

Perspective

Two empires or three?

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In the last 100 years, several spectacular discoveries were made in the world of biodiversity, discoveries such as the Okapi in the Congo forest, the only living relative of the giraffes, or Latimeria in the Indian Ocean, a living coelacanth fish, believed to have been extinct for 60 million years. But these were merely small white spots on the world map of biodiversity. By contrast, Carl Woese's discovery of the archaeobacteria was like the discovery of a new continent. Where should one place this new group of microorganisms?

From antiquity until the twentieth century, the traditional division of the living world was into animals and plants. Even today, biology is taught in zoology and botany departments in many colleges and universities, particularly abroad. Botany, for a long time, was defined as including anything living that is not an animal. As a result, the study of fungi and bacteria was assigned to botany departments. Indeed, in the nineteenth century, some of the leading bacterial taxonomists had their professorships in botany departments. The subdivisions within animals and plants were equally unbalanced.

After 1859, the study of phylogeny produced great advances in our understanding of the relationship of animals. The recognition by Grobben (1) that "above" the coelenterates there are two major groupings of animals, the Protostomia and the Deuterostomia, was a great leap forward. The most important recent development, of course, was the use of molecular methods in the field of classification. Although usually confirming the results of morphological analysis, molecular methods are all-important in all cases of controversy and/or uncertainty. It is now only a question of time until the true relationship of all phyla and classes of animals is firmly established.

Although foreshadowed by suggestions made by earlier authors, by far the most important advance made in our understanding of the living world as a whole was the realization by Chatton (1937) (2) that there are two major groups of organisms, the prokaryotes (bacteria) and the eukaryotes (organisms with nucleated cells). This classification was confirmed and made more widely known by Stanier and van Niel (3), and it was universally accepted by biologists until recently.

As far as the eukaryotes are concerned, it was soon realized that the fungi are not plants; in fact, molecular studies showed that they are actually more closely related to animals. All single-celled eukaryotes were at first placed into the phylum Protista. Although recent studies, particularly molecular analyses, have shown that the Protista are a very heterogeneous assemblage, consisting of single-celled algae (formerly plants), protozoans (formerly animals), water molds (formerly fungi), and members of many other groups, it is still convenient to speak of unicellular eukaryotes in the vernacular as protists. The number and kind of higher taxa of eukaryotes one should recognize for the various types of protists, in addition to the

kingdoms of plants, fungi, and animals, are still under discussion.

The classification of the prokaryotes was chaotic until very recently. Woese (4) considerably clarified by molecular analysis the relationship of the various kinds of bacteria to each other and determined what kind of classification one should adopt. By far his most important discovery was that the prokaryotes actually consist of two major groups: (i) the traditional bacteria, at first best known from the study of human diseases, and (ii) a previously unrecognized group of bacteria, named by Woese (5) archaeobacteria. This group is not only quite different from the eubacteria, as Woese renamed the traditional bacteria, but also of special interest for two reasons. The first reason is that they contain all sorts of highly specialized organisms that can live in very unusual environments, which at first sight would seem totally unsuited for life, such as hot springs, sulfur springs, brines, etc. However, in recent years it has been found that archaeobacteria are also common in many normal environments, such as sea water, rice fields, and marshes (6). Even more interesting was the discovery that the archaeobacteria share many important genes with the eukaryotes; indeed, further studies proved that the eukaryotes had an archaeobacterial root.

Woese baptized the newly discovered organisms archaeobacteria, thinking they would have been the first organisms on the newly habitable earth because of their ability to live in an anoxic atmosphere and in hot springs, sulfur springs (thermoacidophiles), brines (halophiles), and other unusual habitats, presumably common on the new earth. However, when it later appeared probable that they were not the most ancient bacteria and might have a common stem with the eubacteria, Osawa and Hori (7) suggested replacing the misleading name archaeobacteria by metabacteria. Neither Woese (8) nor other microbiologists accepted this change of name. Instead Woese renamed them Archaea, retaining the inappropriate component—*archae*—and discarding the informative component—*bacteria*, which revealed their prokaryote nature.

Woese's discoveries and interpretations were widely acclaimed and accepted, with one exception. Woese was so impressed by the distinctness of this new group of bacteria that he proposed to give the same categorical rank to the archaeobacteria as to the totality of all eukaryotes. Instead of recognizing the two traditional taxa of organisms, the prokaryotes and the eukaryotes, he proposed to recognize three domains, the eubacteria, the archaeobacteria, and the eukaryotes (8). It was this proposal that created considerable opposition, particularly outside microbiology. Woese in his early work apparently based his decision on two assumptions, that the eubacteria and archaeobacteria had independently arisen from the progenote, his hypothetical universal ancestor of life, and that "on the molecular level [the archaeobacteria] resemble other prokaryotes, the eubacteria, no more (probably less) than they do the eukaryotes" (8). Both assumptions were soon refuted, and Woese now bases his three-domain arrangement on

different arguments. These arguments and my objections to them cannot be understood without a few comments on current taxonomic theories. Here it must be remembered that Woese was not trained as a biologist and quite naturally does not have an extensive familiarity with the principles of classification. Virtually all previous discussions on the ranking of the archaeobacteria were made by microbiologists; therefore, the present comments by a student of the eukaryotes may help to achieve a more equitable balance.

Systems of Ordering

Taxonomists currently recognize two systems of ordering taxa: Darwinian classification and Hennigian cladification (9). In a classification, organisms are grouped into taxa on the basis of two criteria, similarity and genealogy. A higher taxon recognized by these criteria is composed of a group of similar and/or related species descended from their nearest common ancestor. Such a taxon is called monophyletic. In a cladification, favored by cladists, only genealogy is considered. It recognizes branches (clades) of the phylogenetic tree, composed of the stem species of such a branch together with all of its descendants. The difference between the two methodologies can be illustrated by the following examples. The mammals arose from a branch of the reptiles (therapsids, pelycosaurs) and the stem species of this synapsid branch of the reptiles lived in the Paleozoic. Similarly, the birds were derived from another branch of the reptiles, the archosaurians (which includes the dinosaurs), and the stem species of this branch lived in the early Mesozoic. In both cases, the cladist removes the branches that gave rise to the mammals or birds from the reptiles, thereby making the reptiles, a taxon used in our every-day grouping of animals, a "paraphyletic group,"[†] not permissible as a formal taxon in a strictly cladistic arrangement. In both cases, the Darwinian taxonomist, who deals with groups rather than with branches, retains the ancestral groups within the Reptilia and recognizes as mammals or birds only those assemblages of species which by their diagnosis are characterized as mammals or birds. It was on this basis that Stanier and van Niel recognized two empires, the prokaryotes and the eukaryotes.

Branching points of clades are all-important in a cladification, but degree of similarity of the branches is not considered. Only derived (apomorph) characters are used by the cladist in the recognition of the branches. By contrast, taxa in a Darwinian classification are ordered by their similarity, provided that each recognized taxon is monophyletic.

A number of bacteria (methanogens and halophiles) were known to Stanier and van Niel when they accepted the distinction between prokaryotes and eukaryotes. Because of the archaeobacteria's phenotypic similarity to the other bacteria, these authors unhesitatingly included these archaeobacteria among the prokaryotes. However, after Woese had pointed out the differences between eubacteria and archaeobacteria, the question was raised whether these differences are sufficiently great to require the replacement of the traditional two taxa classification by a three-domain[‡] classification, as proposed by Woese.

On the basis of which ordering system did Woese switch from the two taxa to the three domain arrangement? Or to

phrase this question somewhat differently, did Woese follow the principles of Darwin's classification or of Hennig's cladification when adopting three domains? Woese makes it quite clear in several statements that he favors "a phylogenetic system," a terminology Hennig frequently uses for his cladification. He follows cladistic principles in his rather strict reliance on the joint possession of derived (synapomorph) characters by the archaeobacteria and eukaryotes and in his emphasis on the location of the branching points. He also adopts another cladistic principle. The archaeobacteria and the eukaryotes are sister groups as a result of the branching-off of the eukaryotes from the archaeobacteria. According to the original Hennigian principles, sister groups must always be given the same categorical rank. Hence the archaeobacteria must be removed from the prokaryotes and given the same high rank as the eukaryotes. Woese does not seem to realize that this sister group ranking principle of Hennig had led to so many difficulties that, as a principle, it was quickly abandoned by most cladists.

The most fateful decision Woese made was to follow the cladistic tradition and largely ignore autapomorphic characters. This term pertains to derived characters found in only one of two sister groups. This procedure is an application of Hennig's principle to base classification only on genealogy and to ignore degree of difference. In the present case, it means to ignore the derived characters that are so diagnostic for the eukaryotes (see below). On the whole, Woese, thus, follows cladistic principles.

But Woese is not consistent in the adoption of these principles. For instance, he does not reject "paraphyletic" taxa, as one must in a cladification. The archaeobacteria consist of several branches. "The evidence suggests that the primary group archaeobacteria underwent several early radiations resulting in genetically distant lineages" (10). There is no consensus yet as to which of these lineages gave rise to the eukaryotes. This event might have occurred quite early or rather late in the history of the archaeobacteria. If the eukaryotes evolved out of one of the later lineages, the archaeobacteria would be a paraphyletic taxon for a cladist. If the branching had occurred very early, it is remarkable how long and to what extent the two kinds of prokaryotes retained their bacterial similarities. Also, archaeobacteria and eukaryotes are derived from the same stem species, and a cladist would have to combine them into a single clade. In both of these situations, Woese does not follow the principles of cladification.

The traditional definition of the prokaryotes was that they "[had] anucleate cells, without membrane enclosed organelles of respiration or photosynthesis, divided by fission not mitosis, and used peptidoglycan to strengthen their cell walls" (3). Although this definition was based primarily on the eubacteria, it is equally true for the archaeobacteria except that archaeobacteria lack peptidoglycan. Woese, seemingly having largely adopted the cladistic principle that only derived characters should be used in a system of ordering, considered the prokaryotes to be an unnatural group because they were based on "negative" characters, the absence of a nucleus and of mitosis. This argument is, of course, not valid at all for a Darwinian classification system. To be sure, only derived characters can be used in a cladistic analysis, but in a traditional classification, as many characters are to be used as are available. The nonpossession of a character is as positive a character in any traditional classification as is its possession (except in cases when the loss of a character can be determined with certainty). As I have remarked elsewhere (9), the possession of certain ancestral characters is often the most characteristic feature of a taxon.

How Different Are the Archaeobacteria from the Other Bacteria?

In contrast to a Hennigian cladification, the Darwinian classification uses two sets of criteria. Although all taxa must be

[†]Paraphyletic is a term given by the cladists to an otherwise monophyletic group of species, from which a clade (branch) had been removed because it subsequently gave rise to a derived group. For instance if the branch consisting of the pelycosaurs and therapsids that eventually gave rise to the mammals is removed from the Reptilia, the latter become "paraphyletic." Paraphyly does not occur in Darwinian classification.

[‡]The highest rank in the Linnaean hierarchy has never been formally named. Woese uses the word domain, others have referred to it as superkingdom or empire. Being one step higher than kingdom, I like the term empire.

monophyletic, that is, descended from the nearest common ancestor, they are ranked according to the degree of difference from each other. Therefore, one must ask, are the archaeobacteria as different from the eubacteria as from the eukaryotes or are they much more similar to the eubacteria, thus justifying the inclusion of both kinds of bacteria in the prokaryotes and confirming the two-empire classification?

Some of the more specialized archaeobacteria differ strikingly from other bacteria, yet when the first methanogens and halobacteria were discovered, their describers did not realize that they were different from the other bacteria because they are to such a degree similar phenotypically to the eubacteria. The acquisition of a nucleus (and all the associated features, such as mitosis and multiple chromosomes) by the eukaryotes was perhaps the most important evolutionary event in the whole history of life. It created a deep cleavage between two kinds of organisms, the bacteria and the eukaryotes. A skeptic might ask, are there any other indications of a great similarity of the archaeobacteria to the eubacteria? Yes, more and more such similarities continue to be discovered. Viable cell fusion (opening and closing of membranes) in feeding and sexuality, and intracellular motility clearly distinguish prokaryotes from eukaryotes (see below).

Furthermore, the genomes of two archaeobacteria have now been sequenced completely (11, 12), and it was possible to determine what part of their genomes was more eubacterial and what part more eukaryotic. The figures for the two kinds of archaeobacteria are remarkably similar.

Koonin *et al.* (11) found that 44% of the *Methanococcus jannaschii* gene products show "significantly higher similarity to bacteria than to eukaryotic proteins as their closest homologues, compared with 13% that have eukaryotic proteins as their closest homologues (the rest of the proteins show approximately the same level of similarity to bacterial and eukaryotic homologues or have no homologues)." Very similar percentages were found by Smith *et al.* (12) for the genome of *Methanobacterium thermoautotrophicum*. "When the *M. thermoautotrophicum* ORFs are compared with sequences from only the eucaryal and bacterial domains, 786 (42%) are more similar to bacterial sequences and 241 (13%) are more similar to eucaryal sequences" (12). Combining the two data sets, one can say that of proteins or sequences that can be definitely assigned, about 77% are more eubacterial and less than a quarter (23%) are eukaryotic. These percentages reinforce the conclusion that the two kinds of bacteria are far more similar to each other than are the archaeobacteria to the fully evolved eukaryotes.

How Did the Archaeobacteria Originate?

If the archaeobacteria had originated from the eubacteria by the conventional process of budding, one might have expected that they would have acquired new genes (characters) randomly throughout their genome, but this does not seem to be the case. Entire functional gene complexes of the archaeobacteria seem to be either eubacterial or eukaryotic. For instance, the transcription, translation, and splicing machineries of the archaeobacteria resemble those of the eukaryotes, while the majority of the functional genes, coding primarily for metabolic enzymes, transport systems and enzymes of cell wall biogenesis, resemble the eubacterial ones. Microbiologists have reviewed a number of possible explanations for this mosaic constitution, but none of them seems to be, at the present time, particularly convincing. Koonin *et al.* (11) for instance have come up with the suggestion that the archaeobacteria might have originated by a fusion event similar to that which later in evolution gave rise to the eukaryotes. If true, an eubacterium-like organism would be one of the ancestral genotypes of this chimera, but what would be the other? Koonin *et al.* suggest "an ancestral cell from the lineage that

gave rise to the eukaryotic nucleocytoplasm." This conjecture has so far no factual support, and no living organism is known that would fit this postulate. Also, how had this organism originated? Thus, the origin of the archaeobacteria is still an unsolved puzzle. It is evident from all recent discussion that the (geological) time of the origin of the archaeobacteria is also highly controversial. Some authors, like Woese, would place it very early, let us say, before a concrete taxon eubacteria had evolved; other authors believe that the archaeobacteria can be derived from one of the lineages of the eubacteria. It depends on whether one ascribes the eubacterial components of the archaeobacteria to an original common root or to frequent gene transfer in later times. Ultimately, however, what are now the eubacteria and the archaeobacteria are derived from a common root. So much about the prokaryotes is still unknown that the ultimate solution might be quite different from any current conjecture. We are in the happy situation of a still wide-open frontier. Actually, the answer to this question is of little relevance for the theme of this paper. We are concerned only with the question whether the archaeobacteria, as they now exist, are as different from the eubacteria as the bacteria as a whole are from the eukaryotes.

What Is the Objective of a Classification?

A classification is an information storage and retrieval system. Its aim is to permit you to locate an item with a minimum of effort and loss of time. This is as true for a classification of books in a library or goods in a store as for taxa of organisms. This objective is optimally achieved by arranging the to-be-classified items in a hierarchy of classes, ranked by degree of similarity. In the traditional Linnaean hierarchy, we distinguish species, genus, family, all the way up to phylum, kingdom, and empire. Each of these levels is called a category, and each taxon (group of similar and related organisms) is given a position (rank) at one of these levels.

One of the basic principles of a good classification is the principle of balance, which states that the retrieval of information is greatly facilitated if the taxa at a given categorical rank are, as far as possible, of equal size and degree of diversity. Alas, nature is far too disorderly to permit the taxonomist always to live up to the ideal of this principle. The number of monotypic higher taxa is evidence for this difficulty. Yet, at all times a balanced equivalence of taxa is an ideal the taxonomist tries to approach as closely as possible.

The question then is does a two-domain or three-domain arrangement of the living world agree better with the principle of balance? So far, I believe only about 175 different archaeobacterial groups have been described. It is quite likely that further research will find thousands, but hardly more than that. Approximately 10,000 eubacteria have been named. The number of species of eukaryotes probably exceeds 30 million; in other words, it is greater by several orders of magnitude. The number of species of birds alone is $\approx 10,000$, and there are many millions of species of insects.

The same is true for phenotypic diversity. From the viewpoint of fundamental biology, genetics, and development, all archaeobacteria are nearly indistinguishable. Even when combined with the eubacteria, as prokaryotes, this group does not reach anywhere near the size and diversity of the eukaryotes. The principle of balance, thus, clearly favors combining eubacteria and archaeobacteria in the empire Prokaryota.

Phenotypes and Evolution

Evolution is an affair of phenotypes. It is phenotypes, not genes, that are the objects (targets) of selection. This is now generally accepted by evolutionists after 50 years of controversy. Indeed, the significance of a molecular change is usually best indicated by its phenotypic consequences.

Here it must be remarked that the phenotypic difference between the two kinds of prokaryotes is minimal as compared with the difference between, let us say, a bacterium and a plant or animal. There is simply a huge gap between a prokaryote and any of the eukaryotes, even the simplest protists. How similar the two kinds of bacteria are to each other is well illustrated by the fact that, as mentioned, none of the microbiologists who described the first archaeobacteria (before their naming by Woese in 1977) realized that these bacteria were anything very special. Phenotypically they simply were bacteria.

Woese bases his support of cladification on that small, even though functionally highly important, piece of genome that the archaeobacteria share with the eukaryotes. However, this is only a small fraction of the eukaryote genome. Even excluding all the noncoding nucleic acids, the eukaryote genome is larger than the prokaryote genome by several orders of magnitude. And it is precisely this part of the eukaryote genome that is most characteristic for the eukaryotes. This includes not only the genetic program for the nucleus and mitosis, but the capacity for sexual reproduction, meiosis, and the ability to produce the wonderful organic diversity represented by jellyfish, butterflies, dinosaurs, hummingbirds, yeasts, giant kelp, and giant sequoias. To sweep all this under the rug and claim that the difference between the two kinds of bacteria is of the same weight as the difference between the prokaryotes and the extraordinary world of the eukaryotes strikes me as incomprehensible.

That the prokaryotes (consisting of both kinds of bacteria) are a natural group is documented not only by their overall similarity but also by other evidence. Because the prokaryotes, in contrast to most eukaryotes, do not have sexual reproduction, they maintain their genetic variability by unidirectional gene exchanges among different lineages, even between rather distant lineages. The close relationship between eubacteria and archaeobacteria is documented by the discovery "that genes have been exchanged between eubacteria and archaeobacteria on numerous occasions" (13). This makes it very difficult to reconstruct the origin of the archaeobacteria.

Rank in a classification is based on degree of difference. If the degree of difference between eubacteria and archaeobacteria were chosen as the yardstick for the recognition of an empire, I wonder into how many empires the highly diverse eukaryotes would have to be broken up? Among the protists alone ≈ 50 natural groups can be distinguished (e.g., ciliates, diatoms, red algae, etc.). It has produced an altogether unbalanced classification to remove the archaeobacteria from the prokaryotes. I cannot see any merit at all in a three empire cladification. To preclude misunderstandings, let me emphasize that I support the dichotomy prokaryotes vs. eukaryotes not owing to a philosophical preference for a dichotomous division but because this is where the great break is in the living world. Most higher taxa actually have multiple subdivisions. In the eukaryotes, for instance, even based solely on 18S RNA criteria, at least six major lineages can be distinguished and within the animals ≈ 30 phyla.

Conclusion

The evidence presented by me shows clearly that the archaeobacteria are so much more similar to the eubacteria than to the eukaryotes that their removal from the prokaryotes is not justified. The eukaryotes differ from the prokaryotes (including the archaeobacteria) not only by the possession of a nucleus and mitosis but also by individual protein-rich chromosomes, meiotic sexuality (including viable regular cell fusions), cellular organelles, highly complex sets of regulatory genes, and all those genes that permit the marvelous world of biodiversity. Of course, after their origin, eukaryotes continued to evolve, and some of the most primitive protists do not yet have the complete set of eukaryote characteristics (e.g., mitochondria, *Giardia*). However, when a biologist speaks of eukaryotes, he has in mind palms, oaks, and orchids; mice, bats, and whales; and hummingbirds, chickens, and ostriches. And this world of highly evolved eukaryotes is simply an entirely different world from the world of the two kinds of bacteria, the Prokaryotes.

Ranking, in any scheme of classification of items (living or not), is by necessity based on degree of difference. The two kinds of bacteria, in the vast majority of their characteristics, are exceedingly similar to each other and fundamentally so different from the eukaryotes that they have to be ranked as a single taxon, the prokaryotes, different from the only other taxon of this rank, the eukaryotes. Only a two-empire classification correctly reflects this structure of the living world. It has the additional virtue that it is a far superior information retrieval system and was therefore adopted by nearly everybody as soon as it was carefully argued by Stanier and van Niel (3).

I am not a microbiologist and could have never undertaken this analysis without a great deal of help from a number of specialists. Earlier drafts were seen and commented on by Francisco Ayala, Ken Kinman, E. V. Koonin, James A. Lake, Lynn Margulis, and Frederick Neidhardt. I am deeply grateful to these readers for numerous constructive suggestions. They greatly helped me to eliminate errors and improve my presentation.

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