Woese and Fox: Life, rearranged

In April 2011 an international team led by researchers at the European Molecular Biology Laboratory in Heidelberg, Germany announced in *Nature* that the mind-boggling mix of microbes in the human gut could be neatly grouped into categories called enterotypes (1). Hailed as a finding that might someday help researchers address the long-intractable problem of antibiotic resistance, the discovery of gut microbial signatures in people raised the possibility that individuals might have a defined enterotype, like a blood type, regardless of age, sex, or ethnicity (1).

The study, which garnered attention in scientific and journalistic circles, follows a long-running initiative funded by the National Institutes of Health called The Human Microbiome Project, whose goal is to catalog the genetic diversity of the trillions-strong microbial communities that inhabit our bodies. The hope is to determine how changes in the microbiome—the genetic endowment of our microbial selves—might influence health.

The microbiome project turns on researchers' ability to compare evolutionarily conserved gene sequences in human-associated microbes. Such a comparison might yield signatures that can help foretell how our bodies might respond to diets, diseases, and drugs. "With the recognition that the human body is an ecosystem that is host to ten times as many microbial cells as human cells, the prospects of the project for personalized medicine become clear," says Nigel Goldenfeld, a professor of physics at the University of Illinois at Urbana–Champaign who has worked on the evolution of biological complexity. Recent advances in DNA sequencing technology have no doubt accelerated the effort, but the microbiome project, like others aimed at documenting biological diversity, has its roots in a once-controversial discovery now enshrined in the annals of evolutionary biology.

Memorialized in a 1977 PNAS article by biologists Carl Woese and George Fox (pictured in Fig. 1), the discovery helped reclassify cellular life into three distinct domains, upending conventional views on biological classification and offering deep insights into the origin of life on Earth. To this day, *Phylogenetic structure of the prokaryotic domain: The primary kingdoms*, which courted controversy and challenged the reigning dogma of its day, remains a breakthrough—one that emphatically retraced the branches on the tree of life to better reflect its evolutionary roots (2).

"Without Woese's 1977 report, today's microbial sequencing efforts would not be meaningful. Woese put a framework of organization on microbial diversity," says University of Colorado, Boulder molecular biologist Norman Pace, a self-avowed follower of Woese.

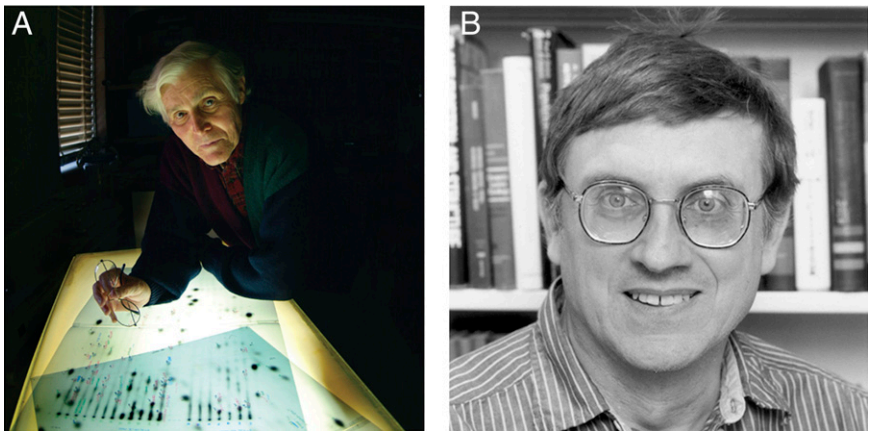

Fig. 1. (*A*) Carl Woese examining film on which ribosomal signatures are displayed (2003). (Photo by Jason Lindley; used with permission of the College of Liberal Arts and Sciences, University of Illinois at Urbana–Champaign.) (*B*) George Fox (1999). (Used with permission of the Department of Biology and Biochemistry at the University of Houston.)

Biology, by Way of Physics

A child of the 1930s Depression era, Woese was born in Syracuse, New York, where he was raised under straitened circumstances. Ever in search of comforting, objective truths, he was drawn to the reassuring consistency of mathematics' often-categorical laws. That is partly why Woese graduated with a bachelor's degree in physics from Amherst College, Massachusetts in 1950. There, he was inspired to pursue science as a career by physicist William Fairbank. Later, Woese began doctoral studies in biophysics under the guidance of Yale University physicist Ernest Pollard, whose contributions to the use of radar in World War II earned him a permanent place in the history of radiation physics. "Pollard came from a respectable lineage of physicists," Woese says, referring to an academic pedigree replete with physics heavyweights like J. J. Thomson, Ernest Rutherford, and James Chadwick. For his doctoral thesis, Woese studied how radiation and heat could inactivate viruses like Newcastle disease virus, which afflicts poultry. In 1953, a standout year in molecular biology's history that was marked by the discovery of the double helical structure of DNA, Woese graduated from Yale. After an inspired but unsuccessful foray into medicine that lasted 2 years, he returned to postdoctoral research in Pollard's laboratory, focusing on the molecular changes underlying the germination of dormant spores of the bacterium *Bacillus subtilis*.

In the next 5 years, Woese documented the formation of parts of the bacterial protein-synthesizing machinery—the ribosome—as the slumbering bacteria emerged from the spores, and studied how radiation could be used to inactivate the spores (3). At the end of his postdoctoral stint, in the fall of 1960, Woese set up his own laboratory at General Electric's Knowles Laboratory in Schenectady, New York, where he continued to explore the molecular biology of spore germination. While waiting for his laboratory equipment to arrive, Woese read voraciously on a challenge that increasingly preoccupied the decade's leading molecular biologists in the wake of the discovery of DNA structure: *cracking the genetic code*.

On the eve of the molecular biology revolution, the question of how cells made proteins began to intrigue many researchers. Of particular interest was the process by which the assemblage of the four nucleotide bases that make up DNA was interpreted by the cell into the sequence of amino acids that make up proteins. Although RNA's role as a messenger in the protein-making process was suspected at the time, the mechanics of protein synthesis was a mystery. Before long, the existence of transfer RNA, a molecule that bridged messenger RNA and amino acids, came to light, and the molecule was thought to act as an adaptor in accordance with the hypothesis advanced by Francis

See Classic Article "Phylogenetic structure of the prokaryotic domain: The primary kingdoms" on page 5088 in issue 11 of volume 74.

See Classic Perspective on page 1011.

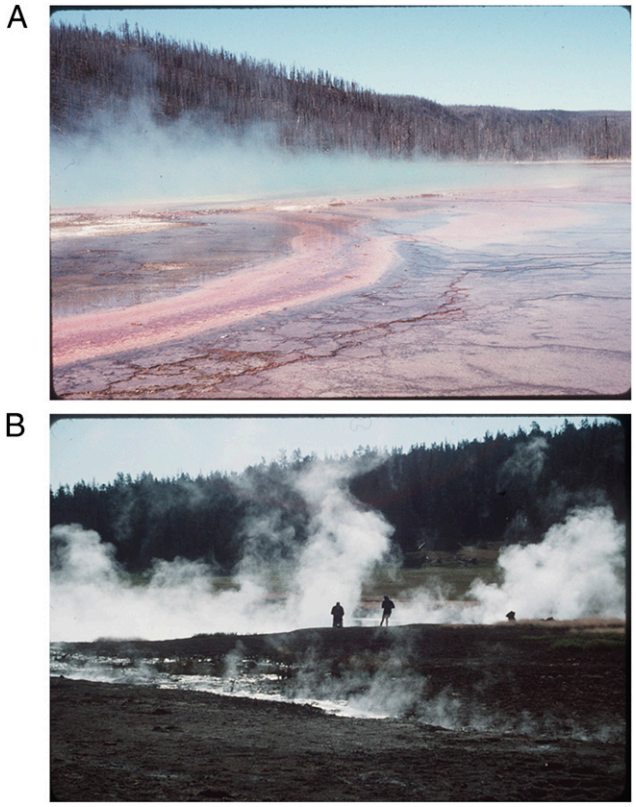

Fig. 2. (*A*) Grand Prismatic Hot Spring, Yellowstone National Park. The red, orange, and green pigments around the spring are microbial mats fueled by photosynthesis and geochemicals from the hot spring. (*B*) Microbiologists preparing to sample a hot spring in upper Hayden Valley, Yellowstone National Park. (Photo courtesy of Norman Pace.)

Crick, codiscoverer with James Watson of DNA's double helical structure.

Then, molecular biologists banded together to decipher how the 20 amino acids found in cells corresponded to triplet codes of bases in the messenger RNA. A mechanistic understanding of the genetic code became one of molecular biology's bedeviling challenges, and sure enough, the ensuing years saw a spate of discoveries that unraveled the molecular mechanism of protein synthesis. Yet despite the attention lavished on the code, Woese laments, its evolutionary origin was largely ignored. "Evolution was dismissed as a historical accident that didn't need to be invoked to explain the code, which was seen merely as a series of chemical interactions between molecules," Woese recalls.

To truly crack the code, Woese held, the question of how codon assignments evolved or why, for example, the triplet nucleotide code CCC encodes the amino acid proline, had to be addressed from an evolutionary perspective–a profound but radical view that might shed light on the evolution of the cell itself. To that end, he adapted sequencing techniques, developed by molecular biologist Frederick Sanger, to compare the sequences of RNA in the ribosomes of a range of microbes.

Ribosomal RNA: Life's Timekeeper

By then, Woese had accepted a faculty position in microbiology at the University of Illinois at Urbana–Champaign on the invitation of molecular biologist Sol Spiegelman, whom he had met during a sabbatical at the Pasteur Institute in Paris. Soon, Woese set out to catalog ribosomal RNA sequences in a range of microbes. One form of rRNA—named 16S for the rate at which it sediments in laboratory experiments—turned out to be the yardstick of choice for evolutionary comparison, largely because the molecule forms a part of the protein-making machinery at the heart of all cells. So, the reasoning went, unlike other adaptive embellishments in cells, it was likely to be conserved over evolutionary time and large enough for meaningful genetic comparisons among organisms.

"The conserved nature of ribosomal RNA makes it an ideal molecule to trace a vertical line of descent," Pace says. Further, the molecule's universality meant that it was unlikely to be shuttled laterally among organisms, unlike other genes that were likely swapped freely in a still-evolving evolutionary soup.

Together with then-postdoctoral fellow George Fox, graduate students Mitchell Sogin and William Balch, technician Linda Magrum, and others, Woese painstakingly assembled a database of differences in 16S rRNA among a laundry list of microbes that included both eukaryotes, a group of organisms defined by the presence of a membrane-enclosed nucleus, and prokaryotes, a group defined solely on the basis of its differences from eukaryotes. Meanwhile, the scientific community's attention was consumed by other developments in molecular biology's early days, and Woese's labors went largely unnoticed. Not for long.

By 1976, Woese's team had developed genetic signatures for dozens of different microbes, including methane producers that thrived in oxygen-starved environments like sewage and cow intestines. "It was a heroic enterprise to develop RNA catalogs for representatives of the different forms of life as we knew it then," says Fox, now at the University of Houston, Texas. As a picture emerged, Woese realized that the methane producers were not bacteria. In fact, their 16S rRNA signatures suggested that they were fundamentally different from life forms then known as prokaryotes or eukaryotes. Years later, Woese named the group of extremophiles, which included heat- and salt-loving microbes that occupied extreme niches like deep sea vents and thermal springs, *archaea* (see Fig. 2). Other biochemical signatures of archaea unearthed by researchers in Germany and elsewhere lent support to the group's uniqueness.

Through calculations of similarities between the 16S rRNA sequences of bacteria, eukaryotes, and methane producers, Woese and Fox proposed in the 1977 PNAS report that the methanogens represent a separate kingdom of life, suggesting that "[the living world] is not structured in a bipartite way along the lines of the organizationally dissimilar prokaryote and eukaryote. Rather, it is (at least) tripartite."

The report unleashed a minor controversy among microbiologists even as *The New York Times* announced in November the same year, "Scientists discover a form of life that predates higher organisms" (4). A few notable researchers denounced the proposal of a phylogenetic classification of life into three kingdoms as a misguided move to impose a new order, citing microbiologists' long unsuccessful attempts at classifying prokaryotes, an endeavor written off as Sisyphean.

Yet the reigning bipartite division of life was largely utilitarian, relying on differences of little evolutionary significance. As evidence of archaea's uniqueness mounted, the group's ranks swelled, and its evolutionary differences from bacteria became established, pointing to a three-pronged tree of life with a still-mysterious but common root. "Archaea could not

have been prokaryotic outliers, because Carl's team was finding the same differences between bacteria and different members of archaea," says University of Illinois at Urbana–Champaign evolutionary biologist Gary Olsen, whose research interests were shaped by Woese's discovery. Archaea, it turned out, were more closely related to eukaryotes than bacteria (See Fig. 3).

"Woese did not set out to uncover a third domain of life; he was interested in the evolution of the protein synthesis machinery and ribosomal RNA," says Pace. Archaea, then, were a revelation to Woese as well as the rest of the scientific community.

Woese says part of the skeptical stance to the 1977 report could be traced back to classical microbiology's quiet divorce from the decade's evolutionary thinking. "There was a disconnect between Darwinists, who had taken over evolution, and microbiologists, who had no use for Darwinian natural selection," he says. To make matters worse, molecular biology's application-oriented approach, he adds, smothered evolutionary considerations of life. "There was a tacit agreement between evolutionists and molecular biologists—*en entente curieuse*—that neither group would criticize the other." Add to these reasons microbiologists' reluctance to abandon the existing classification of life into eukaryotes and prokaryotes, and Woese's phylogenetic classification met with widespread resistance. "By then, the concept of prokaryotes had become firmly entrenched. When the phylogenetic classification was proposed, it was as if a crutch had been taken away," he recalls.

Enduring Legacy

Despite the initial criticism leveled at the 1977 paper, its findings have stood the test of time. Nearly 15 years later, the editors of *The Prokaryotes*, a touchstone textbook of microbiology, proclaimed, "The pioneering work of Carl Woese in cataloguing and sequencing the rRNA of prokaryotes has, for the first time in the history of biology, provided a means of establishing a truly phylogenetic system for living organisms—a goal previously thought impossible" (5). For his findings, Woese won a MacArthur Foundation grant and the Royal Swedish Academy of Sciences' Crafoord Prize.

"Carl's deep understanding of the universality of ribosomal RNA and the pragmatic ease of getting abundant RNA for analysis were key to the revised view of life," says Olsen.

The three-kingdom view of life is now widely accepted, save some muffled opposition. To boot, 16S rRNA sequencing has become a mainstay in the molecular biology toolbox, helping researchers classify biodiversity in a range of environments, including the human body. The field of microbial ecology gained steam in the early 1980s, when Pace found that the technique could be used to identify individual microbes in a naturally occurring assemblage without the need to grow them in the laboratory (6). "The realization that we could get RNA sequences from ecological samples was a singular moment that still makes me high," Pace recalls. Of the more than 100 phyla of bacteria known today, only about two dozen have been successfully grown in the laboratory, a fact that puts Pace's finding in perspective.

Today, the Human Microbiome Project, like others aimed at identifying microbes in terrestrial, aquatic, and aerial environments, is making strides thanks to the advent of rapid and inexpensive genome sequencing technology, which can help researchers sequence entire microbial genomes for less than $1,000. Yet the field of microbial genomics owes an incalculable debt to Woese's trailblazing cataloging technique formalized in 1977.

"The 1977 paper is one of the most influential in microbiology and arguably, all of biology. It ranks with the works of Watson and Crick and Darwin, providing an evolutionary framework for the incredible diversity of the microbial world," says Stanford University microbiologist Justin Sonnenburg, who studies the relationship between human diet and gut microbes.

Edward DeLong, a microbiologist at Massachusetts Institute of Technology who explores biodiversity in oceans, adds that the "paper and its technique provided a quantitative metric for understanding the phylogenetic relationships among all cellular life. That's foundational."

Like the discovery of archaea, Woese's contribution to molecular taxonomy, propelled by the ardent embrace of an evolutionary view of life, might be his enduring legacy. "Woese continues to champion the central place of evolution in biology, and with the biomedical establishment now facing challenges, such as the evolution of antibiotic resistance, the field is finally beginning to heed his words," says Goldenfeld.

Prashant Nair, *Science Writer*

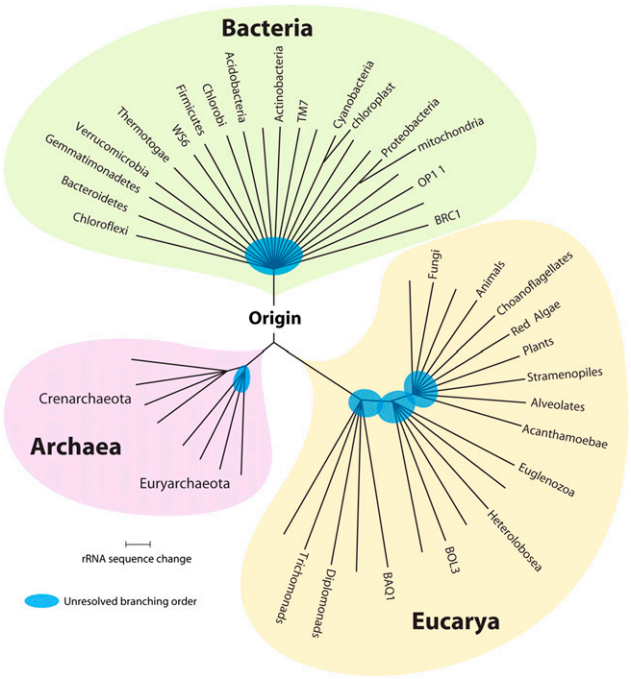

Fig. 3. A molecular tree of life. The diagram compiles the results of many rRNA sequence comparisons. (Reproduced from Microbiol. Mol. Biol. Rev., 2009, vol. 73, 565–576, doi: 10.1128/MMBR.00033-09 with permission from American Society for Microbiology.)

1. Arumugam M, et al. (2011) Enterotypes of the human gut microbiome. *Nature* 473:174–180.
2. Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *Proc Natl Acad Sci USA* 74:5088–5090.
3. Woese CR (1958) Comparison of the x-ray sensitivity of bacterial spores. *J Bacteriol* 75:5–8.
4. Lyons RD (November 3, 1977) Scientists discover a form of life that predates higher organisms. *NY Times*, Section A, p 1.
5. Balows AHG, Truper M, Harder DW, Schleifer KH (1992) *The Prokaryotes* (Springer, New York), 2nd Ed, Vol. 1, p vii.
6. Lane DJ, et al. (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci USA* 82:6955–6959.