SCIENCE CHINA
Earth Sciences

SPECIAL TOPIC: Frontiers of Geobiology
• REVIEW •

May 2014 Vol.57 No.5: 869–877
doi: 10.1007/s11430-014-4858-8

Life in extreme environments: Approaches to study life-environment co-evolutionary strategies

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Received October 30, 2013; accepted February 11, 2014; published online March 20, 2014

The term “extreme environments” describes the conditions that deviate from what mesophilic cells can tolerate. These conditions are “extreme” in the eye of mankind, but they may be suitable or even essential living conditions for most microorganisms. Hyperthermophilic microorganisms form a branch at the root of the phylogenetic tree, indicating that early life originated from extreme environments similar to that of modern deep-sea hydrothermal vents, which are characterized by high-temperature and oxygen-limiting conditions. During the inevitable cooling and gradual oxidation process on Earth, microorganisms developed similar mechanisms of adaptation. By studying modern extremophiles, we may be able to decode the mysterious history of their genomic evolution and to reconstruct early life. Because life itself is a process of energy uptake to maintain a dissipative structure that is not in thermodynamic equilibrium, the energy metabolism of microorganisms determines the pathway of evolution, the structure of an ecosystem, and the physiology of cells. “Following energy” is an essential approach to understand the boundaries of life and to search for life beyond Earth.

energy, extremophile, adaptation, coevolution

Citation: Xiao X, Zhang Y. 2014. Life in extreme environments: Approaches to study life-environment co-evolutionary strategies. Science China: Earth Sciences, 57: 869–877, doi: 10.1007/s11430-014-4858-8

An organism is an “orderly” object (far from maximum entropy); the essential rule in metabolism is that the organism must release all of the entropy that it produces while alive by continually drawing energy from its environment (Schrödinger, 1944). Microorganisms, as the most ancient life form on Earth, have been going through an evolutionary process driven by both environmental impacts and self-maintenance while not in thermodynamic equilibrium. The reactions that build peptides and proteins from amino acids and build nucleic acids and chromosomes from nucleotides cannot occur spontaneously according to thermodynamic law. However, these are the most common reactions seen inside of all types of organisms, even the simplest unicellular microorganism. The key is “energy”: the organism remains alive by consuming and transferring energy from its surroundings. The dependence of life on energy is widely acknowledged (Hoehler, 2007; Hoehler, et al., 2007; Schrödinger, 1944). Biologists have observed that the need for energy includes a core set of applications that are conserved across all known organisms (Baross, et al., 2007). However, the understanding of the mechanisms that convert biological energy is limited and remains a key challenge to astrobiologists. In this essay, we “follow the energy” toward a general understanding of the life-environment co-evolution at the most fundamental level, leading us to define the boundaries of life.

1 Energy metabolism connects life and its surroundings to co-evolve

Metabolism is the signature of life. Energy conversion accompanies substrate utilization. Virtually all non-equilib-

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rium electron transfers on Earth are driven by a set of nano-biological machines that are composed primarily of protein complexes (Falkowski, et al., 2008). During catabolism, cells convert substrates into products through photocatalytic or redox reactions, where energy is gradually released. During anabolism, the large molecules recognized as building blocks for cellular structure are intentionally constructed from small molecules using energy (Wang, et al., 2008). Energy is needed in every biological activity. Depending on the energy supply, the status of life can be distinct. For example, the microorganisms in the deep biosphere can maintain any of three statuses: survival, maintenance, or growth (Price and Sowers, 2004). There is a difference of three orders of magnitude in the energy requirement for each status (Valentine, 2007). How the microorganism can obtain energy from its surroundings, and how much it can obtain, determines the distribution and evolution of the microorganism.

For each specific type of microorganism, there is a minimum unit needed to store and utilize energy, which is called the microbiological energy quantum (MEQ). The free energy in the environment, specifically referred to as potential energy, which is released from photocatalytic or redox reactions, must be greater than or equal to this MEQ before it can be usefully harnessed by microorganism. To understand the MEQ, we have to be aware that ATP is the only energy currency used by microorganisms. Thus, the microorganism has to obtain a sufficient amount of energy to synthesize ATP from ADP to sustain life. Some microorganisms are able to store energy temporally through the proton/iron (e.g., Na^+) gradient that is built across the membrane, and this gradient can later be converted into energy for ATP synthesis. The accumulation of an iron gradient can start from a single iron atom. Therefore, the theoretical minimum MEQ is the energy that is released from the cross membrane gradient created by a single proton/iron. Meanwhile, there must be a continuous energy supply from the environment, which is termed the substrate amount (SAM), for it to be possible for the cell to perform the electron transfer that transports energy along the respiration chain. Hence, the MEQ defines whether the microorganism can effectively harness the energy from its surroundings, whereas the SAM defines how large the population size is and how long the population can last. Once the SAM is limited, maintenance of life is balanced between the population size and lifespan. To summarize, the MEQ and SAM determine: 1) the microbial diversity and community structure within a given ecosystem (Takai and Nakamura, 2011); 2) the habitable zone and evolutional direction of a given type of microorganism (Valentine, 2007); and 3) the condition of survival, maintenance, or growth of a given cell (Price and Sowers, 2004).

"Everything is everywhere, but the environment selects"—Baas-Becking. Given the wide distribution of organic matter on Earth, energy is everywhere as long as a biological conversion process occurs. Thus, the mechanisms of energy conversion and utilization, particularly the MEQ values, are developed through the life-environment co-evolution process. Early life originated after the Earth-Moon system. Before photosynthesis, the free energy in the environment was low, meaning that the MEQ of early life was correspondingly low. Because the harnessing of energy was poor in such a relatively reduced environment, the resident microorganisms, such as methanogens, which we will describe in more detail below, suffered from a chronic energy shortage. The adaptation strategy to this chronic energy shortage drove the general adaptation strategy and the ecological distribution among bacteria and archaea (Valentine, 2007).

Over the historical record of Earth's biosphere, in terms of time and space, the vast majority of life has been suffering from a chronic energy shortage. Even in the modern geological settings, the energy present in the environment is still insufficient to satisfy the entire ecosystem. Based on geological differences, the deep biosphere is a typical low-energy ecosystem: it is permanently dark, with one or more steps disconnected from photosynthesis, and these differences distinguish it from the surface ecosystem. A recent study indicates that 0.18%–3.6% of the biomass on Earth exists in the deep biosphere (Kallmeyer, et al., 2012). Based on climate differences, all of the cold environments can be considered as low-energy ecosystems: 85% of surface ecosystems have average temperatures below 5°C. When the temperature is low, metabolic rates are decreased and pathways are shifted, leading to a reduction in the amount of energy that is harnessed and an alteration of energy allocation. Moreover, under certain extreme conditions, cells must spend additional energy to stay alive. Examples of this process are: 1) maintenance of a moderate intracellular pH while the extracellular pH is severely low or high; 2) repair of cellular material that is damaged while the cell is exposed to stress, such as radiation; 3) compensation for the loss of cellular material, e.g., higher temperatures result in faster cleavage of macromolecules (Hoehler, 2004). Some archaea can tolerate acidity up to pH=0 and high temperatures simultaneously. These cells must consume a substantial amount of energy to sustain a proton gradient that is up to five orders of magnitude higher than normal (Macalady, et al., 2004). Another archaea species can tolerate extracellular salinity up to 150–200 g/L. Salt can cause electrochemical and osmotic stresses that disturb and immobilize nucleic acids and proteins. Because there is always a tendency to maintain thermodynamic equilibrium between intra- and extra-cellular salinity, halophilic microorganisms must maintain a relatively low intracellular Na^+ concentration at an energy cost. The archaeal membranes are less permeable than bacterial membranes, and this lower permeability saves energy and counters the invalid ion circulation. Thus, archaea have a survival advantage at high temperatures and high acidity. A recent study demonstrated that only archaea were able to survive in environments with a pH of less than

2 and temperature above 60°C or a neutral pH and temperature above 90°C (Valentine, 2007).

By "following energy", we propose a diagram that illustrates the life-environment co-evolutionary process (Figure 1). There are three major aspects of this process: Environment (environmental factor and energy quantum), Cell (respiration chain and cellular structure), and Ecosystem (ecological distribution and limit of life). Environmental factor describes the substrate and energy bio-availability, which includes chemical-physical gradients. Energy quantum describes the minimum energy unit, or the MEQ. The energy quantum is taken by the cell through the respiration chain, which is also called the electron transfer chain. The electron transfer chain is composed of a series of electron carriers. The reduced forms of coenzymes (including NADH and $FADH_2$) from the degradation of intracellular organic compounds, such as hydrocarbons, lipids, and amino acids, are re-oxidized through the respiration chain. Dissociated protons are transported to oxygen molecules or oxidized proteins by the electron carriers. Energy that is generated from this process is stored as ATP. The electron transfer chain is normally composed of four parts: NADH-Q reductase, succinate-Q reductase, cytochrome reductase, and cytochrome oxidase. Additionally, flavins, iron-sulfur clusters, hemes, and copper are all cofactors that can carry electrons and complete the electron transfer in cooperation with enzymes (Wang, et al., 2008). Every component in the electron transfer chain has a different sensitivity to temperature. For example, there is a strong association between the use of non-heme iron proteins instead of NAD (P) and microbial thermophiles. NAD and NAD(P) are unstable at 95°C ($t_{1/2}$ is 2 min), whereas non-heme iron proteins are stable and functional at 100°C (Daniel and Cowan, 2000). The limit of life depends on the efficiency with which the cell takes up energy from the environment and the stability of its biological macromolecules. The boundary of the habitable zone is an environment where sufficient energy is bio-available and bio-usable. Thus, the environmental parameters can be estimated by considering the mechanism of biological energy metabolism. For example, methane, carbon monoxide, and magnetite are ranked lower than hydrogen as microbiologically preferred electron donors in acidic hydrothermal systems, whereas the oxidation of iron, sulfide, sulfur, and carbon monoxide all can supply more energy than hydrogen oxidation in slightly basic hot springs (Windman, et al., 2007). When the environmental temperature increases from −20 to 120°C, the energy required for cell maintenance will increase by five orders of magnitude (Tijhuis, et al., 1993). The above evidence and hypothesis provide us with a theoretical basis to build a model for assessing the boundaries of habitable zones.

2 Temperature is the core environmental parameter in microbial adaptation process

Although the temperature of the Precambrian ocean, where life originated, remains in dispute, oxygen isotopic data on early diagenetic charts indicate that the surface temperature was between 55 and 85°C throughout the Archean period (Knauth, 2005). In addition, hyperthermophilic microorganisms branch off at the root of the phylogenetic tree. Therefore, early life likely originated from a "hot" environment, and the evolutionary process in general was adapting to low temperatures. This "cold" adaptation trend has left a notable mark on modern microorganisms. For example, the maximum growth temperature is always close to the optimal temperature for microbial activity, but it is much higher than the *in situ* temperature. In contrast, the minimum growth temperature is lower but similar to the *in situ* temperature of where the isolate originated (Cavicchioli, 2006). This physiological phenomenon suggests: 1) that life originated in a warmer environment and 2) that microbial metabolism is at its limits in cold environments.

The hypothesis that "life is sensitive to temperature" has a molecular basis because life harnesses and utilizes energy through biochemical reactions. First, according to Arrhenius equation, temperature has an exponential correlation to the chemical reaction rate. A reaction with a higher activation energy has an increased reaction rate when the temperature rises, meaning that this reaction is more sensitive to temperature change. Reactions with different activation energies have different sensitivities to temperature. A microbial metabolic pathway is typically composed of several enzymes

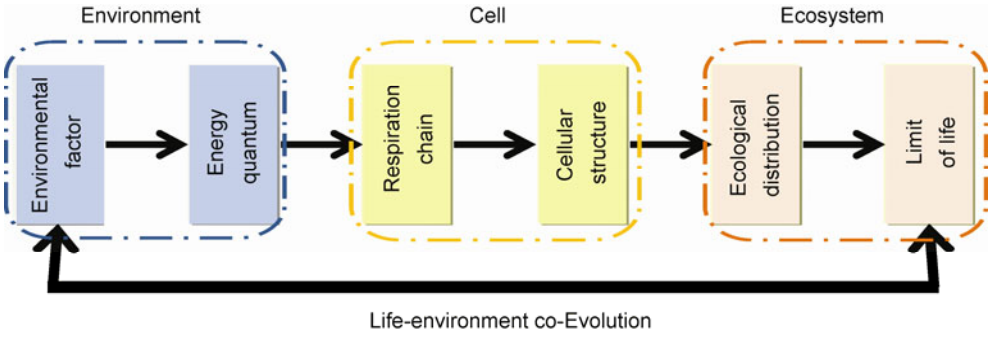

Figure 1 A diagram that illustrates the life-environment co-evolutionary process.

that catalyze dissimilar reactions. If the activation energies of these reactions are highly unequal among each other, then this pathway will be highly sensitive to temperature variation, and the intermediate products will be easily accumulated during temperature fluctuations, which can cause toxicity to the cell. Furthermore, one microorganism possesses hundreds of metabolic pathways that have various temperature sensitivities. The microorganism has to develop strategies to resist the imbalance caused by temperature variation to balance the reactions in one pathway with other pathways in the cell, which is essential for consistent performance of metabolic activities. These strategies are crucial for the cells to set temperature limits in the habitat. Based on experimental cultivation data, the temperature range needed for one microorganism to grow is generally less than 45°C (Cavicchioli, 2006). It is of great interest to find microorganisms or metabolic pathways that have larger temperature ranges because this finding will allow us to better investigate the limits of life.

The stability of biological macromolecules (e.g., lipids, DNA, RNA, proteins) is another crucial constraint that can determine how well a cell can tolerate the extremes and thus provides a theoretical boundary of life. The stability of biological macromolecules is strongly correlated with temperature, solution properties, and other environmental parameters. Under extreme conditions, microorganisms apply various strategies to keep their macromolecules stable, such as intracellular environmental adjustment, ionic modification, enzyme substitution, and enzyme activity alteration. With regard to thermopiles, archaea that have been isolated from deep-sea hydrothermal vents live in temperatures from 85 to 122°C, and their minimum growth temperatures are typically above 50°C. Under such hot conditions, their biological molecules and intermediates are not stable *in vitro* without biological assistance. The temperature tolerance of nucleic acid duplex structures can be enhanced by increasing salinity, the polycationic polyamines concentration, the positive-charged protein concentration, and the amount of supercoils without changing G+C content (Daniel and Cowan, 2000). The RNA stability depends on posttranscriptional modifications, although the secondary structure is also important (Daniel and Cowan, 2000). In contrast, psychrophiles (which are able to proliferate at less than 0°C but are restricted to less than 30°C and able to maintain metabolic activity in snow and ice (Siddiqui and Cavicchioli, 2006)) have other challenges, such as the reduced enzyme activity and membrane fluidity, the altered transportation efficiency of nutrients and wastes, the suppressed transcription, translation, and cell division, the cold induced protein denaturation or inappropriate folding, and the intracellular ice formation. In addition, the production of reactive oxygen species (ROS) increases significantly at low temperature, and ROS are toxic to the cells. To counteract this effect, *Colwellia psychrerythraea* and *Desulfotalea psychrophila* enhance antioxidant activity with the expression of several genes that encode catalases and superoxide dismutases at low temperature. *Pseudoalteromonas haloplanktis*, however, represses the production of ROS by eliminating the entire molybdopterin metabolism pathway at low temperatures (D'Amico, et al., 2006).

Methanogenesis is an ancient biological process (Blank, 2009). Methanogenic microorganisms are widely distributed from deep-sea hydrothermal vents, where the temperatures are above 110°C (and *Methanopyrus kandleri* resides), to Antarctic lakes, where the temperatures are below 0°C (and *Methanococcoides burtonni* resides). As mentioned above, at the cellular level, the temperature range needed for a specific type of microorganism to grow is generally less than 45°C. However, the conserved energy converting and biosynthetic pathways in methanogens are not restricted to the same temperature range as that for cell growth. In this case, it is not necessary to import or develop new metabolic pathways or cellular processes to adapt to the extreme temperatures (Cavicchioli, 2006). The values of the standard Gibbs free energy in all seven steps of methanogenesis are comparable to zero (−30 to 6 kJ/mol) (Thauer, 2011). All of these reactions are easily reversible, and the energy balance in all of the steps is easy to reach. These features ensure that this ancient microbial pathway endures a wide temperature range. Recently, using a single cell technique on an ANME-2 enrichment, we detected the expression of the *Mer* gene in ANME-2. We then demonstrated that the anaerobic oxidation of methane (AOM) mediated by ANME-2 is a reversed process of methanogenesis (Wang et al., in press). Because the AOM process can occur through a direct reversal of methanogenesis and because it was able to tolerate high temperatures in the early ocean (in a modern environment, the AOM activity has been found with temperatures ranging from −1 to 95°C) (Biddle et al., 2012; Rossel,et al., 2011), the hypothesis that the AOM process could be traced to the Archean period is supported (Battistuzzi et al., 2004).

According to the laws of thermodynamics, an equivalent amount of energy is gained from the same metabolic pathways under the same conditions, and the utilization of substrates and the energy distribution inside the cells differ among organisms. This divergence is an adaptation strategy for life that resides in diverse ecological niches. When three methanogens are compared, those that originated earlier, such as *Methanothermobater marburgensis*, *Methanobrevibacter arboriphilus*, and those that originated later, such as *Methanosarcina barkeri*, the actual energy yields per ATP are 0.3, 0.5 and 1.5 g/mol, the actual biomass yields per mole of methane are 1.3, 3 and 7.2 g/mol, and the doubling times are 7, 2 and 13 h, respectively (Thauer et al., 2008).

3 The significance of anti-oxidation in adaptation and evolution

Life originated on the Earth's surface immediately after the

ocean formed and the asteroid impacts that caused extinction stopped. Early life drew nutrients and energy from the environment and excreted waste, including methane. From surviving in a barren environment to being the most significant player influencing the global environment, life had to overcome two bottlenecks during its development: establishment of a bio-recycling mechanism and accumulation of bio-available energy. Bio-recycling refers to the waste generated by one organism that is able to feed another. Considerable energy accumulation only began when oxygenic photosynthetic bacteria appeared on Earth 2.7 billion years ago. However, oxygenic photosynthesis promoted the oxidation of the early atmosphere only 2.4 billion years ago (Bekker et al., 2004; Partin et al., 2013). This delay indicated that other factors were also involved. This oxidation process might have required a novel plate tectonic shift to change Earth's surface structure, such as the formation of a shallow sea, where the reduced organic carbon could be deposited and buried (Lenton et al., 2004). Although the oxidation process has brought an abundant energy supply to life, the organisms that originated from the anoxic environment had to overcome this oxidative stress.

Both aerobic and anaerobic organisms had to face endogenous and exogenous oxidative stress. Flavin, quinine, and metal centers have low redox potentials that neutralize O_2^- and H_2O_2, and the respiration chain is one of the few sites in the cell that is resistant to oxidative stress. For example, a mutation in *E. coli* cytoplasm catalase can induce the inactivation of a Fe-S cluster in dehydrogenase, which further inhibits the biosynthesis of amino acids and the TCA cycle. The same phenomenon is found in yeast superoxide dismutase. This mutation is of great interest because the Fe-S cluster is believed to be the oldest non-biological cofactor, and it is found in all microorganisms. The most common form of Fe-S cluster is diamond (2Fe-2S) or cubic (4Fe-4S), which carries ferric iron (Fe^{2+}) or ferrous iron (Fe^{3+}) and sulfide (S^{2-}), respectively. Because of its chemical flexibility, the Fe-S cluster participates in various metabolic pathways (over 150 in *E. coli*) as a catalyst or electron accepter to proteins, including in respiration, central metabolism, DNA repair, and gene regulation (Roche et al., 2013). O_2^- can shatter $(4Fe\text{-}4S)^{3+}$ into $(3Fe\text{-}4S)^{+}$ (Imlay, 2013). Oxidative stress can induce a series of physiological responses, such as metal transport and nutrient transport. Metal transport, and particularly iron transport, can cause the production of the highly reactive OH^- through a Fenton reaction. It is generally accepted that the mitochondrial electron transport chain is the major site for ROS generation. When the cell failed to eliminate the ROS accumulation, it has to face the damage on nucleic acids, proteins, carbohydrates, and lipids. Oxidative stress is recognized as an important player in many diseases, such as cancer, and in the aging process.

Aerobic and anaerobic organisms developed distinct strategies to address oxidative stress. Aerobes commonly use superoxide dismutase (SOD) and catalase as their primary defense system, whereas anaerobes seldom express these enzymes or their homologs (Imlay, 2013). Instead, the majority of anaerobes contain a non-heme iron-containing superoxide reductase (SOR), which performs the detoxification of superoxide without creating oxygen. And they generate peroxidases instead of catalases to scavenge H_2O_2. In *Pyrococcus furiosus*, a superoxide-reducing system based on SOR, was identified. Other proteins in *P. furiosus* have been implicated in the ROS response, including the peroxidase rubrerythrin (Rbr), electron carrier rubredoxin (Rd), and NADP rebredoxin oxidoreductase (NROR). The mononuclear iron site of SOR is reduced by Rd, and Rd is then reduced by flavoprotein NROR, which uses NAD(P)H as an electron donor. Rbr reduces H_2O_2 to H_2O at the end. The advantage to this process is that O_2, which may cause additional toxicity, is not generated. Recently, many of anaerobic organisms were found to have SOR, which reduces H_2O_2 and alkyl peroxide to H_2O and a corresponding alcohol. This process often reduces ATP production. Theoretically, 1.2 ATP out of a total 3.2 ATP that are gained from converting one glucose molecule to acetate are generated from H_2 production by MBH. The amount of ATP that is lost from decreased H_2 production can be estimated to be approximately 19% (Thorgersen et al., 2012). Recently, phosphorothioate DNA was found to act as an antioxidant in bacteria. A diverse set of bacteria contain DNA with sulfur incorporated stereo-specifically into their DNA backbones at specific sequences. *In vitro* oxidation of phosphorothioate DNA by hydrogen peroxide (H_2O_2) has been observed, and two possible outcomes were proposed: a cleavage in the DNA backbone or the removal of sulfur that results in restoration of a normal DNA backbone. Moreover, resistance to H_2O_2 was abolished when each of the three *dnd* genes, which are required for phosphorothioation, was inactivated (Xie et al., 2012). These findings indicated that phosphorothioation modifications endowed DNA with chemical-reducing properties, which could protect the host bacteria against peroxide. The evolutionary origin and significance of phosphorothioate are still being explored.

Other environmental factors can cause oxidative stress through an electron transport imbalance. A study on an Antarctic obligate anaerobic isolate, *Methanococcoides burtonii*, has demonstrated that ROS are generated under low temperature stress (−2°C). This study also found that multiple copies of oxidative stress proteins that specifically target ROS are expressed at low temperatures, including catalase SOR, iron sulfur flavoprotein, Rbr and related proteins (Cavicchioli, 2006). In piezophilic bacteria, such as *Shewanella benthica* and *Shewanella violacea*, the composition of the respiration chain is shift to respond to oxidative stress under high pressure. Under 60 MPa of pressure, a novel membrane bound ccb- type quinol oxidase displaces the bc1 complex and cytochrome C oxidase. They also give up cytochrome C-552 but keep cytochrome C-551 (Kato and Qureshi, 1999).

By reviewing the natural history of Earth, we have a better systemic understanding on the adaptation strategies used by microorganisms in response to modern extreme environments (Figure 2). Genes originated approximately 3 billion years ago. After that event, life started to develop under extreme conditions, such as temperature, pressure, pH, oxidative stress, radiation, drought, heavy metal, and poison (David and Alm, 2011). All of these environmental factors influenced the development of metabolic pathways that are still present in the modern microorganisms. Temperature is the core environmental factor that microorganisms must address through the adaptation process. The microbial membrane plays an essential role during adaptation because of its (im) permeability and transmembrane transportation capacity. In extreme environments, the ROS generated from an imbalance in oxidation and reduction can cause damage. By changing other environmental parameters, we can enhance the capacity of the cell to handle other types of stress. For example, increasing pressure has a similar effect as decreasing temperature. A decreasing pH may decrease the membrane permeability and shift temperature, pressure, and salt tolerance. This hypothesis has been supported by experimental data. It is difficult to define the specific limits of polyextremophiles under multiple forms of stress because the highest, lowest, and optimal growth temperatures, pressures, and salinities all correlate with each other and are strongly dependent on the growth medium.

4 Understanding the extremophiles and predicting the limit of life

4.1 Living fossils of early life processes

Microorganisms can record a wealth of information about the environmental changes they have experienced (Boussau and Daubin, 2010). However, we have hardly discovered this information in studies of early geological history. Fossil records and biomarkers from before the Precambrian period have rarely been found. The limited information carried by prehistoric biomarkers cannot be compared to the information carried by modern microbial genomes. If we can find "living fossils" that represent early life, then we can better understand some basic processes of evolution and trace the external physicochemical conditions necessary for the formation of the internal structures of the genome. This information would allow for the construction of kinetic models of metabolic networks in combination with environmental simulations (Ao et al., 2008). By comparing the physiological and biochemical characteristics of "living fossil" cells with geological records, we could improve our knowledge on the environment of early Earth and its changing processes. We could also look for new biomarkers by learning from the metabolism of "living fossil" cells. The development of modern microbial metagenomics and synthetic biology provides new opportunities for studying the origin of life and the evolution of early Earth.

The hypothesis that life comes from an underwater hydrothermal environment has been supported by considerable evidence since it was proposed in the late 1980s (Stetter, 2006a). Hyperthermophiles have a slow rate of evolution, and they branch off at the root of the phylogenetic tree. Studies have confirmed that the abiotic formation of short chain fatty acids, such as formic and acetic acids, does exist in hydrothermal systems (Lang et al., 2010). Furthermore, co-precipitated (Ni, Fe)S can act as a catalyst and reduce CO_2 and CO to amino acids (Huber and Wächtershäuser, 1998), which can then be converted into peptides (Huber et al., 2003). The discovery of the reversed tricarboxylic acid cycle, which is induced by FeS without enzymes, also provides us with a model of chemoautotrophy (Huber and Wächtershäuser, 2006). Underwater hydrothermal systems are distributed in mid-ocean ridges worldwide. They contain a variety of reducing gases, maintain high chemical and temperature gradients, and are similar to the original envi-

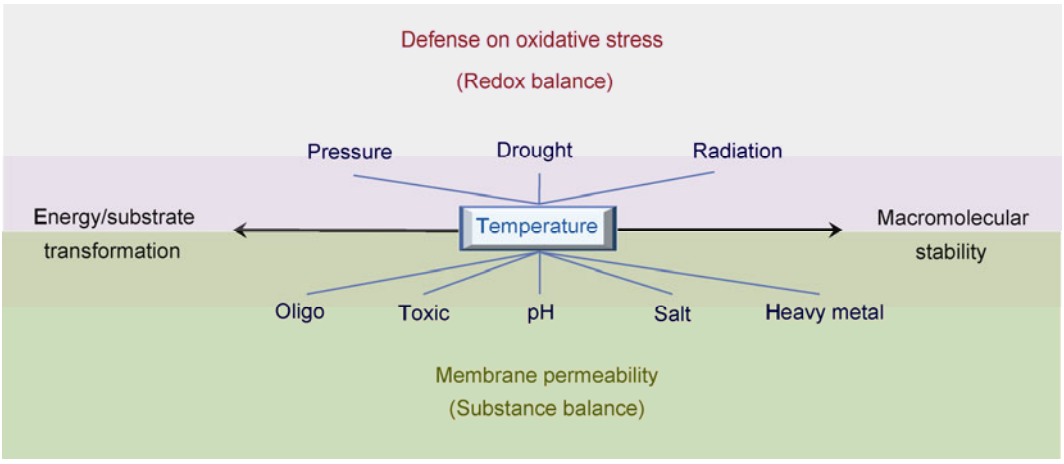

Figure 2 A model to illustrate microbiological adaptation to the extremes.

ronment of early Earth (Martin et al., 2008). The "window" of deep biosphere, the hydrothermal system, did not experience major geological events, such as "snowball", or a significant gradual cooling process. The lack of those experiences prevented the early housekeeping genes (genome) from being eliminated or mutating. Therefore, the microorganisms in this habitat may have a similar genome structure to that found in early life, and they could be "living fossils". Nevertheless, identifying the metabolic systems that are similar to those in early life remains challenging due to the complexity of modern genomic information.

Many researchers have been using existing extremophiles as model organisms to study the early life in the past decades. However, the evolutionary analysis that was based on a molecular calculation was not well incorporated with the study on microbial metabolism (David and Alm, 2011; Gribaldo and Brochier-Armanet, 2006; Stetter, 2006b). We believe that we can use hyperthermophilic archaea (autotrophic methanogenic archaea or heterotrophic thermococcus) in the typical deep-sea hydrothermal area as model organisms. We combine bioinformatics and genetic manipulations, starting from the core metabolic pathways, to elucidate the boundaries for life and to illustrate the environment of early Earth. As shown in Figure 3, we consider the early Earth environment and life processes as objects of research and attempt to analyze ancient DNA. However, a reliable time scale to analyze fossil DNA is limited to 10000 years by the present technology, so we must investigate ancient DNA in modern microbial genomes and then track back the evolutional history in billions of years. The modern microorganisms hosting ancient DNA only exist in the modern environments that are similar to early Earth, such as in an underwater hydrothermal environment. For example, Thermococcales has only been discovered in hydrothermal areas and in deep anaerobic environments. Therefore, it is a good candidate to be a "living fossil" (Auguet et al., 2012). The discovery of the most simple energy converting pathway (formic disproportionation reaction) and autotrophic CO fixation pathway in Thermococcales (Bae et al., 2012; Kim et al., 2010) confirmed our above logic.

4.2 Low-energy life processes and the limit of life

Earth is currently the only planet known to support life that uses carbon as a skeleton, water as a solvent, and solar radiation as primary energy source. However, our understanding of the habitable boundaries for life forms is far from being completely explored. The total cell count of the deep biosphere, where the bioavailable oxidant is insufficient, is approximately 2.9×10^{29} (Kallmeyer, et al., 2012), and organic carbon deposit only accounts for 1% of the carbon fixation by photosynthesis. Microorganisms from the deep biosphere are not phylogenetically close to the cultivatable microorganisms, and most of their biochemical and physiological features are unknown. These deep biosphere microorganisms are characterized by a long doubling time (or so called turnover time, with an intracellular carbon turnover rate of 1000 years (Hoehler and Jorgensen, 2013)), a low cell density (approximately 10^2–10^8 cell/cm^3 at 1000 mbsf (Kallmeyer et al., 2012)), and a low respiration rate (the respiration rate of microorganisms at 20 mbsf in North Pacific Gyre remains is at 10^{-3} fmol cell^{-1} day^{-1}, which is 10^{-3} lower than that of the laboratory-cultivated heterotrophic anaerobes (Roy et al., 2012)). A crucial question to understand the ecosystems in such environments with extremely low energy is how ecosystems remain stable. Specifically, how do cells retain their genotypic characteristics? Are all of the cells at a maintenance status, or are most of the cells at a survival status while very few of them are active? Another crucial question is that whether low-energy-adapted microorganisms indeed exist. If they do, how do they reproduce and influence the cycling of biogeochemical elements? According to Canfield, studies on anaerobic microbial metabolism will provide a new angle for understanding the carbon isotope compositions of different geologic periods (Canfield and Kump, 2013). In addition, sulfur isotope fractionation can be considered a "reliable" measurement for determining the atmospheric oxidation state in Earth's

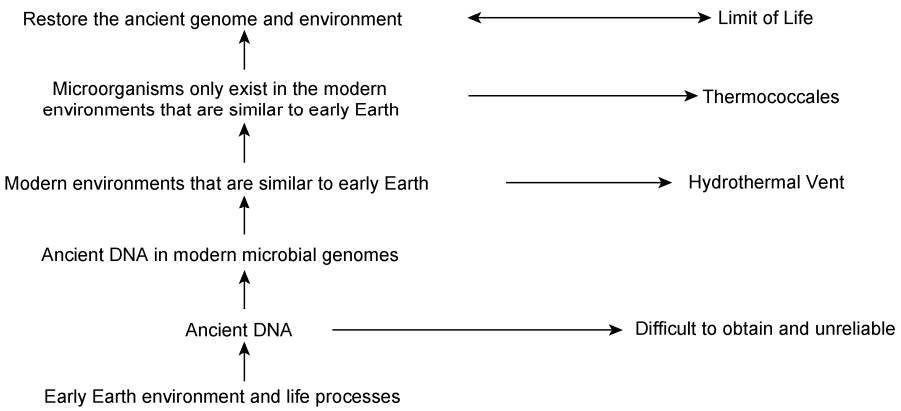

Figure 3 The use of Thermococcales as a "living fossil" to study early Earth's environment and life processes.

early history (Farquhar et al., 2000). Therefore, isotope fractionation analysis on microorganisms with typical low energy metabolism, together with studies in paleobiogeography and ecology, will deepen our understanding on the biological effects, responses, and evolution during major geological events.

The physiochemical environmental parameters for effective and sufficient energy supplies play a major role in defining the limit of life. These parameters can be quantified and calculated, which makes it feasible to construct mathematical models of biological evolution based on energy metabolism. Environmental changes can be quantified through substrate concentration gradients and redox potentials, as described by the MEQ. Meanwhile, biological macromolecules, such as the components in electron transport chains in anaerobic respiration, also transfer and utilize energy in certain energy units. The quantitative feature present in MEQ is the basis for constructing evolutionary models by using mathematical approaches. The purpose of quantification is to create an aggregate function, and the returned values could be used to describe the nonlinear processes of biological evolution. Mathematical modeling can quantitatively describe the process of energy metabolism. A complicated process of life-environment coevolution can be converted into a mathematical problem through modeling. A model describing early life processes and the boundaries of life can be developed after deductive reasoning solving and computing the constructed mathematical model. By integrating computational predictions and experimental verifications, e.g., using Thermococcales as model organisms, we will understand that a balance between efficient energy utilization and the coordination of metabolic pathways must be reached to maximize the stability of macromolecules and to minimize energy requirements. This approach can be applied to determine the habitable zone of early life.

5 Summary

(1) By reviewing microbiological adaptation mechanisms to extreme environments, we can conclude that the inevitable cooling and gradual oxidation processes on Earth are the most significant geological events during the evolution of life.

(2) Adaptation and adjustment to the redox potential of the environmental surroundings by organisms is a crucial step during life-environment co-evolution.

(3) Energy metabolism determines the path of evolution, the structure of an ecosystem, and the physiology of a cell.

(4) Microbial genomes in underwater hydrothermal vents record information on the life and environment of early Earth. A wealth of information is stored in these "living fossils"; this information must be discovered and verified with molecular clock and conventional microfossils or biomarkers.

(5) A "follow energy" strategy may assist us in the construction of a mathematical model that illustrates the nonlinear co-evolutionary processes between life and the environment.

We acknowledge the fruitful discussion with colleagues Wang Fengping, Xu Jun, He Ying while writing. Peng Mengjun assisted on the proofreading. This work was supported by National Natural Science Foundation of China (Grant No. 31290232), National High-Tech Program (Grant No. 2012AA092103-2), and National Basic Research Program of China (Grant No. 2011CB808800).

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