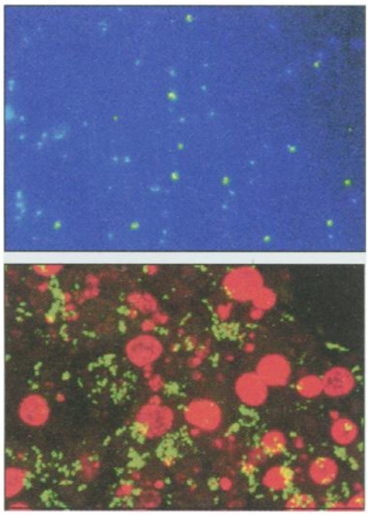
MICROBIOLOGY

Archaeal Means and Extremes

Edward DeLong

In these days of gene chips and genome sequencing, it is only natural to assume that most of the organisms inhabiting Earth are well known. Yet in truth, biology is still discovering basic information about the most abundant, widely distributed, and biochemically versatile organisms on the planet—the prokaryotes. Prokaryotes (microorganisms that lack a membrane-bound nucleus) have been thriving on Earth for more than 3.5 billion years. Their morphological simplicity belies a rich and complex biological heritage. In part because of their venerable evolutionary history, prokaryotes mediate most of the key chemical transformations of carbon, nitrogen, and sulfur in our biosphere. During their evolution, some have been engulfed by their neighbors and transformed into subcellular workhorses, the mitochondria and chloroplasts. And even now, prokaryotes thrive in bizarre and extreme habitats, from polar deserts to geothermal springs. Yet despite their antiquity, ubiquity, and ecological significance, prokaryotes are arguably the least well understood component of Earth's biota.

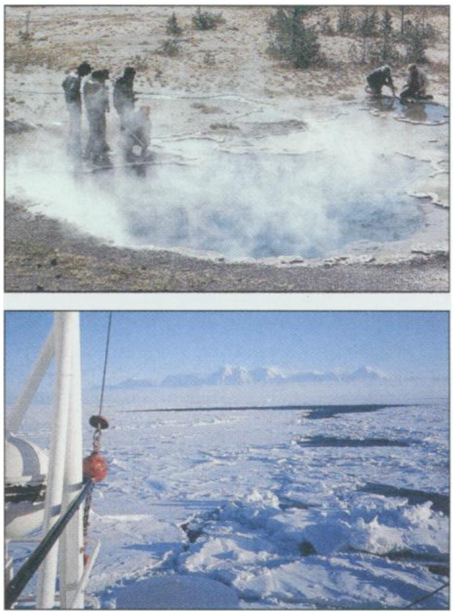

SPONGE MICROGRAPH COURTESY OF CHRISTINA PRESTON

Portraits in situ. Cold-dwelling Crenarchaeota: (**Top**) Planktonic archaea from Monterey Bay (green cells) surrounded by bacterial neighbors (blue cells). (**Bottom**) The symbiotic *Cenarchaeum symbiosum* (green cells) in its sponge host. Red spheres are the nuclei of the sponge host.

Fortunately, several conceptual and technological advances have opened new views on the microbial world. Comparisons of universally conserved nucleic acid and protein sequences now allow the construction of global genealogies encompassing all living things (1). For the first time, all cellular life can now be clearly viewed in one unified evolutionary vista. This molecular evolutionary perspective has also unexpectedly invigorated the field of microbial ecology (2). Microbial biologists have traditionally depended heavily on the isolation and cultivation of microbes one by one from the environment. But these cultivation approaches are not always optimal for ecological studies, because the isolation procedure obviates the community, and many microbes simply resist cultivation. Today, comparison of conserved gene sequences plucked directly from the environment is fast becoming a standard method for surveying natural microbial communities. Nearly every study applying these molecular techniques reveals new microbial groups, many still undetectable by cultivation (2). Comparative molecular approaches in microbial evolution and ecology are bringing a previously invisible world into much better focus.

Molecular phylogenies have also proved the existence of a major new evolutionary lineage: the Archaea (3). Genealogies of ribosomal RNA (rRNA) genes showed that the Archaea, although prokaryotic in cellular ultrastructure, are evolutionarily quite distant from their prokaryotic cousins, the Bacteria. In fact, Archaea appear to share a common ancestor with eukaryotes (4), organisms like us that possess cellular organelles and a membrane-bound nucleus. This distinctiveness of the Archaea precipitated a recategorization of life into three major domains: the Eucarya (all eukaryotes) and the two prokaryotic domains, Archaea and Bacteria (5). Recent breakthroughs in genomics tend to support this tripartite organization of life, with whole genome sequences now testifying to the status of Archaea as one of life's three main evolutionary branches (6).

At the time of their discovery in 1977, the Archaea (then archaebacteria) were an odd collection of beasts, united mainly by their evolutionary heritage. The known habitats of cultured Archaea were brines five times as salty as the oceans, geothermal environments that would cook other organisms to a crisp, and anaerobic habitats where even trace amounts of oxygen prove lethal. In the vernacular, cultivated Archaea are "extremophiles" par excellence. One main branch of Archaea, the Crenarchaeota, is especially notorious for growth at high temperature. All cultivated Crenarchaeota originate from geothermally heated habitats like submarine vents and terrestrial hot springs. Most are hyperthermophiles, requiring temperatures in excess of 80°C for optimum growth. The crenarchaeon *Pyrolobus fumarii* holds the current record for life at high temperature, growing at temperatures as high as a scalding 113°C (7).



Where they live. Contemporary Crenarchaeota can inhabit geothermal springs (**top**) or surface waters off the Antarctic Peninsula (**bottom**).

Until recently, Archaea, especially Crenarchaeota, were not thought to contribute significantly to the ecology of aerobic marine or terrestrial habitats. It was therefore surprising when close relatives of Crenarchaeota started cropping up in surveys of cold aerobic marine plankton off both coasts of North America (8). These sitings reflected the now apparent fact that several major groups of Archaea (group I and group II, see the evolutionary tree in the figure) are common and abundant components of marine plankton. The list of archaeal sightings in nonextreme marine habitats is growing rapidly, with most marine plankton samples yielding evidence for an archaeal presence. Shallow and deep marine waters at polar, temperate, and tropical latitudes, the guts of abyssal sea cucumbers, and marine sediments are all crawling with the cold-adapted cousins of hyperthermophilic Crenarchaeota (9). With this distribution and abundance in such an expansive habitat (ocean waters below about 100 m), these Archaea are one of the more abundant prokaryotic cell types on Earth.

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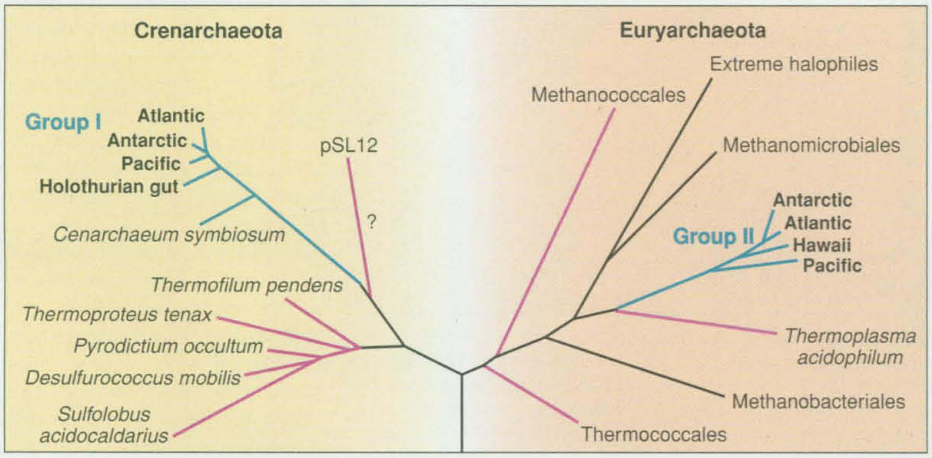
The planktonic archaea have so far resisted cultivation, and their specific physiology remains unknown. In theory, these new microbes could be vagrant thermophiles, swept from their natural habitats with venting hydrothermal fluids and plumes. But accumulating data do not support this scenario. The widespread habitats of planktonic Crenarchaeota, and their steady-state abundance, suggest that they do not live at hydrothermal vents. If vents were indeed a point source for the new archaea, then cultured hyperthermophilic Archaea like *Thermococcus*, *Pyrodictium*, or *Archaeoglobus* should co-occur with them. Yet cultured vent archaea are not commonly encountered in marine plankton, except in hydrothermal plumes sampled immediately after volcanic eruptions (*10*). Finally, the discovery of a symbiotic cold-loving crenarchaeon (*Cenarchaeum symbiosum*) (see the figure below) (*11*), and closely related archaea from rice paddies, terrestrial soils, freshwater lake sediments, and even winery by-products, provide yet more evidence that these archaea thrive in temperate, polar, marine, and terrestrial habitats worldwide (*11*).



Some like it cold. A phylogenetic tree showing abundant, uncultivated marine archaeal groups, relative to cultivated archaea. Blue lines, cold-dwelling archaea; red lines, predominantly thermophilic groups of cultivated archaea. pSL12 is an rRNA sequence recovered from a presumed thermophile at a Yellowstone hot spring (*12*).

In universal phylogenetic trees, cultured Crenarchaeota tend to group together on short, deep branches, close to the hypothetical common ancestor of all cells. This phenomenon has been interpreted by some as support for a high-temperature origin of life, and so the specific relationship between cold-dwelling planktonic archaea and the thermophilic Crenarchaeota is particularly intriguing. As a group, contemporary Crenarchaeota thrive at the temperature extremes for life, from 113°C for *Pyrolobus fumarii* to –1.8°C for Antarctic archaea. Was the common ancestor of cold-dwelling Crenarchaeota thermophilic? The answer, tentatively, is yes, mainly on the basis of a study of microbes collected from a Yellowstone Park geothermal spring (*12*). There, rRNA gene sequences specifically related to cold-dwelling archaeal rRNAs were recovered (pSL12) (see the figure below). If these sequences are truly derived from thermophiles, then planktonic Crenarchaeota are descendants of thermophiles and share a common ancestor with organisms like pSL12. If planktonic archaea did indeed originate in geothermal environments, their adaptive radiation into colder climes must have occurred millions of years ago. Yet these microorganisms share a surprising number of features with their hyperthermophilic cousins. Their history encompasses an incredible evolutionary odyssey, from life at the boiling point to life at subzero temperatures.

Despite their utility, molecular sequence comparisons do not provide much detail on phenotype. So what are the biological properties of the new marine archaea? Because the planktonic archaea are so widely distributed and abundant, they certainly exert some influence on oceanic biology and biogeochemistry. Variables that affect their physiology and distribution can be identified by tracking the organisms in their environment with molecular tools. The sheer abundance of these microorganisms allows their harvest en masse. Such bulk collections provide the biomass for biochemical and genetic studies, which can include characterization of membrane lipid composition (*13*), genomic architecture (*14*), and protein structure and function (*15*). Of course, attempts to culture these organisms should not be abandoned. So-called "uncultivable" microbes pose serious but exciting challenges to contemporary microbiologists. Molecular phylogenetic approaches are not a panacea, but they can provide tools to monitor and optimize enrichment cultures and to facilitate cultivation attempts.

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The discovery of common and abundant cold-dwelling Crenarchaeota is emblematic of the rapidly changing perspective on the true extent of natural microbial diversity. One recent study of a single habitat revealed a large variety of new bacterial groups, nearly doubling the number of phylum-level taxa within the domain Bacteria (*16*)! It is likely that similar discoveries will continue for some time as the vast and largely unexplored microbial world becomes more visible.

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