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Problems with "Procaryote"[∇]

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Conjecture plays an important role in scientific experimentation, but scientific foundations need to be based on facts, not conjectures. Biologists of the last century prematurely embraced a conjecture that turned out incorrect, what I will call the procaryote-eucaryote model of biological organization and evolution.

The procaryote-eucaryote model, as is explicit in the language, posits that fundamentally there are two kinds of organisms, procaryotes and eucaryotes. Furthermore, the prefix "pro" injects the connotation that procaryotes preceded eucaryotes because "pro" means before. This procaryote-eucaryote model dominates our textbooks and discourse in matters of taxonomy and deep evolution.

The thesis of this article is that the notion of procaryote is now obsolete and counterproductive. Here, I (i) trace the source of the procaryote-eucaryote model, (ii) show why it is wrong scientifically, and (iii) comment on the damage it has done to our perception of important biological issues. I conclude that "procaryote" needs to be retired from the lexicon of biology.

THE ESSENCE OF THE ARGUMENT

The procaryote-eucaryote concept was a specific hypothesis, and it has been proven wrong by 3 decades of results in molecular phylogeny and biochemistry. These results, summarized below, show that life's diversity falls into three fundamentally distinct phylogenetic domains: *Bacteria*, *Eucarya* (eucaryotes), and *Archaea*. Moreover, the results show that archaea and eucaryotes evolved independently of the bacterial line of descent. Thus, there is no such thing as a "procaryotic" relatedness group of organisms in any phylogenetically based taxonomy. It makes no sense to lump bacteria with archaea phylogenetically. Furthermore, the molecular tree reveals no group of organisms that preceded eucaryotes.

The accompanying diagrams contrast the procaryote-eucaryote and three-domain models for the large scale of biological organization and the course of evolution.

WHERE PROCARYOTE STARTED

The problems with procaryote are not new. The history of the procaryote concept and its incorporation into the conventional wisdom of biology has been reviewed (4, 8, 9).

In 1962, R. Y. Stanier and C. B. van Niel, seeking a "concept of a bacterium," proposed that "The distinctive property of bacteria and blue-green algae is the prokaryotic nature of their cells . . ." (5). By "prokaryotic nature" they meant their postulated "common cellular organization." Thus, by negative definition, lack of a nuclear membrane meant procaryote. The choice of language, procaryote and eucaryote, explicitly invoked the evolutionary model.

Beyond selection of terminology, Stanier and van Niel did not specify an evolutionary model. Stanier, with colleagues M. Doudoroff and E. A. Adelberg, did so subsequently in the second edition of their popular textbook *The Microbial World* in 1963 (6), where they stated that "It is not too unreasonable to consider that the bacteria and blue-green algae represent vestiges of a stage in the evolution of the cell which, once it achieved a eucaryotic structure in the ancestors of the present-day higher protists, did not undergo any further fundamental changes through the entire subsequent course of biological evolution." (p. 85). They concluded that "All these organisms ["lower protists"] share the distinctive structural properties associated with the procaryotic cell (chapter 4), and we can therefore safely infer a common origin for the whole group in the remote evolutionary past . . ." (p. 409).

The procaryote-eucaryote model was welcomed by biologists and hailed—finally there was a scientific definition of bacteria. But procaryote was never described scientifically because the properties that define the two primary taxonomic groups were exclusionary, not specific. The fact remains that no one can tell you what a procaryote is, only what it is not. The origins of the two groups were guesswork. Yet, the procaryote-eucaryote model was adopted as the foundation of biological classification and evolution with little question and without test. I speculate that easy acceptance by biologists occurred because it neatly fit 19th century concepts of evolution prevalent in textbooks even today, with "monera" (also known as procaryotes) at the base of a tree of complex eucaryotes.

EXPERIMENTAL DISPROOF OF THE PROCARYOTE CONCEPT

The first experimental test of the procaryote-eucaryote hypothesis came with the introduction of molecular phylogeny and Carl Woese's landmark comparisons of small-subunit (16S) rRNA sequences in 1977 (7). In contrast to the physiological and morphological properties traditionally used in microbial taxonomy, comparison of gene sequences provided an objective metric for evolutionary diversity. Simply put, the extent of sequence difference between orthologous genes in different organisms is a measure of evolutionary distance and relationship. Consequently, comparisons of sequences can be used to extract maps of evolutionary relatedness of organisms, phylogenetic trees. rRNA sequences, because of their ubiquity and high degree of conservation, became the "gold standard"

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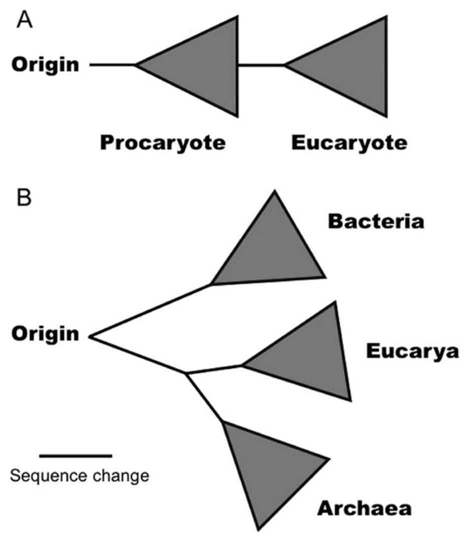

FIG. 1. Models of the pattern of life's evolution. The wedges represent the genetic radiations from the base of the respective primary relatedness groups. Panel A represents the procaryote-eucaryote model, and panel B represents the three-domain model based on molecular properties.

for relating all life. Additionally, the rRNA genes have not undergone appreciable lateral transfer, as have some genes. The other elements of the nucleic-acid-based information processing system are phylogenetically congruent with the rRNA. Consequently, the rRNA tree tracks the evolution of the cell's genetic machinery, at the very least.

The results of rRNA-based molecular phylogenetic studies have been reviewed extensively (e.g., see references 2, 3, and 9). Analyses of many rRNA, and now genome, sequences have solidified Woese's early findings of three primary relatedness groups, the phylogenetic domains of *Bacteria*, *Eucarya*, and *Archaea*. Woese originally dubbed this latter group "archaebacteria," although the sequences and biochemical correlates would show that the traditional bacteria and archaea are fundamentally distinct kinds of organisms.

The pattern of the three-domain tree (Fig. 1) shows that there is no phylogenetically coherent group that can be described as procaryotic. The pattern of the molecular tree also shows that none of the primary domains is derived from another. The molecular results prove that the major eucaryotic organelles, mitochondria and chloroplasts, had their origins among bacteria (not shown in Fig. 1). But the eucaryotic nuclear line of descent was primordial in origin, as old as the archaeal line and derived from neither bacteria nor archaea.

THE RELATIONSHIPS OF THE PHYLOGENETIC DOMAINS

The location of the "root" of the universal tree, the position of the last universal common ancestral state, is critical to the legitimacy of the term procaryote. This root cannot be inferred from rRNA data. However, other phylogenetic results put the root of the molecular tree on the line between the bacterial radiation and the separation of the eucaryal and archaeal lineages (1). This is indicated as "origin" in the diagram of the three-domain tree in Fig. 1. The position of the root of the tree fundamentally separates bacteria and archaea. There is no phylogenetically based grouping that contains bacteria and archaea. There can be no such thing as a procaryote in any phylogenetic classification.

The phylogenetic differentiation of the bacterial, archaeal, and eucaryal lines of descent is supported by many biochemical correlates. For instance, whereas bacteria use sigma factors to control transcription initiation, both archaea and eucarya use a different mechanism, TATA-binding proteins. As another example, bacteria wrap their DNA in a variety of basic proteins, while eucaryotes and many archaea both use histones. The early common history and such similarities do not mean that archaea are rudimentary eucaryotes. A vast evolutionary history separates archaeal and eucaryotic cells, and they have fundamental differences. Eucaryotes (and bacteria), for instance, make their membranes from ester-linked lipids, whereas archaea use ether-linked lipids.

THE DAMAGE OF PROCARYOTE: IT'S MORE THAN TERMINOLOGY

The conflict between the procaryote-eucaryote and three-domain models is not simply one of terminology, the names we give to organisms. So what does it matter if we lump together bacteria and archaea? Maybe, some would argue, we can use the term "procaryote" informally, to refer to small organisms that are not members of the domain eucarya, noneucaryotes. However, this is not satisfactory terminology because it confuses an invalid scientific term with a colloquial one. "You know what I mean" is no sensible foundation for biological classification. Moreover, the language imparts an incorrect model of evolution.

The phylogenetic organization of biological diversity and the course of evolution are foundational biological concepts. Intellectual progress in biology requires a proper perception of life's natural organization and evolution. The procaryote notion distorts and misleads. Taken literally, it would have stipulated that experiments to probe distinctions between archaea and bacteria would not be necessary. Procaryote smothered interest in microbial evolution and obstructed acceptance of archaea, the first test of the procaryote hypothesis. The procaryote-eucaryote dogma has paralyzed thought and teaching on the origin of the eucaryotic nucleus. It permeates our textbooks and journals with subtle and not-so-subtle misinformation. Thus, the procaryote-eucaryote dogma damaged and continues to damage microbiology by retarding, even denying, progress. It elicits false concepts and misdirects inquiry. There is no place for procaryote in modern scientific discourse.

WHAT TO DO ABOUT IT

The facts are on the table, but it will take time for the terminology of procaryote to disappear from our textbooks and language. Nonetheless, the process needs to start and it needs to be catalyzed. We microbiologists need to take the lead in rectifying the misconceptions because we are closest to the problem of how to understand and describe highly disparate organisms.

An early challenge to microbiologists is to stop using the term "procaryote." This is hard to do because of long conditioning. Those tempted still to use it must realize, however, that they saddle their students with misconception and muddy their thinking about important biological problems. An alternative, catch-all descriptive (not taxonomic) term that I use and recommend is "microbe," which captures the microbial quality of size and includes, as well, the poorly acknowledged microbial eucaryotes. But beyond that, it is necessary to be more specific. The adjective "procaryotic" is almost always misleading; for example, it is scientifically more correct and far more illuminating to distinguish bacterial and archaeal transcription than it is to lump them together into "procaryotic transcription."

How should we teach this issue in the context of the currently pervasive use of "procaryote" in textbooks and journals? In fact, the discordance between currently emerging data and the conventional thought on deep evolutionary relationships is a wonderful example of how science, biology in this case, is an ongoing, living process. Bringing the subject to students shows them how new information based on experimental evidence can change in fundamental ways how we understand important natural processes. Dealing with the procaryote-eucaryote issue provides a good example of weighing specific models, hypotheses for testing in the face of experimental data. Phylogenetic trees, maps of evolutionary relationships, are not hard to understand in essence. They are abstract, to be sure, but provide graphic information that is readily interpreted by students. The three-domain concept, of course, poses many questions, but it also provides a solid foundation for progress toward answering those questions.

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