Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya

(Euryarchaeota/Crenarchaeota/kingdom/evolution)

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ABSTRACT   Molecular structures and sequences are generally more revealing of evolutionary relationships than are classical phenotypes (particularly so among microorganisms). Consequently, the basis for the definition of taxa has progressively shifted from the organismal to the cellular to the molecular level. Molecular comparisons show that life on this planet divides into three primary groupings, commonly known as the eubacteria, the archaebacteria, and the eukaryotes. The three are very dissimilar, the differences that separate them being of a more profound nature than the differences that separate typical kingdoms, such as animals and plants. Unfortunately, neither of the conventionally accepted views of the natural relationships among living systems—i.e., the five-kingdom taxonomy or the eukaryote–prokaryote dichotomy—reflects this primary tripartite division of the living world. To remedy this situation we propose that a formal system of organisms be established in which above the level of kingdom there exists a new taxon called a "domain." Life on this planet would then be seen as comprising three domains, the Bacteria, the Archaea, and the Eucarya, each containing two or more kingdoms. (The Eucarya, for example, contain Animalia, Plantae, Fungi, and a number of others yet to be defined.) Although taxonomic structure within the Bacteria and Eucarya is not treated herein, Archaea is formally subdivided into the two kingdoms Euryarchaeota (encompassing the methanogens and their phenotypically diverse relatives) and Crenarchaeota (comprising the relatively tight clustering of extremely thermophilic archaebacteria, whose general phenotype appears to resemble most the ancestral phenotype of the Archaea).

Need for Restructuring Systematics

Within the last decade it has become possible to trace evolutionary history back to the (most recent) common ancestor of all life, perhaps 3.5–4 billion years ago (1, 2). Prior to the mid 1970s evolutionary study had for all intents and purposes been confined to the metazoa and metaphyta, whose histories at best cover 20% of the total evolutionary time span. A sound basis for a natural taxonomy was provided in these cases by complex morphologies and a detailed fossil record. The evolution of the microbial world—whose history spans most of the planet's existence—was at that time beyond the biologist's purview, for, unlike their multicellular equivalents, microbial morphologies and other characteristics are too simple or uninterpretable to serve as the basis for a phylogenetically valid taxonomy (3, 4). The sequencing revolution, by making accessible the vast store of historical information contained in molecular sequences (5), has changed all that. As a result, the biologist finds that textbook descriptions of the basic organization of life have become

outmoded and so, misleading. The time has come to bring formal taxonomy into line with the natural system emerging from molecular data.

This revision, however, is not accomplished simply by emending the old system. Our present view of the basic organization of life is still largely steeped in the ancient notion that all living things are either plant or animal in nature. Unfortunately, this comfortable traditional dichotomy does not represent the true state of affairs. Thus, as a prerequisite to developing a proper natural system we have to divest ourselves of deeply ingrained, cherished assumptions, as regards both the fundamental organization of life and the basis for constructing a system of organisms. The system we develop will be one that is completely restructured at the highest levels.

Haeckel in 1866 (6) formally challenged the aboriginal plant/animal division of the living world. He recognized that the single-celled forms, the protists, did not fit into either category; they must have arisen separately from both animals and plants. Haeckel saw the tree of life, therefore, as having three main branches, not two. Copeland (7) later split out a fourth main branch, a new kingdom accommodating the bacteria, and Whittaker (8) created a fifth, for the fungi. While Haeckel's original proposal and its two more recent refinements did away with the idea that animal/plant was *the* primary distinction, they left unchallenged the notion that it is *a* primary distinction (by representing it at the highest available taxonomic level). The last of these schemes (Whittaker's), which divides the living world into Animalia, Plantae, Fungi, Protista, and Monera, is the most widely received view of the basic organization of life today (8, 9).

It has been apparent for some time, however, that the five-kingdom scheme (and its predecessors) is not phylogenetically correct, is not a natural system. There are sound logical grounds for presuming that the two eukaryotic microbial taxa (Protista and Fungi) are artificial. It is generally accepted that the metaphyta and metazoa evolved from unicellular eukaryotic ancestors; the extant groups of eukaryotic microorganisms, therefore, comprise a series of lineages some (or many) of which greatly antedate the emergence of the Plantae and Animalia. This is confirmed by the fossil record, wherein recognizable eukaryotic unicells appear about 200 million years before the first primitive algae, and over a billion years before the first animals and higher plants (10). There are thus good reasons in principle to presume that the Protista and perhaps also the Fungi are paraphyletic at best.

More seriously, in giving the kingdom Monera the same taxonomic rank as the Animalia, Plantae, Fungi, and Protista, the five-kingdom formulation ignores the fact that the differences between Monera (prokaryotes) and the four other kingdoms are far more significant, and of a qualitatively different nature, than the differences among these four. In

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other words, a primary division of life must lie between the bacteria and the eukaryotic forms; the animal/plant distinction is definitely secondary.

This realization is by no means new. Microbiologists acknowledged it more than 100 years ago (11), and, of course, Chatton (12) codified it with his famous eukaryote–prokaryote proposal, dividing all life into these two primary categories. This view of life, strangely, has coexisted for some time now with the five-kingdom scheme, despite their basic incompatibility, and despite the fact that the evidence overwhelmingly supports the former. However, the eukaryote–prokaryote concept itself has been seriously misunderstood and, consequently, wrongly interpreted.

The problem here arises because the eukaryote–prokaryote concept is fundamentally cytological, and only secondarily, and by inference, phylogenetic. The presumption that the eukaryotic form of cellular organization defines a meaningful phylogenetic unit is a reasonable one; organisms with this cytology are united by possession of a series of complex properties. The same is unfortunately not true of prokaryotes, which are united as a class by their *lack* of the characteristics that define the eukaryotic cell. The definition is consequently a negative one that is empty of meaningful internal phylogenetic information. Microbiologists have long recognized this (even before the articulation of the eukaryotic–prokaryotic concept): e.g., Cohn in 1875: "Perhaps the designation of *Schizophytae* may recommend itself for this first and simplest division of living beings . . . even though its distinguishing characters are negative rather than positive" (11); Pringsheim in 1923: ". . . the possibility of . . . convergent evolution [among bacteria must] be seriously considered" (13); and Stanier in 1971: "Indeed the major contemporary procaryotic groups could well have diverged at an early stage in cellular evolution, and thus be almost as isolated from one another as they are from eucaryotes as a whole" (14).

As the molecular and cytological understanding of cells deepened at a very rapid pace, beginning in the 1950s, it became feasible in principle to define prokaryotes positively, on the basis of shared molecular characteristics. However, since molecular biologists elected to work largely in a few model systems, which were taken to be representative, the comparative perspective necessary to do this successfully was lacking. By default, *Escherichia coli* came to be considered typical of prokaryotes, without recognition of the underlying faulty assumption that prokaryotes are monophyletic. This presumption was then formalized in the proposal that there be two primary kingdoms: *Procaryotae* and *Eucaryotae* (15, 16). It took the discovery of the archaebacteria to reveal the enormity of this mistake.

On the cytological level archaebacteria are indeed prokaryotes (they show none of the defining eukaryotic characteristics), but on the molecular level they resemble other prokaryotes, the eubacteria, no more (probably less) than they do the eukaryotes (1, 17). *Procaryotae* (and its synonym *Monera*) cannot be a phylogenetically valid taxon.

Basis for Restructuring

What must be recognized is that the basis for systematics has changed; classical phenotypic criteria are being replaced by molecular criteria. As Zuckerkandl and Pauling (5) made clear many years ago, it is at the level of molecules (particularly molecular sequences) that one really becomes privy to the workings of the evolutionary process. Molecular sequences can reveal evolutionary relationships in a way and to an extent that classical phenotypic criteria, and even molecular functions, cannot; and what is seen only dimly, if at all, at higher levels of organization can be seen clearly at the level of molecular structure and sequences. Thus, systematics in

the future will be based primarily upon the sequences, structure, and relationships of molecules, the classical gross properties of cells and organisms being used largely to confirm and embellish these.

It is only on the molecular level that we see the living world divide into three distinct primary groups. For every well-characterized molecular system there exists a characteristic eubacterial, archaebacterial, and eukaryotic version, which all members of each group share. Ribosomal RNAs provide an excellent example (in part because they have been so thoroughly studied). One structural feature in the small subunit rRNA by which the eubacteria can be distinguished from archaebacteria and eukaryotes is the hairpin loop lying between positions 500 and 545 (18), which has a side bulge protruding from the stalk of the structure. In all eubacterial cases (over 400 known) the side bulge comprises six nucleotides (of a characteristic composition), and it protrudes from the "upstream" strand of the stalk between the fifth and sixth base pair. In both archaebacteria and eukaryotes, however, the corresponding bulge comprises seven nucleotides (of a different characteristic composition), and it protrudes from the stalk between the sixth and seventh pair (18, 19). The small subunit rRNA of eukaryotes, on the other hand, is readily identified by the region between positions 585 and 655 (*E. coli* numbering), because both prokaryotic groups exhibit a common characteristic structure here that is never seen in eukaryotes (18, 19). Finally, archaebacterial 16S rRNAs are readily identified by the unique structure they show in the region between positions 180 and 197 or that between positions 405 and 498 (18, 19). Many other examples of group-invariant rRNA characteristics exist; see refs. 2, 18, and 19. [The reader wishing to gain a broader and more detailed appreciation for the molecular definition of the three groups can consult refs. 2, 20, and 21 and the proceedings of the most recent conference on archaebacteria (22).]

Molecular characterizations also reveal that the evolutionary differences among eubacteria, archaebacteria, and eukaryotes are of a more profound nature than those that distinguish traditional kingdoms, such as animals and plants, from one another. This is most clearly seen in the functions that must have evolved early in the cell's history and are basic to its workings. All eubacteria, for example, exhibit nearly the same subunit pattern (in terms of numbers and sizes) in their RNA polymerases; however, this pattern bears little relationship to that seen in either the archaebacteria or the eukaryotes (23). On the other hand, eukaryotes are unique in using three separate RNA polymerase functions (24).

The fossil record indicates that photosynthetic eubacteria (and by inference, therefore, archaebacteria and possibly eukaryotes) were already in existence 3–4 billion years ago (25), so that the evolutionary events that transformed the ancestor common to all life into the individual ancestors of each of the three major groups must have occurred over a relatively short time span early in the planet's history. Both the relatively rapid pace of, as well as the profound changes associated with, this early evolutionary transition argue that this universal ancestor was a simpler, more rudimentary entity than the individual ancestors that spawned the three groups (and their descendants) (26).

Fig. 1 is a universal phylogenetic tree, showing the relationships among the primary groups. The root of the tree is seen to separate the eubacteria from the other two primary groups, making the archaebacteria and eukaryotes specific (but distant) relatives. A relationship between archaebacteria and eukaryotes is not overly surprisingly, for with few exceptions (the rRNA being one) the archaebacterial versions of molecules resemble their eukaryotic homologs more than their eubacterial ones (24, 29, 30). Among the ribosomal proteins there are even cases where the archaebacterial and
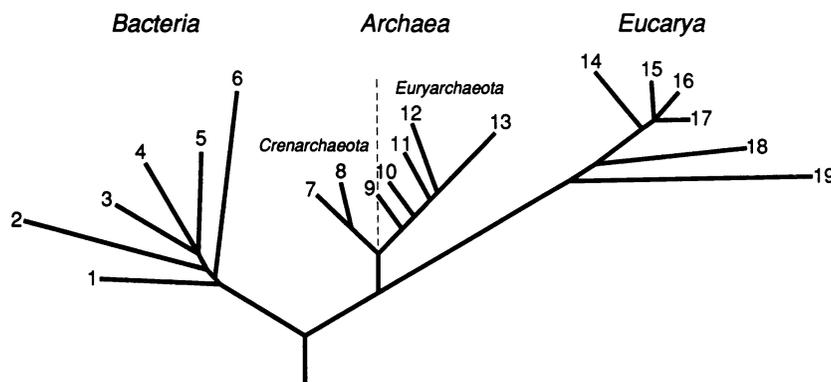
FIG. 1.   Universal phylogenetic tree in rooted form, showing the three domains. Branching order and branch lengths are based upon rRNA sequence comparisons (and have been taken from figure 4 of ref. 2). The position of the root was determined by comparing (the few known) sequences of pairs of paralogous genes that diverged from each other before the three primary lineages emerged from their common ancestral condition (27). [This rooting strategy (28) in effect uses the one set of (aboriginally duplicated) genes as an outgroup for the other.] The numbers on the branch tips correspond to the following groups of organisms (2). Bacteria: 1, the Thermotogales; 2, the flavobacteria and relatives; 3, the cyanobacteria; 4, the purple bacteria; 5, the Gram-positive bacteria; and 6, the green nonsulfur bacteria. Archae: the kingdom Crenarchaeota: 7, the genus *Pyrodictium*; and 8, the genus *Thermoproteus*; and the kingdom Euryarchaeota: 9, the Thermococcales; 10, the Methanococcales; 11, the Methanobacteriales; 12, the Methanomicrobiales; and 13, the extreme halophiles. Eucarya: 14, the animals; 15, the ciliates; 16, the green plants; 17, the fungi; 18, the flagellates; and 19, the microsporidia.

eukaryotic homologs have no apparent counterpart among the eubacteria (29, 30).

From a systematic perspective the specific relationship between eukaryotes and archaebacteria does not require taxonomic recognition; these two groups are sufficiently dissimilar, and they diverged so early, that little would be gained by defining a taxon that encompasses both. In other words, the archaebacteria and eukaryotes themselves show the kind of profound molecular differences that distinguish either from the eubacteria.

Proposal for a New Highest Level Taxon

> The only truly scientific foundation of classification is to be found in appreciation of the available facts from a phylogenetic point of view. Only in this way can the natural interrelationships [among organisms] . . . be properly understood. (31)

A phylogenetic system must first and foremost recognize the primacy of the three groupings, eubacteria, and archaebacteria and eukaryotes. These must stand above the conventionally recognized kingdoms, Animalia and the like. This raises the question of whether the term "kingdom" should be used for the taxon of highest rank, with the traditional kingdoms being assigned to a new, lower-level taxon. For two reasons we feel this is not the correct solution: From a scientific perspective, the distinctions among eubacteria, archaebacteria, and eukaryotes are more profound than those customarily associated with kingdoms. Furthermore, two centuries of association of the label "kingdom" with the animals and (green) plants constitutes a tradition that would be most difficult and divisive to change. The most flexible and informative (and least disruptive) approach would appear to be to add a new rank at the top of the existing hierarchy. The name we propose for this new and highest taxon is "domain" (whose Latin counterpart we take to be *regio*). The formal suffix that we would associate with names of domains is -*a*, chosen for its simplicity.

Naming of the individual domains has been guided by several general considerations: (*i*) maintaining appropriate continuity with existing names; (*ii*) suggesting basic characteristics of the group; and (*iii*) avoiding any connotation that the eubacteria and archaebacteria are related to one another, which, unfortunately, is implied by their common names. For

the eubacteria the formal name *Bacteria*, based upon a traditional common name for the group, is suggested. The term *Eucarya* derives from that group's common name and captures its defining cytological characteristic—i.e., cells with well-defined encapsulated nuclei. The archaebacteria are called *Archaea* to denote their apparent primitive nature (vis a vis the eukaryotes in particular). The formal names for the domains are simple enough that they can also serve in common usage (note that this requires that "bacteria" be used in a sense that does *not* include the archaea). Additionally, "eukaryotes" will continue to be an acceptable common synonym for the Eucarya. However, we recommend abandonment of the term "archaebacteria," since it incorrectly suggests a specific relationship between the Archaea and the Bacteria.

We will not at this time address the matter of the individual kingdoms within the domains, with the exception of the Archaea. For the others, suffice it to say that there will be numerous kingdoms within each domain, and their formal structuring will require a more detailed analysis than is possible here. We anticipate that such an analysis of the Eucarya will preserve the kingdoms Plantae, Animalia, and Fungi (with the last somewhat restructured to reflect new molecular insights), and will replace Protista with a series of kingdoms corresponding to the various ancient protistan lineages. For the Bacteria, we expect that the majority of the described "phyla" (2) will deserve elevation to kingdom rank.

There are, however, two reasons for suggesting formal names for the kingdoms that constitute the Archaea at this time: One is that the phylogenetic structure of the domain seems relatively simple and well defined at the kingdom level. The other is that the kingdoms within the Archaea have never had appropriate names of any kind.

Phylogenetically the Archaea fall into two distinct groups, two major lineages (refs. 2 and 32; see Fig. 1). One, the methanogens and their relatives, is phenotypically heterogeneous, comprising extreme halophiles, sulfate-reducing species (the genus *Archaeoglobus*), and two types of thermophiles (the genus *Thermoplasma* and the *Thermococcus-Pyrococcus* group), in addition to the three methanogenic lineages (2, 33). The proposed formal name for the methanogens and their relatives is *Euryarchaeota*. For this kingdom we use the common name euryarchaeotes or, more casually, euryotes.

The other archaeal kingdom comprises most of what have been variously called the "thermoacidophiles," "sulfur-dependent archaebacteria," "eocytes," or "extreme thermophiles." It is a physiologically relatively homogeneous group, whose niches are entirely thermophilic (2). Since thermophily is the only general phenotype that occurs on both major branches of the Archaea, it is presumably the ancestral phenotype of the Archaea (2). For this kingdom we suggest the name Crenarchaeota. In common usage crenarchaeotes or crenotes would be acceptable.

Definitions

Domain Eucarya [Greek adjective εὗ (good; true in modern common usages); and Greek noun κάρυον (nut or kernel; refers to the nucleus in modern biological usage)]: cells eukaryotic; cell membrane lipids predominantly glycerol fatty acyl diesters; ribosomes containing a eukaryotic type of rRNA (2, 18, 19).

Domain Bacteria [Greek noun βακτήριον (small rod or staff)]: cells prokaryotic; membrane lipids predominantly diacyl glycerol diesters; ribosomes containing a (eu)bacterial type of rRNA (2, 18, 19).

Domain Archaea [Greek adjective ἀρχαῖος (ancient, primitive)]: cells prokaryotic; membrane lipids predominantly isoprenoid glycerol diethers or diglycerol tetraethers; ribosomes containing an archaeal type of rRNA (2, 18, 19).

Kingdom Euryarchaeota (Archaea) [Greek adjective εὐρύς (broad, wide, spacious), for the relatively broad spectrum of niches occupied by these organisms and their varied patterns of metabolism; Greek adjective ἀρχαῖος (ancient, primitive)]: ribosomes containing a euryarchaeal type of rRNA (2, 18, 19).

Kingdom Crenarchaeota (Archaea) [Greek noun κρήνη (spring, fount), for the ostensible resemblance of this phenotype to the ancestor (source) of the domain Archaea; and Greek adjective ἀρχαῖος (ancient, primitive)]: ribosomes containing a crenarchaeal type of rRNA (2, 18, 19).

Conclusion

The system we propose here will repair the damage that has been the unavoidable consequence of constructing taxonomic systems in ignorance of the likely course of microbial evolution, and on the basis of flawed premises (that life is dichotomously organized; that negative characteristics can define meaningful taxonomies). More specifically, it will (*i*) provide a system that is natural at the highest levels; (*ii*) provide a system that allows a fully natural classification of microorganisms (eukaryotic as well as prokaryotic); (*iii*) recognize that, at least in evolutionary terms, plants and animals do not occupy a position of privileged importance; (*iv*) recognize the independence of the lineages of the Archaea and the Bacteria; and (*v*) foster understanding of the diversity of ancient microbial lineages (both prokaryotic and eukaryotic).

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