features in the mid-IR. At these wavelengths, the presence and concentration of O_2 must be inferred from detection of O_3, which models indicate rises to appreciable concentrations even at relatively low O_2 levels (Kasting and Donahue, 1980). Inferring O_2 abundance from O_3 may or may not be robust in atmospheres with different chemical composition and incoming solar flux to our own.
2) CH_4 is difficult to detect in the presence of water vapor without high signal to noise (S/N~100) and spectral resolutions higher than R=1250 because the absorption features are strongly overlapped (Figure C.1.2d).

Sensitivity to Surface Features: As is graphically demonstrated in Figure C.1.2c, although the mid-IR region is sensitive to both surface temperature differences and trace gas abundances, it is extremely insensitive to underlying surface composition. This is mainly due to the lack of spectral features and the uniformly high emissivity of virtually all surface types (ocean/land/vegetation) at these wavelengths. (In analysis of mid-IR remote-sensing data of the Earth, the emissivity of virtually all surfaces is routinely set to 1.0 as an acceptable approximation.)

However, as shown in Figure C.1.2a the optical and near-infrared regions of the spectrum display a rich array of spectral features due to surface composition. With optical observations taken at a set of discrete wavelengths, we could detect chlorophyll on the surface and distinguish between a world dominated by oceans, desert, or forest.

Although current technology still favors the mid-infrared region for observing extrasolar terrestrial planets, more robust indicators of habitability and life may be available in other spectral regions. The detailed study proposed here, which will explore planetary features and biosignatures available over a broad wavelength range will allow us to identify the best wavelength choices for determining the atmospheric and surface characteristics of a planet, including the presence of life. The proposed work will also allow us to make an informed decision as to whether more robust biosignatures in other wavelength regions compel us to develop the more challenging technology required to observe terrestrial planets at optical and near-IR wavelengths.

C.2.3 Determining False Positives

Early TPF support studies indicated that the presence of ozone may be the best biosignature available in the targeted mid-IR region. Angel et al, (1986) first pointed out the significance of ozone as a potential biosignature. Unlike O_2, O_3 absorbs in the thermal IR, with a strong band at 9.6um. In the Earth's atmosphere ozone has a nonlinear dependence on the atmospheric O_2 abundance. Models indicate that it rises to appreciable concentrations even at relatively low O_2 levels (Kasting and Donahue, 1980). Hence, the presence of ozone, and the consequently inferred presence of oxygen, while being highly suggestive, and an excellent first step target, is not on its own an unambiguous or comprehensive indicator of life.

There are several known mechanisms by which oxygen can be produced abiotically in a planet s atmosphere. These include photodissociation of water vapor on modern day Earth, and potentially during the loss of Venus ocean in its early history (Schindler and Kasting, 2000), photodissociation of CO_2 (on present day Venus and Mars), and reduction of

the normal planetary sinks for oxygen on small planets that lack volcanism and weathering (Mars).

To guard against misinterpreting spectra of extrasolar planets, we will use the VPL to better understand the plausible atmospheres likely for terrestrial planets that do not contain life, and to understand under what conditions an abiotic planet could mimic a biosignature (i.e. produce a false positive). In the course of this study, we will also be able to determine any auxiliary information needed to distinguish between abiotic or biological sources (such as planet size, planet-star separation, and the presence of other gases in the spectrum).

C.2.4 Expanding the Types of Life and Their Environments

Current studies of the definition and detectability of astronomical biosignatures have also concentrated almost exclusively on biosignatures for life that produced oxygen, on a planet orbiting a star with the same spectral characteristics as our own Sun. A search for life that is targeted principally on oxygen-producing life may fail to recognize planets that are inhabited by organisms that produce different biogenic gases like those produced by early life on Earth. However, even though we are developing an understanding of the biology and chemistry of this early life, the atmospheric and surface signatures of this form of life are not fully understood.

Similarly, the climate and atmospheric temperature structure of a planet's atmosphere is strongly affected by the spectrum of radiation output by the parent star. Assumptions made about the detectability of biogenic gases may no longer be valid when dealing with a planet orbiting a star of very different spectral type than our own, with a different atmospheric temperature structure and reflected stellar spectrum.

With the proposed work we will expand upon existing work to consider different forms of dominant carbon-based life on a planet s surface, and will the understand the chemistry and climate of planets orbiting stars other than our own. These studies will greatly expand the likelihood of recognizing life on a wider variety of extrasolar planets.

C.3 Technical Approach and Methodology

To achieve our research objectives, we propose an innovative, comprehensive, systematic, multidisciplinary modeling study. This research program will consist of 5 major modeling tasks. These 5 tasks allow us to progressively build a suite of models into a tool that we call the Virtual Planetary Laboratory (VPL). We will use the VPL to derive plausible surface and atmospheric environments for extrasolar planets, and generate high-resolution spectra of the radiation that they reflect and emit. These spectra will be studied to discover and characterize new astronomical biosignatures. This work will help drive the design and search strategies for planet detecting and characterizing instrumentation.

Once completed and validated, the VPL will be made available to the community as a multipurpose tool. This tool will be able to assimilate ongoing research on geological and life processes from members of the NAI and visualize the effect of our new understanding on the atmospheric composition and spectral signature of a terrestrial planet.

The VPL will incorporate a set of existing, highly sophisticated radiative transfer, climate, and chemical models that were developed and validated for studies of the atmospheres of the Earth and other planets in our solar system. These component models provide significant improvements over those that have been used in earlier astronomical biosignature studies. The combined model will contribute completely new capabilities for defining and detecting astronomical biosignatures.

Each of our 5 research tasks will incorporate the tools developed in all previous tasks, and will include a series of experiments to validate and utilize the integrated product. This approach is illustrated in Figure C.1.4. In each step, the number of parameters that must be specified a-priori will be reduced, as more processes are included explicitly in the models.

Task 1, Globally Averaged Synthetic Spectra of Terrestrial Planets: The objective of the first task is to understand the information content of disk-averaged spectra of terrestrial planets. To do this, we will use a radiative transfer model and atmospheric and

surface data for Earth, Mars, Venus and Titan to generate spatially resolved synthetic spectra. These spectra will then be spatially averaged to simulate a range of illumination conditions (phase angles) and viewing geometries. These results will be processed with an observing system simulation tool, that simulates the spectral and spatial resolution, signal levels, noise sources, and other properties of planned space-based observing systems, including nulling interferometers and coronographs.

Our radiative transfer modeling tools have a number of advantages over those used in the pioneering studies of astronomical biosignatures by Schindler and Kasting (2000), Traub & Jucks (2000) and others. Specifically, our spectrum-resolving (line-by-line) model provides an explicit treatment of solar and thermal radiation in realistic, planetary atmospheres, where both gas absorption and multiple scattering contribute to the radiation field. Terrestrial spectra will be derived for a range of surface cover types (ocean, desert, grassland, bog, forest) atmospheric and surface temperature distributions, cloud cover, and trace gas abundances. Spectra of Venus, Mars, and Titan will be produced as abiotic controls. These results will be analyzed with instrument simulators to quantify the instrument parameters required to detect known biosignatures in the Earth's atmosphere, over a spectral range from the UV to the far-IR.

Task 2, The Spectral Signature of Thermodynamically Balanced Terrestrial Planets: One limitation of the methods described in Task 1 is that they require a significant amount of information about the physical and chemical properties of the planetary environments to be investigated. Environmental properties that contribute to the spectra of a terrestrial planet include the range of surface types and their areal coverage, the atmospheric thermal structure and composition, presence and spatial coverage by condensate clouds and photochemical aerosols, stellar illumination intensity and distribution, etc (Figure C.1.2). These methods are therefore best suited for studies of planetary environments whose physical and chemical properties are well constrained by observations. A broader range of environmental conditions can be used, but their properties must be specified a-priori, and substantial care is needed to insure that the combination of conditions is physically and chemically plausible.

In this task, we will develop realistic physical models to simulate environments that are roughly in physical, chemical, and biological equilibrium, such that they require a minimum number of ad-hoc parameters. This approach to an investigation of biosignatures is consistent with the conjecture that life can be detected as a thermodynamic and/or chemical process that forces environment conditions away from their abiotic equilibrium. Tasks 2 through 4 provide the tools needed to simulate equilibrium abiotic environments. Task 5 then introduces biological processes that perturb these environments, producing new equilibrium states whose spectra can be investigated to identify biosignatures.

In Task 2, a one-dimensional (1D) Radiative-Convective-Equilibrium (RCE) climate model will be used in conjunction with an energy balance climate model to construct a range of planetary environments that are thermally self-consistent with their prescribed chemical composition. Methods described in Task 1 will then be used to study their spectral signatures.

1D RCE climate models have been widely used for studies of the Earth (Manabe and Wetherald, 1967) and other planets in our solar system (Pollock et al. 1980; Crisp, 1989; Gierasch & Goody, 1968). These models provided valuable insight into the physical processes that control the thermal structures of these planets. They have also been used to simulate the paleoclimates of these atmospheres (Kasting, et al, 1984; Pavlov et al. 2000). However, they have not yet been used widely for studies of plausible extrasolar planet atmospheres.

The 1D RCE model adopted here also has several advantages over those used in earlier studies because it employs the comprehensive radiative transfer models described in Task 1, and includes a more realistic treatment of convection, condensation, and other physical processes. This model will first be validated through simulations of the current environments of Venus, Earth, Mars, and Titan. This validation exercise will include both comparisons of the equilibrium thermal structures of these environments and detailed comparisons of their reflected solar and emitted thermal spectra. The information content of the globally averaged, radiative equilibrium spectra generated for these bodies will also be compared to that of the spatially resolved and disk-integrated spectra of these terrestrial planets that were generated in

Task 1.

Task 3, The Spectral Signature of Chemically and Thermodynamically Balanced Extrasolar Planetary Atmospheres: In the third task, we will add even more to the range of realistic environments that can be investigated for the detectability of Earth's paleoatmospheres and biosignatures by generating spectra of extrasolar planets atmospheres that are in thermodynamic and chemical equilibrium with specified surface and exogenic boundary conditions. These environments will be simulated combining the 1D RCE climate model with an existing 1D photochemical model. With this combined model, we will explore the detectability of the currently accepted list of potential biosignatures in both oxidizing and reducing atmospheres, over a broad wavelength range, and for a range of stellar types. The high-resolution solar and thermal spectra generated in these experiments will be used to further constrain the instrument parameters and capabilities needed to retrieve the physical and chemical properties of these environments from whole-disk observations of extra-solar planets.

Task 4, The Range of Plausible Atmospheres for Abiotic Planets: The range of environments that can be studied with the models developed in the three Tasks is still somewhat limited, because ad-hoc surface and exogenic boundary conditions are still needed. The relevant surface boundary conditions include sources and sinks of volcanic effluents (gases and aerosols), surface weathering processes, and the exchange of volatiles between the atmosphere, land, ocean, and the planet interior. Exogenic processes that define the upper boundary conditions of the system (along with the incoming stellar flux and outgoing thermal flux) include the influx of meteoritic material and atmospheric loss.

Mechanistic models for these processes will be incorporated into the global 1D chemical/ climate model in Task 4, to produce a versatile, self-consistent tool for simulating a broad range of abiotic planetary environments, and the spectra that they produce. (The Abiotic Planet Model). This model will first be validated through simulations of the present-day environments of Venus, Mars, and Titan. It will serve as the testbed for interdisciplinary studies of the plausible range of climate and chemistry for extrasolar terrestrial planets and for implications the habitability of these planets. We will use these results to determine likely "false positive" detections of life from abiotic planets. These studies will also include investigations of the equilibrium surface and atmospheric conditions of the abiotic early Earth, and early Mars and Venus, as well as a range of other highly oxidized and reducing environments with a range of surface and exogenic inputs.

Task 5, The Influence of Biology on the Composition and Spectral Signature of and Extrasolar Planet: As the final step in our effort to simulate the effects of life on extrasolar planets, we will incorporate a mechanistic model of a biosphere into the abiotic planet models developed in tasks 1-4. The biosphere model will modify the surface boundary fluxes of volatile species. This comprehensive, coupled, 1D climate, chemical and biological model (The Inhabited Planet Model) will be used to derive the age-dependent thermodynamic and chemical characteristics of planetary atmospheres and surfaces for a range of plausible biological inputs. At this point the IPM will support studies of the unique attributes of a habitable terrestrial-type planet that result from the presence of life, and the detectability of these attributes by remote-sensing spectroscopic observations. This will allow us to identify potential new biosignatures. The IPM will also provide insight into the possible ways that life can sustain the habitability of a planet.

Why Not Use a 3D Model? An important aspect of the VPL is the decision to use a one-dimensional framework. 3D models of the terrestrial climate system could be used as the basis for a generalized climate model. However, we believe that uncertainties in the application of these models to other systems, the vast increase in computational expense and complexity resulting from the use of these models, and the ability to constrain the key predictions resulting from such models (such as pole-to-equator temperature contrast, and species mixing) mean that application of a 3D framework at this stage is unnecessary-and indeed limiting.

Several factors compromise the utility of 3D tools for modeling the climates and biosignatures of extrasolar planets. First, even though the very best of these models do a very good job of simulating the Earth's present climate, they have been much less successful at modeling the circulation and spatially-dependent thermal

structure of other planets. Specifically, these models have not yet been proven able to simulate the dynamics of planets with rotation rates that are slower (Venus) or faster (Jupiter, Saturn) than those of Earth. These shortcomings have been attributed to both the physics (vertical distribution of solar and thermal energy, treatment of free and forced convection, surface friction, *etc.*), numerics (numerical diffusion and dispersion, resolution) as well as the inherent assumptions made in these models (*e.g.* the Phillips thin atmosphere approximation, hydrostatic approximation, *etc.*). While there has been some recent progress on the slow and rapid rotation limits (*e.g.* Del Genio and Zhou, 1996; Showman and Dowling, 2000), these models have not yet been fully validated even for the easier problem of dynamics and the related pole-to-equator temperature contrast, let alone the more difficult problem of species transport.

The primary advantage of a mature and validated 3D circulation model would be its ability to self consistently determine the heat and species transport from system parameters such as the rotation rate, gravity, and surface properties (albedo, topography, etc.) Ultimately, such a modeling system should be developed to refine calculations undertaken with the Abiotic Planet Model. However, in most cases of yet-to-be-discovered extrasolar terrestrial planets, we will not have access to these important input parameters. For the APM, we will use prescriptions for the pole-to-equator temperature contrast and mixing which are based upon eddy parameterizations and/or global entropy considerations. While these parameterizations will be just as limited by the lack of input data and likely (at least) as erroneous in predicting mixing, they allow mixing to be treated as a free parameter for each case and allow vastly more APM experiments to be undertaken for a given amount of computing time. In short, the results of 3D calculations would not be significantly more rigorous, accurate, or dependable than ones derived using ad-hoc assumptions of these properties, based on observations of planets in our solar system.

The following 5 sections describe each task in greater detail. In each section, we will review the background and previous work pertinent to that task. We will also describe the technical approach (models), the research methodology, and the expected significance and products.

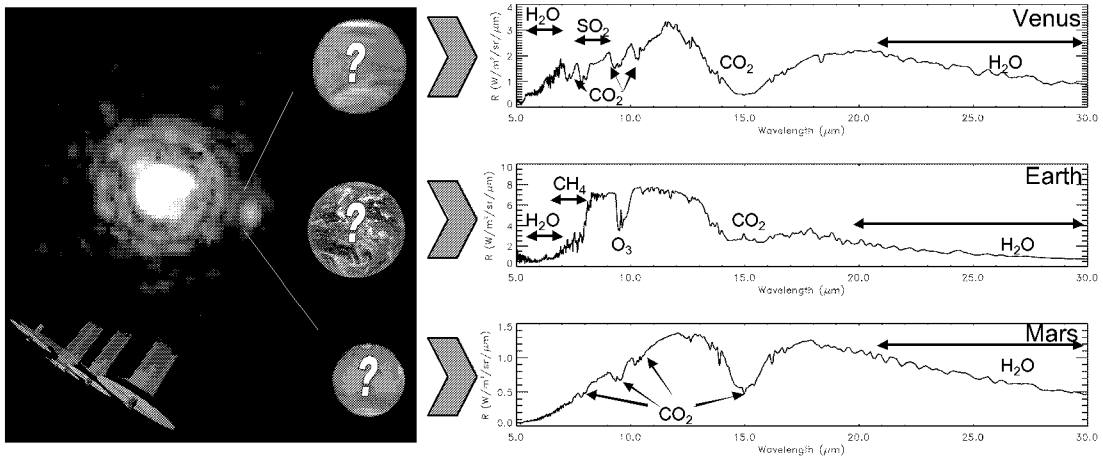


Figure C.3.1 Moderate-resolution spectra can be used to probe the atmosphere and surface of an extrasolar planet. Synthetic thermal IR spectra of Venus (top), Earth (middle), and Mars (bottom) obtained from the Spectral Mapping Atmospheric Radiative Transfer (SMART) model are shown at a resolution of 5 cm^{-1}. These spectra show the major absorption features in the CO_2-dominated atmospheres of Venus and Mars, and those in the $H_2O/CO_2/O_3/CH_4$ dominated terrestrial atmosphere. The CO_2 hot band at 9.4 μm overlaps the ozone 9.6 μm band, reducing its detectability in CO_2 atmospheres.

C.3.1 Task 1: Sensitivity to Environmental Properties in Globally-Averaged Synthetic Spectra of Terrestrial Planets.

Spectroscopy is the most powerful technique available for retrieving the characteristics of planetary surfaces and atmospheres by remote sensing. Spectra of the Earth and other planets, acquired over a wide range of wavelengths, have yielded a great deal of information about the structure and composition of the atmosphere and the properties of the underlying surface (Figure 1). However, the first generation instruments for studying extrasolar planets are expected to provide only disk-averaged spectra with modest spectral resolution and signal to noise. The objective of this task then is to understand the information available from disk averaged spectra of terrestrial planets. To do this, we propose to implement a suite of realistic atmospheric radiative transfer models and observational system simulation tools. The radiative transfer models will be used to generate high spectral resolution, spatially resolved spectra of the Venus, Earth, Mars, and Titan (Figure C.3.1). These spectra will then be averaged over the planet s disk, and then processed with the observational system simulators to provide a quantitative assessment of their information content.

The following sections summarize the types of information that can be retrieved from the spectrum of a planet. We then describe the radiative transfer algorithms, their data requirements, and the observational system modeling tools adopted for this investigation. The role of ultracomputing is then defined and justified. Finally, we summarize the specific experiments and products, and their significance.

C3.1.1 What Can We Learn About An Extrasolar Planet From Disk Averaged Remote-Sensing Spectroscopy?

Remote sensing spectroscopy has provided the primary tool for documenting the properties of the surfaces and atmospheres of planets and their satellites in our solar system. This experience suggests that disk-averaged observations of an extrasolar planet can yield information about the following properties:

- The presence of an atmosphere and its bulk chemical composition
- The nature of the emitting/reflecting layer (solid/liquid surface vs. an opaque cloud deck) and its wavelength-dependent optical properties (albedo, thermal emissivity, temperature)
- Atmospheric pressure at the surface (or over an opaque cloud deck) and total atmospheric mass above that surface
- Atmospheric structure (variation of temperature and pressure with altitude).
- Trace gas mixing ratios and their spatial distribution.

Astronomical Biosignatures

In addition to the basic parameters described above, which are common to all planets with atmospheres, a planet which harbors life may also exhibit astronomical biosignatures, that is, spectral features that are indicative of life.

The concept of an astronomical biosignature was introduced as early as 1965 when James Lovelock proposed that the presence of chemically based life on a planet would change the composition of its atmosphere away from the abiological steady state, and that the change would be recognizable even at astronomical distances (Lovelock, 1965; Hitchcock and Lovelock, 1966). Lovelock went further to argue that on Earth at least, the interaction between life and the atmosphere was so intense, strongly driving our atmosphere away from the chemical equilibrium state, that the atmosphere could be regarded as an extension of the biosphere (Lovelock and Watson, 1982). If this is generally the case, studies of a planetary atmosphere should provide valuable clues to the presence of otherwise undetectable life on the planet's surface.

Lovelock also identified what is now perhaps considered to be one of the most conspicuous astronomical biosignatures, the simultaneous presence of an oxidized gas, such as O_2, and reduced gases such as CH_4 and N_2O at their present concentrations in the Earth's atmosphere. Earth's O_2 and CH_4 are in violation of the rules of (abiotic) equilibrium chemistry by orders of magnitude (Lovelock and Margulis, 1974). This disequilibrium pair can be detected via remote-sensing spectroscopy, given adequate spectral resolution and signal-to-noise, as was successfully demonstrated during the flyby of Earth by the Galileo spacecraft en route to Jupiter (Sagan et al., 1993).

The instrument on board Galileo that detected this biosignature was the Near- Infrared Mapping Spectrometer (NIMS), operating at 0.7

to 5.2 microns. Within that wavelength range, with a modest spectral resolution ($\lambda/\Delta\lambda$) of no more than ~100, NIMS was able to detect and quantify CO_2, CH_4, H_2O, O_2 and N_2O. The Galileo Ultraviolet Spectrometer (UVS) was also able to put a limit on the abundance of O_3.

In Task 1, we will undertake the first systematic exploration of sensitivity to changes in atmospheric and surface properties and the detectability of biosignatures in the globally averaged spectrum of the Earth s atmosphere. High-resolution synthetic spectra will be generated for a range of conditions, and then averaged over the disk from a range of viewing geometries to mimic what Earth would look like to the first (and probably second) generation extrasolar terrestrial planet detection missions. Similar results will be derived for Venus, Mars, and Titan, which will serve as abiotic controls.

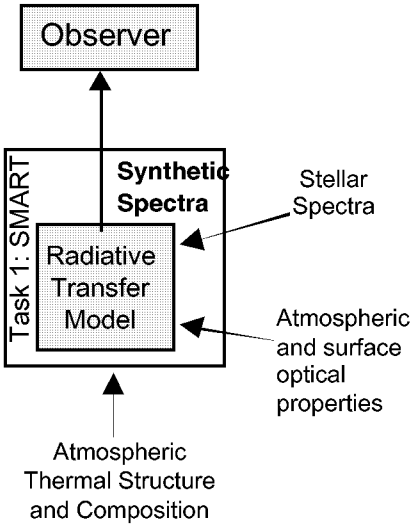


Figure C.3.2 Diagram showing the model components and inputs for Task 1. The radiative transfer model will be used to generate synthetic radiances, which will be analyzed by the observing system models. This task focuses on spectra of the Earth and other planets in this solar system because aspects of the thermal structure, composition, and optical properties of the planet's environment must be specified from observations.

The Earth s atmosphere and surface have been observed from a broad range of remote sensing instrumentation since the dawn of the space age. These data have placed important constraints on the Earth s thermal and solar radiation fields, but are not directly applicable to studies of the detectability of biosignatures in the atmospheres of extrasolar planets because (1) the spatially-resolved, LEO viewing geometry is substantially different than that for disk-integrated observations of distant worlds, and (2) these observations cover only a fraction of the solar and thermal spectra.

The models and methods described below will be used to explore the following questions concerning the detection of astronomical biosignatures from extrasolar planets:

- What does the spectrum of Earth look like from the UV to the far-IR (beyond the range of current Earth-based satellite data)?
- What information on atmospheric physical parameters and chemical composition can we derive from these spectra, and at what spectral resolution and signal to noise do we start losing this information?
- What is the spectral signature of biogenic processes in the Earth s atmosphere?
- At which wavelengths are biosignatures most easily detected?
- Can these spectral signatures be detected and discriminated in observations by existing or planned terrestrial planet detecting instruments?
- How does the presence of clouds and aerosols affect the detectability of biosignatures in the Earth s atmosphere and the optimum spectral regions for detection?
- What spectral resolution do we require to distinguish between possible sources for the biosignature molecules, and to determine the level of disequilibria?
- Can we distinguish the difference between an atmosphere with a boundary layer of rock, or a boundary layer of vegetation (chlorophyll)?
- What common spectral features in abiotic planetary atmospheres (Venus, Mars) can mask or masquerade as biosignatures (false positives)?
- To what extent does the spatial integration over the disk of the planet reduce the detectability of physical, chemical, and biological information in its spectrum?
- How do these disk-averaged spectral signatures change with observing geometry (day/night, pole/equator) or season?

Even though the available observational data is not adequate to directly address these questions, it is more than adequate to constrain at-

mosphere-surface radiative transfer models of this system. Here, we propose to use a comprehensive suite of radiative transfer models, and the best available constraints on the terrestrial environment, to produce synthetic spectra of the Earth s atmosphere over the largest possibly wavelength range. These results will be analyzed with the aid of sophisticated instrument models that can simulate the sensitivity of the current planned instruments to be used for detection and characterization of extrasolar planets. These models and their inputs are described in the following section.

C.3.1.2 MODELS

The Radiative Transfer Models

A comprehensive suite of atmospheric radiative transfer algorithms have been developed as part of our ongoing NASA Planetary Atmospheres and Planetary Astronomy tasks. These models have been extensively tested in applications with atmospheric compositions and temperature structures as diverse as the deep atmosphere of Venus (Meadows and Crisp, 1996), the atmospheres of the Earth (Crisp 1997) and Mars (Bell et al. 1993), and the upper atmosphere of Neptune (Crisp, 1995). They have also been used for studying cometary atmospheres (Brooke et al. 1996) and for determining the effects of the impacts of Comet Shoemaker-Levy 9 with Jupiter (Meadows et al. 2000). The radiative transfer models and supporting programs are described in detail below.

SMART

The Spectral Mapping Atmospheric Radiative Transfer (SMART) model, is the principal model that will be used to generate the high-resolution synthetic spectra of planetary atmospheres for this task, and for all the subsequent tasks. It will also be used to generate solar and thermal radiative fluxes and heating rates for the climate and chemistry modeling calculations proposed in Tasks 2-4.

SMART is a multi-stream, multi-level, spectrum-resolving (line-by-line) multiple-scattering algorithm. Using a high-resolution spectral grid it completely resolves the wavelength dependence of all atmospheric constituents, including absorbing gases (infrared absorption bands, UV pre-dissociation bands, and electronic bands) and airborne particles (clouds, aerosols) at all levels of the atmosphere, as well as the wavelength-dependent albedo of the planet's surface, and the spectrum of the incident stellar source. In addition, SMART employs innovative high-resolution spectral mapping methods (c.f. Crisp and West, 1992; Meadows and Crisp, 1996; Crisp 1997) to minimize the number of monochromatic multiple scattering calculations needed to generate high resolution synthetic spectra for broad spectral regions (c.f. Figures C.1.2 and C.3.1).

SMART generates a high-resolution, angle-dependent radiance spectrum through the following series of steps. It first defines the composite (gas and particulate) optical depths, single scattering albedos and scattering phase functions for each atmospheric layer, at each spectral grid point, in a multi-layer, scattering, absorbing atmosphere. Surface albedos and bi-directional reflection functions are also specified as the lower boundary of the model, and solar (stellar) fluxes are determined at the top of the atmosphere at each spectral grid point. Next, the spectral mapping algorithm employs a user-defined binning criteria to identify all spectral grid points that have optical properties that remain similar at all levels of the atmosphere and at the surface (c.f. West et al. 1990). Similar monochromatic intervals are collected into bins. SMART records the bin number associated with each spectral grid point to create a spectral map. SMART then uses the multi-level, multi-stream discrete ordinate algorithm, DISORT (Stamnes et al. 1988), to compute the angle-dependent radiances for each spectral bin. Finally, it uses the spectral map to distribute these radiances back into their original spectral positions to create a high-resolution radiance spectrum. The results of this model for remote-sensing observations have been tested and shown to be highly accurate, because it uses all of the available information about the atmospheric and surface optical properties, and employs a minimum number of approximations.

The innovative spectral mapping algorithm allows high accuracy with enhanced computational efficiency. In broad-band radiance and heating rate calculations for the atmospheres of Venus, Earth, and Mars, a direct, line-by-line calculation requires about 10^7 monochromatic spectral grid points to resolve the solar and thermal spectra. When compared to a brute force line-by-line model with the same capabilities, SMART usually requires only about 1% as many monochromatic multiple scattering cal-

culations to completely resolve the wavelength dependence of the surface and atmospheric optical properties. The radiance fields generated by this model rarely differ from those produced by the full- line-by-line models by more than 1 to 2% in spectral regions wider than 1 cm^{-1}. SMART therefore provides both the speed and accuracy needed for all of the radiative modeling applications envisioned for this program. Examples of SMART spectra for Venus, Earth and Mars are shown in Figures C.3.1.

In summary, SMART provides the following improvements over the existing models used to study biosignature detectability:

- simulates both solar/stellar and thermal sources,
- includes a rigorous (monochromatic) treatment of multiple scattering by gases, clouds, and aerosols, at both solar and thermal wavelengths (Figure C.1.2),
- can use all available constraints on the wavelength-dependent optical properties of gases, airborne particles, and a reflecting surface,
- can incorporate all available constraints on the vertical distributions of temperatures, trace gases, and airborne particles (clouds, aerosols),
- provides a detailed, angle-dependent description of the radiation field, facilitating the derivation of disk-integrated results from any viewing angle,
- evaluates spectrally-integrated fluxes throughout the atmosphere, facilitating the derivation of solar heating rates, thermal cooling rates and photochemical reaction rates.

The Line-By-Line Model For Gas Absorption, LBLABC:

A spectrum resolving (line-by-line) radiative transfer model like SMART require a comprehensive, wavelength-dependent description of the absorption by gases. To address these needs, we developed the state of the art line-by-line model, LBLABC. Unlike the line-by-line algorithms used by the terrestrial radiative transfer modeling community, LBLABC was designed to compute the absorption by rotational, vibration-rotation, and pre-dissociation lines over a very broad range of pressures (10^{-5} to 100 Bars), temperatures (130 to 750 K), and line-center distances (10^{-3} to 10^{3} cm^{-1}). LBLABC completely resolves these features at each level by evaluating the gas absorption coefficients on a series of nested spectral grids. This multi-grid approach requires only 100 to 200 evaluations of the line-shape function for each spectral line, even when very high resolutions are needed (10^{-3} cm^{-1}) and lines contribute significant absorption at distances as large as 1000 cm^{-1} from the line center (as they do in the deep atmosphere of Venus). Once the absorption coefficients have been obtained for all lines that contribute to the spectral interval of interest, contributions from each grid are interpolated to a single high-resolution output grid, summed, and then saved to disk.

To accurately simulate the absorption throughout a planetary atmosphere, LBLABC employs a different line shape function in the line-center, near-wing, and far-wing regions of spectral lines. For line-center distances less than 40 Doppler halfwidths, a Rautian line shape is used. Like the commonly-used Voigt line shape, the Rautian line shape incorporates Doppler broadening and collisional (Lorentzian) broadening, but it also includes collisional (Dicke) narrowing, which can be important for lighter molecules. At greater line-center distances, LBLABC uses a van Vleck-Weisskopf profile for all gases except for H_2O, CO_2, and CO (c.f. Goody and Yung, 1989). The super Lorentzian behavior of the far wings of H_2O lines is parameterized by multiplying the Van Vleck-Weisskopf profile by a quasi-empirical, wavenumber-dependent χ factor. The χ factor recommended by Clough et al. (1989) has been adopted in most of our published work, but a new ab-initio far-wing line shape formulation recently developed by Ma and Tipping (1992) will also be tested for the simulations proposed here. These profiles provide significant absorption at line-center distances exceeding several hundred wavenumbers. This absorption accounts for the water vapor continuum absorption seen at thermal infrared wavelengths, and even produces a weak continuum throughout much of the visible and near-infrared spectrum (Crisp 1997).

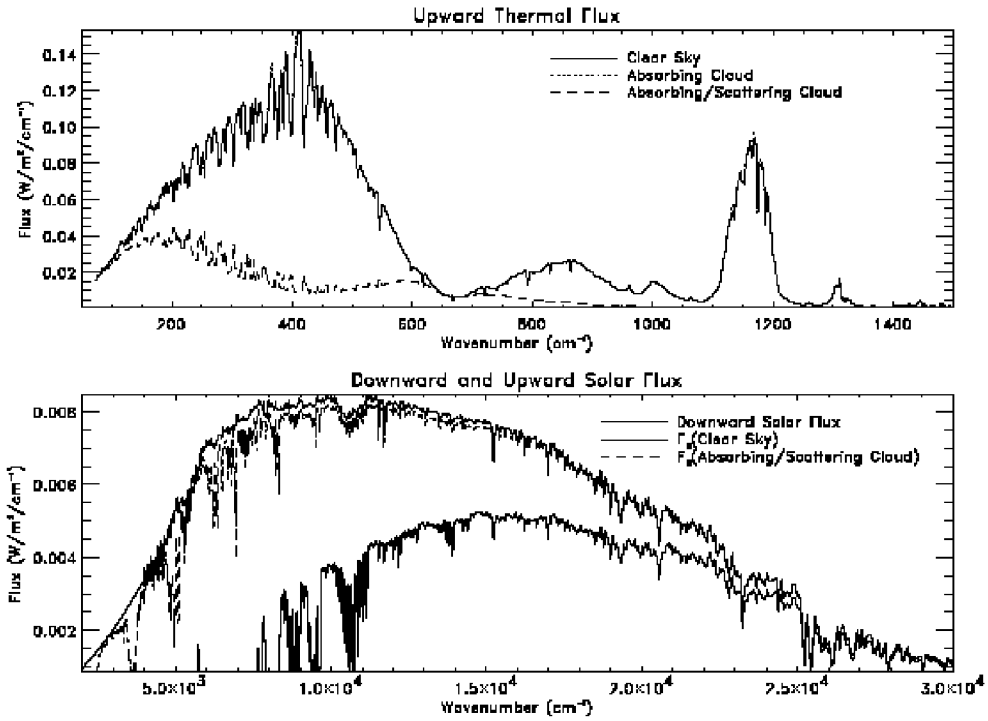


Figure C3.3 The effects of neglecting scattering when modeling CO_2 clouds. Top: The upward thermal flux emitted by a 5-bar $CO_2/H_2O/CO$ atmosphere for a clear sky case and for cases with CO_2 clouds that absorb only, and absorb and scatter. When scattering is neglected, the clouds have very little effect on the upward thermal flux at the top of the atmosphere. However, the more realistic absorbing and scattering cloud produces a significant reduction in the upward thermal flux over both the clear sky and absorbing cloud cases. Bottom: A comparison of the upward solar fluxes for the clear sky (narrow solid line), and CO_2 cloud (dashed line) cases, shown here relative to the incident downward solar flux (thick solid line). Compared to the clear sky case, CO_2 clouds reflect back the vast majority of the incident flux, thereby increasing the planetary albedo. Realistic modeling of planets with CO_2 clouds cannot be achieved with models which neglect the effects of scattering.

The CO_2 line profile can be more accurately simulated by assuming a sub-Lorentzian line shape (Burch et al. 1969). Recent theoretical work (c.f. Levy, 1992) has now confirmed that this behavior is primarily a consequence of a vibration-rotation energy redistribution process called *collisional line mixing*. LBLABC includes an explicit physical line mixing algorithm for CO_2 Q-branches, where these effects are most pronounced (Hartmann and Boulet, 1991), and a semi-empirical technique is needed for CO_2 P- and R-branches (c.f. Meadows and Crisp 1996). These methods should provide the accuracy needed for the calculations proposed here, but more rigorous methods are continuously being sought to support our ongoing planetary atmosphere modeling tasks.

Single Scattering Optical Properties Of Cloud And Aerosol Particles:

To provide an accurate description of the solar and thermal radiation fields in cloudy or aerosol laden atmospheres, spectrum-resolving multiple scattering models like SMART require a detailed, wavelength dependent description of the single scattering optical properties of the cloud and aerosol particles. This information is essential both for the retrieval of cloud and aerosol properties from remote sensing observations, and to estimate their contributions to the solar and thermal radiative forcing in climate models. For the simulations proposed here, we will use our existing suite of single scattering particle codes. These include Mie scattering models for spherical particles (Wiscombe,

1980), T-matrix models for axisymmetric non-spherical partiles (Mishchenko, 1991), and Geometric Optics/Modified-Kirchhoff models for large ice crystals (Muinonen et al. 1989). This suite of models can produce an accurate description of the particle optical properties over the broad range of sizes, shapes, and wavelengths needed in this investigation.

C.3.1.3 Model Input

In addition to the planet-specific input parameters, such as the horizontal and vertical distributions of surface and atmospheric temperature, trace gas concentration, clouds and aerosols, and surface cover, the radiative transfer models require a number of laboratory data sets. These include:

- molecular line databases needed to generate the line-by-line description of infrared vibration-rotation bands of gases,
- UV and visible cross-sections of gases associated with electronic and pre-dissociation transitions in gases (e.g. ozone)
- the wavelength-dependent dielectric properties of photochemical aerosols and condensate cloud droplets needed to generate their single scattering properties.

The last two of these databases have been compiled over the years for simulations of the Earth and other planets in our solar system, and should be adequate for the work proposed here. We also have compiled extensive molecular line databases, but these must be augmented for the proposed modeling tasks.

Molecular Line-list Databases

Comprehensive, up-to-date molecular line lists are needed to provide the most complete wavelength coverage for molecular spectral features. This information is important to ensure that we miss as few spectral features attributable to an atmospheric constituent as possible. Less complete line lists may cause us to fail to identify a potential biosignature or to make a mistake in assessing the detectability of a biosignature. Although much work has been done on line lists for many of the common bulk atmospheric constituents, potential biogenic molecules, e.g. OCS, SO_2, CH_3SCH_3 (dimethyl sulfide) and S_8, will be specifically targeted for extra study in this project.

Complete wavelength coverage throughout the infrared is also advantageous because it allows us to correctly model the radiative balance in a planetary atmosphere for bulk constituents that are strong absorbers. Also, for the work proposed in this task, it allows us to generate *predictive* synthetic planetary spectra for wavelength regions for which we have no observational data.

C.3.1.4 What is a line list?

Line lists are compilations of molecular parameters for spectral lines that allow us to recreate line intensities, shapes and positions with line-by-line models, such as LBLABC. Line list parameters include the wavelength of the line center, lower state transition energies, transition line intensities, and line shape parameters as a function of temperature. Typically, many tens of thousands of ro-vibrational transitions contribute to the spectral signature of a particular molecule.

Table C.3.1: Summary of available molecular line-list databases

DATABASE[+] and WEBSITES	region[#] cm^{-1}	number of species	millions of transitions	Ref.
1996 JPL catalog http://spec.jpl.nasa.gov	0 - 330 -	331 "entries"	1.5	14
1997 SAO-rotational http://firs-www.harvard.edu/dir/sao92	10 - 1600	49	0.5	16
1997 GEISA http://ara01.polytechnique.fr	0 - 22,600	50	0.7	13
1996 HITRAN http://CFA-www.Harvard.edu/HITRAN	0 - 22,600 - 58,500	46	1.0	11
1996 HITEMP	0 - 23,000?	3 or 4 ?	2.3	12
1995 ATMOS	0 - 10,000	49	0.8	15
1996 JPL VUV Reference Data http://remus.jpl.nasa.gov/jpl97	Vis-UV	≈ 65	cross sections	7
1993 Hanst Digitized IR Spectra	500 - 4000	250	lab spectra	17

The most widely used collection of molecular parameters available in electronic form is the HITRAN (High resolution Transmission) database (Rothman et al. 1998). However, there are a number of other spectroscopic databases available, as summarized in Table C.3.1 The HITRAN database is updated every two to five years using data collected in new laboratory studies. To obtain the information required to create line lists for new molecules or new regions of the spectrum, one or two spectra of different optical densities are recorded with a high-resolution spectrometer. The quantum numbers of the observed features are then identified (quantum assignments), and theoretical quantum mechanical models are manipulated in an attempt to reproduce the energy levels. If the modeling is successful, a reliable prediction of positions, lower state energies and relative intensities can be made for the molecular databases. However, the absolute intensities are still required before the predictions can be used for atmospheric measurement and modeling. These intensities and widths are usually measured using least squares curve-fitting techniques (Brown et al. 1983) and analyzed as separate efforts that involve from 5 to 50 spectra. Thus the information needed for a good compilation usually comes from several different studies. Therefore a key step in forming a more-comprehensive compilation for a given molecule is collating the available lab data and merging relevant sources. For heavy molecules like the freons or organic molecules with more than six atoms, the spectra are unresolved bundles of transitions that are not readily characterized through a line-by-line approach. To provide some limited information about the molecular absorption features, laboratory spectra are analyzed to yield wavelength-dependent absorption cross-sections. The Hanst and Hanst catalog (Table C.3.1) is a commercially available collection of low resolution laboratory data which provides these approximate cross sections. Figure C.3.4 shows a low resolution spectrum of dimethyl sulfide from this catalog. Better parameters for some heavier species can also be obtained using a collection of high-resolution spectra recorded to support the ATMOS experiment (Brown et al. 1987).

In the type of planetary modeling envisioned here, it is also important that we obtain line lists for a wide range of temperature regimes, and in particular that we understand the so-called "hot bands" in atmospheres that are near the limits of the habitable zone. We already have extensive experience modeling the remotely sensed atmosphere of Venus, and spectra of the several hundred to several thousand degrees Kelvin impacts of Comet SL-9 with Jupiter. For these tasks, we have collated HITEMP databases for CO_2 (Wattson and Rothman, 1992) and H_2O (Paltridge and Schwenke, 1997), and also have high temperature line databases for CH4 (c.f. Hilico et al., 1994) and NH_3.

During the course of the modeling work the science team will identify important species whose molecular parameters are too poorly characterized to generate reliable extrasolar planet spectra, or will require molecular parameters for temperature regimes not currently supported. If no information exists for these species, we will record new laboratory spectra using the McMath Pierce Fourier transform spectrometer. This instrument is maintained by the National Solar Observatory at Kitt Peak National Observatory near Tucson, Arizona and is described in more detail in the facilities section. In summary, as input to the radiative transfer model, we will obtain the best possible spectroscopic databases for all important species, and create customized databases where necessary. Most of the information will be taken from existing databases (Table 1) and reported laboratory studies in the literature, but we will acquire new data, where necessary, using the McMath FTS. The objective of this activity will be to obtain a database of molecular parameters that is as complete as possible with intensities accurate to 25% or better

Planetary Insolation-The Spectrum of the Parent Star

For Task 1, we will model only planets within our own solar system. For these simulations, we will use a high-spectral-resolution solar spectrum compiled from satellite observations at UV wavelengths (Atlas-2 SUSIM) and stellar atmosphere models at longer wavelengths (Kurucz, 1995). In Task 3 and beyond, high-resolution spectra of other stellar types will be used.

Astronomical Instrument Simulators

The generation of synthetic spectra of planetary atmospheres with the radiative transfer models is the first step in determining the de-

tectability of biosignatures by astronomical instrumentation. The next step is to input these spectra into a model simulator that can explore the detectability of these biosignatures as functions of telescope and instrument design and the stellar environment surrounding the extrasolar planet.

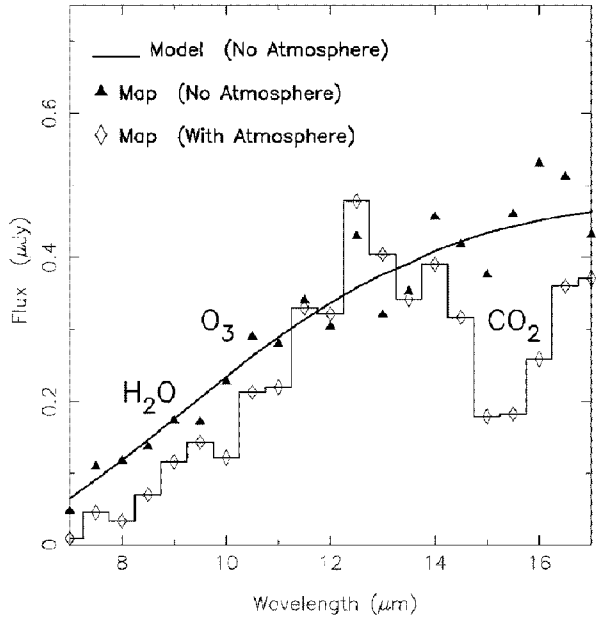


Figure C.3.4 Example of a simulated TPF spectrum of a Earth-like planet at 10 pc. The black body spectrum (no atmosphere) is indicated by the solid line. The symbols (filled triangle and diamond) represent the fluxes measured at each wavelength from the simulated TPF images. The simulated data corresponds to a dual 3-element nulling interferometer and 20 days of integration. Note that the atmospheric features are clearly visible in the simulated spectrum

To this end, we will modify an existing model which is being used to produce S/N estimates for a range of instrument configurations and designs for first generation telescopes and instruments such as Terrestrial Planet Finder (TPF). TPF is currently based on a nulling-interferometric design, and is optimized for observing in the mid-infrared, where the parent star is "only" a million times brighter than an orbiting terrestrial planet (rather than a billion times brighter as is the case at optical wavelengths). However, we will explore the detectability of biosignatures not only at the mid-infrared wavelengths available to the nulling interferometers (Figure C.3.4), but also in the near-infrared and optical, where extrasolar planet detection may be achievable using coronagraphic instrumentation.

Simulations for Nulling Interferometers

Recent simulations suggest that nulling interferometers operating in a 1AU orbit with four 3.5-m telescopes can detect Earth-like planets at distances as far as 15 pc and characterize their atmospheric emission for biosignatures. A smaller system using ~2.5-m apertures could still detect Earth-like planets but would not be able to search for the molecular gaseous absorption features that may be signs of life, such as ozone, in any but the nearest planetary systems.

These first generation instruments could be implemented in wide variety of configurations, constrained by the number of telescopes, the necessary degree of starlight and background suppression, and the required accuracy of the reconstructed images. There is a performance trade-off between combining many telescopes into a single array, to provide a deep and wide null to suppress starlight, and the ability to chop rapidly and to suppress large-scale diffuse emission from a zodiacal cloud by chopping between sub-arrays with relatively shallow nulls (Velusamy, Shao and Beichman, 1999). TPF may be designed with the ability to optimize its performance on-orbit using a number of separate beam-combiner modules which will allow chopping to be selected as needed. Many different instrument configurations are possible, and some of the key essential astronomical and instrumental factors affecting the ability of TPF to meet its goals include

- The size, temperature, and atmospheric composition of the target planet.
- The distance to the stellar system
- The luminosity and physical size of the star
- The distribution and amount of material of the zodiacal dust in the target system.
- The inclination of the orbital plane to the line of sight.
- Observatory properties such as the size and temperature of the telescopes and associated optics, interferometer baseline and nulling configuration, pointing jitter, etc.
- Instrument properties such as optical efficiency, detector dark current, depth and stability of the null.
- Local background due to dust in the solar system at the observing location and in the direction of the target.

Each of these factors will be included in the instrument simulator models to best determine the detectability of identified biosignatures using these first generation designs.

Simulations for Coronagraphs.

In addition to determining detectability for nulling interferometer designs, we will also explore the detectability of biosignatures for coronagraphic instrumentation. Coronagraphic architecture could provide direct imaging access, hence direct photometric and spectroscopic access to terrestrial planets orbiting nearby stars to distances of 10 pc and more. Depending on the wavelength range selected for the observations, the essential contrast requirements for a planetary companion may range from 10^{-6} to 10^{-10} times the star's peak intensity within a few tenths of an arcescond from the star.

The proposed investigation will be supported by computer simulations of coronagraphic telescope performance, based on computational tools developed for a number of recent JPL instrument concept studies. We will take advantage of an optical modeling capability developed to combine experience to date from space and the laboratory, with new optical designs for future space telescopes. These include a coronagraphic mode for the NGST near-infrared (1-5 microns) camera, a single filled-aperture telescope for TPF (1-15 microns wavelength), and a Discovery-class coronagraphic telescope of modest aperture working in the visible wavelength range (0.5-1.0 microns).

The simulation software developed for these studies is configured for a wide range of instrument designs. The telescope s architecture

- may include monolithic or multi-segment primary mirrors of arbitrary shape,
- can be configured with wide range of active wavefront correction technologies and their corresponding correction accuracies,
- may exhibit a range of mirror surface figure and polish characteristics scaled from a database of existing telescope surface characteristics, and
- includes a detailed simulation of wavefront phase retrieval from star images for alignment of active optics as implemented for NGST.

The sources of scattered light within the first few arcseconds of an isolated star image are well understood as a combination of diffraction from the pupil-defining obscurations, scatter from shallow mid-frequency surface figure errors (essentially the residual polishing marks on the 4- 40 cm scale), and stray scattered light traceable to inadequate baffling and dust in the optical path. In broad terms, these three sources are controlled by three technologies, respectively (1) a coronagraphic camera to reduce diffracted light, (2) telescope design and high-order active optical correction to reduce mid-spatial-frequency scatter, and (3) careful baffling and contamination control to mitigate unwanted stray light.

The software further includes the essential sources of background noise. The effects of optical scattering and diffraction in the telescope are combined with Zodiacal and exo-zodiacal light to estimate the background level against which a planet must be detected. Shot noise on this background and detector noise are included. The effective background noise from optical instabilities, including pointing jitter, focus drift, and thermal drift of the optical alignment, can be included to identify the outstanding specifications for optical system stability.

With these tools, we will be able to determine the sensitivity of coronagraphic observing instruments to biosignatures over the wavelengths covered by the study. We will also evaluate a number of feasible telescope configurations, and will provide recommendations for telescope design options and specifications required for biosignature detection.

C.3.5 Increases in Computational Efficiency

The radiative-transfer modeling tools to be used for this task are computationally intensive. Therefore, increasing the computational throughput is essential to achieve our modeling goals. This will also be true of most of the models to be used in the subsequent tasks. In the course of this project we expect to generate several thousand simulations.

Currently the synthetic spectrum generating code, SMART, when modeling realistic, absorbing, scattering planetary atmosphere from only 1 to 2.5 microns takes about 3 hours running on a modern desktop workstation. Dramatic increases in computational efficiency can be obtained by moving to an appropriate parallel architecture. We therefore propose to implement these algorithms on two systems available in our

Ultracomputing Technology Research Group at JPL.

The first is a dedicated, 32-node BEOWULF parallel machine where each node is a Pentium II. This low-cost, dedicated machine is well suited for Data Parallel type of computation, that is, for computation with a very large degree of coarse grain parallelism and minimal inter-processor communication.

The second machine is a highly parallel Multi-Instruction-Multi-Data (MIMD) machine. This is a SGI Origin 2000 machine with 128 processor nodes. It is suitable for large-scale parallel computation and it is much more efficient for communication-intensive parallel computation.

For both machines, the approach is to first identify the computation-intensive kernels in the existing FORTRAN code and try to parallelize and optimize these kernels first. However, for a better result, and depending on the application, a more detailed algorithmic analysis might be needed and novel parallel algorithms may need to be developed. The central task for the first year of the proposed project will be an initial analysis of the existing FORTRAN code to choose the optimal hardware and software configuration for each simulation module.

C.3.1.6 Research Applications of These Tools

The first step in Task 1 is to analyze and upgrade SMART and LBLABC to increase their computational efficiency. With these upgraded radiative transfer tools, we will run a global grid of individual SMART simulations as a function of latitude, longitude and underlying surface. These simulations will generate angle-dependent radiance spectra extending from the UV through to the far-infrared. . These spectra will then be averaged over the planet s disk to produce a disk-averaged view that will simulate that available to astronomical instrumentation that does not explicitly spatially resolve the extrasolar planet.

To validate the accuracy of SMART over large wavelength ranges, the synthetic spectra will be averaged over the appropriate fields of view and compared to the available results from Earth-observing satellites (e.g. NIMBUS 4 IRIS, ADEOS IMG; ERS GOME, Galileo NIMS, and hopefully EO1 Hyperion).This validated technique will then be used to produce disk-averaged Earth spectra over a continuous wavelength range from the UV to the far-IR for changes in the following parameters:

- seasonal changes in trace gas distribution (solstices and equinoxes)
- changes in surface albedo for visible hemispheres dominated by land, ocean or snow/ice
- a range of cloud distributions from fully clear to fully cloudy
- a range of viewing phase angles (fractional illumination of the visible hemisphere).
- a range of viewing geometries (sub-Earth points) from equator-on to pole-on views.

These spectra will be used to determine astronomical sensitivity to variations in these parameters. The spectral resolution will be successively degraded to determine at which point we lose sensitivity. These spectra will then be processed with the astronomical instrument simulators to determine the sensitivity of these specific first generation designs to variations in these parameters.

We will then use this set of high-resolution spectra to identify potential biosignatures from the atmosphere and surface. We will then successively degrade the spectrum to note at which spectral resolution these features can no-longer be detected. We will also look for the detectability of seasonal variations in biosignatures. Finally, to identify possible false positive signatures, we run validation comparisons of synthetic spectra for Venus, Mars, and Titan, using the best available globally averaged data for these objects.

C.3.1.7 Products And Significance For Task 1

The primary products of the first task in this investigation include:

- The conversion of the radiative transfer models, SMART and LBLABC, for use on JPL s ultracomputing facilities. This is not only important for timely execution of this Task, it is the first step towards creation of the Fully-Coupled Virtual Planetary Laboratory.
- These models will be used to generate high-resolution, spatially resolved, spectra of Venus, Earth, Mars, and Titan that extend from the UV to the far IR.
- These spectra to will be studied to determine the effects of spatial and spectral averaging on the detectability of biosignatures and other spectral features that provide important

constraints on a planet s physical and chemical state and habitability.

These spectra will also be processed with observing system models for nulling interferometers and coronographs to determine how well the space-based instrumentation can detect and characterize extrasolar Earth-like planets, and discriminate them from abiotic planets (e.g. Venus and Mars). These results will be used to provide recommendations for the minimum and optimum spectral resolution, wavelength range, and signal to noise required for determination of atmospheric and surface physical and chemical parameters, for a range of terrestrial planets, and the detection of biosignatures in an Earth-like planet.

C.3.2 Task Two: The Spectral Signature of Thermodynamically Balanced Terrestrial Planets.

As the next step in the development of the Virtual Planetary Laboratory, we propose to implement a simple climate model to simulate the surface temperatures and atmospheric temperature profiles of extrasolar planets (Figure C.3.5). This is a logical next step because the thermal structure plays a dominant role in the infrared spectrum emitted by the planet. If the atmosphere includes condensable species, such as water, the atmospheric thermal structure will also control the distribution of clouds, which will affect the solar/stellar flux reflected by the planet and the vertical extent of the atmosphere that can be observed. Finally, the atmospheric thermal structure can affect the distribution of reactive trace gases of biological and abiotic origin through its effects on chemical reaction rates.

A variety of tools exist for determining the temperature distribution in terrestrial planetary environments. These tools fall into three major categories:

- 1D Energy Balance (1D EB) climate models (North et al. 1981),
- 1D radiative convective equilibrium (RCE) models (c.f. Manabe and Wetherald, 1967; Gierasch and Goody, 1968; Pollock et al. 1980; Crisp and Titov, 1997), and
- 3D General Circulation Models (GCM s).

The classical, diffusive, energy balance climate models provide a one-dimensional description of the pole-to-equator temperature distribution. These models have been widely used for studies of the effects of albedo on the energy balance, but they provide no direct constraints on the vertical distribution of atmospheric temperature or composition, or the details of the emitted spectrum.

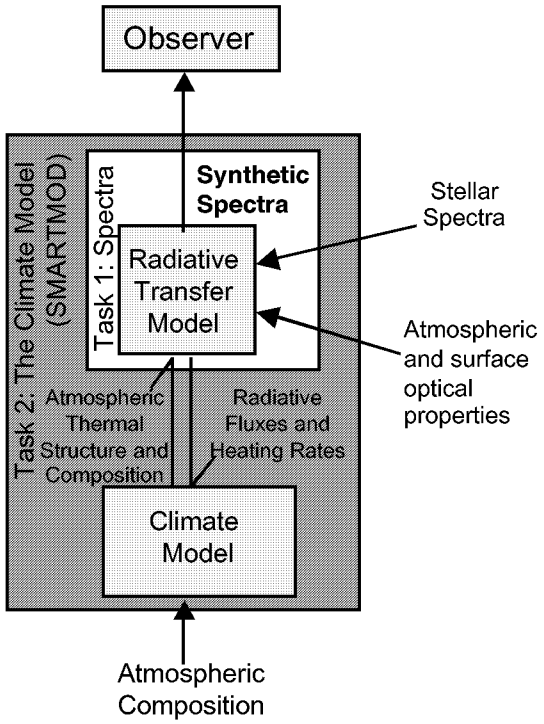


Figure C.3.5 Components of the Virtual Planetary Laboratory included in Task 2 are shown. The radiative transfer models are integrated with a climate model to produce a surface and atmospheric temperature distribution (and emitted spectrum) that is in thermal equilibrium with the specified atmospheric composition.

In contrast, 1D RCE climate models describe the globally averaged equilibrium temperature distribution with altitude in a planetary atmosphere with a given composition and incident solar flux. However, these models provide no direct constraints on the horizontal (equator to pole or sub-solar to anti-solar) temperature distribution. These horizontal variations can introduce changes in disk-integrated spectra of a planet viewed from different directions. For example, assume that the spectrum associated with the globally averaged RCE thermal profile is an excellent match to the disk-averaged spectrum of an extrasolar planet viewed from a point over mid latitudes (this works amazingly well for Earth). This spectrum will probably provide a much less satisfactory fit to the spectrum of

the same planet viewed from other angles (ie. a polar view). Furthermore, the single global average spectrum may not adequately simulate the results of the spatial convolution of spectra from different parts of the planet. These arguments suggest that some estimate of the horizontal temperature variations is needed for studies of biosignatures on extrasolar planets.

In principle, a 3D General Circulation Model can provide constraints on both the horizontal and vertical distributions of temperatures in a planetary atmosphere. However, these models are much more complex and computationally intensive than the simple 1D climate models described above. Also, as we noted in the introduction to the task section, the reliability of these models depends crucially on the validity of a wide range of variables that will not be known for extrasolar planets for several decades. These parameters include the planetary rotation rate, axial obliquity, surface friction, topography, surface albedo distribution (ocean/land/ice), and many others. In addition, because these models must evaluate a host of parameters at thousands of horizontal grid points at every time step, they typically use relatively simple approximations for radiative forcing, convection, and other sub-grid-scale processes. These parameterizations can be tuned to yield good results for systems that are well constrained by observations, but they produce much less reliable results when applied to arbitrary environments. These factors, combined with the computational requirements of these models, suggest that, at this time, 3-D GCM s provide little real benefit to the study of extrasolar planetary environments.

For this investigation, we propose to use an efficient, hybrid climate model that incorporates advanced 1-D RCE and 1-D EB models to derive estimates of the temperature distributions in extrasolar planetary environments. A state-of-the-art 1-D RCE model will be used to derive solar radiative heating rates, thermal radiative cooling rates, and vertical convective heat fluxes for selected latitudes and solar longitudes. An EB climate model employing baroclinic adjustment (c.f. Stone 1978; Stone and Nemet, 1996) will then be used to estimate the exchange heat between these soundings. This approach has been widely used for studies of the Earth s present climate, terrestrial paleoclimates, and the atmospheres of other planets. These methods are described in greater detail below.

C.3.2.1 MODELS

The Radiative-Convective Equilibrium Model

We are currently in the process of incorporating the Spectral Mapping Atmospheric Radiative Transfer (SMART) model (Task 1) into an existing 1D RCE, model, called RTMOD (Crisp 1989), as part of Meadows' ongoing NASA Exobiology task for modeling the effect of clouds on planets at the limits of the habitable zone. The combined RCE model, SMARTMOD, is currently undergoing testing and will be ready for routine use in this task by February of 2001.

SMARTMOD employs a number of innovative features that enhance its versatility and accuracy. First, unlike most 3-D climate models, which use highly simplified radiative transfer schemes, SMARTMOD derives a detailed description of the solar and thermal radiation fields using the full SMART algorithm (Task 1). It then uses an explicit time-stepping scheme to solve the 1-D thermodynamic energy equation as an initial value problem to yield the radiative equilibrium temperature profile consistent with the atmospheric composition and solar forcing. This time-marching approach is ideal for this application because it can accommodate changes in the optical properties of the atmosphere. For example, it can explicitly include changes in gas mixing ratios, cloud formation and dissipation, and surface albedo variations (ice accumulation and melting) that might occur as the temperature profile evolves.

SMARTMOD also employs a more physically realistic treatment of vertical heat transport by free convection. Most existing RCE models (Manabe and Wetherald, 1967; Pollack et al. 1980; Crisp 1989) use a simple "convective adjustment" to simulate the effects of convective heat transport. In contrast, SMARTMOD uses an explicit mixing length formulation (c.f. Gierasch and Goody, 1968) for moist or dry convection. This approach permits temperature discontinuities between the surface and the lowest atmospheric layer, and produces much more realistic temperature profiles near the surface. It also provides estimates of the vertical eddy diffusion rates, for use in atmospheric chemical models.

In addition to globally averaged tempera-

tures, SMARTMOD can generate high-resolution spectra of the resulting equilibrium atmosphere with output parameters identical to those produced by SMART. The SMARTMOD results can therefore be analyzed by the observation system modeling tools employed in Task 1.

The Energy Balance Climate Model

To simulate horizontal (equator to pole or sub-solar to anti-solar) heat transport by atmospheric dynamics, SMARTMOD, will be incorporated with a simple, energy balance (EB) climate model. In the integrated model, SMART algorithm will be used to find the instantaneous solar heating and thermal cooling rates at several locations on the planet at each time step. These soundings can include clear and cloudy conditions and a variety of surface albedos over a range of latitudes and solar longitudes. The simple EB model will then use linear diffusion (c.f. North et al. 1981) to distribute heat between the soundings, and modify the atmospheric thermal structure for the next time step. This process will be repeated until the system reaches equilibrium.

On moderate to rapidly rotating planets (e.g. Earth, Mars), the meridional (equator to pole) heat diffusion coefficients will be parameterized by Baroclinic Adjustment (c.f. Stone, 1978; Stone and Nemet, 1996). In this approach, the zonal mean meridional atmospheric temperature gradients are compared to the critical gradient for baroclinic stability at each time step. If it exceeds this gradient, the down-gradient heat diffusion coefficient is increased, and heat is more efficiently transported downgradient to stabilize the horizontal temperature contrast. For slowly rotating planets, like Venus (or Titan), or in very statically stable regions in the atmospheres of more rapid rotators (i.e. the Earth s stratosphere), an empirical horizontal diffusion coefficient will be specified, and validated by comparing simulations with available observations.

C.3.2.2 Model Inputs

Like the radiative transfer model described in Task 1, the climate models described above require a comprehensive description of the incident stellar flux, atmospheric composition, surface properties (albedo, thermal inertia) and the distribution of absorbing gases and aerosols. They also need the comprehensive line lists developed in Task 1. However, they only need relatively crude initial estimates of the atmospheric and surface temperatures to initialize the thermal equilibrium calculations.

Computational Efficiency

As part of this task, we will adapt SMARTMOD to run on the ultracomputing facilities at JPL. This is extremely important for achieving our stated goals in a timely manner, while maintaining the rigorous physical treatment of gases and aerosols provided by the SMART algorithms embedded in SMARTMOD.

C.3.2.3 Research Tasks

SMARTMOD Validation

The 1D RCE component of SMARTMOD is currently being developed independently of this CAN using Meadows' NASA Exobiology funds. The preliminary integration of SMART to RTMOD was completed this August, and the full integration of the two programs is expected to be complete by February 2001. The RCE component of SMARTMOD will be validated by generating globally averaged RCE temperature profiles for Venus, Earth, Mars, and Titan, and comparing these results to existing observations. In addition, the high-resolution synthetic spectrum of the equilibrium atmosphere produced by SMARTMOD will be validated against globally, annually, averaged observations for Venus (Venera 15 Spectrometer), Earth (NIMBUS 4 IRIS) and Mars (Mars Global Surveyor TES) to determine how well these spectra preserve important spectral features.

In parallel with this effort, SMARTMOD will be integrated with the EB climate model to provide a spatially varying description of the surface and atmospheric temperature distribution. The combined model will then be implemented on the JPL ultracomputing facilities. This integration effort should be completed before the end of FY 2001.

To validate this combined model, spatially resolved radiative-convective-diffusive temperature distributions will be generated for Venus, Earth, and Mars. These temperature distributions will be compared to the available observations of these planets to refine the horizontal diffusion coefficients, and to identify simple mechanistic relationships between these coefficients and the planet s bulk parameters (e.g. rotation rate, atmospheric density, surface al-

bedo and thermal inertia, etc.). Finally, because the fundamental objective of this project is to identify biosignatures in disk-integrated, planetary spectra, SMARTMOD s spatially resolved, high-resolution spectra for the simulated atmospheres of Venus, Earth, and Mars will be averaged over the planet s disk for the range of viewing conditions considered in Task 1. These results will then be compared to the disk-integrated spectra derived using observed atmospheric properties in Task 1, to determine how well the climate model can simulate disk-integrated atmospheric spectra. This validation effort should be completed before July 2002.

SIGNIFICANCE AND PRODUCTS FOR TASK 2

Upgrade of SMARTMOD to incorporate a RCE and EB model, and the implementation of these tools on JPL s ultracomputing facility is an essential step toward the development of the Virtual Planetary Laboratory. The validation effort will also provide a number of products that will be of value to NASA s Astrobiology Program. For example, these experiments will produce an important advance in our understanding of the Venus greenhouse mechanism. This is important for planets near the limits of the habitable zone since earlier models included much a much less sophisticated description of radiative processes (e.g. band models, neglect of scattering at thermal wavelengths, etc., c.f. Crisp and Titov, 1997). The comparison of viewing-angle-dependent, disk-averaged spectra of Venus, Earth, and Mars obtained from SMARTMOD, and those produced directly from observations (Task 1) will also show how well the environment of an extrasolar planet can be simulated with a simple, efficient climate model.

C.3.3 TASK THREE: THE SPECTRAL SIGNATURE OF CHEMICALLY AND THERMODYNAMICALLY BALANCED ATMOSPHERES OF EXTRASOLAR PLANETS

To increase the versatility of the Virtual Planetary Laboratory, Task Three incorporates the radiative transfer and climate modeling tools developed in the first two tasks with a comprehensive, atmospheric photochemical model (Figure C.3.6). The resulting climate-chemistry model will allow us to create a wide-range of plausible model extrasolar planet atmospheres that are radiatively, thermally, and chemically self-consistent. With this tool, we will:

- initiate a study of biosignatures in the Earth s early atmosphere
- explore the detectability of the currently accepted list of potential biosignatures in both oxidizing and reducing atmospheres, for a variety of stellar types
- provide recommendations for instrument parameters and capabilities required to detect these biosignatures.

To address these goals, we need a versatile, modular, atmospheric photochemical model that can reliably simulate a wide range of conditions in the atmospheres of terrestrial planets. The comprehensive, state-of-the-art Caltech/JPL photochemical model will be ideal for this application. The following two sections describe the background and implementation approach of the chemical model. We then summarize the types of input data sets needed, and propose a series of specific experiments that address the goals listed above.

C.3.3.1 BACKGROUND:

NASA has conducted a nearly complete reconnaisance of the Solar System, having acquired close-up observations of every planet, except Pluto, and many moons. From these spacecraft missions and from ground-based and earth-orbiting telescopes, a detailed catalog of the chemical composition has been developed for every significant atmosphere. Atmospheric photochemical models have been able to quantitatively reproduce the essential compositional measurements of these atmospheres (Yung and Demore, 1999). The stability of the primarily CO_2 atmosphere of Venus and of the ozone layer in the Earth's stratosphere are maintained by catalytic reaction cycles involving oxygen-, hydrogen-, and chlorine-containing radical (highly reactive) chemical species that exist in these atmospheres in trace amounts. The stability of the CO_2 atmosphere of Mars and of ozone in the terrestrial mesosphere are maintained similarly by reactions involving primarily oxygen- and hydrogen-containing radical species. Complex hydrocarbon species are formed in the molecular hydrogen-dominated atmospheres of Jupiter, Saturn, Uranus, and Neptune as a result of the photolytic decomposition of methane and secondary reactions following the photolytic decomposition of acetylene, a primary product of methane dissociation. The cold nitrogen-dominated atmospheres of Titan and Triton have both methane photochemistry and the chemistry

of reactive nitrogen species that leads to the formation of nitrile compounds (containing carbon-nitrogen chemical bonds).

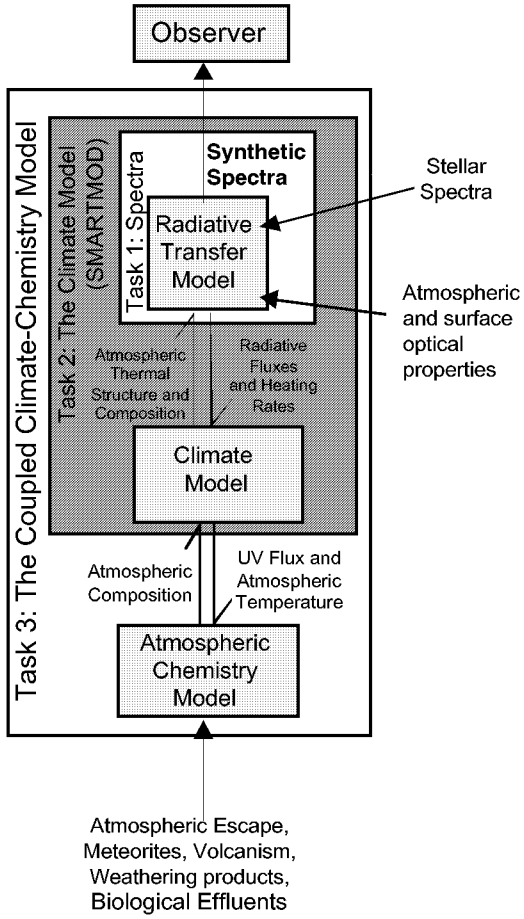


Figure C.3.6 Components of the Virtual Planetary Laboratory included in Task 3 are shown. The radiative and climate models are integrated with an atmospheric photochemical model to yield spectra of environments that are in radiative, thermal, and chemical equilibrium.

Yung and DeMore (1999) show that the chemical kinetics of the solar system can be reproduced with about two thousand reactions relating the interactions among several hundred species. One can point to the success achieved in explaining the observed composition of the primary atmospheres in the solar system as evidence of the success of this program. In addressing the question of the atmospheres of extrasolar planets, the planets of our Solar System can serve as a guide. The terrestrial and Jovian atmospheres represent the end members of oxidizing and reducing atmospheres, respectively. Titan is an intermediate case between Earth and Jupiter. In all cases a quantitative assessment is possible if the disequilibrium chemical forcing on the system can be quantified.

The Caltech/JPL planetary atmosphere model has been used to simulate and quantitatively reproduce modern observations: Venus (Yung and Demore, 1982; Mills, 1999), Earth stratosphere (Froidevaux et al., 1985; Allen and Delitsky, 1991), Earth mesosphere (Allen et al., 1981, 1984), Mars (Nair et al., 1994), Jupiter (Allen et al., 1992; Gladstone et al., 1996), Saturn (Moses et al., 2000a, b), Titan (Allen et al., 1980; Yung et al., 1984), Neptune (Moses et al., 1992), Triton (Lyons et al., 1992), and the Jovian planets (Lee et al. 2000).

C.3.3.2 MODELS

The 1D Chemical Model

The Caltech/JPL general planetary atmosphere model is a multidimensional model that simulates the chemical composition of a planetary atmosphere, accounting for the coupling of photochemistry and atmospheric transport. The modular design of the code provides great flexibility in simulating a wide variety of chemical environments. In addition to the atmospheric chemistry of the Earth and other major Solar System bodies, the model has been used to simulate cometary comae and interstellar molecular clouds.

The Caltech/JPL planetary atmosphere model includes a one-dimensional (1D) mode. In this mode, it produces a vertical distribution for each selected chemical constituent by solving the time-dependent (steady-state in the case of long timesteps) continuity equation consisting of terms for the local production and loss of the chemical species and vertical diffusive transport (Allen et al., 1981). Vertical transport includes eddy, molecular, and thermal diffusion (Banks and Kockarts, 1973). The complete, coupled set of continuity equations (one for each constituent) is solved simultaneously using a finite-difference iterative algorithm adopted from Richtmeyer (1957).

When this chemical model is used in isolation, the physical structure of the atmosphere is prescribed. Once coupled with the climate model, developed in Task 2, the physical structure can be self-consistently determined. Calculations are performed on a vertical grid with an arbitrary number of levels and at each level total atmospheric pressure, temperature, and eddy diffusion coefficient are defined. The physical structure can be updated during a chemical model run.

The model can handle an arbitrary number of chemical constituents interrelated by an arbitrary number of chemical reactions. Processes occurring above and below the boundaries of the modeled atmospheric column and processes occurring at these two spatial interfaces are parameterized by fixing the abundance of a constituent at a boundary or by selecting one of several formulations for mass flux into or out of each boundary. These boundary conditions also can be updated in the course of a model run.

C.3.3.3 Model Input

The photochemical model requires a library of kinetic reaction rates, as well as constraints on UV fluxes and the atmospheric thermal structure. It can also accommodate arbitrary sources or sinks at the upper and lower boundary. The chemical kinetic data needed for the experiments proposed here has been compiled over a number of years, and are readily available to the team (c.f. Demore et al. 1992). The atmospheric physical properties can be obtained from the coupled radiative and climate models.

However, the addition of a comprehensive atmospheric photochemistry model provides opportunities to initiate our investigation of the chemistry in the atmospheres of terrestrial planets around stars with spectral types that are different than that of the sun. Task 3 therefore includes a focus on obtaining realistic spectra of these stars. This input data is also needed to refine our understanding of the Habitable Zone and the detectability of biosignatures by using realistic stellar spectra as the radiation source at the top of extrasolar planetary atmospheres.

Stellar Spectra

Another critical input to radiative transfer, climate, and chemical models of terrestrial planet atmospheres is the wavelength-dependent radiative input from the parent star. Traditional calculations of stellar "habitable zones" (HZs) have represented the stellar emission spectrum as black bodies for all but the G2V case, where the Sun's spectrum was used (Kasting 1993). Over the past decade, there has been a major effort to establish accurate, detailed, absolute calibration stars in the infrared, spanning the 1-30 μm range, with extensions to 300 μm to support specific space-based instruments. This work graphically demonstrates that no star radiates as a blackbody and that stellar spectral energy distributions (SEDs) are invariably mutilated, even at low spectral resolution, by strong absorption bands. Planets have now been discovered around stars with spectral types ranging from F7 to M2V. Examples of the extreme departures of realistic stellar spectra from blackbodies over this spectral range can be seen in Figure C.3.7.

The use of black body approximations for spectra of stellar types other than our own Sun may be adequate to indicate crudely the scale of the HZ around a variety of stars (e.g. Cohen 1999, Kasting 1993). However, more realistic stellar spectra would improve our ability to assess the correct integrated luminosity incident on a planetary atmosphere. Improved estimates of the spectral distribution of the stellar energy incident on a planet would also improve the accuracy of both radiative balance calculations and photochemistry in planetary atmospheres. In addition, the spectrum of the parent star may affect the detectability of features in a planetary atmosphere. For example, many of the molecular signatures of the terrestrial atmosphere can be found as absorption features in the spectra of the host stars, e.g. H_2O, CO, CO_2. Consequently an effort to detect CO_2 (in the red wing of the CO fundamental) or O_3 (within the SiO fundamental) might become quite difficult, especially at low spectral resolution. These considerations suggest that state-of-the art representations of stellar radiation spectra are an essential part of a rigorous investigation of the detectability of astronomical biosignatures.

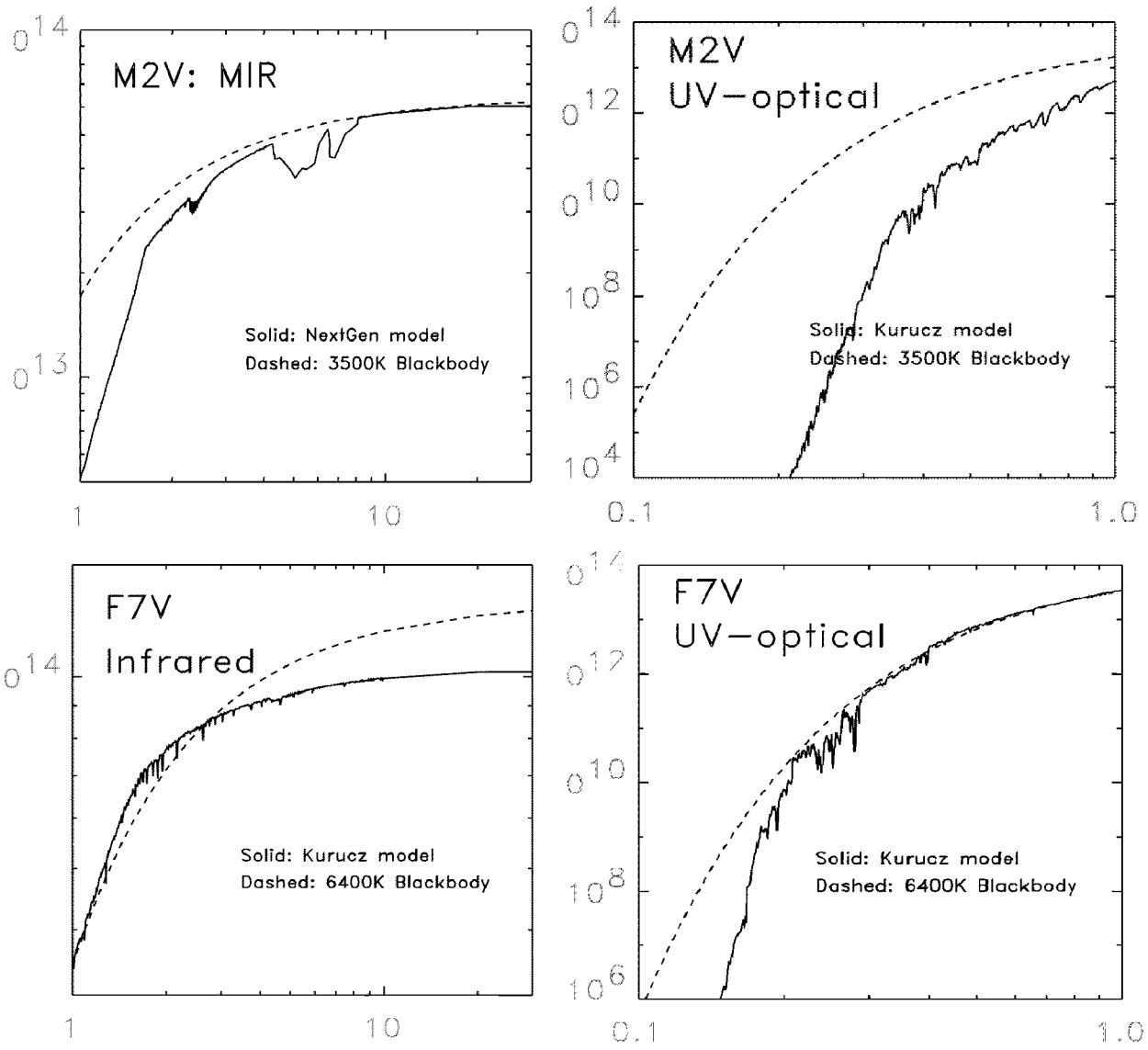


Figure C.3.7 Comparisons between Kurucz and NextGen model spectra and photospheric blackbodies for F7V and M2V stars. The blackbodies are presented simply to show deviations of the shape of real stars from those of blackbodies. The blackbody has been normalized to one point on the entire UV-mid-IR spectrum for each spectral type, so the separate UV and mid-IR diagrams show consistent scaling. Large deviations from a blackbody can be seen for both spectral types, especially for the UV region of the stellar spectrum. The features near 2.3 and 4.7μm are CO 1st overtone and fundamental bands, but the dominant opacity from 5-8μm is water vapor, with a small contribution from the SiO fundamental.

There is now a network of about 450 absolute calibrators derived from observations whose low-resolution ($\lambda/\Delta\lambda$~100) absolute spectra have supported several recent satellites (Cohen et al. 1999 & references therein). A major legacy of the recent ESA International Space Observatory is a set of higher resolution ($\lambda/\Delta\lambda$~1500) stellar spectra emphasizing a range of stars in which molecules play a dominant role. There has also been a corresponding growth in the creation of model grids for a wide diversity of stars, with associated synthetic spectra with resolutions ($\lambda/\Delta\lambda$) in the range ~100-100,000. The primary resource for years has been the set of computations by Kurucz (1992), which cover stellar classes from B to early K-types. For later-type stars, his models have traditionally lacked strong IR bands whose presence in stellar spectra was unrecognized for many years. For example, the SiO fundamental has a prominent head near 7.5 μm but extends beyond 11 μm.

To synthesize meaningful spectra for cooler stars, NextGen grids of models (Hauschildt et al. 1999a,b) are preferred to the standard Kurucz models. NextGen includes many more sources of molecular opacity than does Kurucz. To explore spectra of cool stars, the NextGen models now incorporate 313 million lines of water vapor (Partridge & Schwenke 1997), 12 million TiO lines (Jorgensen 1994), and the best available line lists for relatively common molecules such as CO (Goorvitch & Chackerian 1994a,b) and SiO (Langhoff & Bausclicher 1993).

In the wake of the ISO mission, and in preparation for SIRTF, there is currently a major effort to compare and contrast synthetic and observed IR spectra, and to interpret the differences. Leveraging this work, we will use these improved stellar models to represent the stellar radiation fields used as input to the Virtual Planetary Laboratory. We will use the Kurucz models for warmer stars (above ≈4000K), and the NextGen models for cooler stars. All these models provide synthetic spectra extending from the far-UV through to the far-IR. However, we will also make an effort to absolutely calibrate ISO observed spectra for cool stars (now archived) at resolutions of ~1500 in the 2-12 μm region.

Although these observations cover a smaller range of stellar types than the model grids, this approach would provide more realistic spectra over this wavelength range, in comparison to models that rely on parameterized atmospheres and molecular line lists.

C.3.3.4 Upgrading the Chemical Model to Ultracomputing.

The computational efficiency of the Caltech/JPL model will be improved by re-coding certain portions of the model to take advantage of the capabilities of the JPL massively parallel-processing computers. The evaluation of the internal atmospheric radiation field at each wavelength is independent of the other wavelengths. Similarly the evaluation of the chemical production and loss terms at each atmospheric level is independent of the other levels. Both sets of computations occur many times during the course of a single model run. We therefore expect significant improvements in the performance of this model.

C.3.3.5 Research Tasks

This task includes 2 sub-tasks. The first focuses on simulations of the Earth s paleo environment. The second involves preliminary simulations of a range of extrasolar planets around stars with different stellar types.

Biosignatures of the Early Earth

The chemical history of Earth's atmosphere currently provides our only record of the evolution of an atmosphere that has been influenced by biology. Over the past several years considerable progress has been made in reconstructing the first half of this record (prior to 2 billion years ago) by: (1) tightening empirical constraints on oxygen (Rye and Holland, 1998), (2) establishing the first empirical constraints on CO_2 (Rye and Holland, 2000a; Rye et al., 1995) and (3) suggesting plausible constraints on methane levels (Rye and Holland, 2000b). This work points to an early Earth radically different from today's Earth and has helped drive the push to understand how microbially-active planets with oxygen-poor atmospheres might appear from space.

The geologic record is consistent with the notion that atmospheric oxygen levels were very low during the first half of Earth history and rose sharply sometime around 2 billion years ago (Ga) (for early insights into this history see: Cloud, 1968; Holland, 1958; Holland, 1962; Holland, 1984). The behavior

of Fe during weathering of paleosols (ancient soils) that formed before 1.7 Ga indicates that atmospheric oxygen levels were ~ 3 x 10^{-4} atm before 2.3 Ga and 0.03 atm after 2.0 Ga (Rye and Holland, 1998). Several other compelling lines of evidence from the sedimentary record support these conclusions. The presence of detrital pyrite (Ramdohr 1958a,b; Rye and Holland, 1998 and references therein) as well as of detrital siderite (Rasmussen and Buick, 1999) in sedimentary rocks that formed before 2.3 Ga indicate that oxygen levels were very low prior to 2.3 Ga. The worldwide emergence of red beds around 2.2 Ga indicates oxygen levels were already fairly high about 2.2 Ga (Rye and Holland, 1998 and references therein). A prolonged excursion to strongly positive values in the marine $\delta^{13}C$ record indicates that a large amount of oxygen was added to the atmosphere between 2.22 and 2.06 Ga (Karhu and Holland, 1996). For an alternative view see Ohmoto (1996 and 1997) and Watanabe et al. (1997), in which Ohmoto and his colleagues have concluded that the sedimentary and paleosol record contains no evidence of a major change in atmospheric oxygen levels over the past 3 to 4 Ga. However, the preponderance of evidence, including evidence of the sorts cited by Ohmoto and colleagues in the references cited above, is consistent with the hypothesis that oxygen was low until about 2 Ga.

Rye et al. (1995) found that atmospheric CO_2 was ~0.02 bar between 2.75 and 2.2 Ga. Their conclusions were based on the observation that Fe and Mg were distributed in paleosols of that era in a manner consistent with the precipitation of Fe-rich smectites and inconsistent with the precipitation of more than trace amounts of Fe-carbonate, siderite. Rye and Holland (2000a) found that the distribution of Ni, Co and Mn in the circa 2.2 Ga Hekpoort paleosols also was consistent with the precipitation of Fe-smectite in the lower portions of early paleosols. Siderite would have been the dominant Fe-mineral if atmospheric CO_2 was > 0.02 bar.

One-dimensional climate models suggest that greenhouse forcing equivalent to CO_2 > 0.1 bar was necessary to compensate for the fainter sun at 2.75 Ga (Kasting, 1987 and 1998). Another greenhouse gas that might have made up for the deficiency in CO_2 is CH_4, which is primarily a biogenic gas on Earth. There is isotopic evidence for non-marine methanotrophy ca. 2.75 Ga in lacustrine sediments (Buick, 1992) and at the top of a paleosol (Rye and Holland, 2000b). These observations suggest that atmospheric CH_4 may have been >20 ppm at that time and that biogeochemical processes involved in both the production of methane (methanogeny) and the consumption of methane (methanotrophy) may have helped to regulate the redox state of Earth's atmosphere as well as Earth's climate.

As well as using the best available constraints on the Earth's paleoatmosphere derived from the geological record, we have also initiated a collaborative agreement to work closely with the Carnegie Geophysical Lab. The Geophysical Lab will be working in parallel with this proposed effort using lab data and framework theoretical modeling to understand the suite of planetary atmospheric composition types that might be expected before the development of a significant oxygen fraction as occurred for the Earth about 2 Ga.

The objective of this paleoatmospheres subtask is to compile the best available assessments of the atmospheric bulk composition at selected times early in the Earth s evolution. For these periods, we will produce radiatively, thermally, and chemically self-consistent model atmospheres using the integrated chemistry-climate model. The earliest period we will model will be a nominally prebiotic Earth at 4.0 Gyr ago. That environment is thought to have been characterized by higher CO_2 and lower CH_4 than the present day atmosphere. We will also model a late Archean atmosphere (2.8 Gyr ago). For this period we have carbon isotopic evidence for a thriving biosphere with a large methanogenic community, consequently producing a relatively methane-rich atmosphere. After 2.3 Gyr ago, O_2 rose dramatically in the Earth's atmosphere, but the exact amount of oxygen present in a given time period is still contentious. For early atmospheres with oxygen, we will therefore run a suite of models for each period, varying that constituent abundance over a reasonable range. These models will be validated by comparing the predicted surface temperature for a given atmospheric composition, and the known surface temperature for these periods inferred from the geological record.

We will then output the synthetic spectra for a family of Earths at different stages in its evolution. For periods known to support life, we will search for potential biosignatures in the spectra, and make assessments of their detectability using the Astronomical Instrument Simulators as described in Task 1.

Biosignatures of a Range of Extrasolar Planets

In this sub-task, we will generate self-consistent solutions for the physical and chemical state of a range of planetary atmospheres with specified surface and exogenic sources and sinks.

For inert atmospheric constituents like molecular nitrogen, the vertical distribution will be fixed in the chemical model. For major source constituents like carbon dioxide, water, and biological emission species (putative biosignatures), the abundances at the lower boundary will be fixed (assuming that the abundance at the surface is buffered by atmosphere/surface processes) or an upward flux will be prescribed. Photochemically-derived minor constituents will have lower boundary conditions reflecting whether or not surface loss occurs. For most planetary scenarios, mass loss to space primarily will consist of escape of atomic or molecular hydrogen, with the lower atmosphere cold trap ultimately determining the limiting escape flux.

Once a thermodynamic and chemical equilibrium state has been reached, we will run tests for the detectability of varying amounts of the *currently accepted* biosignatures. These include H_2O, O_2, O_3, CO_2, CH_4 and N_2O against the bulk atmospheres of modern Earth and representative early Earths that we derived in the previous task. This work will provide an improvement over existing detectability studies in the following areas:

First, all model atmospheres included in this simulation will represent conditions that are radiatively, thermally, and chemically self-consistent. Second, these simulations will generate spectra over the broadest wavelength range possible to facilitate the identification of the most advantageous wavelength regions for detecting these biosignatures. For example, extension down into the near-infrared may be particularly valuable because this spectral range hosts many features from the classic "disequilibrium pairs" of biosignatures, including O_2 and CH_4. Also, features confused by overlap with another spectral feature in the mid-IR may be better separated and observable at near-IR wavelengths. A wide wavelength range will also allow us to probe to different depths in a planetary atmosphere and therefore allow a more comprehensive evaluation of the detectability of biosignatures on the surface and in the atmosphere. For example, mid-IR wavelengths are often sensitive only to a planet's stratosphere, and may therefore provide incomplete information on trace gas abundance, potentially hiding biosignatures

Third, our radiative transfer model can realistically include the effects of clouds on the radiative balance of the planet. Models can therefore include different types of cloud at different levels in a planetary atmosphere and fractional cloud-cover can be simulated with combination of separate runs for a clear and cloudy case. We will therefore also explore the detectability of biosignatures as a function of cloud cover on a terrestrial planet.

Finally, we will run these simulations for a range of different stellar types using realistic stellar spectra, not blackbody approximations. The effect of varying UV fluxes on the photochemistry of the atmosphere, as well as the effects on the insolation can be determined for different stellar types, and the effect on detectability of trace constituents in different wavelength regimes will be explored.

With this study, we will be able to make recommendations on the optimum wavelength and spectral resolution for detection of the currently known biosignatures. However, spectral regions that look promising for biosignature detection from a planetary scientist's point of view may not be quite as appealing when the hard realities of astronomical observing are taken into consideration. While one goal of this project is to simply determine the biosignatures themselves, and then to drive the design of the instrumentation that would be required to detect these, we are also acutely aware of the limitations of the currently proposed first generation of these instruments. Therefore, once the synthetic spectra for this task have been generated, we will first successively degrade the spectra to simulate the anticipated resolutions of future instruments, and to determine at what spectral resolution crucial biosignature information is

lost. We will then also run them through the astronomical instrument simulators to determine detectability as a function of instrument detector sensitivity, spectral resolution, aperture size, and against environmental noise factors such as a zodiacal disk.

Statistical Analysis Tools

As part of Task 2, we will also develop a set of sophisticated statistical tools to most efficiently analyze the biosignatures spectral catalog. These tools will provide a probabilistic estimate of the likelihood that a simulated planetary system falls into one of three categories: inhabited, habitable, or sterile, and will allow us to understand and explore the relative importance and interaction of specific features in the model. These database analysis tools are described in more detail below.

Probabilistic Classification of Spectra

Leveraging from work underway on galaxy morphological (Storrie-Lombardi, Lahav et al 1992) and spectral (Ronen, et al) classification, and stellar spectral classification (Storrie-Lombardi et al 1994), we propose to incorporate a set of non-linear, stochastic neural network techniques capable of transforming the multiple data outputs of our model into classifications with co-existent Bayesian probabilities for the correctness of those classifications. Such networks have been previously easily trained to mimic the pattern matching abilities of human experts in galaxy and stellar classification tasks. Their ability to serve as Bayesian estimators makes it possible to attach probabilities to each classification decision in an automated fashion. The algorithms permit the input of multiple data types including (but not limited to) atmospheric spectra and stellar classification of the parent star.

Identifying Critical Biosignature Features

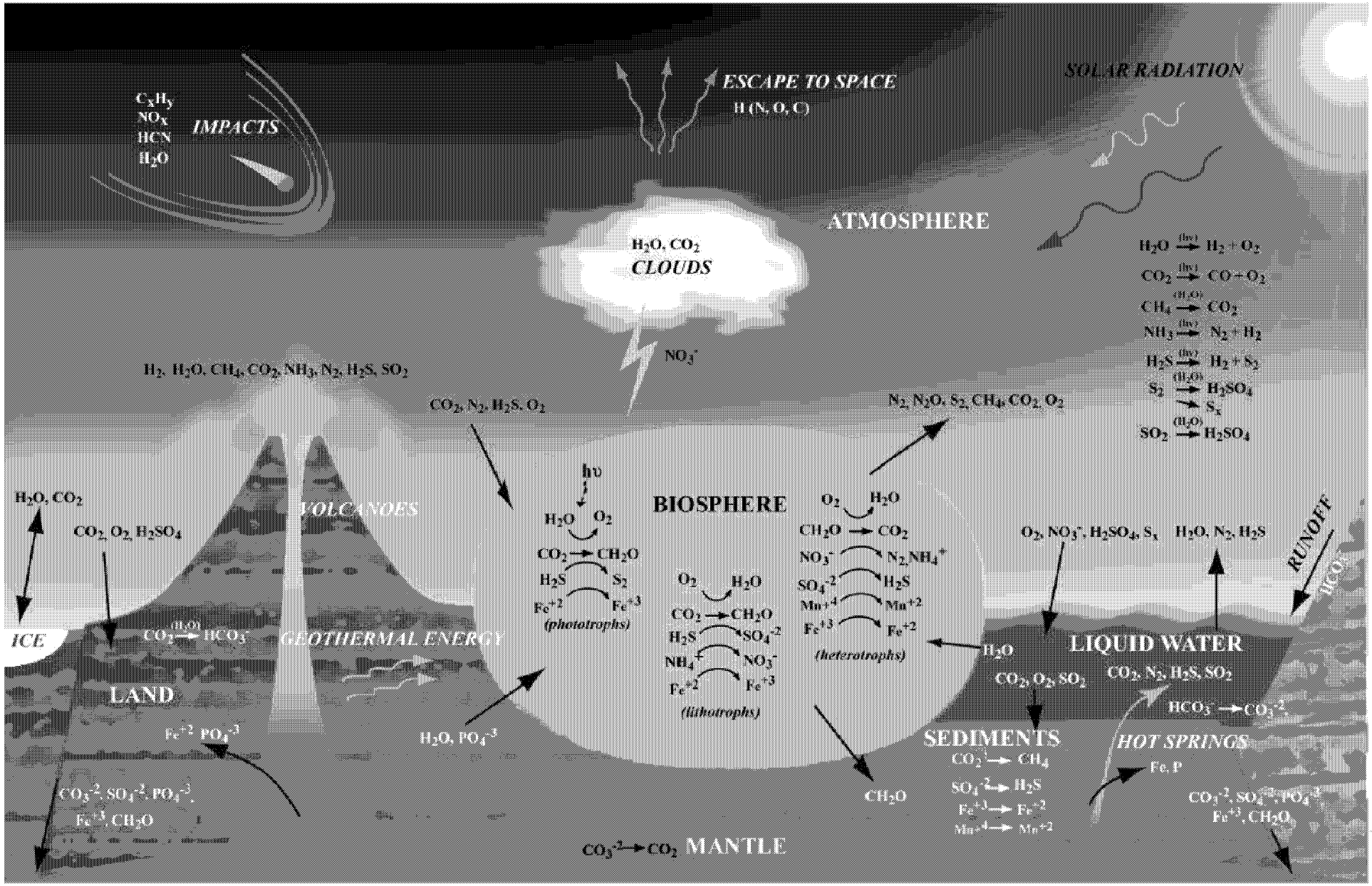
These same non-linear algorithms can be employed to assess both linear and non-linear contributions and interactions amongst the data feature set and have been employed to assess spectral biosignatures (Storrie-Lombardi 1997). The basic technique involves iterative training of a network on data sets devoid of a specific feature or set of features contained in the putative biosignature. For example, the spectra continuum information or specific spectral bands might be removed from training and test sets and the resulting network classification efficiency determined. In other experiments, information about stellar type might be removed. Analysis of both the linear and nonlinear portions of the networks can be accomplished to determine the nature of the interaction between specific features.

C3.3.5 Significance and Products of Task Three

This task generates the following products:

- Physically, radiatively and chemically self-consistent atmospheres for a suite of Earth s paleoatmospheres based on new constraints provided by laboratory data and geological and biological modeling.
- Physically, radiatively and chemically self-consistent atmospheres for planets around stars other than our own in which abundances of currently identified biosignatures are varied as a test of spectral sensitivity to these constituents.
- Spectra of realistic surfaces and atmospheres (including the radiative effect of clouds and aerosols) produced by the coupled radiative chemical climate model. This work will produce a catalog of spectra, which can be used to characterize the detectability of these biosignatures against a range of atmospheres and for stars of different spectral type.
- Recommendations for detectability of trace gases with no a priori assumptions for instrumentation
- Recommendations for detectability of biogenic trace gases for spectra generated under this task for instruments based on both nulling interferometer and coronagraphic design.
- Development of statistical tools to analyze the resultant comprehensive spectral catalog to determine abundances and best available wavelength regions, sensitivities and spectral resolutions for detection.

As a by-product of this Task, we will produce the best available estimates of the Habitable Zone around stars other than our Sun using a coupled radiative chemical climate model that realistically models the radiative effects of cloud near the limits of the Habitable Zone. The radiative-chemical climate model produced here will be used as the basis for the Abiotic and Inhabited Planetary Models described in the following Tasks.


C_xH_y
NO_x
HCN
H_2O
IMPACTS
ESCAPE TO SPACE
H (N, O, C)
SOLAR RADIATION
ATMOSPHERE
H_2O, CO_2
CLOUDS
NO_3^-
$H_2O \xrightarrow{(hv)} H_2 + O_2$
$CO_2 \xrightarrow{(hv)} CO + O_2$
$CH_4 \xrightarrow{(H_2O)} CO_2$
$NH_3 \xrightarrow{(hv)} N_2 + H_2$
$H_2S \xrightarrow{(hv)} H_2 + S_2$
$S_2 \xrightarrow{(H_2O)} H_2SO_4$, S_x
$SO_2 \xrightarrow{(H_2O)} H_2SO_4$
H_2, H_2O, CH_4, CO_2, NH_3, N_2, H_2S, SO_2
CO_2, N_2, H_2S, O_2
N_2, N_2O, S_2, CH_4, CO_2, O_2
H_2O, CO_2
CO_2, O_2, H_2SO_4
VOLCANOES
hv
H_2O O_2
$CO_2 \rightarrow CH_2O$
H_2S S_2
Fe^{+2} Fe^{+3}
(phototrophs)
BIOSPHERE
O_2 H_2O
$CH_2O \rightarrow CO_2$
NO_3^- N_2, NH_4^+
SO_4^{-2} H_2S
Mn^{+4} Mn^{+2}
Fe^{+3} Fe^{+2}
(heterotrophs)
O_2 H_2O
$CO_2 \rightarrow CH_2O$
H_2S SO_4^{-2}
NH_4^+ NO_3^-
Fe^{+2} Fe^{+3}
(lithotrophs)
O_2, NO_3^-, H_2SO_4, S_x
H_2O, N_2, H_2S
RUNOFF
HCO_3^-
ICE
$CO_2 \xrightarrow{(H_2O)} HCO_3^-$
GEOTHERMAL ENERGY
LAND
H_2O
LIQUID WATER
CO_2, O_2, SO_2
CO_2, N_2, H_2S, SO_2
$HCO_3^- \rightarrow CO_3^{-2}$
Fe^{+2} PO_4^{-3}
H_2O, PO_4^{-3}
SEDIMENTS
$CO_3^{-2} \rightarrow CH_4$
$SO_4^{-2} \rightarrow H_2S$
$Fe^{+3} \rightarrow Fe^{+2}$
$Mn^{+4} \rightarrow Mn^{+2}$
HOT SPRINGS
Fe, P
CO_3^{-2}, SO_4^{-2}, PO_4^{-3}
Fe^{+3}, CH_2O
CO_3^{-2}, SO_4^{-2}, PO_4^{-3}
Fe^{+3}, CH_2O
CH_2O
$CO_3^{-2} \rightarrow CO_2$ MANTLE

C.3.4 Task Four – The Range of Plausible Atmospheres for Abiotic Planets

In this task we will determine the range of plausible physical and chemical states for abiotic terrestrial planet atmospheres with active geological and exogenic sources. This study is important to the issue of biosignature detection, because even in the absence of life, a planetary atmosphere can be sustained in a disequilibrium state by a range of abiotic processes. By understanding these abiotic disequilibrium states, we will be able to identify the special disequilibrium states induced by life (Lovelock, 1965; Lederberg, 1965; Hitchcock and Lovelock, 1967; Sagan et al., 1993). This study will also define the range of contexts that result in a habitable planet, and in atmospheric states that might falsely be interpreted as indicating an inhabited planet. Possible false positives might include ab iotically elevated levels of molecular oxygen, an oxidizing atmosphere with elevated levels of methane, or substantial levels of nitrous oxide.

Current ideas about the climate and composition of lifeless terrestrial planets come from geological records of the early Earth and from present day observations of Venus and Mars. However, it is likely that atmospheres on planets around stars of different spectral type will differ from terrestrial atmospheres in our own solar system. The model proposed here will allow us to explore the abiotic states of a wide range of extrasolar planets.

In this Task we will combine the Coupled Climate-Chemical Model developed in task three with realistic parameterizations of geological and exospheric fluxes to develop the Abiotic Planet Model (APM). This innovative model will provide the most realistic simulation of a terrestrial planet to date.

A planetary atmosphere is a part of a larger planet system which includes processes on land and in liquid surface water (if either exists) and in the planetary interior. The volatile composition in the atmosphere is a result of exchange with this system. The carbonate-silicate cycle is a classic example of the connection between atmospheric composition (and in turn the thermal state of the atmosphere) and the other domains (Walker et al., 1981). In a pioneering paper, Hart (1978) documented the factors influencing the size of the Habitable Zone, making use of a computer simulation of the Earth that accounted for a variety of solar and planetary factors and processes, including connections between the atmosphere and interplanetary space, land, ocean, and interior. Schubert et al. (1989) reviewed the processes that couple interiors and atmospheres. Lasaga et al. (1985) and Sleep and Zahnle (2000) provide examples of a mechanistic model simulating cycling of carbon through atmosphere/geophysical domains to yield best estimates for atmospheric composition and its climate implications.

The APM we propose to develop will include more planetary processes than these previous works, including treatments of processes that, in simpler forms, were limitations on the results derived in previous work. The Abiotic Planetary Model is designed to be a testbed for interdisciplinary studies of the factors determining the plausible range of climate and chemistry for extrasolar terrestrial planets and implications for the habitability of these planets. For each computed atmospheric state, synthetic spectra will be generated to support the study of the detectability of biosignatures. The flexible design of the APM will support many collaborations with a wide range of investigators, both scientists associated with the NAI and others in the general astrobiology community.

C.3.4.1 Development of the Abiotic Planetary Model

As illustrated in Figure C.3.4.1, the Abiotic Planetary Model will add two components to the Coupled Climate-Chemistry Model described in Task 3:

- more sophisticated treatments of atmospheric processes at the upper boundary of the model (meteoric influx, and atmospheric escape)
- a mechanistic description of the cycling of volatile constituents between the atmosphere, land, ocean, and planet s interior.

At the upper boundary, detailed simulations of gas escape (particularly hydrogen escape with its impact on the oxidation state of the planet) will be implemented. We will explore the effects of small solar system objects entering the atmosphere introduction of new material, transformation of the existing atmospheric climate and composition, and ero-

sion of the atmosphere. We will explicitly model the processes controlling the fluxes of volatile species between the

- atmosphere and land (weathering, etc.),
- atmosphere and ocean (air-sea exchange, etc.),
- atmosphere and interior (volcanic emissions),
- land and oceans (runoff and sedimentation), land and the interior (subduction, etc.), and
- oceans and interior (subduction, deep oceanic vents, etc.).

The model also will treat processing of volatiles within the ocean (aqueous chemistry, etc.) and the interior (change in oxidation state of volatiles as a function of oxidation state of interior, etc.). More details on each of these development subtasks will be provided below.

Atmospheric Processes at the Upper Boundary

In this section, we describe atmospheric processes at the upper boundary that will be included as part of the APM. These include volatile escape and impact processes.

Escape of gas to space

The APM will provide improvements over the best existing coupled climate/atmospheric chemistry models (e.g. Brown 1999) by providing improved hydrogen escape formalism. In the Brown (1999) and other existing coupled-climate models, the extent of hydrogen escape is the central determinant of the oxidation state of the atmosphere. However, if hydrogen escape is treated as simple diffusion-limited escape, as it is in these models, overestimates of the extent of hydrogen escape and incorrectly high estimates of atmospheric oxidation can occur. The development of a new, mathematically-tractable hydrodynamic simulation of hydrogen escape as part of the APM advances the current state of planetary modeling.

Gas escape to space from a planetary atmosphere can have two major consequences. First, gas escape can result in reduction in atmospheric elemental composition. The reduction in the nitrogen content of the Martian atmosphere as a result of the non-thermal loss of nitrogen over the age of the planet (Brinkmann, 1971; Nier et al., 1976; Fox and Hac, 1997) is an example of this. Second, the escape of hydrogen from a planetary atmosphere following photolytic dissociation of atmospheric water can result in an increase in atmospheric oxygen, repartitioning elemental oxygen between the source water and the reservoir molecular oxygen without the participation of biology. For example, early in the history of Venus the enhancement of water in the upper atmosphere, and the subsequent photolysis and loss of hydrogen to space, could have produced a transitory high abundance of molecular oxygen (Kasting, 1988).

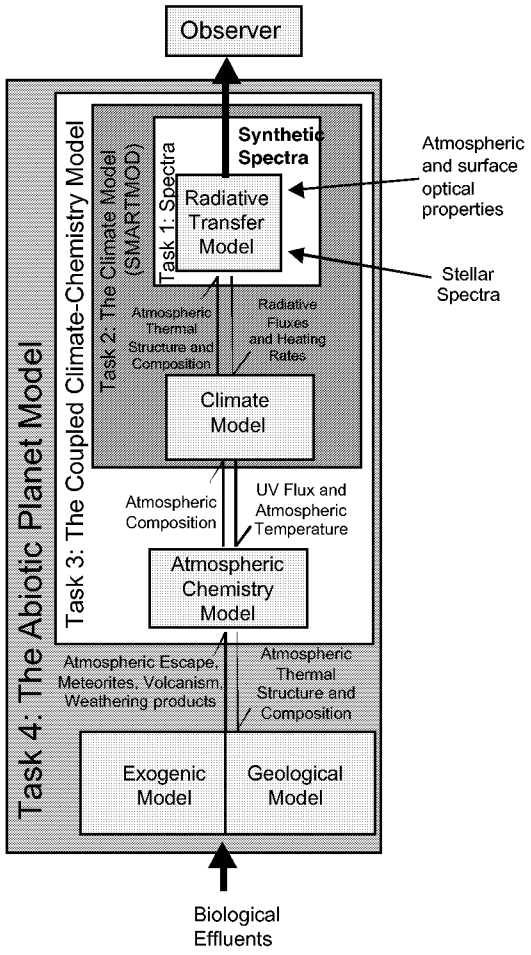


Figure C.3.4.1 The Abiotic Planet Model combines the Coupled Climate-Chemistry Model developed in Task 3 with more sophisticated treatments of gas escape, the effects of impacts, and the cycling of volatiles between the atmosphere and the land, ocean, and planetary interior.

For inclusion in the APM, we will develop a comprehensive model of atmospheric escape. This model will include treatment of gas evaporation (or Jeans loss) and hydrod y-namic escape of hydrogen where the hydrogen

abundances are great enough to generate or contribute to a planetary wind. In the latter case, we will develop an improved treatment that better deals with transonic winds. In both cases, the flow of hydrogen through other (potentially major) atmospheric gases will be considered, including the case of hydrodynamic drag and blow-off of heavier species. Non-thermal escape mechanisms will also be treated (which, for example, may be especially important for the evolution of atmospheric oxygen and nitrogen). These non-thermal (ion-exchange, sputtering) processes provide an important example of the coupling between the atmosphere and the interior since the planetary magnetic field plays a role in controlling the non-thermal escape rates.

THERMAL LOSS

Thermal evaporation of gases occurs above the exobase, where the mean free path becomes comparable to the scale height of the overlying atmosphere: particles that attain escape velocity will likely not collide with another particle and will escape. The escape rate can be determined from integration of the upward Maxwellian flux (e.g. Yung and DeMore, 1999). In many cases including the loss of hydrogen from the current terrestrial atmosphere the loss rate will be limited by the flow of escaping gas(es) through the non-escaping components. This is diffusion-limited loss and is readily calculated from the diffusion equation for a multi-component gas (Hunten, 1973; Zahnle and Kasting, 1986).

An evaporative model of loss is appropriate when a sufficiently small fraction of the velocity distribution of the escaping gas is above the escape velocity that the escaping species does not significantly perturb the vertical structure of the atmosphere. When the loss rates become higher, the gas escapes as a continuous fluid. In this case, the pressure force becomes important (Watson et al., 1981; Kasting and Pollack, 1983), and the loss rate is over-predicted by both the Jeans and diffusion-limited models. In hydrodynamic escape, the escape flux is determined partly by energy considerations and partly by the density of hydrogen at the base of the flow. The escape is powered by solar EUV energy (Watson et al., 1981) and perhaps by interaction with the solar wind as well (Chassefiere, 1996). Sufficiently vigorous hydrodynamic escape can lead to loss of heavier atmospheric constituents. These elements can be dragged or blown-off along with the hydrogen, both modifying the atmospheric inventory of heavier elements and modifying the outflow of the planetary wind (Hunten et al., 1987; Zahnle et al., 1990; Chassefiere, 1996).

Within the APM, we will treat evaporative escape, diffusion-limited evaporative escape, and hydrodynamic escape, dependent upon the physical conditions of the planetary atmosphere and incident external heating. A major development for this model will be incorporation of a new treatment for hydrodynamic loss. Although planetary wind models have been created in the past (Watson et al., 1981; Kasting and Pollack, 1983; Chassefi re, 1996), the numerical methods that have been employed to date are not sufficiently robust to allow the incorporation of detailed physics, and to simultaneously explore a broad range of possible primitive atmospheres. Mathematically, one needs to integrate the coupled energy and momentum equations for the escaping gas from the homopause out to infinity. A major difficulty arises because of the existence of a singularity, or critical point, in the one-dimensional, steady-state solution at the distance where the outflow becomes supersonic. Various methods have been applied to provide solutions to the planetary wind equations (e.g. Watson et al., 1981; Kasting and Pollack, 1983; Chassefiere, 1996). However, these methods generate solutions which are either ill conditioned, or else provide solutions for a limited subset of cases. For example, Kasting and Pollack (1983) used an iterative method, in which one alternates back and forth between the momentum and energy equations, but were unable to make this work for supersonic solutions. Chassefi re (1996) developed perhaps the most elaborate numerical technique, but one that is only valid when the exobase occurs below the critical point, which excludes a whole range of possible H_2-rich primitive atmospheres.

Fortunately, a stable numerical method exists for such problems that allows a generalized treatment of hydrodynamic escape. It is called Godunov s method (Godunov, 1959) and it has been successfully used in one dimension to study cometary winds (Gombosi et al., 1985). Godunov s method overcomes

the instabilities inherent in modeling subsonic-supersonic transitions by solving shock relations at each grid point instead of using standard finite differences. A detailed description of a first-order Godunov scheme is given by Gombosi (1984). This solution method will be applied to the planetary wind energy, m o-mentum, and continuity equations. The interaction between different (escaping and non-escaping) atmospheric gasses will be treated within this system by carrying continuity equations for each species and modifying the momentum and energy equations as composition evolves.

NON-THERMAL LOSS

Non-thermal loss processes involve the ejection of atmospheric gases through interactions with ions or electrons above the exobase, or by photodissociation. Dissociation processes (e.g., electron recombination or electron impact) result in the atomization of molecules, where the resultant atoms may have sufficient velocity to escape. An example of dissociation loss is provided by nitrogen loss from Mars (e.g. Yung and DeMore, 1999). In order to reconcile the observed nitrogen fractionation ratio ($^{15}N/^{14}N$) of roughly 1.62 times terrestrial, roughly 75%+ of the original atmospheric nitrogen must have been lost (Fox and Hac, 1997). Similar dissociative loss of oxygen should have occurred, but no fractionation relative to terrestrial is observed, suggesting that oxygen is buffered by a large, non-fractionated oxygen reservoir (Owen, 1992).

Impact of energetic ions and atoms provides an additional loss process. Solar wind particles are capable of removing (sputter - ing) hydrogen molecules, and compete with Jeans thermal loss in the current terrestrial atmosphere (Hunten et al., 1989). A related process, indirect solar-wind induced sputtering, can eject heavier species and will likely be important on planets without a magnetic field. Here, EUV produced O^+ ions are accelerated by the draping of the solar wind magnetic field over the ionosphere. Some of these ions are directed back into the atmosphere, where they impact molecules and/or atoms, providing some with sufficient energy to escape (Luhmann et al., 1992; Kass, 1999). Such sputtering models have been used to estimate loss rates for CO_2 from the Martian atmosphere (Kass and Yung, 1995, 2000) resulting in loss estimates of between 0.2 and 0.8 bars of CO_2 (as compared to a current atmospheric inventory about $6x10^{-3}$ bars).

For the APM, we intend to incorporate dissociative recombination and particle impact loss mechanisms. Treatment of dissociative recombination will follow Fox and Hac (1997). The production of candidate loss ions in the upper atmosphere, as well as the structure of the upper atmosphere, will be calculated by the APM photochemical and radiative models and passed as input to the loss model (following a similar approach as Fox, 1993). Based on these distributions, the dissociative recombination yields will be calculated and used as input for a Monte Carlo loss calculation (Fox and Hac, 1997). These calculations use Maxwellian initial velocity distributions and random initial directions to calculate loss probabilities and hence rates. While Fox (1993) and Fox and Hac (1997) concentrated on nitrogen, we will also treat oxygen loss.

Treatment of sputtering will follow Kass (1999). Again, the composition and structure of the upper atmosphere will be taken from the APM photochemical and radiative models. In this case, a model for the incident O^+ ions is required, which depends strongly on the assumed planetary magnetic field. Given a magnetic field produced by the geophysical (interior and planetary formation) model, we will use the work of Luhmann et al. (1992) and Luhmann and Kozura (1991) to estimate O^+ production and impacting populations. The Monte Carlo sputtering model then follows the incident oxygen ion and maps impact histories using a library of impact cross sections.

Effects of impacts

Depending on the size of an object entering a planetary atmosphere, the impact can chemically modify or add to the target atmosphere by the reprocessing (mostly by shock heating) of air already present, by the ablation of new exogenic material from the impactor, or by volatilization of planetary materials (e.g., it can liberate CO_2 from carbonate rocks, or it can evaporate water). Some classes of impacts can remove more volatiles from a planet than are added. The objective of this proposal activity will be to develop globally averaged rates for atmospheric processing as a function of the flux and composition of impactors.

ATMOSPHERIC MODIFICATION

Impacts can change the chemical speciation of the existing atmosphere either by shock heating air or by directly reacting with the air. In a strongly shock-heated gas, temperatures get so high that ordinarily stable molecules react to form a thermochemically equilibrated mix; rapid cooling of the gas leaves many chemical species quenched at relatively high abundances, far from equilibrium because their destruction is kinetically inhibited. The disequilibrium products that survive depend most strongly on the elemental composition of the shocked gas. A general rule of thumb is that CO forms until either C or O is exhausted. If O>C, oxidized species result, if C>O, reduced species result. Impact shock chemistry has been addressed as a source of nitrogen oxides in a modern terrestrial atmosphere (Prinn & Fegley 1987; Zahnle 1990) and as a source of HCN in more reduced atmospheres (Fegley et al. 1986, Fegley & Prinn 1989, Chyba & Sagan 1992).

Methods developed elsewhere for impacts on Earth (Zahnle 1990; Toon et al 1997) and for impacts on Jupiter (Zahnle 1996) can be applied to impact shock chemistry in atmospheres in general. For impacts on planets with solid surfaces, there are 4 major shock processes:

- the inbound passage of the impactor through the atmosphere;
- the expansion of the ejecta plume through the atmosphere;
- the reentry into the atmosphere of ejecta; and
- radiative heating of the entire atmosphere and surface.

These processes become progressively more important in the order listed as the impact energy is increased.

The first process is relatively well understood (Park and Menees 1978). For objects small enough to stop in the atmosphere, we can approximate each individually by a terminal explosion that processes a predictable amount of atmosphere at an altitude above a quench temperature. These objects will contribute ablated vapors to the atmosphere according to the impact velocity and the chemical composition of the impactor. Objects large enough to reach the surface without slowing down deposit relatively little energy or ablated material into the atmosphere before they hit the ground. The net effect on the atmosphere of the first process can be usefully approximated by global average rates as functions of altitude.

The second process is more important because it harnesses a greater fraction of the energy of the larger impacts (Prinn & Fegley 1987). Zahnle (1990) used an analytical approximation suggested by Zel'dovich & Raizer (1967) to model the superheated rock vapor (and/or water vapor) ejecta plumes to estimate the mass of strongly shocked air. The amount of atmosphere shocked above a given quench temperature is computed from the momentum of the ejecta plumes. As with the first process, the effect on the atmosphere can be usefully approximated by global average rates.

Examples of the third process are provided by the 1994 impacts of comet D/Shoemaker-Levy 9 with Jupiter and the leading model (Melosh et al. 1990) for starting global wildfires at the K/T boundary. Air can be shock heated either by gas-on-gas shocks as the plume falls or by reentry of myriad tiny ejecta particles. Zahnle (1990) used this model to estimate NO production for the K/T impact. For impacts of this scale, it is most useful to regard the materials of the impactor itself as being dispersed at the highest ejecta velocities. Much of this will escape if these are high enough. The SL9 events provided an opportunity to test models of impact-induced atmospheric chemistry. Zahnle (1996) devised a chemical kinetics model for the H, N, C, O, S system appropriate to SL9. The model traces the evolving chemical composition of a parcel of gas by directly integrating the web of chemical reactions. Pressure and temperature histories of the parcels are modelled after those calculated by numerical hydrodynamic simulations of the ejecta plume. In general, a given parcel is shocked twice; i.e., a parcel strongly shocked near the impact site is ejected at high velocity and is therefore shocked again when it reenters the atmosphere. Hence the final state of the gas often depends on the second shock. Shock products are computed for a range of different peak shock temperatures, ejecta velocities, and chemical compositions. The latter can include material from the impactor.

The fourth process only becomes important for the largest impacts. Essentially the entire atmosphere is radiatively heated by infalling ejecta, and so the atmosphere is globally "reset". For Earth's present atmosphere this occurs for events comparable to the impacts that formed the lunar Orientale and Imbrium basins ca. 3.8 Ga. There were hundreds of similar impacts on Earth ca. 3.8-4.2 Ga, and thus all four process are likely to affect inhabited planets.

To put impacts into an atmospheric chemistry code requires that we specify the effects of shock-heating of ambient air as well as the chemical content of the impactor. Because high altitudes are affected more often than low altitudes, and because more air is processed at low temperatures than at high temperatures, the most useful description of shock heating of ambient air is to specify how often the atmosphere above a certain altitude z gets heated above a quench temperature T. Quench temperature is a weak function of the cooling time scale, but it is sensitive to chemical composition and will need to be specified according to air composition. Photolytic chemistry driven by thermal ultraviolet radiation can be important to lightning in some atmospheres, but is less likely to be important in impacts because radiating temperatures in impacts are much lower. Vapor ablated from the impacting bodies themselves can also influence the redox budget of the atmosphere (Kasting 1993); this effect will also be included in the atmospheric chemistry model.

IMPACT EROSION.

Impacts cannot be regarded solely as a source of volatiles. Provided it is big enough and energetic enough, an impact can expel more volatiles from a planet than it adds. A popular example in this solar system is the planet Mars, for which the leading hypothesis is that its atmosphere was mostly stripped by impact erosion (Melosh and Vickery 1989). The mass of atmosphere stripped by impacts depends upon the velocity of the impactors, the size of the planet, and the size of the impactors. The dependence on planetary size is illustrated by the analytic fit to numerical calculations derived by Melosh and Vickery (1989). Above a specific impactor mass, which is determined by the atmospheric pressure and scale height, as well as the planetary radius and gravity, all the atmosphere above the impact tangent plane is lost. However, there is some disagreement over how efficient impact erosion actually is. Walker (1986) suggest that only air directly interacting with the bolide will be ejected, while Newman et al. (1999) use numerical models to show that only the entry column of atmosphere is significantly accelerated. In the proposed work, we will use descriptions of impact erosion described by Zahnle et al (1992) to address the efficiency with which atmospheres are supplied or lost as a function of planetary parameters (escape velocity, principally) and of the parameters describing the population of impactors (composition and impact velocity, principally). Zahnle et al. (1992) considered both aggressive impact erosion as proposed by Melosh and Vickery, and the less aggressive forms of impact erosion proposed by Walker (1986) and Ahrens and O'Keefe (1987).

IMPACT FLUXES

The problem of impact flux populations is a significant one. Ideally, we would simulate the impacting flux populations as a function of the star and the location of the planet in the system. However, the credibility of such an effort would be dubious given the observational constraints. Instead, we can take a number of different approaches. The lunar record of impacts provides an impactor flux history that is scaled and applied throughout the solar system (e.g. Melosh, 1989; Neukum and Ivanov, 1994). Insofar as this represents an impactor flux for a system consisting of a mix of terrestrial and gas-giant planets, we will used a scaled version of this flux to generate the individual impactor consequence results for generation of atmospheric histories (see above). The lunar impact record can be used to construct a chronology of impact intensity because surfaces of different ages, with differing crater densities have been geochemically dated. For a given crater size range, the cumulative impact density on a surface can be derived as a function of age. For example, Neukum and Ivanov (1994) provides an analytic formulation that can be differentiated to give an impact rate function which decays exponentially. Lunar and planetary impact craters also provide some constraints on impact size distributions. The distribution appears to follow a power law with exponent of -3 for <4km (Neukum and Ivanov, 1994). Estimates

of size distributions of near-Earth crossing asteroids provides an additional source of information. Rabinowitz (1993) finds a power law of -5.4 for > 3.5 km, increasing to -3.5 to -2 for smaller objects. However, the lunar impact flux history and near-Earth asteroid populations are likely strongly affected by the specific arrangement of our solar system, most directly to the existence of Jupiter at its location. To test the sensitivity of results to the chosen impactor record, we will use the impacting flux history as a free parameter.

Geophysical Cycling of Volatiles

The Earth provides an initial analog for the interaction of solid planetary surfaces with planetary oceans and atmospheres. Research on this topic involves many branches of Earth Science. We can extrapolate to other planets by examining how terrestrial geochemical cycles work and how conditions elsewhere are similar to or different from those on the Earth.

Much can be learned about the Earth simply by considering the size of reservoirs and the magnitude of the fluxes between them. It is convenient to divide planets from top down into atmosphere, ocean, crust, mantle, and core, with some grouping and subdivision. Since the core interacted chemically with the rest of the Earth only during its formation, we define the global reservoir of an element to exclude the core. The crust and mantle are the silicate part of the Earth. Crust, ocean, and atmosphere are shallow geochemical reservoirs; sedimentary rocks, ocean, and atmosphere are surface reservoirs.

The mantle is about 2/3 of the mass of the Earth compared with 0.05% for the shallow reservoirs. However, some biologically significant elements are strongly concentrated in the shallow reservoirs. A sizable global fraction of the global H, C, N, and Cl are in various surface reservoirs. Ocean water is the dominant reservoir for H and Cl. The atmosphere is a significant reservoir for N and rare gasses. Sedimentary rocks contain massive carbonate deposits. S and P, in contrast, have remained mainly in the mantle. Their abundance in surface reservoirs is limited by the rate that they are liberated, ultimately from plentiful quantities in igneous rocks, by weathering and by hydrothermal circulation. That is, there is plenty of P and S in reasonable igneous rocks.

We discuss two specific issues in more detail to show how our understanding of the Earth and other solar planets can be used to infer conditions elsewhere. We begin with the chemistry of igneous rocks. The global carbon cycle is an example where the efficacy of crustal traps determines the partitioning between the mantle and surface reservoirs.

Composition of reactable igneous rocks.

Weathering of rocks on land and the hydrothermal circulation at depth involve rock/water chemical reactions. Vented fluids and altered rocks at oceanic and land sites have been extensively studied. The long term composition of the ocean and the atmosphere are determined by such reactions, and by reactions that follow the depostion of sedimentary rocks.

The most abundant igneous rock on the surface of the Earth is the mid-ocean ridge basalt (MORB), which upwells from the mantle and covers the floors of the ocean basins (60% of the Earth s area). Massive amounts of hydrothermal circulation through the basalt occur near midoceanic ridge axes. The process has significant effects on the chemistry of the modern ocean. For example, there is a net flux of Mg into the rock and a net flux of Ca into the water. The basalt becomes somewhat oxidized, removing dissolved O_2 and sulfate from the water. As discussed below, the erupted basalt carries CO_2 from the mantle to the surface and later becomes carbonatized so that subduction returns CO_2 to the mantle.

The composition of the silicate mantle ultimately is determined by the composition of the stellar nebula out of which a planet forms. This is not planet specific. Basaltic rocks are expected to be the dominant rock type in terrestrial-class planets with modest variations in composition. Sampling within our solar system indicates that this is true.

Global carbon cycle.

The terrestrial carbon cycle couples the atmosphere to interior processes. Its surface reservoir, mainly sedimentary carbonates, has a limited capacity. On an abiotic planet, carbonate can be sequestered in both shallow water and deep water.

The effect of atmospheric CO_2 on climate has been studied extensively. It has been suggested that a clement climate has always re-

quired high levels of atmospheric CO_2 (Owen et al., 1979, Kasting, 1993), but the evidence from the geological record prior to 2.2 Gya appears not to support this hypothesis (Rye et al., 1995). Sleep and Zahnle (2000) present a new argument, based on CO_2 fluxes in to and out of the mantle, that weighs against high levels of CO_2 on early Earth during the Archean and the Hadean eras.

In the carbon cycle, there are five significant reservoirs: the atmosphere, free carbonate in the ocean, carbonates lying upon or veined within oceanic basalt, carbonates on continental platforms, and CO_2 in the mantle. Of the vast amount of terrestrial CO_2 that is not in the atmosphere, roughly 6×10^{21} moles are currently in the crust and a comparable or larger amount is in the mantle (e.g., Zhang and Zindler, 1993). The current CO_2 mantle outgassing rate, $\sim2.5\times10^{12}$ moles/yr (ibid.), is fast enough to double the surface carbonate inventory in 2.4 billion years, and the mantle connection would have been stronger during the Archean. To first approximation, under plate tectonics ocean crust is recycled as the square of the heat flow. On early Earth, with a geothermal source 2-3 times that of today, ocean crust would have been created and subducted 4-9 times faster than today. Outgassing increases proportionately, so that the time scale for doubling crustal carbonate drops to 300-700 Ma. Evidently the mantle cannot be neglected on Earth's longest time scales. In particular, ingassing is needed to close the mantle cycle. Possibilities include subduction of carbonates directly deposited on the ocean floor (pelagic carbonates); subduction of continental carbonates that are scraped off and dragged down; and subduction of carbonates formed by seawater alteration of the oceanic basalt itself. The latter in particular depends on the amount of free CO_2 in the ocean, and hence can act as a buffer on atmospheric CO_2 levels. The atmosphere and ocean are linked by the effective solubility of CO_2. There are currently 6.2×10^{16} moles of CO_2 in the atmosphere and 3.3×10^{18} moles in the oceans; the ratio is 54:1. These reservoirs are tightly coupled on geological time scales, so that the air is refreshed in a thousand years.

Sleep and Zahnle (2000) have modeled the carbon dioxide cycle, focussing on the mantle reservoir and the exchange between surface reservoirs and the mantle, using the language and concepts of plate tectonics to extrapolate the CO_2 cycle into the past. Their first step is to construct a specific albeit much-simplified model of the modern CO_2 cycle as the Earth's CO_2 cycle is complicated and not fully understood. For purposes of presentation, they organize their reservoirs and fluxes following Tajika and Matsui (1992). They use arc fluxes modified from Plank and Langmuir (1998) and Sano and Williams (1996) and ridge fluxes from Zhang and Zindler (1993). They then accelerate the cycle into the Archean, taking into account the greater influence of the mantle and the diminished influence of the continents. Finally, they consider the effects of abundant impact ejecta on Hadean cycles.

The flux equations are then solved numerically. The procedure for modeling other real or hypothetical planets is similar. To illustrate the parameterization procedure, we discuss fluxes at midoceanic ridges.

The flux of CO_2 from the mantle to ridges is an example where the flux depends only on interior processes. At the relatively shallow depth where midoceanic ridge basalt forms, CO_2 is nearly quantitatively extracted from the mantle into the magma. Most of the CO_2 down to about 56 km (Langmuir et al., 1992) is extracted. The basalt ascends to crust depths where most of the CO_2 escapes into the ocean. The CO_2 concentration in modern magmas is estimated from He/CO_2 ratios and the known flux of He into the ocean. The mantle concentration then obtained has the average fraction of melting known independently of CO_2. The process in the past differed from that of the present in that the mantle was hotter causing the extraction depth to be greater and the mantle reservoir was probably larger, as less CO_2 was within crustal carbonates.

In contrast, the CO_2 from the carbonatization of midoceanic ridge lavas by hydrothermal circulation depends on shallow processes and the concentration of CO_2 in the ocean. That is, the amount of CO_2 taken up in the rock depends on the oceanic concentration (to a power less or equal to 1). The amount of water circulated through a given surface area of oceanic crust is expected to change little over time. The magnitude of the modern flux is calibrated by monitoring the composition a

vented water which reacted with the rock and the composition of reacted rock samples.

This model is a traditional treatment of climate buffering via the update of CO_2 by weathering of silicates on land if the mantle cycles are ignored. However, mantle fluxes are important over long geological times. The dependence of CO_2 flux from carbonatization of oceanic basalt on the amount of it in the ocean and hence the amount of it in the air acts as a buffer. Cold climates are expected particularly early in the Earth s history unless another greenhouse gas was important.

Modeling of any planet with an ocean is analogous to the Earth, although planets with no large standing bodies of water can also be modeled. The ground water in the shallow subsurface can be treated either as part of the mantle box or as a separate shallow box closely coupled to a deeper mantle box.

Modeling the cycling of other volatile species

A mechanistic model of the cycling of carbon will be similar to the approaches taken by Lasaga et al. (1985) and Sleep and Zahnle (2000). The abiotic version of the water cycle (see below) also will be implemented. The analysis for nitrogen is similar to that for H2O: both have a long residence time in surface reservoirs.

Time-dependent planetary evolution scenarios

The coupled planetary system intrinsically varies with time due to as number of factors. The brightness of the central star varies as it ages. The planet interior cools with time. The character of the impact flux changes with time and, in particular, large, planet-altering impacts may occur only once in a planet s history.

Consequently the Abiotic Planetary Model will produce time-varying planetary evolution sequences, forced by the natural variation of stellar and planetary processes. For example, in the carbon cycle which will be based on the Sleep and Zahnle (2000) model, there are time-dependent specifications for mantle degassing, ocean basalt hydrothermal circulation, subduction rate, subduction efficiency, continental metamorphism, and solar luminosity.

Since the model will be a time-dependent simulation, we will be able to investigate the consequences of singular events in a planet s history such as a major impact.

Model validation

The ability of the Abiotic Planetary Model to simulate what is known about the physical and chemical state of the atmosphere of the early Earth and also the current states of the atmospheres of Venus and Mars serves as the key validation test of the APM.

Venus and Earth seem to have comparable amounts of carbon dioxide and nitrogen, taking into account the carbonate reservoir on the Earth. It is not unreasonable to consider that both planets formed with comparable amounts of water. At the elevated temperatures corresponding to its closer position to the Sun, the early Venus upper atmosphere could easily be more humid than the case for the Earth. With more water in the upper atmosphere, the loss of hydrogen to space is enhanced sufficiently to lose the planetary inventory of water. In the absence of water, the processes that transform atmospheric carbon dioxide to carbonate would be much suppressed on Venus relative to the Earth situation, sustaining the high atmospheric abundance of carbon dioxide.

Mars also may have started out with amounts of carbon dioxide and water comparable to the Earth s. Being smaller than the Earth, Mars s gravity cannot hold atmospheric gases from escaping thermally or nonthermally as effectively as the Earth can. Also a result of the smaller planetary radius, the interior of Mars has cooled sufficiently to shutdown tectonic activity. If tectonic activity subsided while Mars still had a warm, humid atmosphere, the atmospheric burden of carbon dioxide would be converted to carbonate, but recycling back to the atmosphere would be suppressed. In time, the Martian atmosphere would drop to the level observed today.

These scenarios that explain the current differences between the atmospheres of Venus and Mars and that of the Earth, considering that these three terrestrial planets may have had initial atmospheres very similar to that for the early Earth, provide a good opportunity to test the Abiotic Planetary Model. The validation tests are designed to confirm that the processes in this model controlling the partitioning of volatiles amongst the atmosphere, land, ocean, and interior domains are a reasonably complete set and that the parameter-

izations have some appropriate level of realism. One experiment would be to move an early Earth to the distance of Venus and see if the computed atmosphere relaxes to a physical and chemical state similar to what is observed today for Venus. Does the model produce a runaway greenhouse or a moist greenhouse, which in either case increases the atmospheric water content, enhances loss of hydrogen to space, and diminishes the surface water reservoir?

A second experiment would be to move an early Earth to the distance of Mars from the Sun and scale down the planetary size to that of Mars. The test then would be to see that the APM so initialized would relax to the atmospheric state currently observed on Mars. To what extent is impact erosion necessary to explain today s thin Martian atmosphere?

Successfully demonstrating that the APM provides a reasonable simulation of the planetary-scale cycle of volatile species for the three terrestrial planets in the Solar System sets the basis for use of the APM to model terrestrial planets in other solar systems.

C.3.4.2 Research Tasks

Once validated, the APM will be used to explore the range of plausible atmospheres for a wide variety of extrasolar planets to address the issue of false positives . Specific experiments and questions we will address using this model would include:

- Determining the range of molecular oxygen abundance in an extrasolar planet atmosphere that can be produced by abiotic sources. A planet with a CO_2 atmosphere and little water can have a large fraction of O_2 (Nier et al., 1976), especially if a planet is larger than Mars and oxygen escape is suppressed. A planet such as an early Venus with a lot of water vapor in the atmosphere and, in the presence of intense stellar ultraviolet radiation, may have an especially O_2-rich atmosphere following the escape of hydrogen (Kasting, 1997). How long would the O_2-rich atmosphere last?
- Can any abiotic planet have detectable levels of both CH_4 and O_2 (or the O_3 proxy)?

For atmospheric compositions and surface properties derived from these model runs, we will also output high resolution synthetic spectra and assess these spectra for detectability of spectral features using the instrument simulators and statistical methods developed in Tasks 1-3.

Additional Research Tasks

In addition to the principal tasks described above, there are a number of other investigations that will be supported by the APM. As time permits, we will run as many of these experiments as possible.

- Model planets for a range of central star stellar types, considering a range of planetary size, composition, rotation rate and obliquity , impact history, H_2O inventory (follow through on consequences of H_2O and CO_2 clouds and UV-shielding photochemical hazes).
- Can a planet warmup from a cold start? I.e., the planet starts out frozen (surface frozen with CO_2 clouds) at low stellar flux, but because of its high albedo the planet might not warm up when stellar flux increases (Kasting 1991, Caldeira and Kasting 1992). However, clouds also might produce warming (Forget and Pierrehumbert, 1997). What about warming from a massive impact or extensive volcanism?
- The sensitivity of planetary evolution to the initial volatile inventory. If the initial water content of a planet is half that of the Earth, there might be no surface water. If double that of the Earth, there might not be any continents. In the later case, the carbonate cycle might still be possible with aquatic chemistry, but much slower.
- To what extent can a single impact significantly change the volatile inventory of a planet? Is a planet with enough water to be habitable just a coincidence?
- With reduced solar flux levels, CH_4 will have a longer lifetime relative to photolytic decomposition. At low stellar luminosities, can a tectonically active planet with a reduced mantle produce enough methane to maintain a clement environment?
- How big would a planet have to be to retain its H_2 inventory and remain in a reduced oxidation state for its whole lifetime?
- The sensitivity of climate to the N_2 abundance. More N_2 on the Earth would moderate daily temperature fluctuation. Less of it would cause nightly frost. It appears that N_2 behaves like H_2O in the sense that only so much can go into the mantle. Like hydrogen, nitrogen is more abundant in the accreting material than it is in the final silicate plus surface reservoirs of the Earth.

• What is the lower size limit to a habitable planet? Is this limit restricted to a sufficient size to maintain the carbonate-silicate cycle that is thought to be the key to clement climate stability (Walker et al. 1981, Kasting 1988)?
• Role of impacts in allowing any particular planet to be habitable. How can a major impact lead to a deviation from the planetary evolution sequence?
• At what planetary distances from a central star, do moist and runaway greenhouses suppress a habitable planetary state for a given solar flux? Previous work (Kasting et al. 1993) parameterizes clouds with high surface albedo, rather than the more realistic treatment in the APM. Real clouds may allow surface water at higher solar flux (Chyba et al., 2000).
• Outer limit of Habitable Zone: how far out can a planet the size of the Earth remain habitable? a planet the size of Mars? To properly address this question, one needs a realistic treatment of CO_2 clouds (Chyba et al., 2000).
• For faint stars, would planets have to get so close to be habitable that they end up tidally locked? Then without a day/night cycle and if there is only a thin atmosphere, a planet might be frozen on one-side (Joshi et al. 1997).
• To what extent is the range of the Habitable Zone around a specific stellar type changed if one considers an expanded definition that includes the presence of liquid water at some depth below the surface (Chyba et al., 2000)? Sub-surface life could survive intense stellar radiation. On Earth there is a subsurface biosphere feeding off geothermal energy, not surface photosynthesis.

C.3.4.3 Products and Significance

The Abiotic Planetary Model is a tool for investigating the variety of planetary climates and atmospheric composition that can arise from a combination of stellar type and planet context and provides a valuable tool for furthering our understanding of the Habitable Zone.

This task will produce a comprehensive suite of physically, radiatively and chemically self-consistent atmospheres for a range of uninhabited planets. We will analyze the synthetic spectra produced to investigate the relationship between spectral features and the climate/chemistry of a planet, and determine what features unique to a particular planetary state are detectable.

We will also use modeling results for a range of abiotic planets around stars other than our own Sun to determine the plausible range of atmospheric compositions for abiotic planets. We will then determine the likelihood of false positives , abiotic planets that may produce spectral signatures that could be mistaken for life. We will also determine what combination of auxiliary parameters, if any, should be looked for to remove the potential ambiguity in these cases.

The APM will be a multipurpose tool for use by the science team and a forum for collaborations between the science team and NAI member scientists and members of the broader astrobiology community. The APM will be programmed in a modular fashion to allow easy exploration of alternative formulations of processes integrated in the APM simulation of a planet.

C.3.5 Task Five—The Influence of Biology on the Composition and Spectra Signature of an Extrasolar Planet

The objectives of this task are to determine the unique attributes of a habitable terrestrial-type planet that result from the presence of life and the detectability of these attributes by remote-sensing spectroscopic observations.

To address these objectives we will add processes involving life to the Abiotic Planetary Model developed in Task 4 to create an Inhabited Planetary Model (IPM). For each simulation of a planet with life, synthetic spectra will be generated to determine whether the influences of biology on the observable environment are detectable. As with the APM, the IPM has a flexible design that will allow it to support many collaborations with a wide range of investigators, both scientists associated with the NAI and others in the general astrobiology community.

C.3.5.1 Development of the Inhabited Planet Model

Life can affect the observable planet at - mosphere and surface by mediating the cycling of volatiles between the atmosphere and land, water, and interior. One example of this is the role life can play in the carbonate-silicate cycle discussed in detail in Task 4. Plants can enhance the partial pressure of CO_2 in surface soils, accelerating the formation of bicarbonate, and can enhance the rate of car-

bonate precipitation in the oceans through the secretion of plankton shells, for example. We will augment the Abiotic Planet Model developed in Task 4 with a suite of biospheric processes to develop the Inhabited Planet Model (Figure C.3.5.1). Figure C.3.8 (pg. 34) now illustrates the range of processes that will be addressed in the Inhabited Planet Model.

The following sections describe in detail the life processes that will be parameterized and added to the APM to produce the IPM. These are broken into two principal subclasses: geophysical-biological cycles, and the direct effect of life on an atmosphere.

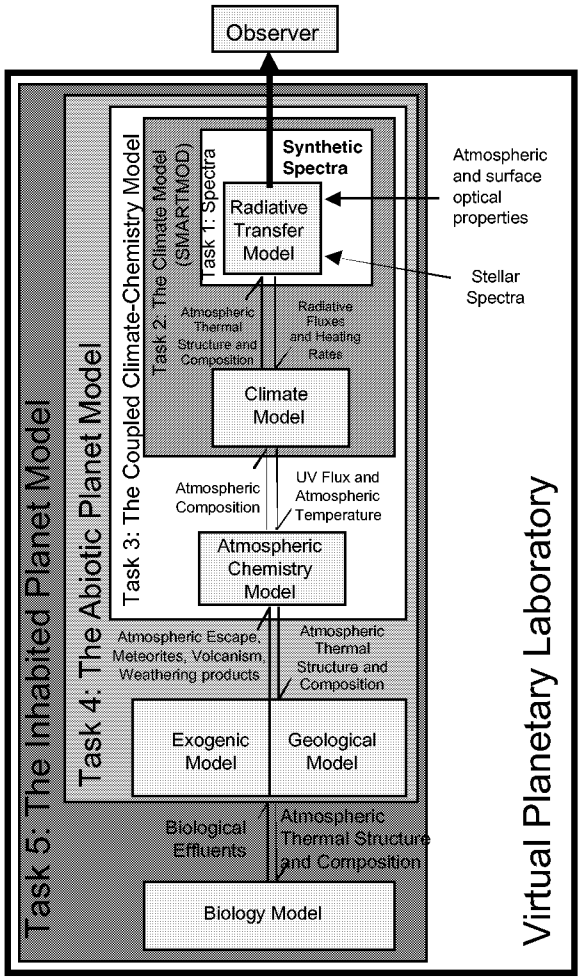


Figure C.3.5.1. Components of the Virtual Planetary Laboratory included in Task 5 are shown. The Abiotic Planet Model is augmented with biological processes to produce The Inhabited Planet Model.

Geophysical-biological cycles of volatiles

The mechanistic descriptions of cycling of volatile species between the atmosphere and land/ocean/interior will be expanded to include processes introduced by the presence of life.

The global water cycle is an example where the mantle reservoir can hold only so much of an element and is an example of coupling with the surface oxygen cycle. In this case, the oxygen cycle is fundamentally biological, while life does not grossly effect the water cycle. Even within the habitable regions of the Earth s crust (including soils), biology serves mainly to speed up chemical reactions which would occur anyway. Consequently, some of the processes discussed here will be treated in the mechanistic model developed for the APM (Task 4).

In some cases biological processes can dynamically overwhelm the buffering of the ocean and atmospheric composition. In regard to the Earth the issue of how atmospheric O_2 is maintained when it is grossly out of equilibrium with the FeO within igneous rocks is of interest because there is no sufficient abiological source.

The gross features of our oxygen atmosphere are well known. The O_2 which we breath has built up over geological time from photosynthesis, which is simply expressed as the reaction: $CO_2 \rightarrow C + O_2$. The burial of organic carbon within sedimentary rocks has inhibited the back reaction to CO_2 allowing O_2 to persist in the air. A global microbial ecology, where the back reaction went to near completion, worked fine on the early Earth.

Using the Earth as the best available analogue gives the chain of linked processes that lead to reduced carbon burial. Around 70-80% of the reduced carbon in terrestrial sediments is in shales (Holser et al., 1988). The reasons for this are fairly evident. Shales form from deposits of clay-rich mud. They are relatively impermeable, preventing extensive contact with circulating oxygen-rich surface waters. Shale beds retard the circulation of water into interbedded sandstones and limestones, protecting the remainder of the reduced carbon in sediments.

The ultimate source of shales is Al-rich igneous rocks. Significant Al_2O_3 is present in most common igneous rocks and clays form from their weathering. The complicating factor is that FeO consumes oxygen by the reaction, $4FeO + O_2 \rightarrow 2Fe_2O_3$. The mantle derived mafic rocks, basalt and gabbro, have

about 10% FeO and 15% Al_2O_3, while continentally derived granites have only 2% iron as FeO (some of which is already oxidized) and 15% Al_2O_3 (Krauskopf and Bird, 1995). The basaltic sink is potentially significant given that seafloor spreading renews the oceanic crust every 100 m.y. For example, there is enough FeO in the uppermost kilometer of the oceanic crust to consume 3×10^{20} moles of O_2. This compares with the 0.4×10^{20} moles of O_2 in the air (Garrels and Perry, 1974). Other surface reservoirs of free oxygen are more massive. Fe_2O_3 in sedimentary rocks comprises the equivalent of 1×10^{20} moles of O_2 and sulfate in the ocean, and sediments comprise the equivalent of 4.8×10^{20} moles (Garrels and Perry, 1974). Oxidation of the uppermost kilometer of oceanic crust would remove the O_2 and sulfate reservoirs in under 200 m.y. A significant amount of ridge basalt has in fact been altered over geological time to form Fe_2O_3, which was eventually subducted. This process has left more reduced carbon in sediments, equivalent to 10.4×10^{20} moles of O_2, than the sum of the surfacial free oxygen reservoirs (Garrels and Perry, 1997). Conversely, the small relative size of the atmospheric O_2 reservoir indicates that subtle variations in the basalt/granite ratio of sedimentary sources could have profound effects.

For the present situation to occur, granite, which makes clays, must dominate over basalt, which removes O_2, in the global (igneous) sedimentary source. That is, continents, where granite is a significant rock type, were exposed to weathering and erosion to a much greater extent than mid-oceanic ridges, which are mostly basalt. There seems to be just the right amount of water in the Earth s oceans. Much more would cover all land precluding erosion and much less would leave the ridge axes exposed. Increasing or decreasing the global surface volume of water by a factor of 2 would greatly change the Earth s surface and a factor of 4 would render it unrecognizable.

The amount of water for each case is obtained because the elevation distribution is known on the Earth (Sandwell and Smith, 1997). The current ocean is equivalent to a 2.5-km thick global layer and ~1/10 of this amount is buried in sediments. Beginning with the amount of water needed to cover dry land, the highest large uplifted region on the Earth is the Tibet plateau with an elevation of 5 km; the Altiplano has 3-km elevation. Adjusting for the load of the water, an additional 7-km thickness of water would cover Tibet. Only 3.8% of the Earth s surface is above 2-km elevation. An equivalent water layer of 1.1-km thickness lies beneath the current ridge axis. Further reducing the global amount of water to an equivalent area of a few hundred meters would produce conditions like early Mars where the bulk of the water at any one time was buried in sediments.

Surface topography is the result of the combined effects of tectonics and erosion by water. The topography in turn has a significant influence on climate, for example, the amount of rainfall, and ultimately impacts the composition of water runoff from the land mass to the ocean.

The surface plus mantle reservoir of water was determined by processes during accretion of the planet. The mantle can accumulate only so much water and the rest ends up in the ocean. Water degasses at midoceanic ridge axes at a rate proportional to the amount in the mantle. Hydration and eventual subduction of oceanic crust returns water to the mantle. A fraction of the water is deeply subducted and the remainder is returned to the surface at island arc volcanoes. Higher mantle temperatures enhance degassing, while lower temperatures favor deep subduction of water.

The steady state amount of water in the mantle is independent of the global spreading rate and should increase in time as the Earth s interior cools. The time to reach steady state depends on the time to circulate the volume of the mantle through the melting regions of midoceanic ridge axes, which now extend to a depth of ~56 km (Langmuir et al., 1992). This time is now quite long as can be seen from the behavior of argon.

Viewed in a more sophisticated way, the water in the Earth s mantle is self-fluxing. Water when present in small amounts acts as a trace element and does not affect the total amount of melting or the fluidity of the solid mantle. When present in greater amounts, water increases the amount of melting at given conditions and makes the mantle more fluid. Island arc magma forms when water from the subducted slabs lowers the melting temperature in the overlying mantle wedge (Stolper

and Newman, 1994). The preferred amount of mantle water tends to that for transition from trace to major element behavior.

The accretion of water to the earliest Earth is not well understood, but a reasonable mixture of accreting material brings in 20 to 40 oceans. Of that, present mantle water and ferric iron produced from ferrous iron account for around an ocean. Oxidation of the iron metal associated with Pt-group elements in the mantle to ferrous iron consumed another ocean. The fate of the remaining water involves loss of H_2 into the core and loss of H_2 to space (Fukai and Suzuki, 1986). There is enough ferrous iron in the mantle to consume a few oceans.

Other Earth-sized planets need not have the right amount of water to have the familiar oceans and continents. The balance results from internal processes that limit the amount of water in the mantle and violent random processes during accretion that set the total mantle plus surface inventory. The tuning of erosion and tectonics produces the familiar ebb and flow of the sea over continental platforms, which has greatly influenced the evolution of life. A less obvious but more profound effect on complex life is that the right amount of water exists to cover the reducing rocks of the ridge axis but not to cover continental granites, which are the sources of clay in shale. Without a burial ground in shale, organic carbon would have reacted with O_2 and O_2 would not have accumulated in the atmosphere.

Coupled parameterized models for water, oxygen, CO_2 , and other volatile species will be useful for seeing how hypotheses relate to the early Earth and to other planets. The influence of planet size on tectonics can be included by explicitly modeling thermal history and having the rate of tectonics depend on the interior temperature of the planet. The effect of pressure (and hence planetary gravity) on melting is included easily. Again planets with limited planet-wide surface water are simplifications of Earth models.

The Imprint Of Life On The Observable Environment

A non-Earth-centric approach to identifying biosignatures is to consider a fundamental feature that is common to all life. For the purposes of this proposed investigation, the relevant feature of life is that it consumes energy and produces disequilibrium products. In particular, life will consume and add volatile species to and from the planetary environment. The manifest evidence of either process may be detectable in the atmosphere of a planet and serve as a signature of an inhabited planet. Therefore, a second approach we will adopt to model the environmental implications of the presence of life, will be to simulate consumption and emission of gases by various biological mechanisms at appropriate stages in the computed planetary evolution sequence. We will then simulate the fate of biologically emitted gases in the atmospheric-chemical system, including both homogenous gas-phase and heterogeneous processes.

Very few organisms exist that do not consume and/or produce copious quantities of gases, many of which would not be found, even in small quantities, without the catalytic activities of life. Alternatively, the biosphere may result in steady state levels of gases which individually might exist in abiotic atmospheres, but should otherwise not be found together at the observed levels.

Should our search of extrasolar planetary atmospheres encounter evidence of life, that evidence will most likely be the gaseous products of microorganisms. Our biosphere was exclusively microbial for over 80 percent of its history (Schopf and Klein, 1992) and, even today, microbes strongly influence atmospheric composition. Life's greatest environmental impact arises from its capacity for harvesting energy and creating organic matter. Microorganisms catalyze reactions between C, S and transition metal species at temperatures where such reactions can be very slow in the absence of life (e.g., S redox reactions, Des Marais 1996). Sunlight harvested by photosynthesis has created enormous energy repositories in the form of coexisting reservoirs of reduced, organic C and S stored in Earth's crust, and highly oxidized species (oxygen, sulfate and ferric iron) stored in the crust, oceans and atmosphere (Garrels and Perry, 1974).

Ecological processes determine life's distribution and survival and therefore determine our ability to detect life (Des˚Marais et al., 1998). The evolution and survival of the early biosphere depended upon the efficient coordination of resources and processes among

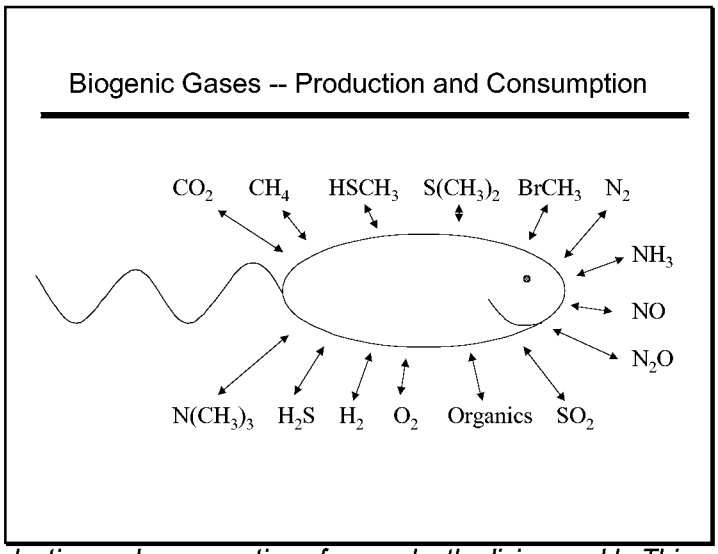


Figure C.3.5.2. Production and consumption of gases by the living world. This cartoon represents some of the known reactions that occur by living organisms with regard to gas production and consumption. All the gases shown are both consumed and produced by some living organisms on Earth, and many are key to the existence of certain groups of organisms. (see Nealson, 1997; Nealson and Conrad, 1999).

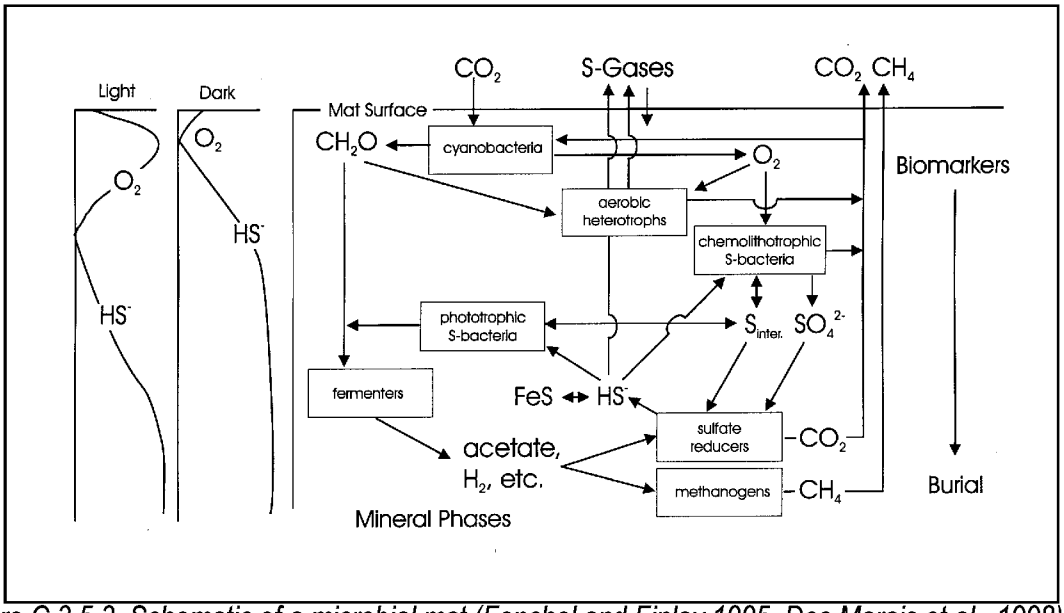


Figure C.3.5.3 Schematic of a microbial mat (Fenchel and Finlay 1995, Des Marais et al., 1998). Boxes denote functional groups of microorganisms, and arrows denote flows of chemical species into or out of microorganisms. S_{inter} indicates S in intermediate oxidation states.

diverse microbial populations. Microbial mats offer an example of such coordination (Fig. C.3.5.3), and they are particularly important because they are the oldest-known ecosystems, as evidenced by their 3.4+ billion-year fossil record. Photosynthetic microbial mats are key because, today, sunlight powers more than 99 percent of global primary productivity (Des Marais 1997; 2000a). For most of Earth's history, photosynthetic ecosystems have affected the atmosphere profoundly and have created the most pervasive fossils. Therefore, extraterrestrial surface-dwelling biospheres that derive energy principally from starlight will be most amenable to astronomical detection (Des Marais, 1999).

While photosynthetic bacteria dominate microbial mats numerically and in terms of productivity, many aspects of the system's emergent behavior may ultimately depend on

the associated nonphotosynthetic, including anaerobic, microbial populations (Figure C.3.5.3). The anaerobic zone of the mat represents the ultimate biological filter on chemical, isotopic, and geologic biomarkers passing into the fossil record. Transformation of photosynthetic productivity by anaerobic bacteria may contribute diagnostic "biosignature" gases that could represent search targets for remote spectroscopic life detection efforts. To understand the overall structure and function of mat communities, it is thus critical to determine the nature and extent of interaction between phototrophic and anaerobic microorganisms.

The broadest approach to defining life's role in planetary atmospheres recognizes that planets, environments and biospheres evolve and change (Des Marais, 1999). For example, a tectonically more active early Earth hosted a biosphere that accommodated elevated temperatures (at least locally), was potentially non-photosynthetic, and coexisted with a mildly reducing, CO_2-rich and O_2-poor atmosphere (Veizer, 1994). Microorganisms acquired energy by consuming H_2 and sulfide and producing a broad array of reduced C and S gases, most notably CH_4. Diverse types of bacterial photosynthesis developed that indeed enhanced global productivity but could not yet split the water molecule to produce O_2 (Xiaong et al., 2000). Later, but still prior to 2.7 billion years ago, oxygenic photosynthesis developed (Schopf and Packer, 1987; Des Marais, 2000a). In a planet s evolutionary track, it is possible that the nature of biogenic gas emission will be indistinguishable from the background atmosphere.

We can expect to encounter distant biospheres that represent various stages of evolution and that coexist with atmospheric compositions ranging from reducing to oxidizing. Accordingly, we must be prepared to interpret a broad variety of atmospheres, all potentially containing signatures of life. Apart from what the discussion in Task 3 regarding evidence for the composition of the paleoearth atmosphere in the geological record, remarkably little is known about the composition of our own earlier atmosphere, particularly prior to the rise of O_2 levels some 2.0 to 2.2 billion years ago (Kasting, 1993). Thus, field and laboratory observations are being conducted to examine the relationships between the structure and function of microbial ecosystems and their gaseous products. Ecosystems that are analogs of our ancient biosphere (e.g., based upon chemosynthesis and various types of photosynthesis, thermophilic and subsurface communities, etc.) are, or will soon be, studied. Because key environmental parameters such as temperature, seawater composition, and levels of H_2, CO_2 and O_2 varied during planetary evolution, their consequences for microbial ecosystems are being explored (Des Marais, 2000a, 2000b). Studies of a number of sites that mimic key attributes of Earth's early environment focus upon evolutionary adaptations to long-term changes in the global conditions (Des Marais et al., 1998). These include marine platform settings (carbonate-depositing; hypersaline) similar to those that nurtured ancient stromatolites, and also thermal springs in Yellowstone National Park that resemble analogous ancient thermal habitats (Ames Group). To the extent that rocky planets experience parallel evolutionary trajectories (Des Marais, 1999), this work is also relevant to studies of extrasolar habitable environments.

We propose to integrate a fully parameterized biology component into the IPM. Biological processes directly affect the atmosphere via fluxes of biogenic and bioavailable gases. In turn, local and global fluxes of biogenic gases are affected by changes in the physico-chemical characteristics of the local and global environment. In particular, gas fluxes change in response to changes in length of day, solar insolation, temperature, wind stress, atmospheric composition, rainfall and nutrient availability. At present, data on how particular environmental conditions affect biogenic gas fluxes are relatively sparse, though ongoing research is rapidly changing this situation. We will parameterize the response of the biosphere to changes in these variables at various length- and time-scales using whatever data are available from studies such as those described above. As data are gathered regarding fluxes in and out of microbial mats, the mixed layer of the ocean, soils and other environments, these parameterizations will be improved accordingly. On a global scale these parameterizations will depend in part on assumptions about the fraction

of the biosphere in each of the various ecological niches mentioned, which will in turn depend on the configuration of continents and on climate. By scaling up to global fluxes from local experiments and analyses given an assumed continental configuration and climate state, we can investigate how biogenic gas fluxes affect the composition of the atmosphere. In turn, the consequent changes in composition will affect all the relevant parameters and so forth from time step to time step in the model. With a better and better parameterized model we will become increasingly good at estimating the true global impact of life on the atmosphere of Earth, throughout its history, and on the atmospheres of any postulated extrasolar planet.

Time-Dependent Planetary Evolution Scenarios

Like the Abiotic Planet Model, the Inhabited Planet Model will produce time-varying planetary evolution sequences. The processes related to life will be treated as a function of the time-varying planetary context.

C.3.5.2. Research Tasks

The Inhabited Planet Model is a tool for investigating the influence of life on an environment, and the detectability of that influence using astronomical techniques. Model runs will focus on stellar/planetary combinations that were determined in Task 4 to provide a habitable environment. For each IPM output, a globally-averaged synthetic spectrum will be produced and comparison between these spectra and corresponding abiotic planet spectra will be used to investigate the relationship between life and unique, detectable spectral features. This study will allow us to identify new potential biosignatures, initially for different types of carbon-based life, but with the potential to expand to other forms of life as parameterizations for these forms become available.

We will also use the IPM to address the extent to which the presence of life sustains a habitable environment, and results in a widening of the Continuously Habitable Zone beyond previous estimates. We will also use the IPM to address and explore potential mechanisms for the rise of oxygen on our own planet.

In the following sections, we briefly expand upon the latter two research tasks.

Life And Climate Stability

Climate modelers have long relied on the Walker feedback mechanism to explain the long-term stability of Earth s climate and its operation remains the most plausible explanation for why the Earth s climate has remained relatively stable over the last 2 billion years. However, the empirical constraints on CO_2 levels suggest that there was not enough CO_2 in the atmosphere prior to ca. 2.2 Ga to support this sort of mechanism and that another greenhouse gas, e.g. CH_4 may have played a critical role in warming the Earth (Rye et al., 1995). There is no well-established or widely accepted mechanism to explain how Earth s climate would have been stable for hundreds of millions of years under a CH_4-CO_2-H_2O greenhouse. Nor do we understand why this system failed and shortly thereafter O_2 rose dramatically, essentially forcing the system to rely on CO_2. The model we will develop will allow us to test a series of possible explanations for both of these geological events.

A detailed understanding of the ways that climate and atmospheric chemistry affect life *and* vice versa are essential to any explanation of climate stability in which a biogenic gas plays a key role. As an example of the integrated nature of the system we are attempting to model and the possible complexity of the cycles that may drive it, we describe the following experiment, which we will test with the IPM.

For an Earth atmosphere with 20 atm CH_4 , < 2000 atm CO_2 as well as H_2O (with CH_4 largely biogenic in origin) we ask whether the following feedback loop could maintain a stable climate for hundreds of millions of years. Let CH_4 accumulate until CH_4/CO_2 equals 1. A UV absorbing haze will form. Biogenic NH_3 will begin to accumulate in the atmosphere. At high enough NH_3 levels, rain pH will increase above 7. Weathering essentially will cease. Phosphate delivery to the ocean will shut down. Over a time scale of a few thousand to tens of thousands of years, primary productivity will drop dramatically. Biogenic methane production will eventually follow. As CH_4 falls and CO_2 accumulates CH_4/CO_2 goes to < 1. The UV screen and the NH_3 will disappear. Rain pH will fall and weathering will restart. CO_2 levels will also begin to fall. Primary productivity recovers. CH_4 begins to accumulate

again. And so forth. Many questions arise immediately. Could long-term outgassing provide enough H_2 to reduce enough CO_2? How long would it take before loss of H_2 to space shut down the system? Would a UV screen really form? How would the NH_3 abundance really respond to changes in UV flux, when one included the effects of changes in nutrient availability? How would CH_4 production be coupled to global primary productivity in such a world? etc. Such a scenario can only be fully tested by a fully coupled model, though of course, if any point in the loop simply cannot work the whole loop would have to be modified or discarded. The point is that, when biogenic gases play a key role in postulated climates, we need not only to account for the radiative effects of the gases but also keep track of the impact of a variety of changes, including rain chemistry, weathering rates and consequent nutrient availability, cloud and/or aerosol microphysics and so forth. This scenario is illustrative of the sorts of interactions that could be of importance in understanding Earth s present and past as well as the atmospheric chemistry of any postulated inhabited extrasolar planet. Understanding these interactions and modeling their consequences will allow us to address what Earth s atmospheric spectrum may have looked like from space throughout Earth history and what other planets with non-Earth like histories might have looked like as well.

The Rise Of Oxygen

An understanding of the rise of oxygen in Earth s early history provides a basis for simulating the rise of oxygen in other inhabited worlds, and understanding what these planets would look like spectroscopically during various stages in their evolutionary history. Two of the current ideas on why the Earth seems to have had little free O_2 before about 2.3 Ga are best supported by available evidence.

(1) O_2 is controlled by the disproportionation of a more or less fixed CO_2 inventory, and the extent of disproportionation increases over time (i.e. the reservoir of buried reduced carbon increases with time). Carbon isotope data provide support for the hypothesis that a massive pulse of reduced carbon burial accompanied the first rise of O_2 (Karhu and Holland, 1996). Increasing disproportionation of CO_2 could be driven by geological or biological changes. A geological change might consist of continents growing larger and less subject to metamorphism and weathering (processes that liberate buried carbon). This is probably the leading hypothesis, and one that one can hope to model. A related geological argument is based on the observation that carbon is buried in clays. Clays derived from basalt contain a great deal of ferrous iron, which can easily consume the oxygen derived by burying reduced carbon. Clays derived from granite contain little ferrous iron, so when reduced carbon is buried, free O_2 is left behind. A progressive change from basaltic to granitic weathering is documented in the strontium isotope record, with the transition occurring towards the end of the Archean (Godderis and Veizer, 2000). Both geologically-based hypotheses are tied to the origin of continents and therefore are potentially generalizable to other terrestrial planets. A biological (or Gaian) argument would be the progressive development of better pumps to dynamically separate the C from the O_2. In effect Gaia gets ever better at pushing the C down or pushing the O_2 up. Again, if the driving force is biological, it provides a challenging situation to model.

(2) Hydrogen escape from an early reduced atmosphere changes the redox state of the Earth near the surface (Catling et al 2000). Circumstantial evidence in favor of biogenically-supported methane-rich ancient atmospheres on Earth provides intriguing context. An effective methane greenhouse would make secular oxidation inevitable. In this hypothesis all accessible reduced reservoirs are titrated, and eventually become poorer at removing photosynthetically produced O_2 than the oxidized reservoirs are at removing CH_4. (One key photochemical issue is the degree to which CH_4 and O_2 can coexist.) This differs from pure disproportionation of CO_2 because it posits that the oxidation state of the crust determines the lifetime of the oxidant generated by disproportionation. This process can be heuristically generalized to other terrestrial planets by calibrating the titratable reservoirs to Earth.

C.3.5.3 Products and Significance

This task will produce a comprehensive suite of physically, radiatively, chemically and biologically self-consistent atmospheres for a range of possible extrasolar terrestrial planets both with and without different forms of dominant life.

The synthetic spectra generated from these atmospheres will be used to define new potential biosignatures using astronomical instrumentation, and will be offered to the community as a reference source for the design of instruments for planet detection and characterization.

We will also provide specific recommendations and guidelines derived from these spectra for the best spectral range, spectral resolution and signal to noise ration required to detect these biosignatures, and test for their detectability using a variety of likely instrument and telescope designs.

The IPM, and the entire VPL, will be a tool for use by the science team and a forum for collaborations between the science team and NAI member scientists and members of the broader astrobiology community. The IPM will be programmed in a modular fashion to allow easy exploration of alternative formulations of processes integrated in the IPM simulation of a planet.

Relevance to NASA Interests

The proposed work will expand the scope of existing research being undertaken in the NASA Astrobiology program. It will provide a comprehensive, systematic study of capabilities for the remote-sensing detection of extrasolar life, to augment existing NAI focus on detection of life within our own Solar System.

In so doing, this research program will provide practical, timely, and much-needed recommendations and guidelines for development of future NASA missions that seek to detect and characterize extrasolar terrestrial planets, such as TPF and its second generation follow-on, Life Finder. This work will not only drive the design and survey strategies of these missions, it will also provide an initial solid theoretical basis for the interpretation of the results of these missions. The following table maps the VPL Products and Tasks into the NAI Goals and objectives.

The proposed work, including the modeling tools and spectroscopic results, will also be valuable for research in many other NASA research programs including Planetary Astronomy, Planetary Atmospheres, Sun-Earth Connection, and the Origins program. These tools and results may also be of use to the design of future Discovery proposals that seek to discover or characterize extrasolar planets

NAI ROADMAP GOALS AND OBJECTIVES	VPL PRODUCT	TASK
Does Life Exist Elsewhere in the Universe? Goal 7 Determine How to Recognize the Signature of Life on Other Worlds. To understand remotely sensed information from planets circling other stars, we should develop a catalog of possible signatures of life.	Development of Spectral Catalog of Habitable, Uninhabited and Inhabited Planets	Tasks 1-5
Does Life Exist Elsewhere in the Universe? Goal 7 Essential to learn to identify the chemical signatures of life on a distant world through remote sensing of its atmosphere or surface. For the previous several billion years during which Earth had life, the atmospheric and surface signatures are not fully understood. In exploring other worlds, it is critical that we generalize the process of coevolution of planet and life.	Better Understanding of the Spectral Appearance of Earth's Paleoatmospheres	Tasks 2-5
Extrasolar Biomarkers Objective 13 Define an array of astronomically detectable spectroscopic features that indicate habitable conditions and/or the presence of life on an extrasolar planet. We must develop the database for interpreting those spectra, both for evidence of habitable conditions (e.g., the presence of liquid water) and for evidence of life. Aspects of the strategy include developing appropriate observational approaches that optimize sensitivity and spectral and spatial resolution, creating models of atmospheric chemistry and its evolution, and achieving an understanding of the factors that control the composition of biological gas emissions to the atmosphere. We must develop the ability to discriminate between those environmental conditions and gas compositions that indicate a geologically active but "lifeless" planet, versus those conditions and compositions that compel a biological interpretation. The requirements for detecting extrasolar biospheres in association with a range of atmospheric compositions will be key drivers behind the designs of interferometric telescopes that will obtain spectra of extrasolar planets. The astrobiology research program therefore must contribute substantially to the optimization of those designs. The program must lead the continuing search for novel methods to detect remote biospheres spectroscopically. Provide recommendations for instrument capabilities and search strategies to optimize designs of future instruments that will obtain spectra of extrasolar planets.	Provide atmospheric compositions of habitable planets that lack biospheres. Determination of potential false positives Emission of biogenic gases as a function of planetary environment Develop global models for the composition of Earth's early reduced atmosphere Calculate synthetic spectra of a range of plausible extrasolar planetary atmospheres, both with and without free O_2. Identify a menu of biologically-produced volatile atmospheric species. The spectral signatures of plausible extrasolar planetary surfaces both with and without life that might be detected remotely.	Tasks 3-5 Task 4 Task 5 Tasks 3-5 Tasks 2-5 Task 5 Tasks 1-5
Effects of Climate and Geology on Habitability Objective 12 Define an array of astronomically detectable spectroscopic features that indicate habitable conditions and/or the presence of life on an extrasolar planet.	Best available estimates of the habitable zone using physically, chemically and radiatively self-consistent atmospheres with realistic clouds. Better models for how hydrogen escapes Realistic paleoatmospheres for early Earth Effects of geology on planetary atmospheric composition and effects on habitability Effects of biology on planetary atmospheric composition and the limits of the habitable zone.	Task 3 Task 4 Task 2-5 Task 3-4 Task 5

C.5 Work Plan And Schedule

TableC.5.1 shows the five major Tasks, the sub-task components and the members of our team working each component.

Table C.5.1
Allocation of Personnel to the Five Major Tasks and sub-task components
Leads responsible for each overall Task, and then for each subtask are indicated with an L.

	Meadows	Allen	Brown	Cohen	Crisp	Fijany	Huntress	Kasting	Nealson	Richardson	Rye	Sleep	StorrieLombardi	Velusamy	Yung	Zahnle
Task One Lead Scientist - Meadows																
Conversion of SMART and LBLABC to ultracomputing					♦	♦							L			
SMART global grid simulations for Earth	L		♦		♦											
Validation of SMART spectra with Earth data	♦				L											
Experiments to determine sensitivity to known changes in trace gas abundance, surface albedo, cloud distribution, phase angles, viewing geometries	L				♦											
Astronomical instrument simulator assessments of sensitivity to these parameters	L													♦		
Identification of potential biosignatures from the atmosphere and surface	L				♦											
Mars, Venus and Titan global grid simulations and validation.	♦				L											
Task Two Lead Scientist - Meadows																
Completion of SMARTMOD	L															
Upgrade SMARTMOD to ultracomputing	♦				♦	♦							L			
Validation of SMARTMOD	♦				L											
Task Three Lead Scientist - Meadows																
Incorporate 1-D chemical model into SMARTMOD	L	♦			♦										♦	
Upgrade combined model to ultracomputing		♦			♦	♦							L			
Determination of the Habitable Zone for these atmospheres	L		♦	♦	♦			♦								♦
Synthetic spectra of Earth paleoatmospheres	L		♦		♦		♦	♦			♦					
Synthetic spectra for modern and early Earth around other stars	L		♦	♦	♦		♦	♦			♦					
Run tests for the detectability of varying amounts of the currently accepted biosignatures in a range of plausible habitable atmospheres around stars of different spectral type	L			♦	♦			♦								
Determine the optimum wavelength range and spectral resolution for detection	L				♦											
Run through instrument simulators	♦													L		
Determination of detectability of biosignatures for paleoearth and planets around other stars	L							♦						♦		
Development of statistical tools for analysis of the biosignature catalog	♦												L	♦		
Task Four Lead Scientist - Allen																
Ultracomputing		♦				♦				♦	♦		L			
Escape of gas to space								L		♦						♦
Effects of small bodies entering the atmosphere										♦						L
Land/Ocean/Interior		L					♦	♦			♦	♦			♦	
Determination of false positives	L				♦			♦		♦	♦			♦	♦	
Task Five Lead Scientist - Allen																
Ultracomputing		♦				♦				♦			L			
Biology			♦				♦		L		♦	♦				
Determination of new biosignatures	♦	L			♦			♦	♦	♦	♦					
Spectral catalog for physically, radiatively, chemically and biologically self-consistent atmospheres	L				♦			♦								
Recommendations for instrument parameters, designs and search strategies	L				♦									♦		

C.5.1 Schedule of Research Tasks

To implement the tasks outlined in Section C.5, we will begin work on Tasks 1, 4 and 5 in the first year of this effort. Task 1 will determine sensitivity to environmental characteristics in the globally averaged synthetic spectra of terrestrial planets using a radiative transfer model, and will provide the first step for development of the subsequent modeling tools. This task is scheduled for completion 6 months after the start of this effort (the receipt of funding). The design and development of models for the geological, exospheric and biological sources and sinks of volatiles will be done in Tasks 4 and 5 and will start simultaneously with Task 1, continuing for the next 4-5 years. Task 2, the next step in the development of the modeling tools, will validate a climate model and the 1D globally averaged approach used in our modeling. This task starts immediately after completion of Task 1 and will take 9 months to complete. Task 3, integration of a chemical model with the climate model validated in Task 2, completes the mechanistic core of the planet models to be developed in Tasks 4 and 5 and finishes in the 2nd quarter of CY03. Work in the final two years of this effort will concentrate on integration of the ongoing parameterization of geological and exospheric sources and sinks of volatiles into the coupled radiative-climate-chemical model to produce the Abiotic Planet Model at the end of Task 4. Integration of the biological model components into the Abiotic Planet Model will produce the Inhabited Planet Model. At all stages of model development, scientific results, synthetic spectra, and recommendation for instrument parameters will be produced.

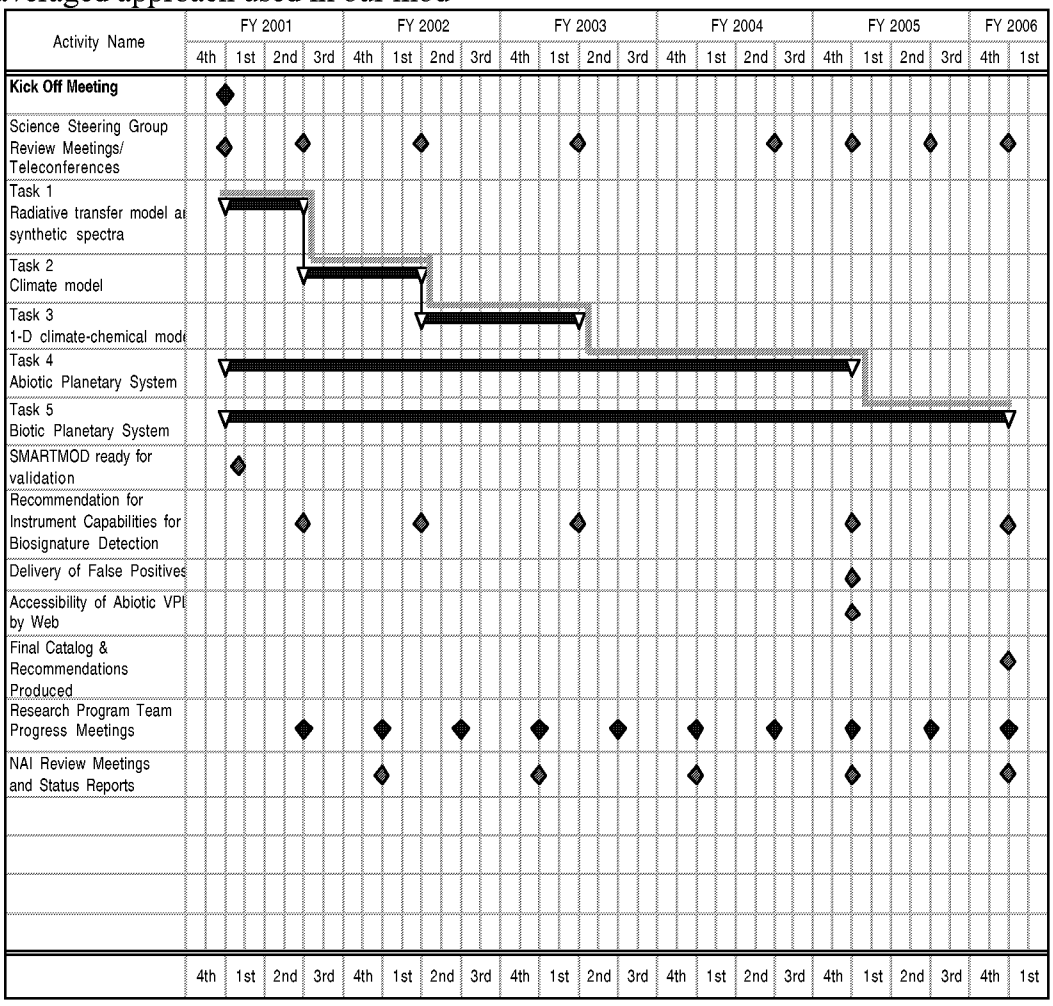


Figure C.5.1 Astronomical Detection of Biosignatures key tasks and milestone schedule

C.6 Management Plan

This research program is a cooperative effort consisting of a 17 member multidisciplinary team that leverages the resources and knowledge from 7 academic and research institutions. The majority of the computational research will be accomplished at JPL/Caltech, although all institutions will provide investigators' scientific expertise. Coordination and integration of the efforts of the research team members, in a timely manner, is the focus of this management activity. The framework in which this will be accomplished will be addressed in three areas: Organization and Decision Making; Schedule (discussion in Section C.5.1); and Tracking, Review and Reporting.

C.6.1 Organization and Decision Making

The organizational structure of the research program is shown in Figure C.6.1 (Org Chart). The PI, Dr. Victoria Meadows, is accountable to NAI for the implementation, outcome, and scientific integrity of the research and its products. The overall direction is the responsibility of the PI, however, when unavailable the PI will temporarily delegate management authority to a member of the Science Steering Group.

The research efforts of the program will be accomplished within five major task groups, each with a Lead assigned. Issues that are not resolved between or within the task groups will be resolved by the PI, who is the final arbiter. In addition to other responsibilities discussed (Section C.6.1.2), a Science Steering Group, consisting of lead scientists from this effort, will advise the PI and provide additional assurance of the integrity and focus of achieving the research objectives on schedule. The organizational structure also shows the EPO effort and the administrative support, which will provided by the PI's home institution. The EPO effort will be managed in manner similar to the research tasks (Section C.6.1.1).

C6.1.1. Task Groups

The individual research tasks performed under the proposed effort are coordinated within task groups, maintaining the focus of the work on the program s objectives. Each task group has a leader whose responsibility is to maintain this focus and coordinate the overall work with other task groups and with the NAI. Each task group has subtask components and each component has been assigned a lead scientist, (Table C.5.1).

C.6.1.2 Science Steering Group

The Science Steering Group will assist Dr. Meadows in performing her many responsibilities as Principal Investigator. The SSG consists of the following senior personnel: Allen, Crisp, Huntress, Kasting, Nealson and Sleep and has the primary function of integrating the work carried out in all the task groups.

The specific functions of the SSG will be to assist Dr. Meadows in:

The review and assessment of overall progress towards the goals of the research program

The coordination of tasks within the research program

Arranging and conducting general co-investigator meetings

The promotion and implementation of interactions with the NAI

Facilitation of communication among the team members and with the NAI

Development and implementation of a policy for the publication of scientific results from tasks performed by the team members

Assuring all reporting requirements are met

In addition, the SSG will assume such responsibilities as may be delegated by the Principal Investigator.

The SSG will interact by teleconference and will meet to carry out its responsibilities.

C6.1.3. Administrative Support

Dr. Meadows will draw upon JPL s Earth and Space Sciences Division for administrative support. The Earth and Space Sciences Division maintains Offices of Scientific Administration (OSA) that support routine administrative needs of PI tasks within the division at no direct cost to these tasks. This support includes the handling of procurements, contracts, travel arrangements and account management. Upon the award of the contract, JPL will execute required contracts with participating institutions for non-government funded Co-I support.

C.6.2 Tracking and Reporting

The PI will establish and implement as simplified financial tracking and reporting system. This will be used to assure that the research planned is accomplished within the allocated funding and schedule profile. Status of these

activities will be assessed quarterly and be available for NASA review and will be rolled up into the final report.

We will provide annual and final reports as specified in Section A (revised October 1997) of the NASA Grant and Cooperative Agreement Handbook. Annual progress reports provided by each of the task leaders will be integrated by the PI and used as the technical basis for these reports. These elements will be supplemented with financial and schedule data accumulated over the annual reporting period as discussed above.

C.6.3 Research Reviews

A Co-Investigators Meeting will be held biannually to facilitate scientific collaborations among the members of this research program and to assure progress towards a common goal. All Co-Is and post-docs will be invited to this meeting and all interested NAI members. The task leaders will report annual progress for peer review at this meeting. Our research program will also support NAI s annual integration workshop and we will participate in the preparation of workshop reports. All research products will be documented in the open literature and available for the NAI/scientific community for review.

C6.3.1. Next Generation Internet

The Next Generation Internet (NGI) will be used heavily to manage the research activities outlined in this proposal. Since all co-investigators are at or near institutions already having a NAI-supplied videoconferencing area, most of the monthly science advisory group meetings and the quarterly co-investigator meetings will be held as videoconferences. In addition, we will utilize extensively collaborative software that runs on desktop computers to allow simultaneous visualization and analysis of model results by multiple members of the science team sitting at their home institutions. There are several applications programmed in Java that would facilitate such interactions because a common program could operate under a variety of host computer operating systems. The Internet will be used to allow members of the science team from their home institutions to execute model runs on the JPL supercomputers and retrieve results.

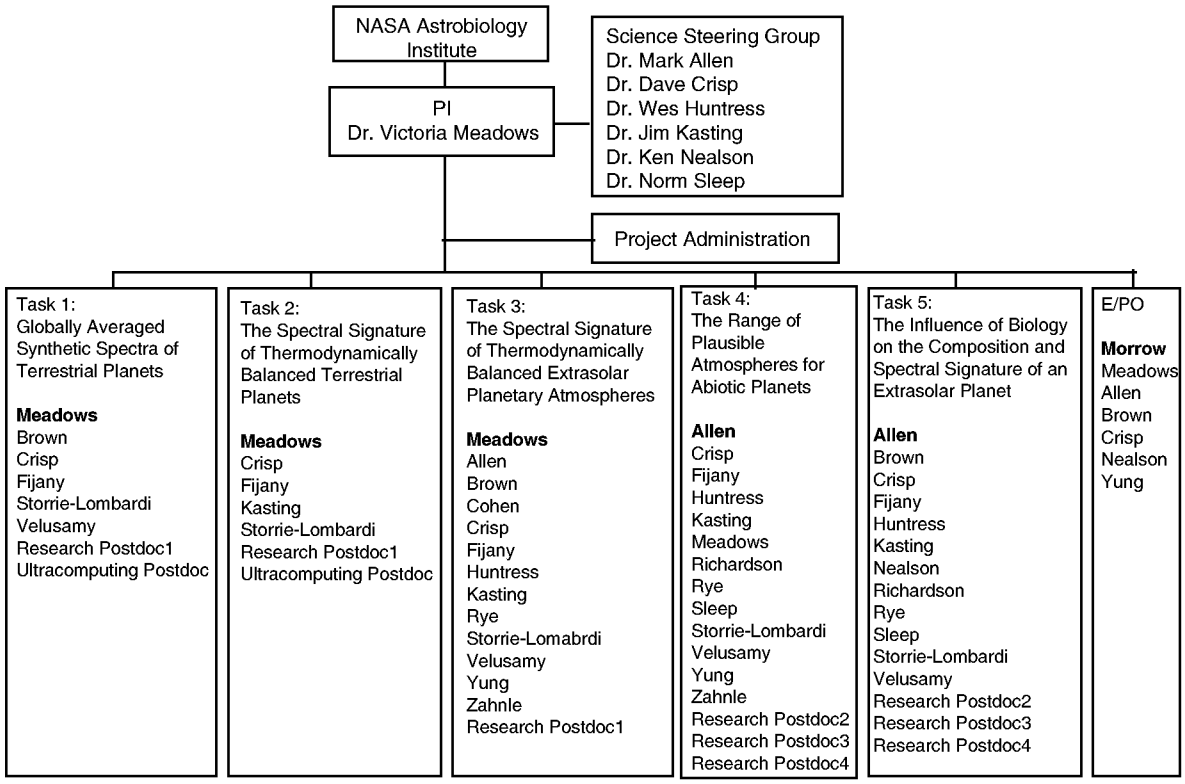


Figure C.6..1 Organization Chart

C.7 Statement of Contributions by PI & Co-Is

Dr. Victoria S. Meadows, (JPL) is the Principal Investigator and is accountable to NASA for the overall research program. Dr. Meadows has had extensive experience on mission design and development work and understands the importance of schedule and delivery and integration of multiple components. She is committed to working in a team environment to accomplish a common goal.

Dr. Meadows will assure that the scientific goals of the program are met on schedule, and the members facilitate the overall objectives of the NAI. Dr. Meadows will be supported in this role by the Scientific Steering Group , whose members will be: Allen, Crisp, Huntress, Kasting, Nealson and Sleep.

C.7.1 The Multidisciplinary Team

This multidisciplinary team is composed of 17 scientists from Caltech, JPL, Penn State, Berkeley, Ames, Stanford, Carnegie and Univ. of Colorado, all who are experts in the respective fields. The majority of the research program activities occur at JPL/Caltech. The scientists include not only atmospheric chemists, physicists, and radiative transfer specialists, but astronomical observers with experience in ground-based and space-based observing, and spacecraft mission design and support. This team understands both the atmospheres of terrestrial planets, and the realities and limitations of astronomical observing.

Dr. Vikki Meadows (JPL), the team leader exemplifies the multidisciplinary qualities of JPL/Caltech by being trained both as an observational astrophysicist and as a planetary atmospheric scientist. Her work has focused on remote-sensing retrieval of gases and aerosol properties from infrared observations of planetary atmospheres. She will lead the development of a suite of radiative-transfer tools to characterize the detectability of astronomical biosignatures and make recommendations to drive future instrument designs. Expertise in modeling atmospheric chemistry and photochemistry for the global model will be provided by Prof. Yuk Yung (Caltech), a world leader in this field, and by Dr. Mark Allen (Caltech/JPL) will serve as the lead for the Planetary Models experience with incorporating disparate modules into a global planetary model will be provided by Dr. Mark Richardson (Caltech). The radiative-transfer components that will be part of the global model, as well as the means to synthesize spectra of extrasolar planet atmospheres will be provided, updated and maintained by Dr. David Crisp, who is a world leader in state-of-the-art, highly accurate, spectrum-resolving radiative transfer models. Up-to- date and custom spectroscopic molecular line parameters will be provided as input to the radiative transfer and climate models by Dr. Linda Brown (JPL). The best available stellar spectral energy distributions will be collated for us by Dr. Martin Cohen (UC- Berkeley) as input to the radiative transfer, chemistry and climate components of the model.

Caltech/JPL will provide the central expertise in atmospheric science and modeling, we have also recruited additional experts from around the nation to provide us with input on the many processes that have to be considered in creating a global model. Information on hydrogen escape processes, and proven expertise in coupled chemical and thermal radiative convective equilibrium models will be provided by Astrobiologist, Prof. Jim Kasting (Penn. State). Geological sources and sinks of volatiles and the impact of plate tectonics on planetary climate and atmosphere will be provided by Prof. Norm Sleep (Stanford). Available information on plausible abiotic and biotic paleoatmospheres, and the chemistry and products of subsurface processes, including hydrothermal vents, will be brokered by Wes Huntress in collaboration with the Carnegie Geophysical Lab. The impact of biological processes on planetary atmospheres (and in particular the likely biogenic gases produced by different forms of life) will be collated and provided by Prof. Ken Nealson (Caltech/JPL) and Dr. David DesMarais (Ames) and their Astrobiology research teams. Dr. Rob Rye will provide expertise in paleoatmosphere and biogeochemical cycles. The effects of impacts on the chemistry of a planet, and detailed models of hydrogen escape will be provided by Dr. Kevin Zahnle (NASA Ames). Detailed understanding and modeling of the detectability of planetary biosignatures in the context of their planetary system with realistic astronomical tools will be provided by Dr. Thangasamy Velusamy (JPL).

This large and innovative modeling effort will clearly require a great deal of computing performance. Dr. Michael Storrie-Lombardi is an astrobiologist in the JPL ultra computing group. He will act as our liaison through Dr. Amir Fijany, as we work with them to adapt the planetary model to run on JPL's suite of high performance machines.

C.7.2 Collaboration with Other Institutions

In addition to the personnel who are identified explicitly on this proposal, we have set up collaborative agreements with the Carnegie Geophysical Laboratory and with other members of the NAI based at Carnegie and at Ames. Huntress will act as broker for collaborative work with CGL and the Carnegie Astrobiology Group, and Nealson will act as interface and broker for work being done by Dave Des-Marais Astrobiology Group at NASA Ames. Each of these collaborative agreements allows us a window into the research being done at these institutions, and will provide important constraints and input to several of our proposed models, using instrumentation and facilities that would not be available at JPL.

C7.2.1. GL collaboration

The Geophysical Laboratory conducts state-of-the-art laboratory research in fundamental geochemical and biochemical processes relevant to understanding the current and early state of planets. The following listing includes some of the processes being studied at GL (with their relevance to this proposal given in parentheses).

1. Elemental, isotopic and chemical analysis of extrasolar materials (exogenic source materials) and geologic samples (biogenic processing of geomaterials).

2. Laboratory and theoretical work on melt processes and rock-volatile chemical equilibrium (coupling of interior chemistry with the atmosphere).

3. High-pressure chemistry (internal source and evolutionary processing of planetary volatiles).

4. Hydrothermal vent chemistry (pre-biotic chemistry and early biochemistry, evolution and cycling of planetary volatiles).

5. Mechanisms of biological metabolism (evolution of energy sources for planetary biology, and evolution of global volatiles).

6. Biological diagenesis (cycling of biological elements and biogenic volatiles).

The GL is currently one of the founding elements of NASA's Astrobiology Institute and is carrying out fundamental and significant work on hydrothermal systems and the origin of life. Recent laboratory work has shown the synthesis of simple biochemical molecules important as initiators in ubiquitous biochemical cycles by the interaction of reduced carbon (carbon monoxide) with venting iron sulfides at elevated pressures and temperature as in hydrothermal vents. The development of life during a vigorous phase of hydrothermal activity on a young planet will have a significant effect on trace and perhaps major components of the atmosphere. These processes will consume early inorganic reduced gases in the atmosphere and convert them into more complex organic components inevitably altering the oxidation state and trace volatile inventory in the atmosphere. These altered components and their relationship may provide an atmospheric signal for microbial biology.

The questions being pursued at GL in hydrothermal geochemistry relevant to the proposed work include the following. What range of planets develop hydrothermal systems and what are the sources and distribution of volatiles within these systems? To what extent and by what mechanisms do organic compounds form in hydrothermal systems and what is the role of mineral catalysts? What processes of self-organization can occur and what steps lead from non-life to life? The work being proposed here would provide a theoretical modeling construct to quantify the effect of global hydrothermal processes on the atmosphere and provide a context for understanding the external detectability of pre-biological and early biological on young planets.

Dr. Huntress will act as a broker between the Geophysical Laboratory (GL) and the research team to integrate the expertise and facilities at GL with the proposed work on chemical characteristics of planetary atmospheres and surfaces. The laboratory work on chemical processes at GL in hydrothermal vents will be an important contribution to the proposed theoretical modeling work on extrasolar planetary atmospheres. Dr. Huntress will provide the interface to the modeling work in

this proposal. To facilitate this collaboration, he will attend the SSG meetings.

C7.2.2. ASTROBIOLOGY AT AMES

The team s biology co-investigators will be in close contact with the NAI Ames team of microbiologists and ecologists to define key life-related processes that influence the state of a planetary atmosphere. The team at NASA Ames is led by Dr. David DesMarais, and is actively involved in data gathering related to biogenic gas production and consumption at a number of sites. They have a number of foci of activity and their data, as well as their models for global fluxes will be of great value to the overall success of our proposed modeling activities. Their considerable efforts and expertise demonstrate the way in which the Astrobiology virtual institute can lead to valuable collaborations in areas of immense importance to the scientific community and to the agency.

The Ames NAI Team's current research in microbial ecology is directly applicable to defining life's contributions to the atmosphere during a planet's history.

Key members of the Ames team include the following: David DesMarais (Ames), team leader, coordinated a 10-year field study of the mat ecosystems at Guerrero Negro, Mexico, and is an authority on the biogeochemical carbon cycle. Brad Bebout (Ames) has conducted extensive biogeochemical analyses of microbial mats. Pieter Visscher (University of Connecticut) has conducted studies in biogeochemistry, mat ecology, and the production and consumption of reduced biogenic gases. Chris Potter (Ames) has constructed several ecosystem models, some of which integrate local biogenic gas emissions into estimates of global fluxes. Robert Chatfield (Ames) has developed multidimensional models for atmospheric chemistry, with specific attention to trace gases. Linda Jahnke (Ames) has extensively studied biomarker compounds and carbon isotope discrimination in bacteria. Tori Hoehler (Ames) is a biogeochemist with extensive experience in anaerobic processes in coastal marine environments.

The Ames team is examining the roles played by ecological processes in the early evolution of our biosphere, as recorded in geologic fossils and in the macromolecules of living cells. (1) It is defining the microbial mat microenvironment, which was an important milieu for early evolution. (2) The team will compare mats in contrasting environments to discern strategies of adaptation and diversification, traits that were key for long-term survival on early Earth. (3) Ongoing studies of budgets of solutes and gases in mats will contribute to better estimates of biogenic gases in Earth's early atmosphere.

In collaboration with the team of Dr. M. Sogin, Marine Biology Laboratory, the Ames team proposed an "Ecogenomics Focus Group" (EFG) within NAI. This project was recently funded, and it will begin to define the relationships between microbial diversity, complex gene expression patterns, and biogeochemical processes that shape planetary environments. In this work, 1) the EFG team will characterize biogeochemical patterns in the microbial mats with special emphasis upon gradient location and shape, 2) the team will employ molecular techniques to develop a quantitative assessment of microbial population structure in this microbial ecosystem, 3) EFG will use DNA microarrays to measure gene expression patterns for genes of known function at different locations in the environment, and 4) the team will explore responses to transient and periodical environmental perturbations imposed by diel cycles. Finally, based upon the results of these empirical measurements, 5) EFG will model feedback loops between microbial gene expression and key biogeochemical processes, including gas production, that influence planetary environments.

The model will be verified with observations and rate data determined in the laboratory and field: namely the production, consumption and flux estimates of volatile C and S compounds made in a variety of microbial mats (gas fluxes: VOSC, CH_4 from hyperaline mats; measurements: DMS, MSH, DMDS, ESH, CH_4 from temperate mats [e.g., Visscher and Taylor, 1994, Visscher et al., 1994, 1996, and other papers]). Data on microbial metabolic rates that produce and consume gases and/or volatile compounds, that are required as input parameters for the model, will be supplemented with results from ongoing studies and mat manipulations by the Ames NAI team. Alternate or successive physical conditions of the earlier-Earth surface environment will be evaluated in simulation trials. These will determine the pos-

sible outcome of competition for light and gaseous and mineral substrates in the microbial mat ecosystem, and related effects on predicted bio-emission estimates of O_2 and CO_2 gases and other volatile species. In order to accommodate the implications of the long-term evolution of inhabited worlds, it will be important to compare these simulations with careful manipulations planned by the experimentalists : e.g., exposing mats to environments that represent plausible extrasolar planetary atmospheres, as identified by atmospheric scientists.

C.8 Training Plan

Our proposed research program will provide many opportunities for training of undergraduates, graduate students, and postdoctoral fellows. The training will provide exposure to many disciplines supporting astrobiology and in methods for multidisciplinary research. Our specific programs in education and training are described in the following sections.

C.8.1 Undergraduate Education

There are several undergraduate education avenues that will provide opportunities to participate in astrobiology-related research. These are important parts of our program, as they are one of the major ways in which undergraduates will be attracted to graduate work in the general area of space sciences, and astrobiology in particular.

C.8.1.1 JPL and Caltech

Summer Undergraduate Research Fellowship (SURF) and Minority Undergraduate Research Fellowship (MURF) Programs are directed at providing significant research opportunities for students at the undergraduate level. The MURF program is aimed at improving the representation of African-Americans, Hispanics, Native Americans, Puerto Ricans, and Pacific Islanders in science and engineering. The competition is open to undergraduates around the country, and is advertised through the Caltech Homepage. Interns are selected competitively. We will participate in both programs by submitting a short description of the proposed astrobiology research program and its co-investigators, and selecting students for the summer internships.

The biology component of our work has some excellent opportunities for undergraduate training. We expect that students will be particularly interested in combining biological field work funded by other programs with the physical and chemical modeling activities in our research program. A student who is comfortable in translating information from one discipline into an appropriate context for another discipline is the sort of student that we hope to produce as a result of our program.

Dr. Victoria Meadows, the Principal Investigator, has supervised 2 SURF students, 1 for one year and the other for 3 years during the past 3 years. Her students have gone on to graduate student programs in extrasolar planet detection and atmospheric remote-sensing.

The JPL/Caltech team members have collectively supervised 17 SURF students over the past three years.

Dr. Ken Nealson has directed at Harvey Mudd College a special undergraduate program in astrobiology the Harvey Mudd Clinic Pr o-gram in which 5-6 undergraduates are trained per year in a research-intensive program.

The Caltech Division of Geological and Planetary Science (GPS) offers undergraduate courses in astrobiology. Dr. Ken Nealson teaches three different classes. The first is a course in microbial diversity and extremophiles, the second is a course in geobiology, called Microbes and Minerals , and the third is a course taught with Professor Yuk Yung, called Habitable Planets . This team will augment the existing curriculum by providing coursework highlighting remote-sensing detection of biosignatures and multi-disciplinary studies of the climate, chemistry, and habitability of terrestrial planets. Using the interconnective abilities of the Next Generation Internet (NGI) system, we will endeavor to offer this course in all of the member institutions of this research program, and even in institutions of other members of NAI. We are fully committed to interactions within NAI, and this approach will be a focal point for attracting undergraduates. We will explore the possibility of providing tapes of these lectures, thus building a library of educational materials at the undergraduate level. We will also explore the possibility of using the Internet for course offerings.

C.8.1.2 STANFORD UNIVERSITY

Planetary habitability is presented in both introductory and advanced undergraduate courses.

C8.1.3. CARNEGIE INSTITUTION OF WASHINGTON

CIW science staff teach at universities, and many staff members work in collaboration with university colleagues. These interactions generate opportunities, on an individual basis, for undergraduate research work at CIW. The advantages to the students are the interaction with additional science staff there and access to state-of-the-art research facilities. There are generally 2-3 undergraduates working at any one time.

In addition, there is an active summer intern program for undergraduates that are supported jointly by the National Science Foundation and CIW. About 15 students participate each summer.

C.8.1.4 ALL PARTICIPATING INSTITUTIONS

NASA Planetary Biology Internship (PBI). The PBI program offers outstanding undergraduates (and beginning graduates) the opportunity to spend summers in NASA-related research laboratories. For example, Dr. Nealson had two PBI interns doing interdisciplinary science in his Caltech laboratory. We will add the members of our program to the list of labs that are qualified to accept PBI interns.

The proposed research program will complement the existing activities of NAI members. Also detailed in this proposal are explicit collaborations already planned for the science team to work with the Ames Research Center and Carnegie Institution of Washington NAI members. However, in addition to actively participating in NAI workshops, science meetings, and committee deliberations, the science team will provide access to the research models developed in the proposed effort via the Internet and the Web for NAI member scientists and others in the larger Astrobiology community to explore ideas about the evolution of the Earth and other terrestrial planets. Many astrobiologists have ideas about how some portion of a planet works. Our flexible models will provide the opportunity to those with ideas to explore the implications of their hypotheses in the context of the virtual planet simulations. Lastly, results of science team activities will be communicated to other members of the NAI using NGI via videoconferencing and Web postings.

C.8.2 GRADUATE EDUCATION

The universities involved in our research program, (Caltech, Stanford University, Pennsylvania State University, and University of California at Berkeley) have histories of graduate education, while JPL and the Carnegie Institution are more research- or mission-oriented institutions, with histories of training graduate and postdoctoral fellows on individual projects. This combination offers outstanding opportunities in graduate education.

We will participate in the NASA-sponsored Graduate Student Researchers Program (GSRP). In this program proposals for up to 12 months of research support must be written by the students and submitted with the approval of a potential research sponsor. This is an ongoing, competitive program that will include sponsors from the major NASA centers, and will be available to the members of our Astrobiology Team.

The members of the science team have collectively supervised 27 graduate students in the past 3 years.

C.8.3 POSTDOCTORAL EDUCATION

The postdoctoral fellows supported by this research program will be trained in interdisciplinary science by members of the science team. Their primary training will be on the job, working with the co-investigators and doing research. The majority of these postdoctoral fellows will reside at JPL/Caltech, where they will have a JPL/Caltech mentor within our team. One postdoctoral fellow will be resident at Stanford University and will be supervised by Prof. Sleep. The postdoctoral fellows will be supervised jointly by other members of our team at academic institutions (Caltech, Stanford, Pennsylvania State) and research institutions (JPL and Carnegie Institution of Washington) and will have the opportunity to spend some time at different institutions. Given the interrelatedness of the tasks outlined in this proposal the postdoctoral fellows will need to interact frequently with each other, and with many of our team members, resulting in truly multidisciplinary training.

The NAI program offers an unprecedented opportunity for participation to multidiscipli-

nary research. In particular, our postdoctoral fellows will have the opportunity to work with the NAI groups at JPL, Ames, Penn State, and CIW and be exposed to current work in microbiology, field biology, field geology, and theoretical studies in planetary evolution. Providing young scientists with an understanding of the coupling of these disciplines with physical, chemical, and mathematical simulations of planets will be our contribution to astrobiology in the next decade.

In addition, JPL and Ames actively participate in the National Research Council Resident Research Associateship program that supports postdoctoral and senior researchers to come to these institutions. This is a competitive program, advertised by the NRC, that attracts the highest quality researchers. JPL and Ames members of our research program will each write descriptions of their projects for inclusion in the NRC RRA announcement of opportunity.

At CIW, postdoctoral fellows will have access to a diverse spectrum of scientists (geochemists, geophysicists, biologists, material physicists, bio-organic chemists, mineral scientists and planetary scientists), and open access to the wide spectrum of instruments and facilities. Carnegie Institution of Washington funds a Fellows program. There are funds available for 5-6 of these each year. They are one-year appointments, generally renewable for a second year. These are competitively selected on the basis of doctoral thesis research, recommendations, and a research proposal. A seminar visit is generally required also. The selected Fellows are free to carry out the research of their own choosing. They are given open access to the staff and their laboratories and are encouraged to carryout individual cross-disciplinary research.

NAI postdoctoral fellows will be available to us, and we expect having applicants assigned to our research program. We will welcome visiting postdoctoral fellows from other members of NAI to either spend time working with appropriate members of our group, or to interact on research projects via the NGI.

The JPL/Caltech members of the science team have collectively supervised the research of 13 postdoctoral fellows in the past 3 years

C.8.4 Additional Science Team Training Experience

Dr. Vikki Meadows supervises 3 junior scientists at JPL as part of her ongoing NASA Exobiology and Planetary Astronomy tasks.

Dr. David Crisp holds a Bachelor of Science Educational Curriculum and Instruction, in addition to a Ph.D in Geophysical Fluid Dynamics, and is a certified Secondary Education teacher (Texas).

Dr. Ken Nealson directed the NASA Planetary Biology and Microbial Ecology Course for a decade. This course was given at NASA Ames, and featured the teaching of space sciences to biologists, and biology to space scientists. It annually attracted about 20 students and was taught by Nealson and a distinguished group of faculty. The course was originated by Dr. Nealson, and was funded through the exobiology program.

C.8.5 Minority Faculty Program

JPL has an active program called NASA/ASEE, which offers summer faculty fellowships (SFF) for faculty from Historically Black Colleges and Universities (HBCU) and other minority-serving institutions. The SSFs are competitive, and selection is made by the NASA SFF selection committee.

C.8.6 Recruitment

Existing experience within the JPL Astrobiology group indicates that it has been very easy to attract students to multidisciplinary areas like this, and we expect no problems recruiting talented individuals. The opportunity to build an interdisciplinary career working with people of high caliber, and the opportunity to be involved with future space missions are two reasons that recruiting has been and will continue to be rather easy. This, coupled with the network of experts already in place in the Astrobiology community, will help with recruiting.

The recruitment will be different at each level. For undergraduates, we will use established methods, such as the SURF announcements for summer interns, the advertised programs of the PBI program, and college catalogs for course announcements.

At the postdoctoral level, past experience indicates we will attract the highest quality candidates into the program. The existing JPL Astrobiology group has been successful in us-

ing advertisements for postdoctoral fellows in astrobiology and has received an overwhelming response in the past from highly regarded institutions. The National Research Council-Resident Research Associateship (NRC-RRA) program will be valuable in both recruitment and selection.

Recruitment of minorities will be achieved via cooperation with JPL s Educational Affairs Office and its Minority Science and Engineering Initiatives. We will also focus some of our efforts on HBCUs and the Hispanic-dominated universities of the Southwest. Finally, we will utilize JPL s Co-op program, which focuses on underrepresented minorities, to engage undergraduates.

C.8.7 Women, Minorities, and Disabled

Recruitment and retention of female, minority, and disabled students will be an important part of our program. Some details have been included in the previous discussion.

Of the two postdoctoral fellows supervised by Prof. Sleep in the past few years, one has been a woman and one was disabled.

The majority of the participants in CIW special programs summer interns, undergraduate students, postdoctoral fellows, and visiting scientists are in fact minorities or women at the present time, and CIW actively recruits minority and women candidates.

Finally, as one of the few female principal investigators of an NAI member team, Dr. Meadows will be an important role model for aspiring women astrobiologists.

C.9 Integration of Activities and Role Statements

C.9.1 Principal Investigator

Dr. Victoria Meadows (JPL/Caltech)is the Principal Investigator and is accountable to NASA for the overall research program proposed by this team. Dr. Meadows will ensure that the scientific goals of the program are met and will facilitate interactions and coordination within her team and with other members of the NAI. She will also lead the radiative transfer and climate modeling (Tasks 1-3) and will supervise two post-docs at JPL. She will spend a total of 0.6 WY/yr on these tasks

C.9.2 Co-Investigators

Each co-investigator listed here has participated in the preparation and review of this proposal and has endorsed his or her participation as described here in a letter to JPL (a file of such letters is available on request). In addition to specific investigation responsibilities described below, investigators have allowed time for and will attend the annual meeting and participate in other NAI activities, as appropriate.

Dr. Mark A. Allen (JPL) is the Lead Scientist for Task 4 and 5. He will focus on coordinating the land/ocean/atmosphere interface chemistry and will provide chemical modeling expertise in Task 3. He will spend 0.5 WY/yr of his time on this task.

Dr. Linda R. Brown (JPL) will create a customized database of molecular line parameters which will be used as input for the model calculations. Part of this will be achieved by recording new laboratory spectra for molecules requested by the team members. She will spend 0.23 WK/yr on the task.

Dr. Martin Cohen (UC-Berkeley) will contribute the photospheric spectral energy distributions of main-sequence stars, covering a variety of spectral types, as relevant inputs to the radiative transfer modeling under this proposal. He will also support requisite CAN Team meetings and NAI workshops/ conferences. He will spend 0.12 WY/yr on this effort, for a total of 0.63 WY over the 5-year period.

Dr. David Crisp (JPL) will contribute to the radiative transfer and climate modeling efforts described in Tasks 1 and 2. Specifically, he will take the lead role in the implementation of the radiative transfer models SMART and SMARTMOD. He will participate in the formulation, interpretation, and publication of the experiments using these models in Tasks 1 and 2. He will also collaborate in the efforts to implement these codes on the JPL Ultra-Computing Facilities, and the efforts to integrate these methods into the Virtual Planetary Laboratory (Tasks 3-5). These tasks will require .4WY/yr during each of the 5 years of the proposed effort.

Dr. Amir Fijany (JPL) will collaborate on the analysis of the existing simulation code and its conversion to the appropriate parallel architecture. He will spend a total of .05WY/yr on this task.

Dr. Wesley T. Huntress (CIW) will be the interface for our proposed activity with current activities at the Geophysical Laboratory in early planetary geochemical processes and hydrothermal vent chemistry. The proposal includes 0.05 WY/yr at no cost for Dr. Huntress.

Dr. James F. Kasting (Penn State) will be responsible for supervising the hydrogen escape calculations. These will actually be performed by a postdoc working at JPL. Dr. Kasting will also supply information from his ongoing photochemical modeling work that is being carried out under separate funding from NASA Astrobiology and Exobiology. These two efforts combined will occupy about 30 percent of Dr. Kasting's time. He anticipates spending a minimum of 5% of his time to this research program. However, there is no direct charge to this proposal, other than the support requested for the JPL postdoc.

Dr. Cherilynn A. Morrow (Space Science Institute) will work with the PI to manage the education and public outreach (EPO) aspect of the proposal. The plan involves educator workshops, public symposia, and web-based resources. Implementation will engage proposal scientists in partnership with several other EPO organizations whose involvement will be coordinated by Morrow. She will spend approximately .1WY/year on this task.

Dr. Ken Nealson (JPL) will be responsible for providing constraints on the production of biogenic gases by various forms of life. He will also act as liaison with Dr. Dave DesMarais group at NASA Ames. He will spend a total of .05WY/yr of his time on these tasks.

Dr. Mark Richardson (Caltech) will lead the development of atmospheric loss models for the Global Model and the Virtual Planetary Laboratory. He will also provide general support for coupled global model development and testing, and support requisite CAN Team meetings and NAI workshops/ conferences. He will spend 0.25 WY/yr on this effort, for a total of 1.25 WY over the 5-year period.

Dr. Rob Rye (Caltech) will be responsible for the development of parameterization schemes for global fluxes of various biogenic gases as a function of environmental variables. He will spend 0.5 WY/year on this task.

Dr. Norm Sleep (Stanford) Prof. Norman Sleep will supervise the development of mechanistic descriptions of global geochemical cycles and their effects on planetary atmospheres for inclusion into the Virtual Planetary Laboratories. He will devote .1WY/yr of his time to this activity.

Dr. Michael Storrie-Lombardi (JPL) will implement the multi-sensor Bayesian classification and analysis algorithms for the biosignatures spectral catalog. He will also coordinate analysis of the modeling software for migration to a parallel ultracomputing architecture. He will spend .20 WY/yr on this task.

Dr. Thangasamy Velusamy (JPL) will investigate the detectability of the biogenic spectral features by modeling the performance of future space missions such as Terrestrial Planet Finder (TPF). He will spend 0.1 WY/yr on this task.

Prof. Yuk L. Yung (Caltech) will participate in the development and application of the photochemical and evolution models for studying the interactions between the atmosphere and the biosphere. While most of the chemical modeling tools have been built by his group in the last twenty years, he will have to modify them for application to Astrobiology, with particular emphasis on the coupling between the atmosphere, ocean, biosphere and escape processes. He will spend 0.1 WY/yr of his time on this task.

Dr. Kevin Zahnle (NASA-Ames) will supply descriptions of impact processing of planetary atmospheres for incorporation into atmospheric chemistry models. He will also work with others in parameterizing geophysical cycles that in part control the atmospheric abundances of important volatile compounds. He will spend 0.1 WY/yr on this task.

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Subsurface Biospheres

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Executive Summary

A better understanding of the Earth's deep biosphere is essential because it can serve as a model for life on other planets and it is a critical component of the Earth's biogeochemical cycles. This proposal is aimed at gaining a fundamental understanding of the life in deeply buried marine sediments. We propose to conduct an interdisciplinary set of projects that takes advantage of our considerable expertise in marine sedimentary microbiology, sedimentary biogeochemistry and deep ocean drilling. Our objectives are to understand the subsurface microbial ecosystems of marine sediments, their role in Earth's biogeochemical cycles, and their relevance to the search for life on other planets. We will focus on three major projects.

The first project will explore the taxonomic composition, metabolic activity and geochemical consequences of buried microbial ecosystems in marine sediments with widely different physical and chemical characteristics. Environments will include hot, deeply buried anoxic sediments where life may exist independently of the photosynthesis-based ecosystem at Earth's surface, and old, deeply buried sediments where life may be limited by the availability of electron donors or key nutrients. The ecosystems of such subsurface habitats are potentially representative of the ecosystems that may exist on other planets.

The second project will document the nature, extent and perturbation-sensitivity of microbial activity in marine sediments and the effect of that activity on Earth's biogeochemical cycles of various chemical species, particularly sulfate and methane. Sulfate, the second most abundant anion in seawater, is the dominant terminal electron acceptor in marine sediments. Methane is climatically active and marine methane deposits comprise the largest untapped hydrocarbon reservoir on Earth. The biogeochemical cycles of these and other chemical species are potentially sensitive to centennial to millenial changes in Earth's surface temperatures, nutrient fluxes to the ocean, and the structure of marine ecosystems.

The third project will focus on identifying signatures of present and past microbial processes in Earth's subsurface as a guide to predicting such signatures in extraterrestrial subsurface environments. The molecular-isotopic based data that will be generated as part of this project, will complement the objectives of the first two projects. For example, the study of biomarkers produced by deeply buried methanotrophic microbes will give us an indication of both past and present activity of this specific taxonomic group.

We have already obtained extensive sample sets from deep Pacific drill-holes for analysis. We developed procedures for identifying contamination of samples, spearheaded the drive to instrument a microbiology laboratory on the Ocean Drilling Program (ODP) drill-ship *JOIDES Resolution*, and compiled relevant global geochemical and physical databases. We proposed and will participate in the first ODP Leg (Leg 201) dedicated to Subsurface Biosphere research scheduled for 2002. These and other developments provide the framework for collecting, analyzing and interpreting microbiological, biogeochemical and physical data from a wide range of deep subsurface samples. This framework leaves us poised to greatly advance Subsurface Biosphere objectives.

Our approach is fundamentally interdisciplinary and we will use advanced techniques from various fields, including microbiology, molecular biology, organic and inorganic biogeochemistry, isotope geochemistry, large data set integration and computational modeling. The work will be field-, laboratory-, and model-based. In order to effectively accomplish the program's goals, we will build teams of investigators, post-doctoral researchers, graduate students and select undergraduate students with diverse skills.

We are fully committed to implementing the collaborative and networking concepts of the NASA Astrobiology Institute.

Integrated Research Plan

The research plan of this proposal is divided into three sections. Each section is focused on one of the proposal's three major projects. The first project will explore the taxonomic composition, metabolic activity and geochemical consequences of deeply buried microbial ecosystems in marine sediments with widely different physical and chemical characteristics. The primary goal of this project will be to identify the nature of individual taxa, microbial communities and limits to subsurface life under such conditions as high burial temperatures, and extremely low availability of electron donors or nutrients. The second project will document the nature and extent of marine-sedimentary microbial control of Earth's cycles of sulfur, nitrogen, and carbon (particularly methane). The primary goal of this project will be to understand how the dominant microbial processes in deep-sea sediments control (1) the flux of sulfate (the second most abundant anion in seawater) from the ocean, and (2) the creation and destruction of marine sedimentary methane deposits (the world's largest untapped fossil fuel reservoir and an extremely large greenhouse-gas source in close proximity to the Earth's ocean and atmosphere). The third project will focus on identifying organic and inorganic signatures of present and past microbial processes in Earth's subsurface. The primary goal of this project will be to help inform the search for microbial signatures in extraterrestrial subsurface environments and to add complementary molecular-isotopic information to resolution of objectives stated in projects one and two.

The first and third projects "address the general issue of the nature and/or search for habitable worlds outside the solar system, as well as methods for detecting the presence of life thereon" [CAN preference (a)}. The second project "explores the role of ecosystem processes (e.g., large-scale episodic disturbance, nutrient dynamics and trace gas production, biogeographic change) in the global carbon cycle and other biogeochemical cycling processes on centennial to millennial time scales with a goal of contributing understanding that could advance our ability to predict future conditions" [CAN preference (b)].

Background.

Advances in microbial monitoring and identification, and increasing knowledge about the metabolic capacities of microorganisms provide a solid basis for addressing fundamentally important questions about deep marine subsurface microbiota. Significant progress has already been made in extending our knowledge about the diversity of microbially inhabitable subsurface environments and microbial types. Cruise-of-opportunity studies have documented the existence of a widespread deep biosphere in marine sediments (*e.g.*, Parkes *et al.*, 1994). Recent studies have pushed the maximum burial depth of that deep biosphere to more than 807 meters below seafloor (Ocean Drilling Program Leg 180 Preliminary Report).

Recent microbiological investigations of sediments in the deepest Mariana Trench, close to 11000 m depth, have made clear that depth and hydrostatic pressure *per se* do not preclude vigorous and highly diverse microbial life (Tamegai et al., 1997; Takami et al., 1997). The finding of microbial "burrowing" structures in volcanic glass (Fisk et al., 1998), and the isolation of a new strain of the thermophilic bacterial genus *Thermus* from 3.5 km deep, geothermally heated ore of a South African gold mine (Kieft et al. 1998), indicate that both deep sediments and oceanic crust, including their geothermally heated portions, provide potential habitats for microbial life.

Meanwhile, novel forms of bacterial metabolism with subsurface potential are being discovered. As an example, systematic studies of sulfate- and sulfur-reducing bacteria and archaea have shown that many representatives of these organisms, among them an astonishing set of phylogenetically very deep lineages (Vargas et al. 1998), share an unexpected capacity for Fe-III reduction (Lonergan et al. 1996). Respiration of metal oxides could allow bacteria and archaea a respiratory

mode of life even after other electron acceptors, including oxidized sulfur species, become extinguished with increasing distance from the oxidized biosphere. Given such novel metabolisms, it is likely that temperature, not lack of respiration substrates or carbon, will pose the ultimate limits to life in the deep subsurface. The record growth temperature for a microbial culture in the laboratory is currently 113°C, for the microaerophilic and denitrifying, autotrophic Crenarchaeon *Pyrolobus fumarius* (Blöchl et al. 1997). The success of this culture indicates that temperature regimes between 110 and 120°C are still compatible with microbial life.

The study of the deep microbial biosphere is becoming an essential and interdisciplinary research focus of the geological and biological sciences. The important questions are as varied as the viewpoints. Geochemistry: What are the relationships between microbial activity, pore-water chemistry, and post-depositional alteration of marine sediments? What can be learned from the isotopic record of microbially mediated processes in deep sediments? What are the organic-biomarker and isotopic signatures of deeply buried microbial activity? Microbial physiology: What are the physiological adaptations of microorganisms under the various extreme conditions in the subsurface? Genetics and Evolution: Are there new and possibly ancient types of bacteria to be found? And from the most far-reaching perspective, Astrobiology: If some extremophilic bacteria are known to survive in deep sediments, in hot or cold crust, could similar life forms also survive or thrive on other planets? Can we recognize unprededented biomarkers in the earth's crust in order to be prepared for elusive evidence of life in non-terrestrial samples?

First project: Taxonomic composition, metabolic activity and geochemical consequences of microbial ecosystems in marine sediments with widely different physical and chemical characteristics.

Sediment chemistry, microbial activity and community composition.

The primary goal of our research on sediment chemistry and microbial communities will be to document the limits to life imposed by the availability of electron donors and electron acceptors. The availability of electron donors (i.e., H_2, CH_4, organic matter) may ultimately control the presence or absence of life in some deeply buried sediments. In contrast, the presence or absence of specific electron acceptors (such as Fe(III)-oxides, Mn(IV)-oxides, NO_3^-, SO_4^{2-}) may control taxonomic composition and the nature of microbial activity, but is not likely to ultimately determine whether life is present or absent, because the electron acceptor CO_2 is always present in marine sedimentary porewaters.

Chemical and sedimentary data from Ocean Drilling Program (ODP) and Deep Sea Drilling Project (DSDP) drill holes can be used to identify regions and burial depths where this goal can be most readily met (Fig. 1). For example, geographic regions and stratigraphic (burial depth) intervals of extremely low microbial activity can be predicted from the vertical profiles of dissolved sulfate and methane concentrations in sedimentary porewaters of ODP and DSDP drill holes.

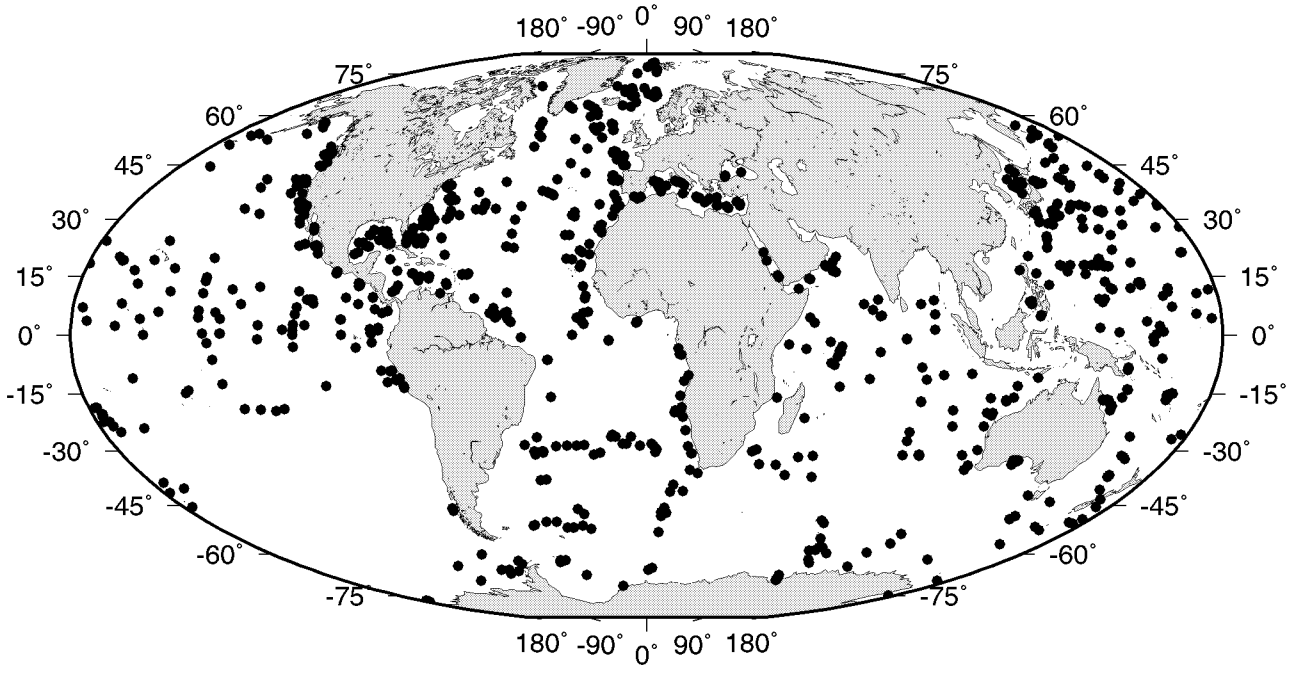

Figure 1. Global map indicating all Ocean Drilling Program (ODP) and Deep Sea Drilling Program (DSDP) sites.

We can infer that sulfate reduction and methanogenesis are the dominant microbial processes in deeply buried marine sediments, because (1) in deep-sea porewaters, initial concentrations of dissolved sulfate are nearly 50 times as great as concentrations of all other electron acceptors combined, (2) depending on the flux of organic carbon to the sediments, the other external electron acceptors typically disappear within the first few centimeters to tens of meters of burial depth (Froelich et al., 1979), and (3) when all external electron acceptors have been reduced, fermentation and methanogenesis are the only known avenues of microbial activity that remain.

Typical downhole profiles of dissolved sulfate and methane are presented in figure 2 a & b. The concentration of dissolved sulfate in sediments results from the balance between (1) diffusion of sulfate from the overlying ocean and (2) reduction of sulfate by microbial activity in the sediments. Because the sulfate diffuses down from the overlying ocean, peak sulfate concentrations typically occur at the sediment-water interface. In contrast, methane is produced by buried microbes and typically diffuses up toward more shallowly buried sediments, where it is consumed by sulfate-reducing microbial consortia (e.g., Hinrichs et al., 1999; D'Hondt et al., 2000). Consequently, peak methane concentrations usually occur at some depth below the seafloor.

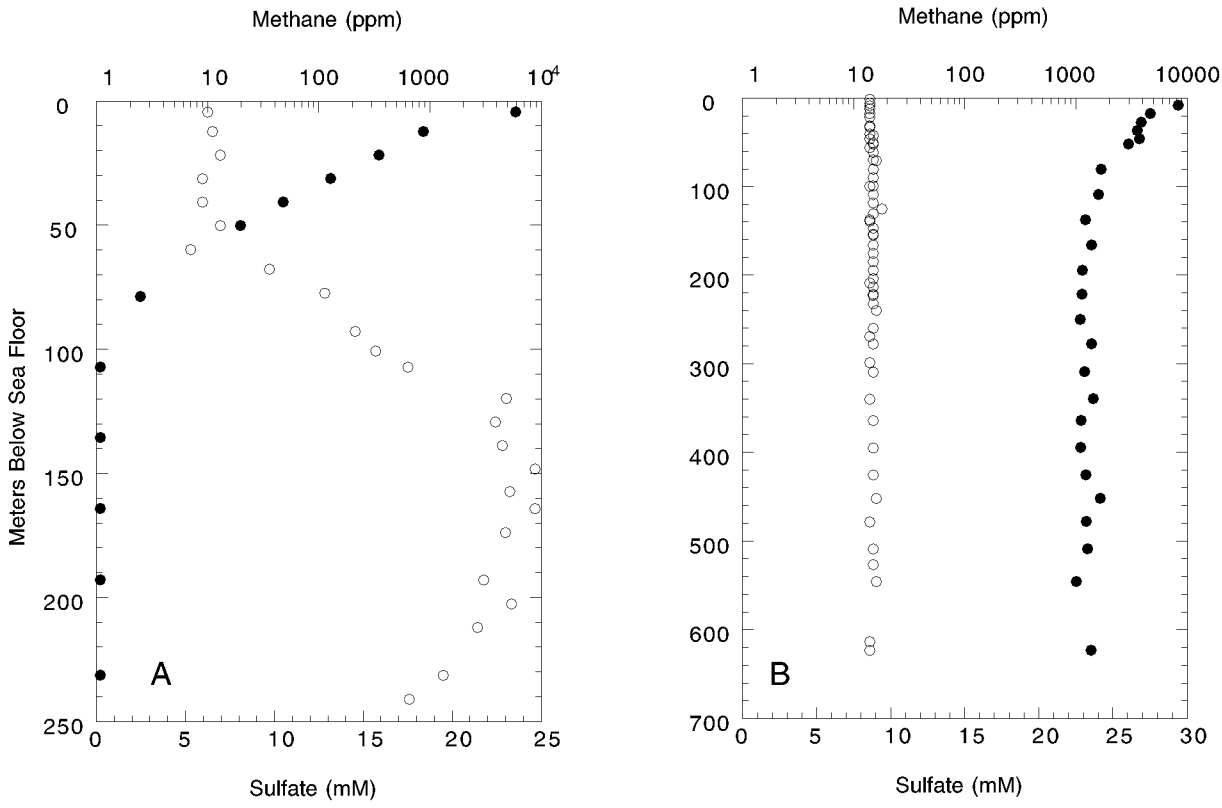

Figure 2a. Profiles of downhole methane and sulfate concentrations at a) a typical coastal site and b). a typical open ocean site.

In open-ocean regions, dissolved sulfate profiles (and where present, methane profiles) typically reach a stable non-zero value within the first few meters to few tens of meters below the seafloor (D'Hondt et al., 2000) (Fig. 3 a & b). Below this burial depth, any effect of microbial activity on dissolved sulfate profiles is indistinguishable from analytical uncertainty in the sulfate measurements. Most deep-sea sediments that are characterized by such stable downhole sulfate profiles contain very low concentrations of organic carbon and are relatively cool. Hence, life in these sediments is likely to be limited by electron donor availability. The nature of microbial assemblages in these intervals of stable sulfate concentrations remains largely unknown. Are they unique communities of slow-living microbes with extraordinarily low nutrition requirements? Or do they merely represent a dead or relatively inactive ecosystem whose microbial components exhibit greater levels of activity in organic-rich sediments at shallower sediment depths and in other regions of the world ocean? If slow-living, dead and inactive microbial assemblages all occur in deep-sea sediments, under what physical and chemical conditions does each type of assemblage occur?

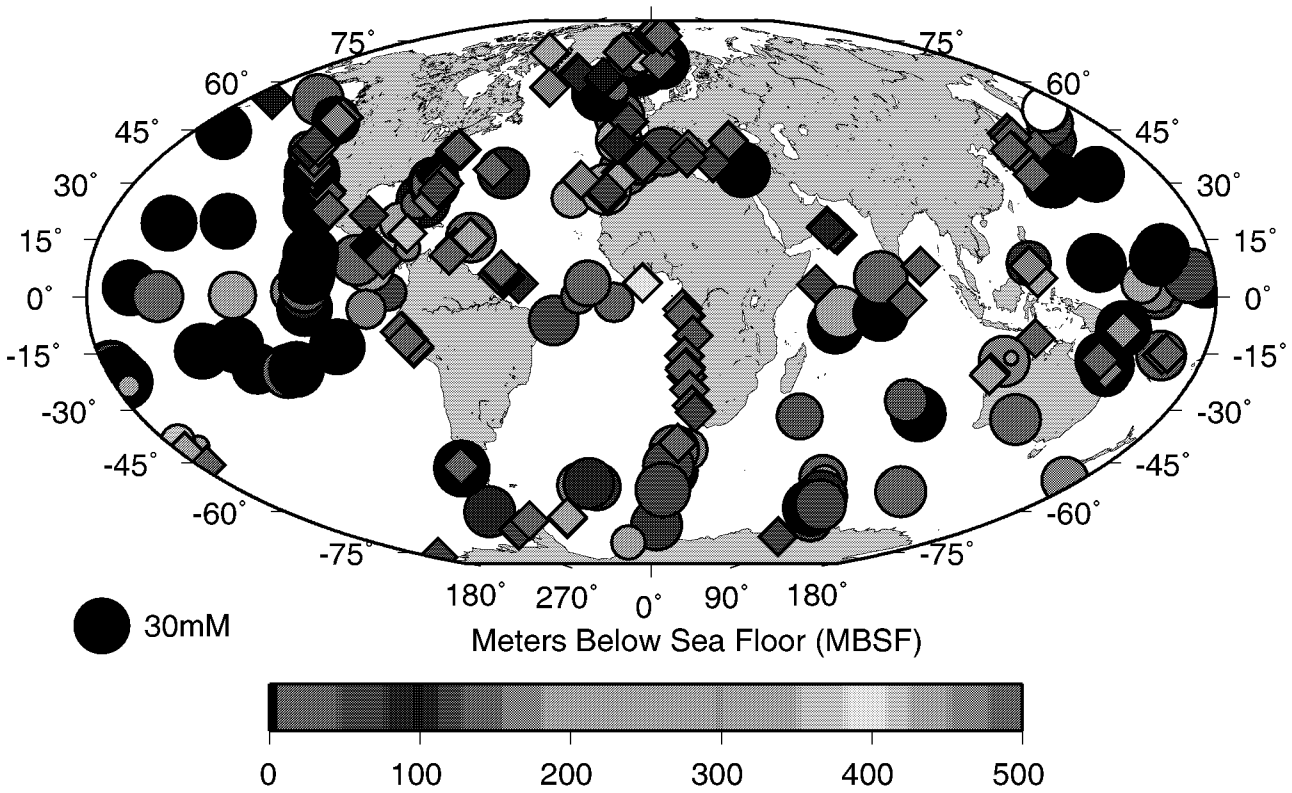

Figure 3a. Global map of dissolved sulfate concentrations in deep sea sediments. Color of symbol indicates the sediment depth at which dissolved sulfate concentrations reach a stable value. Size of dot indicates sulfate concentration at the depth of stabilization. Diamonds mark sites where dissolved sulfate disappears at the depth of stabilization.

Characterization of these subsurface microbial communities and the sedimentary environments in which they occur will have a host of benefits for our understanding of subsurface life on Earth and other planetary bodies. Most obviously, it will provide us with models for estimating (1) the lowest levels of electron donor concentrations that can sustain life, (2) how long microbial communities can remain active and still be reactivated, and (3) what kinds of metabolic and other cellular adaptations, if any, are required to sustain life at extremely low concentrations of electron donor or nutrients.

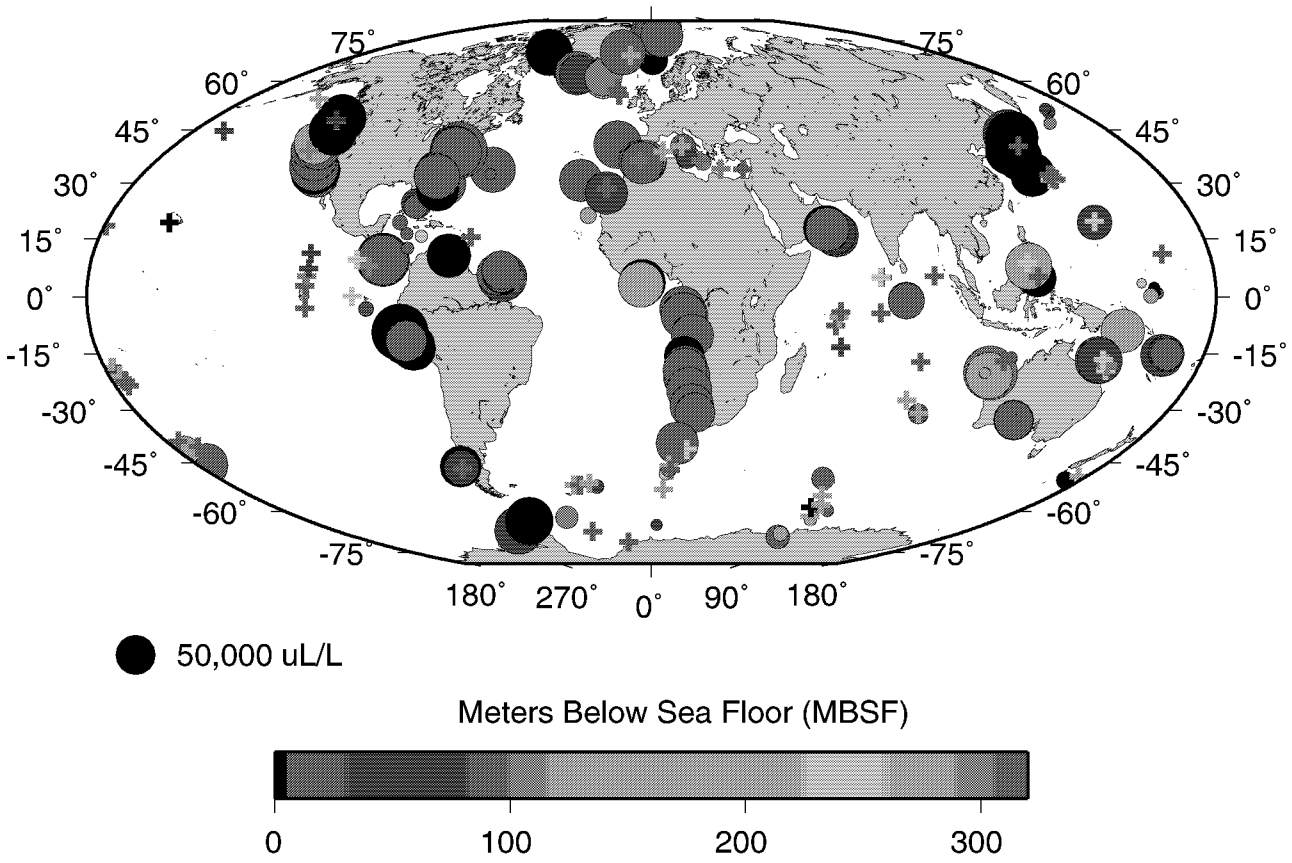

Figure 3b. Global map of methane concentrations in deep sea sediments. Color of symbol indicates the minimum sediment depth at which methane rises above the detection limit. Size of dot indicates the peak subsurface methane concentration at each site. Crosses mark sites with no subsurface methane.

Dissolved nitrogen species in subsurface ecosystems.—

In addition to sulfate and methane a well-developed understanding of the ecology of the deep biosphere requires quantifying the role of nitrogen. Traditionally, the study of sedimentary microbial activity has focussed on net dissimilatory activity, in effect the unidirectional decomposition of detrital organic compounds supplied from the overlying water column. However, there are a number of observations that point to the significance of the uptake, assimilation and recycling of material through the buried microbial biomass. The significance is twofold; it is directly related to the controls on the activity and structure of sedimentary ecosystems and the global geochemical cycle of nitrogen.

The existence of an active microbial nitrogen cycle is pointed to by two observations, the existence of deeply buried bacteria and sedimentary pore fluid ammonium profiles. Clearly, if bacteria are active they require reduced nitrogen or must actively fix N_2. In shallowly buried anaerobic sediments there is abundant dissolved ammonium and microbial activity is associated with a net conversion of organic nitrogen to dissolved ammonium. However, in deeply buried sediments, microbial activity appears to be a net sink of dissolved ammonium. At many ODP sites (e.g. Site 1173, fig. 4) there is a mid-core maximum in dissolved ammonium.

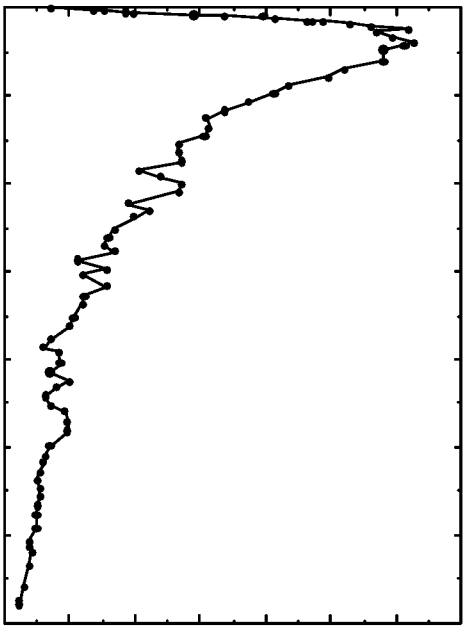

Figure 4. Concentration of dissolved ammonium as a function of depth, ODP Site 1173, Nankai Trough.

This decrease with depth has often been attributed to inorganic ion exchange reactions because deep microbial activity was not previously recognized and ammonium is not involved in significant authigenic mineral formation. We, however, argue that net uptake via ion exchange is unlikely as it is not consistent with the profiles of the major ions. Our alternative hypothesis is that there is active microbial uptake of ammonium in the depth interval of the ammonium decrease and that the decrease is effectively due to the sequestering of nitrogen into refractory compounds synthesized by microbes. For example, Tanoue et al. (1995) showed the ubiquity of a trans-outer-membrane protein (porin P) in vertical profiles in the water column. This protein, produced by Gram negative bacteria, is well know to be resistant to degradation by protease (Lugtenberg and Alphen, 1983). This sequestration of nitrogen appears to be effective enough to drive deep pore fluid ammonium to nearly zero concentrations. This depletion may lead to the nitrogen limitation of deeper microbial activity and require deeper metabolic activity to be dependent on nitrogen fixation. In this regard, it is interesting to note the universal presence of nitrogen fixing enzymes in sulfate reducing bacteria has been considered a "mystery" as the possibility of microbial activity in deeply buried ammonium depleted sediments was not recognized (Fenchel et al., 1998).

A complete understanding of the dynamics of nitrogen cycling in these deep food webs will require quantifying gross rates of 1) ammonium production from detrital organic matter, 2) assimilation of ammonium into both labile and refractory organic nitrogen compounds, 3) recycling of microbially produced organic nitrogen and 4) nitrogen fixation. These effectively are the same issues being addressed by scientist studying the role of microbes in the oceanic water column and soil food webs. To quantify these rates, nitrogen isotopes ($^{15}N/^{14}N$) have been used in two ways. Tracer enrichment studies have been conducted and natural abundance variations are measured and modeled (e.g., Tietema and Vandam, 1995; Sigman et al., 1999). Our calculations indicate that the turnover time of ammonium is too long to allow for tracer studies using stable isotopes in deeply buried sediments. However, a combination of net fluxes derived from diffusion modeling of pore fluid profiles and measured variations in the nitrogen isotopic composition of ammonia should allow for the determination of the individual fluxes that are components of the deep biosphere nitrogen cycle.

The presence or absence of subsurface life on Earth and other planetary bodies is commonly recognized to depend on the presence or absence of water, electron donors and electron acceptors. The potential role of nutrients in limiting subsurface life—and in shaping the evolutionary history of subsurface cellular machinery—is more rarely considered. Studies of the nitrogen cycle in deeply buried marine sediments and their microbial communities will document the effect of nutrient availability on subsurface life help to shape predictive models for the nature of subsurface life on other planetary bodies.

Dissolved hydrogen in subsurface ecosystems.—

Dissolved hydrogen plays a critical and unique role in the biology of deeply buried sediments. It is involved in almost every transformation catalyzed by anaerobic bacteria. Hydrogen is an end product of fermentation and it is well documented that hydrogen consumers are necessary for the survival of hydrogen producers via interspecies hydrogen transfer. Bacteria that perform the complete oxidation and bioremineralization of organic compounds to CO_2, in shorthand, terminal oxidizers, consume hydrogen as electron donor for their energy metabolism. In this way, dissolved hydrogen concentrations represent a balance between microbial production and consumption of hydrogen, with very fast hydrogen turnover times and low steady-state concentrations (Jørgensen, 1989). Recent work on shallow near-shore sediments supports the hypothesis that terminal oxidizers that use a specific electron acceptor regulate hydrogen concentrations to gain a competitive advantage over bacteria that utilize other electron acceptors (Hoehler et al., 1998). For example, sulfate reducers regulate hydrogen to a concentration that is too low for it to be of use to methanogens.

In addition to this role in influencing community structure via competition, hydrogen has also been suggested as a substrate that may support chemolithotrophic communities, a process that has been identified as clearly relevant to the possibility of life on other planets. However, despite its clear biogeochemical importance, previous published studies of sedimentary hydrogen have been limited to shallow, near-shore sediments; there are no published data on the distribution and dynamics of hydrogen in deeply buried sediments. From an astrobiology perspective, this lack of information must be remedied as soon as possible, because the most deeply buried sediments and rocky crust are the farthest removed from the photosynthetic world—and the likeliest model for life-bearing subsurface environments on other planetary bodies.

During ODP Leg 190 we established and tested procedures for the measurement of hydrogen in deep-sea sediments recovered on the ODP drillship *JOIDES Resolution* (Spivack et al., 2000). In addition, we analyzed a limited number of sediment samples covering a depth range of a few meters below the seafloor (mbsf) to 1,100 mbsf and spanning an estimated temperature range of 3 to 140°C. Hydrogen concentrations in the upper sections of the sediment columns were similar to those in shallow near shore sediments suggesting similar microbial control as argued for in near shore sediments. Concentrations generally increased with *in situ* temperature as predicted by thermodynamic calculations. Preliminary results from sterilization experiments indicate the possibility of significant abiological hydrogen production at higher temperatures. However fundamental questions remain concerning the relationship between hydrogen, pore fluid composition (e.g., total dissolved organic carbon, specific organic compounds such as acetate and formate, and electron acceptors), sediment composition (e.g., organic carbon content and iron abundance), temperature and microbial community structure.

Effect of sedimentary temperature on microbial community composition and activity.

The primary goal of our research on sedimentary temperature and microbial communities will be to (1) document the nature of subsurface life at a wide range of ambient temperatures, and (2) identify sedimentary microbial communities that are fully independent of photosynthesis. The latter communities may serve as a reasonable model for the subsurface life that may exist on other planetary bodies.

In approaching these goals, we expect to address a number of closely related questions. What is the effect of temperature on the phylogenetic composition of the deep-sea sedimentary biosphere? Where might the upper thermal bound to life be encountered in deep-sea sediments? What is the

importance of temperature for independence from photosynthesis of the deep-sea sedimentary biosphere? For example, is sustenance by abiological H_2 production an effective growth strategy in or near hot (deeply buried) anaerobic sediments? Are these hot sedimentary environments reasonable analogues for environments on extraterrestrial bodies—or for environments inhabited by Earth's early biosphere?

Except in hydrothermal vents, seafloor temperature approximates that of the overlying deep sea (about 1°C in most of the ocean). The diffusive thermal balance between the cold seafloor and geothermal heat below causes sedimentary temperatures to increase with burial depth. This can be expected to affect microbial activity and community composition in two ways. First, temperature sets a physical boundary to the kinds of microbes that can survive at increasing burial depths. The ultimate high-temperature limit to buried microbial communities is the temperature above which no microbes can survive.

Microbial studies of the surface Earth demonstrate that normal marine bacteria (mesophiles) grow best at ca. 20 - 25°C and do not survive prolonged exposure to 40°C or higher (ZoBell, 1946). Normal thermophiles, which include numerous gram-positive spore-forming anaerobes, fermenters, and sulfate- and sulfur reducers, exhibit a temperature range of ca. 50 to 70°C (examples in Hippe et al., 1992; Widdel, 1992). Most known hyperthermophilic Archaea have an optimal temperature range of 85 to 95°C (Blöchl et al., 1995). At present, the upper thermal limit to life appears likely to be in the range of 110 to 120°C; the most heat-tolerant microbes that are presently known were isolated from hydrothermal vent chimneys and can grow and divide at 113°C (Blöchl et al., 1997).

These generalizations provide a basis for predicting the composition of subsurface microbial communities at different sedimentary temperatures. For example, most sediment in the world's ocean basins is cooler than 30°C (Fig. 5) (Rutherford and D'Hondt, unpublished). Consequently, it is likely, but largely untested, that the microbes in most deep-sea sediments are not particularly adapted for thermal extremes. The relative coolness of most deep-sea sediments largely stems from their relative thinness; throughout most of the ocean, the 30°C isotherm is actually in the oceanic crust that underlies the sediments. However, much higher temperatures occur within sediments in regions of high geothermal heat flux, such as active plate tectonic margins, and relatively thick sediment cover, such as near continental margins. For example, the 120°C isotherm occurs in the first kilometer of burial depth in many regions of the world ocean (Fig. 6) (Rutherford and D'Hondt, unpublished). Although the taxonomic composition and metabolic activity of the highest-temperature deep-subsurface microbial communities are presently unknown, such communities are likely to occur close to the burial depth of the 120°C isotherm in deep-sea sediments.

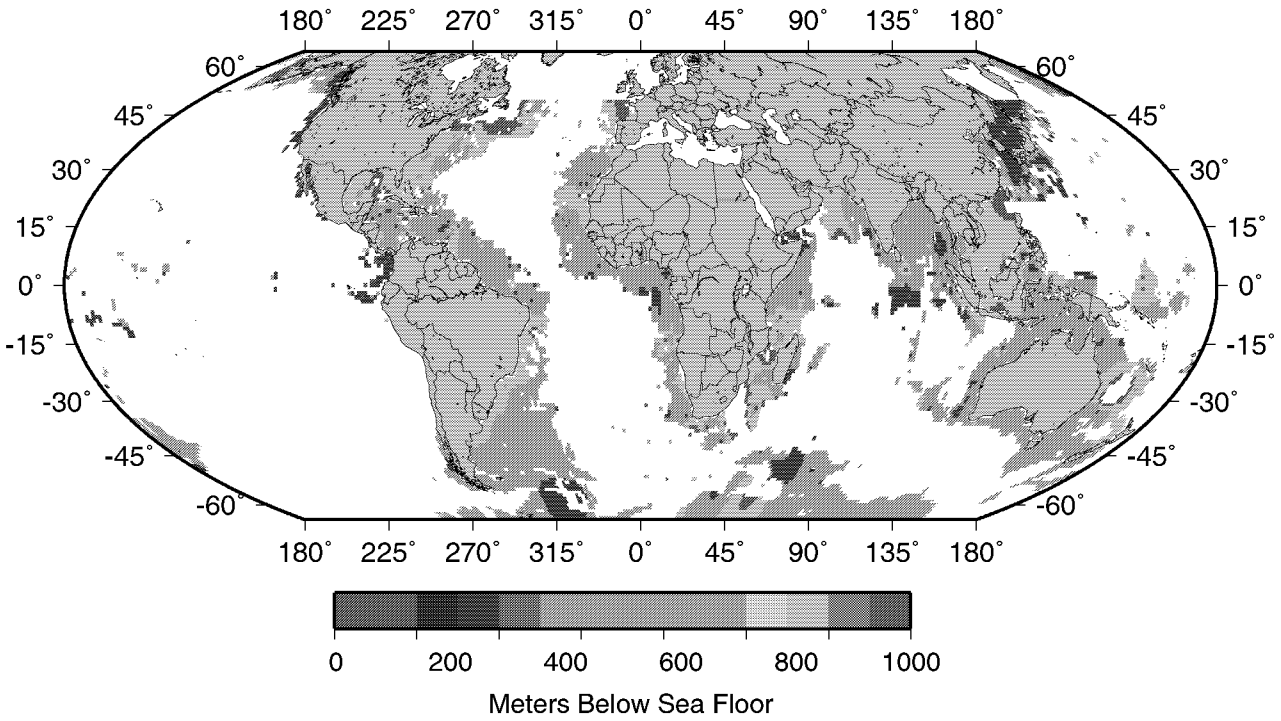

Figure 5. Global map indicating the depth (meters below seafloor) of the 30 °C isotherm in ocean sediments. In white regions, the isotherm typically occurs within the underlying crust (rather than the sediments).

The second way that burial temperature can affect microbial communities is by changing the availability of electron donors. Increased temperature can increase electron donor availability by enhancing degradation of buried organic matter (Wellsbury et al., 1997) and by enhancing the reaction rates and equilibrium concentrations of inorganic electron donors, such as H_2. These two processes have different implications for the nature of subsurface microbial communities. The first process, enhanced degradation of refractory organic carbon, may not be truly independent of photosynthesis, since most of the organic carbon in deep-sea sediments was originally synthesized in the overlying surface ocean. However, the second process, enhancement of the abiological release of electron donors, may support subsurface microbial communities that are fully independent of photosynthesis. In at least one respect, these hot subsurface communities are even farther removed from the photosynthetic world than hydrothermal vent communities, which have access to highly oxidizing electron acceptors, such as dissolved oxygen, nitrate and sulfate (these electron acceptors are relatively abundant in seawater because high oxygen concentrations are maintained in Earth's atmosphere by photosynthesis). Consequently, the anaerobic chemolithotrophic communities of hot deeply buried sediments may provide a reasonable terrestrial model for (1) Earth's early life, and (2) the subsurface life that may exist on other planets and planetoids.

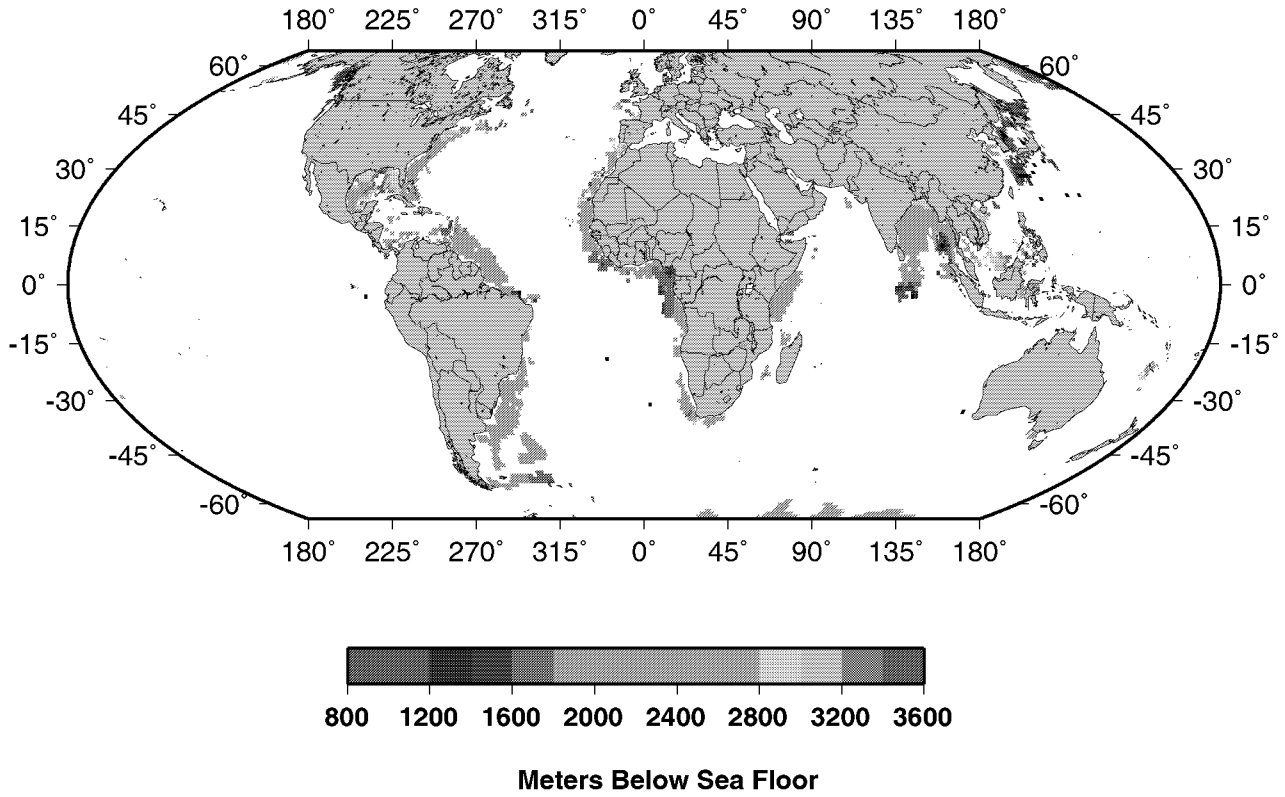

Figure 6. Global map indicating the depth (meters below seafloor) of the 120 °C isotherm in ocean sediments. In white regions, the isotherm typically occurs within the underlying crust (rather than the sediments).

Second project: Nature and extent of marine-sedimentary microbial control of Earth's cycles of sulfur and carbon (particularly methane).

In addressing this theme, we propose to focus primarily on the microbial communities responsible for sulfate reduction, methanogenesis, anaerobic methanotrophy, and the sequestration of nitrogen and phosphorus in deep-sea sediments. Numerous studies suggest that these sedimentary microbial processes are respectively responsible for (1) the dominant flux of sulfate (the second most abundant anion in seawater) from the ocean, (2) the creation of the world's largest methane deposits (which incidentally constitute the world's largest untapped fossil fuel reservoir), (3) the ongoing natural destruction of those methane deposits by microbial methanotrophy, and (4) the dominant fluxes of bioavailable nitrogen and phosphorus from the oceans. Consequently, focusing on these processes will allow us to better understand the dominant microbial processes in deep-sea sediments, the ways in which those processes affect the surface earth, and the sensitivity of those processes to changes in Earth's surface conditions. Conditions to which those processes may be sensitive include: marine ecosystem structure, sea-surface temperature, deep-ocean temperature and fluxes of dissolved nutrients to the surface ocean.

Nature of the relevant (sulfate-reducing, methanogenic and methanotrophic) microbial consortia.

The processes of sulfate reduction, methanogenesis, and methane oxidation in marine sediments are tied to each other in several ways; together, they perform the complete bioremineralization of organic compounds in the seabed. Organic compounds are oxidized by sulfate reducers as long as sulfate is available. In deep layers of marine sediments where sulfate is depleted, the terminal steps of carbon remineralization are taken over by methanogens, which can utilize a variety of carbon sources; besides CO_2 and H_2 also acetate, ethanol, formate, methanol and methylamines are metabolized. Methyl compounds such as methanol or methylamines are preferentially utilized by genera within the Methanosarcinales (*Methanosarcina*, *Methanosaeta*, *Methanococcoides*, *Methanomethylovorans*, and *Methanolobus*) which possess methyl-disproportionating pathways for concomitant oxidation of methyl carbon to CO_2 and reduction to methane (Whitman et al., 1992). These methanogens may be viewed as preadapted to anaerobic methane oxidation to CO_2, since they lack only a methane-activating enzyme system to bridge the gap between methane and methyl group oxidation (Hoehler and Alperin, 1996). While the responsible microorganisms have not yet been cultured, the process of anaerobic methane oxidation dominates sulfate reduction / methanogenesis transition layers in marine sediments on a global scale. Here, sulfate reducing bacteria occur in association with methane-oxidizing organisms and oxidize methane stoichiometrically, most likely by remineralization and removal of acetate and hydrogen, the presumed intermediates of methane oxidation (Valentine and Reeburgh, 2000). Understanding the composition of sulfate-reducing, methanogenic, and methane-oxidizing microbial communities in marine sediments and in the subsurface is essential to grasp the microbial physiology and therefore the effective environmental controls behind these pervasive processes that shape the anaerobic portion of Earth's carbon cycle. Here we give a summary of the relevant microbial communities in marine sediments and in the subsurface.

Sulfate-reducing bacteria extend deeply into marine sediments. Bacterial sulfate reduction is the dominant terminal anaerobic oxidation process in marine sediments, and in coastal sediments this process can be responsible for more than 50% of the organic matter degradation (Jørgensen, 1982). The importance of sulfate reduction decreases in deeper-water sediments, where the diagenetic zones are greatly extended (Froelich et al., 1979; Jørgensen, 1983) and sulfate can be present to a depth of several hundred meters in some pelagic sediments (Canfield, 1991; D'Hondt et al., 2000) (Figs. 2, 3). Although the peak activities of sulfate reduction were always found within the uppermost meters of the sediment column, the process was still measurable at depths of ca. 100 m in diverse organic-rich marine sediments (Cragg et al., 1990, 1995). Microbial sulfate reduction has been recorded at sediment depths of at least 425 m (Parkes et al., 1994).

At present, we do not have a working inventory of sulfate-reducing bacterial diversity in cold deep-sea sediments, the geothermally heated subsurface, or generally subsurface habitats; few species have been described in any detail. The barophile *Desulfovibrio profundus* from 500 m depth in sediments of the Sea of Japan remains so far the only described species from deep marine sediments (Bale et al., 1997). Its unusually wide growth temperature range (15 – 65°C) has no counterpart in any other sulfate-reducing bacterium and is a unique physiological feature of this subsurface organism. The new species *Desulfomicrobium hypogeium* was isolated from deep sandstone in Mew Mexico (Krumholz et al., 1999), and the thermophile *Desulfotomaculum putei* from 2.7 km deep terrestrial sandstone (Liu et al., 1997). Sulfate-reducing microorganisms exist that can cope with several of the environmental extremes of the subsurface. For example, the sulfate-reducing Archean *Archaeoglobus* grows at temperatures of 85 - 90°C, and has been found in geothermally heated, deep oil reservoirs (Stetter et al., 1993). In thermal gradients of hydrothermal vent sediments, different suites of sulfate reducers perform sulfate reduction over a continuous temperature spectrum of 10 - 102°C (Elsgaard et al., 1994). Members of the thermophilic sulfate-

reducing genera *Thermodesulfobacterium* and *Thermodesulfovibrio* are possible players (Henry et al. 1994, Zeikus et al. 1983).

Methanogenesis is a pervasive process in the Earth's subsurface. In deep marine sediments, porewater methane may accumulate over depth gradients of several hundred meters (Parkes et al., 1994), and is subject to in-situ reoxidation processes within the sediment (Cragg et al., 1995). Methanogenesis in the subsurface was originally regarded as the most likely chemolithoautotrophic basis for a deep microbial biosphere that is fuelled by hydrogen and CO_2 (Gold, 1992). An unexpected dimension was added by the discovery that methanogenesis in deep marine sediments can utilize acetate - and probably other low-molecular weight carbon compounds - that is released from recalcitrant organic matter during burial and reheating (Wellsbury et al., 1997). In concert with other microbial populations, even extremely recalcitrant carbon compounds such as alkanes can be degraded and finally fuel methanogenesis (Zengler et al., 1999).

Although this large body of evidence emphasizes the importance of methanogenesis for the subsurface biosphere, the record of methanogenic isolates from the subsurface is surprisingly spare. Most-Probable-Number enumerations of methanogens in deep marine sediments have yielded cultured methanogens in much smaller numbers than sulfate-reducing bacteria (in the Peru margin; Cragg et al., 1990) or not at all (in the Japan Sea; Cragg et al., 1992). These surveys have, to our knowledge, not lead to the description of new methanogen species from the marine subsurface. This may, in part, be a problem of slow growth of methanogens, or their extreme sensitivity to elevated redox potential during shipboard handling (Rothe and Thomm, 2000). Candidates for subsurface methanogens are versatile acetate- and methyl compound-utilizing methanogens (Methanosarcinales), that were found down to 112 m below sea level, and chemolithoautotrophic methanogens from 45 to 446 m below sea level, both from cold granitic groundwater (Kostelnikova and Pedersen, 1998). A different group of deep-subsurface methanogens is associated with hydrothermal vents. Isolations of extremely thermophilic, chemolithoautotrophic *Methanococcus* spp. from moderately warm, mixed vent fluids which are too cold to permit their growth suggest that these thermophiles thrive in the hydrothermal vent subsurface (Holden et al., 1998); their actual occurrence depth remains unknown. Chemolithoautotrophic methanogens with subsurface potential also include the hydrothermal vent genus *Methanopyrus*, the current most thermophilic methanogen with a growth temperature of up to 110 °C (Kurr et al., 1991).

Sulfate reduction and methanogenesis interact with each other in the process of anaerobic methane oxidation which takes place in a distinct sulfate-methane interface in the sediment. Methane produced in deep sediment layers diffuses upward and is oxidized in a microbial process where sulfate acts as the terminal oxidant; sulfate reduction deep in the sediment can be driven by methane oxidation (Niewohner et al., 1998). Although most studies of anaerobic methane oxidation have focused on near-shore shallow sediments, the process is also documented in deep marine sediments, including methane hydrate sites (Cragg et al., 1995, 1996). The working hypothesis that a microbial consortium of methane-oxidizing Archaea and sulfate-reducing bacteria performs anaerobic methane oxidation (Hoehler et al., 1994) is generally consistent with the available geochemical evidence. The intermediates of methane oxidation, either hydrogen alone (Hoehler et al., 1994), or - with improved energy yield - hydrogen and acetate together, can be utilized and removed by syntrophic sulfate-reducing bacteria (Valentine and Reeburgh, 2000).

These anaerobic methane-oxidizing microbial populations have not been cultured, but small subunit RNA surveys have identified a number of candidates among methanogen-related Archaea. Whenever possible, sequence profiles of anaerobic methane-oxidizing communities are corroborated with diagnostic Archaeal lipids. By their unusually light stable carbon isotopic composition, these lipids reveal their origin from biogenic methane that has been assimilated, oxidized to the level of a coenzyme-bound acetyl group, and channeled into lipid biosynthesis. Several examples exist here: 1) A combined study of 16S rRNA and lipid biomarkers at the Eel River methane seeps offshore northern California identified a novel phylogenetic cluster among the

methanogens (ANME-1) that is not specifically related to any cultured methanogen. The sequence signature was complemented by isotopically light archaeal signature lipids, which included archaeol, the general lipid of nonthermophilic methanogens and their relatives, and (2-sn-hydroxyarchaeol) that is diagnostic for members of the Methanosarcinales (Hinrichs et al., 1999). 2) Sediment cores from the Guaymas Basin hydrothermal vent sites, an unusual, sediment-covered vent site with ammonia- and methane-enriched vent fluids, yielded sequences of the ANME-1 cluster, a new side branch of the ANME-1 cluster, and members of the Methanosarcinales (Teske et al., 2000). The sequence profile was consistent with the parallel data set of isotopically light archaeol and *sn*-2-hydroxyarchaeol signatures. 4) 16S rRNA sequence signatures of a methane clathrate from the Gulf of Mexico, a methanogenic sediment from the sea floor near the Cascadia Margin, and borehole fluid from ODP site 891 offshore Oregon at the Cascadia margin, yielded predominantly sequences related to members of the *Methanosarcinales* (Lanoil et al., 2000). A fluorescent-in situ hybridization study of methane-rich Cascadia margin sediments visualized tight clusters of methanosarcinales and sulfate-reducing bacteria, the first direct look at methane-oxidizing consortia (Boetius et al., 2000). While the involvement of members of the methanosarcinales in anaerobic methane oxidation is increasingly well supported, the role of the novel ANME-1 cluster that is detected frequently in anaerobic methane oxidation sites remains open; it could be an active participant in anaerobic methane oxidation, or have an altogether different function.

Quantifying rates of sulfate reduction, methanotrophy, methanogenesis—and the total activity of the deeply buried biosphere.

Sulfate reduction by subsurface microbes is a major sink of sulfate from the world ocean. Methanogenesis by buried microbes is the principal source of methane over the 70% of Earth's surface that is covered by oceans. Anaerobic methanotrophy by more shallowly buried microbes appears to be the primary mechanism of methane destruction over that same 70% of Earth's surface (e.g. Reeburgh, 1996; D'Hondt et al., 2000). Despite their importance to the global cycles of methane and sulfate, the rates of sulfate reduction, methanogenesis and methanotrophy in deep-sea sediments are poorly known. Furthermore, although the total biomass of microbes in deeply buried marine sediments and crust has been estimated to approximate the biomass of Earth's surface, the total activity of those deeply buried microbes remains poorly constrained.

Rates and kinds of microbial activity in deep-sea sediments are primarily controlled by the availability of electron donors (usually organic matter) and electron acceptors. The most concentrated microbial activity occurs in the shallowest sediments, where organic matter and the most energy-yielding electron acceptors [O_2, Mn(IV)-oxides, NO_3^-, Fe(III)-oxides) are most abundant. Rates of sulfate reduction are highest in deeper sediments, where electron acceptors with higher energy yields are absent. Rates of methanogenesis are highest in deeper organic-rich sediments that have already been stripped of dissolved sulfate by microbial activity. Because sulfate diffuses down into the sediment from the overlying seawater, the rate of sulfate reduction increases where the distance to the sulfate-reducing zone decreases and concentrations of organic matter in that zone increase. Similarly, the rate of methanogenesis is higher where the concentration of organic matter in the methanogenic zone is greater.

In relatively cool sediments, to a first approximation, total microbial activity varies directly with the flux of organic matter to the sediment surface from the photosynthetic zone of the overlying surface ocean; when the organic flux to the sediment is high, sedimentary microbial activity in the sediment is also high. The flux of organic matter to the sediment surface varies as a function of (1) the distance from the upper water column (the photosynthetic zone) to the sediment surface (Berger et al., 1989), (2) biomass production in the photosynthetic zone (Eppley and Peterson, 1979), and (3) the trophic structure of the marine ecosystem in the upper water column (Michaels and Silver,

1988). The second and third variables are closely linked. Biomass production and ecosystem structure are highly correlated and appear to be affected by dissolved nutrient availability. A recent modeling study suggests that, at least in the modern ocean, these biological variables are united by sea-surface temperature (Laws et al., 1999); in that study, 86% of the variance in the proportion of total production that sinks from the surface ocean is correlated to temperature.

At least some of these properties can change on human timescales and due to human activities. For example, the long-term warming of Earth's atmosphere over the last few decades has been paralleled by ocean warming (Levitus et al., 2000) and may eventually warm large areas of the ocean surface. Warming of the ocean surface may in turn cause widespread changes in marine trophic structure and biomass production. Even on short timescales, in relatively large key areas, such as large river deltas and other nearshore environments, the organic flux to deep-sea sediments may be changed by human agricultural activity. Such changes are due to both the increased organic load of agriculturally influenced rivers and the increased flux of dissolved nutrients to the sea. For example, the annual flux of dissolved phosphate to the ocean has doubled since the advent of widespread fertilization (Pilson, 1998).

The total subsurface activity that relies on any single microbial metabolic pathway, such as sulfate reduction or methanogenesis, depends on (1) how much organic matter survives O_2-fueled microbial activity at the sediment surface and gets buried, and (2) how much sulfate diffuses into the sediment to be reduced. The first parameter depends on both the dissolved oxygen concentration of the deep sea and the rate of sediment accumulation. The second parameter depends on the diffusivity of the sediment and the distance over which the ion must diffuse. The diffusional distance depends on sedimentation rate, diffusivity depends on the porosity and tortuousity of the sediment, and the oxygen content of the deep sea varies with both the temperature of the deep sea and the amount of oxygen that has already been stripped from the deep water by microbial activity.

At any given site, depth-integrated rates of sulfate reduction can be estimated from porewater geochemical profiles and core-based estimates of effective diffusivity. After local rates have been determined for large numbers of sites, a predictive model can be developed by correlating those rates to relevant site-variable properties (such as water depth, sediment type, productivity of the overlying water column, seasurface temperature, etc.) (e.g., Canfield, 1991). Such models could be used for estimating global or regional rates by using the fit of rate estimates to geographically continuous variables (i.e., water depth, sediment type, productivity of the overlying water column, seasurface temperature) to extrapolate over the area of the entire deep seafloor. These models could also be used to predict the sensitivity of subsurface sulfate reduction to widespread (global or regional) changes in such oceanographic properties as seasurface temperature, deep-sea temperature, or ecosystem structure or nutrient availability in the upper water column.

In principle, similar techniques could also be used to estimate local, regional and global rates of methanogenesis and anaerobic methanotrophy in deep-sea sediments. Unfortunately, measured methane concentrations in recovered drill cores often under-represent in situ concentrations because methane is outgassed as the cores are brought up through the water column and ambient pressure decreases (Dickens et al., 1997). Fortunately, the recent development and ODP deployment of a downhole high-pressure sampling apparatus now allows this problem to be circumvented (albeit at a moderately high cost in drilling time and effort) by recovering cores at *in situ* pressures (Dickens et al., 1997). Over the next few years, utilization of this apparatus on selected ODP cruises should allow measurements of methane concentrations that are accurate enough to directly estimate rates of methanogenesis and methanotrophy for key regions of the world ocean. Furthermore, two recent intellectual developments collectively indicate that it should be possible to indirectly estimate rates of anaerobic methanotrophy from downhole profiles of dissolved sulfate. The first of these developments is the recent recognition that almost all of the methane diffusing up through deep-sea sediments is destroyed by sulfate-reducing microbial consortia at the buried interface between

methane-rich sediments and overlying sulfate-rich sediments (e.g., Hinrichs et al., 1999; D'Hondt et al., 2000). The second development is the recognition that sulfate concentrations linearly decline from the sediment-water interface to the depth of anaerobic methanotrophy in regions of abundant subsurface methane (such as methane clathrates) (Borowski et al., 1999). This relationship implies that virtually all of the sulfate entering the sediment diffuses to the sulfate/methane interface, where it is reduced by anaerobic methanotrophy.

The census of DSDP/ODP data by Borowski et al. (1999) and our more recent census of nearly 600 ODP sites suggest that this linear relationship of downhole sulfate and methane profiles is characteristic of most geochemical records in all the major methanogenic regions that have been drilled by ODP (Fig. 3). The constancy of this relationship effectively indicates that local, regional and global rates of anaerobic methanotrophy may be estimated from rates of subsurface sulfate reduction in methane-rich regions.

Almost all microbial activity in deeply buried marine sediments is supported by either sulfate reduction or methanogenesis [because dissolved sulfate is nearly 50 times as abundant as all other external electron acceptors combined in deep-sea sediments (data from Froelich et al., 1979 and Pilson, 1998) and fermentation and methanogenesis are the only avenues of microbial activity that remain after all external electron acceptors have been reduced]. Consequently, to a first approximation, total rates of microbial activity in the buried biosphere of deep-sea sediments can be estimated from subsurface rates of sulfate reduction and anaerobic methanotrophy, if subsurface methanotrophy is assumed to be in balance with methanogenesis in the underlying sediments.

The geographic density of Deep Sea Drilling Project (DSDP) and ODP pore-water data appears sufficient to estimate profiles of sulfate and methane concentrations in deep-sea sediments throughout most of the world's ocean basins. Our preliminary census of DSDP Leg 1 through ODP Leg 170 indicates that pore-water sulfate data are available from well over 600 DSDP and ODP Sites. Furthermore, because the methane concentrations of recovered sediments are routinely measured for safety reasons on all ODP drilling legs, methane concentration data (measured as headspace ppm) are available for nearly all ODP Sites (there are nearly 600 ODP Sites). Methane concentrations in deep-sea sediments range from 0 ppm in shallow sediments and at many mid-ocean sites to values approaching 1,000,000 ppm in sub-surface sediments of some near-shore basins (at least 30 ODP Sites have peak headspace methane values of 900,000 ppm or higher).

Pore-water concentrations of inorganic electron acceptors other than sulfate are more poorly documented in the DSDP and ODP databases (see fig. 3 for a subset of the ODP data coverage). Our preliminary census of those databases shows that they include sedimentary manganese data for approximately 100 sites and sedimentary iron data for a few tens of sites. The sedimentary records of only seven ODP Sites are represented by nitrate data in the DSDP and ODP geochemical databases. It should be possible to find in the peer-reviewed literature more data on the concentrations of these electron acceptors in pore waters from DSDP and ODP Sites. However, it will probably be necessary to measure downhole profiles of dissolved NO_3^-, Mn(IV)-oxides, and Fe(III)-oxides at many more sites before robust global estimates of subsurface nitrate, manganese and iron reduction can be developed.

Third Project: Organic signatures of present and past microbial processes in Earth's subsurface.

Chemical approaches will complement traditional isolation and characterization of microbes inhabiting the deep marine subsurface and their characterization by small subunit 16S rRNA sequence techniques. In principle, two analytical avenues with distinct target compounds – (1) geologically relatively stable biomarkers and (2) their mostly unstable biological

precursors - promise to provide independent insights on microbial ecology, on patterns of carbon flow, and on populations of living microorganisms. Chemical techniques for the study of environmental microbiology provide information on a large variety of microorganisms whereas traditional isolation techniques are limited to the small percentage of cultivable organisms.

The first group of compounds comprises extractable, volatile lipids that are derived by slight chemical transformation from their polar and, under environmental conditions, highly labile biological precursor molecules produced by archaea and bacteria. The volatile lipids are commonly analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Many of these compounds serve as chemotaxonomic markers for the detection of certain microbes in complex communities. Their mostly conveniently determinable carbon isotopic composition (e.g. Hayes et al., 1989) provides information on their carbon fixation pathway and on the carbon source utilized (Hayes, 1993). For example, archaea and a few deeply-branching bacteria synthesize distinct glycerol diether lipids with isoprenoid and non-isoprenoid alkyl moieties, respectively (Langworthy et al., 1982; 1983). Bacteria synthesize a large variety of fatty acids as part of their phospholipid membranes. Their structural features can often provide important insights on the structure of the microbial community, for example on environmental populations of sulfate-reducers (e.g. Taylor and Parkes, 1983). An additional group of exclusive prokaryotic products is the hopanoids, again structural elements of cell membranes from many bacteria. Although this group of compounds has to date exclusively been isolated from aerobic bacteria (Rohmer et al., 1984; Farrimond et al., 1998), their occurrence is probably not categorically limited to aerobes. In addition, recent developments of new analytical protocols (Hopmans et al., 2000) now allow determination of intact archaeal tetraethers by liquid-chromatography-mass spectrometry (LC-MS). This technique has revealed a previously unknown diversity and abundance of these potentially powerful biomarkers for the study of the deeply buried biosphere (Schouten et al., in press).

Lipid-biomarker approaches to the understanding of microbial processes and ecology have been particularly fruitful in near-surface environments such as Hot Springs (e.g. Zeng et al., 1992a,b), hydrothermal vents (Teske et al., 2000), microbial mats (Ward et al., 1989) and methane seeps (e.g. Hinrichs et al., 1999; 2000; Pancost et al., 2000). Especially in methane-rich environments, the structural features of archaeal and bacterial biomarkers in combination with their carbon isotopic compositions provided a previously unobtainable view of complex consortia of anaerobic methane-oxidizers (Hinrichs et al., 1999; 2000) which have escaped cultivation in spite of multiple attempts. These findings set the stage for a new era in the study of these biogeochemically significant microbes. Particularly the combination of molecular-isotopic lines of evidence with modern, culture-independent microbiological techniques has proven to be fruitful in the elucidation of the anaerobic oxidation of methane (AOM) and the participating microorganisms (Hinrichs et al., 1999; Boetius et al., 2000).

In deep-subsurface sediments, however, such an integrated approach faces potentially significant challenges that are caused by the decline of microbial activity with burial depth. Such a decline with depth should lead to an increasingly disadvantageous signal-to-noise ratio for the study of microbial products produced by a presently active *in situ* community. This signal-to-noise issue is exacerbated by the accumulated remnants of microbial biomass from earlier (nearer-surface) microbial activity. One means of overcoming these problems is to search for molecular biomarker signals related to active microbial communities at sediment horizons with distinct chemical features. In such microenvironments, the presence certain types of microbial activity can be predicted with some confidence. One example for such a focused strategy is the successful search for products of anaerobic methanotrophic microbes at the transition zone at ~ 2 m depth in anoxic sediments at Kattegat (Bian et al., submitted) – the sediment horizon defining the boundary between sulfate-reduction zone and methanogenesis where anaerobic oxidation is commonly observed (e.g. Iversen and Jørgensen, 1985). Methane-oxidation rates in that horizon are several orders of magnitudes lower than at methane-hydrate-related seep sites, where near-surface sediments contain significant populations of active methanotrophic communities which facilitate the study of their products.

Nevertheless, in those sediments with presumably less-active communities of lower density, the combination of geochemical search criteria and the very distinct isotopic signature allowed the characterization of methanotrophic products such as crocetane with a carbon isotopic composition of ~-100‰. This lipid has never been isolated from a cultured microorganism, but is presumably of archaeal origin and has been found to date only in environments with active AOM.

The understanding of the role of deeply buried anaerobic methanotrophic communities and their role for their importance in the deep biosphere is one major objective in our research plan. In the case of methanotrophy the "isotopic labeling" caused by consumption of strongly ^{13}C-depleted methane (e.g. Kvenvolden, 1995) at sediment horizons in which the chemical environment favors AOM, e.g. the deep, sulfate-rich brine incursion intertwining into methane-laden sediments (targeted during Leg 201; D'Hondt et al., 1999), will guarantee the distinction of methanotrophic products from other microbial biomarker products that are unrelated to *in situ* activity.

In contrast, the group of relatively stable biomarkers may be only of limited use in the study of microbial processes that are not linked to methane-consumption and therefore do not possess a distinct isotopic signal. In fact, the commonly praised stability of biomarkers impedes the differentiation between a signal related to an active community and one that was active possibly a million years ago in a much shallower sediment or even in the water column. Therefore, we envision an approach that combines the analysis of established, stable biomarker as well as more labile microbial products that, to a first approximation, will represent the living stock of prokaryotes thriving in deeply buried sediments.

One particularly labile group of abundant building blocks for bacterial cell membranes – phospholipids – is widely used in environmental, microbiological and biogeochemical studies to assess the amounts of living bacterial biomass (e.g. White et al., 1979, Balkwill et al., 1988) and to characterize microbial communities based on chemotaxonomic information embedded in these compounds. These compounds are associated with living microbes because the phospholipid-head groups are labile to hydrolysis soon after cell death (White et al., 1979). In addition to information on the amount of living biomass, the distribution and structural features of phospholipids carry important information on the structure of a microbial community. Traditional approaches to the analysis of bacterial phospholipid isolate and quantify the phospholipids to provide estimates of living biomass (e.g. White et al., 1997). The ester-linked fatty acids become GC-amenable as fatty acid methyl esters (FAMEs) following mild alkaline trans-methylation (Fang and Findlay, 1996). Their structural features, such as double-bond configuration, methyl-branching patterns, and chain lengths of FAMEs provide chemotaxonomic information on participants in microbial consortia (e.g., Boschker et al., 1998). An analogous approach is often taken for archaeal phospholipids such as glycerol di- and tetraethers, the corresponding membrane elements in archaea, which possess a diverse group of ether-linked, often genera-specific, isopranyl moieties (e.g. Langworthy et al., 1982; Kates, 1993). In environmental studies in marine and sedimentary habitats, however, only the ether-linked isopranyl moieties are commonly analyzed upon chemical isolation and degradation procedures, with both the phospholipid head group and the glycerol being removed (e.g. DeLong et al, 1998, Hoefs et al., 1997).

However, these procedures do not permit coupled analysis of the distinct structural features of the associated phospholipid head groups of bacterial and archaeal products, which themselves bear an additional wealth of chemotaxonomic information (White et al, 1979; Kates, 1993). The bacterial fatty acids and archaeal ispopranyl moieties determined as such are a mixture of compounds derived from an assemblage of distinct intact phospholipids from different microbial populations. As a result, information on the microbial population is lost, and the specificity of the phospholipid-bound microbial marker determination to estimate biomass and determine community structure is lost.

Recently, a different path was chosen for the analysis of intact bacterial phospholipids by direct coupling of liquid chromatography to mass spectrometry (LC-MS; e.g. Kim et al., 1994; Karllsson et al., 1996; Fang and Barcelona, 1998; Fang et al., 2000). This approach allows analysis of the intact phospholipids and therefore simultaneous determination of the ester-bound fatty acids and the phosphorus-containing head group, an approach which, due to its increased specificity, is superior to traditional phospholipid analysis protocols as outlined below (Fang et al., 2000). The phospholipid head group is a phosphate linked to the *sn*-3-glycerol position (*sn*-1 in case of archaeal phospholipids) by an ester bond and, by a second ester bond, to a variety of additional substituents such as amino acids (phosphatidylserine, PS), amines (phosphatidylethanolamine, PE; phosphatidylcholine, PC), sugars (phosphatidylinositol, PI), and glycerol (phosphatidylglycerol, PG). Analogous rapid approaches using LC-MS for the analysis intact archaeal membrane phospholipids have not yet been attempted, although the chemical and chromatographic features of these compounds predict successful analysis by very similar analytical protocols. For example, Hopmans et al. (2000) have successfully used LC-MS techniques to analyze intact archaeal tetraethers from which the polar head groups had been removed. More recently, this techniques has been successfully applied to the study of archaeal and bacterial diversity in marine and lacustrine sedimentary environments (Schouten et al., in press). Notably, in a single study, this advancement revealed previously unknown details about the structural diversity of intact archaeal ether lipids.

The analysis of intact bacterial phospholipids has been pioneered by H. Rütters (University of Oldenburg, Ph.D. thesis in progress, pers. communication, 2000), who demonstrated how their characterization in anoxic, near-surface sediments allows a chemotaxonomic description of a complex community of sulfate-reducing bacteria. The extent of information about the composition of a complex natural community that can be derived in this way is directly linked to the extent of available information about the distribution of phospholipids in isolates and to an appropriate numerical treatment of complex data (Fang et al., 2000). Despite the present paucity of data derived from cultured organisms (the diversity of intact phospholipid distributions may resemble that found in assemblage patterns of DNA), other features of phospholipids confirm their importance as analytical targets. Specifically, the phospholipids provide a chemical indicator for living biomass and provide access to isotopic information about that material. Such points are of great importance in the thorough exploration of an unknown microbial habitat. Integrated in a program with other molecular-isotopic and culture-independent microbiological tools discussed earlier, the analysis of intact bacterial and archaeal phospholipids has great potential to open new dimensions for the study of environmental microbial habitats. For example, interesting information can be expected from comparisons of the sedimentary inventories of archaeal glycerolethers and of bacterial acyl glycerides with the respective head group-containing phospholipid precursors. Since the head group is readily lost after an organism's decay (White et al., 1979), these relative amounts may bear important details on the turnover rate of certain microbes and/or microbial communities and thereby on their overall activity.

The unique analytical facilities being assembled at WHOI will allow the study of complex lipids such as intact phospholipids and their head-group-liberated analogs. The recently acquired moving-wire interface coupled to an isotope-ratio mass spectrometer will allow on-line and off-line analysis of these microbial biomarkers (n. b., the moving-wire interface is performing well in the analyses of intact nucleic acids; the successful analysis of sub-nanomole-C quantities of phospholipids can be predicted with confidence). The moving-wire interface in Woods Hole (Brand and Dobberstein, 1996) is a prototype and is the only such instrument available in institutional laboratories, world-wide. The analytical capability to study simultaneously the structural and isotopic compositions of classes of compounds that were previously largely ignored or analytically inaccessible in studies of environmental microbiology and biogeochemistry opens new doors to the study of microbial habitats, not only in deeply buried sediments, and is of major relevance for astrobiological research. No other single analytical approach offers a comparable amount of information in the study of unknown microbial habitats in terms of microbial diversity, amount and possibly activity of living biomass, and possible substrates and metabolic pathways.

In principle, each of the targeted biomarkers can serve as an indicator for extraterrestrial subsurface life. In the case of a hypothetic living extraterrestrial biosphere, tests for life using biomarkers such as those described in this section would likely be trivial. This task would be more challenging for past extraterrestrial biospheres that may have become extinct billions of years ago. Here, examples of recoveries of chemotaxonomically diagnostic biomarkers from 2.7 billion years old, highly metamorphic rocks on earth (Summons et al., 1999; Brocks et al., 1999) suggest that such information may be preserved on other planets as well.

Microbiological approaches to these research projects.

Contamination tests.—

Contamination tests by ODP Leg 185 shipboard scientists now provide robust shipboard protocols for assessing whether or not the microbial assemblages recovered from cored deep-sea sediments are indigenous to those sediments or are potential contaminants. These protocols use perflorocarbons as contamination tracers and are based on continental drilling protocols (Harvey *et al.*, 1989; Senum and Dietz, 1991) introduced to the ODP by its Deep Biosphere Program Planning Group. Two proponents of this Astrobiology Institute proposal, David Smith and Arthur Spivack, successfully pioneered these tests on Leg 185 (Smith *et al.*, 2000 a & b) and again successfully applied them on Leg 190. Their studies demonstrated that core interiors are typically uncontaminated. With their shipboard colleagues, they also demonstrated the feasibility and power of paired microbiological and geochemical studies on-board the *JOIDES Resolution.*

Sample retrieval.—

Fresh ODP cores will be sampled within one or two hours after arrival on the ship for cultivable microorganisms. Cultivations and molecular analyses require fresh samples, the main reason why a microbiology lab is indispensable directly on the ship. Within a few hours of core recovery, microbial communities undergo drastic alteration in composition (Rochelle et al., 1992), and do not allow inferences about their original microbial populations.

Subcores from sediments will be placed in an anaerobic chamber filled with an oxygen-free N_2/CO_2 gas mixture and subsampled with sterile metal or plastic sampling cores. The inner portions of the subcores which do not show the presence of contaminant tracers (i.e. fluorescent dye, perfluorocarbon or microscopic beads) will be selected for cultivations and molecular and organic biomarker analyses. -Solid rock and highly compacted sediments will be sampled with the use of a rock-splitter. The rock splitter will be placed in the anaerobic chamber and subsamples from the interior of the rock will be used in further analyses.

Cultivations.—

Cultivations are our principal handle to demonstrate microbial diversity, not only under a taxonomic viewpoint (different isolates with different 16S rRNA sequences), but actually as a proof that bacteria of specific metabolic types, according to the specificity of the media, exist in deep sediment and rock samples. 16S rRNA surveys are not always convincing in that respect, especially if the connection between sequence type and metabolism is ambiguous. Cultivations will focus on anaerobic prokaryotes, since oxygen disappears a few centimeters under the sediment-water interface. Inoculations and transfer of oxygen-sensitive anaerobes into anaerobic medium must proceed without delay from freshly retrieved cores, unaffected by prolonged oxygen exposure. For example, mesophilic sulfate-reducing bacteria begin to show oxygen damage after a few hours of air exposure (Cypionka et al. 1985, Fukui and Takii, 1990).

Quantification of cultivable microbial populations will be done with the Most Probable Number (MPN) technique (American Public Health Association, 1989). In short, homogenized sample materials will be serially diluted, and the pattern of positive samples in three or more parallel dilution series will be used to infer the most likely population density of bacteria in the original sample. The cultures in the highest positive dilutions will represent bacteria which occur in relatively high densities in the sampled habitat. Sometimes these dominant populations are novel and unexpected (Teske et al., 1998). We will use Hungate techniques (Breznak and Costilow, 1994) and anaerobic media under N_2/CO_2 gas mixtures for cultivations of sulfate reducers (carbonate-buffered as described by Widdel and Bak, 1991), iron reducers (specifications in Vargas et al., 1998), and methanogens (Balch et al., 1979).

If surplus deep sediments are available for this purpose, sterilized sediment portions can be added to MPN culture tubes on the shipboard laboratory. Such additions increase the sensitivity of MPN counts by factors of 100 to 1000 and thus push MPN counts close to the actual *in situ* bacterial densities (Vester and Ingvorsen, 1998). The reason for this increased efficiency of natural media is not only the presence of a substrate spectrum that matches the needs of the *in situ* populations, but also the availability of particles which facilitate growth of many "difficult", surface-associated or clump-forming bacteria which do not grow in homogeneous suspension (Widdel et al., 1983).

Temperature is a decisive factor in cultivations. The heat sensitivity of most mesophilic marine bacteria was recognized early (ZoBell, 1941). Generally, incubation temperatures over 20-25°C have to be avoided, except in cultivations which aim explicitly at thermophiles and hyperthermophiles. Cold can be tolerated much more easily. Thermophilic anaerobes cease to grow during cold spells and also become less sensitive to oxygen exposure; they disperse widely through cold water and colonize new habitats (Stetter et al., 1993), or they survive as spores (Isaksen et al., 1994).

Pressure is an important factor in cultivations from deep-sea cores. Obligately barophilic bacteria, so far isolated from depths greater than 6000 m, require cultivation under elevated pressure (Yayanos et al., 1981), and the ability to reproduce at high pressure is considered a hallmark characteristic of barophilic bacteria (Yayanos et al., 1982). Facultatively barophilic bacteria also grow at atmospheric pressure, but have their growth optimum at elevated pressure—frequently the *in situ* pressure of the depth they were isolated from (see compilation in DeLong et al., 1997). Acquisition of high-pressure culture vessels will allow for the cultivation of microbes from samples collected from extreme depths. Samples collected at more moderate depths may not require the use of such vessels.

Molecular approaches.—

In order to detect microbial populations which do not grow under the culture conditions employed, cultivation surveys have to be complemented with molecular, DNA- and rRNA-based approaches. Samples for molecular work have to be taken within a short time after core retrieval, in parallel with samples for cultivations. After just a few hours, cored sediments undergo drastic alteration of their microbial community composition (Rochelle et al., 1992), and do not allow inferences about their original microbial populations. Core samples for nucleic acid isolation can be kept deep-frozen at - 80°C or in liquid nitrogen for months until arrival in the home laboratory and further processing.

The potentially large number of ODP core samples will require high-throughput techniques for nucleic acid based microbial community analysis. We plan to use Denaturing Gradient Gel Electrophoresis (DGGE) as a means to monitor changes in the microbial community structure over the extended spatial gradients of ODP cores. DGGE separates homologous double stranded DNA

fragments according to their melting domain stability and primary sequence. DGGE is most commonly used to separate mixtures of double-stranded PCR products from PCR-amplification of environmental DNA mixtures (Muyzer et al. 1993). The PCR products migrate into an acrylamide gel of increasing denaturing concentration, melt at different points in the gradient, stop their migration, and sort themselves into distinct bands of one sequence type each, thus avoiding the cloning bias inherent in shotgun cloning separation of mixed PCR products (Muyzer et al. 1993, 1997). Side by side comparisons of DGGE patterns from different samples over the length of a core make it possible to track and identify individual microbial populations in a spatially changing pattern of microbial community composition; as demonstrated with millimeter layers of a microbial mat core (Teske et al. 1998). Individual DGGE bands can be eluted from the gel, reamplified, and sequenced for phylogenetic identification, or DGGE patterns can be transferred to a nitrocellulose carrier membrane for hybridization with genus- or group-specific probes (reviewed in Muyzer and Smalla 1998).

A variety of other nucleic-acid based survey techniques can be applied to nucleic acids extracted from core samples. These techniques include terminal restriction fragment length polymorphism (T-RFLP; Liu et al., 1997). This method is suitable for side-by-side comparisons and identification of metabolic key genes such as dissimililatory sulfite reductase, the key gene of sulfate reduction (Wagner et al., 1998) which remains inaccessible to DGGE due to insufficient sequence conservation (D. Stahl, pers. comm.).

These molecular analyses do not have to be performed in the shipboard laboratory. For practical reasons, core samples for nucleic acid isolation can be kept deep-frozen for months until arrival and further processing in the home laboratory. Here, the specialized, expensive, and partially fragile equipment will be available, along with more time than the sample retrieval schedule of the JOIDES Resolution can accommodate. However, good -80°C and liquid nitrogen storage facilities will be necessary on board to preserve samples for nucleic acid extraction and molecular analyses.

Microscopic observations.—

Direct microscopic observation is an indispensable source of information about microbial community composition. It requires a well-equipped microscope on the ship. Generally, direct observation is one of the first steps (never the only step) in identifying microbial community members, using clues from morphology or chemotactic behavior for a tentative identification. Some microbial groups are recognizable by autofluorescence. For example methanogens show a distinct blue-green autofluorescence. Different fluorescent stains have to be used to detect non-fluorescent and inconspicuous microbial cells. General DNA-stains such as Acridine orange allow total community cell counts (Hobbie et al. 1979). When microbial activities and intracellular rRNA levels are high, fluorescent in-situ hybridization (FISH) of individual cells with rRNA-targeted fluorescent oligonucleotide probes (Amann et al. 1995) allows to analyze the phylogenetic community composition of sediment samples (Llobet-Brossa et al. 1998). The caveat has to be kept in mind that many cells in natural microbial assemblages show significantly diminished FISH fluorescence, due to low activity and rRNA content (Ramsing et al. 1996); this could apply especially to deep sediments with low nutrient content.

Microbial activity determinations.—

The use of radioisotopes in microbial ecology has led to both qualitative and quantitative discoveries which were possible only due to the extreme sensitivity of these detection methods. Radioisotopes can be used to determine which individual cells are active by the use of microautoradiography [a technique that Brock (1966) introduced into the field of microbial ecology]. In addition to cell-specific assays, radiolabeled compounds can be used to determine the rates of bulk processes such as methanogenesis and sulfate reduction. ^{3}H-thymidine has been widely used to determine DNA synthesis rates of a variety of microorganisms. While there is evidence that

many anaerobes do not incorporate thymidine there are cases where it has been successfully employed (Winding 1992, Wellsbury et al. 1993). Other radiolabeled precursors have also been used to measure microbial growth (e.g., ^{3}H -leucine for protein synthesis rates).

Biogeochemical approaches to these research projects.

Quantitative and isotopic analysis of microbial molecular biomarkers.—

The molecular biogeochemical component of the proposed research plan will focus on the extractable organic fraction of microbial origin and will be combined of analyses of relatively stable biomarkers and on intact phospholipids and their head-group liberated derivatives. Always, these analyses will be performed on samples for which complementary traditional and culture-independent microbiological data will be generated in parallel.

For the analysis of stable biomarkers, we will employ established protocols developed in our laboratories (e.g. Hinrichs et al., 1999, 2000). These compounds are largely GC-amenable. Presumably, this group of compounds will consist of archaea-specific ether lipids such as archaeol and hydroxyarchaeol (e.g., Hinrichs et al., 1999), irregular isoprenoid hydrocarbons (Elvert et al., 1999; Thiel *et al.*, 1999; Hinrichs et al., 2000; Bian et al., submitted), and products of sulfate-reducing bacteria such as mono- and dialkylglycerolethers (Hinrichs et al., 2000), and free fatty acids (e.g., Taylor and Parkes, 1983; Dowling *et al.*, 1986). In addition to environments with active anaerobic methanotrophy, bacterial ether lipids are expected to be particularly abundant in geothermally heated subsurface environments, where phylogenetically deeply-branching relatives of known producers may be abundant (e.g. L'Haridon et al., 1995). The compound-specific isotopic compositions (Hayes et al., 1989) of these groups can indicate mechanistic interactions within microbial communities. For the study of deeply buried methanotrophic communities – one major objective on the forthcoming Peru margin expedition ODP Leg 201 - the isotopic composition will unambiguously identify products from methane-consuming microbes. In such a case, the structural features of ^{13}C-depleted compounds in combination with phylogenetic analyses will allow the culture-independent characterization of deeply buried anaerobic methanotrophs and their comparison to near-surface communities.

The isotopic compositions can also be used, in combination with those of total organic carbon and carbon-containing solutes in pore waters, respectively, to identify carbon flows within the microbial community. As analyses accumulate from a substantial number of samples and environments, abundances of biomarkers (incl. ntact phosholipids) will be tested for correlation with abundances of organisms represented by specific 16S rRNA sequences, and MPN-dilutions. These tests should allow identification of relationships between biomarkers and phylogenetic types. In addition, products of cultivation experiments of deep subsurface microbes will be continuously monitored for biomarkers incl. phospholipids in order to allow both chemotaxonomic assignment of biomarker distributions observed in environmental samples to certain microorganisms.

All necessary equipment is available for these analyses. Non-phospholipid biomarkers will be extracted from aliquots of the freeze-dried and homogenized sediment by accelerated solvent extraction (ASE, Dionex) with a solvent mixture of DCM/MeOH (9:1). The solvent will be evaporated under a stream of N_2, using a Zymark –turbo evaporator™. When needed, extracts for analysis will be separated into fractions (hydrocarbons, ketones and esters, alcohols, multifunctional compounds, and fatty acids) using commercial glass cartridges (Supelclean™ LC-NH2) eluted with solvent mixtures of increasing polarity according to a protocol developed in our group (Hinrichs et al., 2000). Prior to gas-chromatographic analyses, fractions containing alcohols and fatty acids will be derivatized to produce TMS-ethers and TMS- or methyl esters, respectively. Analytes will be quantified relative to internal standards added to samples prior to extraction.

Analyses of non-phospholipid biomarkers cultivated biomass will employ protocols developed in our group and successfully tested with sub-mg amounts of biomass from cultured archaea and bacteria. Briefly, they involve cell lysis by sonication of cell material with combusted, analysis-grade sand in MeOH (2.5% HCl) and subsequent heating of the mixture to 70°C for 16h to remove the phospholipid-head groups, with subsequent extraction by sonication in DCM/MeOH (9:1, four times). Novel and previously uncharacterized compounds (in particular those related to glycerol-ether lipids) will be identified by comparison to reference standards and to materials from pure cultures of archaea and deeply-branching bacteria.

Starting in year 2002, distribution and abundance of intact phospholipids of archaeal and bacterial origin will be monitored on a regular basis from samples retrieved from different boreholes. Their abundance will provide independent information on the density of deeply buried microbial communities. Complementary monitoring of the abundance and distribution of related analogs that lost their head group already in the sediment (representative for decayed biomass) will establish a data base that may contain valuable information on the activity of a microbial community. For example, in an extremely slow growing community under low-energy conditions, the ratio of phospholipids to their analogs without head group is expected to be large relative to the ratio in a fast-growing community. Statistical approaches will be employed to relate their distribution to those of relevant cultured organisms (Fang et al., 2000; H. Rütters, pers. commun., 2000) and to other relevant data indicative for the microbial ecology, which will be generated from comparable samples. Stable carbon isotopic compositions of phospholipids and glycerolether and –acyl derivatives will be determined on-line by transferring the LC effluent to the moving-wire interface (Brand and Dobberstein, 1996) coupled to a Finnigan MAT 252 isotope ratio mass spectrometer, and off-line when poor LC compound resolution requires additional clean-up steps by solid phase extraction or thin layer chromatography.

Since no published material exists on studies of microbial polar lipids such as intact phospholipids in marine sediments, in year 1 a particular focus will be given to development of analytical protocols under use of standards, cultured microorganisms, and marine sediments with abundant microbial biomass. The specification of the Thermoquest Deca XP liquid chromatograph coupled to an ion trap mass spectrometer allows two ionization modes, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Phospholipids have been successfully studied using ESI (Kim et al., 1994; Karlsson et al., 1996; Fang and Barcelona, 1998), whereas for bacteriohopanepolyols (Fox et al., 1998) and archaeal tetraethers from which the head groups had been removed (Hopmans et al., 2000) good experience has been made using APCI, in the latter case even in sediment samples (Schouten et al., in press). Additional development is planned for the optimization of extraction procedures of intact phospholipids. To keep up with high numbers of samples we intend to establish focused protocols for rapid analysis like those applied for non-phospholipid biomarkers. For that purpose we will compare established extraction procedures for the analysis of intact phospholipids (modified Bligh and Dyer: e.g., White et al., 1979a) with the automated extraction system in our laboratory, which allows variation of temperature, pressure, and solvent mixtures. Different LC stationary phases will be tested for the optimization of analytical conditions for different analytes.

Hydrogen analyses and experiments.—

There are two interrelated components to the proposed work involving hydrogen. The first component includes the quantification of hydrogen in deeply buried sediments. In order to understand the dynamics of hydrogen and its relationship to environmental conditions, a number of other chemical constituents must be determined in addition to the hydrogen. Therefore, we intend to measure pore fluid acetate, formate, methane, alkalinity, sulfate, sulfide, and calculate pH (based on in situ T, P, alkalinity and Ca^{+2}) as part of our planned ODP drilling leg. We will also determine the isotopic composition (D/H) of the pore fluid hydrogen in order to quantify the relative roles of

biological and abiological hydrogen production. While the existing protocols allow for the reliable determination of dissolved sedimentary hydrogen, further refinements of the methods in a shore-based laboratory are warranted. These methodological studies will focus on the development of vessels for the quantitative storage of hydrogen gas produced during ship-board incubations and the development of low-level hydrogen gas standards for both ship-board and shore-based analysis.

The second component involves experimental manipulations of sediment samples to determine the factors critical in controlling hydrogen concentrations in sediments. The experiments have two principle goals, 1) quantifying the role of hydrogen production via fermentation, inorganic reactions, and abiological diagenesis of organic matter, and 2) testing the theoretical relationships between hydrogen concentration, pore fluid composition and temperature. Specifically we intend to quantify the relationship between abiological hydrogen production, temperature and sediment composition. We will employ two general approaches to this problem.

The first approach involves inhibiting biological production (sterilizing samples by autoclaving) followed by purging of all the pre-existing hydrogen. The samples will then be heated at in-situ temperatures and hydrogen production will be quantified. We conducted preliminary experiments of this type during ODP Leg 190 and, as stated above, observed the production of hydrogen at higher temperatures (Spivack et al. 2000). We intend to refine and expand these experiments based on the results of the method development. In these experiments we also intend to determine the hydrogen isotope systematics that are associated with abiological production. This information will be used in the interpretation of the pore fluid hydrogen isotope data to differentiate biogenic (produced via fermentation) and abiogenic hydrogen (as separately produced from both inorganic and organic substrates).

In the second group of experiments we intend to apply techniques that have been successfully employed in near shore shallowly buried sediments (Hoehler et al., 1998). Specifically, we will conduct incubation enrichment experiments to test the dependence of pore fluid hydrogen on the concentration of specific substrates (sulfate, sulfide, acetate, carbon dioxide and methane), pH and temperature.

Analyses of nitrogen species and isotopes.—

The long-term goal of the proposed work on nitrogen is to quantify rates of nitrogen transformations and net fluxes in the deeply buried sediments and to relate these rates to bacterial abundance and community structure. Our approach will be fourfold involving the measurement of dissolved ammonium, ammonium nitrogen isotope ratios, and dissolved dinitrogen (N_2). In addition we will look for evidence of active biological nitrogen fixation utilizing molecular techniques. Estimates of net fluxes and reaction rates will be based on the diffusion modeling of measured ammonium profiles (Berner, 1980). Since ammonium behaves as a strong ion exchanger, this modeling will require the determination of ammonium adsorption coefficients along with porosity and tortuosity. Tortuosity will be inferred from sediment electrical resistivity measurements. Resistivity, ammonium concentrations and porosity are routinely determined on the *JOIDES Resolution*. Adsorption coefficients will be determined in shore-based experiments.

As described above, nitrogen isotopes may enable the quantification of gross rates. Our first goal with regards to the development of nitrogen isotopes for this purpose will be the determination of a pore fluid ammonium nitrogen isotope profile. This will allow us to evaluate the sensitivity of this approach for partitioning net transformations into its components (degradation of detrital nitrogen, uptake of ammonia, and microbial production of refractory nitrogen containing compounds). We will use the results of this initial work as a basis for laboratory studies aimed at determining the isotopic fractionations associated with specific transformations, such as the bacterial uptake of ammonium. Nitrogen concentrations will be determined by gas chromatography with thermal conductivity detection and by mass spectrometry (N_2/Ar ratios). The GC analyses can

be done on the *Resolution* during core recovery and mass spectrometric analyses will be conducted shore-based.

Where nitrogen fixation is occurring we expect dissolved dinitrogen concentrations to be depleted relative to atmospheric equilibrium concentrations. Based on measured profiles, uptake rates can be modeled. This method should be sensitive to low levels of nitrogen fixation as the equilibrium concentration of dissolved dinitrogen is only approximately 600 micromolar. While we expect nitrogen fixation to occur in intervals where ammonium is depleted we will examine regions of high ammonium as well for comparative purposes. Nitrogen concentrations will be determined by gas chromatography with thermal conductivity detection. These analyses can be done on the *Resolution* during core recovery.

As noted above, nitrogen fixation genes are found in sulfate reducing bacteria, thus the presence of these genes in the sediments is expected whether there is active nitrogen fixation or not. Therefore, we will look for the evidence for the transcription of these genes by using reverse transcriptase polymerase chain reaction (RT-PCR) analysis to detect the presence of mRNA specific to nitrogen. The presence of this mRNA would indicate active nitrogen fixation activity.

Other sedimentary and porewater chemical analyses.—

A variety of measurements on interstitial waters and solid phases are necessary to characterize the chemical environments of buried microbial communities. Many of these chemical measurements are already routinely undertaken on ODP cruises. These include measurements of: pH and alkalinity (via titration); ammonium (by spectrophotometry); sulfate (by ion chromatography); total organic carbon (TOC) and total inorganic carbon (TIC) (by coulometry); headspace methane and other evolved gases; total Fe and Mn (by atomic absorption spectrometry); dissolved silica and phosphate (both by spectrophotometry); Na, Ca, and Mg by ion chromatography; and Sr, Li, Rb, and other metals by atomic absorption.

More complete characterization of microbial environments would require additional measurements to be made. These include measurements of nitrate (by spectrophotometry), total CO_2 (by coulometry), total H_2S (by spectrophotometry), H_2 (by gas chromatography), and dissolved organic carbon (DOC) (by Shimadzu analyzer). In general, these measurements can be done in the *Resolution's* shipboard chemistry laboratory during relevant cruises. Some of these analyses, such as the H_2S measurements, can be done on preserved samples.

Computational approaches.—

The computational analyses required for this Subsurface Biospheres Program fall into two distinct categories: 1) the integration and analysis of existing data to help target investigations to particular geographic regions and subsurface depths, and 2) the modeling of geochemical profiles and calculations of the global impact of microbiological processes in deep sea sediments. An example of the first category is provided by our use of over 24,000 existing heat-flow measurements (Pollack et al., 1991) to estimate the depth of microbially important isotherms in marine sediments (Figs. 4 and 5). During ODP Leg 190 the results of these isothermal mapping exercises served as a guide for targeting microbiological sampling. Through analysis of existing sulfate, methane, ammonium, and organic carbon measurements in deep sea drill holes, we can similarly expand and refine our ability to target specific geochemical regimes conducive to or indicative of different microbiological processes (as described in the section on the First Project of our Integrated Research Plan).

As described in the subsection on our Second Project, one-dimensional modeling of down-hole geochemical profiles can provide local estimates of microbial influence on cycles of sulfur and methane. These models must at least incorporate the open-ocean and coastal-ocean profile types

shown in Figure 2, and in reality must be flexible enough to embrace a wider range of profile types, including those influenced by anhydrite ($CaSO_4$) precipitation and subsurface brine flow. With sufficient geographic coverage, we can combine the results of these one-dimensional models with global fields of primary production at the sea surface, water depth, and distance from shoreline, for example, to link the subsurface microbial biosphere with surface processes. These quantitative links can provide the basis for estimating the global impact of microbial processes (e.g. sulfate reduction) occurring in deep sea sediments.

Initial data compilation and quality assessment will be performed with a combination of the Unix tools Sed and Awk in addition to FORTRAN programs. For global and regional color contour maps we will use The Generic Mapping Tools (GMT) (as in Fig. 3). GMT will also be used to create downhole profiles. One of the main advantages of GMT over other mapping/plotting utilities is its ability to work within Unix shells or Perl programs to automate mapping and profile plotting. These tools are also applicable to World Wide Web based applications suitable for outreach and communication with other researchers.

Geochemical modeling may be best accomplished with Matlab and its associated optimization and statistics toolboxes. Matlab also provides basic environment for solving inverse problems that can encompass multiple spatial fields and is flexible enough to be adapted for almost any computational requirement.

Sample acquisition through deep-sea drilling.

The microbiological and geochemical samples that are required to directly study the deeply buried biosphere of marine sediments can only be recovered by deep-sea drilling. Such samples are recovered by the Ocean Drilling Program (ODP) on a near-daily basis, using the ocean drillship *JOIDES Resolution*. The *Resolution* spends approximately 340 days at sea every year, drilling and recovering sediments and crust from every part of the world ocean. At water depths ranging from 100 meters to several kilometers, the ship routinely recovers deep-sea sediments and oceanic crust from burial depths in excess of one kilometer. All that has been needed for routine global collection of microbes from those sediments and crust is (1) a well-equipped microbiology laboratory on the *Resolution*, and (2) the presence of microbiologists on the shipboard scientific crew. Since the microbial populations of samples change irreversibly and beyond recognition within a few hours, it is mandatory that microbiological work be carried out onboard immediately after sample retrieval.

A well-equipped microbiology laboratory has recently become part of the permanent scientific facilities on the *Resolution*. Basic instrumentation for that lab was purchased by ODP for western Pacific Leg 185 (April – June, 2000). In order to further advance studies of the deep biosphere in oceanic sediments and crust, a wide range of additional microbiological and geochemical instrumentation was purchased by a grant from the U.S. National Science Foundation (NSF) Life in Extreme Environments (LExEn) Program to the proponents of this Astrobiology Institute proposal (Teske *et al.*, 1999). The shake-down cruise for this fully equipped laboratory was ODP Leg 190 (May – July, 2000).

We have already acquired many of the deep-biosphere samples necessary for our proposed Research Plan through the participation of co-proponents David Smith and Arthur Spivack on western Pacific ODP legs 185 and 190. We propose to acquire future deep-biosphere samples in the same manner. Because the scientific participants on each cruise must undergo a competitive ODP application and selection process, we cannot guarantee that we will be allowed to participate on any individual future ODP cruise. Nonetheless, we do not anticipate any serious trouble participating in future cruises on a fairly regular basis. The Ocean Drilling Program has just scheduled the first-ever scientific ocean-drilling cruise to focus primarily on deep-biosphere objectives. That cruise

(ODP Leg 201, scheduled for Feb – April, 2002) is based on a drilling proposal by the proponents of this Astrobiology Institute proposal (D'Hondt et al., 1999/2000). Furthermore, microbiologists and geochemists can apply to participate as shipboard scientific staff on any ODP cruise, regardless of its primary scientific objective. This open application process effectively renders every ODP cruise a cruise of opportunity for scientists interested in deep-biosphere studies. Cruises scheduled for the next two years will collectively sample every deep-sea sedimentary environment mentioned by this proposal, including: deep hot anoxic sediments; organic-poor open-ocean sediments where life is likely to be limited by electron-donor availability; and organic-rich sediments with very active sulfate-reducing, methanogenic and methanotrophic microbial communities.

The Ocean Drilling Program does not provide significant support for large shore-based scientific efforts (such as the Astrobiology Institute Subsurface Biospheres Program that we propose here). However, ODP does pay all of the operational costs associated directly with the ocean-drilling cruises of the *JOIDES Resolution*. These operational costs include the shipboard salaries of participating scientists. Furthermore, shipboard scientists and technicians on every ODP cruise routinely measure a host of geochemical, geophysical and sedimentary properties for each recovered core and drilled hole. These routine measurements facilitate close integration of microbiological, geochemical and geophysical studies. This close integration will allow scientists to routinely document the environmental limits and biogeochemical effects of the deep biosphere, at no real cost to the proposed Astrobiology Institute Deep-Biosphere Program.

Roles of individual participants

As described in the relevant sections of this proposal, all of the Investigators will be expected to participate in all aspects of the proposed program, including Research, Training, and Education / Public Outreach.

The PI, Steven D'Hondt, will bear the ultimate responsibility for guiding and coordinating all aspects of the proposed Subsurface Biospheres Astrobiology program, including its Research, Education and Outreach activities. The WHOI Institutional PI, Andreas Teske, will bear the primary responsibility for guiding and coordinating the research, education and outreach activities of the Subsurface Biospheres program at Woods Hole. We anticipate that both D'Hondt and Teske will spend at least one month per year on these activities.

The major components of the Integrated Science Plan can be grouped into three broad categories: microbiological studies, porewater and inorganic biogeochemical studies, and organic-biomarker biogeochemical studies. Research in each of these categories will be undertaken by teams of Investigators, post-doctoral scholars and graduate students. For short periods of time, summer undergraduate research fellows and visiting (guest) undergraduates and (at WHOI) select high-school students will also be members in those research teams.

Andreas Teske and GSO Co-investigator David Smith will coordinate and oversee all of the microbiological studies. In particular, Andreas Teske will tutor the microbiology postdocs and coworkers on anaerobic cultivation, ribosomal RNA sequence analysis and microbial systematics and phylogeny. David Smith and Andreas Teske will take care of and supervise shipboard cultivation of bacteria and Archaea from fresh deep subsurface samples Co-Investigator Arthur Spivack and Steven D'Hondt will coordinate and oversee the porewater and inorganic biogeochemical studies. Spivack will bear primary responsibility for the analytical aspects of those biogeochemical studies. D'Hondt will bear primary responsibility for the computational aspects of those and other studies (such as the mapping of sedimentary isotherms). Both Spivack and D'Hondt will actively participate in the modelling and interpretation of those

data. Co-investigator Kai-Uwe Hinrichs will coordinate, oversee, and bear primary responsibility for guiding and interpreting all of the organic-biomarker biogeochemical studies.

Spivack and D'Hondt will work closely with Smith to keep the microbiological studies closely integrated with the porewater and inorganic biogeochemical studies. Hinrichs will work closely with Teske to maintain integration of the microbiological and organic biogeochemical studies. As discussed in the Management Plan, all five Investigators will meet regularly to coordinate the overall program and all program participants will meet regularly to share their results and advance their collective understanding of (and enthusiasm for) Astrobiology and Subsurface Biospheres.

If this proposal is funded, we anticipate that all of the Investigators will spend at least three months per of their NASA-funded time on the Research activities of this Astrobiology Program. Most of that time will be spent on personal research efforts. However, much of that time will also be spent guiding the post-doctoral scholars, graduate students and summer undergraduate research fellows in their respective efforts.

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Habitable Planets and Evolution of Biological Complexity

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EXECUTIVE SUMMARY

SETTING THE STAGE Designing and carrying out the search for evidence of extraterrestrial life is widely regarded as one of the most significant, intriguing, and difficult of scientific undertakings. As part of this effort, this proposal brings together a wide-ranging suite of talented researchers to address the questions:

What are the characteristics of planets that can evolve complex organisms? Where might such planets occur? How does biological complexity evolve on a planet?.

Earth is our only example so far of a life supporting planet. We therefore begin with studying the history of Earth as a planet (formation, bombardment, plate tectonics, evolution of surface and atmosphere), and likewise the history of its life over billions of years: early evolution, continual adaptation to changing conditions, extinctions, and resurgences. We then try to generalize as much as possible to other possible sites for life and how life in those environments would compare to Earth's.

EXAMPLES OF SPECIFIC ISSUES TO BE STUDIED

- Earth-like planets may preferentially form around stars that fall within a particular range of chemical compositions and also have one or more gas giant, Jupiter-like planets.
- Since many planets form at first without water or organic materials, accretion of cosmic dust, comets, and asteroids may be critical delivery mechanisms.
- Without the right crustal and core composition, or without sufficient water, an otherwise habitable planet may never develop plate tectonics and therefore the long-term climate stability provided by the Earth's carbon cycle.
- Mass extinctions would seem to be harmful to life, but in fact a certain number of them may well promote greater biocomplexity.
- Transfer of genes in the past may have been critical for the development of biocomplexity, including the very origin of cells with nuclei.

OUR TEAM These introductory remarks only touch on the complexities involved in determining the envelope of a planet's "habitability." It will take a diverse and multidisciplinary team to address such a Gordian Knot with any chance of success. We have assembled such a team, primarily at the University of Washington (UW). This team is not ad-hoc: the group began working together four years ago, and has a significant history, including obtaining large-scale support for Astrobiology from NSF for the nation's first formal graduate program in Astrobiology (now starting its second year), as well as permanent support from the UW for creation of our Center for Astrobiology and Early Evolution. We have already learned how to work together across normally yawning disciplinary chasms, and need no 'spin-up' time to become productive. We include specialists in fields as diverse as Galactic chemistry, solar system dynamics, interplanetary dust, the planetary evolution of Mars, plate tectonics, biomarkers and fossils from the oldest rocks, mass extinction events, the evolutionary trajectory

of Earth's life as revealed through genomics, the origin of complex cells, and life in the most extreme environments on Earth today.

OUR TOPICS We cannot attack every question, so have selected areas where three criteria apply: (1) our group has expertise, (2) data exist or can be developed, and (3) there is enough knowledge to suggest that applicable theoretical models show promise of yielding insight. We propose to study a suite of topics in an highly interrelated manner, always intertwining the research with our pioneering graduate educational program. Topics here and in the proposal are arranged to flow roughly down the overall scales of both physics and time.

LARGE SCALES (Milky Way, solar system, and planets). We seek to set physical constraints on formation of habitable planets at Galactic through planetary scales. We will study astrophysical constraints such as Galactic chemical composition and evolution, the likely composition and size of extraterrestrial planets, and consequences of these parameters for development of life. At solar-system scales, we will study the composition of interstellar and solar-system dust, orbital dynamics of gas giants and terrestrial planets, the orbital stabilities and compositions of comet clouds and asteroid belts, and the delivery of volatile and organic material to planetary surfaces. At planetary scales, we will do modeling of plate tectonics on Earth-like planets and its consequences for habitability. Our sister planets Venus and (especially) Mars supply valuable case studies as approximately Earth-like planets that have nevertheless evidently traveled very different paths over the past 4.5 billion years.

LOCAL SCALES (Life itself, on one planet). We do not propose new investigations on the origin of life itself, rather to study how life moved from its undoubtedly simple beginnings to the blooming, buzzing complexity of species, communities, and ecosystems of today. We will search for geochemical evidence of the nature of life and of the atmosphere at the time of the oldest Archaean rocks. Impacts of large bodies onto a planet may be both critically important and devastating: on Earth they supply both water and extinctions. We will study Earth's known mass extinction events to determine whether they are generally caused by impacts, and also whether there is a critical number of such impacts needed both to drive evolution and allow it to continue. Using a specific microorganism, we will try to determine the minimum genome necessary for life on Earth, on the presumption that similar requirements will be imposed elsewhere (energy transformation, reproduction, etc). We will study how complexity was generated from simple life, through combinations and transfers of genetic information. Genomic studies will be combined with field and laboratory work on Earth's simplest and most ancient extant lineages, found in communities as contrasting as deep-sea vent systems (high temperature, high pressure) and microbial mats (rapidly varying salinity and acidity).

OUR INTELLECTUAL MIXMASTER: EDUCATION Our proposed research is based at the UW, but includes several Co-Is at other institutions and within NAI. In order to strengthen the ties among the member NAI institutions, we also propose a Summer Institute in Astrobiology and an inter-institutional Graduate Student Exchange Program. Our existing NSF-funded Astrobiology graduate program, with its variety of students, new classes, laboratory rotations, and inter-campus internships will be the intellectual mixmaster for our research program.

SUMMARY We are proposing research on habitable planets and the evolution of biological complexity that is central to NAI's program in Astrobiology. At the UW we already have a

history of success and strong, committed support from our faculty and administration. Together, NASA and we can develop a powerful synergy and symbiosis between the research efforts proposed here, our UW Astrobiology educational program, and NASA's efforts to establish NAI as an effective research/education entity. NASA's investment in this proposed research program will profit from this intellectual leverage and be extraordinarily productive for NAI's overall goals.

Section I: INTRODUCTION

A. Primary goal- research
B. Second goal- education
C. Problems we will address
- **1. formation of habitable planets: where, when, role of plate tectonics**
- **2. causes of mass extinctions: testing impact models**
- **3. fossil evidence about evolution of Archaean complex life**
- **4. extant life as evidence about evolution of complex life: origin of eukaryotes**

PREAMBLE The surge of interest in Astrobiology has resulted in new information about many aspects of early evolution of life on Earth and the range of conditions needed for a habitable planet. Far less understood, however, is the range of conditions under which more complex organisms, including metazoans, might occur and survive for long periods. Astrophysical and geophysical processes (cometary and asteroid impacts, volcanism, (variable) solar insolation, plate tectonics, greenhouse effects, etc.) provided (1) the basic molecules leading to life, (2) the carbon and energy sources to sustain life, and (3) the diverse and changing environments on Earth in which evolution and biocomplexity have occurred.

Increasing biocomplexity - microbes, microbial communities, eukaryotes, metazoans - has been characteristic of evolving life on Earth and is the result of a co-evolution of organisms with their environment and with each other. The details of the interchanges in this co-evolution are simply not known. Yet recent advances in astronomy, geosciences, microbiology, and genomics have made it possible to begin the task of understanding how biocomplexity evolved, providing deeper insight both into Earth's history and into gauging the prospects for simple or complex extraterrestrial life.

This proposal on "Habitable Planets and Evolution of Biological Complexity" is designed as an integrated approach, extending in time from the earliest days of the Milky Way and the solar nebula, to the Archaean age of the oldest biomarkers, to the mass extinctions of metazoans, to today's extant microbes that through genomic analyses allow inferences about their evolutionary past. In terms of size scales, our research spans from the Milky Way to planets to interplanetary dust particles to microbes to genes. Techniques to be applied include computer models of orbital dynamics and geophysics, astronomical observations of extrasolar planetary systems, geological and paleontological field work on several continents, microbiological field work ranging from hydrothermal vents to microbial mats, and genomic analyses.

We are not an *ad-hoc* group assembled just for this proposal, but an interdisciplinary group of faculty who have been together for over four years. Over this time we, the members of the UW Astrobiology Program, have been committed to interdisciplinary communication, research, and education centered around the key issues of Astrobiology, in the process defining the nascent field and securing its future through our students.

In the remainder of this introductory section, we set out our two major goals: (1) to carry out research on planetary habitability and the evolution of biological complexity, and (2) to further develop our existing Astrobiology Educational Program. We next provide brief descriptions of our four major research themes. Subsequent sections then detail our proposed research (Secs. II and III) and the educational program and other institutional commitments to Astrobiology (Sec. IV).

I.A. PRIMARY GOAL: To implement a research program focused on planetary habitability and the evolution of biological complexity. We propose an integrated multidisciplinary effort concentrating on four broad questions, each of which is discussed more fully in Secs. II and III below:

1. How often, where, and under which conditions do habitable planets form and persist, and what role does plate tectonics play?
2. How do mass extinctions and impacts affect the evolution and survival of complex organisms, i.e., the long-term habitability of planets?
3. What can we learn from the geological and fossil record about the evolution of eukaryotes and metazoans?
4. What can we learn from the physiology and molecular characteristics of extant life about the evolutionary pathways by which microbes and their communities evolve, and by which complex organisms originate?

I.B. SECOND GOAL: To further develop our Astrobiology Education Program (Sullivan, Staley)

The second major goal of our program deals with education. Our educational program will be tightly integrated with the proposed research. We will use the NASA-funded research as leverage to greatly enhance our existing educational program in Astrobiology; conversely, the education program and the cohesiveness it has already brought to our group will strengthen the quality of our research and of our contribution to the NASA Astrobiology Institute.

The UW Astrobiology group formed several years ago with a group of faculty having a common interest in habitable planets and extraterrestrial life. Actual funding of our pioneering graduate program in Astrobiology began with an award in 1998 from the NSF Integrative Graduate Education and Research Training (IGERT) program ($2.2M, 5 years). Our highly interdisciplinary enterprise has been further enhanced by recent funding from the UW's competitive University Initiative Fund (UIF), which has provided major permanent funding for a Center of Astrobiology and Early Evolution, including two new faculty members (one of these positions has recently been filled by R. Buick from Sydney University) and two new post-docs in Astrobiology. We currently have 12 graduate students in our program and a faculty comprising 21 astronomers, chemists, planetary scientists, geologists, microbiologists, oceanographers, and engineers (for a listing of all participating faculty and students, see the appendix "Personnel of the UW Astrobiology Program" in Vol. II).. We have developed a curriculum spanning all of the disciplines and as a group have made every effort to interact across disciplines and identify research problems that can be addressed only through an interdisciplinary approach. Further details of the UW Astrobiology Program and other UW institutional commitments are in Sec. IV.

The IGERT and UIF awards have provided a firm foundation, but they focus on personnel, teaching, and infrastructure. What is missing is strong research support with a direct emphasis on Astrobiology. The proposed scientific program and membership in the NAI will provide much needed research support and enable powerful synergy and leverage among NASA, UW, and NSF.

I.C. PROBLEMS WE WILL ADDRESS

I.C.1. How often, where, and under which conditions do habitable planets form and persist, and what role does plate tectonics play? (Gonzalez, Quinn, Brownlee, Kress, Leovy, Solomatov)

We define a habitable planet as a solid body capable of supporting life as we know it on Earth - carbon-based, requiring liquid water, and capable of self-replication and evolution. The study of extra-solar habitable planets involves a broad interdisciplinary approach that extends from understanding how planets are formed to understanding the conditions that allow such life to originate, survive, and evolve.

Stellar and Galactic environment. Through detailed spectroscopic analyses of the parent stars of extrasolar giant planets, we will study the abundances of chemical elements essential in the construction of both gas giant planets. The basic result that parent stars have significantly higher abundances of the heavy elements is proving to be powerful: Co-I Gonzalez has already successfully predicted the presence of planets later discovered about two stars.

Our exploration of habitability also will consider processes that span the size and time scales of the Milky Way galaxy. Known radial gradients of elemental abundances in the disk will be examined for their effects on the structure of any planets formed around stars at given locations and times. We will also explore the effects of nearby transient radiation events (supernovae, gamma ray bursts, active galactic nucleus outbursts), as well as how Galactic-scale perturbations of the probable Oort cometary clouds around other stars would enhance the rate of collisions with any planets present. We will make use of Galactic chemical evolution models to study the time evolution of key elements likely to be required to build habitable planets. Our goal is to determine the optimal places and times in the Milky Way for the formation and sustenance of habitable planets.

Orbital dynamics and impacts. There exists a huge gap (a factor of 100 in mass) between the extra-solar giant planets discovered with current observational techniques and Earth-like planets. Although gas-giant planets are not habitable (but their moons may be), they greatly affect planetary system formation and evolution, and thus their presence may prove important for the existence of habitable worlds. We will explore the dynamical connections between gas giants and possible habitable planets. These connections include the overall dynamical stability of the planetary system, the influence of gas giants on planet formation in the terrestrial zone, and the gravitational influence that giant planets have on small bodies such as comets and asteroids (and therefore on where and how they impact other bodies). All these issues profoundly affect the type of environment that any potential life must face. Examples of the factors involved include (a) size of the planet (large enough to retain volatiles, as well as drive long-term active tectonism and volcanism that could support microbial life), (b) early delivery of water and organic compounds to the planet via comets, (c) presence or absence of a stable orbit and obliquity in order to maintain a relatively stable climate, and (d) periodic catastrophic events such as bolide impacts that are perhaps necessary to create and maintain high variability of habitable conditions, producing increased biodiversity and biocomplexity (see Sec. I.C.2).

- How do planetary size, mass, and orbital parameters affect impact rates?
- What minimum size of a gas giant can effectively screen terrestrial planets from impacts? (Can two large planets act in this way?)

- How do impact rates vary with time and location in planetary systems?
- What percentage of impacts on terrestrial planets have been caused by comets as opposed to asteroids, and how does this affect the delivery of organic and volatile material?
- Do most of the impacting comets come from the Oort Cloud or Kuiper Belt?
- How common are asteroid belts? Could our solar system have a higher than average impact rate because of the presence of our asteroid belt?

Delivery and retention of organics and volatiles. The volatile and organic composition of impacting bodies is a key factor in the evolution of habitable planets. What are the relative roles of large comet and asteroid fragments and interplanetary dust particles (IDPs) in bringing these materials to a planet? While the role of IDPs may be important, it is poorly understood. Detailed measurements of IDPs in our own solar system, combined with interpretation of infrared-emitting dust clouds observed to surround young solar-like stars, will be the basis for developing a detailed theoretical model of the delivery of organics to Earth-like planets. Not only must a habitable planet be supplied with volatile materials, it must also retain them. Retention of volatile materials is a function of a planet's size, internal evolution, and orbital parameters. Because of its small size, distance from its parent star, and relative accessibility, Mars provides an excellent case for investigating the limits of habitability. Retention and subsequent evolution of volatile materials on Mars is one of the key issues that we will investigate with the aid of existing observations and atmospheric circulation models.

Plate Tectonics. The importance of plate tectonics in planetary habitability is controversial. For microorganisms it is unclear, but for complex metazoans there is evidence suggesting that plate tectonics may be a key to their appearance and preservation. Since the ground-breaking paper of Walker et al. (1981), it has been understood that plate tectonics provides a climate feedback mechanism allowing relatively stable global temperatures over long periods, and may have been the single most important mechanism allowing the Earth to maintain liquid water on its surface for the past f4 Byr. Plate tectonics is also the dominant force causing changes in sea level, creating continents, and even perhaps maintaining in an indirect way magnetic fields of the terrestrial planets (Nimmo and Stevenson 2000). Our study of the role of plate tectonics in the formation and maintenance of planetary habitability will focus on the following questions:

- Which planetary conditions allow the presence of plate tectonics (the range of planetary mass, lithospheric strength, mantle viscosity, mineralogy, crustal thickness, abundance of water)?
- What role does the coupling between plate tectonics, melting, volatile cycling and climate play in regulating the environment of a terrestrial planet?
- Which planetary conditions allow the presence of a magnetic "shield" for a terrestrial planet?
- We will also examine the controversial question (McKay 1996): Could plate tectonics hinder, rather than enable, evolution to complex organisms?

The major thrust of this work will be on modeling the interaction of key tectonic processes; insights into the role and mechanisms of plate tectonics may also be gained from our studies of Archaean rocks (Sec. I.C.3) and of the evolution of the surface of Mars, as an example of a

planet where plate tectonics is no longer active, but could have taken place very early in the planet's history (Sec. II.A.4). Our research does not directly address the important roles of tectonics in the maintenance of climate or in the evolution of biocomplexity, but we expect to interact with other members of the NAI in these areas.

In summary, our approach will be to use astronomical observations (Gonzalez, Sec. II.A.1 below), dynamical modeling (Quinn, Sec. II.A.2), observational and modeling studies of IDPs and dust clouds (Brownlee, Kress, Sec. II.A.3), models of plate tectonics (Solomatov, Sec. II.B), and focused studies of the evolution of the atmosphere and surface of Mars (Leovy, Sec. II.A.4) to help understand important astrophysical and planetary constraints on the formation, evolution, and properties of habitable worlds.

I.C.2. How do mass extinctions and impacts affect the evolution and survival of complex organisms, i.e., the long-term habitability of planets? (Ward, Kring, Farley, Quinn, Buick)

Mass extinctions are short-term events that kill off a significant proportion of a planet's biota, and on Earth have been of greatest consequence to more complex organisms such as metazoans. Surface life is vulnerable to major planetary catastrophes, for example, impact of a large comet or asteroid, radiation and particles from a nearby supernova, or catastrophic climate changes such as intense intervals of greenhouse heating or Snowball-Earth type episodes. It may even be that life on the surface of Earth was repeatedly sterilized during the period of heavy bombardment about 4 billion years ago (= 4 Ga), only to be re-seeded by a possible "reserve" of deep-Earth microbes. But if the animals and higher plants are ever wiped out by a catastrophe, they cannot be immediately restocked from some underground reserve; rather, biocomplexity must "re-evolve", a process lasting at least hundreds of millions of years.

Judging from the history of life on Earth, mass extinction events (MEEs) have the potential to end animal life on any planet where it has arisen. On Earth there have been about 15 such episodes during the last 500 Myr: five eliminated more than half of all species then inhabiting our planet. There were also an unknown number during the Archaean and Proterozoic. The frequency and severity of MEEs thus influence the biocomplexity of a planet, and are appropriate areas of astrobiological study.

MEEs have significantly affected the evolutionary history of Earth's biota in two competing ways. After each of the major events of the Phanerozoic, biotic diversity was substantially reduced for several Myr. Yet these same extinctions and diversity depressions were each followed by rapid periods of diversification, composed of different assemblages of organisms, that resulted in more global biodiversity than prior to the MEE. MEEs thus seem linked to diversity enhancement as well as formation of biotic novelty.

Major questions about MEEs. Today there are two major unanswered questions about MEEs.

(1) The first deals with their relative importance as large-scale evolutionary phenomena. MEEs on this planet have acted as important instigators to biotic evolution by removing incumbent taxa (McKinney 1998) and thus opening the way for evolution of new species. Too few or too weak MEEs on a planet may retard its biodiversity (Raup 1985). Yet too many or too severe MEEs will reduce diversity or even lead to the removal of complex life. Is there a critical number of MEEs necessary for the development and/or subsequent diversification of metazoans?

(2) The second major question about MEEs deals with their causation. Of the so-called "Big Five" (the Ordovician, Devonian, Permian, Triassic, and Cretaceous (K/T) events), only the K/T event has consensus as to its cause, namely by asteroid impact. Following the hypothesis of Alvarez et al. that (a) the Earth was hit by an asteroid ~10 km in diameter 65 Myr ago, and that (b) the environmental effects of this impact event caused the K/T MEE, it appeared that a general theory of MEEs as caused by asteroid impact was imminent. Yet by the late 1980s it had become apparent that the clear-cut impact signature recognizable at numerous K/T boundary sites around the world could not be unambiguously identified in any of the other MEE boundary horizons (summary in Hallam and Wignall 1998). Over the last several years, however, there is renewed interest in the possibility that one or more of the other major MEEs are at least partially caused by the environmental effects of large-body impact on the Earth. In particular, new evidence concerning the Permian and Triassic MEEs indicates that they may have been to some degree caused by an impact. In this area we will address the following questions:

- What percentage of Earth's MEEs were generated by impact?
- After an MEE, how does biodiversity re-emerge?
- Is there a critical number of MEEs necessary to maximize biological diversity?

Field studies by Ward, Kring, Farley, and Kyte addressing these questions are described in Section II.C. Their results will be integrated with Quinn's orbital dynamics models in order to connect models of impacts with the resultant effects on the Earth's biota.

I.C.3. What can we learn from the geological and fossil record about the evolution of eukaryotes and metazoans? (Buick)

Precambrian geology and paleontology. Though we can extrapolate back from existing organisms or build theoretical biogeochemical models, the only robust empirical data that shows how early life on Earth evolved and interacted with its environment comes from the study of early Precambrian rocks. Despite popular misconceptions, globally there is in fact a moderate abundance of well-preserved Archaean (>2.5 Ga) and Palaeoproterozoic (2.5-1.6 Ga) rocks, which can serve as our clearest windows on the events that occurred shortly after life's origin. Some of these rocks formed during the period when Mars, the best current candidate for having once developed extraterrestrial life, was evidently warm and wet, potentially allowing life to radiate there too. Thus, the primordial geological, geochemical and paleontological records of Earth might serve as proxies for what will be encountered on the initial astrobiological missions to Mars. Moreover, the advent of eukaryotes, a major step in the development of biological complexity, now appears to have occurred during this time interval (Brocks et al. 1999). It is important to understand the state of the biosphere before, during and after this evolutionary transition, especially with regard to studying the relationship between oxygenation of the surficial environment around 2 Ga and the introduction of eukaryotes.

We propose to investigate this ancient empirical record to discover what sorts of organisms inhabited the early Earth, how they lived, where they lived, what sorts of conditions they tolerated, and how they modified their physical and chemical environment by their activities. The project will investigate well-preserved early Precambrian successions in Australia, South Africa and Greenland. We will concentrate on critical intervals in the evolution

of the biosphere and the terrestrial environment such as (1) the earliest Archaean, when life first radiated, and (2) the mid-Palaeoproterozic, when oxygen proliferated and large, complex organisms apparently rose to ecological dominance.

Biogeochemical investigations. One research direction will be biogeochemical in focus, as this provides the most continuous proxy record of biological activity. Though preserved fossils are invaluable for interpreting the history of the primordial biota, they are only rarely preserved and thus hardly representative of the state of the total biosphere. By contrast, biogeochemical evidence survives much better and can be used to infer both local and global biotic activity. By comparing signatures from different depositional settings at different times, an animated picture can be constructed of metabolic, ecological and evolutionary change through time.

Geological investigations. Our second research direction will concentrate on the physical environment, because this provided the stage upon which the evolutionary drama was played out. As the biota and environment seem to have interacted with reciprocity throughout later Earth history, it seems reasonable to expect that they also did so early on. Along with other important influences on life's evolutionary trajectory (plate tectonics, impact history) considered in detail elsewhere in this proposal, here we examine changing conditions in the atmosphere and hydrosphere. This work, led by Co-I Buick, is described in Sec. III.A.

I.C.4. What can we learn from the physiology and molecular characteristics of extant life about the evolutionary pathways by which microbes and their communities evolve, and by which complex organisms originate? (Baross, Deming, Leigh, Stahl, Staley)

Lateral gene transfer and species symbioses in the past. All extant life on Earth arose from a common genetic ancestor. While there may have been competing genetic codes, only one code survived. The very first stage in creating biocomplexity was the building of the microbial genome to a size that would allow independent growth and reproduction. This may well have been accomplished by extensive lateral transfer of genes between "cells" that were dependent on other "cells" for growth and survival because of limited genetic information. The 0.5 Byr period prior to 3.5 Ga probably experienced extensive evolutionary experimentation and very limited physiological diversity (Woese 1998). Recent evidence also points to lateral gene transfer between Bacteria and Archaea as the main mechanism involved in the formation of eukaryotes. Subsequently, symbioses between specific kinds of bacteria and eukaryotes contributed to the rise of oxygen-respiring and oxygen-producing multicellular biota. Today, both lateral gene transfer and interdependence of different species living in communities are ancient processes that continue in the microbial world.

While the above provided the mechanisms for biological innovation, other factors fostered increased biodiversity and biocomplexity. These factors included multiple and changing environmental conditions - from possibly a hot, anaerobic, volcanically active Earth to a more temperate, oxygen-rich habitat. As discussed in previous sections, environmental conditions over the past 4 By have been modulated and punctuated by changes such as widespread anoxia in marine environments, varying atmospheric composition and temperature, changing volcanism, growth of continents, and widespread glaciation. The *Methanococcus* lineage, for example, may have evolved over a gradual cooling period. The instigators of these changes are not completely understood, but large-scale tectonic events and impacts by large bolides certainly played a

significant role. It is becoming increasingly clear that these marked changes in environmental conditions coincided with drastic changes in the eukaryotic communities; usually mass extinction of dominant genera was followed by the emergence of new taxa. It is also apparent that Earth's biota has had strong feedback effects on the physical state of the Earth's surface, ocean and atmosphere. Evolution appears to have been profoundly influenced by these events - they may have been the most important factors in creating the high biodiversity and biocomplexity of the present-day Earth.

That microorganisms may have been affected by these eukaryote extinction events and that particular phylotypes became extinct at various times throughout Earth's history is rarely considered. The present dogma holds that the global tree of life based on small subunit ribosomal RNA genes represents all 4 Byr of evolution. This assumption is probably mostly correct in that there is evidence in the early Precambrian for specific kinds of metabolisms, such as photosynthesis, methanogenesis and sulfate reduction. However, many of the specific taxa of these metabolic groups of organisms may have become extinct along with the eukaryotes without significantly affecting the conclusions drawn from the phylogenetic tree. A more difficult scenario is that there was greater metabolic diversity in the Precambrian microfauna then at present, but that many of these taxa became extinct or retreated to more restrictive habitats either not affected by catastrophic events or less affected by evolutionary competition.

Extant analogues to the past. Today there are microbial communities that likely resemble those of the Precambrian. These communities include those found in (a) anaerobic and photosynthetic microbial mats and biofilms, (b) the sub-seafloor associated with deep-sea hydrothermal vents, and (c) water ice. It is possible that hidden in the presently unknown diversity of these ecosystems there exist organisms with metabolic pathways that are relics of common metabolisms of the past.

There is also a connection between these environments and the possible existence and kinds of life on other habitable planets. Today's microbial mats are surely ancient expressions of microbial growth from an era with an abundance of nutrients or energy sources and a lack of animal predators. These mats consist of multiple species of organisms that have adapted to the variable conditions associated with tidal and seasonal cycles. Microbial mats and biofilms are common in extreme environments germane to this proposal including the sub-seafloor associated with hydrothermal vents, surface hot springs, dry-valley and desert rocks, and sea ice. Many of the Co-Is on this proposal work with microbial communities from these ancient environments, or with other microorganisms that are believed to be among the most ancient of extant organisms.

Genomics. Genomics has proven to be extremely useful for addressing questions of early life and evolution of eukaryotes including metazoans (Katz,1999; Lake 1991; Roger,1999). To date, more than 50 bacterial and archaeal genomes have been sequenced or are in progress. One of the conundrums from the genome sequences is that up to 50% of the open reading frames in genomes from microorganisms encode for unknown proteins. Moreover, some of the enzymes involved in metabolic pathways known to be present in the organism are absent in the genome, suggesting either that the pathways are incomplete or that enzymes with unknown sequences or low sequence homology are involved (Cordwell 1999). There are also genes that encode for enzymes associated with pathways not observed to function in the organisms, such as the ribulose-bisphosphate carboxylase in *Methanococcus jannaschii* (Bult et al. 1996).

Lateral gene transfer and symbioses must have been hallmarks of all evolving life forms. Understanding these mechanisms using targeted organisms, microbial communities, and specific genes will be a focus of this proposal. The organisms will include a methanogen, subsurface hyperthermophilic Archaea, and sulfide-rich microbial mat communities in which sulfate respiration is a dominant process. We will search for genes that reflect the evolution of metabolic pathways that are primordial and thus prime candidates for lateral transfer to early eukaryotes, as well as genes that reflect the co-evolution of organisms with their environment.

The invention of multicellularity was a major biological innovation contributing to new states of biocomplexity. This was preceded, however, by the invention of eukaryotic cellular organization. The genome sequences from Bacteria have yielded many surprises, including the presence of genes thought only to be present in eukaryotes. Recently, the tubulin gene was discovered by Co-I Staley in a group of bacteria belonging to the Phylum *Verucomicrobia*. Tubulin in eukaryotes is involved with the cytoskeleton, as well as the separation of chromosomes during mitosis and meiosis. We propose to use specific molecular methods to determine if the tubulin genes evolved in bacteria and were transferred to eukaryotes, or vice versa.

We also propose to study the genomics of the *Methanococcus* lineage to gain insight into the evolution of biocomplexity. We will mine the genomic data to understand the role of lateral gene transfer in the evolution of the lineage, to help determine the structures and functions of proteins, and to determine mechanisms that allowed proteins to adapt to changing temperatures.

Metabolism in extreme environments. Another approach to understanding the habitability of planets is to consider the range of extreme environmental conditions on Earth that support life. For example, the detection of water ice and/or submarine hydrothermal vent systems on another planetary body would satisfy some of the key criteria for habitability. Other criteria needed to support life would include a source of carbon, nitrogen and phosphorus. The sub-seafloor environment associated with present day hydrothermal systems harbors hyperthermophilic microbial communities (growth temperatures > 80° C) that could be analogous to the microbial communities present on the early Earth.

We do not understand the nitrogen and other mineral cycles in these environments or the source and nature of the organic-C assimilated by these microbial communities. Nitrogen gas is the most abundant form of nitrogen in sub-seafloor fluids, and organic-N and ammonia are usually not present at detectable levels. The nitrogen-fixation genes are believed to be very ancient, implying that nitrogen fixation may have been a common characteristic of early Precambrian microbial communities. We propose to use molecular methods to determine the overall diversity of subseafloor microorganisms that harbor and express the nitrogen-fixation gene (*nif*). We will also sequence these genes in search of evidence for ancestral sequences in sub-seafloor Archaea. Similarly, CO_2 is the most common carbon source in vent environments although there is evidence from experiments and models that a variety of organic compounds can be abiotically synthesized under vent conditions. Recently, the sub-seafloor has been found to harbor a new group of anaerobic hyperthermophilic Archaea that can oxidize organic acids using iron as the electron acceptor. In the process they make magnetite. Isolates of this new genus of Thermococcales can also assimilate CO_2 in conjunction with the oxidation of hydrogen, using what appears to be a novel metabolic pathway. These organisms will be tested for their ability to grow anaerobically on organic compounds commonly synthesized abiotically or thought to be present in extraterrestrial sources, as well as on actual meteoritic material.

We will address the problems outlined in this section through a variety of integrated field and laboratory biological approaches, as detailed in Sec. III.B, with studies led by Co-Is Baross, Deming, Leigh, Stahl, and Staley.

Summary of the Introduction section. We propose to implement a wide-ranging, but coordinated research program focused on planetary habitability and the evolution of biological complexity. A powerful team of astronomers, geoscientists, and microbiologists has been brought to bear on several key questions for Astrobiology. In addition, if this program is funded, the NAI will greatly profit from the participation of UW's Astrobiology graduate program, now in the first stages of producing the first generation of true Astrobiologists.

Section II: Habitable Planets

II. Habitable Planets

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II. A. Astrophysical and planetary constraints

We will explore astrophysical processes that relate to habitability on galactic, interstellar, and interplanetary time and space scales. The empirical foundation will be our knowledge of extrasolar planetary systems, the Solar System, and Earth-Moon system, but we will extrapolate as far as our theoretical understanding will allow. We will explore several key phenomena: (1) the chemical signatures that are specific to the parent stars of extrasolar planets; (2) the role of galactic chemical evolution in constraining the timing and location of the formation of habitable planets; (3) the role of transient radiation events (active galactic nucleus (AGN) outbursts, supernovae, gamma ray bursts); (4) formation and dynamical history of terrestrial and giant planets; and (5) formation and dynamical history of the Oort comet cloud and asteroid belt.

II.A.1. Galactic chemical evolution and extrasolar planets (Gonzalez)

II.A.1.a. Extrasolar planets.

Our group (UW Astronomy Dept.) has led the study of physical characteristics of parent stars of extrasolar planets discovered using the Doppler method (Marcy and Butler 1998). Recently Gonzalez and Laws (2000) showed that parent stars have a higher (~2x) average "metallicity" than stars without known planets. Here we use the astronomical definition of metallicity: the abundance of elements heavier than Helium. Figure 1 compares the distribution of [Fe/H] -- defined in caption], a quantitative measure of a star's metallicity, among field stars and the parent stars of extrasolar planets. In addition to metallicity, Gonzalez et al. (2000) summarize preliminary evidence that parent stars also differ from field stars in some abundance ratios (C/Fe, Na/Fe, Al/Fe). These comparisons with field stars are possible only via use of optical spectra of high signal to noise ratio and high resolution. With our analysis techniques, we can derive stellar atmospheric parameters with smaller uncertainties than can competing groups. High precision is critical, because the trends are subtle. Recently, we showed that the binary pair 16 Cygni A and B (B has a planet) differ in metallicity by 6% ± 2% (Laws and Gonzalez 2000); this is the smallest claimed real difference in metallicity between two stars to date.

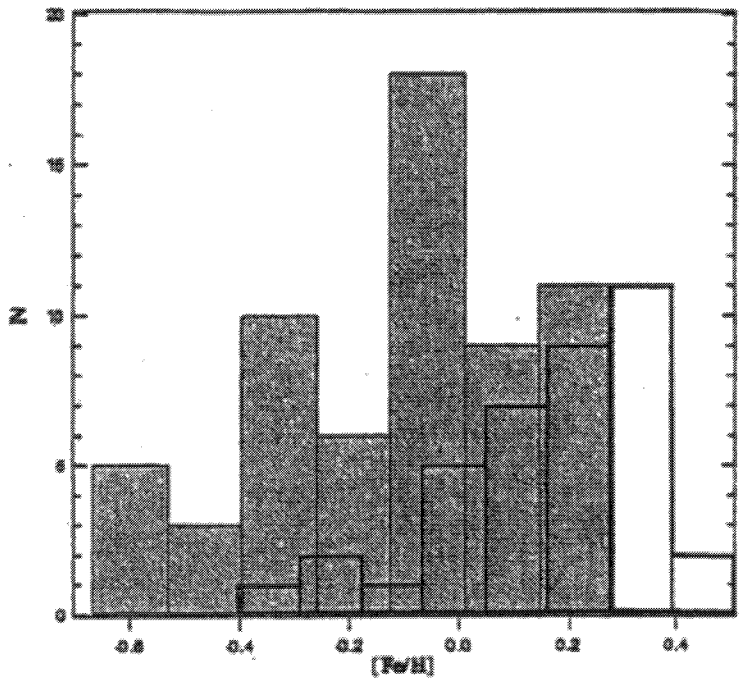


FIGURE 1. [Fe/H] values for field stars without known planets (from Favata et al. 1997) - shaded. [Fe/H] values for 38 extrasolar planet parent stars with high precision spectroscopic [Fe/H] determinations – bold outline. (Note: [Fe/H] is the logarithmic number abundance of Fe relative to H in a star's atmosphere, relative to the same ratio in the Sun.)

Our methods. Briefly, our method involves: 1) obtaining high quality optical spectra with a moderate size telescope equipped with an echelle spectrograph (currently the McDonald Observatory 2.7 m), 2) calibration and reduction of the spectra, 3) measurement of line strengths in the spectra, 4) derivation of stellar atmospheric parameters and abundance ratios. At the current rate of discovery of extrasolar planets, we anticipate that about 100 new systems will be announced in the next three years. We can keep up with this discovery rate with two observing runs per year, separated by about 6 months for full sky coverage.

Using the trend data immediately. Even before we understand what causes such observed trends, we can use them to constrain searches and to concentrate observational resources on the most likely stars. For instance, our group has successfully predicted planets around stars HD 89744 and BD-10 3166. Also, Laughlin (2000) employed the planet-metallicity correlation to identify candidate planet-bearing stars. Possible explanations for the correlation include: (1) high initial metallicity of the parent cloud, which is more likely to lead to the formation of giant planets; and/or (2) in some systems, migrating giant planets scatter disk material (in the form of planetesimals or even planets) into the parent star during early stages of planet formation. Careful observations might separate these two hypotheses. One possible test is to search for a chemical abundance trend with stellar mass, since the depth of the outer convection zone correlates inversely with mass for main sequence stars. More massive stars would be more likely to display the effects of accretion of disk material given their shallower convection zone. If pollution of a stellar atmosphere is eventually demonstrated, we can turn the problem around and try to identify the accreted objects via the observed abundance anomalies in the star. There are

several candidates, each of which will leave its own peculiar signature in the atmosphere of a star: gas giant planets, terrestrial planets or planetesimals, or comets.

Metallicity and planet migration. We will also explore the role of metallicity in planet migration: planets in "hot Jupiter" systems (like 51 Pegasi) may have experienced large migration to reach their present observed positions (Lin et al. 1996). As yet we have too few (<40) high quality spectroscopic abundance analyses to definitively test these hypotheses, but we will continue to observe newly-discovered parent stars until we accumulate enough data to do so. Once we determine the physical mechanisms behind metallicity trends, models of galactic chemical evolution can be used to estimate the probability of forming giant planets at any place and time in the Milky Way.

II.A.1.b. Galactic chemical evolution

The Solar System and extrasolar planetary systems formed within the broader context of the Milky Way galaxy. The Milky Way is a dynamic system, with variations of chemical and dynamical properties on kiloparsec and Byr scales. Compared to the Solar System, a planetary system forming in a different place and time in the Milky Way will have a different composition throughout, and a different dynamical environment. For example, a system forming closer to the center of the Milky Way will have a higher abundance of metals, and will exist in an environment of higher stellar density and velocity dispersion than the Solar System. Both differences will have profound consequences on habitability of any terrestrial planets that form. We will study theses specific Galactic-scale links to habitability:

(1) Radioactivity for a planetary interior. Elements with a likely role in habitability include long-lived radioactive isotopes of K, Th, and U, primary sources of Earth's present interior heat. That heat drives plate tectonic processes that lead to continent formation, the CO_2 - carbonate cycle, and help deep-sea vent formation (although vent formation does not require it), a likely locus for the origin of Earth's life. We will study the evolution of the abundances of these isotopes in the interstellar medium. Their abundances must decline with time, because the sources (massive star supernovae) have been decreasing in frequency since the early history of the Milky Way (Portinari et al. 1998). We will explore the effects of varying the abundances of these isotopes in the interior of a terrestrial planet (Sec. III.B). These considerations can constrain the search for habitable planets to certain ages of the host galaxy. More specifically, we will set limits on the time during which a terrestrial planet must form in order to have sufficient radiogenic heat to maintain plate tectonics.

(2) Mass of planet. The most direct effect of metallicity on habitability is on the mass of a terrestrial planet. All else being equal, the mass of a terrestrial planet forming in a proto-planetary disk should scale with the surface density of solid material to the 1.5 power, and surface density should be directly proportional to the metallicity. Terrestrial planets near the center of the Milky Way will be much larger than those in the outer edge.

(3) Mass ratio of core to mantle. Earth is composed primarily of O, Mg, Si, and Fe (Kargel and Lewis 1993). The mantle is primarily O, Mg, and Si, while the core is primarily Fe. Therefore, variations in the abundances of Mg and Si relative to Fe will alter the mass of the core

relative to the mantle (O can be considered a free parameter, since its abundance is determined by the degree of oxidation of the other abundant species). If the relative mass of the core to the mantle is altered, many global geophysical properties will be affected (e.g., mantle convection, magnetic field generation, plate tectonics). Since Mg, Si, and Fe have similar condensation temperatures, in a given planetary system their relative abundances in the condensation step to solid grains and rocks will closely follow the initial gas phase abundances. The ratios of Mg and Si to Fe vary in the interstellar medium due to the varying contributions of massive star supernovae to white dwarf supernovae.

(4) Elemental ratios and water. We will also study the effects varying the C/O ratio on forming both giant and terrestrial planets. Gaidos (2000) and Gonzalez (1999) identified the C/O ratio as a potentially critical parameter in formation of giant planets, because it is critical in determining how much water forms in a planetary system. We will examine the range of variation of all these elements in the interstellar medium across time and location, using available observations and theoretical Galactic chemical evolution models.

II.A.1.c. Galactic habitable zone

We will also consider possible threats to life from transient radiation events and impacts. Because both types of threats depend on a planetary system's location and on the time since formation of the Milky Way, understanding them might help quantify constraints on where and when one might find habitable planets. When combined with the chemical constraints (above), we can conceptualize a Galactic Habitable Zone (GHZ), analogous to the circumstellar habitable zone (HZ) which describes the region of habitability around a star. We will apply the latest knowledge of supernova rates, gamma ray bursts, and Active Galactic Nuclei (AGNs) to constrain the GHZ.

Another important constraint on the GHZ is the Galactic radial metallicity gradient. The metallicity in the outer disk is much lower than that of the solar neighborhood, hence habitable terrestrial planets are much less likely to form there. We will quantify the probability of forming a terrestrial planet at any time and place in the disk of the Milky Way, given the observed metallicity gradient and its dispersion.

We will link the rate of impacts from asteroids and comets to broader galactic environment by using (1) the dependence of the initial inventory of asteroids and comets in a planetary system on metallicity, and (2) effects of the Galactic environment on dynamics of Oort cloud comets. The dynamical aspects of asteroids and comets within a given planetary system will be treated by Co-I Quinn (Sec. III.A.2).

II.A.1.d. Work statement: We will (1) determine the physical parameters (including abundances) of the parent stars of extrasolar planets and compare them to stars without known planets, (2) identify key elemental abundance ratios likely to be relevant to habitability and determine their variation with time and location in the Milky Way, and (3) apply the latest data on supernova rates, gamma ray bursts, and AGN outbursts to constrain the GHZ.

II.A.2. Dynamics of comets, asteroids, and planets (Quinn)

BACKGROUND: We pointed out (see introduction) the very large gap between the general search for extra-solar planets and the possible discovery of habitable worlds. We propose to reduce the gap through theoretical studies of the influence of gas giants on habitability. Current techniques for detecting extra-solar planets can only find those of Saturn's mass or greater (Marcy et. al. 2000), not the Earth-size bodies usually expected to indicate possible habitability. (A list of extra-solar planets is at http://exoplanets.org/planet_table.html.) Nevertheless we expect strong connections between the two processes: a Saturn- or Jupiter-size body in a planetary system has a dramatic influence over the entire system, including many factors that bear on habitability. In our own Solar System, Jupiter and Saturn obviously influence both (1) long term stability of the orbits of all other planets (for a review, see Duncan and Quinn 1993) and (2) the orbits of various smaller bodies (e.g., comets, asteroids) whose impacts may have made Earth's life possible (by providing water and other volatiles and organics), and certainly have significantly altered its evolution (Alvarez et al 1980).

Our program will make real connections between current discoveries of extra-solar giant planets, and the possibility of finding habitable worlds associated with them. We will address, inter alia:

II.A.2.a Question #1: How stable are other planetary systems? Single-planet systems are trivially stable. The first known extra-solar multiple-planet system (*Upsilon Andromeda, aka "Ups And"*) is just barely stable (Barnes and Quinn 2000). A small planet in the classic habitable zone around *Ups And* cannot long remain in a stable orbit (Rivera and Lissauer 2000). As we find more multiple planet systems, stability needs to be addressed for each in turn. In probing the *Ups And* system, we developed a program (using a cluster of workstations) for quickly exploring the possible parameter space for stability, and will use it to study new systems as discovered.

II.A.2.b. Question #2: How do giant planets influence formation of terrestrial planets? We will investigate the twin possibilities (not mutually exclusive) that gas giants (a) may *suppress* some potential formation of habitable planets, and (b) may be *necessary* for that formation. In the standard model of the formation of the Solar System, gas giants form relatively early and quickly (Lissauer 1993), and significantly influence subsequent formation of terrestrial planets, which they may promote and/or hinder. For example, Jupiter may have suppressed formation of a planet where we find our asteroid belt (Richardson et al. 2000). But perhaps a little chaos generated by giant planets is needed for the proto-planets in the habitable zone to start colliding with one another and to build up into larger bodies. We will address the critical issue of how to produce terrestrial planets with nearly circular, coplanar orbits like those of Venus and Earth. (Chambers and Wetherill 1998) Of course, if the gas giants migrate, either by a small amount (Malhotra 1993), or all the way through the habitable zone (Lin et al. 1996), it will dramatically influence formation of terrestrial planets. We will investigate these possibilities using our unique massively parallel tree code for planetesimal simulations.

II.A.2.c. Question #3: How do giant planets influence development of reservoirs of small astronomical bodies (e.g., the asteroid and Kuiper belts; Oort Cloud)? Giant planets have strong, observable effects on solar-system structure, particularly on the reservoirs of small bodies that are important in forming and determining subsequent impacts on terrestrial planets. The Kirkwood gaps in the asteroid belt occur at mean motion resonances with Jupiter, and the

Kuiper Belt has many objects at the 3:2 mean motion resonance with Neptune. Our current model of formation of the Oort Cloud involves scattering planetesimals from the giant-planet region (Duncan et al. 1987). The influence of small solar-system bodies on the evolution of life on Earth (e.g. providing volatiles, causing extinctions) closely connects gas giants to habitability of terrestrial planets. We will explore these issues relative to known extra-solar planetary systems.

II.A.2.d. Question #4: How do giant planets influence impact rates of asteroids and comets on terrestrial planets? Habitability may require a time-changing frequency of comet and other impacts. They should be common early in planetary evolution (to provide water and volatiles), but rare later (to avoid extinctions.... except that extinctions may be necessary to development of complex life). Giant planets affect both formation of reservoirs of small bodies and the collisions of small bodies with habitable planets. In our System, the 3:1 and other mean motion resonances and the secular resonance with the gas giants deliver main-belt asteroids into Earth-crossing orbits (= extinction promotion). Conversely, gas giants may act as "planetary protectors" (Wetherill 1994), drastically reducing the number of Oort Cloud comets that develop Earth-crossing orbits (= extinction avoidance). Furthermore, gas giants increase the flux of Kuiper Belt objects into the inner Solar System. We will consider the balance among all these processes, which have not yet been studied through detailed numerical simulations such as we will undertake. To ascertain gas giants' possible roles as "planetary protectors", we will simulate formation and evolution of the Oort Cloud (per Duncan et al. 1987), using a variety of masses for the giants.

II.A.2.e. Question #5: How is the rate of impact of small-bodies on habitable planets affected by stellar companions, local stellar density, and the galactic environment? Galactic and stellar environments influence impact rates on habitable planets. For example, the galactic tidal field changes the orbits of some Oort Cloud comets into Earth-crossing orbits, thus raising the impact rate. Our Sun is in a particularly quiet part of the galaxy (Gonzalez and Ward 2000): more active areas (e.g., star clusters, molecular clouds, and areas closer to the galactic center) might be uninhabitable simply due to increased impact rates. We will investigate impact rate as a function of increased disturbance by passing stars and molecular clouds.

II.A.2.f. Methods: To tackle these questions we will bring to bear our expertise in computational gravitational dynamics. Our massively parallel tree code for planetesimal dynamics (Richardson et al. 2000) has pushed the state-of-the-art by being scalable to millions of particles and hundreds of processors. We also have a proven track record in modeling long term stability of planetary orbits and evolution of the orbits of small bodies. For the large parameter studies, we have the infrastructure to harness large numbers of individual workstations.

II.A.2.g. Work statement: specific goals and tasks

1. Integrate orbits of multi-planet systems as they are discovered, to determine their stability on Byr time-scales.
2. Perform million-particle simulations of the growth of planetesimals in the terrestrial region, with various assumptions about gas giants in the system.
3. Simulate formation of Oort clouds and Kuiper belts with gas giants of various masses.

4. Calculate impact rates on a terrestrial planet using the above simulations of Oort cloud and Kuiper belt formation.
5. Add the effect of larger stellar density to the above models to determine how it changes the small body reservoirs and impact rates.

II. A.3. Delivery of organic materials to planets (Brownlee, Kress, Zahnle, Quinn).

BACKGROUND: The first objective of NASA's astrobiology roadmap is to determine the sources of organics on the Earth. Broadly speaking, reduced carbon compounds have two possible initial sources: they can be synthesized via geological and atmospheric processes in the planetary environment (such as hydrothermal vents), and they can be exogeneously delivered via impacts of asteroids, comets, meteorites and interplanetary dust particles (IDPs). Planetesimals that form in the habitable zone (HZ) of protoplanetary disks are likely to be intrinsically bereft of these materials. Habitable planets thus require exogenous delivery of volatiles (carbon, nitrogen and water) and they also may need surviving organic compounds. It is an irony of planet formation that HZ accretion occurs interior to the regions in protoplanetary disks where volatiles and organics are abundantly contained in solids. It appears that the majority of the Earth's volatiles and perhaps a major fraction of its pre-biotic inventory of organic materials were derived from regions of the solar system beyond the HZ. This is a situation that is likely to be repeated in other planetary systems. If HZ terrestrial planets themselves cannot generate adequate supplies of pre-biotic molecules then exogenous sources will play a critical role leading to the formation and evolution of life on habitable zone planets.

The delivery of outer planetary system organics to HZ planets is likely to have interesting and important variations between different types of planetary systems. In some cases the supply may be inadequate to seed life and in others it may provide a much richer supply than was obtained by Earth. In the proposed work we will examine the general role that exogenous sources of organic materials may play in seeding HZ planets with organic materials and we will examine the implications for our own planet. This work will also provide insight that will be valuable for evaluating which types of planetary systems and HZ planets might evolve life. We propose a two part approach: a) study of the origin, dynamics, evolution and survival of organic rich materials that impact HZ planets and b) laboratory analysis of organic materials currently impacting the Earth and implications for accretion of organic materials by other HZ planets

II.A.3.a. Delivery of organic compounds to habitable zone planets

Sources. Organic materials from outside the habitable zone can reach HZ planets in three forms; a) meteorites (rocks), b) larger chunks (meter to kilometer) of asteroids and comets and c) interplanetary dust particles smaller than a millimeter. Meteorites carry organic materials in their unheated interiors, but this delivery system works only for rocks that are both perturbed to orbits that intersect the HZ and ones that are sufficiently strong to survive atmospheric entry. Meteoroids that are too large to decelerate in the atmosphere and produce meteorites, impact at high velocity and produce explosion craters. Hydrocode computations by Pierazzo and Chyba (1999) indicate, that in-spite of the severity of these large events, that there should be some survival of organic material including amino acids. This is a potentially very significant source of pre-biotic organic material on HZ planets. The third source of organics, and one that must operate in all planetary systems, is delivery by dust. Dust particles must exist in all planetary systems and they spiral inwards towards the central star due to the action of light pressure drag

(the Poynting-Robertson effect). This process inevitably delivers organic-rich outer planetary system materials to inner planets in the HZ. Once at a planet, dust is decelerated at the top of the atmosphere and particles receive a brief thermal pulse. Particles below a critical diameter (depending on entry speed, and atmospheric scale height) are only moderately heated and survive with their organic contents intact. Recent work (Galvin and Bada 2000) has shown that even the most strongly heated particles can also provide organic materials. When strongly heated, particles sublime a fraction of their volatile organics, including some amino acids, directly into the atmosphere. This is an interesting source of molecules because they are in a more bio-available form than molecules that might be otherwise be trapped inside masses of meteoritic kerogens. Dust may be the dominant source of exogenous organics accreted by Earth (Anders 1989) and it may also be the major source for planets in the HZs of other stars as well.

Interplanetary dust. Dust is the major source of organic material currently falling to Earth and this source was surely much larger early in the history of planetary system evolution. Both the asteroidal and Kuiper belt sources of interplanetary dust must have been much greater in the past. Habing et al. (1999) have shown that the majority of nearby main sequence stars are surrounded by spectacular dust disks during their first 400 million years. Presumably the dust in the disks is generated by degradation of comets in the inner regions of Kuiper Belt comet disks, a process that declines after 400 million years. Dust spiraling inwards from these disks would provide an intense source of organics for nearly half a billion years. The duration of this effect is potentially highly significant, because it is similar to the time when Earth's period of heavy bombardment ended and when evidence of life first appears in the fossil record. When life began on Earth it is likely that this dust source was a major supplier of pre-biotic organic compounds. With few continents, little sediment formation and anoxic conditions, it is possible that the seafloor was highly enriched with meteoritic materials. We will use the observed data for young disks around main sequence stars to evaluate the timing and quantity of organic material that might plausibly accrete onto HZ planets.

Extrasolar dust, small-bodies. Extrasolar analogs to the Kuiper belt, our zodiacal dust cloud, even the planetary system in its early stages of evolution are indeed observable. Observations of dust around other stars will also be able to tell us about the particulate environments of other planetary systems at various times analogous to important solar system milestones; a) when the terrestrial planets formed, b) at the time life originated on Earth, and (c) at the time of the late heavy bombardment. Infrared observations of these debris disks can tell us about the sizes and composition of the dust; radial velocity observations can tell us whether there are giant planets in these systems; dynamical models can then tell us whether habitable planets might exist (Quinn). We will use data from these observations to tell us about the flux of material into the HZs of these (candidate) planetary systems, giving a cosmic perspective of what the Earth was like before, during and immediately after life originated.

Dynamics and transport processes. We will examine the dynamics and transport process from the disks to HZ planets and evaluate how the dust disk sources compare with other sources of organic materials. We will also evaluate how these processes might vary in different planetary systems. Although the focus in this portion of the proposal is on dust, there is a strong connection to the dynamics portion of the proposal (section I.A.2). The high dust production rates in young disks is linked to the interaction of Kuiper belt comets and outer planets. The

magnitude and time-scale of the dust source may vary significantly with the masses, spatial distribution, and evolution of outer planets.

Accretion: here and elsewhere. We will also examine how the general accretion of organic materials onto HZ planets may vary in different planetary systems. For example, in the solar system it is likely that a large fraction of the Earth's early exogenous organics came from the asteroid belt, a region that surely produced orders of magnitude more rocks and dust in its early phases than its present rate of over 30,000 tons per year. We will investigate what happens in planetary systems that do not have an asteroid belt. No cometary meteorite has ever been identified on earth and it is possible that comets do not produce debris larger than dust that is strong enough to survive atmospheric entry and thus be able to carry organics to HZ planets. Without an asteroid belt there may be no primitive meteorites (rocks) landing on HZ planets and there certainly would be no asteroid samples. This might be very significant to the evolution of life for a very interesting reason: asteroids may have a richer complement of organics than comets.

Sources of organics. Organic materials in primitive bodies are derived from three major sources; a) pre-solar materials, b) compounds produced in the nebula and c) compounds produced inside parent bodies by aqueous reactions with nebular and pre-solar products. Comets are likely to contain mainly organic materials carried with presolar materials, while asteroids are likely to have organic materials produced by all three sources. Evidence from meteorites indicates that many of the asteroids had warm interiors that melted ice and produced water that altered silicates and formed other alteration products. It is a fascinating possibility that some of the key pre-biotic compounds were formed in aqueous environments (chemical factories of minerals, water and organic matter) that occurred inside asteroids early in the history of the solar system. It has been suggested that amino acids in carbonaceous chondrites formed in this manner. It is possible that moderately heated and internally wet asteroids are a critical step leading to life on HZ planets.

Comets and asteroids: systems without giant planets. In addition to investigating the dust effects in planetary systems without asteroid belts, we will also investigate systems without Jupiters or with Jupiters of smaller mass or larger radial distance. Jupiter plays several well known roles in influencing the impact rate of large objects on Earth, but it also has profound effects on dust. Jupiter's gravity strongly affects particles spiraling inwards from the outer regions of the solar system and may prevent particles of certain sizes from reaching HZ planets. Jupiter also plays a huge role in providing comet dust to Earth. It "produces" short period comets, the source of most of the comet dust in bound orbits in the inner solar system. Short period "Jupiter family" comets are Kuiper belt comets that were placed into short period orbits due to gravitational interactions with the giant planet. Some of the comet dust that reaches Earth may come directly form the Kuiper belt but most of it comes from Kuiper belt comets that have become temporally trapped in short period orbits as Jupiter family comets. In planetary systems without giant planets of the right mass in the right places there would be no short period comets.

This is a critical issue for delivery of comet dust because long period comets cannot effectively inject dust into bound orbits in HZs. Dust ejected from long period comets with highly eccentric orbits is blown out of the planetary system because of light pressure effects. These orbits have near parity between potential and kinetic energy and even tiny effects of light

pressure place released particles on unbound orbits. Short period comets, trapped by Jupiter have smaller eccentricities and much higher ratios of potential to kinetic energy. Nearly all solar system comet dust in bound orbits in the HZ was released from Jupiter family comets. Without Jupiter there would only be long period comets and there would not be a strong source of comet dust for HZ planets. A planetary system without a Jupiter and an asteroid belt would only be able to get dust by long distance spiraling directly from the Kuiper belt.

Effects of particle size. The effectiveness of delivery of organics from dust will vary with particle size, planet size and planetary position due to differences in entry speeds and other factors. The sizes of surviving particles will also vary. On the present Earth the bulk of the extraterrestrial material annually accreted is in 200μm size particles while the surviving organic matter may be carried in sizes of about 25μm. In other situations the surviving particle sizes may be quite different. A good example is Mars whose lower surface gravity and larger distance from the Sun leads to atmospheric survival of much larger particles. The organic input from dust should be much higher for mars-like planets than those similar to Earth and Venus. Larger planets even closer to the central star might not be able to accrete these materials without severe thermal alteration and destruction of organics.

II.A.3.b Work statement. Work on this section will be led by M. Kress with participation by K. Zahnle (at Ames), T. Quinn and D. Brownlee. All team members are very experienced with delivery of primitive materials to planets.

II.A.3.b.1. First, we will develop a time-dependent model for the delivery of organics over the lifetime of the Earth. Its parameters will be constrained by observed and/or measured characteristics of the Earth and solar system: the geological record, studies of IDPs, cratering record preserved on the Moon, etc.

II.A.3.b.2. Second, we will generalize this model to extrasolar planetary systems, to determine the extent to which possible habitable planets may acquire organics. The parameters of the generalized model will be constrained by observed characteristics of extrasolar planetary systems and debris disks around main sequence stars, including the size distribution of dust grains in the disk; disk mass; age, luminosity and mass of the star; and the positions, eccentricities, orbital inclinations and masses of any giant planets in the system. We will compare model results for the sources, delivery and survival of exogenous organics to Earth-like planets, and compare these results to those for the Earth. We will quantify the amount of organic material that would have accumulated at their surface, and when, and compare these results to those for the Earth.

II.A.3.b.3. Third, the model will consider:

- size/mass of the impacting objects (micron- to meter-scale meteoroids)
- velocity at which these objects encounter the Earth (slower is better, therefore habitable planets further from the Sun fare better)
- composition and physical properties of meteoroids
- source of the meteoroid (and hence its potential organic inventory, its velocity, other properties related to source).
- effect of atmospheric entry of the composition, density, etc., of the Earth's atmosphere on the survival of the meteoroid's organic inventory.

- effect of giant planets on the effectiveness of transfer of material from large heliocentric distances
- effect of giant planets on the location of organic reservoirs (asteroid belts, Kuiper belts)
- change in luminosity of the Sun over time
- change in optical depth of the Zodiacal cloud over time, as dust spirals in, gets blown away by the solar pressure, destroyed by collisions, or incorporated into planets or the sun
- effect of collisions, sublimation, radiation pressure, and Poynting-Robertson drag on dust grains in the disk.

II.A.3.c. The nature of organic matter accreted by Earth: Dust

BACKGROUND Dust is a major carrier of organic material to HZ planets. We plan to use collected samples of interplanetary dust in the 3 μm to 35 μm range to directly investigate the nature of organic matter carried to Earth. The 40,000 tons of extraterrestrial dust accreted by Earth each year contains samples of all of the asteroids, as well as samples of short period comets. NASA's ER-2 aircraft routinely collect IDPs: many samples are in hand for this study. These samples are likely to be very similar to typical dust particles accreted by HZ planets in other planetary systems. If comets are largely composed of interstellar grains, then it is likely that comet dust will have common properties across planetary systems.

Goals. The ultimate goal is to determine the range of organic materials carried to HZ planets by interplanetary dust of cometary and asteroidal origin. Although analysis techniques are rapidly evolving, the main problems are sample size and heat effects from atmospheric entry, which alters some components. The practical near-term goal is to apply the best techniques available to learn as much as possible about IDP organic content. While this work might not reproduce the full shopping list of materials in "primordial soup," it will provide highly significant insight into the origin of organic materials. If this work can provide powerful constraints on the origins of pre-biotic organics, then modeling and experiments can try to predict the full range of compounds that might or might not be delivered to HZ planets. For example, we should provide evidence that the organics were produced by one or more of the following: irradiation in the interstellar medium, irradiation in molecular clouds, catalytic reactions in the solar nebula, or irradiation in the solar nebula. Messenger (2000) provided D/H isotopic evidence that some organics in IDPs formed in molecular clouds. We will work on both asteroidal and comet particles, and may find that organic materials significantly differ between these "outer solar system" and "inner solar system" sources.

Samples. We will identify and use those rare IDPs that show minimal atmospheric heating. We will also use atmospheric heating, particle mass, and particle density to determine which particles most likely come from comets. We routinely use the stepped He release method (Joswiak et al. 2000) developed by A. Nier at the University of Minnesota. These efforts will give us a set of samples with identified cometary and asteroidal origins and particles with minimal thermal alteration.

Analytical techniques. The technology for examining the organic contents of these small samples is rapidly evolving as are the methods of handling and preparing IDPs for organic studies while minimizing contamination. Typical IDPs are highly complex, fine grained

mixtures of organic material, glass and thousands to millions of small silicate, sulfide and metal grains. The bulk of this work will be done on 10μm IDPs that will be microtomed to produce 100 nm sections for electron microscopy and various microbeam studies. Sulfur will be used as embedding medium for microtomy (it sublimes in vacuum and does not interfere with carbon analysis, as does conventional epoxy mounting media). These special techniques were developed by our analysis team. Some samples will be demineralized in hydrofluoric acid to remove silicates, leaving only carbonaceous matter and sulfides.

Sublimation. We will also do a special project to analyze the organic content of IDPs that sublimes into the atmosphere during atmospheric entry heating. The goal of this effort will be to provide insight into organic sublimation source from IDPs that was suggested by Galvin and Bada (2000)at last year's Astrobiology Science Conference. Galvin and Bada heated carbonaceous chondrites grains and demonstrated that glycine can successfully escape strongly heated particles by sublimation. We will extend this work to individual IDPs. We will carefully select weakly heated IDPs and place them on 20 nm thick silicon monoxide films mounted on standard TEM grids. We will then place a second grid with film on top of each IDP forming a sandwich with a space of only 10's of microns between the films. Each IDP will then be heated with a 1μm electron beam (in the SEM, TEM or microprobe) until the particle reaches a temperature > 500C. We have previously used this method to heat individual 10μm IDPs to temperatures as high as 1300C. Volatiles which sublime from the spot-heated IDPs will re-condense on the surrounding cold thin films, only 10's of microns from the particles, and form a highly concentrated deposit. We will analyze the condensed sublimate with the same procedures that will be used for IDP thin sections. It needed these experiments can be done over a range of temperatures.

Organic content of IDPs. IDPs contain, on average, about 12% by weight carbon (Thomas et al. 1994), about 4x the carbon content of the most carbon-rich meteorites. In some particles it ranges up to 50wt % and is very easily observed in microtome sections. Clemett et al. (1993) identified organic carbon, particularly polycyclic aromatic hydrocarbons, in some IDPs. Messenger (2000) worked on the isotopic composition of nitrogen and hydrogen, and showed that IDP organic matter is more primitive than in any meteorite class, in the sense that there is a higher preservation of pre-solar molecular cloud material. To determine types and abundances of organic compounds we will use we will use electron energy loss spectroscopy and imaging in the electron microscope and two synchrotron-based instruments at the National Synchrotron Light Source (Brookhaven National Laboratory). These instruments are a Fourier Transform Infrared (FTIR) Spectrometer (it detects the 3 micron C-H stretching vibrations of organics); and a Scanning Transmission X-ray Microscope (STXM: this allows us to map the carbon distribution and perform Carbon-X-ray Absorption Near Edge Structure (C-XANES) spectroscopy).

II.A.3.c.1. FTIR Examinations of the 3 micron Region The 3.4 μm feature due to C-H stretch vibrations (aka the "organic feature") is observed in some comets and other astronomical sources. It is one means that allows comparisons of organic materials in meteoritic samples with astronomical sources. The NSLS delivers an infrared flux ~1000x that of conventional infrared sources, and can readily provide high-quality FTIR spectra for IDPs. Flynn et al. (1998) and others examined the 3.4μm feature in IDPs. He found an absorption feature at 2926 cm^{-1}, and a second (weaker) at 2854 cm^{-1}. This pair is characteristic of C-H_2 symmetric and asymmetric

stretching vibrations of aliphatic hydrocarbons. The FTIR spectra of the examined IDPs are very similar to the 3.4 micron absorption detected in the interstellar medium by Sandford et al. (1991). We will extend this work to well-analyzed microtome sections from weakly heated IDPs representative of cometary and asteroidal origins.

II.A.3.c.2. STXM Examinations We will map the absorption of ultramicrotome thin sections at x-ray energies of ~275 eV and 310 eV. Since the absorption coefficient of carbon increases sharply at the carbon K-edge (~290 eV), while other elements have roughly constant absorption coefficients over this energy range, spots showing increases in absorption at 310 eV are carbon-rich. Carbon-rich regions will be examined by C-XANES spectroscopy, which detects absorptions that correspond to photon-induced transitions by core level electrons into various bound and virtual excited states. Variations in electron density surrounding the photoexcited states leads to well-resolved absorption bands corresponding to carbon in different functional groups.

II.A.3.c.3. Work statement This work will be led by D. Brownlee, with participation by J. Bradley (Georgia Tech and MVA Inc.), G. Flynn (SUNY Plattsburgh) and M. Kress. This team has extensive experience in IDP analysis and has pioneered most of the techniques used in the study. The following activities will be done in sequence:

- Select rare IDPs that show evidence for only mild thermal alteration.
- Identify particles with likely cometary and asteroidal origins with stepped He analysis.
- Produce microtome sections under non-contaminating conditions.
- Determine carbon distribution by imaging in the TEM, EELS, and STXM.
- Do IR analyses on the 3.4 μm feature.
- Do C-XANES and EELS analyses of carbonaceous regions to investigate molecular speciation and local atomic bonding environments.
- Other analyses as appropriate- ion microprobe, UV spectroscopy, two pulse laser mass spectroscopy
- Other analyses as appropriate- ion microprobe, UV spectroscopy, two pulse laser mass spectroscopy
- Select rare IDPs that show evidence for only mild thermal alteration.

II.A.4. Evolution of the surface and atmosphere of Mars (Leovy, Warren)

BACKGROUND Delivery of volatile materials to a planet is a necessary condition for habitability, but it is not sufficient. These materials must be retained in a planetary atmosphere, hydrosphere, or crust if life is to originate and evolve. Not only is liquid water a key requirement of life, but also liquid water percolation through the crust seems to be required to produce Earth-like plate tectonics (Sec. I.B.3.). Earth-like plate tectonics are linked to efficient cycling of CO_2, hence to maintenance of a stable temperature regime via a long-term greenhouse effect (e.g., Kasting 1989). Our solar system has three rocky planets whose orbital parameters potentially allow liquid water retention on or near the surface. Earth is well endowed with liquid water and life. Venus is hot and has apparently had no liquid water over at least the past 0.5 Byr. Mars is cold, and has little or no liquid water at the surface today, but there is evidence that liquid water has existed at and near the surface in the past.

Retaining water on Mars: problems. Water delivered to the surface of Mars faced two serious retention hazards. The first of these was impact erosion. Because of Mars' small mass and gravity, its atmosphere was far more susceptible to erosion by early heavy bombardment than was that of either Earth or Venus (Melosh and Vickery 1989). The second hazard involves core formation and the related timing of volatile material delivery. If reduced iron reacted efficiently with water-bearing minerals prior to core formation, much of the initial water would have converted to hydrogen and rapidly escaped (Dreibus and Wanke 1987). Water available near the surface may have been limited to impact sources that followed core formation.

Why study Mars? Careful study of Mars' surface and atmosphere can reveal much about its water history and give insight into the broader problem of water retention for rocky planets in thermally benign orbits. We propose to investigate aspects of the evolution of the atmosphere and surface of Mars that will help to constrain models of water retention. An improved understanding of the history of water will also help develop a sharper strategy for the biological exploration of Mars.

II.A.4.a. How much liquid water has there been on Mars?

Evidence FOR lots of water on Mars. Water is certainly present today on Mars in the form of ice and vapor. Several lines of evidence also point to liquid water, at least in the past. But there are still very large uncertainties about the abundance, distribution, and timing of liquid water. Evidence for abundant past liquid water exists, e.g., outflow channels and valley network features (Carr 1996). Evidence of very high erosion rates during the Noachian period (before ~3.8 Ga) has been interpreted in terms of abundant flowing water and a warmer climate at that time (Craddock and Maxwell 1993). Delta, shoreline, and flow features have been interpreted as evidence of paleo-lakes, some extant during the most recent ("Amazonian") period (Cabrol and Grin 1999). Detailed topographic data from the Mars Global Surveyor Mars Orbiter Laser Altimeter (MGS MOLA) reinforce the hypothesis that large lowland areas of the northern plains have been occupied by liquid water seas, quite possibly covered with ice (Parker et al. 1993; Head et al. 1998; Zuber et al. 2000). Head (2000) discussed features that closely resemble terrestrial eskers in the south polar region. Martian meteorites show evidence of modification by liquid water within the past 1.3 Byr (Swindle et al. 2000). The most compelling evidence for running water at the surface is from Mars Orbiter Camera (MOC) images from MGS, which clearly show gullies originating in alcoves and terminating in debris aprons that resemble alluvial fans on steep slopes (Malin and Edgett 2000). Remarkably, these gullies are very recent and occur predominantly at middle and high latitudes on slopes that face away from the Sun, where present-day temperatures rarely if ever exceed 250 K.

Problems with the evidence. However, there are difficulties with interpreting all of these features in terms of liquid water. Perhaps the most troubling issue is the sheer scale of the outflow channels. Their vast scale and ubiquity seem to require an available surface water inventory of at least several hundred meters of equivalent planet-wide ocean at about 3.8 Ga (Carr 1996), much later than the termination of heavy bombardment. Yet abundances of noble gasses (particularly ^{84}Kr) and N suggest that the volatile inventory of Mars was small at 3.8Ga. Since ^{84}Kr has not been subject to escape to space or interaction with the surface over the past

3.8 Byr, its abundance is a particularly significant indicator of volatile abundance prior to outflow channel formation. In one estimate based on ^{84}Kr scaling and a model of comet composition, Owen and Bar-Nun (1995) obtain a total CO_2 inventory (expressed as global mean surface pressure) of only 85 hPa at the end of heavy bombardment. Water in ice deposits may have been protected from impact escape before that: up to 750 meters may have been preserved at the end of heavy bombardment according to Owen and Bar-Nun (1995), but this amount is highly uncertain (e.g., Chyba 1990, Chyba et al. 1994). The high atmospheric D/H ratio (5.6x terrestrial) is also unsettling. At face value, it suggests that >90% of water available in exchangeable near-surface reservoirs was lost by mass-selective escape during the period of outflow channel formation. Mass-selective escape rates of H, C, and O may have been significant, but are uncertain (Luhmann al 1992, Jakosky and Jones 1997, Johnson et al. 2000). However, some of the difference between sources of D and H on Mars and Earth or temporary sequestration of water from exchange with the atmosphere (Carr 1990) may contribute to the high ratio.

Where are the sinks for water and CO_2? Adequate sinks for large volumes of water and CO_2 also pose problems. While CO_2 may be adsorbed in the regolith, and a small amount resides in the permanent south polar cap, large amounts of surface liquid water together with abundant CO_2 should have generated large carbonate reservoirs (Kieffer and Zent 1992; Pollack 1979; Pollack et al. 1987). Carbonate may have been destroyed by reactions with sulfur, which is abundant in Martian soil, but the failure to detect substantial carbonate deposits in MGS Thermal Emission Spectrometer (TES) spectra is disturbing (Bandfield et al. 2000). Moreover, while TES has provided unambiguous evidence of hematite in two restricted locales (Bandfield et al. 2000; Christensen 2000), there is little evidence of the widespread aqueous-phase weathering products that are expected if large volumes of water have flowed across the surface. Valley network channels and some interpretations of paleo-lakes require warmer climates in which surface liquid water could persist over significant time periods, but it has proven difficult to account for a sufficiently powerful climate warming mechanism. The most promising mechanism is the "scattering CO_2-ice cloud" model (Forget and Pierrehumbert 1997), but its efficacy has not yet been fully demonstrated. It depends on the sizes and distribution of the ice particles and seems to require CO_2 surface pressures of at least 1000 hPa.

II.A.4.b. Alternatives to massive flows of liquid water While none of these issues rules out either formation of outflow channels or putative paleo-lakes by massive water flows or past existence of warm wet climates, they motivate careful consideration of other possibilities.

Volcanism, water ice, or CO_2 ice? Some, but not all, of the apparent indications of liquid water may arise from volcanism. Features closely resembling Martian outflow channels and valley networks are found on the surface of Venus, and are attributed to exceptionally fluid lava flows (Komatsu et al. 1993, Baker et al. 1997). Lava erosion, previously proposed (Cutts et al., 1978), has not been widely accepted as a major mechanism for Mars channel formation, but images from the MGS-MOC camera show strong evidence of fluid lava flows on Mars (Hartmann 2000, Keszthely and McEwen 2000). Morphology of some flow features is also suggestive of glacial action (Lucchitta, 1982). Hoffman (2000) proposed an entirely different mechanism for forming outflow channels and valley networks. Calling attention to morphological similarities to gas-driven pyroclastic flows and to terrestrial submarine channels generated by turbidity currents, he suggests that sudden release of CO_2 from pressurized

subsurface clathrate, CO_2 ice, or CO_2 liquid deposits could have produced some of the putative water channels in the present climate regime. In the light of the data from Venus, new observations of the surface of Mars, and Hoffman's hypothesis, mechanisms for forming channels on Mars require re-examination.

Wind? The role of wind may be more important than usually recognized (but see Cutts and Blasius, 1981). Turbidity currents occur today during Martian dust storms that are characterized by sharp lobate fronts. Spacecraft images clearly show such storms and indicate that wind erosion and deposition are acting today and have played very important roles in modifying the surface. At middle and low latitudes and in many high-latitude regions, dunes and drifts are ubiquitous, locally concentrated in low areas, and strongly controlled by topography. Wind erosion could account for the "clean-up" and removal of fine materials accumulating from slumping of crater, canyon, and valley walls. Wind transport of sand-sized particles may take place especially rapidly in low areas such as the northern plains (Anderson et al. 1999). If atmospheric pressure was ever a few tens of hPa or higher, the frequency of dust storms and the rate of fine particle transport by wind could have been one or two orders of magnitude greater than today (Fig. 2).

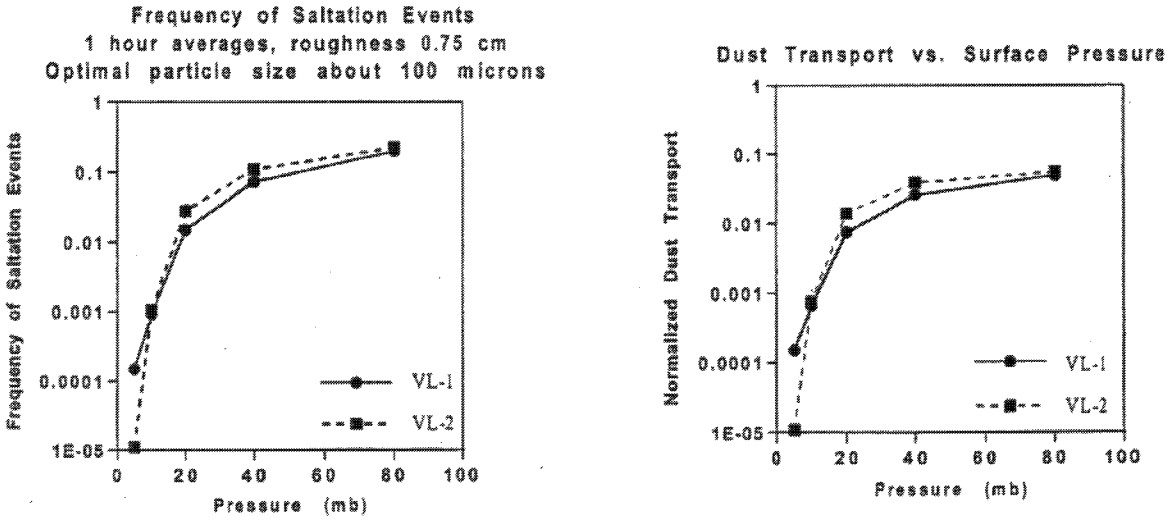


Figure 2. Left: Frequency of saltation events, 1-hour averages, roughness 0.75 cm. Optimal particle size about 100 microns. Right: Dust transport vs surface pressure.

The threshold wind speed for surface grain saltation drops rapidly as surface pressure increases. Surface roughness increase can also decrease the threshold wind speed. These two mechanisms provide potentially powerful feedback for surface erosion of fine particles. As fine material is removed from the surface by wind, larger resistant material remains, roughening the surface and lowering the threshold for further wind erosion. Regions of deep regolith whose initial surface elevation is several km below the planetary average would be particularly susceptible to erosion. This mechanism is potentially powerful and can explain the high positive correlation between rock abundance and low elevation and the tendency for winds simulated by a Mars general circulation model to rapidly sweep sand from the northern lowlands (Christensen 1986, Anderson et al. 2000). The fallout rate of suspended dust is substantial in the present regime, amounting to about 10 meters per Myr at the Pathfinder landing site (Golombek and Bridges 2000). It is likely that there has been a substantial redistribution of fine material over the past several billion years. The role of wind erosion over the past 4 Byr is unclear, but high resolution MGS MOC images clearly show that it must be taken into account in the interpretation of Martian surface evolution.

II.A.4.c. Research questions: We propose to address two related questions about the evolution of the Martian atmosphere and surface.

(1) To what extent can wind erosion and redistribution of fine materials account for observed properties of the surface of Mars?

(2) To what extent is wind erosion implicated in the formation or subsequent modification of outflow channel and valley network features?

II.A.4.d. Work statement: We will combine modeling tools with the wealth of new observations to better constrain the roles of wind and water in surface evolution over the past 3.8 Byr and to contribute to the study of the implications of Mars' evolution for the general problem of planet habitability. Specific tools include: (1) the NASA Ames General Circulation Model

(AGCM) in collaboration with collaborator R. Haberle, (2) a mechanistic simple climate model incorporating wind redistribution to be developed here, (3) Co-I Quinn's dynamical calculations of Mars' orbital parameter statistics, and (4) the MGS data sets, in particular, a tool-based MGS data retrieval system being developed at NASA Ames (G. Briggs, pers. comm.). This research will be closely linked to our research efforts on impacts and the delivery of volatile materials (Sec. I.A.2 and I.B.1), and to our work on fundamental processes underlying plate tectonics (see I.B.3). Improved understanding of the distribution of biologically favorable habitats will link this effort to physical aspects of biocomplexity (Sec. IV).

(1) Application of Mars orbital parameter statistics to climate evolution. Co-I Quinn has completed dynamical calculations of the orbital parameters of the solar system planets for time spans ranging up to 1 Byr. We will use these results to develop statistics of obliquity, eccentricity, and argument of perihelion. These will guide studies of wind erosion and transport using our simple climate model (below) and the AGCM, and will be provided to Haberle et al at NASA Ames for additional studies of Martian climate variations.

(2) Simple Climate Model Studies. We will develop a simple climate model along the lines of Haberle et al. (1994) to explore the parameter space of surface pressure, surface topography, and orbital variations. In addition to exchanges between volatile reservoirs included in that model (also see Kieffer and Zent1992), we will incorporate a simple model of wind erosion and dust redistribution dependent on surface wind stress, topography and roughness. We will adapt the transport parameterization developed by White (1979) and used by Anderson et al. (1999). The model will be driven by Quinn's orbital parameter statistics. Incorporation of surface wind speed and stress distributions will be guided by the AGCM runs (below). R. Haberle (NASA Ames) will collaborate on this work.

(3) Studies using the AGCM. We will use the AGCM to selectively explore wind erosion and dust redistribution in the parameter space of surface pressure, orbital parameters, and surface roughness. A key issue for both the AGCM and the simple climate model is the effectiveness of the roughness and surface pressure feedback mechanism for the distribution of wind erosion. The model will include a parameterization of saltation and will allow for transport and fallout of dust. We will follow the approaches of Murphy et al. (1995) and Anderson et al. (1999), and will also incorporate a parameterization of dust lifting by dust devils. The AGCM is currently being upgraded (at NASA Ames) with an improved transport algorithm. This effort will rely heavily on our collaboration with R. Haberle.

(4) Analysis and interpretation of MGS data. Our ultimate objective is to combine the power of the modeling tools and the new observations to better constrain the roles of wind and water modification of the Martian surface over the past 3.8 Byr. Many investigators are using these data to interpret the evolutionary history of Mars. We will relate spatial distributions of surface features seen by MOC, TES, MOLA, and the MGS Magnetometer experiment to patterns of wind erosion and dust redistribution. Our modeling efforts (above) will provide a powerful interpretative tool for integrating disparate observations, and can contribute to an integrated view of the processes affecting the surface which will account more completely for the role of the atmosphere. We have already begun to work with several undergraduates to systematically examine high resolution MOC images for wind-related features.

II.B. Plate tectonics on the terrestrial planets (Solomatov)

Background While most major factors allowing planetary habitability such as distance from a star and the presence of liquid water have long been known, only in the past two decades has the presence (or absence) of plate tectonics been considered of importance as well. The landmark 1981 paper by Walker et al. and subsequent work by Kasting and others have led to a new appreciation of the importance of plate tectonics for maintaining the long term temperature equilibrium apparently necessary for the maintenance of complex life. The basic chemical equation of this system, $CaSiO_3 + CO_2 = CaCO_3 + SiO_2$, in effect allows a temperature feedback on planets with crustal recycling that will not take place as efficiently on fixed lithosphere planets. Walker et al. pointed out that as atmospheric CO_2 increases (causing warming), the rate of chemical weathering on its surface increases. As the rate of weathering increases, more silicate material is made available for reaction with the atmosphere, and more CO_2 is removed, causing cooling. Yet as the planet cools, the rate of weathering decreases, and the CO_2 content of the atmosphere begins to rise, causing warming. Thus, Earth's temperature oscillates between warmer and cooler due to these carbonate-silicate weathering and precipitation cycles.

Many astrobiologists are coming to see the presence of plate tectonics on a planet as a necessity for allowing long-term temperature stability (which is assumed to be a necessity for complex life), but this view is not universally accepted. It may be that plate tectonics causes sufficient recycling of reductants to inhibit for long periods of time the formation of an oxygen atmosphere. Thus while plate tectonics may allow the long term stability necessary for complex life, at the same time it may inhibit the also-necessary rise of atmospheric.

The astrobiological study of plate tectonics is in its infancy. While the study of plate tectonics on Earth is a mature discipline, terrestrial geologists have largely ignored factors dealing with crustal composition and planetary size that would lead to a more general theory of plate tectonics that would permit a deeper understanding of the necessary and sufficient conditions for stable plate tectonics on Earth and other planets. Some geologists have speculated that plate tectonics can only take place on a world with oceans, and that some critical depth of water is necessary to allow the hydration of basalt that makes subduction possible.

The astrobiological study of plate tectonics thus requires a multidisciplinary approach. We believe that this study should be an NAI priority, and that no one member institution can supply all necessary expertise. The questions are enormously varied, and include:

- What are the planetary conditions that allow the formation of plate tectonics, in terms of size, compositions, and accretion history of a planet?
- Are oceans necessary for plate tectonics?
- How does plate tectonics begin on a world (and how and when did it begin on Earth?)
- How does plate tectonics affect the rise of oxygenation and atmospheric history?
- What are biological consequences of plate tectonics?
- What aspects of the different origins and evolutionary histories of Earth, Venus, and Mars are responsible for their very different tectonic styles and histories?

In the section below, we will examine several of these questions. We foresee a fruitful collaboration between our planetary modeling group (Quinn, Gonzalez) and our plate tectonic modeling group (Solomatov); between Solomatov and our early Earth group (Buick), between

Solomatov and Leovy on comparing the different histories of Earth and Mars, and between Solomatov, Buick and Ward on early biological consequences of plate tectonics.

Among the terrestrial planets, only Earth has plate tectonics. The other terrestrial planets and Earth's Moon are covered by immobile lithospheres ("one-plate" planets). Debate on whether or not plate tectonics occurred on other planets in their pasts is fueled by two major observations. First, the surface of Venus is only 300-800 Myr old (Basilevsky et al. 1997): this might mean cessation of plate tectonics or of its most recent episode (Schubert et al. 1997). The second is the magnetic lineations on Mars discovered by Mars Global Surveyor (Connerney et al. 1999). These could have been formed if plate tectonics occurred on Mars during the planet's first 500 million years. Completely different arguments for Martian plate tectonics were suggested earlier by Sleep (1994). Plate tectonics may not be the only explanation: Reese et al. (1999a) suggested that Venus could have undergone an episode of widespread melting and volcanism relatively late in its history. The search for alternative explanations for magnetic lineations on Mars, however, has not yet produced any competitive hypotheses. In any case, plate tectonics, if it did take place in the Martian history, had a relatively short life. Long-lived, stable plate tectonics has happened (in our system) only on Earth. Earth is also the only planet that has complex life. These two phenomena are unique in the Solar System and there is little doubt that there is likely some connection between them.

As discussed above, plate tectonics affects the evolution of life through providing chemical exchange and creating diverse geological environments, which promote biodiversity. On the other hand, the fluctuations in plate motion are relatively gentle compared, for example, with the dramatic changes in the tectonic and volcanic regimes of Mars or Venus, and do not sterilize the entire planet. Plate tectonics is also essential for the generation of the magnetic field which serves as a shield against cosmic radiation and solar wind. It seems that to understand the role of plate tectonics in the formation and maintenance of complex life in the Universe, we should first understand how plate tectonics works on this planet. Is it something that most planets would inevitably have during some period of their evolution, or is it a rare phenomenon requiring special conditions?

Mechanisms of plate tectonics: Rephrasing W. Saslaw's statement regarding galaxies we can say that "If plate tectonics did not exist we would have no difficulty in explaining the fact". Discussion of initiation of plate tectonics on Earth started with McKenzie (1977) and Turcotte et al. (1977). McKenzie (1977) wrote: "The argument above suggests why the Earth might be unique. The initiation of consumption depends on the shear stress on fault planes being as small as 100 bars, and on the existence of a low viscosity layer below the plates to permit their motions to be decoupled from the mantle. It is now believed that both are due to the existence of water, which is thought to reduce the friction on fault planes and to produce the low viscosity layer through partial melting of about 1% of the mantle." Full realization of how difficult it is to initiate plate tectonics on a planet came much later. Until recently pate tectonics appeared to be a "normal" phenomenon, and researchers were mainly concerned with the question "Why don't other terrestrial planets have plate tectonics?"

Crust and subduction Many workers have studied local subduction processes under terrestrial conditions. Crust (or some other buoyant residuum) was often considered to be a factor preventing subduction, and explanations of why other terrestrial planets do not have plate tectonics usually involve crust. Although crust certainly helps stabilize a lithosphere,

temperature-dependent viscosity with a high resistance to brittle failure would immobilize the lithosphere in any case (Solomatov and Moresi 1996). One suggested mechanism of plate mobilization, non-Newtonian viscosity (stress-weakening), is ineffective: a stagnant lithosphere develops for both Newtonian and non-Newtonian viscosity, if viscosity parameters are realistic (Solomatov and Moresi, 1997). Other mechanisms, such as sediments (Lenardic and Kaula 1994) or granitic continents (Turcotte 1996) cannot mobilize the surface either. With all the uncertainties in the rheological parameters, fluid dynamical models, and mineralogical and thermal structure of the planets, temperature-dependent viscosity would certainly not allow plate tectonics to occur.

Brittle failure Brittle failure seems to be critical in plate tectonics. The importance of brittle deformation was recognized by researchers struggling to explain features of terrestrial plate tectonics such as toroidal/poloidal energy partitioning, plateness and transform faults (Zhong and Gurnis, 1996; Bercovici, 1998; Tackley, 2000). Incorporation of brittle failure into convection models (Moresi and Solomatov, 1998) showed that the tectonic regime of a planet depends on the magnitude of the yield stress for brittle failure: plate tectonics occurs only if the yield stress is very low. Tentatively, the friction coefficient on the modeled faults must be ~0.03-0.13 (vs ~0.6-0.8 suggested by laboratory experiments). Although surprising from a point of view of rock mechanics, it was not so from the point of view of seismic data and observations of actual faults. Those data indicate a very low stress level in Earth's lithosphere, consistent with results from convection models. This consistency, and approximate agreement with theoretical predictions by Fowler (1993) suggest that the lithosphere of the Earth is unusually weak.

II.B.1. Research topic #1: What planetary conditions allow for plate tectonics? First we will complete and extend our study of conditions allowing plate tectonics on a planet. We will find mechanical criteria separating various convective regimes (stable plate tectonics, strongly fluctuating plate tectonics, stagnant lid convection, etc.) as a function of rheological laws governing mantle and lithosphere behavior. The two main laws are temperature- and pressure-dependent viscosity, and a depth-dependent yield stress. We assume a continuum model of brittle deformation, and ignore details of the fault system (e.g., number of faults, their orientation and size distribution). To describe faults' macroscopic influence, we need only the yield stress. This approach captures the correct physics and is at the moment the only way to study general aspects of plate tectonics. It also reproduces such features as two-scale flow (small-scale convection and plumes) and plateness. Shear strain (or strain-rate) dependent yield stress can produce well-developed shear localization zones (Tackley 2000), but it does not much change the criteria for initiation and cessation of plate tectonics.

We will examine, e.g., pressure-dependent viscosity, the presence of a weak asthenosphere (which may affect stability of plate motion: Richards and Baumgardner 2000), the presence of a buoyant, rheologically distinct crust (Lenardic and Moresi 1999) and small-scale convection (which stabilizes the thickness of lithospheric plates). Using 2-D studies Solomatov and Moresi (2000) showed that it is also important to consider large aspect-ratio boxes, because simple square boxes cause artificial wall effects and do not indicate much about the stability of the solutions. These 2-D studies will use the finite element code CITCOM (Solomatov and Moresi, 1996) and will be used as a guide for fully 3-D simulations in a spherical geometry. The 3-D simulations will use TERRA (Reese et al. 1999b). The results will be summarized with the help of scaling laws to show how initiation of plate tectonics depends on planetary mass (small

planets have low gravity and thus low driving force for plate tectonics), viscosity, yield stress (these two are affected by mineralogy; for example, high viscosity of the pyroxene-dominated mantle of Mars could be the reason why the planet does not have plate tectonics), crustal thickness, surface temperature, mantle temperature, heat flux from the core, etc. This approach has succeeded in studies of small-scale convection (Solomatov and Moresi 2000).

The choice of variables on which the "plate tectonics law" depends on cannot be arbitrary. For example, these scaling relationships can depend on the mantle temperature but not on the internal heat production rate as one might think. At any given moment of evolution, mantle dynamics ``knows'' little about how much heat it is currently generating and depends mainly on the current temperature (which is a product of the previous heat production history). To address the effect of internal heat production rates one must consider the global thermal evolution of the planets. Although some aspects of thermal evolution can and will be studied using fully 3-D models, a much broader parameter range with various additional processes will be explored with the help of parameterized convection models described below.

II.B.2. Research topic #2: Parameterized models. What role does coupling between plate tectonics, melting, climate and volatile cycling play in regulating earth's environment? The next step will be to develop a model which goes beyond the limits of fluid dynamics and includes other processes that affect and are affected by plate tectonics: melting, degassing and volatile cycling. A fundamental problem is the complexity of Earth's system, which makes it difficult to treat all physical and chemical processes from "first principles" (although some interactions can be so modeled). One possible approach is to understand and describe every individual process as simply as possible and then combine all processes into a single system, with all necessary interactions between individual elements. This principle of simplicity in treating individual components of a complex system is widely used in other areas of science (including modeling of climate, early magma oceans, material processing, etc.) and seems reasonable here. It may not catch the very fine details of the system's behavior, but the large effects can be identified and understood.

In studies of planetary evolution this philosophy led to the development of a "parameterized convection model". Initially it was used mainly for fluid dynamical calculations and was very attractive because of its simplicity (Turcotte et al. 1979; Schubert et al. 1979). It allowed for exploration of planetary evolution in a broad parameter range, using simple laws obtained with the help of numerical simulations. This approach can simulate various non-linear interactions between fluid dynamic processes like melting, crustal formation, degassing, magnetic field generation, etc. (Stevenson et al. 1983; Schubert et al. 1997; Reese et al. 1999a; Nimmo and Stevenson,2000). It can also predict the correct convective regimes and the transition between them during planetary evolution (Solomatov and Moresi 1996; Schubert et al. 1997; Reese et al. 1999; Sleep 2000). Individual aspects of the parameterized model can and should be tested in first-principle models. We will continuously improve the parameterized model by means of advances from studying individual components and interactions.

Parameterized models. Our parameterized model of mantle dynamics will include scaling laws governing mobility of lithospheric plates; plumes and small-scale convection; melting; degassing; and circulation of volatiles (especially water and CO_2). Scaling laws governing mobility of lithospheric plates are those from the numerical simulations (above). Magmatism due to melt generation in supersolidus regions can affect initiation of plate tectonics and is the most

efficient outgassing mechanism. Magmatism can be parameterized in both plate tectonics and stagnant lid convection regimes (Reese et al. 1999a). This allows us to calculate crustal thickness and buoyancy of lithospheric plates. Experimental data can constrain reduction in viscosity due to melting (Hirth and Kohlstedt 1995a,b). Mantle dynamics, magmatism and the climate of a planet can be strongly interconnected (Walker 1981; Solomon et al. 1999). Reese et al (1999a) suggested that magmatism and the associated increase in surface temperature and lithospheric weakening can even initiate plate tectonics. Feedback between climate and interior dynamics can be parameterized and included (Phillips and Bullock 1999).

Water Surface water is important for the global water budget of the Earth, and abundance of water in the lithosphere and mantle is the major factor affecting plate tectonics. The effect of water on the viscosity of rocks can be parameterized using experimental constraints (Karato and Jung, 1998; Mei and Kohlstedt 2000a,b). Water also reduces the yield stress for brittle failure (Blanpied et al. 1995; Bercovici 1998). We suspect that serpentinization could be a critical factor, because it produces weak minerals (serpentinites) via interaction of water with olivine. This is a likely candidate because serpentine is the only rock with a friction coefficient low enough to allow plate tectonics (Escartin et al. 1997). Rates of extraction of water from the mantle due to partial melting (e.g., Ito et al. 1999) as well as the reverse process, percolation of water into the lithosphere and its recycling back to the mantle (e.g., Kasting and Holm 1992) can be parameterized and included in calculations of water cycles.

We will use the parameterized model approach to understand complex interaction between plate tectonics, melting, and volatile recycling. This will show which factors help to initiate and maintain plate tectonics and which factors inhibit plate tectonics or make it unstable and strongly fluctuating with time.

II.B.3. Research topic #3: What conditions permit generation of a planetary magnetic field? Parameterized convection approach will also help to understand better the relationship between plate tectonics and magnetic field of a planet which is believed to be due to convection in the highly conductive metallic cores of the terrestrial planets. Although the problem of generation of magnetic field is rather complex, simple parameterizations of convection in the core and the mantle of the terrestrial planets allow to predict the presence or absence of the dynamo and even estimate the temporal changes in its magnitude (Stevenson et al. 1983). This approach was recently used to evaluate the role of plate tectonics in the formation of magnetic lineations on early Mars (Nimmo and Stevenson 2000). We will investigate the conditions that allow a stable, long-lived magnetic field (which, in addition to the conditions controlling plate tectonics include also the composition of the core). Generation of the magnetic field in other convective regimes (e.g., stagnant lid convection) appears to be out of question (the mantle easily becomes hotter than the core so that the driving force for convection in the core disappears). However, there could be situations when the magnetic field can be sustained for long periods of time due to a large initial temperature of the core and a relatively small abundance of radioactive isotopes in the mantle. Such situations would be interesting to explore as well.

II.B.4. Research topic #4: Is there a role for living organisms in the regulation of plate tectonics? This is very speculative at this point, but feedback could exist between biological

processes and plate tectonics. This problem is related to one of the most fundamental questions in the origin of life: are we here because of a fortunate sequence of physical and chemical processes that created a habitable environment, or because living organisms have been active participants in the planetary evolution? Although this is quite a difficult question, we believe that it is time to invest a small portion of our efforts to address it.

There are some indications that microbial life can affect plate tectonics. First, it is well known that living organisms affect climate (e.g., own species, which is responsible for global warming). Climate affects surface temperature and volatile recycling, and thus tectonic processes. Also bio-amplification of rock weathering enhances the uptake of atmospheric CO_2 and global cooling. Second, microbial life has been found as deep as 3.5 km below the Earth's surface. This "biospheric depth scale" is not drastically different from the length scale required to weaken the lithosphere (~20-40 km). We do not know to what depth microbial life extends, or extended in the past: this means that 3.5 km is a lower bound. It is also important that the interplay between variations of temperature and pressure with depth is such that the maximum rate of all transport processes, including those that control microbial life, is below the surface rather than at the surface. Since the search for mechanisms responsible for the weakness of the lithosphere has not yet succeeded, some "bio-weakening" is at least a possibility worth considering.

Given huge uncertainties in the problem, what specific questions will we address? An improved understanding of plate tectonics (topic #1) will let us put constraints on how deep the microbial life must reach, how quickly the chemical reactions must occur, and to what level the yield stress must be reduced for plate tectonics to be substantially affected by microbial life. Parameterized models (topic #2) can help explore the role of microbial life in the stability of the entire system (through the coupling between plate tectonics, melting, degassing, volatile recycling, climate, surface temperature, and microbial activity.

II.C. Causes of Mass Extinction: Testing impact models (Ward, Kring, Farley)

BACKGROUND The frequency of complex life (metazoans and higher plants or their equivalents) on planets within a habitable zone must be some function of how often it evolves from simpler life, and then how long it survives after evolving. The frequency and intensity of mass extinction events ("MEEs") may significantly influence both of these factors.

The document *"The evolution of complex and higher organisms"*, (NASA SP-478; 1985) notes:

> "... existing NASA programs in Planetary Biology have not addressed some important questions. Previous research work focused upon the biological evolution of microorganisms over most of the Pre-Cambrian time, but did not consider evolutionary processes operative after the appearance of eukaryotic cells. In other words, the biological evolution of the most recent 1 billion years including that of the entire Phanerozoic has not been examined by NASA planetary biology program."

and

> "... what is proposed in this report is a study of complex life in a context that differs from that considered by contemporary evolutionary biologists. These mechanisms are usually intrinsic terrestrial ones, stemming from genetic properties of organisms, their competitive and cooperative relationships, and their

relationships with particular terrestrial environments. This report examines the possibility of studying the evolution of complex biological systems in another context, that of space. Specifically, it considers the physical properties of the universe as whole upon the evolutionary processes occurring on a planetary surface...No systematic and comprehensive analysis of these potentially important factors has been made, to date."

Of all factors mentioned in this report, mass extinction caused by impact was considered the most important and least studied area. Fifteen years later, few of the report's recommendations have been followed. We believe that the UW Astrobiology group, and its allies, are in an ideal position to tackle whether or not the impact-caused K/T MEE was an exception, or typical of MEE causation during the last 500 Myr.

II.C.1. Research Question #1: Besides the K/T extinction, is there any evidence for impact as a mechanism for other major mass extinction?

There have been five major MEEs over the past 500 Myr (Ordovician, Devonian, Permo/Triassic ("P/T"), Triassic/Jurassic ("T/J"), and K/T events). Here, we will concentrate on the latter three, and compare K/T boundary sites with P/T and T/J sites. We propose to test the hypothesis that either one or both of the P/T and T/J events was caused by asteroid or comet impact. We will do so by examining paleontological and geochemical evidence at exceptionally complete P/T and T/ J boundary sites.

II.C.1.a The P/T Event: The P/T MEE was the most consequential of the five major (> 50% species extinction) Phanerozoic events. The P/T event of 251.4 Mya (+/- 0.1 Myr; Bowring et al. 1998) is estimated to have caused between 80% (Erwin 1998) and> 90% (Raup 1978) of all species on Earth to go extinct. In marine environments 48% of families went extinct (Sepkoski1992), compared to 63% of terrestrial tetrapod families (Benton 1992).

The paleontological record of the P/T event in marine strata now shows that the duration of the event was at most 10kyr (at some locations) to 100 kyr (at other locations) (Bowring et al 1998). This boundary interval is marked by geochemical events, including an unusual direct precipitation of $CaCO_3$ from seawater (Grotzinger and Knoll 1995, Knoll et al. 1995) and a large negative spike in $d^{13}C$ at the paleontologically-determined boundary in both marine (Holser et al. 1989, Hsu and McKenzie 1990, Magaritz et al. 1992, Stanley and Yang, 1995, Xu and Yan 1993) and non-marine strata (Morante 1997, MacLeod et al. 1997). At many non-marine sections the event is marked by floral signatures (including a "fungal spike"), suggesting substantial disruption of terrestrial floras during and after the boundary interval (Eshet 1995, Retallack 1995,Visscher et al. 1996).

The possibility that the P/T extinction was due to bolide impact was raised soon after the Alvarez et al (1980) study on the K/T extinction. Various Chinese workers (Sun et al 1984, Xu et al. 1985) claimed Ir spikes of 8 ppb from the late Changxingensis Zone of the Meishan section of China, and at an equivalent section at Shangsi in Sichuan Province. Another report of elevated iridium from P/T sections was made by Dao-Yi et al. (1989. Subsequent work could not replicate this result (Clark et al. 1986, Zhou and Kyte 1988). However, new work in marine sections raises again the possibility of impact: Bowring et al (1998) suggest that the P/T extinction in the Chinese sections occurred so quickly as to preclude other proximal causes. New work at various Tethyan sections may also lend support to an impact hypothesis. Eight

sections show a minor Ir anomaly, associated with a substantial delta-^{13}C excursion at the paleontologically defined (biomass loss, extinction) P/T boundary (Dao-Yi et al 1989).

Proposed P/T Study Sites We will study (a) well-exposed P/T sites in non-marine facies in the Karoo Basin in South Africa and (b) Sydney Basin of Australia, and (a) a deepwater chert P/T boundary site in Japan. These have been chosen because they seem both complete and expanded (i.e., yielding high temporal resolution).

P/T site #1. Karoo Basin, South Africa. The P/T boundary is marked by the disappearance of the Dicnodon-Zone fauna, which coincides with a characteristic lithological succession transition (Ward et al. 2000). The base of a thin bedded mudstone unit (op cit) is the P/T boundary. The bed itself shows evidence of deposition under reducing conditions, and a lack of bioturbation. This is in contrast to beds above and below it, which are oxidized and bioturbated. No fossils of any type have been collected from this bed, which will be our main target for a search for ejecta deposits indicative of impact. We will study and sample in four widely spaced regions of the Karoo basin.

P/T site #2. Non-marine P/T boundary site #2, the Sydney Basin, Australia. The P/T boundary in the Sydney Basin is identified through palynology (pollen studies). Preliminary carbon isotopic studies show that a profound disturbances in terrestrial biogeochemical cycling began some time prior to the boundary and continued up to it. This contrasts with the single carbon isotopic excursion noted in marine sections and other terrestrial sites, and most probably reflects accelerated depositional rates and hence accentuated stratigraphic resolution. The excursions cannot be readily ascribed to changing paleogeographical settings, physiographic conditions or organic matter source. Clearly, a single impact could not have been responsible. The most obvious interpretations are repeated environmental perturbations, such as methane hydrate melting pulses, repetitive overturn of a stratified ocean, persistent prodigious volcanic exhalations, or serial extra-terrestrial impacts. Our aim is to resolve which is the most viable explanation. To clarify the meaning of these complex interaction of signals in both the Karoo and Sydney Basin record, we propose:

a) to establish a high resolution geochronological framework, to provide a separate chronological correlation tool to accompany the existing chronostratigraphic system. Little zircon U/Pb dating has been done over the boundary interval in the Karoo and Sydney Basin, but abundant felsic tuffs suggests the possibility. This should allow better integration with the growing global chronometric data-base for marine P/T sections.

b) to investigate more closely the palynological record of the critical interval, in order to constrain paleoecological changes and isolate leaf cuticle. Previous palynology has been biostratigraphic, but the relative abundance of pollen and spore-bearing rocks in a small time interval should allow monitoring of environmental change at finer scales. In the Sydney Basin, leaf cuticle will be examined for stomate size and abundance, a potential measure of pCO_2 and/or $\delta^{13}C_{atmos}$.

c) to generate a more complete secular $\delta^{13}C_{org}$ curve for both bulk kerogen and discrete plant organs (wood, cuticle, palynomorphs). With the advent of rapid, small-sample elemental-analyser/mass-spectrometry techniques, one can now determine just what parts of plants were being most affected by the biogeochemical turmoil. This should provide a better idea of the source of trauma: atmosphere, water ,or soil.

P/T site #3. (Deep-sea site) Japanese Red Chert sections in the Inuyama (dog-mountain) area, Central Japan. Although they were subjected to tight chevron folding and local brittle

deformation, micropaleontological investigations show that these Japanese deep-sea chert sections preserve a remarkably complete microfossil zonation, particularly rich in conodonts and radiolaria (Matsuda and Isozaki 1991, Isosaki 1997). Complete sections spanning both the P/T and T/J MEEs are exposed continuously along the Kiso river near Inuyama (NW of Nagoya).

II.C.1.b. The T/J Event: Less work has been done on this MEE than on any of the others (Hallam and Wignall 1998). Raup and Sepkoski (1988) found it to be less catastrophic than the P/T event, but more catastrophic (defined by % elimination of taxa) than the Ordovician, Devonian, and K/T events. In contrast to the other mass extinction events there is a marked paucity of geochemical data for T/J boundary strata. Carbon isotopic examinations have only been conducted on three sections worldwide, and all were compromised by diagenesis. Here we propose to sample three previously unstudied sections that appear from stratigraphic evidence to be highly appropriate candidates for testing hypotheses about the duration and cause of the end Triassic extinction event.

Possible causes of the T/J event. Causes of the T/J MEE have been narrowed to climate change, sealevel change and anoxia, and bolide impact. The first two would have produced extended intervals of extinction, a pattern not seen in the T/J microfossil record (Hallam and Wignall, 1998). The presence of the large Manicouagan Crater in Quebec dated at ~T/J boundary time has long led to speculation that like the K/T event, the T/J MEE was caused by the impact a large asteroid impact. The strongest supporting evidence came from Bice (1992), who found shocked quartz grains at a T/J boundary section in Italy. The recent report of a series of craters of T/J age (Spray et al. 1998) [and the reply by Kent (1998) that the supposed five correlative craters leading to this multiple impact event were diachronous in age] has increased the possibility that this MEE was impact-induced. What we lack are both a search for isotopic anomalies, and evidence of impact from stratigraphic sections. Our study sections should remedy this: if an impact or impacts occurred, at least one of our sections will likely show it.

Proposed T/J study sites:

T/J study area #1: Top of *Tritylodon* Zone, Elliott Formation, Karoo, South Africa. The T/J has been identified in expanded non-marine strata in the Karoo in the Elliott Formation exposed in the eastern Orange Free State (Smith and Kitching 1997). This locality is exposed as a series of nodule rich fluvial deposits rich in vertebrate fossils of the *Tritylodon* Acme Zone. The Elliott Formation is made up of continental redbeds deposed mainly in fluvial environments. It is rich in floodplain mudrock with concretions. The concretions contain organic material and will be analyzed for their carbon isotopic record across the paleontologically-defined T/J boundary. The boundary itself will be sampled and analyzed for evidence of impact. Finer fraction silts and clayrocks spanning the boundary will be sampled for ^{3}He.

T/J study area #2: Kennicott Point, Sandilands Formation, Queen Charlotte Islands, BC. Ward has studied the Norian (Triassic) to Pliensbachian (Jurassic) Sandilands Formation in the Queen Charlotte Islands, British Columbia, at two sites: Kunga Island, (a candidate for the Triassic/ Jurassic boundary global stratotype), and Kennecott Point (~100km NW of Kunga) . These sections are important because (1) an expanded T/J boundary is present, recently dated at 201Myr of age. We will test for evidence of impact at this site. (2) Abundant ashes in a thick succession of strata exposed below the T/J boundary will allow us to search for impact evidence

resulting from the 214 Myr-old Manicouagan Impact. It is important to know if this event resulted in either a world wide ejecta layer, or caused a paleontologically discernible extinction.

T/J study area #3: Pelagic Red Chert Tr/J sections, Inuyama area, Central Japan

The deep-sea chert sequence in Central Japan (above) also spans the T/J MEE. Paleontological investigations of this interval reveal nearly 60 species of conodont and radiolaria, with the boundary horizon being located to within a single chert/siltstone couplet of a 23 Kyr Milankovitch cycle (Matsuda and Isozaki 1991). One study shows a slight (and noisy) elevation in the background flux of cosmic spherules (Hori et al. 1993): we can do a much more accurate and higher resolution study with the ^{3}He technique (below).

II.C.1.c. Sampling protocols

Impact debris: Once the boundary zones have been identified using stratigraphic techniques, Kring, Ward and Buick will collect detailed samples across them for integrated geochemical, isotopic, and mineralogical analyses. Kring will separate mineralogical components and search for impact ejecta. Because we do not know *a priori* whether the impact hit land or sea, the search will target a broad spectrum of potential ejecta components. For example, shocked quartz will indicate a continental impact, and microspherules and spinel will indicate ocean (the best marker will be components that condense from the vapor-rich impact plume). We will pay particular attention to those sections where volcanic ashes have been described, because impact ejecta have often been mistaken for volcanic debris.

If impact ejecta are found, the mineralogic and petrologic properties will be fully characterized to determine the material's provenance (Kring et al. 1994). The goal will be to characterize the impact event to the fullest extent possible. As work with the Chicxulub impact event at the K/T boundary has shown, analyses of impact debris can potentially lead to good estimates of source crater location and source crater dimensions (Kring 1995). This information can, in turn, be used to begin evaluating environmental effects of the event, at which task Kring's group is particularly adept (Kring 1993, 1997, 2000; Pierazzo et al. 1998). Not only will we be able to determine whether one or more impacts occurred, we will also be able to begin investigating environmental effects of the events and correlating them with Ward's paleontologic record.

The same tactics can be used at all P/T and T/J sites. Sample processing depends on lithologies. We can process limestones quickly because we can dissolve carbonate. Lithified mudstones and/or shales take longer, because we must mechanically separate clays from the silt- to sand-sized particles of potential impact debris. (We have a bank of ultrasonic baths to help us process up to a dozen samples at a time.)

Extraterrestrial ^{3}He: constraints on impacts and sedimentation rates

Why Helium? Collected boundary strata will be sent to Co-I K. Farley for Helium measurements which can provide important insights into possible causes of our MEEs. Helium in sediments derives from two sources: fine-grained interplanetary dust particles (2-35 microns dia.) characterized by very high [^{3}He] (typically 10-5 cc STP/g), and terrestrial matter with very much lower [3He] (typically <10-15 cc STP/g). Given this strong isotopic contrast, it is easy to calculate the extraterrestrial fraction of ^{3}He (Patterson and Farley 1999). In many seafloor sediments this fraction is >90% and often approaches 100% (Patterson and Farley 1999, Farley

et al. 1998). Thus ^{3}He is an extremely sensitive and unambiguous proxy of extraterrestrial matter in sediments.

The concentration of extraterrestrial ^{3}He (called "^{3}HeET") in a sediment is related to the flux of ^{3}He from space and the sediment mass accumulation rate (a): ^{3}HeET=f/a. This relationship has two important applications for our proposed investigations. The most obvious is that major impact events may be associated with substantial increases in interplanetary dust flux. For example, the two large impacts in the Late Eocene (Popigai and Chesapeake Bay) are associated with a several Myr period of elevated ^{3}HeET flux (Farley et al. 1998). The observed temporal variation in ^{3}He flux and a precise coincidence of its maximum with the two impacts provide strong evidence that an impulsive shower of long period comets occurred about 35 Myr ago. Similarly, it is possible (though not required) that asteroid collisions may lead to enhancements in the interplanetary dust (and ^{3}HeET) flux to Earth (Kortenkamp and Dermott 1998). A large increase in [^{3}HeET] associated with a MEE would provide support for occurrence of a synchronous extraterrestrial event of some type, provided the increase is large enough to rule variations in sedimentation rate.

Alternatively, ^{3}He can be used to constrain sedimentation rate and therefore the duration of events that are otherwise poorly constrained temporally (e.g., duration of a MEE). If the flux from space is constant (or nearly so), then [^{3}HeET] is a measure of sedimentation rate. For example, [^{3}HeET] is identical in carbonates above and below the K/T boundary but ~3x higher in the K/T impact clay itself. This is most simply interpreted as invariance in both ^{3}He flux and sedimentation rate in the carbonate sediments that bound the K/T clay, and an ~3x slower sedimentation rate in the clay itself. Coupled with the known thickness of the clay layer, this rate constrains the K/T clay deposition interval to about 10 Kyr, with an uncertainty of perhaps 20% (Mukhophadhyay et al. in prep.) This constraint is considerably more precise than can be obtained from any direct radiometric method, wherein one establishes an age difference by subtracting two large numbers. In addition, the absence of a significant increase in ^{3}HeET associated with the K/T impact argues against a Late-Eocene style comet shower at this time. The K/T impactor was apparently not associated with a substantial component of interplanetary dust.

One possible outcome of our measurements is that we will be unable to clearly distinguish sedimentation rate from ^{3}HeET flux changes. However, in both intervals we have explored to date, results have been quite unambiguous. We thus believe that measurements of ^{3}HeET may provide important clues to the origin and duration of MEEs.

II.C.2. Research Question #2: Is there some critical number of mass extinctions necessary to maximize biological diversity?

While it is usually assumed that diversification following mass extinction events caused an increase in evolutionary tempo, there has been to date no systematic study rigorously examining the proposition. One surprise from Raup and Boyajian (1988) was that a relatively small proportion of total extinction through time was due to MEEs, contrary to expectation. Here we will invert the usual thinking to ask whether mass extinctions are actually of **little** consequence in biological evolution.

II.C.2.a. Methodology: a new metric: This question may be the most central and pertinent to understanding the relationship between habitability and the rise of metazoans (Figure 3). It is also one of the most difficult to test. There are presently no clearly defined field tests that bear

on this question. However, examination of published synoptic generic level data sets (Sepkoski 1992) may help. We propose a new metric: "background origination rates" for species, and compare these rates to origination rates of species immediately before and after MEEs. Raup and Boyajian (1988) used an analogous metric (their "background extinction rate") to consider what percentage of species through time went extinct due to mass extinction. Here we will use the Sepkoski (1992) data to look at the percentage of new genera that originate immediate after a mass extinction and compare that to origination in non-extinction times. Using these computed values, we can then simulate diversity through time.

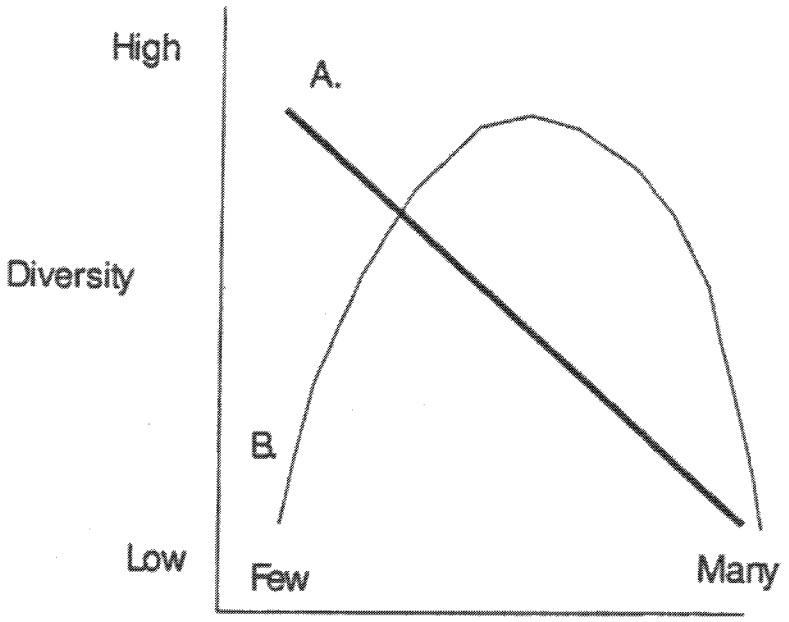


Figure 3. Two competing models of the effect of mass extinction on planetary biodiversity. In A, increasing the number of mass extinctions causes diversity to drop monotonically. In B there is some critical number of mass extinctions necessary for maximum diversity.

II.C.2.b. Modeling diversity. We will model diversity through time, assuming that the P/T, T/J, and K/T extinctions did not take place. An average background origination rate will be assigned to the major skeletonized taxa in the Sepkoski data, and major clades that did go extinct in each of MEE will be allowed to continue onward through time. At 10 Myr after each MEE, the number of genera from the model will be compared to the number of genera actually present (real data) at that time. For example, Ward and Signor (1983) and Ward (1984) have computed background origination rates for all major clades of ammonoid cephalopods. In our model, instead of undergoing wholesale extinction at each MEE boundary (as actually happened), the clades will be allowed to survive and diversify at pre-extinction rates. The total number of genera will then be compared to the genera actually produced where

$$D_T = D_s + D_{no}$$

With D_T =total diversity ten million years after extinction
D_s = diversity of surviving taxa
D_{no} = diversity of new origination rate taxa

These studies will take place at the University of Chicago in association with Dr. M. Foote and Dr. D. Jablonski (not listed in the proposal), with whom we have discussed the planned work.

Section III: Evolution of Biocomplexity and Biogeochemical Cycles

III. Evolution of Biocomplexity and Biogeochemical Cycles
- **A. building a habitable planet: geological record**
  - **1. biogeochemistry of early precambrian sedimentary rocks**
    - **a. phosphorus distribution and abundance**
    - **b. nitrogen isotopic ratios and abundances**
    - **c. sulfur isotopic ratios from extreme environments**
  - **2. environmental evolution of the Archaean Earth**
    - **a. paleo-aeronomy (redox sensitive detrital minerals)**
    - **b. paleo-oceanography (wave and tide energy)**
    - **c. paleo-barometry (basalts sensing atmospheric pressure)**
- **B. Genomic, cellular and community evolution**
  - **(1) geochemical cycling**
  - **(2) lateral gene transfer**
  - **(3) responses of organisms to environmental variations**
  - **1. evolution of biocomplexity from an ancient autotrophic lineage**
    - **a. minimal essential genome**
    - **b. evolution of mesophiles from thermophilic homologues**
    - **c. loss and lateral gain of genes**
  - **2. origin of the eukaryotic cell: implications from bacterial tubulin**
    - **a. hypotheses to be tested**
    - **b. goal #1: assess the distribution of tubulin genes in Verrucomicrobia**
    - **c. goal #2: asses whether tubulin genes were part of a large genetic exchange**
  - **3. Structure and evolution of biological communities**
    - **a. microbial mat communities**
      1. **goal #1: map chemical and bio structure across time and space**
      2. **goal #2: map key functional groups at high genetic resolution in time and space**
      3. **goal #3: relate gene expression to species' distribution and environment**
      4. **goal #4: map sulfate respiration genes within populations**
    - **b. sub-seafloor hydrothermal vent microbial communities**
      1. **topic #1: nitrogen fixation by hyperthermophiles**
      2. **topic #2: anaerobic oxidation of organics, coupled with Fe reduction**

III.A. Building a habitable planet with biological complexity: the historical record and ancient Earth analogs (Buick)

BACKGROUND: Earth is the only inhabited planet that we know of. It is certainly the only one overtly infected with complex organisms. So, to understand how life and complexity might have arisen elsewhere in the Universe, we have only the terrestrial example as evidence. Though we can extrapolate back from existing organisms or built theoretical biogeochemical models, the only robust empirical data that shows how early life on Earth evolved and interacted with its environment comes from the study of early Precambrian rocks, those formed before 1.6 Byr ago. Despite popular misconceptions, globally there is in fact a moderate abundance of well-preserved Archaean (>2.5 Ga) and Palaeoproterozoic (2.5-1.6 Ga) rocks, which can serve as our clearest windows on the actual events that occurred shortly after life's origin. Some of these rocks formed during the period when Mars, the best current candidate for having once developed extraterrestrial life, was evidently warm and wet. Thus, the primordial terrestrial geological, geochemical and palaeontological records can also serve as proxies for what might be

encountered on any initial astrobiological missions to Mars. Moreover, the advent of eukaryosis, a major step in the development of biological complexity, now appears to have occurred during this time interval (Brocks et al. 1999). To understand how such an event might have occurred, it is necessary to understand the state of the biosphere before, during and after this evolutionary transition. If eukaryosis was either a cause or a consequence of the oxygenation of the surficial environment around 2 Byr ago, then the mechanics and dynamics of this major global environmental transformation must also be elucidated.

Overall plans. The logic of our sampling and research is to assemble a collection of best-preserved and well-dated geological material representing a wide range of well-constrained depositional settings, to allow construction of secular trends in biological, biogeochemical and environmental evolution. The data-set will concentrate on critical intervals in the evolution of the biosphere and the terrestrial environment such as the earliest Archaean, when life first radiated, and the mid-Palaeoproterozic, when oxygen proliferated and large, complex organisms apparently rose to ecological dominance.

The empirical record. We propose to investigate this ancient empirical record to discover what sorts of organisms inhabited the early Earth, how they lived, where they lived, what sorts of conditions they tolerated and how they modified their physical and chemical environment. We will investigate well-preserved early Precambrian successions in several parts of the world, concentrating on Australia but also considering well-known successions elsewhere. Several stratigraphic successions from northwest Australia spanning the interval from 3.5-1.7 Ga will be examined: the ~3.52 Ga Coonterunah Group, ~3.45 Ga Warrawoona Group, ~3.25 Ga Sulphur Springs Group, ~3.2 Ga Gorge Creek Group, ~3.0 Ga De Grey Group (all Pilbara Craton), ~2.7 Ga Fortescue Group, ~2.5 Ga Hamersley Group (both Hamersley Basin), ~2.0 Ga Wyloo Group, ~2.2-1.8 Ga Yerrida Group and the ~1.75 Ga Earaheedy Group.

These all contain exceptionally well-preserved sedimentary successions which have been sampled for geobiological purposes. These samples will be augmented by rocks already collected from the ~2.4-2.2 Ga Huronian Supergroup of Canada and the ~3.8 Ga Isua Supracrustal Belt in Greenland, both covering critical intervals in the evolution of the biosphere. We also wish to prospect the well-preserved ~2.1 Ga Animikie Group and ~1.85 Sudbury Group (Canada), and the ~2.85 Ga Witwatersrand Supergroup, ~2.5 Ga Transvaal Supergroup and ~2.0-1.7 Ga Olifantshoek and Waterberg Groups (South Africa).

Research directions. We will follow two research directions, each comprising several discrete projects. (1) The first, specifically biogeochemical, provides the most continuous proxy record of biological activity. Though preserved fossils are invaluable for interpreting the history of the primordial biota, they are only rarely preserved and thus hardly representative of the state of the total biosphere. By contrast, biogeochemical data summarize both local and global biotic activity and are much more readily obtainable, being more robust under traumatic post-depositional conditions. By comparing signatures from different depositional settings at different times, an animated picture can be constructed of metabolic, ecological and evolutionary change through time. (2) The second concentrates on the physical environment, the stage for this evolutionary drama. As biological and environment seem to have interacted with reciprocity throughout later Earth history, it seems reasonable to expect that they also did so early on. Some aspects of early environmental evolution (plate tectonics, impact history) are considered in more

detail elsewhere in this proposal: here we consider equally important influences on life's trajectory, examining changing conditions in the atmosphere and hydrosphere.

III.A.1. Biogeochemistry of early precambrian sedimentary rocks

Biogeochemical cycling of metabolically important elements (C, H, O, N, P, S) reflects both the size and diversity of the biosphere and the complexity of its interactions with the lithosphere, atmosphere and hydrosphere. In extremely ancient environments, evidence of such cycling can act as a proxy for the existence of life itself. Isotopic fractionations of nutrient elements constrain the antiquity of some metabolic processes. Secular changes in the marine budgets of important biogeochemical nutrients mirror the evolution of ecosystems. Hence, study of the abundances and isotopes of these elements in Archaean (3.8-2.5 Ga) and Palaeoproterozoic (2.5-1.6 Ga) sedimentary rocks should allow an understanding of the ecology and metabolism of the primordial terrestrial biota and provide an analog for extraterrestrial biotic systems. Though broad reconnaissance studies of secular trends in several of these biogeochemical systems (particularly C and S in shales, carbonates and cherts) have been undertaken (Strauss and Moore 1992, Des Marais 1997, Canfield 1998), few detailed and environmentally constrained studies have been carried out on the less common elements.

Why phosphorus and nitrogen? Other nodes of NAI are undoubtedly continuing this broad reconnaissance program, so we propose to investigate the cycling of key nutrient elements P and N through time, and to study the isotopic systematics of S in extreme environments, where life may have originated.

III.A.1.a Phosphorous distribution and abundance

In marine clastic sediments, P is liberated from organic matter by microbial degradation during very early diagenesis and then sequestered into stable phosphate and aluminophosphate minerals rich in alkaline-earth and rare-earth metals (Rasmussen et al. 1998). These minerals have been observed in marine clastic sediments back as far as 3.5 Byr ago. Their abundance suggests a fairly consistent clastic reservoir size through time, albeit with poor constraint before the Neoproterozoic.. We will attempt to extend this secular trend by analyzing P abundance in Isua (~3.8 Ga) clastic metasediments. If the biogeochemical P cycle functioned at similar magnitudes and rates to the P budget at later times, then the amount of sequestered P should be similar to that in similar facies of younger age. The relationship between mineralized P and TOC will also be examined to determine if they are inversely correlated, as would be expected if phosphate were in high biological demand in microbial ecosystems of modern complexity. We will also examine P abundance and distribution across the ~2.0 Ga oxygenation event. If this event led to a profound change in biogeochemical cycling, with a major increase in marine productivity as a result of the rise to dominance of aerobiosis, then it should be reflected in the behavior of P across various sedimentary facies and environments. If, on the other hand, productivity was high before eukaryotes rose to ecological dominance, then negligible change in P cycling might be expected.

Research Plan:

a) Investigate polished thin-sections of sedimentary rocks of Archaean and Palaeoproterozoic age by SEM-CL-EDS and XRF to determine sedimentary P abundance and distribution,

concentrating in particular on rocks from Isua and from across the ~2.0 Ga oxygenation event.
b) Results will be compared with our data-base obtained from younger marine clastic sediments.
c) Where poorly constrained, diagenetic ages will be determined by SHRIMP U/Pb in xenotime dating (McNaughton et al. 1999).

Outcomes:

a) A more complete record of the distribution and abundance of diagenetic phosphate through early Precambrian time;
b) A better understanding of the antiquity of biogeochemical phosphorus cycling;
c) Characterization of any change in the size of the sedimentary P sink across the ~2.0 Ga oxygenation event.

III.A.1.b. Nitrogen isotopic ratios and abundances

Nitrogen isotope ratios in kerogen vary through time, with early Archaean values being markedly lighter than those in younger rocks (Beaumont and Robert 1998). This has been interpreted as indicating the late evolution of the modern N cycle, which is isotopically controlled by denitrification. However, this interpretation is based on a very small sample size and many of the rocks analysed were not controlled for depositional environment. It would be interesting to know how robust this pattern is when subjected to denser sampling from a range of environments. It would also be useful to determine if this pattern persists back to Isua times, or if an even more primitive N cycle utilizing dissolved marine ammonia were in existence at the beginning of the geologic record. Any substantial changes in isotopic ratios from previously recorded early Archaean values would probably indicate differences in the metabolic capability of microbial ecosystems, as metamorphism does not greatly affect N-isotope fractionations. In particular, because many cyanobacteria can fix molecular nitrogen, unusual N isotopic values may provide information about the evolutionary history of this important group of prokaryotes. C:N ratios will also be examined for evidence of biological nitrogen fixation, which should be easily detected if nitrification and denitrification were not significant processes in Archaean oceans.

Research Plan:

a) Acid-digestion of large samples (~1kg) of kerogenous shales and cherts to release kerogen;
b) Bomb-combustion and IRMS of representative samples to yield high-precision $\delta^{15}N$ analyses;
c) Continuous-flow isotopic analysis of remaining samples in an elemental analyzer/ mass spectrometer configuration for rapid processing;
d) C:N ratio determination by elemental analyzer.

Outcomes:

a) improve the Archaean and Palaeoproterozoic data-base for $\delta^{15}N$;
b) determine if there are differences in $\delta^{15}N$ based on depositional environment;
c) develop a data-base of early Precambrian C:N ratios; and
d) discover if ~3.8 Ga metasediments at Isua record evidence of biological nitrogen fixation.

III.A.1.c. Sulfur isotopic ratios from extreme environments

Molecular phylogenies of microorganisms suggest that sulfur metabolism is widely distributed among the earlier-branching lineages on the "Tree of Life". Many of these microbes

are also extremophiles, in particular thermophiles or hyperthermophiles. From this, it has been hypothesized that much of earliest biota was sulfur-metabolizing and lived in hydrothermal settings. Recently, the prospect of finding empirical evidence to test this hypothesis has been considerably enhanced with the recognition of microbial sulfate-reduction in early Archaean rocks (Shen et al. submitted) and the discovery of pyritic filaments in early Archaean hydrothermal deposits (Rasmussen 2000). We propose to perform a more comprehensive survey of primordial sulfate oases and hydrothermal environments to discover whether the isotopic signatures of sulfur-metabolism were more widespread on the early Earth than these initial hints suggest.

Research Plan:

a) field and drill-core sampling of early Archaean sulfate-sulfide deposits in northwestern Australia and South Africa;
b) thin-section petrography to determine the hydrothermal paragenesis of sulfate and sulfide phases; and
c) laser-ablation mass-spectrometry for $\delta^{34}S$ in both sulfate and sulfide minerals.

Outcomes:

a) better knowledge of early Archaean hydrothermal deposits;
b) more data on $\delta^{34}S$ secular trends early in Earth's history;
c) potential discovery of sulfur-metabolizing microecosystems in ancient subsurface high-temperature settings.

III.A.2. Environmental evolution of the Archaean Earth

The advent and complexity of life on Earth is arguably the product of its apparently unique environment. But that environment has not stayed static since the inception of life. It has instead evolved along with the biota, partly as a consequence of biological metabolic transformations. It has also changed as a result of extrinsic secular trends, such as the gradual diminution of internal radiogenic heat and the growth of the solar energy flux. One needs to understand the changing state of Earth's physical conditions in order to understand the growing complexity of organisms. Sedimentary and volcanic rocks that were deposited at the interfaces between land, air and water provide the best record of the state of the physical environment through time. Thus, the physical sedimentology, chemical sedimentology and volcanology of shallow marine, fluvial, lacustrine and subaerial successions in Archaean and Palaeoproterozoic terrains can reveal how the atmosphere, hydrosphere and lithosphere have interacted through time.

Atmospheric composition, atmospheric pressure, and ocean tides. Some aspects of these interactions will be covered elsewhere in this proposal (plate tectonics, extra-terrestrial impacts). Here, we propose to examine three less overt processes: how atmospheric composition has evolved through time, if atmospheric pressure has changed, and whether tidal and wave energy was greater in ancient oceans. All of these would have constrained the origin and early evolution of life and some may have influenced the development of biological complexity, in particular the redox state of the atmosphere.

III.A.2.a. Palaeo-aeronomy (redox sensitive detrital minerals)

Rasmussen and Buick (1999) found that careful petrological examination of Archaean sandstones reveals many environmentally diagnostic detrital minerals to occur in small but

significant quantities in sediments that were clearly equilibrated with the contemporary atmosphere. Though detrital minerals have been used before to try to determine the composition of the Archaean atmosphere (uraninite, pyrite), the minerals used were not overly sensitive to variations in the atmospheric redox state. However, the newly discovered mineral suites are much more sensitive and can monitor the abundance of some species other than oxygen. Thus we should be able to obtain a much better picture of the rise of atmospheric oxygen levels and may be able to identify the mysterious greenhouse gas responsible for counterbalancing the "faint young Sun". As these gases were probably biologically modulated, their abundance should provide a monitor for microbial metabolic activity on the early Earth.

We will examine a wide range of Archaean and Palaeoprotoerozic sandstones for their detrital mineral assemblages, concentrating particularly on those rocks deposited in turbulent environments such as fluvial and shallow marine settings. Well-aerated depositories are required to avoid the masking or confounding effects of biological activity and samples from diverse source terrains are needed to ensure a wide spectrum of detrital assemblages. As well as detrital analysis, each sandstone will be subjected to a full sedimentological and geochronological study, to constrain the atmospheric data as closely as possible.

Research Plan:

a) study sedimentary and diagenetic environments of a diverse suite of Archaean and Palaeoproterozoic sandstones;
b) examine polished thin-sections for redox-sensitive detrital oxides, carbonates and sulfides by SEM-EDS;
c) date poorly constrained samples by SHRIMP U/Pb geochronology of detrital zircons and diagenetic xenotime.

Outcomes:

a) develop a clearer picture of the distribution of redox-sensitive detrital minerals through time;
b) determine if there is a marked change in detrital species across the proposed ~2.0 Ga atmospheric oxygenation event

III.A.2.b. Paleo-oceanography (wave and tidal energy from physical sedimentology)

We are currently undertaking a pilot study to determine how effective grain size and density distributions in Archaean sandstones are for monitoring the energetics of wave and tidal currents on the early Earth. Both parameters are controlled by several variables (waves by beach profile, wind strength, wind fetch, etc.; tides by bottom morphology, lunar proximity, bathymetry, etc.), and they do not generally yield unique solutions to questions about the physical environment of the early Earth (such as, how close was the Moon and thus how great was the tidal range; how hot was the Earth's surface and thus how strong were weather systems). However, there is very little other empirical evidence about these important issues pertinent to the origin of life and even imprecise results would be valuable.

We expect that in some instances where basin morphology is well constrained (in some lacustrine settings), more definitive solutions can be obtained. Initial results are encouraging and we have been able to determine what conditions were like on one Archaean shoreface at one time (Buick et al. 1995). We will extend this study to other marginal marine and lacustrine settings in Archaean terrains, to discover whether our initial findings about wave energy can be generalized and to investigate some tide-dominated strandlines. Appropriate sandstone units have already been picked out for further study in the Pilbara Craton, Australia, but comparable units in

southern Africa should also be assessed for corroborative evidence. For each strandline, a full palaeoenvironmental and geochronological study is necessary to validate the palaeocurrent data obtained. Some comparative data from modern environments must also be obtained because the sediment properties recorded in Archaean sandstones differ from those typically reported from unlithified sands.

Research Plan:
a) collect palaeocurrent data from foreshore sediments by measuring sedimentary structures;
b) analyze grain shapes in polished thin-sections using computer image analysis programs;
c) calculate density/size-distribution plots using physical sedimentology software;
d) compare data with known distribution from modern environments of varying wave and tide energy.

Outcomes:
a) develop energy profiles for tide and wave dominated shorelines through time;
b) determine whether wave or tide energy has changed over the course of 2 billion years of Earth history.

III.A.2.c. Palaeo-barometry (amygdaloidal basalts measuring atmospheric pressure)

Currently there are no empirical data available about the pressure of the early atmosphere, though it is a vital parameter in atmospheric evolution. For instance, many models have postulated a denser early atmosphere with a much higher partial pressure of CO_2 to counterbalance the reduced luminosity of the faint young Sun. Any information about its actual pressure would be invaluable for assessing their validity. We have recently located an ideal section for attempting to determine atmospheric pressure in the Archaean. Vesicle size in terrestrial basalts can be used as a barometer, if relative height above sea level at eruption is known. As the latter cannot normally be determined for Archaean basalts, precise atmospheric pressures cannot be obtained. However, we have found stratigraphic successions of amygdaloidal basalts that pass upwards from submarine to subaerial environments in a single eruptive cycle, thus defining Archaean sea level. If the volatile charge and rheological properties of the initial magma can be calculated, atmospheric pressure can be approximately determined. With detailed petrology and physical volcanology, this should be possible, as studies of stratigraphically equivalent basalts show that there are relationships between flow morphology and thickness, amygdale size and distribution, and whole-rock geochemistry.

Research Plan:
a) field sampling of basalt flows;
b) quantification of vesicle size and abundance using computerized image analysis;
c) whole-rock geochemistry to determine initial volatile charge;
d) thin-section petrography to determine diagenetic and metamorphic alterations to calculated volatile charges;
e) comparison of data with modern Hawaiian data sets.

Outcomes:
a) determination of Archaean atmospheric pressure at sea level;
b) determination of approximate pCO_2 by comparison with palaeosol data;
determination if pCO_2 by itself could have provided a greenhouse sufficient to counteract the "faint young Sun" by comparison with palaeoatmospheric models

III.B. Genomic, cellular, and community evolution

BACKGROUND The investigations we propose here address the evolution of biocomplexity at three levels: the individual lineage (with focus on genome evolution), the eukaryotic cellular chimera, and the microbial community. In describing specific projects we devote a separate subsection to each level. However, our investigations are connected by several interrelated themes. These themes, and the levels at which we apply them, can be illustrated using the phylogenetic tree of life (see next page).

THEME #1: Geochemical cycling. At the single lineage level we focus on a group of methanogenic Archaea, *Methanococcus*. Methanogenesis (generation of methane from CO_2) may have been one of the first types of energy-generating metabolisms (Leigh, 2001). The resulting accumulation of methane may have contributed to an early greenhouse effect (Pavlov et al., 2000). *Methanococcus* species (methanococci) also represent early autotrophs, meaning that they may have participated in the early conversion of inorganic to organic carbon. One of our investigations aims to determine how many genes are required for an organism of this type to function. At the community level we address biogeochemical cycling in microbial mats in relationship to microbial population ecology. Carbon, oxygen, and sulfur cycling in mats involves oxygenic and non-oxygenic phototrophs, sulfur oxidizers, and sulfate reducers. At this level we will focus primarily on the community ecology of sulfate reducing populations, known to be the dominant anaerobic respirers in the system.

Microbial mats are recognized to be among the most ancient of communities (as documented in the fossil record) that also have representation in contemporary habitats that restrict metazoan grazing (e.g., high salt or high temperature). Our most general guiding hypothesis is that microbial mat communities (by sustaining highly diverse and highly interactive populations) may have been extremely important in the evolution of biological novelty , i.e., they may have been the site of origin of new metabolic and cellular states. This hypothesis builds upon recent recognition, derived primarily from comparative genome sequencing of microorganisms, of the importance of lateral gene transfer to biological innovation. Thus, mat communities provide a natural context for framing our second general theme - lateral gene transfer.

THEME #2: Lateral (horizontal) gene transfer. The movement of genes across lineages, as opposed to linear inheritance, is a major force in evolution (Doolittle, 1999). An excellent example is the transfer of genes for metabolism of single-carbon compounds from methanogenic Archaea to methylotrophic Bacteria (Chistoserdova et al., 1998). Another example is the joining of two photosystems to give rise to oxygenic photosynthesis (the cyanobacteria), an event that may have occurred in mats. Still other examples occur in the evolution of the eukaryotic cellular chimera, where lateral transfer took the form of massive fusion or endosymbiotic events. The mitochondria and chloroplasts (common eukaryotic organelles) came from Proteobacteria and cyanobacteria, respectively. One theory of the origin of the eukaryotic cell invokes an endosymbiotic event in which a hydrogen-consuming methanogen engulfed a hydrogen producer (Martin and Müller, 1998).

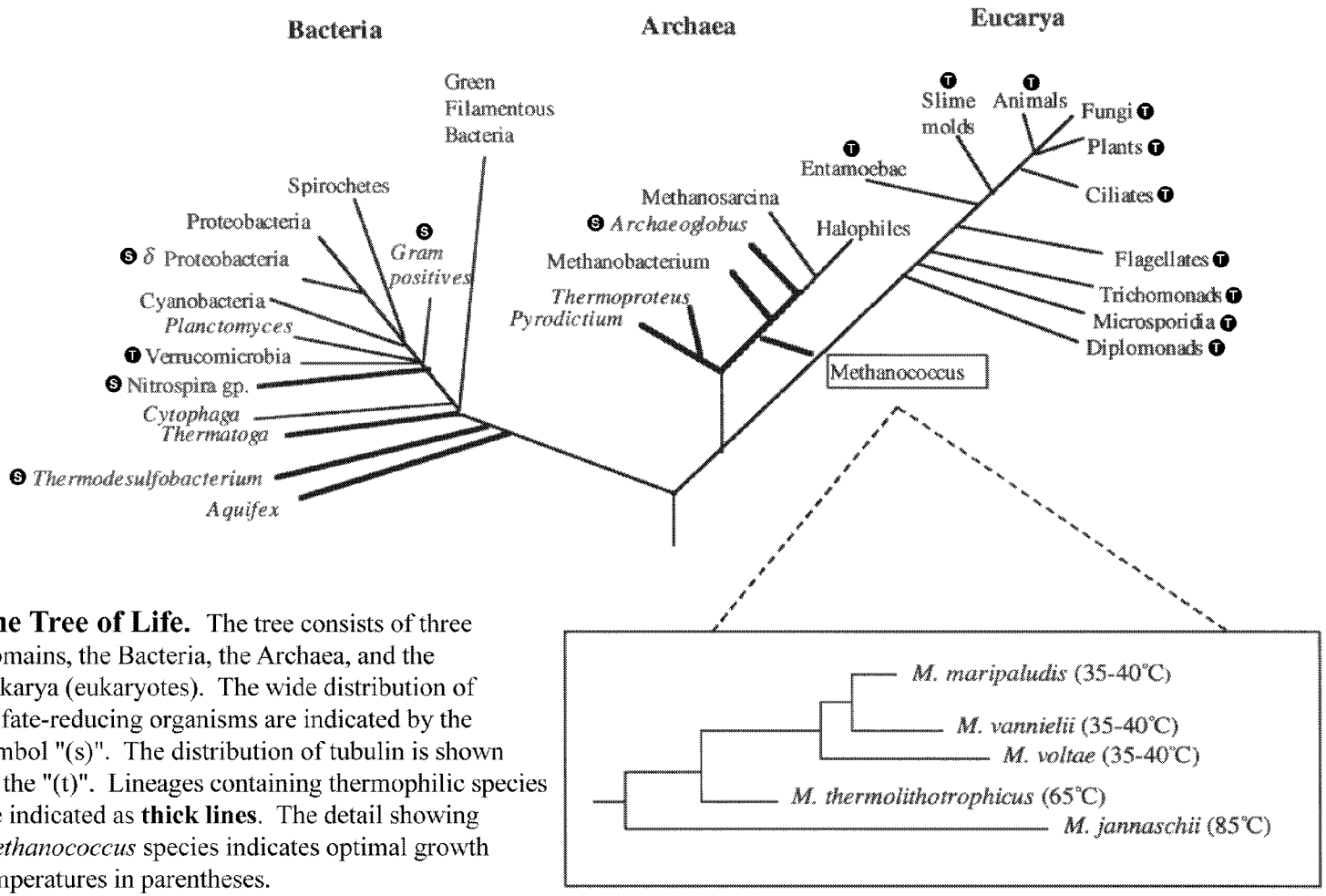
In our work we will assess the occurrence of lateral transfer in the evolution of new species within the methanococci. We will begin to assess its role in the emergence and

diversification of sulfate-respiring organisms. Initial comparative studies of the genes encoding for a key enzyme in the pathway for sulfate respiration have revealed that lateral gene transfer has also played a significant role in the diversification of sulfate reducers. In the evolution of the eukaryotes we will test hypotheses to explain the remarkable discovery (Staley, unpublished) of genes for tubulin in a member of the Bacteria, the Verrucomicrobia. Tubulin was previously known only in eukaryotes as a fundamental component of the cytoskeleton. Cytoskeletal components may have been ancestral to the eukaryotes, but they might also have been acquired laterally, perhaps to facilitate predation in prey-rich ecosystems such as microbial mats.

THEME #3: Response of organisms to temporal and spatial variations in their environment. Rarely have organisms been studied in the context of their native habitat, mostly because appropriate tools to do so have not been available. The combination of complete genome sequence information and expression array technology now provides the technical foundation to more completely understand a genome sequence - by studying its expression both in a controlled laboratory setting as well as the natural context in which it evolved and now functions. At the community level we will characterize the biotic and abiotic features of a microbial mat community that promote and sustain a remarkably high diversity of sulfate-respiring populations. In association with Dr. Mitchell Sogin (MBL Astrobiology Institute) we will study global gene expression patterns that accompany the diurnal cycle. At the lineage level we will use the methanococci as a model to study the apparent evolution of proteins from a high-temperature (hyperthermophilic) ancestral state to a moderate temperature (mesophilic) state.

III.B.1 Evolution of biocomplexity from an ancient autotrophic lineage (Leigh)

BACKGROUND: The accumulation of phylogenetic and genomic information is opening exciting new opportunities to study the evolutionary process at the level of individual lineages as well as at the community level. The study of an individual lineage can yield valuable information on the evolution of genomic complexity, adaptation to physico-chemical factors, and lateral gene transfer. This section deals with a unique opportunity to study an individual lineage, the methanococci. We propose to address three general questions crucial to the evolution of life. First, "What is the minimum genome required for a living system?" Second, "What adaptive changes must occur within a lineage to adjust to changing physical parameters such as temperature?" Finally, "To what degree does the lateral transfer of genes between lineages, as opposed to direct descent of genes, affect the evolutionary process?"



The Tree of Life. The tree consists of three Domains, the Bacteria, the Archaea, and the Eukarya (eukaryotes). The wide distribution of sulfate-reducing organisms are indicated by the symbol "(s)". The distribution of tubulin is shown by the "(t)". Lineages containing thermophilic species are indicated as **thick lines**. The detail showing *Methanococcus* species indicates optimal growth temperatures in parentheses.

The tree should be considered a work in progress. It is incomplete because the vast majority of organisms in nature are not included. It is based on only one gene, the one that encodes the RNA of the small subunit of the ribosome (16S or 18S ribosomal RNA). Many other genes give the same result, but many do not. One explanation for trees that differ for different genes is lateral gene transfer, a theme of this proposal. This diagram is redrawn from Perry and Staley, Microbiology: Dynamics and Diversity, Saunders College Publishing, 1997. Information in part from Pace (1997). *Methanococcus* tree modified from Keswani et al. (1996).

ANCIENT EVOLUTION ON EARTH The genome sequence we are generating for *Methanococcus maripaludis*, together with the completed sequence of *Methanococcus jannaschii* (Bult et al., 1996), offers a unique opportunity to focus on the nature of an ancient evolutionary process within a single lineage. The species *M. maripaludis* and *M. jannaschii* represent mesophilic and hyperthermophilic members, respectively, of the family *Methanococcaceae*, a lineage that branches from the phylogenetic tree at an ancient stage within the Archaea (see Tree of Life above). Within the *Methanococcaceae*, phylogeny based on 16S rRNA suggests that the hyperthermophiles were the first members of the *Methanococcaceae* to evolve, followed by moderate thermophiles, and finally mesophiles (Keswani et al. 1996). Therefore, the *Methanococcaceae* provide a window into the evolutionary process that may have occurred as hyperthermophiles gave rise to mesophiles, an event that would have happened as the Earth cooled or as organisms spread away from high-temperature habitats of origin to colonize cooler niches (see Figure 6: separate page).

Approach and work plan: *M. maripaludis* is ideal: it grows well in the lab, and genetic tools facilitate generation of mutants. Leigh works with it routinely (Kessler and Leigh, 1999) and has an ongoing NIH-funded collaboration with M. Olson (UW Genome Center: see Olson's letter) to obtain its genome sequence. We will use it in the following studies:

III.B.1.a. Minimal essential genome

What is the minimal genetic complexity required for life (Maniloff 1996)? Pressures for stream-lining and genome stability may tend to select for small genomes, while pressures for new functions would lead to larger genomes. Studies of the minimal genome have been carried out with parasitic *Mycoplasma* species. The genome of *M. genitalium* has been sequenced and 517 genes are present. The small genome size is attributed to the organism's reliance on its host for many metabolic functions. Mushegian and Koonin (1996) have deduced a minimal genome size of 250 genes by informatic comparison of *M. genitalium* and *Haemophilus influenzae*. This number comes close to the value of 265 to 350 genes determined experimentally by Hutchison et. al. (1999) for *M. genitalium*. Therefore, in a parasitic bacterium with a small genome, a third to a half of the genes are dispensible. We propose to carry out a similar study of a strikingly different organism, a free-living autotrophic methanogen. The genome content of *M. maripaludis* is estimated at 2,000 genes, fairly typical of Archaea but considerably more than *M. genitalium* and less than a free-living bacterium such as *Escherichia coli* (4,289 genes). Will the free-living and biosynthetic requirements of *M. maripaludis* require a larger percentage of essential genes, or a larger absolute number? What accounts for the lower number of genes in free living Archaea vs. free living Bacteria?

We will model our approach after Hutchison et. al. (1999). We will obtain a large set of viable, random transposon insertion mutants in *M. maripaludis*, determine the genes harboring these mutations by sequencing, and deduce the identity of the dispensable genes. By inference, the remaining genes are essential. In *M. maripaludis* we have implemented a random transposon insertion method (Zhang et al. 2000]), which together with our genome sequence constitutes the tools we need for this analysis. By testing the viability of our mutants under a variety of growth conditions, we will deduce the essential gene set not only for basic viability but for autotrophic growth and for growth under various stringent conditions. These experiments will also yield

valuable hints of function for protein families not previously characterized. (Because the transposon is polar on downstream genes in an operon, we can initially determine only essential operons, not individual open reading frames. Follow-up experiments can produce non-polar mutations to determine the roles of individual protein-coding regions where needed.)

Specific questions, minimal essential genome: Which portion of the total gene complement of *M. maripaludis* (and by extension *M. jannaschii*) appears to be essential for survival and for specific functions?

1. What portion of genes is dispensible for survival under rich laboratory conditions?
2. What genes are essential under stringent conditions (autotrophy, limiting hydrogen, temperature and pH stress)?
3. What are the functions of unknown proteins encoded in the genome, and by inference of homologues found throughout the phylogenetic tree?

III.B.1.b. Evolution of mesophiles from thermophilic homologues

If mesophily evolved from thermophily , then this fact should be reflected in the phylogeny of a variety of protein-coding genes, not just 16S rRNA. Although the genomes of only *M. jannaschii* and *M. maripaludis* have been sequenced among the methanococci, the sequences for a variety of protein coding genes have been generated for a moderate thermophilic and other mesophilic *Methanococcus* species. We will construct phylogenetic trees (with J. Felsenstein, see letter) using a variety of protein families represented in a range of methanococci, with homologues from non-methanococci as outgroups. These phylogenetic trees will be evaluated to see if they confirm the evolution of mesophilic methanococci from hyperthermophilic ancestors.

Several mechanisms might account for hyperthermostability of proteins, including surface ion pairs, loop shortening, and increased compactness (Cambillau and Claverie 2000). No systematic, genome-wide comparisons have been carried out between closely-related mesophilic and hyperthermophilic species. We will carry out computational comparative genomics of *M. maripaludis* with *M. jannaschii*. The work aims to study differences between mesophilic and hyperthermophilic protein homologues from a structural viewpoint. It will be carried out in collaboration with R. Samudrala (see letter), a new member of the Department of Microbiology faculty and an expert on computer modeling of protein tertiary structure (Xia et al. 2000).

We will attempt to produce a structural model for every protein in the genomes of the two *Methanococcus* species, using a three pronged approach: 1. Proteins that have recognizable homologues to known structures in the protein databank using sequence information alone will be modeled using comparative modeling techniques developed by Samudrala. 2. The comparative models will be used to construct sequence alignments that are superior recognizers of distant homologues. This will be used in conjunction with existing fold recognition (threading) methods to build models for homologues where there is no obvious sequence similarity. 3. *Ab initio* methods developed by Samudrala will be used to predict structures for small proteins. An all-against-all structural comparison will be made between the proteins in the two genomes. We will then carry out an analysis of systematic differences that may account for differential temperature adaptation.

Specific questions, evolution of mesophilic from thermophilic homologues: What adjustments occurred in the amino acid sequences of proteins to adapt them to function at new temperatures?

1. Do phylogenetic trees of a variety of genes confirm the evolution of mesophilic from hyperthermophilic methanococci?
2. Will protein structure modeling predict similar three-dimensional structures for closely related hyperthermophilic and mesophilic homologues?
3. Can systematic changes in amino acid composition, such as number of charged amino acids at the protein surface, account for the adaptation of proteins to their respective temperatures? What other temperature-adaptive mechanisms can be discerned?

III.B.1.c. Loss and lateral gain of genes

We have found that while the majority of genes in *M. maripaludis* have closest homologues in *M. jannaschii*, many have closest homologues outside of the methanococci. This observation indicates that lateral gene transfer, or differential gene loss, has occurred during the evolution of these species from their common ancestor. To evaluate this phenomenon more carefully, we will carry out a comparative blast analysis of *M. maripaludis* and *M. jannaschii* as part of our collaboration with M. Olson (see letter). We will catalogue those genes in both species that appear to have been lost or to have arrived laterally. We will carry out blast searches with these genes to determine what organisms harbor homologues. We will then construct phylogenetic trees to determine the likely origins of these genes. From any suggested functions for these proteins, we will try to understand the adaptive significance of these loss and transfer events.

Specific questions, loss and lateral gain of genes: What genes were lost or gained through lateral gene transfer during this evolutionary transition?

1. Which genes in *M. maripaludis* have no recognizable homologues in *M. jannaschii*, and vice versa?
2. Do genes unique to one or the other species have homologues elsewhere, giving hints as to their origin and function?

III.B.2 Origin of the eukaryotic cell : implications from bacterial tubulin in the Division Verrucomicrobia (Staley)

BACKGROUND: One of the major mysteries in biology concerns the origin of the eukaryotic cell. The structural dichotomy that exists between eukaryotic cells and their bacterial and archaeal counterparts is so profound that it raises questions about the origin of eukaryotic complexity. Major structural criteria used to distinguish eukaryotes from Bacteria and Archaea include a large genome size, complex mechanisms of DNA replication and cell division, the presence of intracellular organelles including a membrane-bounded nucleus and the presence of a complex internal cytoskeleton.

Cytoskeletons. Many biologists believe that the acquisition of a cytoskeleton may have represented the greatest evolutionary leap in the transition of Bacterial/Archaeal to eukaryotic cell organization (Stanier 1970, Cavalier-Smith 1987, Doolittle 1995, Faguy and Doolittle 1998). Firstly, the presence of a cytoskeleton may have conferred an endocytic ability on the proto-eukaryote thus allowing engulfment of Bacterial or Archaeal cells and the acquisition of both genetic material (Lake and Rivera 1994) and in some cases entire organelles (Woese and Fox

1977). Furthermore, uncoupling of the processes of chromosome replication and segregation and the development of an intracellular transport system may have allowed for an unrestricted genome size (Faguy and Doolittle 1998) and an increase in structural complexity.

Origins of the cytoskleleton. While the acquisition of cytoskeletal genes is recognized to be an important step in the evolution of cell complexity, the origin of these genes remains unclear since thus far cytoskeletal genes appear to be unique to eukaryotes. One possibility is that these genes arose *de novo* in eukaryotes. Another is that they arose from related but functionally different Bacterial or Archaeal precursors and subsequently diverged (Doolittle 1995). Finally, it is possible that these genes arose early in evolution in either the Bacteria or the Archaea and were transferred to eukaryotes at a later stage via a lateral gene transfer or fusion event. However, neither of the latter hypotheses can explain the presence of cytoskeletal elements in eukaryotes if these genes have never been discovered in the Bacteria or Archaea.

Search to date for origins. Despite the search for a bacterial or archaeal origin for cytoskeletal proteins, to date they have only been found in eukaryotes. At present, the leading candidate for a homolog of a cytoskeletal protein in Bacteria and Archaea is FtsZ; a protein involved in cell division which resembles eukaryotic tubulin in its tertiary structure (Erickson 1995). Nonetheless, no sequence homology between FtsZ and tubulin has been detected, despite the fact that cytoskeletal proteins are known to be highly conserved (Doolittle 1995). Although many "microtubule-like" structures have been observed in bacteria such as *Azotobacter*, myxobacteria, cyanobacteria, spirochaetes (Bermudes et al. 1994) and mycoplasmas (Korolev et al., 1994), no evidence has been found that these structures are homologous to eukaryotic cytoskeletal elements or even that they possess an analogous function.

New discoveries. We have recently discovered two genes for tubulin in the genomes of members of the Bacterial division, the Verrucomicrobia (Jenkins et al. in prep.). The Verrucomicrobia are a relatively uncharacterised group of Bacteria that are commonly found in 16S rRNA clone libraries from DNA extracted from many terrestrial and aquatic environments (Hedlund et al. 1997; Hugenholtz et al. 1998). Despite the fact that these organisms are apparently ubiquitous in the environment, only nine strains have been isolated in pure culture. These include four species of prosthecate bacteria from the genus *Prosthecobacter* which were isolated from freshwater habitats in our laboratory (Staley et al. 1976; Hedlund et al. 1997), a single strain from the genus *Verrucomicrobium* and four strains of anaerobic bacteria recently isolated from soils (Janssen et al. 1997).

Tubulin and microtubule construction. In eukaryotes, the alpha and beta tubulin proteins are the basic subunits of microtubules while gamma tubulin only plays a role in microtubule organizing centers (Zheng et al. 1991). Thus, the lack of a gamma tubulin gene in *Prosthecobacter* strains does not preclude the formation of microtubules in these organisms. We are currently investigating the functionality of the tubulin proteins in *Prosthecobacter vanneervenii* and have preliminary evidence that the tubulin genes are expressed in this organism. Additional evidence that microtubules may exist in members of the Verrucomicrobia comes from reports of microtubule-like bundles in a Verrucomicrobia symbiont (referred to as "epixenisomes") of the ciliate protozoan *Euplotidium itoi* (Rosati et al. 1993; Petroni et al. 2000). The tubular structures of the epixenisomes show a positive immunoreaction to anti-tubulin antibodies, however there is currently no molecular evidence for tubulin in epixenisomes.

Tubulin homologues in Bacteria/Archaea. From the bacterium *Prosthecobacter vanneervenii* we have found the first strong molecular evidence for tubulin homologs in members of the Bacteria or Archaea. This information was derived from the genome of *P.*

vanneervenii which is being sequenced in cooperation with Integrated Genomics (see letter of collaboration from Dr Michael Fonstein). At present, two tubulin gene sequences have been identified from this bacterium. The first is predicted to encode a 450 amino acid protein which according to phylogenetic analysis appears to be a homolog of eukaryotic alpha-tubulin. The second gene appears to encode a truncated 350 amino acid protein also with high sequence homology to tubulin but with no obvious affiliation to alpha, beta or gamma eukaryotic tubulin. Based on these sequences we have designed primers for the polymerase chain reaction (PCR) to determine whether other species of *Prosthecobacter* contain tubulin genes. The tubulin genes were successfully amplified and sequenced from the type strain, *Prosthecobacter fusiformis*, as well as two additional strains, *Prosthecobacter dejongii* and *Prosthecobacter debontii*.

Lateral gene transfer? The discovery of tubulin genes in members of the Verrucomicrobia, plus lack of evidence for these genes in any other Bacteria and Archaea, suggests that a horizontal gene transfer event occurred between a member of the Verrucomicrobia, or an ancestor thereof, and a eukaryote.

III.B.2.a. Hypotheses. This raises several questions about the nature of the gene transfer. Three hypotheses are proposed to explain the presence of tubulin genes in *Prosthecobacter* species:

1. The tubulin genes evolved in the Eucarya and were transferred from a eukaryotic organism to *Prosthecobacter* species via horizontal gene transfer.
2. Tubulin genes evolved in the Verrucomicrobia and were transferred to a eukaryotic organism by horizontal gene transfer.
3. The tubulin genes evolved in Verrucomicrobia and were transferred to a eukaryotic organism as part of a larger gene transfer such as a cell fusion event.

To test these hypotheses we propose the following:

III.B.2.b. Research goal #1. To assess the distribution of tubulin genes within the division Verrucomicrobia

To determine at which stage the gene transfer occurred we propose to assess the distribution of tubulin genes in members of the Verrucomicrobia. We have already confirmed that the tubulin genes are present in all currently cultured members of the genus *Prosthecobacter*. addition, we intend to perform Southern hybridization experiments, using probes designed against *Prosthecobacter* tubulin genes, to assess the presence of tubulin genes in each of the available cultured members of the Verrucomicrobia. These would include one strain from the genus *Verrucomicrobium*, *Verrucomicrobium spinosum,* and four anaerobic strains recently isolated from anoxic rice paddy soil (Janssen et al. 1997).

For those species of the Verrucomicrobia containing tubulin genes, PCR will be used to amplify the genes using specific primers that we have successfully used for *Prosthecobacter* species. If the specific primers do not yield products, then degenerate primers will be designed to attempt to amplify the genes. A phylogenetic tree will subsequently be constructed using tubulin sequences from various lineages of the Verrucomicrobia. If the distribution of tubulin genes within the Verrucomicrobia is limited to members of the genus *Prosthecobacter*, this would suggest a relatively recent transfer of these genes to this group of organisms and lend credence to the first hypothesis.

In contrast, if the tubulin genes are found in all members of the Verrucomicrobia, this would suggest a more ancient gene transfer which occurred around the same time that this lineage diverged from the other Bacteria.

Preliminary phylogenetic analysis of already seems to support this hypothesis. The eukaryotic tubulins form three well-defined clades, alpha, beta and gamma, which are each approximately 30-45% homologous to each other and which code for functionally unique tubulin proteins. In contrast, *Prosthecobacter* only contains two tubulin genes, suggesting that the *Prosthecobacter* tubulin lineage diverged from the eukaryotic tubulins prior to a gene duplication that gave rise to the third eukaryotic tubulin paralog. Further support of this idea is the divergent positions of the *Prosthecobacter* tubulins on the phylogenetic tree. One *Prosthecobacter* tubulin gene appears to be a deeply branching member of the alpha tubulins. The other shows no obvious phylogenetic affinity to alpha, beta or gamma tubulin. More rigorous phylogenetic analyses using tubulin sequences from additional Verrucomicrobia representatives may lend further support to this hypothesis.

III.B.2.c. Research goal #2. To assess whether the transfer of tubulin genes was part of a large genetic exchange such as a fusion event.

To assess whether the transfer of tubulin genes was part of a large genetic exchange we aim to search for additional eukaryote-like genes in the genome of *Prosthecobacter vanneervenii*. If a large genetic transfer occurred between a member of the Verrucomicrobia and a proto-eukaryote or if a fusion event occurred between a member of the Verrucomicrobia and a member of the Archaea which resulted in a eukaryote (chimeric origin theory), then many genes in addition to the tubulin genes would be expected to be present. Two types of genes would be predicted:

1. Eukaryotic genes for which there are no known Bacterial or Archaeal counterparts such as actin and kinesin. (At present, we have preliminary evidence that kinesin-like genes may also be present in the genome of *Prosthecobacter vanneervenii*, although further analyses are required to confirm this.)

2. Genes coding for proteins that are known to be present in all three domains of life. If a member of the Verrucomicrobia represented an ancestral fusion partner which gave rise to eukaryotes then many Verrucomicrobia genes should resemble eukaryotic genes more closely than those of other Bacteria or Archaea do.

Proponents of the chimeric origin theory of eukaryotes suggest that amongst the putative genes contributed by the Bacterial partner, are several genes encoding enzymes involved in metabolism (Brown and Doolittle, 1997; Gupta and Golding 1996). Phylogenetic analyses of metabolic genes from the genome of *Prosthecobacter vanneervenii* may lend support to this theory. Furthermore, the genome analysis will provide more information about the genetic diversity of microbial life and lead to a better understanding of the biochemical and biotechnological potential of the Verrucomicrobia.

III.B.3. Structure and evolution of biological communities

BACKGROUND: Here, our most general questions are fundamental to evolutionary ecology. We will explicitly address the question of what factors (biotic and abiotic) determine and sustain the high species diversity of microbial communities (as represented by contemporary microbial mat systems). Although there is a large body of recently generated molecular data revealing tremendous and previously unrecognized diversity of natural microbial populations, there is virtually no understanding of the ecological framework that sustains that diversity. A related question concerns the evolution of biological novelty. As also discussed in earlier sections, recent studies in comparative genomics have provided new insights into the role of lateral DNA

transfer in the generation of novelty. Although lateral DNA exchange has long been recognized as a significant source of new genetic information, most previously documented examples were of transfer limited to accessory genetic information, for example, the transfer of plasmid encoded genes for antibiotic resistance or catabolism of rare organic compounds. Comparative genomics has now revealed significant transfer of genes encoding core metabolic processes between lineages, including exchange between domains. In fact, the evolution of new physiological types (e.g., oxygenic phototrophs and methanotrophs) almost certainly was an outcome of the melding of disparate genetic elements invented independently by different microbial lineages. Thus, microbes are now recognized to have a tremendous fluidity of genomic content and this fluidity has almost certainly been a fundamental contributor to biological innovation and diversification.

OUR QUESTIONS: We pose two related questions.

(1) What environmental or community conditions promote high species diversity, and therefore high genetic complexity?

(2) Do microbial mat communities, by providing for high species diversity and frequent contact among different populations, promote lateral gene transfer?

We specifically address the first question and will develop a system-level understanding that should constrain future studies of the second question. We hypothesize that microbial mat communities provide both high diversity and the opportunity for intimate interactions among populations. We suggest that it is highly probable that many key biological innovations central to the evolution and diversification of life on Earth occurred within microbial mat systems, as more fully elaborated below. For this reason, we also suggest that the study of mat ecology at high resolution, emphasizing the mapping of population and gene expression distribution patterns is fundamental to a more complete understanding of ecological and genetic contributors.

III.B.3.a. Microbial Mat Communities (Stahl, Dillon)

BACKGROUND Microbial mats are possibly direct descendents of the most ancient biological communities on Earth; communities in which oxygenic photosynthesis may have been invented (Schopf 1983, Des Marais 1990, Risatti et al. 1994, Zavarzin 1994). They are highly adapted as evidenced by extremely high productivity and the occurrence of common structural features among geographically isolated systems (Canfield and Des Marais 1993, Stal and Caumette 1994). They are highly dynamic systems that oscillate through extreme chemical changes on a diel cycle (Frund and Cohen 1992). These complete ecosystems cycle all key biogenic elements and therefore represent global processes. They therefore offer a unique opportunity to define holistic functionality in sample sizes on the millimetric scale.

Study site. Of the many diverse microbial environments that might be appropriate to study, we will focus our efforts on the cyanobacterial mats of the evaporating ponds in the Exportadora de Sal salt works, located in Guerrero Negro, Baja California, Mexico (Canfield and Des Marias 1991). This well-studied system does not experience major seasonal change and it will provide data that can be extended to many other sites (e.g., tidal mat communities). The high population density in cyanobacterial mats provides ample biomass for the molecular studies and high lateral structural homogeneity facilitates experimental replication. Previous molecular, microscopic, and molecular analyses (including our work) have revealed high species diversity among the key functional groups (e.g., oxygenic and non-oxygenic phototrophs, sulfur oxidizers, sulfate-reducers) (Risatti et al. 1994, Nubel et al. 1999, Nubel et al. 2000). Since sulfate is not

limiting at any depth, the non-photosynthetic anaerobes are dominated by sulfate-respiring species.

Factors contributing to high species diversity. We hypothesize that the high species diversity of this microbial mat system is a consequence of variation along two fundamental dimensions of time and space. The diurnal cycle imposes a well-defined photoperiod associated with predictably varying light intensity . Increasing depth is associated with an ever-changing chemical and light environment that is controlled by a combination of biological activity and mass transport limitation. The biology modifies the environment via complex networks of metabolic interactions and feedback controls that create and stabilize additional microhabitats (niches).

Sulfate-respiring organisms as a model functional group. Our studies will focus on sulfate respiring microorganisms to better define factors that sustain high species diversity of a single functional group within an ecosystem. Our focus on this group will also address the evolution of the sulfur cycle and the origins of phototrophs (The genes encoding for the oxidation of sulfide to sulfite by anoxic phototrophs are homologous to the dissimilatory sulfite reductase (DSR), raising the question of the relative order of invention: anoxic phototrophs first or sulfate respiration first?). Key work includes:

- Comparative sequencing of the DSR and isotopic data suggest that sulfate respiration is a very ancient phenotype, possibly predating domain divergence (Schedel et al. 1979, Dahl et al. 1993, Karkhoff-Schweizer et al. 1995, Wagner et al. 1998, Shen et al. submitted)
- Phylogenetic inference based on 16S rRNA and DSR sequence divergence suggests that lateral transfer of these genes has occurred in the past and that independent exchange of the alpha and beta subunit genes has also occurred on at least once occasion (Wagner et al. unpubl). Since all known sulfate-respiring lineages are composed exclusively of organisms having this phenotype (Devereux et al. 1989), the implication is that lateral exchange of these genes has marked the emergence of major microbial lineages.

To systematically address the complex questions relating to the origins and stabilization of high species diversity, we will employ the experimental strategy outlined in objectives 1 - 4 (next paragraphs). These studies will be coordinated with a complementary research program directed by M..L. Sogin (Marine Biological Laboratory, Woods Hole, MA) , a Co-I on an existing Astrobiology Institute (see his letter). The first two objectives listed below will be conducted in collaboration with Dr. Sogin., who has received supplemental funding from NASA to initiate these studies. Research relating to objective 3 will be primarily based at MBL but will enrich interpretation of population distribution patterns determined in objective 4 of this proposal. Objective 4 studies will be conducted at UW.

III.B.3.a.1. Research goal #1. Map the basic chemical and biological structure of this system along the two key dimensions of time and space.. We will use a combination of established microscale analytical techniques (e.g., ion-specific microelectrodes) to map chemical microenvironments and biogeochemical process rates (with special emphasis upon chemical gradient location, shape, and stability) and molecular genetic fingerprinting procedures to map the distribution of the most abundant microbial species. We will use methods based on comparative sequencing of the 16S rRNA and genes encoding the dissimilatory sulfite reductase

to map population structure, as more fully discussed below. This mapping will describe the coupling between simple measures of biological complexity , inferred from comparative gene sequencing, and functional parameters (e.g., productivity, levels of elemental cycling). Initial population mapping, using nucleic acid recovered from different depths and depth intervals, will define appropriate scales for evaluating parameter variation. These studies will be performed in collaboration with the existing research programs of M. Sogin and NASA scientist David DesMarais (NASA Ames; see letters of collaborative support).

III.B.3.a.2. Research goal #2. Map key functional groups at high genetic, spatial, and temporal resolution. The information obtained from goal#1 will be used to refine the mapping of species distribution of three functional groups known to be abundant, to interact, and to control key chemical transformations: (1) oxygenic phototrophs (cyanobacteria), (2) phototrophic and non-phototrophic sulfur (*Thiocapsa* spp., *Ectothiorhodospira*, Chloroflexaceae, *Beggiatoa* spp), and (3) sulfate-reducing bacteria. Ribosomal RNA-based distribution studies will be complemented by analysis of conserved functional genes representative of the groups. The UW research will focus on using the DSR genes to relate sulfate reduction to the distribution of populations having this key enzyme of sulfate respiration. This mapping of both rRNA sequence type and functional gene sequence will provide an essential foundation to explicitly link population structure with gene expression patterns and emergent properties (primary and secondary production, oxygen evolution, S cycling). Research by Stahl et al. provided the foundation to initiate the proposed studies. In particular, comparative sequencing of the dissimilatory sulfite reductase genes from all characterized sulfate-respiring microorganisms provides for selective recovery of the genes encoding this key enzyme from all sulfate respiring microorganisms and a target for gene expression that can be related to a system-level process, sulfate reduction (see goal #3) .

Information gathered from goals #1 & 2 will be used to direct the ultimate objectives of this research (goals #3 & 4) - the fully integrated study of this ecosystem at all levels of system organization. To accomplish this goal we will use DNA micro array technology and laser scanning confocal microscopy to define single-cell distribution in relationship to gene expression patterns. These measures will be also be evaluated in terms of relationship to both local chemical environment (micro sensor studies) and emergent system-level properties e.g., CO_2 fixation). The work conducted at UW will focus upon the distribution of specific sulfate-respiring populations in relationship to expression of the cognate DSR genes. Our previous research has revealed a remarkably high diversity of distinct sulfate respiring populations in the GN system and a comparable system in Egypt (Solar Lake). The proposed studies will provide an environmental and ecological context for this diversity.

III.B.3.a.3. Research goal #3. Use DNA micro array technology to relate gene expression patterns to species distribution and physical/chemical environment.. By treating the total genetic coding capacity of a microbial ecosystem as a complex mixed genome, we can correlate spatial and temporal modulation of gene expression in a series of experimental observations. The mixed environmental genome DNA micro arrays will bridge the link between formation of biogeochemical gradients that shape the planet with underlying complex patterns of gene expression.

This research objective has two experimental elements, as defined by the genetic composition of the DNA micro arrays. These studies will require the construction of two types

of DNA micro arrays: (1) Mixed Environmental Genome Arrays (MEGA) and (2) Genomic DNA micro arrays. MEGA will primarily contain genes that have been randomly collected from genomes represented in selected microbial mat samples. Sequenced genes (or gene fragments) whose function has been inferred will be "printed" along with the set of functional genes characterized in objective 2.

The Genomic DNA micro rays will be derived from pure culture representatives of two major functional populations, a cyanobacterium (*Microcoleus chthonoplastes*) and a sulfate-reducing bacterium (species to be determined). These DNA micro arrays will be fabricated from random genomic libraries without prior sequencing of the cloned fragments. The MEGA and Genomic DNA micro arrays will be used in parallel. The MEGA will provide a holistic and integrated approach to system analysis. The Genomic DNA micro arrays will provide for population-specific analyses of gene expression in the community and in relationship to expression in laboratory pure culture. The UW Astrobiology group will provide the MBL laboratory with DSR genes derived from the GN mat community , recovered using selective PCR amplification as described in goal #4.. Inclusion of these genes on the microarray will allow us to relate differential expression of population-specific DSR genes with the mat population structure (goal #4). Since these studies will be performed primarily at the MBL with approved NASA astrobiology support, we do not provide additional experimental detail here. However, we note that our group has an existing program in microarray technology that will provide some additional technical support to this experimental objective (Guschin et al. 1997).

III.B.3.a.4. Research goal #4. Map single-cell distribution in relationship to population-specific expression of genes in the pathway for sulfate respiration. We anticipate that complete understanding of gene expression patterns will require information of interaction among populations at the single cell level. It is well recognized that close physical associations are essential for certain community activities (e.g. oxidation of fatty acids by syntrophic populations (Warikoo et al. 1996, Zellner et al. 1996)) and these associations are anticipated to be of general importance within these microbial mat systems (Jørgensen 1990, Minz et al. 1999). For these studies we will use the established phylogenetic frameworks inferred from sequences encoding the dissimilatory sulfite reductase and 16S rRNAs (Wagner et al.1998, Minz et al. 1999). We earlier developed PCR primers that amplify the DSR genes from all known sulfate respirers and have used these primers to identify novel lineages in a cyanobacterial mat and other sulfidogenic habitats (Wagner et al. 1998, Minz et al. 1999). We will use this primer set to directly identify sulfate reducers at different depths in the GN mat and to isolate DSR genes for inclusion on the DNA micro array. Since the divergence of DSR generally corresponds to that of the 16S rRNA, sequences encoding for this metabolic attribute can be assigned to populations identified by 16S rRNA sequence. This provides a mechanism to relate population distribution, visualized by fluorescence in situ hybridization (FISH), with population-specific DSR expression.

We will then use fluorescence in situ hybridization (FISH) - using the 16S rRNA as the probe target - to microscopically relate the distribution of single cells of sulfate-respiring populations with expression of the cognate dissimilatory sulfite reductase. Expression analysis will be based on DNA microarray analysis, using RNA derived from corresponding depth intervals of the mat. We anticipate that this will provide sufficient resolution to relate expression patterns to single-cell distribution. However, if the diversity of sulfate reducers at certain depth intervals is too great to establish correspondence between expression patterns and

populations visualized microscopically, we will evaluate published methods for single cell mRNA expression analysis.

The general methods for in situ hybridization are well established in our research group . If intrinsic autofluorescence interferes with direct staining, we will evaluate established enzymatic methods for signal amplification (Amann et al. 1992, Schonhuber et al. 1999). In addition, we emphasize that these studies will be greatly facilitated by the use of a scanning confocal laser microscope (SCLM), providing for optical sectioning of relatively thick mat specimens and the reduction of stray light originating from sample autofluorescence. Confocal microscopy will also provide for mapping the fine features of cellular distribution and cell-cell association, in particular for filamentous organisms that often extend beyond the depth of field of a conventional light microscope.

Comments on the investigators' experience. Our investigators have well established research programs that routinely use the tools and methods of selective PCR recovery and analysis of 16iS rRNA genes from environmental samples, quantitative hybridization, DNA-based fingerprinting techniques, and fluorescent in situ hybridization. Our experience with these methods is well documented in the literature (Stahl et al. 1984; Stahl 1986; Devereux et al. 1989; Devereux et al. 1989; Amann et al. 1990; Amann et al. 1992; Amann et al. 1992; Alm et al. 1996; Minz et al. 1999; Minz, Green et al. 1999; Alm and Stahl 2000).

III.B.3.b. Sub-seafloor hydrothermal vent microbial communities (Baross, Deming)

BACKGROUND One approach to understanding the habitability of planets is to consider the range of environmental conditions on earth that support life. The most extreme environments on earth have received particular attention since analogous conditions exist or existed in the past on other solar bodies. Specifically, the presence of water ice and/or submarine hydrothermal vent systems on another solar body would make it a prime candidate for habitability by microorganisms.

Vent systems. Hydrothermal systems are open systems and thus resemble chemical reactors. They have extensive chemical, temperature and pH gradients, they produce a large suite of elements, volatiles and possibly organic compounds from water/rock interactions, and they provide abundant reactive surfaces and catalysts. Also, impact frustration models of the early earth point to the subseafloor as perhaps the only safe environment for early microbial ecosystems. It follows that the subsurface may also harbor microbes that are slowly evolving and may still contain genetic information and metabolisms that are conserved from the earliest microbes.

Subsurface microbes. Subsurface microbiology has recently been the focus of considerable interest by diverse groups of scientists. Microorganisms have been discovered below the surface of the earth's crust, in environments once thought sterile including deep granitic and basaltic aquifers, oil wells and Cretaceous sandstone cores. The sedimentary layer of ocean crust has also been biologically investigated; microbes have been observed in deep marine sediments, over 500 m below the seafloor. Sulfate-reducers have been cultured from these samples and the sediments show the potential for microbial sulfate-reduction. However, at the present time nothing is known of the possible existence of microbial communities in igneous ocean crust. Though of all the deep environments, the igneous ocean crust contains the most

favorable habitat for microbial life. The near-ridge subseafloor also has characteristics that would have existed in early Precambrian subsurface environments.

Our study area and organisms. Consequently, the nature of the subseafloor habitat in mid-ocean ridge environments is not understood. There is no method of directly sampling this habitat. We have been examining the phylogeny and physiology of high-temperature organisms that come from the subseafloor (hydrothermal fluids from new eruption sites and diffuse flow vents) to determine if these microorganisms could yield insights about the nature of the subseafloor environment. One of the groups of microorganisms isolated from the subseafloor includes a new genera of the Archaeal Family, Thermococcales (Summit and Baross 1999). The Thermococcales are excellent candidates for indicating a hot subsurface biotope. They are cosmopolitan and have been isolated from all hydrothermal environments thus far sampled. They are Archaea and members of the kingdom Euryarchaeota. All are strict anaerobes and heterotrophic and utilize carbohydrates by a glycolytic pathway that includes some unusual tungsten-enzymes (Mukund and Adams 1995). Most species have a requirement for amino acids and peptides, and elemental sulfur. *Pyrococcus* and *Thermococcus* are the two genera within the Thermococcales and at the present time there are approximately 30 species described. *Thermococcus* and *Pyrococcus* spp. are routinely cultured from near-vent sites, from samples of sulfide rocks, alvinellid worms, and occasionally from hot fluid samples. Since the hyperthermophilic Thermococcales are one of the slowest evolving groups of extant microbes, and are capable of growing under environmental conditions that are consistent with some models of early earth environments, they are good candidates for addressing questions about nonphotosynthetic life in the Precambrian.

Basic questions. Two questions discussed in other sections of this proposal include the sources and kinds of organic material on the early earth from both extraterrestrial and abiotic terrestrial sources and the nature of the nitrogen cycle during the Precambrian. We propose to use cultured hyperthermophiles from the subseafloor to address aspects of these questions. Specifically, is nitrogen fixation a significant source of nitrogen for subseafloor hyperthermophiles and can hyperthermophiles anaerobically oxidize organic compounds commonly synthesized abiotically?

III.B.3.b.1. Research topic #1: Nitrogen fixation by hyperthermophiles

The conversion of atmospheric nitrogen to ammonia is widespread in microorganisms. Nitrogen fixation (*nif*) genes have been sequenced and analyzed from a large number of microorganisms. The *nif* genes are highly conserved in both Bacteria and Archaea. Very little is known about the nitrogen cycle at vents. Ammonia has not been detected in subsurface vent fluids except at sites where there is overlying or buried sediments (Lilley et al. 1993). The dominant source of nitrogen to the hot subseafloor biosphere is N_2; however, nothing is known about nitrogen fixation by hyperthermophiles at vents. One of the interpretations from these data is that nitrogen fixation is an ancient trait and may have been present in the common ancestor before the separation of Bacteria from Archaea (Fani et al. 2000). We propose to use molecular methods to search for nitrogen fixing genes (*nif*) in both an existing culture collection of hyperthermophilic Archaea and in archaeal communities from subseafloor environments and identify the groups of organisms, if any, that are actively fixing nitrogen. One of us (JAB) has cruises funded by NSF and NOAA/Sea Grant scheduled for 2001 and 2002 to study the diversity of the microbial communities in the subseafloor. We will use these cruise opportunities to look for the diversity of archaeal *nif* genes and determine if they are expressed *in situ*. We will

compare the sequence of these archaeal *nif* genes with other sequences in the database and determine if they show ancient lineages.

The nitrogenase enzyme is made up of two highly conserved proteins: the iron protein (encoded by the *nifH* gene) and the molybdenum iron protein (encoded by the *nifDK* gene). The *nifHDK* genes form a transcriptional unit. Since the nif operon is regulated by ammonium concentration at the level of transcription, and mRNA is very labile, the detection of nif mRNA is an ideal way of monitoring nitrogen-fixation expression.

Evolutionary conserved amino acid sequences within the nifH gene has been used to design PCR (Polymerase Chain Reaction) primers that can amplify and sequence *nifH* genes (Ohkuma et al.,1999; Zehr and Capone, 1996). We have already designed primers to detect and amplify the *nifH* gene and the *nifH* mRNA transcripts from subseafloor microbial communities and from our culture collection of subseafloor hyperthermophiles. They are 17 base pair degenerate PCR primers that were designed by aligning the *nifH* DNA sequences from several species of nitrogen-fixing bacteria and methanogenic Archaea and choosing two highly conserved regions 200 base pairs apart. These *nifH* primers successfully amplified a 200 base pair fragment of the *nifH* gene from *Methanococcus jannaschii* DNA and a 200 base pair fragment of DNA from a subsurface fluid sample. We will apply the FLT-RFLP (Fluorescently Labeled terminal-Restriction Fragment length Polymorphism) method and the cloning-based sequence methods to quantitatively and qualitatively describe the nitrogen fixing community, and RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) to detect mRNA.

III.B.3.b.2. Research topic #2: Anaerobic oxidation of organic compounds coupled with iron reduction

Organic compounds can be abiotically synthesized by many different energy sources including heat, ultra violet light, electrical discharges, meteor impacts and radionuclide decay. Some of the compounds produced include hydrocarbons, amino acids, organic acids and insoluble polymers of unknown composition. Recently, both acetate (Huber and Wächtershäuser 1997) and pyruvate (Cody et al. 2000) were synthesized abiotically in the presence of metal sulfur compounds under conditions found in subseafloor crustal environments associated with hydrothermal vent systems. To date there are no published reports of subseafloor hyperthermophilic microorganisms capable of growing anaerobically on simple organic acids and hydrocarbons. Recently, we have isolated a group of hyperthermophilic Archaea from both crustal and deep hot sediment environments that can grow anaerobically on acetate using Fe (III) as the electron acceptor. Based on 16S rRNA sequence analysis, these subseafloor isolates are new genera within the Family Thermococcales and they are also the deepest rooted organisms within this group and appear to be using acetate and Fe (III) in classic dissimilatory iron reduction fashion (Lovley and Phillips 1988). Unlike many organisms that can reduce soluble iron-organic compounds, these isolates reduce Fe (III) from insoluble minerals. They accomplish this by first attaching to the minerals where they generate organic polymers and a generous biofilm.

We are proposing to test these isolates for their ability oxidize carbon sources commonly synthesized abiotically using Fe (III) as the electron acceptor. The carbon sources will include low molecular weight acids, hydrocarbons, ketones and other compounds either identified in extraterrestrial bodies or hypothesized to be synthesized abiotically (McCollum and Shock, 1997). We will also continue our efforts to isolate new hyperthermophilic microorganisms that

use other transition metals as electron acceptors and determine the mineral products from microbial reduction and whether or not they could be important biosignatures for detecting life.

Section IV: The UW Astrobiology Education Program

(Sullivan, Staley)

Precis: We wish to make very clear the existing and ongoing intense commitment of the UW to Astrobiology. It takes two main forms: (1) long-term educational support (discussed here), and (2) direct support for this proposal's work (cash, salaries: see mandatory "Institutional Support" section, end of Vol. I). Long-term institutional support includes strong internal UW funding for, and hiring into, the "Center for Astrobiology and Early Evolution" (permanent funding at about $151 k/year), plus major matching support ($500k) for our NSF "IGERT" graduate program in Astrobiology.

In addition to the astrobiological research outlined above, UW's administration and faculty have shown extraordinary institutional support for Astrobiology through establishment of our graduate program in Astrobiology and our Center for Astrobiology and Early Evolution. The programs detailed below will provide tremendous leverage to the research funding requested in this proposal.

Background and Philosophy

The Beginning. Astrobiology at the UW started with an interdisciplinary graduate seminar in the autumn of 1996 entitled "Planets & Life." Enthusiasm from faculty and students led to our responding to an RFP for NSF's new IGERT (Integrative Graduate Education and Research Training) program. Fourteen UW faculty from ten departments were awarded (1998) a five-year $2.2M IGERT grant to train graduate students in Astrobiology; UW matching funds added $0.5M. The grant primarily pays for ~60 "student-years" of RA-ships, a coordinator, seminars and workshops, faculty release time, and establishment of an Extremophile Lab. Our faculty are committed to teaching, and have produced many PhDs in recent years (see appendix for "Recent Students and Teaching" in Vol. II).

Current Status. Now in its second year, this is the first graduate program in Astrobiology (see www.depts.washington.edu/astrobio). We are now developing this cross-disciplinary program that includes departments in the physical sciences, biological sciences, oceanographic sciences, and engineering.

Guiding Philosophy. We relish the opportunity to breach walls between highly specialized disciplines and departments by providing a rigorous, interdisciplinary curriculum with unique features that bond students and faculty from disparate departments. Our goal is to create a cadre of students each with particularly strong expertise in one aspect of Astrobiology as well as sufficient knowledge of other areas to foster interdisciplinary research, especially between the biological and physical sciences.

Research Theme. Our Astrobiology Program's central research theme is study of the extreme forms of microbial life on Earth (now and in the past) so as to inform the search for fossils or extant life at extraterrestrial sites. We strive to fully integrate research and education and create a truly new community of scholars and investigators: the first Ph.D. astrobiologists.

UW Initiatives Fund and new faculty.

Center for Astrobiology and Early Evolution. In 2000 we got permanent funding from the UW College of Arts & Sciences to establish a Center for Astrobiology and Early Evolution.

These funds are for two faculty members in Astrobiology, support for post-docs, an annual summer institute, and development of courses and research jobs for undergraduates.

Faculty new hires. Our first faculty hire is Professor Roger Buick (Co-I here: arrives 2001); he is an international leader in micropaleontology and the search for biomarkers in the oldest geological formations. The second Astrobiology hire will be made during the coming year. Furthermore, departments involved in the Program are making supportive hires. Three examples (all new, fall 2000) who will be intimately involved in Astrobiology (the first two are Co-Is here): David Stahl (dual appt- Civil/Environmental Engineering & Microbiology); Tom Quinn (Astronomy); and Luann Becker (Geological Sciences). Also just now joining the UW team are Astrobiology post-docs M. Kress (Co-I here: from NASA/Ames) and J. Dillon (U. Oregon).

Graduate Education

Overview. Students are admitted to a participating department: they spend about 2/3 of their efforts there and 1/3 outside. RA-ships for two "rotation" quarters are spent outside the home department. In the first or second year of graduate study Astrobiology students take a sequence of courses designed to train them in basics of the "other" side (microbiology for physical scientists, and planetary sciences for biologists); during later years course choices are from existing courses or special topics seminars of an interdisciplinary bent (three are required). After two years a student forms a thesis committee that is highly interdisciplinary, although the Ph.D. will still be in the home department's field (with an additional Certificate in Astrobiology).

New Graduate Courses. New courses are being created for the Astrobiology core curriculum. Students with a biology background take "Planetary Properties and Processes for Astrobiologists", which uses examples from our solar system and extrasolar planets to teach general physical and chemical principles of planetary formation and environments. Physical sciences students take "The Evolution of Life: Biology for Astrobiologists," which teaches the basics of microbiology through an organizing principle of evolution: from prebiotic evolution, through the origin of life, and up to present-day organisms. Finally, all students take "Astrobiology," a team-taught course that integrates and extends principles earlier learned, as well as introduces students to most of the Astrobiology faculty. Our initial cadre of grad students have suggested another format, which we plan to try in the coming academic year, namely a largely student-run course in which students mentor each other in the core basics of their individual fields. These courses differ from the usual graduate courses because of the diversity of student backgrounds and range of topics that must be covered. However, they are not "dumbed-down" versions of "proper" graduate-level treatments: they are intensely challenging for both students and instructors. Based on the Astrobiology faculty's experiences with two existing interdisciplinary graduate courses with some similarities (Geophys/AtmSci 508 on "Biogeochemical Cycles" and Ocean 535 on "Biological Oceanography for Physical Scientists"), we are confident that instructors can maintain a fast pace, introduce only essential background and terminology, and not sacrifice understanding of core principles and concepts.

Astrobiology Seminar and Workshop. Our Astrobiology seminar series has now been running highly successfully for three years, providing a weekly focus for us, drawing in a wide range of audience from all over campus, and importing outside speakers who have enriched our program.

Our annual Workshop, a required two/three-day event held at a field site or laboratory, is another important component. In addition to an in-depth facilities tour and talks, each Workshop

features a prominent non-UW expert and involves hands-on experience for all participants. Our first workshop in October 1999 was hosted by James Fredrickson of Pacific Northwest National Lab and featured a subterranean microbial ecosystems site near Richland, Wash. Hands-on experiments for twenty participating UW Astrobiology grad students and faculty involved obtaining (via deep wells) and examining subterranean samples to assess metabolic activities such as anaerobic CO_2 fixation, sulfate reduction, and hydrogen oxidation. This biological work was further enhanced by geology field tours to understand the context of the microbial ecosystems. Our workshop this coming autumn will be held at NASA/Ames Research Center and focus on Mars simulations in a wind tunnel.

Summer Institute in Astrobiology. Described below, in Sec. V.

Jobs for our Ph.D. s. We anticipate that our graduates' broadly-based backgrounds will open up a spectrum of possible positions in NASA centers, academia, and industry. The last category may seem surprising, but in addition to its intellectual excitement, Astrobiology also has an "applied" side important to Seattle-area firms such as Zymogenetics (a partner in our IGERT grant) and Bioworks-IMS (a partner in a grant to one of our faculty). Microorganisms that grow at extremes of temperature and acidity have considerable commercial potential, producing enzymes active under much more severe conditions than those of typical bacteria and therefore valuable to catalyze commercially important reactions.

Undergraduate Education

New courses. The intrinsic appeal of Astrobiology acts marvelously to attract undergraduates and show them the excitement of science. Moreover, to consider the possibilities of extraterrestrial life is also salutary in that it forces one to study and better understand the one form of life and the one life-bearing planet that definitely *do* exist. We are developing two lower-division courses open to all students. These will discuss those conditions on Earth and elsewhere that supported or may support life's origin and sustained existence, as well as the origin and early evolution of life on Earth and implications for possible life on other planets. Our Astrobiology graduate students will gain invaluable teaching experience as TAs for these courses.

Research participation. We also have funds for in-term and summer research stipends for undergraduates. The intrinsic appeal and tremendous variety of research avenues into Astrobiology make such positions very attractive. We are closely cooperating with the Washington State NASA Space Grant Office, housed in the UW Geophysics Department, whose director in the summer of 2000 agreed to split funding with us for six fulltime summer stipends for undergraduates.

Minority/under-represented students.

We point out the close connection between NASA and the UW on the issue of under-represented groups in science: on campus at UW we have Tom Colonnese, co-PI of the NASA-sponsored American Indian Science and Technology Education Consortium (AISTEC), which seeks to attract undergraduates into the sciences. Dr Colonnese is also Director of the Office of Minority Affairs, and will help us to establish a close connection between AISTEC, our Astrobiology IGERT program (one RA of which is designated for a minority graduate student), and the research effort proposed here. We also point out, in line with NASA's desire that proposing institutions team with minority institutions, that our partner, New Mexico State University, is federally recognized as one such (serving the Hispanic community).

Section V: The UW's Place in the NASA Astrobiology Institute

NAI Integration. The activities set forth in this proposal will allow the UW to significantly help in integrating members of the NAI. We envision ourselves applying the same skills that have allowed us to create the UW Astrobiology Program from many disparate parts to the larger goal of fostering cohesion in NAI. Besides the astrobiological research itself (which interacts with many other groups who are already members of NAI (see, e.g., letter of collaboration from M. Sogin of MBL Woods Hole), the primary ways in which the UW Astrobiology Educational Program and Center for Astrobiology and Early Evolution will serve NAI are:

- the annual Summer Institute in Astrobiology (described in Sec. IV), a marvelous opportunity for a broad range of researchers in Astrobiology to gather and learn from each other in relaxed surroundings designed to promote interaction across disciplines.
- a graduate student exchange program (described below)
- access for the NAI community to the UW Extremophile Lab (described in "Facilities", Vol. II), the UW Mars simulation chamber, the laser confocal microscope (proposed here), and to the other facilities described in Vol. II.
- production of the kinds of Ph.Ds who will be the lifeblood of the future of Astrobiology and of NAI, the first generation of students trained with the new astrobiological outlook
- A pilot graduate student exchange program with present NAI members (below).

Graduate Student Exchange Program. We propose to set up a pilot program for exchange of graduate students between the NAI institutions. Graduate students are the heart of much research and certainly represent the future of the field. Such exchanges between NAI members will be extremely valuable, accomplishing on a larger scale what we do now on our own campus with our students' required two quarters of rotations outside their own discipline. The students will greatly profit from their exposure to new research vistas, while NAI's overall research effort will profit from the cross-fertilization. A given student will spend 3-6 months away from his/her home institution in order to (1) work or study under a specific person or in a specific group, (2) learn a specific technique, (3) be exposed to a discipline not available on the home campus (e.g., geology fieldwork or an astronomical observing run; computer techniques for inferring phylogeny; an oceanographic cruise to hydrothermal vents), etc. Preference will be given to those requests that "stretch" the student's knowledge well beyond its present limits. If this is successful, we will expand it (given more funding) to include students of all NAI members.

Summer Institute in Astrobiology. This will be a magnet for top researchers to visit Seattle, in the process enriching our graduate program, our faculty, NAI, and Astrobiology research in general. The Summer Institute (to start in 2001) will run for three or four weeks, of which a central three days will be a scientific symposium on a specific topic in Astrobiology. The other weeks are available for researchers to remain in residence at the UW and interact with each other and with UW faculty and students on problems of common interest, create new intellectual links, give informal seminars, write papers, etc.

CONCLUSION

We thank persistent and patient reviewers for their work. We believe we have assembled a very strong team to investigate many intertwined aspects of planetary habitability, and to commingle that research with our educational program in Astrobiology. We believe too that our program can fill a very significant gap in the NAI's present investigations, as clearly identified in the CAN. This is, we know, a complex proposal. It is all too easy to lose sight of the forest for the trees. Therefore, we ask that reviewers now reread the executive summary and/or introduction, so as to regain an overall sense of our program.

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