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Habitable Planets and Evolution of Biological Complexity

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Table of Contents

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
  A. astrophysical and planetary constraints: background
       1. galactic chemical evolution and extrasolar planets
           a. extrasolar planets
           b. galactic chemical evolution
           c. galactic habitable zone
       2. dynamics of comets, asteroids, and planets
           a. how stable are planetary systems?
           b. influence of giant planets on formation of terrestrial planets
           c. influence of giant planets on development of reservoirs of small bodies
           d. influence of giants on impact rates of asteroids and comets on terrestrial planets
           e. effects on impact rate of stellar companions, stellar density & galactic environment
       3. delivery of organic materials to planets
           a. delivery of organic compounds to habitable zone planets: comets, rocks, dust
           b. work statement
           c. nature of organic matter in dust accreted by Earth
                1. FTIR examination of the 3u region
                2. STXM observations
       4. evolution of surface and atmosphere of Mars
           a. how much liquid water has there been on Mars: evidence pro and con
           b. alternatives to massive flows of liquid water
           c. wind erosion as possible cause of surface features
  B. Plate tectonics on the terrestrial planets: background
       1. what planetary conditions allow plate tectonics?
       2. interactions of plates, melting, climate, volatile cycling in regulating Earth's climate
       3. what planetary conditions allow the generation of a planetary magnetic field?
       4. what is the role of biota in influencing plate tectonics?
  C. Causes of mass extinctions: testing impact models
       1. Question #1: evidence for impact as cause of mass extinction events
           a. the P/T event
           b. the T/J event
           c. sampling protocols: extraterrestrial ^{3}He
                1. impact debris
                2. constraints on inputs and sedimentation rates
       2. Question #2: is there a critical number of mass extinctions necessary for maximum diversity?
           a. methodology: a new metric for diversity and its changes
           b. modeling diversity

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.
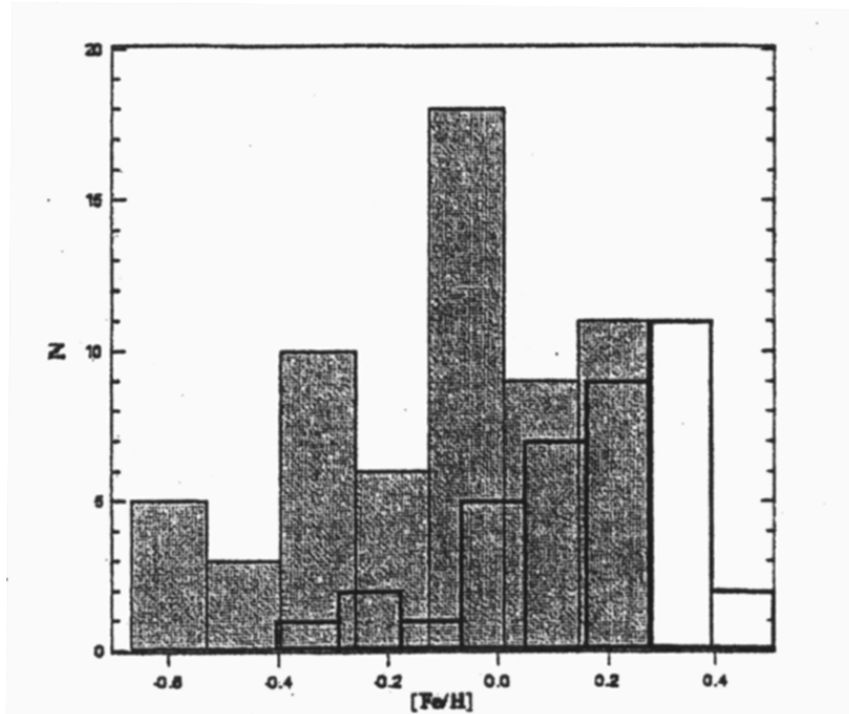
FIGURE 1. [Fe/H] values for field stars without known planets (from Favata et al. 1997) - shaded. [Fe/H] values for 38 extrasolar planet parent stars with high precision spectroscopic [Fe/H] determinations – bold outline. (Note: [Fe/H] is the logarithmic number abundance of Fe relative to H in a star's 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 <u>deliver</u> main-belt asteroids into Earth-crossing orbits (= extinction promotion). Conversely, gas giants may act as "planetary protectors" (Wetherill 1994), drastically <u>reducing the number </u>of Oort Cloud comets that develop Earth-crossing orbits (= extinction avoidance). Furthermore, gas giants <u>increase the flux</u> 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).

Frequency of Saltation Events
1 hour averages, roughness 0.75 cm
Optimal particle size about 100 microns

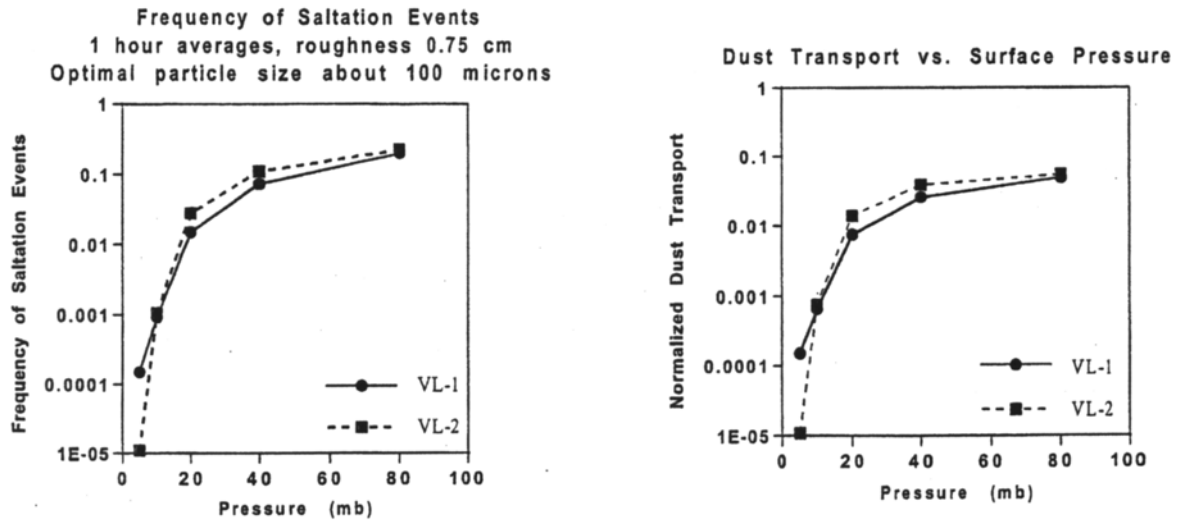**Dust Transport vs. Surface Pressure**

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.

<u>T/J study area #3: Pelagic Red Chert Tr/J sections, Inuyama area, Central Japan</u>

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.
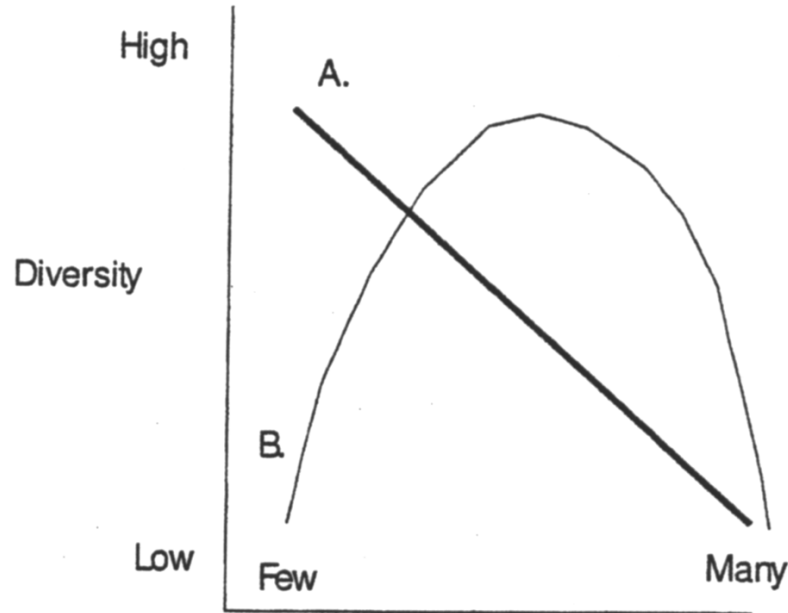

Figure 3. Two competing models of the effect of mass extinction on planetary biodiversity. In A, increasing the number of mass extinctions causes diversity to drop monotonically. In B there is some critical number of mass extinctions necessary for maximum diversity.

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-Palaeoproterozoic, 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.
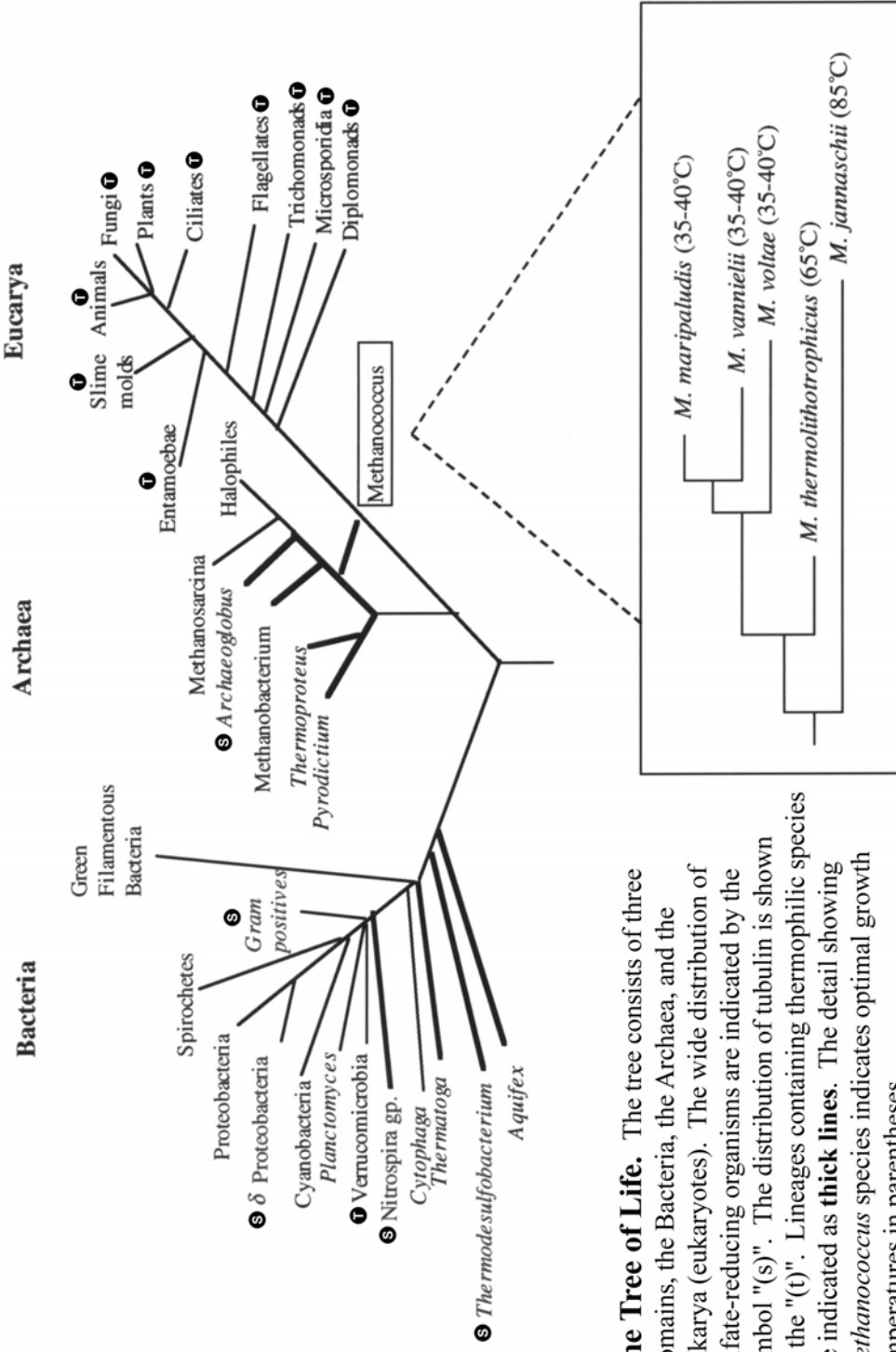
THEME #2: Lateral (horizontal) gene transfer. The movement of genes across lineages, as opposed to linear inheritance, is a major force in evolution (Doolittle, 1999). An excellent example is the transfer of genes for metabolism of single-carbon compounds from methanogenic Archaea to methylotrophic Bacteria (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?"

Bacteria

Green
Filamentous
Bacteria

Spirochetes

Proteobacteria

ⓢ δ Proteobacteria

Cyanobacteria
Planctomyces
ⓣ Verrucomicrobia
ⓢ Nitrospira gp.
Cytophaga
Thermatoga
ⓢ *Thermodesulfobacterium*
Aquifex

ⓢ *Gram positives*

Archaea

Methanosarcina
Methanoeglobus
ⓢ *Archaeoglobus*
Methanobacterium
Thermoproteus
Pyrodictium

Halophiles

ⓣ Entamoebae

Methanococcus

Eucarya

ⓣ Slime molds

Animals ⓣ
Fungi ⓣ
Plants ⓣ

Ciliates

Flagellates ⓣ
Trichomonads ⓣ
Microsporidia ⓣ
Diplomonads ⓣ

M. maripaludis (35-40°C)
M. vannielii (35-40°C)
M. voltae (35-40°C)
M. thermolithotrophicus (65°C)
M. jannaschii (85°C)

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 dispensable. 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 dispensable 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. *Thermococcu*s 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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