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Archaebacteria

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Summary. Experimental work published elsewhere has shown that the Archaebacteria encompass several distinct subgroups including methanogens, extreme halophiles, and various thermoacidophiles. The common chacteristics of Archaebacteria known to date are these: (1) the presence of characteristic tRNAs and ribosomal RNAs; (2) the absence of peptidoglycan cell walls, with in many cases, replacement by a largely proteinaceous coat; (3) the occurrence of ether linked lipids built from phytanyl chains and (4) in all cases known so far, their occurrence only in unusual habitats.

These organisms contain a number of 'eucaryotic features' in addition to their many bacterial attributes. This is interpreted as a strong indication that the Archaebacteria, while not actually eucaryotic, do indeed represent a third separate, line of descent as originally proposed.

Key words: Archaebacteria - Extreme halophiles - *Thermoplasma* - *Sulfolobus* - Methanogens - Progenote

Molecular genealogical analysis – based upon ribosomal RNA sequence homologies – has revealed that the bacteria do not constitute a phylogenetically monolithic grouping (Balch et al., 1977; Fox et al., 1977). What has come to be called the Kingdom Procaryotae actually comprises two phylogenetically distinct groups (Woese and Fox, 1977a). One, designated the 'eubacteria', in effect encompasses the classically recognized bacteria, inclusive of the (true) mycoplasmas and cyanobacteria. The other, designated 'archaebacteria', brings together a collection of little studied microorganisms from diverse and highly specialized niches. The properties and relationships of this latter, rather bizarre assemblage are the subject of the present discussion.

What are Archaebacteria and why are they so named?

The first organisms recognized to be archaebacteria were the methanogens – fastidious anaerobes whose metabolism centers around the reduction of carbon dioxide to

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methane (Fox et al., 1977; Zeikus, 1977; Mah et al., 1977; Wolfe, 1971). A continuing search has now identified the extreme halophiles, *Halobacterium* and *Halococcus*, also as archaebacteria (Magrum et al., 1978). The sulfur oxidizer, *Sulfolobus*, and another thermoacidophile, *Thermoplasma*, share certain biochemical peculiarities with the extreme halophiles (Langworthy et al., 1972; Langworthy et al., 1974; deRosa et al., 1975; Kates, 1972); examination of their (16S) rRNA and tRNA sequences show these organisms to be archaebacteria as well (Woese et al., 1978; Magrum et al., unpublished). Thus, the archaebacteria are a phenotypically varied group.

The full extent of diversity in the archaebacteria needs to be appreciated. In each major phenotypic subgroup a wide variety of species seem to be represented. The methanogens, for example, are known to comprise two orders, containing at least three families (Fox et al., 1977) a classification justified also by the extreme diversity in their cell wall structures (Kandler and Hippe, 1977; Jones et al., 1977; Kandler and König, 1978). Similarly, the minimally characterized *Sulfolobus* subgroup, inclusive of the related 'Ferrolobus' (Brierley and Brierley, 1973) and 'MT strains' (deRosa et al., 1975) encompass a wide range of G-C contents. As a whole, the archaebacteria include anaerobes, aerobes, autotrophs, heterotrophs, thermophiles, acidophiles, and even photosynthetics. Morphologically, they can be rods, cocci, sarcinae, and spirilla. Their overall diversity in G-C content ranges from values at least as low as 28% in one methanogen (Zeikus and Henning, 1975) to ones at least as high as 68% in the halophiles (Joshi et al., 1963; Moore and McCarthy, 1969). In short, the archaebacteria exhibit a degree of diversity roughly comparable to that seen among the eubacteria.

In terms of their *general* phenotypes archaebacteria resemble the ordinary bacteria, which of course they have always been considered to be. However, on the basis of degree of molecular sequence homology – i.e., in terms of a strictly genealogical criterion – the archaebacteria cannot be considered to be ordinary bacteria at all. They show no more relationship to the ordinary bacteria than they do to eucaryotic cells (Woese and Fox, 1977a). Therefore, although the classification of archaebacteria by traditional criteria is at present a moot point, there can be little doubt that in a strictly genealogical sense, these organisms constitute a separate grouping at the highest level – which can be called a 'primary kingdom' or 'urkingdom' (Woese and Fox, 1977a).

The name 'archaebacteria' is obviously meant to connote antiquity. Some of their properties are suggestive of a kind of bacteria one might expect to have dominated the early Archaen ecology. Various of their niches, which appear 'extreme' in terms of modern terrestrial conditions, would seem even normal on a warm planet with a reducing atmosphere, some 3-4 billion years ago. The genealogical difference between the two major methanogenic lines is comparable to that seen between ordinary bacteria as diverse as clostridia and cyanobacteria (Woese and Fox, 1977a). Thus, at least one of the archaebacterial phenotypes, methanogenesis, appears to be very ancient. Methanogenesis is also one of the most unique biochemistries yet discovered (Mah et al., 1977; Zeikus, 1977). Since it appears to have little or nothing in common with the usual, well known metabolic pathways, it may have arisen totally separately from them. Furthermore, methanogenesis is peculiarly well suited to the projected primitive atmosphere of this planet, a mixture of gasses rich in carbon dioxide and hydrogen (discussed in Woese, 1977). Together these considerations make it tempting to speculate that in early Archaen times an 'Age of Archaebacteria' existed, in which methanogenesis was the primordal form of 'respiration'.

The existence of two distinct bacterial urkingdoms brings into focus three new evolutionary issues. Firstly, which, if either urkingdom is the more ancient? Secondly, what are the common ancestral phenotypes in each urkingdom; and thirdly, which phenotypes from the two urkingdoms were contemporaneous at any particular time?

How do Archaebacteria Differ from Normal Bacteria and in what Ways do Archaebacteria Resemble One Another?

If the archaebacteria are so distinct genealogically, it must follow that despite their superficial resemblance to ordinary bacteria, they differ from them profoundly in many ways – i.e., much of their ostensible similarity to ordinary bacteria represents *convergent* evolution, or simply their being unlike eucaryotes. Conversely, in spite of apparent great dissimilarities among the phenotypes within the archaebacterial urkingdom, genuine phenotypic similarities (reflections of *common* evolutionary origin) must exist. Although relatively little is known about the archaebacteria, it is already apparent that these requirements are fulfilled. Consider the following specific points:

The Archaebacterial Cell Wall

Both gram positive and gram negative walls are known among the archaebacteria (Fox et al., 1977). However, there the resemblance to eubacterial walls ends. Archaebacterial walls altogether lack diaminopimelic and muramic acids – i.e., peptidoglycan (Hippe and Kandler, 1977; Brock et al, 1972; Reistad, 1972; Brown and Cho, 1970; Darland et al., 1970; Kushner and Onishi, 1968; Brown and Shorey, 1963; Weiss, 1974). Yet, within the archaebacterial group, wall structures are extremly varied. Methanogens alone exhibit four distinct wall types (Kandler and König, 1978; Kandler and Hippe, 1977; Jones et al., 1977), the halophiles two (Steber and Schleifer, 1975; Kushner and Onishi, 1968). All of the major archaebacterial lines (except of course, *Thermoplasma*) contain at least one genus whose wall is a simple, regular (largely) proteinaceous covering.

The Archaebacterial Translation Apparatus

30S and 50S ribosomal subunits containing 16S and 23S rRNAs again seem to be the extent of specific resemblance between the archaebacterial and eubacterial translation mechanisms (Woese et al., unpublished). Ribosomal RNA sequences are not specifically related between the two bacterial groups (Fox et al., 1977; Magrum et al., 1978; Woese et al., 1978). The archaebacterial 5S RNA secondary structure resembles its typical bacterial counterparts in the same three segments (the 'molecular stalk', the 'tuned helix' and the 'common arm base') that are also found in the eucaryotic 5S RNAs (Fox and Woese, 1975a,b). However, in the remaining region of secondary structure, the so-called, 'procaryote loop', the archaebacterial 5S RNA resembles its typical bacterial counterparts no more than do the eucaryotic 5S RNAs (Luehrsen and Woese, unpublished).

One of the more significant ways in which archaebacterial and eubacterial translation mechanisms differ is in the patterns of posttranscriptional modification of the RNA nucleotides. The patterns of modification for 16S rRNA have almost nothing in common between the two bacterial urkingdoms – in spite of the fact that the very

same locales in the 16S rRNA sequence appear to be modified in the two cases (Magrum et al., 1978). A more striking example is seen in archaebacterial tRNAs. What had previously been considered a universal feature of tRNAs (with minor exceptions), the so-called common arm sequence, ..GTΨCG.., is not found in archaebacterial tRNAs. Instead, they possess unique and distinctive counterparts, GΨΨĊG, GΨΨĊĠ, GU̇ΨĊĠ, or GU̇ΨĊĠ (Fox et al., 1977; Magrum et al., 1978; Woese et al., 1978). [The superscript dot denotes a modified nucleotide, but U̇ ≠ T. This family of sequences is readily identified in that the ĊG (ĊĠ) dimer is insensitive to endonucleases (Woese et al., unpublished.)] Moreover, very few of the modified nucleotides found in eubacterial tRNAs are present in their archaebacterial counterparts (Best, 1978).

The need for base modification in tRNAs and rRNAs is apparent from these comparisons; the need for *particular* modifications is not. The same evolutionary problem seems capable of various solutions even at the molecular level.

Lipids

Lipid comparisons too, underscore both the uniqueness of archaebacteria and the extent of evolutionary convergence at the molecular level. It has been known for some time that the extreme halophiles, *Sulfolobus*, and *Thermoplasma* contain negligible amounts of saponifiable (i.e., ester linked) lipids; (Kates et al., 1966; Kates, 1972; Langworthy, et al., 1972; Langworthy et al., 1974; deRose et al., 1975, 1976); the same has been demonstrated very recently for a methanogen as well. (Tornabene et al., 1978). Nevertheless, archaebacteria have lipid analogs for the major lipid groups found in other organisms – glycolipids, phospholipids, etc. Ether links replace ester links, and straight carbon chains are replaced by polyisoprenoid (branched) chains (Kates, 1972; Langworthy et al., 1974; deRosa et al., 1976; Tornabene et al., 1978). In other words, archaebacteria seem to contain classes of lipids with the same gross physical properties as do other organisms. Yet in molecular structure and mode of biochemical synthesis (which in the archaebacterial case is via a mevalonate pathway (Kates, 1972)), the archaebacterial lipids are completely unrelated to those of other organisms.[1]

Intermediary Metabolism

Over the past several decades, a complacency has developed towards the idea that all organisms employ a cergain universal core of biochemical pathways. This assumption has made the biochemist loathe to investigate many aspects of intermediary metabolism in new organisms. What little experience there is with archaebacterial intermediary metabolism appears to challenge the concept of a universal intermediary metabolism, at least the extent of such universality. Not only do archaebacteria appear to use novel pathways in constructing cell walls and synthesizing lipids, but the methanogens

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[1] These novel lipids are not simply an adaptation to extreme environments, as has been suggested. Eubacterial halophiles or thermoacidophiles, e.g. *Bacillus acidocaldarius*, have ester linked lipids (Langworthy et al., 1976). And some archaebacteria, e.g. methanogens, do not inhabit niches extreme in this sense. Thus, the character of the lipids corresponds with the bacterial kingdom, not the environmental niche.

at least exhibit a spectrum of unique coenzymes (Cheeseman et al., 1972; Taylor and Wolfe, 1974). Moreover, attempts to demonstrate some of the more usual cofactors in methanogens have failed. Clearly a comprehensive examination of the metabolic pathways in the archaebacteria is called for. Given the possible antiquity of their ancestry, the intermediary metabolic patterns of archaebacteria may provide further insights linking intermediary metabolism to prebiotic chemistry.

Archaebacterial Habitats

All the organisms known to be archaebacteria come from 'specialized' or 'extreme' habitats – the hot acidic niches of the thermoacidophiles, the saturated or near saturated salt environments required by the halophiles, and the methanogens' need to grow near the redox extreme defined by molecular hydrogen. Various of the eubacteria also grow in extreme environments. However, in the latter cases, the species in question often appear to be adaptations to the extreme conditions from more moderate ones; at least relatives growing under more moderate conditions can generally be found in profusion. Archaebacteria have yet to be isolated from 'moderate' habitats. If, upon extensive search, this continues to be the case, then one has to consider seriously the possibility that archaebacteria for some reason require 'extreme' habitats.

It is known for the cases of the thermoacidophiles and halophiles that intracellular environments are quite unlike extracellular ones – e.g., the sodium-potassium ratios in the halophiles, (Lanyi and Hilliker, 1976) the pH of the thermoacidophiles (Darland et al., 1970; Brock et al., 1972; deRosa et al., 1975). The methanogens appear to be an exception. One wonders whether adaptation to an oxygen atmosphere is somehow connected with this phenomenon in archaebacteria.

In summary, enough comparative information now exists pertaining to the archaebacteria to make it possible to characterize the kingdom in a positive way. The general properties expected of a putative member of the archaebacteria would include all of the the following: (1) The presence of characteristic tRNAs and ribosomal RNAs in the translation machinery. (2) The absence of peptidoglycan cell walls. (3) The occurrence of ether linked lipids built of isoprenoid subunits. And perhaps (4) an ecological niche that is highly specialized, 'extreme' in the above sense.

The Relationship of Archaebacteria to Eucaryotes

While archaebacteria resemble typical bacteria in many gross characteristics, they differ from them in many ways in their molecular details. On the other hand, archaebacteria bear no gross resemblance whatever to eucaryotic cells; yet they have certain molecular properties that are characteristically eucaryotic. These latter resemblances, mentioned below, could lead to claims that various of the archaebacteria are 'proto-eucaryotes', i.e., are the procaryotes from which the main component (the engulfing, or cytoplasmic, aspect) of the eucaryotic cell arose. Furthermore, there exists a strong tradition in biology that sees life in terms of dichotomies; formerly what was not animal was plant; now what is not eucaryotic is procaryotic. So when archaebacteria *lack* a certain 'procaryotic' (i.e., eubacterial) property, they will for this negative reason, also tend to be seen somehow as eucaryotic. Clearly, caution must be exercised in interpreting any evidence that seemingly relates archaebacteria specifically to eucaryotes.

Archaebacteria do show certain 'eucaryotic' characteristics, however. For one, the halophiles have a photosynthetic pigment very similar to the eucaryotic visual pigment, rhodopsin (Osterhelt and Stoeckenius, 1971, 1973). Archaebacterial tRNAs in general show the high level of modification considered characteristic of eucaryotic tRNAs (Best, 1978), in addition to which the halophile initiator tRNA specifically resembles its eucaryotic counterpart: (a) in carrying nonformylated methionine, and (b) in that the 5' terminal base, in the stalk of the molecule, *is* paired (Baumstark et al., 1978). The archaebacterial translation apparatus is sensitive to certain antibiotics specific for its eucaryotic counterpart (but insensitive to analogous 'procaryotic' antibiotics) (Tsen and RajBhandary 1978). The presence of an actin-like protein in Thermoplasma has been reported (Searcy et al., 1978).

The amount and nature of this evidence, however, does not warrant the conclusion that archaebacteria (especially any specific member of the archaebacteria) are 'proto-eucaryotes'. In addition, there are too many ways in which archaebacteria as a group appear unique – their lipids, their tRNA common arm, and, of course, the detailed comparative analysis of their rRNA primary structures. The sum of the genealogical evidence does not suggest that life comprises two primary lines of descent, one leading to the 'procaryotes', the other (though some 'proto-eucaryote') to the eucaryotes. Instead it suggests there to be three such lines – one represented by the eubacteria, one by the archaebacteria, and one by the 'cytoplasmic aspect' of the eucaryotic cell. Moreover, the three lines of descent separated from one another before the level of complexity we recognize as 'procaryotic' was reached; their (last) common ancestor probably represented a simpler level of organization, called the 'progenote' (Woese and Fox, 1977a, b). This tripartite division of life appears a very deep one – the basic characteristics of each of these three primary kingdoms probably having been established 3-4 billion years ago.

It is in this context that one should view the specific resemblances between eucaryotes and archaebacteria and other such specific resemblances. Since all three lines of descent come from a common ancestry, any specific similarity between two of them would just as readily reflect a loss or change of some property in the remaining one, as it could the evolution of that property in (a common ancestor of) the other two. Moreover, the eucaryotic cell is an evolutionary chimera, whose various components arose either as genealogically distinct free living organisms (e.g., chloroplasts or mitochondria, which were initially eubacteria) or as parts of free living organisms. Some of these eucaryotic components may indeed have arisen in the archaebacterial line of descent.

The real question biology will come to face is not whether two of the three lines of descent are more closely related to each other than to the third. It is, rather, the deeper but ill-defined question (or set of questions) having to do with the nature of progenotes and how they become procaryotes, and how the eucaryotes have formed from various simpler entities.

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