



Extreme Halophiles Are Models for Astrobiology

Tolerance of extremes in salinity, radiation, and temperature may permit halophiles to survive elsewhere in the universe

Shiladitya DasSarma

Studying extremophiles on Earth may provide helpful metrics in our search for life elsewhere in the universe. Species that thrive in hypersaline environments are among the most fascinating models for studying mechanisms of survival away from our planet. The genomic sequences of six such extreme halophiles and post-genomic studies of these microorganisms are providing insights about what it takes to live in environments that were once thought uninhabitable.

A unifying requirement for life is liquid water. However, evaporative forces dissipate liquid water in arid environments on Earth, on dry planets such as Mars, and in space. In brines on Earth, evaporating water concentrates dissolved salts and increases osmotic stress for all life forms. This process, which is common in natural and artificial solar salterns, leads calcium sulfate (gypsum) and sodium chloride (halite) to precipitate from seawater, leaving mainly potassium and magnesium salts in solution. During this process, dense microbial photosynthetic mats initially form but later decline, leaving only a few extremely halophilic archaea and bacteria (Fig. 1). The saturation point of sodium chloride (5.3 M), is about nine times the salinity of seawater and marks the upper limit of resistance of cultivated biological forms.

The Diversity of Hypersaline Environments and Halophiles on Earth

Two of the largest and best-studied modern hypersaline environments are Great Salt Lake in Utah and the Dead Sea in the Mid-

dle East. Of the two, Great Salt Lake at 3,900 km² is larger and shallower, about 10 m deep, and contains salts that closely match seawater in their relative proportions. The Dead Sea covers 800 km², has an average depth of 340 m, and contains salts of nonmarine proportions, with a high concentration of divalent magnesium ions. Both bodies of water contain total dissolved salts close to saturation.

Extremely halophilic microorganisms thrive in these extreme environments, turning the Great Salt Lake into a deep red color that is visible from space (Fig. 2). The archaeon *Halobacterium* sp. NRC-1, the first halophile to have its genome sequence determined, is very similar to a dominant species present in Great Salt

Summary

- Studying extremophiles such as halophiles on Earth may provide insights helpful in our search for life elsewhere in the universe.
- A common characteristic among halophiles is that they contain a preponderance of acidic proteins, better enabling them to dissolve and to function in high-salt environments.
- A model halophile, *Halobacterium* sp. NRC-1, is being studied for its ability to withstand many environmental insults, including damaging radiation, extremes of temperature, low oxygen, and exposure to heavy metals.
- Retinal-based phototrophy, first discovered in halophiles and now known to be prevalent in common marine microbes, may be one of the oldest metabolic capabilities on Earth.

Shiladitya DasSarma is a Professor at the University of Maryland Biotechnology Institute, Center of Marine Biotechnology, Baltimore.

DasSarma Focuses on Halobacteria, Hails Their Versatility

Like many children who grew up in the 1960s during the Apollo missions, Shiladitya DasSarma was fascinated by space exploration and wanted to become a rocket scientist. But his career path veered from what he expected, perhaps his subconscious response following the flight of his first model rocket. Its parachute failed to open, plunging the rocket onto a roof.

“Luckily, no one was hurt, and there was no damage to the house—although the Jupiter [rocket] did not fare so well,” he recalls. “This was the last personal experience I had with model rocketry, though I remained an avid fan of NASA’s launches throughout my formative years.” Moreover, the experience likely “directed my attention away from astronomy and towards the molecular sciences.”

Today Das Sarma, 48 is a professor in the University of Maryland Biotechnology Institute, its Center for Marine Biotechnology, and its Life Sciences Graduate Program, all in Baltimore. His research focuses on, among other things, the postgenomics, genomics, biotechnology, and molecular biology of halophilic Archaea. Halophilic microbes were discovered in spoiled foods that were salted to preserve them before refrigerators came into use, he points out. “In modern times, these organisms pose no danger, but rather are potentially valuable resources. Since they require only sun, seawater, and desert, all of which are in plentiful supply on our planet, they are easily grown and may be genetically engineered to produce unique and potentially valuable biological materials.”

With his wife, Priya, a microbiologist and manager of his lab, he has developed a series of hands-on microbiology teaching kits that are based on using *Halobacterium sp NRC-1*, which they consider safe for teaching basic microbiology to high school and college students. “The ease of culture, the ability to transform and genetically manipulate the organism, and the availability of the complete genome sequence [offer] significant advantages for inquiry-based teaching and learning,” he says. The kits, made in collaboration with Carolina Biological Supply Co., have “really taken hold, and halophiles are being used for teaching microbiology by many schools and colleges.” His wife “champions” this project, and more information is posted on a dedicated website (<http://halo.umbi.umd.edu/~haloed>).

A native of Calcutta, India, DasSarma was 8 years old when his family immigrated to Charleston, W.Va. His father, Basudeb DasSarma, a chemist who had served as the chief chemist of the Geological Survey of India, became a chemistry professor at West Virginia State College. The son regards his father as having had “the most influence in my early life,” particularly through telling his children about his reverence for science and about great Indian scientists whom he knew and admired.

DasSarma was introduced formally to microbiology at Indiana University in Bloomington in the late 1970s. After several years in the chemistry honors program, he sought a research experience, and wound up in the lab of S. Richard

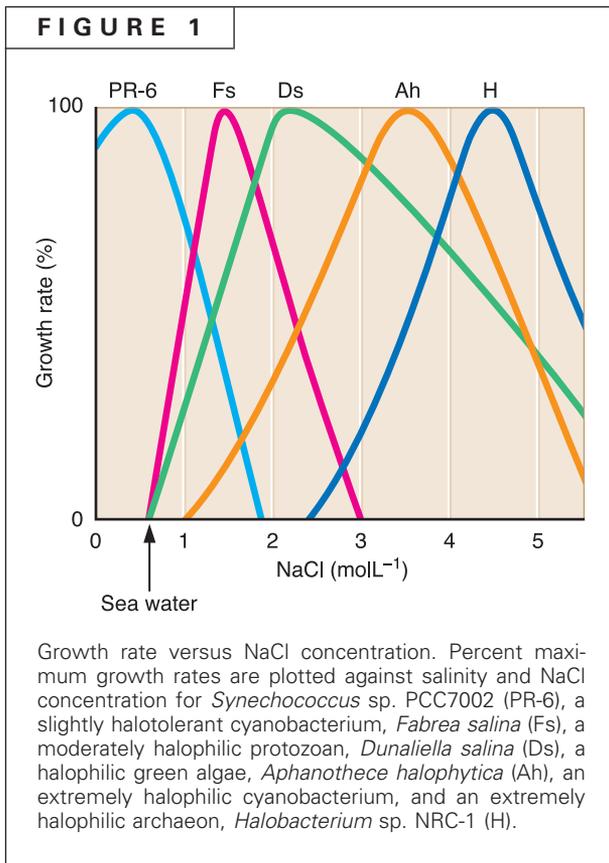
Jaskunas, a microbial geneticist. “I think it was actually the picture of ‘earthrise’ taken during the Apollo 11 mission in his office that first attracted my attention,” he says. “My experience in research with Professor Jaskunas made me realize the incredible power of microbial genetics for deciphering the rules of molecular biology.”

DasSarma received his B.S. in 1979 in chemistry from Indiana University and his Ph.D. degree in biochemistry in 1984 from the department of biology at the Massachusetts Institute of Technology, where he was a student of H. Gobind Khorana and Uttam L. Raj-Bhandary. He did research from 1984–1986 in the Department of Molecular Biology at Massachusetts General Hospital, and in the Department of Genetics at Harvard University School of Medicine. He was a faculty member in the Department of Microbiology at the University of Massachusetts, Amherst, from 1986–2001.

DasSarma and his wife have two children, a boy, 9, and a girl, 6. Priya DasSarma has taught microbiology at both the high school and college level, and conducts her own research on halophiles. “She has an infectious love for the organisms, and adds a lot of fun and excitement to the laboratory,” he says. “This is especially apparent on the kids’ day off, which they frequently want to spend in our spectacular lab in the Baltimore Inner Harbor.”

Marlene Cimons

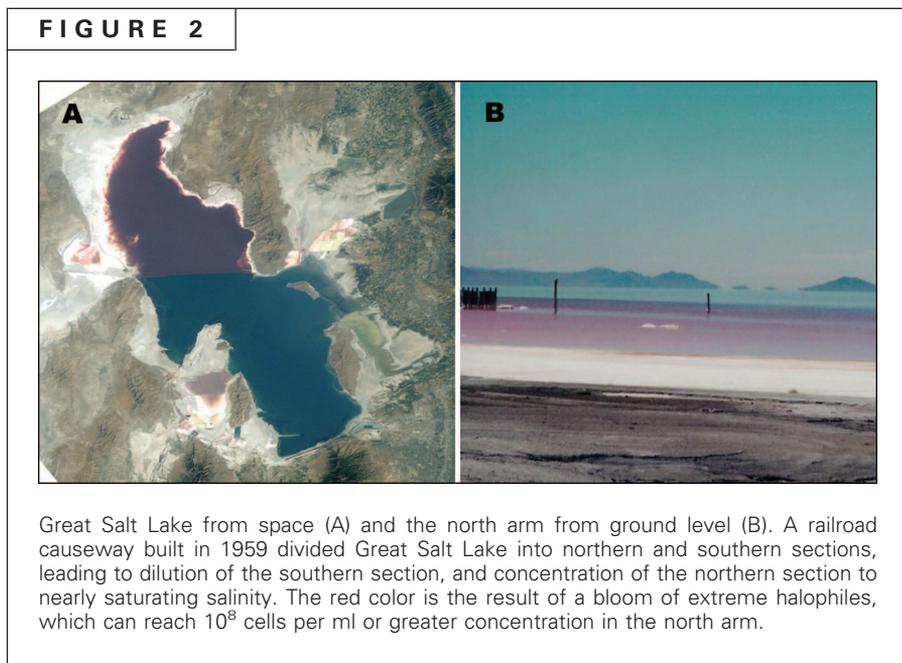
Marlene Cimons is a freelance writer in Bethesda, Md.



Lake. Two related archaea, *Haloarcula marismortui* and *Haloferax volcanii*, whose genomic sequences have also been determined, are from the Dead Sea.

Permanently cold hypersaline evaporation ponds are found in dry regions of Antarctica, including Deep Lake, Organic Lake, and Lake Suribati. The psychrophile *Halorubrum lacusprofundi*, whose genome sequence is being analyzed, was isolated from Deep Lake, which despite surface temperatures of -20°C in winter, does not freeze because of its nearly saturating salinity. Natural lakes that consist of alkaline hypersaline soda brines include Lake Magadi in Kenya, the Wadi Natrun lakes of Egypt, and the Great Basin lakes of the western United States (such as Mono Lake in California). Soda brines lack the divalent cations magnesium and calcium because of their low solubility at alkaline pH. They support the growth of halophilic alkaliphiles, such as *Natronomonas pharaonis*, that can tolerate these two extremes. Halophilic microorganisms are also found in many small evaporation ponds or sabkhas near coastal areas, where seawater penetrates through seepage or via narrow inlets from the sea. Smaller hypersaline pools are especially dynamic environments in which size, salinity, and temperature fluctuate with the seasons.

Hypersaline environments are also found within the Earth subsurface, in deep-sea basins associated with undersea salt domes and in subterranean halite deposits from evaporated ancient seas. Deep-sea brines are relatively stable because of their high densities. They are found in the Red Sea, the Gulf of Mexico, and the Mediterranean Sea—and may also occur under ice crusts on the Jovian moon Europa. On Earth, intense microbial activity is associated with these deep-sea hypersaline basins. Subsurface salt deposits that are more than 10 million years old have yielded bacterial and archaeal DNA as well as culturable microorganisms. Despite skepticism about whether these recovered species are truly ancient, their similarity to modern halophiles reinforces the view that model halophiles will help us to better understand the fundamental limits to life.



Characteristics of Halophile Genomes and Acidic Proteomes

The detailed information about the sequences, for the genome sequence of *Halobacterium* sp. NRC-1, a typical halophilic archaeon widely distributed in hypersaline environments, became available in 2000. Since then, the complete genomes of two halophilic archaea, *Haloarcula marismortui* and *Natronomonas pharaonis*, and a bacterium, *Salinibacter ruber*, were published, and draft sequences of two other archaeal halophiles—*Haloferax volcanii* and *Halorubrum lacusprofundi*, have been determined.

One common characteristic among these halophiles is that their predicted proteomes have dramatically low isoelectric points. The *Halobacterium* sp. NRC-1 proteome, for example, contains proteins with an average pI of only ~4.9, and similar values are found for other extreme halophile genomes (Fig. 3A). By contrast, pIs of nearly all nonhalophilic proteomes are close to neutral, usually with a bimodal distribution of basic (pI ~10) and acidic (pI ~5) proteins.

Proteins from halophiles typically display high surface negative charges, and this pattern of increased negative charge and lowered pI appears to hold true for the vast majority of halophilic proteins (Fig. 3B). The acidic halophilic proteins readily dissolve with enhanced functions in a milieu with high salinity and markedly reduced water activity. These observations help to explain how metabolic functions can be maintained in hypersaline media where cytoplasmic proteins are bathed in saturated potassium chloride.

Halobacterium sp. NRC-1: Useful for Postgenomic Studies

Among extreme halophiles, *Halobacterium* sp. NRC-1 is being widely used for postgenomic studies because its genome sequence is available, it is easily cultured, and its metabolism is highly versatile, with both facultative anaerobic and phototrophic capabilities. This microbe tolerates and responds to many environmental stresses, including high

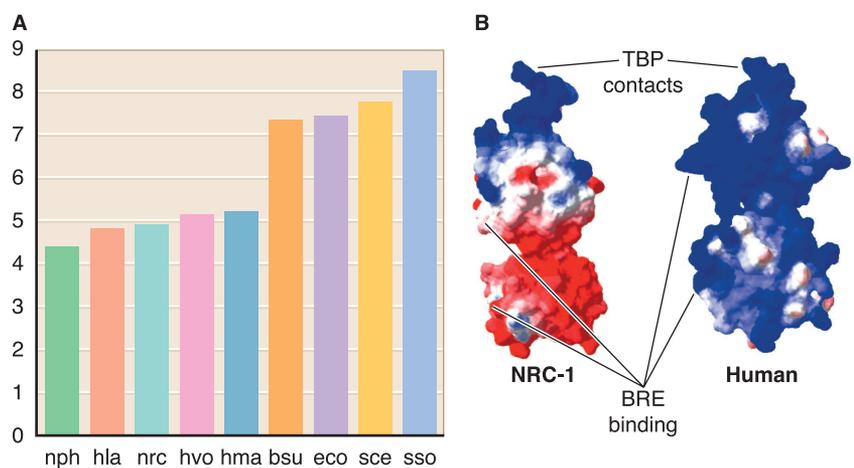
Halobacterium sp. NRC-1: A “New Model Microbe” for Teaching

The model halophile, *Halobacterium* sp. NRC-1, is an ideal example of a microorganism that can be used for teaching basic microbiology to students at high school and early college levels (http://www.carolina.com/life_science/halobacteria/). A primary reason is that it requires a medium that avoids potential growth of contaminants, since the salinity is too high for non-halophilic and potentially pathogenic microorganisms. Additional advantages are:

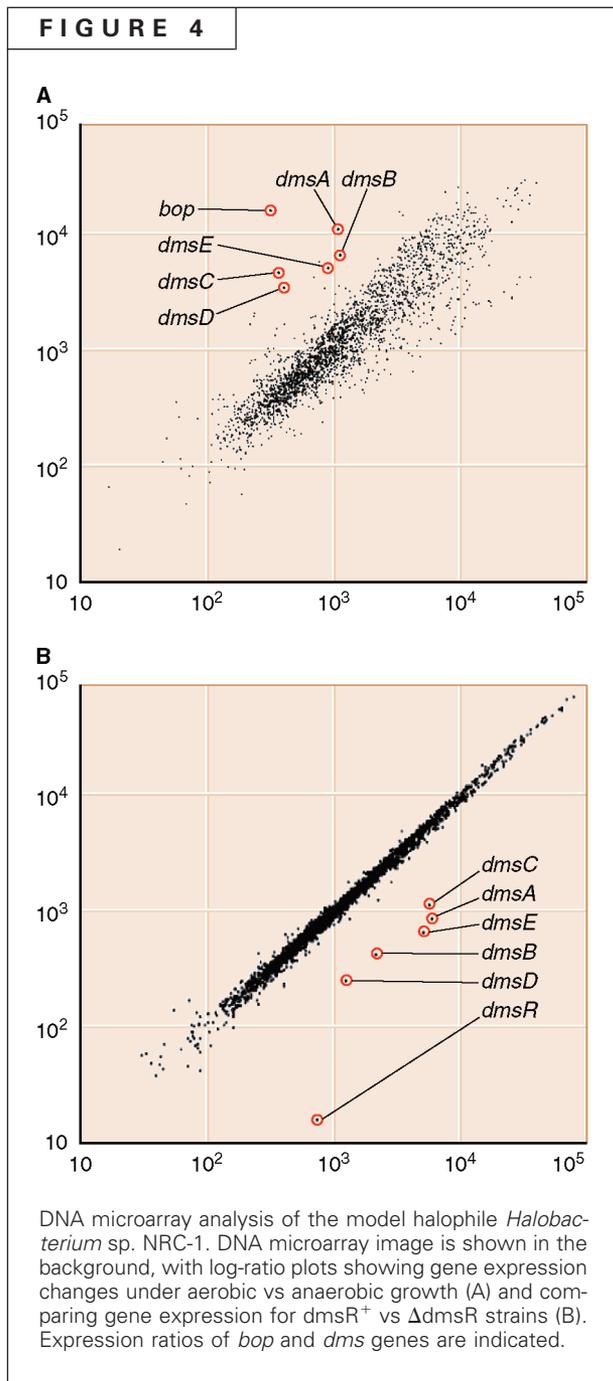
- It does not need special growth chambers.
- It can be stored easily for long periods in salt crystals.
- It requires mainly inexpensive and safe materials to culture.
- It is easily manipulated genetically.
- Its 3- to 7-day culturing period makes it convenient for weekly labs.
- It is well-characterized: the whole genome has been sequenced, and physiology and biochemistry have been studied extensively.

and low temperatures, UV and ionizing radiation, heavy metals, fluctuating ionic concentrations, and microaerobic and anaerobic conditions. Moreover, *Halobacterium* sp. NRC-1 is

FIGURE 3



Protein acidity among halophiles and non-halophiles. (A) The average pI is plotted for 5 halophiles (*Natronomonas pharaonis* [nph], *Halorubrum lacusprofundi* [hla], *Halobacterium* sp. NRC-1 [nrc], *Haloferax volcanii* [hvo], *Haloarcula marismortui* [hma], and other representative nonhalophilic microorganisms (*Bacillus subtilis* [bsu], *Escherichia coli* [eco], *Saccharomyces cerevisiae* [sce], *Sulfolobus solfataricus* [ss0]). (B) Comparison of surface charge in corresponding halophilic TFB (NRC-1) and nonhalophilic TFIIIB (human) transcription factors. Red indicates negative and blue indicates positive charge.



genetically tractable, can be transformed, and has a readily used gene knockout system based on the selectable and counterselectable *ura3* marker. Whole-genome DNA microarrays are being employed to interrogate patterns of gene expression, and biochemical studies are under way using cellular components that are released by hypotonic lysis.

Gene knockouts and DNA microarrays are

being used to analyze *Halobacterium* sp. NRC-1 responses to a variety of environmental conditions, including several relevant to astrobiology. Inkjet technology has been used to synthesize 11,000 60-nucleotide long oligonucleotide probes, representing 99% of the genes, directly onto glass slides. This relatively low-cost analytic approach provides a flexible means for studying cellular responses to environmental fluctuations (Fig. 4). For instance, halophiles such as *Halobacterium* sp. NRC-1 are subject to and can withstand intense solar radiation—in this case, by producing a cyclopyrimidine photolyase (*phr*) that reverses photoproducts of UV radiation. When irradiated with UV, such cells also induce high levels of *radA*, which is related to both eukaryotic *rad51* and bacterial *recA*, indicating the involvement of homologous recombination. Meanwhile, in response to high-energy γ radiation and desiccation, which can lead to extensive double-strand DNA breaks, cells produce membrane pigments, such as bacterioruberin, to shield the genome. These cells also induce *rfa3*, which encodes a single-stranded DNA binding protein that may be important for protecting and repairing damaged DNA.

Toxic metals and fluctuating ionic conditions also can affect *Halobacterium* sp. NRC-1. For instance, cells resist the toxic effects of arsenic through the extrachromosomal gene cluster *arsADRC*, which encodes arsenate reductase, a transporter ATPase component, but not the arsenite pump. When a nearby gene, *arsM*, is knocked out, the cells show increased sensitivity to arsenite, suggesting a second detoxification system involving an arsenite(III)-methyltransferase that *Halobacterium* sp. NRC-1 may use to transform arsenite to a volatile form, trimethylarsine. Changing the concentrations of metal and ionic species affects gene expression among a complex set of regulators, including the arsenic regulator *arsC* and a metal-responsive regulator, *sirR*.

One way *Halobacterium* sp. NRC-1 responds to limiting oxygen levels is to induce gas vesicle production, enabling the cells to float to aerobic zones in water columns. Moreover, under strictly anaerobic conditions, cells induce a bifunctional reductase, which is related to NarG-type nitrate reductases. The reductase, encoded by the *dmsEABCD* operon, uses both dimethyl sulfoxide (DMSO) and trimethylamine N-oxide

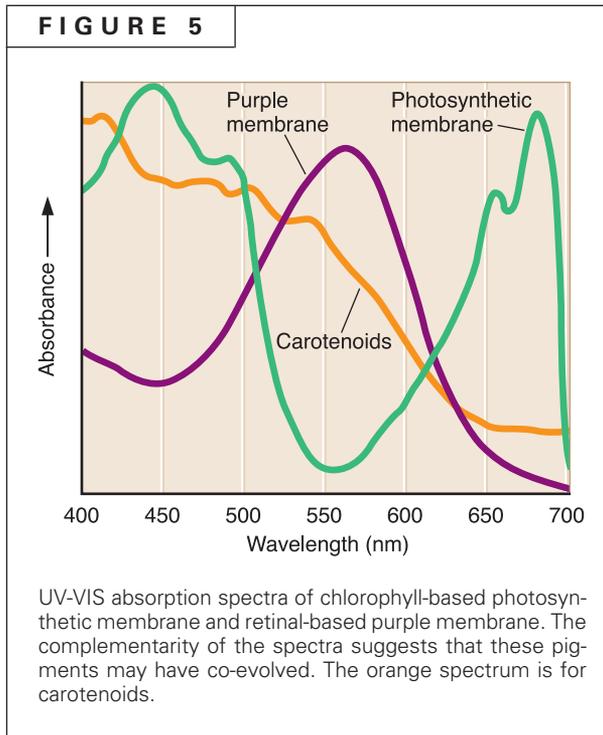
(TMAO) as terminal electron acceptors (Fig. 4A). Transcript levels of the *dms* operon depend on an upstream gene, *dmsR*, coding a transcriptional activator. Knocking out the *dmsR* gene significantly reduces *dms* operon transcription (Fig. 4B), and such cells no longer can grow on TMAO.

Another dramatic response to limiting oxygen occurs when *Halobacterium* sp. NRC-1 cells induce the *bop* gene, encoding bacterio-opsin, the protein component of the light-driven proton pump, bacteriorhodopsin, which allows for phototrophic growth (Fig. 4A). Bacterio-opsin synthesis is coordinately regulated with the chromophore retinal, and the two molecules form a complex with 1:1 stoichiometry. In addition to *bop*, this regulon includes *crtB1* and *brp*, which encode the first and last committed steps of retinal synthesis, and *bat*, the sensor-activator gene.

The eukaryotic-like TATA-box in the *bop* promoter deviates significantly from the consensus version, suggesting that novel factors are involved in its transcription. Multiple *tbp* and *tfb* genes (coding for six TATA-binding Tbp proteins and seven TFIIB-like Tfb factors) are found in the *Halobacterium* sp. NRC-1 genome, suggesting an alternate Tbp-Tfb pair may be used in *bop* transcription, similar to some regulated genes in higher organisms. Mutagenesis defined an upstream *bop* gene regulatory site, UAS, which is also near the co-regulated *brp* and *crtB1* genes, and where the Bat regulator binds. The Bat protein sequence contains GAF (cGMP-binding) and PAS/PAC (redox-sensing) motifs, indicating that it senses both light and redox potential. It also contains a C-terminal DNA-binding helix-turn-helix (HTH) motif like that in DmsR, which mediates binding to UAS. Similarities of the Bat regulator and UAS to analogous regions in diverse organisms, including a plant and a γ -proteobacterium, suggest an ancient origin for this archaeal regulon.

Evolutionary Origin of Retinal Proteins

One interesting evolutionary question concerning halophiles centers on the origin of retinal proteins, like bacteriorhodopsin. This family also includes halorhodopsin and sensory rhodopsins, which are found in *Halobacterium* sp. NRC-1 and in other halophilic archaea, and



xanthorhodopsin in *Salinibacter*. These can act as light-driven ion pumps, allowing cells to grow phototrophically, or as photosensory receptors, conferring phototactic responses on cells.

Although prokaryotic rhodopsins were discovered in halophilic archaea and thought to be unique to this group, metagenomic studies show them to be prevalent in diverse bacteria and eukaryotes. Therefore, these proteins may have arisen before the three domains diverged, or they may have been dispersed through lateral gene transfers to the relatively few clades in which they are found, including oceanic planktonic bacteria, some fungi, and halophilic archaea. Although lateral gene transfers appear to be a major force in the evolution of extreme halophiles, phylogenetic analysis remains inconclusive for retinal proteins. However, their spectroscopic complementarity suggests possible co-evolution of retinal pigments with chlorophyll-based pigments (Fig. 5). If true, retinal-based phototrophy may be one of the oldest metabolic capabilities on Earth.

Halophiles as “Exophiles”

Because halophiles tolerate so many forms of environmental stress, they are candidate “exo-



philes” — organisms that might survive on Mars or other planets. They might also be capable of surviving travel between planets—for instance, encased in salt crystals and thus protected from damaging radiation. When researchers working with the European Space Agency launched the halophile *Haloarcula* strain G, the organism survived for several weeks in deep space without protection, longer than any other vegetative cells. This finding is consistent with the observed capacity of *Halobacterium* sp. NRC-1 to withstand desiccation and radiation in laboratory studies. Moreover, several Martian meteorites, including the Shergotty and Nakhla meteorites, contain halite salt crystals, which could also be a vehicle for the interplanetary transport

of halophiles. Finding entrapped halophiles within brine inclusions in ancient halite deposits on Earth thus is a subject of considerable interest.

Extreme halophiles are remarkably resilient microorganisms with hardy molecular characteristics, including an abundance of extremely acidic proteins that function in the presence of 5 M salt. The discovery of both archaeal and bacterial halophiles with similar strategies for survival, and anaerobic halophiles in subsurface environments such as submarine brine pools provides further evidence of the remarkable physiological capabilities among this family of microorganisms, whose study continues to expand our understanding of the breadth of microbiology on Earth.

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