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Commentary

Archaeal ubiquity

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In the seventeenth century, Antoine von Leeuwenhook used a simple microscope to discover that we live within a previously undetected microbial world containing an enormously diverse population of creatures. The late nineteenth and early twentieth century brought advances in microbial culture techniques and in biochemistry, uncovering the roles that microbes play in all aspects of our world, from causing disease to modulating geochemical cycles. In the last 25 years, molecular biology has revealed the complexity and pervasiveness of the microbial world and its importance for understanding the interactions that maintain living systems on the planet. The paper by Preston *et al.* (1) in this issue of the *Proceedings* provides a clear illustration of the power of these molecular techniques to describe new biological relationships and to pose important questions about the mechanisms that drive evolution.

The analysis of ribosomal RNA gene sequences is one molecular approach that has radically altered our view of microbial diversity. Its application can be extended and expedited by the use of PCR. The confluence of these techniques has stimulated the rapid assembly of sequence information from homologues rRNA gene regions derived from virtually all classes of organisms. The data collected thus far support the scheme first presented by Woese *et al.* (2), which holds that the relationships among organisms can be summarized in the form of a universal phylogenetic tree comprised of one eukaryotic and two prokaryotic domains: the Eucarya, the Bacteria, and the Archaea (Fig. 1).

This approach has particularly benefited the field of microbial ecology by allowing an objective measure to be made of the relationships among rRNA genes from morphologically indistinguishable microbes. Just as importantly, these tools have provided access to the vast array of microbes that are recalcitrant to cultivation. Present estimates suggest that >99% of the microorganisms in most environments are not amenable to growth in pure culture (3). These organisms can, however, be categorized into phylotypes according to their rRNA genes, which can be amplified directly from environmental DNA extracts and subsequently cloned and sequenced. In the past several years, there has been a veritable explosion of new prokaryotic phylotypes described by these techniques, and, in many cases, the predominant organism that can be cultivated from a given environment represents a minor fraction of those that can be detected using molecular methods.

Perhaps the most surprising aspect of the universal tree as described by Woese *et al.* is that the Archaea are actually more closely related to the Eucarya than they are to the morphologically similar Bacteria. Despite the relative phylogenetic proximity to the Eucarya, the Archaea occupy the position closest to the hypothesized root of the universal tree, suggesting that this group may have traits in common with some of Earth's earliest life forms. Compared with the Bacteria, the phenotypic range of cultivated members of this group appears relatively circumscribed (4), and they have generally been characterized as living under extreme conditions of high temperature or high salt and usually under strict anaerobiosis. These are niches largely devoid of other forms of life, and this has led to the notion that the Archaea are relict organisms unable to compete for limiting resources in normal environments populated by metabolically more versatile microbes.

This view was radically changed 4 years ago when DeLong (5) and Fuhrman *et al.* (6) used taxon-specific probes and PCR primers to independently discover evidence of Archaea in coastal marine plankton communities. Quantitative hybridization measurements by DeLong indicate that up to 2% of the bacterioplankton in surface and deep-water samples are comprised of Archaea. More recently, DeLong and coworkers estimated "archaeoplankton" concentrations of up to 34% in Antarctic surface waters (7). Similar phylotypes have been reported in the Adriatic, Mediterranean, and Irish seas, as well as in terrestrial soils (8, 9). All of these novel archaeal phylotypes fall into two coherent clusters: the group I Archaea, related to the Crenarchaeota, and the group II Archaea, related to the methanogens within the Euryarchaeotal cluster. The marine populations appear to partition according to depth, with the group I members most abundant in the cold water below 100 m in temperate regions or in the frigid surface waters of the Antarctic. This is one of the most intriguing aspects of the discovery, because all other members of the Crenarchaeota are hyperthermophiles—organisms with optimal growth temperatures >80°C.

The fact that the group I archaeoplankton root or branch below other members of the Crenarchaeota conflicts with the theme that hyperthermophily is an ancestral trait common to organisms near the root of the universal tree. Was the universal ancestor actually cold-adapted? Probably not. More recent studies from the laboratory of Norman Pace have uncovered a new hyperthermophilic group, the Korarchaeota, that branch below the group I archaeoplankton near the base of the Archaeal domain (10). A more likely explanation is that a hyperthermophilic ancestor of planktonic Crenarchaeota, perhaps originating from a hydrothermal vent habitat, adapted to grow at low seawater temperatures. In support of this is the long branch length giving rise to the planktonic Crenarchaeota, which suggests that this group is evolving rapidly relative to other members of the Archaea. Such rapid evolution could be a result of relieving the selective pressure of life at high temperatures or, in an ecological context, it could indicate that the planktonic Crenarchaeota, unlike their hyperthermophilic relatives, may be competing with eubacteria for limited resources or responding to planktivorous predators.

In the absence of additional information on the biology of the group, such interpretations remain speculative. Furthermore, it is difficult to see what kind of molecular mechanisms would allow an organism to rapidly evolve a large number of proteins so that they all shift the optimal temperature at which they function by 50–70°C. It is generally thought that hyperthermophilic proteins owe their stability to incremental contributions by a large number of intraprotein interactions, and they often function poorly at low temperature. Thus either all of the genes have gone through many rounds of mutation and selection to optimize the function of each of the proteins that they encode, or there is some hitherto unknown mechanism that can modulate the temperature stability of many different proteins simultaneously.

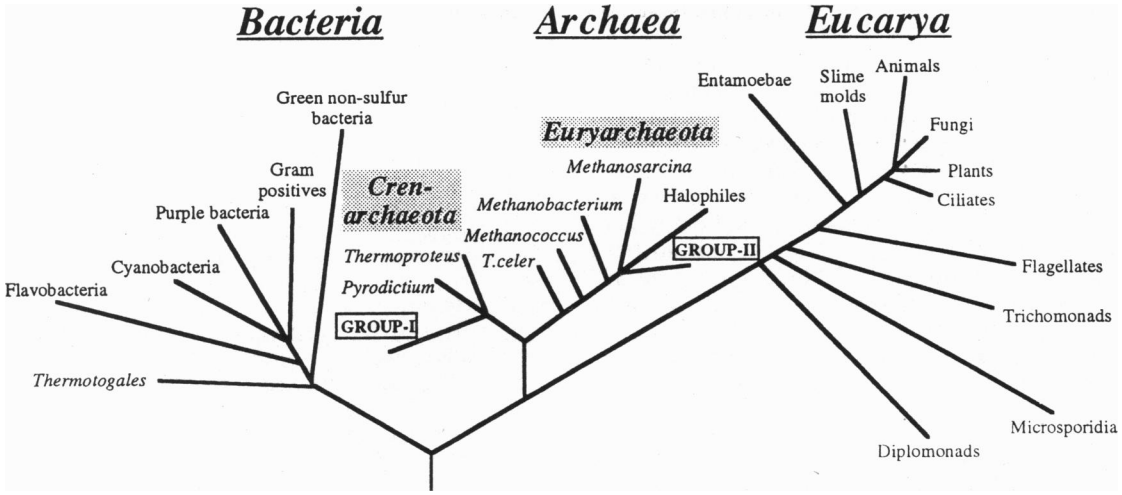

FIG. 1. Rooted phylogenetic tree based on 16S rRNA sequences showing the three domains and the relationship of the cold-adapted groups I and II members within the Archaea.

Clearly, it would be useful to learn more about the metabolism of the cold Archaea to determine whether their proteins retain a "memory" of their hypothesized hyperthermophilic ancestry. The classical approach to study the metabolism of novel prokaryotes is to grow them in pure culture under defined conditions; however, the planktonic Archaea have not yet succumbed to this approach, despite attempts by several groups. Although physiological attributes can often be predicted for uncharacterized phylotypes on the basis of phylogenetic proximity to characterized members (11), this approach is less successful for entirely novel groups and would have suggested that the group I Archaea are hyperthermophiles were it not for the large body of ecological data to the contrary. Alternatively, it would be useful to access the rest of the genome to identify protein coding genes that may provide clues to physiology. This approach was recently used by Stein *et al.* (12) to isolate a 40-kb pair genome fragment of a group I archaeon from a mixed population sample in which the Archaea constituted ≈4% of the total. The fragment contained the entire 16–23S rRNA operon as well as several protein coding genes, two of which were novel to the Archaea. These sequences suggest that the planktonic Crenarchaeota are resistant to the antibiotics streptomycin and erythromycin (missing rRNA target sequences) but are susceptible to diphtheria toxin protein (conserved His residue in elongation factor 2 gene). In addition, the fragment contained a gene encoding glutamate semialdehyde aminotransferase (GSAT), which in higher plants is involved in making the heme used in chlorophyll biosynthesis. Clearly, this is a powerful approach, and it would be very useful to identify genome contigs containing additional informative sequences. However, extracting contigs from mixed population libraries is difficult due to the complexity of the libraries that need be constructed and is potentially risky due to the possibility of generating chimeric contigs from different organisms.

In the latest contribution from the DeLong laboratory, reported in this issue of the *Proceedings*, Preston *et al.* (1) describe a solution to this problem. They have discovered marine Crenarchaeota living as symbionts within a species of Axinellid sponge inhabiting reefs offshore of Santa Barbara, CA. The symbiotic crenarchaeote, named *Cenarchaeum symbiosum*, exists as the sole archaeal phylotype in every individual of the sponge species thus far examined. The same phylotype has not been found in the water surrounding the sponge, suggesting that it is not merely filtered from the environment, and it has been stably maintained in sponges living in laboratory aquaria for over 2 years. By quantitative hybridization analysis, the archaeal rRNA constitutes up to 5% of the total, and, in one individual, this proportion was stably maintained over a period of 6 months. Moreover, whole cell hybridization studies show that some 15% of the symbionts appear to be in a state of division, demonstrating that *C. symbiosum* actively grows within its host. Despite evidence of its growth *in vivo*, *C. symbiosum* has thus far resisted all attempts at cultivation outside its sponge host. This is a trait shared with many other prokaryotic symbionts of marine invertebrates and has been interpreted as an indicator of tight linkage between the physiologies of symbiont and host. Because *C. symbiosum* appears as the sole archaeal phylotype in the sponge, the stage is now set to perform the "genome walking" experiments to identify informative protein coding genes and metabolic pathways that can provide clues to its physiology.

What role does *C. symbiosum* play in the association with its sponge host? Associations between eubacteria and marine invertebrates are common, and, in these relationships, the prokaryote usually possess a unique metabolic pathway that is coopted by the host. For example, hydrothermal vent invertebrates acquire the Calvin–Benson cycle of autotrophic CO_2 fixation through association with their sulfur-oxidizing symbionts, and Myctophid fish can bioluminescence by virtue of the lux operons possessed by their *Vibrio* symbionts. Cultivated Crenarchaeota possess unique pathways of carbon fixation and lipid biosynthesis that could be beneficial to the sponge host; however, it is not yet known which, if any, of these pathways exist in *C. symbiosum*. Another intriguing possibility is that *C. symbiosum* may make bioactive compounds that protect its host against predation or infection by pathogens. Because they live in a nutrient rich aqueous environment, marine invertebrates are subject to constant pathogenic challenge. In response to this challenge, many marine organisms acquire symbionts that manufacture secondary metabolites active against other bacteria or against possible predators. Marine sponges are a well-known source of bioactive compounds, and, in some cases, the source of these compounds has been traced to associated eubacteria (13).

The newly found ubiquity of the Archaea, coupled with their surprising association with a metazoan host, raises other questions regarding the extent of their distribution. A large proportion of eubacterial symbionts are members of the gamma subdivision of the proteobacteria; the same group that contains pathogenic *Salmonella*, *Escherichia coli*, *Vibrio*, and other species. If the ability to establish symbiotic associations is an indicator of predisposition to pathogenicity, then does the association of *C. symbiosum* with its sponge host portend the possibility of an Archaeal pathogen? After all, within the

definition of symbiosis, the line between mutualism and parasitism is often blurred. Unlike the limited range of other Archaea, the widespread distribution of group I and II members certainly provides ample opportunities for contact with higher eukaryotes, including humans. The discovery of the association of the apparently benign *Helicobacter* with human ulcers suggests that there might be other disease-causing microorganisms lurking in our intestinal tract. If any are Archaeal and are difficult to culture, they could easily have evaded detection by standard approaches and would have been resistant to the usual repertoire of antibiotics applied against eubacterial pathogens. These possibilities, in addition to the other roles that these ubiquitous Archaea may play in a variety of environments, suggest that the investigation of microbial diversity will continue to provide surprising revelations.

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