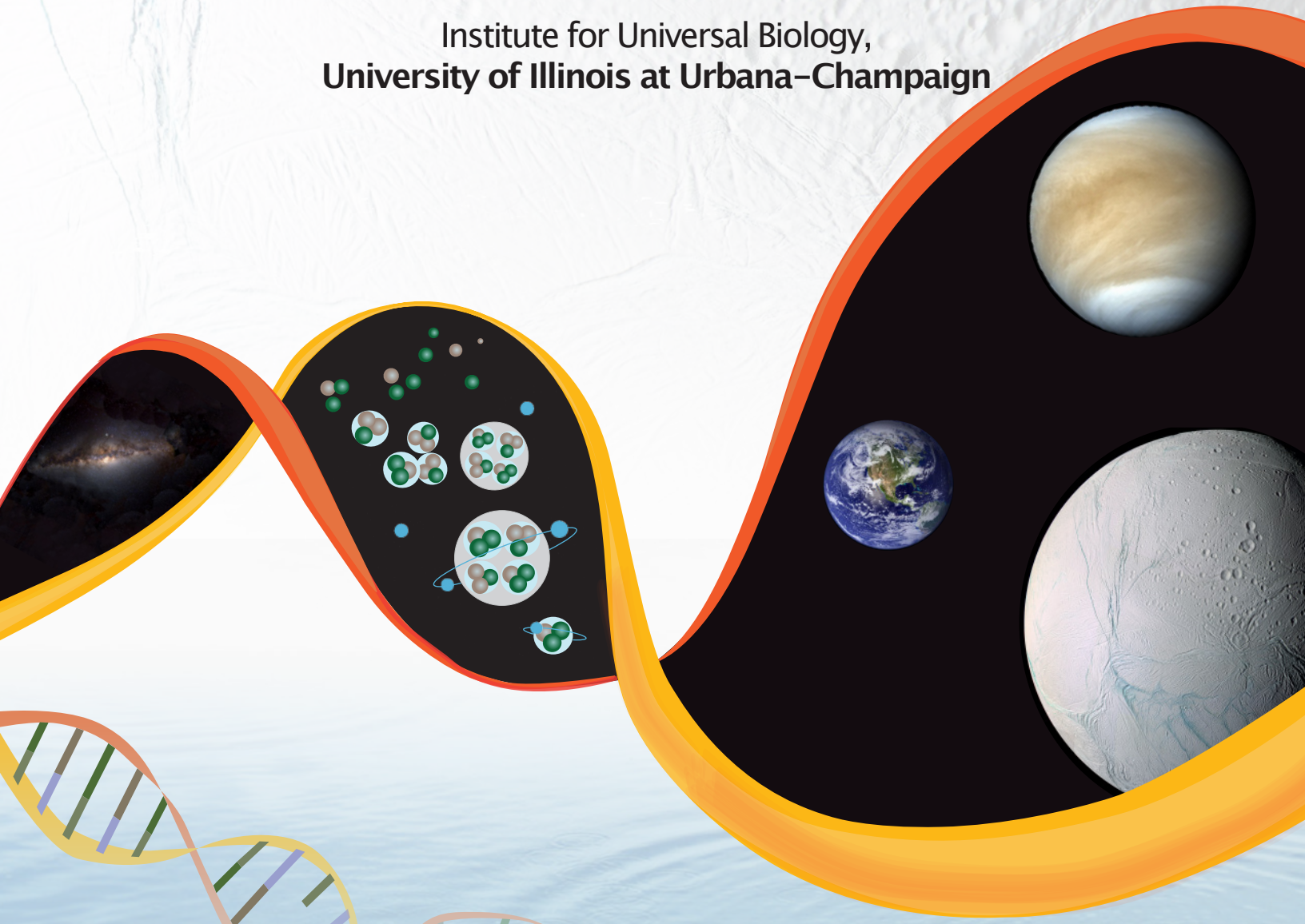
National Aeronautics and Space Administration

NASA Astrobiology Institute 2016 Annual Science Report

Team Report:

Institute for Universal Biology,
University of Illinois at Urbana-Champaign



INSTITUTE FOR UNIVERSAL BIOLOGY

NASA ASTROBIOLOGY INSTITUTE

Lead Institution:
University of Illinois at Urbana-Champaign

Principal Investigator:
Nigel Goldenfeld

Team Overview

"How does life begin and evolve?" This simple question, a fundamental topic within astrobiology, still today remains an enigma. The Towards Universal Biology Team is exploring how molecules come to life by investigating fundamental principles of biology from a multidisciplinary perspective – encompassing microbiology, geobiology, computational chemistry, genomics and even physics. A series of four innovative and cross-disciplinary research themes have been established by this Team in order to define their research program:

- Theoretical understanding of the universal features governing living systems, their operation, evolution and origin;
- Constraints on the nature of life before the Last Universal Common Ancestor (LUCA), in particular to identify new signatures of the collective state of life ("progenote") which enabled the evolution of the cell to occur so rapidly. In this early phase of life, believed to exist before about 3.8 billion years ago, there was neither a "family tree" of life nor species in the modern sense of the word.
- To explore the breakdown of the progenote and the transition to the current epoch of "vertical" evolution;
- Explore the interplay between biological and environmental determinants of the rate of evolution. How do environmental fluctuations influence the nature and speed of evolution?

Team Website: http://astrobiology.illinois.edu

2016 Executive Summary

There are two universal aspects of all life on Earth. One is the genetic code, shared by all known organisms. The other is biological homochirality: chiral amino acids are all left-handed, whilst the sugars are right-handed. The universality of these characteristics in life on Earth could be a special feature of terrestrial life, or it could be a general outcome of the evolution of life, something that would occur if life evolved elsewhere in the universe. Our work strongly suggests the latter possibility. Our explanation of the statistical properties of the genetic code from dynamical systems theory sets the stage for the emergence of complex life, and predicts a network phase of life preceding the "Darwinian Transition" to the current era of vertical dominated evolution.

We have explained theoretically how this transition occurred, accounting for the way in which horizontal gene transfer between primitive life inexorably leads to the network phase of life and its demise. Additionally, we have used an ingenious experiment to transfer replication proteins between the three Domains of Life, finding that the Darwinian Transition has occurred in core replication machinery also.

We have made dramatic progress in understanding the origin of biological homochirality. Our work showed how the property of life being far from equilibrium and the steady progression of the efficiency of autocatalysis inexorably leads to homochirality. The implications of this research are relevant for future missions, because there is significant interest in using homochirality as an *in situ* biosignature, for example by examining the chirality of molecules obtained in powder samples from the surface of Mars. In summary, our work shows that homochirality at the 100% level is a biosignature which can be used to detect life, without false positives, thus providing a theoretical foundation for pursuing homochirality as a biosignature.

Field photo showing the primary flow path of water from a carbonate hot spring in Yellowstone National Park. Source: UIUC

The precise manner in which life self-organizes into this large-scale structure and the details of its evolutionary trajectory are contentious. A resolution would shed light on the question of what sorts of life might exist elsewhere in the universe: for example, would we expect multicellular advanced life to be ubiquitous? Our studies of essential genes for life, based upon Archaea extracted from geothermal hot springs, supports the three Domain picture. Computational studies of methanogens shows that it is possible to build predictive metabolic models of life, potentially allowing future studies to predict the types of life to be expected in missions to extra-terrestrial environments. We have described in detail how the environment of alkaline hydrothermal vents could have been the crucible for early life, by providing the necessary thermodynamic gradients to drive early metabolism and the emergence of cellular membranes.

One of the biggest puzzles in astrobiology is the rapidity by which life emerged on Earth. What factors control the rate of evolution and how can they be accurately measured? We have developed a range of technologies for exploring this question with high precision, using microfluidics and synthetic microhabitats to quantify the emergence of mutants that can survive toxic environments and even succeeding to visualize in real time the activity of jumping genes in living cells. We have used mutagenic break repair assays to measure both point mutation and chromosomal rearrangements, establishing that unrepaired DNA base damage is required for stress-induced mutation, elaborating the role of error-prone polymerase. Overall, we are moving closer to understanding the molecular mechanisms by which point mutation and chromosomal rearrangement occurs so rapidly in response to environmental stress.

Field photo of Angel Terrace at Mammoth Hot Springs in Yellowstone National Park. Source: UIUC

Project Reports

The Evolution of Homochirality as a Universal Biosignature

There are two universal aspects of all life on Earth. One is the genetic code, shared by all known organisms. Its ubiquity is what enables us to do genetic engineering. The other is biological homochirality: chiral amino acids are all left-handed, whilst the sugars are right-handed. The universality of these characteristics in life on Earth could be a special feature of terrestrial life, or it could be a general outcome of the evolution of life, something that would occur if life evolved elsewhere in the universe. Our work strongly suggests the latter possibility. Our explanation of the statistical properties of the genetic code from dynamical systems theory sets the stage for the emergence of complex life, and predicts a network phase of life preceding the "Darwinian Transition" to the current era of vertical dominated evolution.

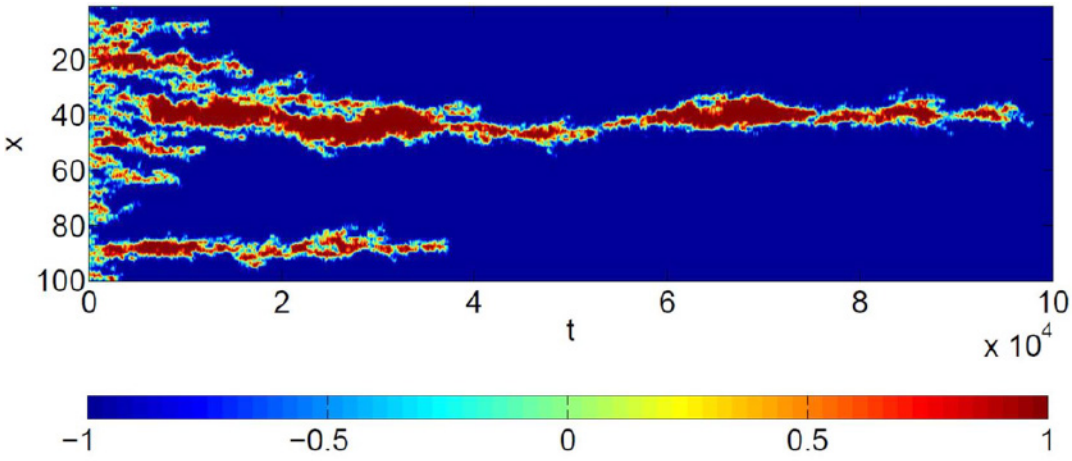


Fig. 1. Evolution of homochirality in a one-dimensional model. Red stands for left-handed and blue for right-handed. The horizontal axis is time and the vertical axis is space. The figure shows how homochirality emerges eventually in this one-dimensional ecosystem.

We have made dramatic progress in understanding the origin of biological homochirality. The only class of theories that had previously been proposed to explain it, in effect put in the answer by the assumptions they make. Our work, published last year in *Physical Review Letters* (widely regarded as the premier broad impact journal in physics), showed how the property of life being far from equilibrium and the steady progression of the efficiency of autocatalysis inexorably leads to homochirality. The mechanism we discovered is intrinsically stochastic, and very different from earlier ideas.

This research motivates the use of homochirality as a biosignature. It can be used in remote imaging of exoplanets, or *in situ*, by examining chirality of molecules in powder samples from the surface of Mars, when heated in an oven. In summary, our work shows that homochirality at the 100% level is a biosignature which can be used to detect life, without false positives, and provides a theoretical foundation for pursuing homochirality as a biosignature.

Evolutionary History of Early Life on Earth

One way to probe the earliest life on Earth is to identify what are the genes essential for life. Previously we reported the creation and sequencing of a transposon library to investigate gene essentiality in the crenarchaea *Sulfolobus islandicus*. Over the past year we have completed the statistical analysis and genetic knockouts to confirm the essentiality of 458 genes in the *Sulfolobus genome*. Phylogenetic reconstruction of essential genes in these organisms compared to essential gene outcomes in other organisms support the 16S ribosomal RNA tree i.e. the monophyletic nature of the Archaea and their common ancestry with the eukaryotes, despite challenges to this viewpoint that have arisen in the last year.

We have developed computational models for the metabolism of early life, likely to be methanogens. The genomes of these archaeal organisms contain many of the most ancient and highly conserved enzymes. Methanogens inhabit many extreme environments—both hot and cold—and can subsist on simple compounds such as CO_2, formate, acetate, methanol, methylamines, etc. that could be found on primordial planets. Thus, these archaeal organisms are likely to be similar to any microorganisms that could inhabit planetary bodies in their early stages of developing life. In order to predict the metabolic behavior of simple organisms that inhabit other planets, and to identify the potential for biosignatures, we have developed accurate metabolic models of methanogens and other related Archaea. Our metabolic construction work began with studying *Methanosarcina acetivorans*, a model methanogen widely used in experimental labs. We have significantly improved a genome-scale metabolic model by updating it with the latest molecular biological characterizations and biomass composition data. The improved model accounts for 807 metabolic genes and 759 reactions and performs better than previous models, nearly completely consistent with experimental growth data. These techniques will be used for less metabolically diverse organisms, such as *Methanobrevibacter smithii*, which would be closer to putative life on Mars, where CO_2 is the single carbon source.

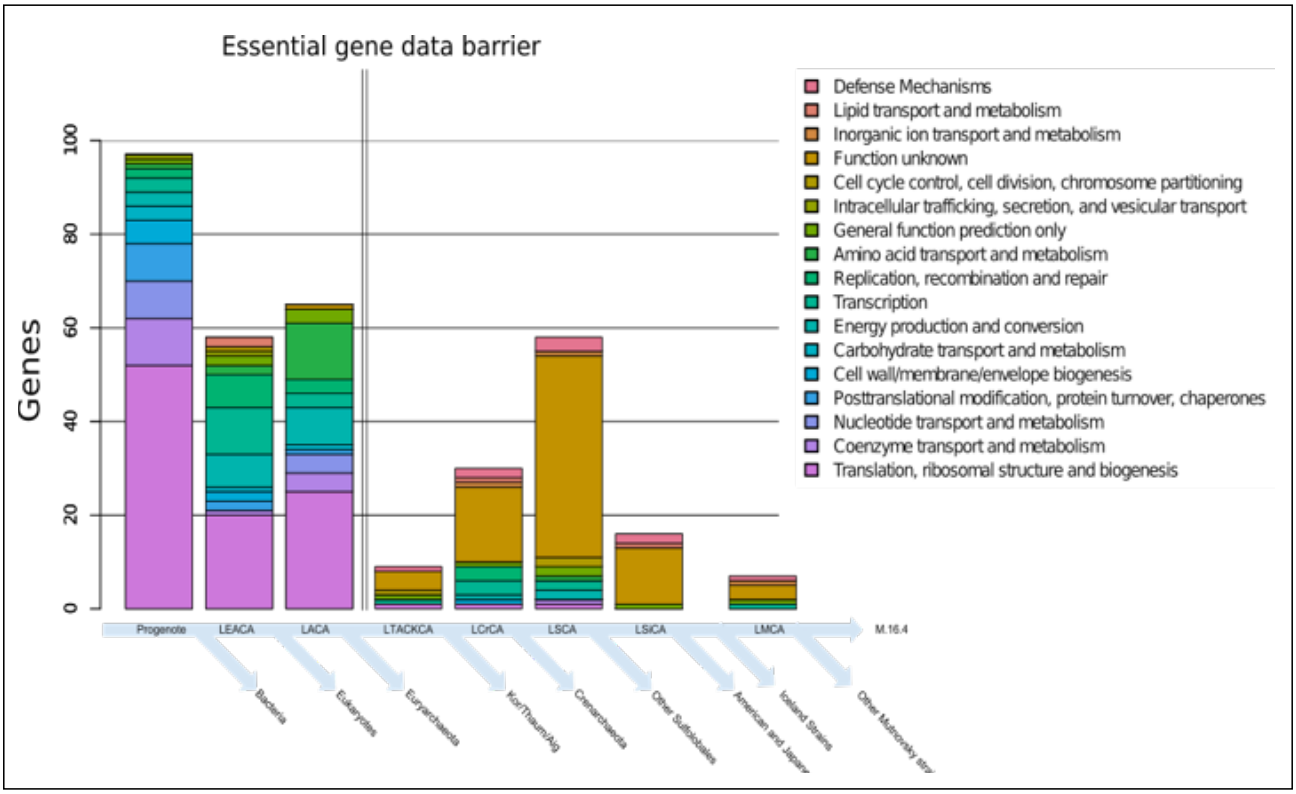


Fig. 2. Inferred timeline of development of essential genes based on phyletic distribution. Number of genes in each arCOG functional category are shown above the hypothesized common ancestor in which they could have served an essential function similar to that observed in modern descendants. Bars for "Progenote, LEACA, and LACA" (last universal common ancestor, last eukaryotic and archaeal common ancestor, and last archaeal common ancestor) contain only genes that have been shown to be essential in their constituent groups. Bars for the last TACK superphylum, crenarchaeal, Sulfolobales, Sulfolobus islandicus, and Mutnovsky common ancestor (LTACKCA, LCrCa, LSCA, LSiCA, and LMCA, respectively) are based only on presence/absence via bidirectional best blast matches.

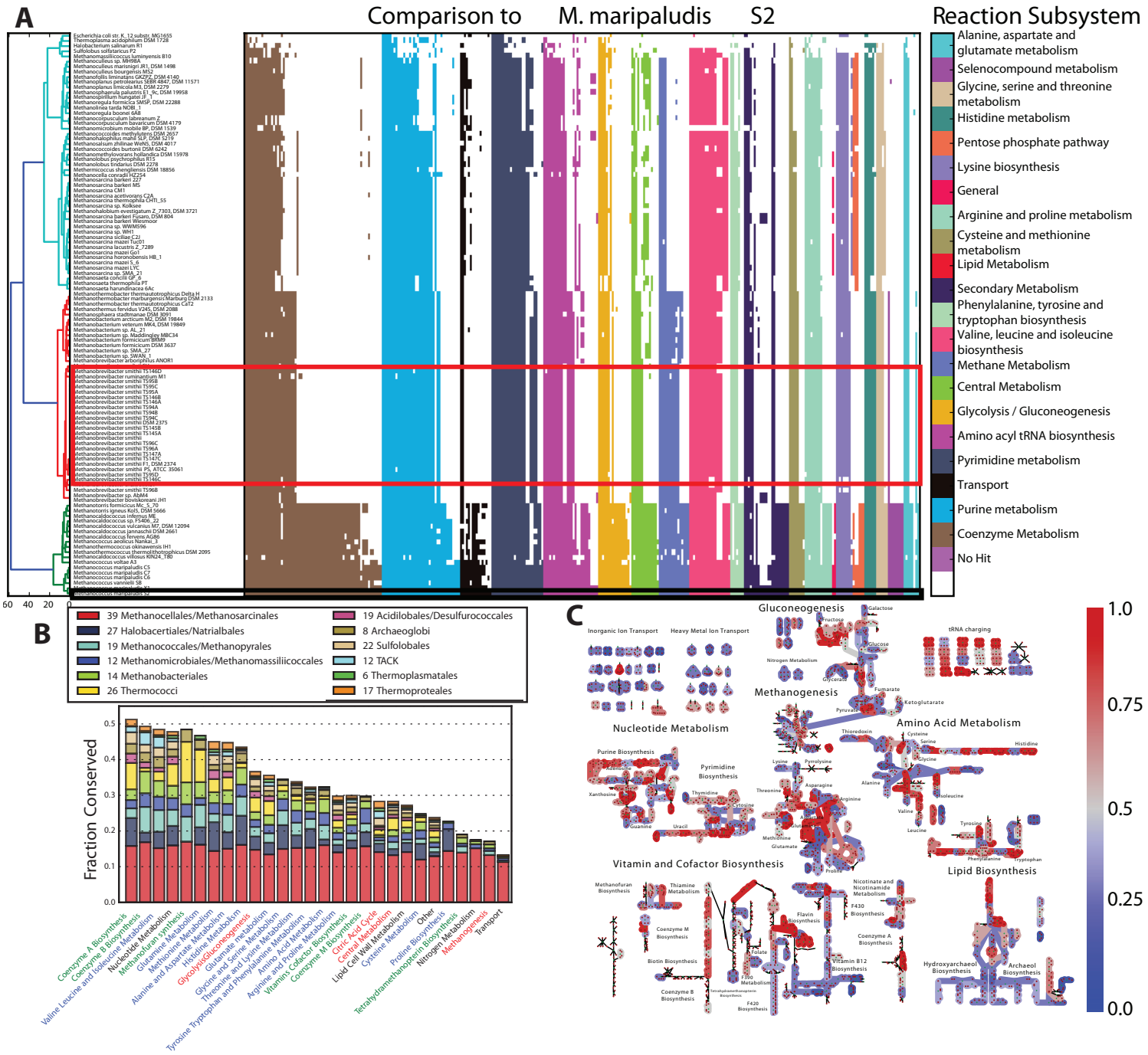


Fig. 3. Conservation of reactions in the M. maripaludis model among other methanogens organized by reaction subsystem (categorized by colors). The red box identifies M. smithii strains while the black box on the bottom indicates the reference M. maripaludis model. B) Fraction of conserved reactions grouped by metabolic subsystems. The overall height of each bar indicates the total fraction of reactions in the metabolic subsystem that are conserved, while the height of individual portions of each bar indicate the relative conservation of reaction in the subsystem from that taxonomic group. The 221 archaea were grouped by taxonomic order, yielding 12 distinct groups as seen in the legend. Metabolic subsystems labels are color coded: amino acid metabolism subsystems in blue, vitamin and cofactor metabolism subsystems in green, central metabolism subsystems in red and other categories in black. Relative conservation of metabolic genes across 221 archaea. C) Conservation of metabolic reactions annotated in the M. acetivorans model among archaea (red – higher conservation, blue – lower conservation).

The Darwinian Transition: From Networks to the Tree of Life

All life on Earth can be associated with one of three Domains, which emerged sometime after the time of the Last Universal Common Ancestor, around 3.8 billion years ago. This Tree of Life was preceded by an earlier phase, deduced to exist from the statistical properties of the genetic code, which was collective and structured like a network rather than a tree. The multiple connectivity of the network accelerates the evolution and allows rapid convergence to a unique, near-optimal genetic code.

With all these advantages of HGT, why would it ever stop?

To address this question, we built a quasi-species model of interacting organisms that includes HGT. We ran the simulation in an environment which exhibited a "Mount Fuji" fitness landscape. We discovered that for early times, the system exhibited a networked phase, with rampant HGT and no unique species. After some time, a transition occurred and HGT switched off, leading to tree-like vertical evolution. HGT is still operative, but the actual effect of it becomes minimal because the population as a whole is now near the fitness peak and the likelihood of an improved gene being transferred becomes correspondingly smaller. The Darwinian Transition occurs without fine tuning or external factors being adjusted, and should be universal and independent of precise chemistry or genes.

We have attempted to understand if the DNA replication machinery in modern organisms has passed through the Darwinian Transition. In vivo experiments used the substitution of the genes for an important protein complex called the sliding clamp across the three Domains of life. The archaeal proteins (sliding clamps) are homotrimers, as also observed in eukaryotic cells. In contrast, the bacterial sliding clamps are homodimers, and thus are different in subunit organization from the archaeal and eukaryotic proteins. We first substituted the yeast sliding clamp with that of the human and this gene clearly could substitute for the yeast gene, and thus demonstrating that the yeast replication machinery will accept this central gene from another eukaryote. Next, we substituted the yeast sliding clamp with an archaeal sliding clamp (Methanosarcina acetivorans) and to our surprise, although the structures and homologies are very high, the archaeal gene failed to complement the yeast gene. The sliding clamp in the yeast cell was also replaced with the homologous gene from a bacterium, i.e., Escherichia coli, and the bacterial gene failed to complement the yeast gene. In conclusion, while the eukaryotic gene (human sliding clamp) could be incorporated in the eukaryotic replication machinery, both the gene products of the archaeal and bacterial genes failed to be functionally incorporated into the eukaryotic replication machinery, and thus clearly demonstrating that eukaryotic replication machinery has crossed the Darwinian Threshold.

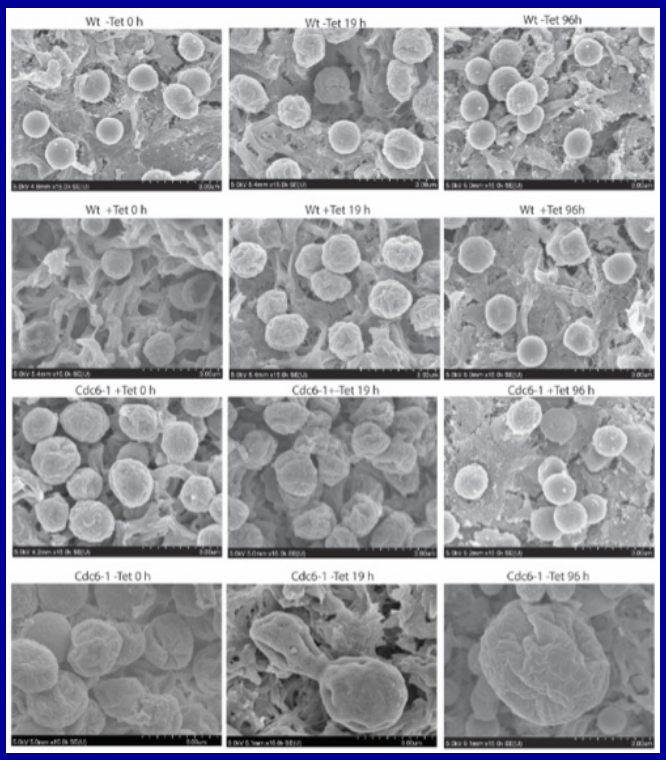


Fig. 4. The ancestors of the methanogenic archaeon Methanosarcina acetivorans acquired a eukaryotic-like DNA replication machinery; however, they couple it with a bacterial type cell division. Normal Methanosarcina cells are usually about 2.5 um in size. However, when their eukaryotic-like DNA replication machinery is blocked, the cells expand close to 5 times the normal size. Our interpretation of the results here is that cell expansion was initiated in this archaeon such that when the genome is duplicated, a copy each can be appropriated to each daughter cell upon cell division. Since DNA replication, the process that makes a copy of the genome is blocked, the cell continued to expand without dividing. The large cells continue to expand until they finally lyse (die). The results also demonstrate that DNA replication in this archaeon is tightly linked to cell division.

Laboratory Evolution of Living Cells in Real Time

A major problem in astrobiology is to understand the rate of evolution, and its dependence on environmental variability. If life emerges rapidly in an environment and colonizes it effectively, efforts to identify biosignatures on nearby planets are all the more likely to be successful. Conversely, if evolutionary rates are sufficiently slow, then the likelihood becomes smaller of life having made enough evolutionary transitions to be prevalent enough to impact the environment and leave observable biosignatures. We have developed three platforms to measure and control evolution at the molecular level in bacteria residing in artificial microhabitats, using tools from the physical and engineering sciences to quantify the evolution of populations.

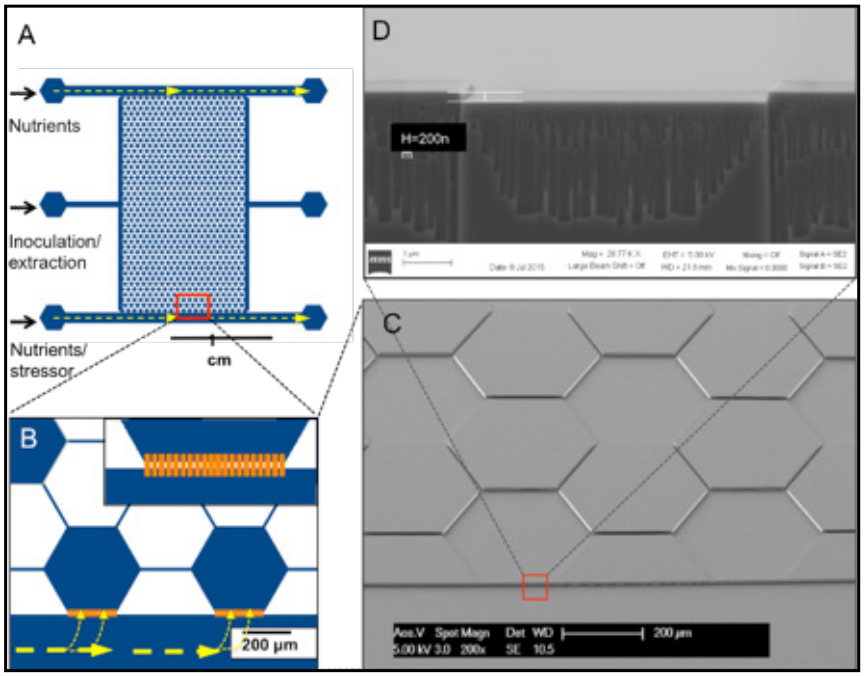
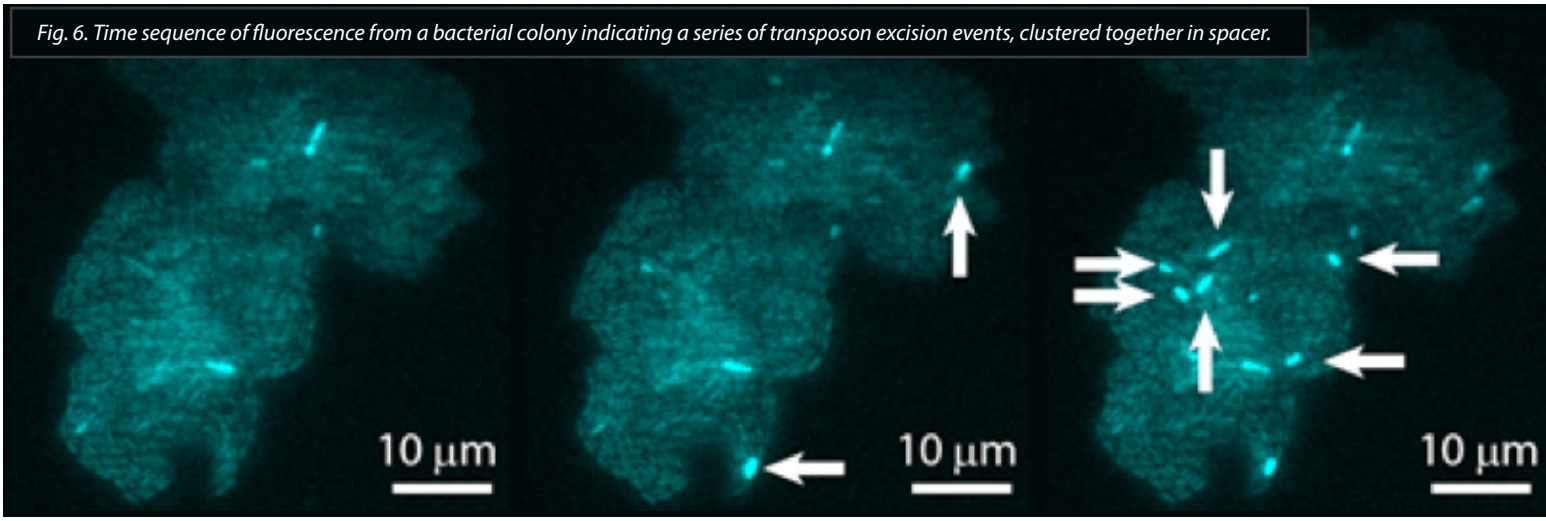


Fig. 5. (A) Top view of microfluidic gradient cell, depicts boundary flow channels (media/ media+stressor) and inoculation/extraction ports. (B) View of hexagonal well system with interconnecting channels and nanoporous barrier separating boundary channel from wells. Wells: wall length 200 µm, depth 10 µm. Channels: length 200 µm, width 10 µm, depth10 µm. Nanoporous barrier: length 10 µm, width 7 µm, depth 200 nm. (C) SEM image of wells, and channels, and nanoporous barrier at 200x magnification. (D) SEM image of nanoporous barrier at 28.77 KX magnification.

We used mutagenic break repair assays to establish that unrepaired DNA base damage is required for stress-induced mutation, and we have identified the way in which error-prone polymerases are regulated to induce genome instabilities in response to stress. Surprisingly, over-expression of error-prone polymerase is not the only mechanism: the regular polymerase must be stalled to allow the error-prone one to take over. Our work also shows how genomes commit to a point mutation pathway or a more global chromosome rearrangement. Overall these results paint a detailed narrative of the sophisticated sensing and response mechanisms that cells have to handle stress.

We have followed in real time how transposons, or "jumping genes", move around in the genome of a living organism through molecular "cut-and-paste" mechanisms. Through quantitative measurement using novel fluorescent reporters, we show that transposable element activity occurs in spatio-temporal bursts, sensitive to the cellular growth state and local environment. This activity is the first definitive example of environmental influence on the rate of mutation and evolution at the molecular level. In a related, theoretical project, we showed how

Fig. 6. Time sequence of fluorescence from a bacterial colony indicating a series of transposon excision events, clustered together in spacer.



certain classes of retrotransposon, jumping genes that use "copy-and-paste" mechanisms, will behave like competing predators and prey in an ecosystem.

We used a microfluidic gradient cell device, known as the "GeoBioCell", to measure the rate of evolution of antibiotic resistant bacteria through controlled stress micro-environments, as well as through temporal gradients of stress. High stress gradients simulate the effects of a highly heterogeneous microenvironment. Increased tolerance to antibiotics is observed within five days, associated with genome plasticity including the disruption of membrane transporter genes by transposons. Apparently the stress causes increased transposon activity, which disrupts the expression of proteins involved in carrying toxins across the cell membrane.

These results indicate the close interplay between environmental stress, spatial and temporal fluctuations, and genome instabilities in the evolutionary dynamics of microbial communities.

Serpentinization and the Emergence of the Universal Engines of Terrestrial Life

This project is concerned with the far-from-equilibrium thermodynamics of cells, and in particular the enabling mechanisms of bioenergetics. Our research is designed to address the question of how free energy disequilibria in planetary environments are exploited by life. The motivating focus is primarily to understand the particular abiotic disequilibria conversion processes that form a causally essential element of the alkaline hydrothermal vent (AHV) model of the emergence of life propounded by collaborator Michael Russell at JPL. This environment is widely considered to be the leading candidate for the origin and emergence of early life, partly because it naturally accounts for the surprising direction of proton gradients in all living cells.

Our recent work has been motivated by the fact that the accepted understanding of free energy conversion does not fully take into account the thermodynamics and sequence of cellular operations that enable energy utilization by the cell. In particular, we have shown how to clarify the essential role of thermodynamic disequilibria, Brownian statistical fluctuations, and the inter-process control of such fluctuations by stochastic "escapement" mechanisms in disequilibria conversions, for example those in which ATP hydrolysis drives an endergonic reaction. A key implication and prediction of the corrected understanding of such processes is that in them, the driving reaction, e.g., ATP hydrolysis, is only allowed to take place after the completion of an instance of the 'up-hill' (endergonic) reaction that it is driving. These events are purely the result of a Brownian fluctuation driven ratchet mechanism. Thus the conversion does not work by the transfer of energy from the driving to the driven reaction, as is usually assumed. In order to establish this mechanism, we have constructed a concrete theoretical mechanism for the way in which a cell functions thermodynamically.

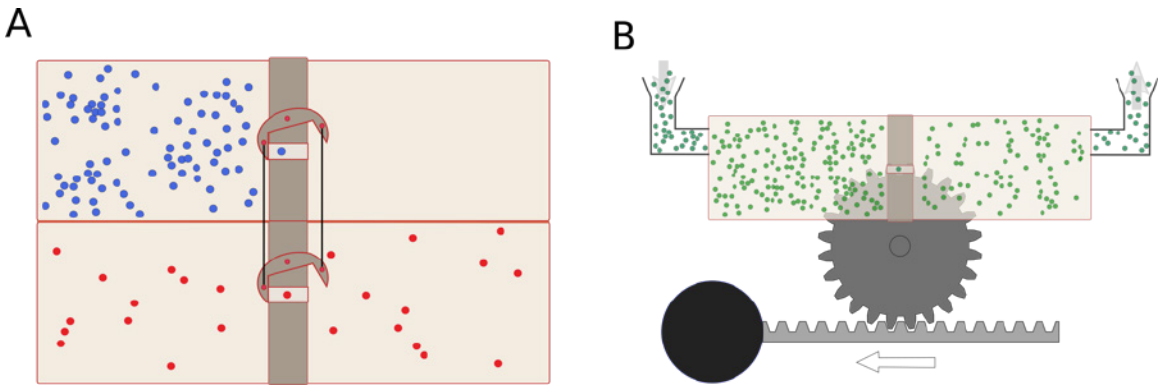


Fig. 7. Disequilibria conversion between two diffusion chambers. The chamber's portals are gated by linked 'Brownian' valves which can flip from being both open-left or open-right but are prevented from DOIng so if only one of the portals is loaded with a particle. This simple rule insures that the blue disequilibrium will create, as it dissipates, and purely as a statistical consequence, a concentration disequilibrium in the red chamber (though in steady state neither chamber is separately in equilibrium). The engine is unbiased as to direction. B: A concentration disequilibrium coupled to mechanical work. Here the rule is that the gear can rotate one 'tooth' (in either direction) only if in so DOIng it conveys a particle from the space between teeth adjacent to the portal, on either side, into the portal. The motion of the load is driven by Brownian impacts, which would, in the absence of the conversion device, trace a random path in space; the statistical disequilibrium of a determined versus a random path is the thermodynamic work produced by the motor. The device is inherently reversible both as to direction of motion and as to which disequilibrium is driving the other.

Field Work

How Cells Evolve in Response to Environmental Gradients

Mammoth Hot Springs, Yellowstone National Park

The natural extension of understanding how cells structure their genomes in response to environmental stress is to ask whether or not such forcing mechanisms have been a major factor in the evolution of cells in natural environments over geological time. An important expression of this type of genome remodeling is the ability of a microorganism to utilize multiple cellular shapes and metabolic pathways (*plasticity*) in response to changing physical and chemical environmental conditions. A striking example of the effectiveness of this plasticity is the broad environmental tolerance of the evolutionarily deeply rooted Aquificales Group Bacteria, which are common as filamentous microbial mats at Mammoth Hot Springs in Yellowstone National Park (Figs. 8 and 9). Samples collected across steep geochemical gradients have been analyzed with next-generation *Omics* molecular analyses (i.e., metagenomics, metatranscriptomics and metaproteomics) to track *in situ* ecophysiology. The reconstructed pan-genome for the 98% dominant *Sulfurihydrogenibium* bacteria, in combination with their over-represented transcripts and expressed cognate proteins, reveals a suite of metabolic strategies (i.e. chaperones, synthesis of more saturated membrane lipids, EPS, Type IV pili, rTCA cycle and oxidation of reduced sulfur species using microbially-concentrated O_2) in turbulent, dysoxic regimes with low levels of organic substrates (Fig. 10). Importantly, these metabolic dynamics play out in the context of calcium carbonate (aragonite) that takes place at astronomically-high rates (10^9-times faster than other environments of planetary mineral precipitation). Thus the rate of mineral precipitation rates emerges as a potential forcing mechanism in thermophile cell evolution.

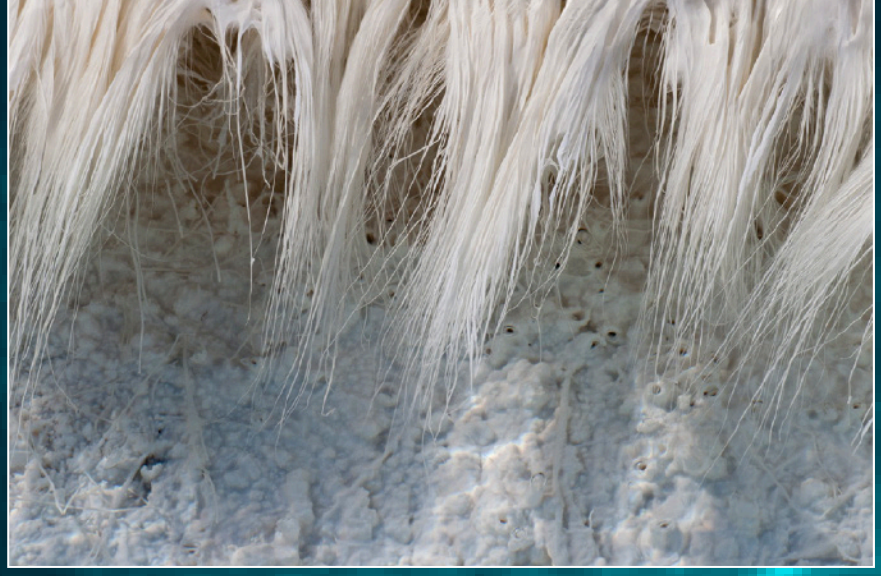


Fig. 8. Filamentous mats of Sulfurihydrogenibium.

Fig. 9. Filamentous mats of Sulfurihydrogenibium entombing travertine terracettes.

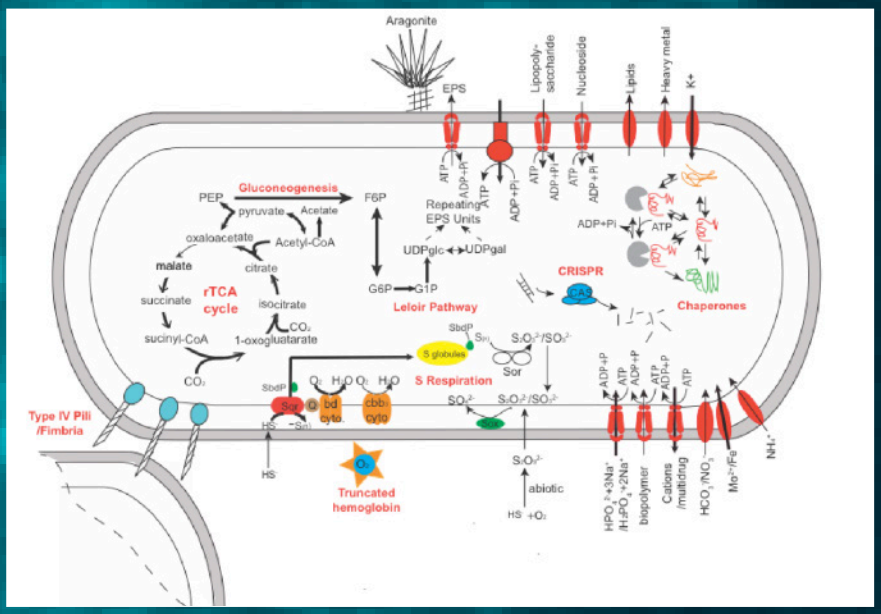


Fig. 10. Reconstructed metabolic functions for the dominant Sulfurihydrogenibium spp. in the ACF Fettuccini microbial community. The names of the metabolic pathways with significantly transcribed COGs and identified proteins were shown in red. The arrows connecting steps of the metabolic pathways were highlighted in thicker lines when both transcripts and proteins were identified.

Institute for Universal Biology: 2016 Publications

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