Classic Spotlight: 16S rRNA Redefines Microbiology

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We cannot imagine current evolutionary biology, taxonomy, microbial ecology, environmental microbiology, and human microbiome studies without phylogenies derived from comparison of small-subunit (16S-18S) rRNA gene sequences. It is equally hard to imagine (at least for a younger generation of scientists) that the fundamental discovery of the three domains of life—*Bacteria*, *Archaea*, and *Eukarya*—made by Carl Woese and George Fox in 1977 using rRNA as an evolutionary marker (1) came nearly 20 years before the first genome was sequenced (2) and, even more impressively, nearly 10 years before a technique for efficient 16S gene sequencing was developed (3). Those who are intrigued should read a classic perspective written by Norman Pace and colleagues (4), who thoroughly discussed the scientific and historical significance of the paper by Woese and Fox (1) and its role in our understanding of the Tree of Life. One of the most interesting parts of this story describes the years of opposition and nonacceptance of the 1977 paper, which finally claimed its place as a pivotal point in evolutionary biology and microbiology. It was only in the early 1990s that a formal designation of the three major phylogenetic groups and the universal Tree of Life were formally introduced (5) and generally accepted by the scientific community.

From this standpoint, two minireviews published in ASM journals in 1994 under symbolic titles can be viewed as a triumphal manifesto: “The Winds of (Evolutionary) Change: Breathing New Life into Microbiology” by Gary Olsen, Carl Woese, and Ross Overbeek appeared in the *Journal of Bacteriology* (JB) (6), and “There Must Be a Prokaryote Somewhere: Microbiology’s Search for Itself” by Woese was published in *Microbiological Reviews* (7). The papers summarized nearly 2 decades of intensive studies by numerous evolutionary biologists who contributed to the validation, improvement, and development of the three-domains-of-life view. By that time, more than 1,500 species of *Bacteria* and *Archaea* had been characterized by 16S rRNA sequencing, and Fig. 1 in the JB paper (a 16S rRNA-based phylogenetic tree) revealed major phyla of *Bacteria* and *Archaea*. Genomic revolution started a year later (2), and for the next 2 decades, comparative genomic studies were guided by this tree and its definition of major bacterial and archaeal taxonomic groups. This minireview also emphasized the role of 16S gene sequencing in revolutionizing microbial ecology (8, 9) and foresaw our ability “to count not just flowers and beetles, but also microorganisms, in taking a census of life on this planet” long before advances in metagenomics truly opened up this new horizon: the current release (May 2015) of the Ribosomal Database Project (10) contains more than 3.2 million annotated 16S rRNA sequences from *Bacteria* and *Archaea*.

That could have been a perfect ending for this story, but there is another interesting twist. Carl Woese, the father of 16S rRNA-based phylogenetics, published several of his early papers in JB. In following the timeline, one can only be amazed at how a biophysicist bombarding *Bacillus subtilis* spores with ion-

izing radiation (11) became interested in RNA properties of germinating spores (12, 13) and, while thinking more and more about the genetic code, developed a method for separating rRNAs (14). In comparison with fundamental discoveries, these studies might appear insignificant; however, without these (and many other) “small steps,” there simply would have been no 16S story.

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