MICROBIAL BIOLOGY

Microbiology's Scarred Revolutionary

Carl Woese revised the tree of life and started a new age in microbial biology by recognizing a third domain of life—but he paid the price for his radical ideas

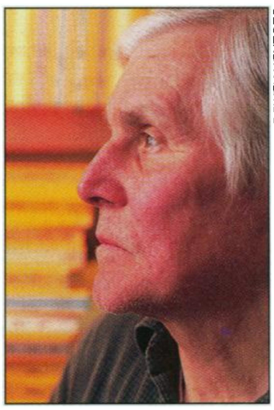
Shortly after dawn on 3 November 1977, evolutionist Carl Woese picked up the morning newspaper from his front lawn in Urbana, Illinois, and thought of how people across the country were doing the same thing. "Soon," he remembers thinking, "they'll be reading about me and my discovery." And he wondered how much his world would change. For Woese (pronounced "woes") had just announced his discovery of the Archaea—a group of one-celled organisms so different from all other living things, including bacteria, that he had placed them in a separate domain of life. It was as if a colony of alien creatures had suddenly been discovered living secretly in the backyards of suburbia. That day, as Woese had expected, the Archaea hit page one of newspapers from the *Urbana News Gazette* to *The New York Times*.

However, Woese soon found that the world doesn't stop even if you've created a new paradigm for understanding life on Earth. Later that same day, he ordered coffee from a young person at a fast-food counter and asked if she knew who he was. She shook her head, so he offered some clues: "I'm Carl Woese, and I discovered the third domain of life." Then she smiled in recognition. "Oh yeah," she said. "You're Bob's dad."

Telling this story today in his paper-strewn office, Woese tilts his head back and laughs: "That [comment] put everything in perspective for me." Keeping things in perspective has been a challenge for Woese, in part because after the first burst of publicity, not only the general public but also most microbiologists ignored his tripartite tree of life. Few researchers in the United States followed up on his work; grad students did not clamor at his door; and his funding stayed modest. "People didn't understand the size of my contribution," says the slightly built 69-year-old scientist, who still works in the same converted lab, full of rusty sinks and pipes, where he made his discovery. "They had no appreciation for what not having a microbial phylogeny meant and therefore no appreciation for what having one would mean."

By now, Woese has gotten his due: a MacArthur Foundation grant, election to the National Academy of Sciences, and honors including the Leeuwenhoek medal, microbiology's top honor. There are even rumors of a Nobel Prize in the offing. Yet he still appears to feel the sting of that first rejection, perhaps because he never received praise from microbiology's leading figures when he needed it most. His story dramatically illustrates the price paid by those who start a scientific revolution—even a spectacularly successful one. "Carl knew he had seen the Truth, that he'd seen God in his table of gene relationships, and I knew it when I saw it," recalls Norman Pace, an evolutionary microbial biologist at the University of California, Berkeley, who has been a friend and colleague of Woese's since the 1960s. "But very few other people in the field were convinced. They dismissed him or ignored him, and maybe because Carl is a shy, introverted person, he took this very hard."

> Microbial biology is undergoing a renaissance, and in this special section we explore bursts of progress in areas ranging from how pathogens invade cells to the origins of life. News reports beginning on this page focus on the extremophiles unusual microbes that flourish in harsh conditions while the Articles that follow probe a broad range of topics, including plant-microbe interactions and the astonishing diversity of microbes.



DEWEY HENTGES

Carl Woese

Most microbiologists today have just about forgotten the initial skepticism. Their science is bursting with discovery, and much of the ferment is due to what many now term the "Woesian revolution": his single-handed revelation that microbes, far from being just one of life's five major kingdoms, are actually two of its three broad domains: Bacteria, Archaea, and Eukarya (which includes all multicellular organisms, from plants to people). The stunning implication: Most life is one-celled, and all Eukarya are but a twig on what amounts to a great microbial tree of life. Perhaps most important, Woese's research—triumphantly confirmed last summer by DNA sequencing of a complete archaeal genome—has enabled microbiologists to place their organisms in the Darwinian fold. With an evolutionary tree in hand, biologists can look at microbes as they do the rest of life—as organisms with histories and evolutionary relationships to one another, and to all other organisms.

"It's as if Woese lifted a whole submerged continent out of the ocean," says Günter Wächtershäuser, an evolutionary biologist at Germany's University of Regensburg. "Now we can look at the continent—the microbial biosphere—in detail and with purpose." That, he says, "finally makes biology a complete science, because for the first time the study of evolution includes all organisms."

From physics to RNA

Perhaps part of the reason Woese had a hard time convincing fellow microbiologists about his work was that he wasn't officially a microbiologist, or even a biologist for that matter. As an undergraduate at Amherst College in Massachusetts, he studied physics, and his Yale University doctorate was in biophysics—the term first used to describe molecular biology. He stumbled into the microbial world as a postdoc at Yale in the mid-1950s; there he investigated the development of ribosomes (the cell's protein-synthesis machines) and became interested in the origin of the genetic code. After stints at General Electric in New York and France's Louis Pasteur Institute, he got a job at the University of Illinois in 1964 and has been there ever since.

Woese wanted to unravel the evolutionary history of DNA and RNA; to do this, he knew he needed a family tree, or phylogeny, that encompassed all organisms. At the time, most of the microbial world was lumped into a single group known as the prokaryotes, defined as organisms that lack a nucleus. "We had a real evolutionary understanding of the plants and animals, but that left out the whole world of bacteria. So I thought that's what I would do first: Bring in the prokaryotes," says Woese.

He was not the first to tackle this problem. In the 1930s, the leading microbiologist C. B. van Niel of Stanford University's Hopkins Marine Station had cited the classification of the bacteria as the key unresolved

Tracing the Mother of All Cells

When evolutionist Carl Woese unveiled the once-hidden land of the Archaea in 1977, he not only revolutionized microbial classification but also ushered in a new era in one of biology's grandest, if most problematic, pursuits: understanding the origins of life. Researchers had been exploring this question with test-tube experiments on organic molecules for years, but those studying living microbes had little to add to the debate. By sketching the first complete family tree of the microbes, however, Woese allowed researchers to search for organisms near the tree's base—close to the ancestral one-celled form. The features of those organisms may open a window on the era that preceded cells, when free-floating molecules first developed the ability to replicate and evolve.

"Before Woese, microbiologists simply wouldn't touch the question of what the last common ancestor looked like," says Alan Weiner, a molecular biologist at Yale University. Now hardly a month goes by without a fresh insight or meeting on the subject, from an April Nobel forum in Stockholm to an American Chemical Society symposium in June. The results so far suggest that the first organisms were probably not bacteria but archaea. And instead of evolving in a mild soup of organic molecules—as was first suggested by Charles Darwin—these organisms may have been born in what humans would consider marginal environments, such as boiling, sulfurous pools or hot, mineral-laden, deep-sea volcanic vents. "For the first time, we can go into the deep, totally unobservable past to test our predictions about the early evolution of life," says Günter Wächtershäuser, an evolutionary biologist at Germany's University of Regensburg.

The leading theory has long held that life began when lightning charged a warm soup of organic molecules with energy and caused them to begin replicating; variations include the possibility of a cold ocean as the cradle of life, an idea now advocated by longtime origin-of-life expert biochemist Stanley Miller of the University of California, San Diego.

But to many researchers, Woese's tree points to a different conclusion. Originally based on RNA and now confirmed by other genetic sequences (see main text), the tree separates all living organisms into three domains: Archaea (microbes that often inhabit extreme environments), Bacteria, and Eukarya (all multicellular animals and plants). By comparing sequences from the three domains, Woese could trace where various groups of organisms had branched off. And by following the branches back down the tree, Woese, and later other scientists, could identify organisms close to the shared common ancestor for the archaea and bacteria (see diagram on next page). Most of these turn out to be thermophiles or hyperthermophiles—Archaea and Bacteria that thrive at 80°C or higher.

That suggests that the last common ancestor was a hyperthermophile, says John Baross, an evolutionary microbiologist at the University of Washington, Seattle. Extrapolating back from the first fossil evidence of microbes at 3.8 billion years ago, he and others estimate that this organism lived about 4.3 billion years ago. Going even further back, researchers such as Everett Schock of Washington University in St. Louis conjecture that life began in an anaerobic, "nasty and hot" environment much like Yellowstone's sulfurous hot springs or a deep-sea vent. In addition, many of the most ancient organisms on the Woese tree are autotrophs, with metabolism based not on organic compounds but on inorganic material, such as carbon dioxide or hydrogen sulfide. Some scientists argue that the first cell was an autotroph, too.

That scenario matches many scientists' view of the early Earth. For example, Stanford University geochemist Norman Sleep has proposed that the ancient atmosphere contained high, greenhouse levels of carbon dioxide and was constantly being bombarded by meteorites and asteroids; some of these were as "big as Mount Everest" and sparked enough heat to boil off a 3-kilometer-deep ocean, says Baross. "That would have caused the death of any organism," he adds, "so where are the safe places on early Earth? In deep-sea volcanic vents, or subsurface sea-floor cracks"—just the places the primitive hyperthermophiles love today.

Trying to divine the branching pattern of the tree—and hence which organisms lie near the base of it—from the genetic sequences of living microbes has pitfalls, however. Chief among them is that early in history, "life was not chaste," as Antonio Lazcano, an evolutionary biologist at the National University of Mexico in Mexico City, puts it. Clues from living bacteria suggest that, like characters in a John Updike novel, different species freely swapped genetic material or simply picked up exogenous free-floating DNA, leaving a confusing trail.

Still, Wächtershäuser has recently argued that some traits are not easily swapped—notably those related to temperature. He theorizes that it is impossible for "a hot organism to exchange genes with a cold organism, and vice versa," because the proteins regulating cellular functions are tuned to work at a specific temperature. The implication is that the first cell did indeed like it hot. What's more, because it is easier to activate proteins at warm-to-hot temperatures, it makes sense that life evolved in a heated soup, then adjusted to lower temperatures as Earth cooled, he says.

But even if the first cells inhabited hot springs, the molecules that preceded them could have originated in a milder, "Club Med" environment, say Miller and others. They point out that even more than 4 billion years ago, the ancestral cell was already sophisticated, with a genome and the ability to replicate. "The hyperthermophiles may be ancestral to later life, but they are hardly primitive," says Miller. "They are as complicated as we are." He and Lazcano argue that life could have as easily started in warm, balmy seas—or even cold ones—and later given rise to both hyperthermophiles and lower temperature organisms. A megameteor strike might then have killed off all but the heat-loving organisms, leaving them to give rise to all later ones, says Miller.

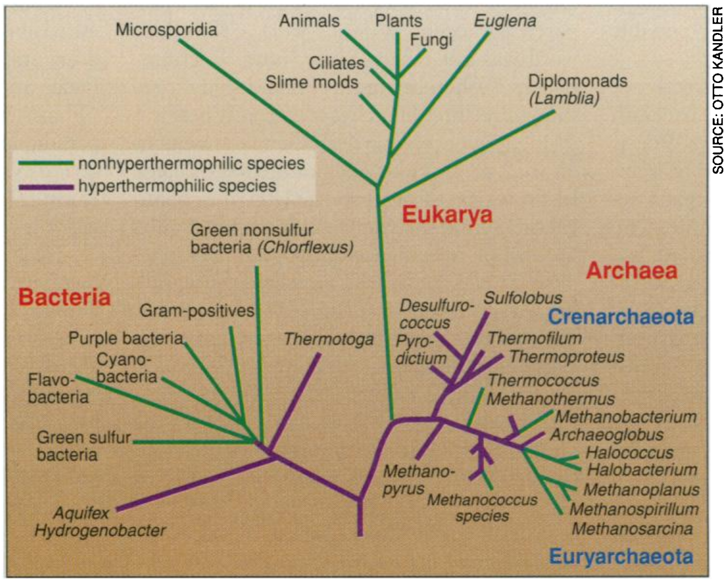
Those who favor an archaeal origin of life admit that they need to go back further into the past. Baross argues that hydrothermal vents themselves are windows into the nature of the early Earth—and that the genes of some vent organisms may therefore be living relics. Indeed, Wächtershäuser and Claudia Huber, a chemist at the Technical University of Munich in Germany, took their scenario further back into the past by showing that chemical reactions at vents could lead to the synthesis of key biological molecules (*Science*, 11 April, p. 245).

Then again, none of these scenarios may be right. Perhaps life evolved elsewhere in the solar system and came blasting into Earth on a bolide. Not even that would knock down the new tree of life, says Norman Pace, an evolutionary microbial biologist at the University of California, Berkeley. "I wouldn't be surprised that if we dredge up some martian creature, we'll find it rooted in Woese's big tree"—making that tree truly universal. **–V.M.**

issue in microbiology. But after decades of fruitless labor trying to classify bacteria by their shape and metabolism, he and his former student, Roger Stanier of the University of California, Berkeley, decided that the bacteria simply could not be phylogenetically ordered. "The ultimate scientific goal of biological classification cannot be achieved in the case of bacteria," Stanier wrote in the second edition of his leading textbook, *The Microbial World*. In Woese's view, that attitude consigned microbial research to the Dark Ages. "It was as if you went to a zoo and had no way of telling the lions from the elephants from the orangutans—or any of these from the trees," he says.

Yet that is where bacterial classification stayed for the next 2 decades. Woese, however, didn't think the problem insoluble. "I hadn't been trained as a microbiologist, so I didn't have this bias," he explains. His physics background led him to believe that "the world has deep and simple principles, and that if you look at it in the right way," you can find these. What's more, he was convinced that the molecular revolution, then in its infancy, offered just the tools to decipher the problem. He turned to ribosomal RNAs (rRNAs), nucleic acid sequences found in ribosomes. Woese knew from his previous research that these sequences are among the most conserved elements in all organisms, making them excellent recorders of life's evolutionary history. They are also abundant in cells, so that they're fairly easy to extract. And because they are found in all organisms, from *Escherichia coli* to elephants, their similarities and differences could be used to track every lineage of life. Woese calls them "the ideal tool." But this, too, went against the prevailing scientific tide, says Wächtershäuser, because everyone else was building family trees using proteins, which were then much easier to work with than other tools were.

In 1966, of course, none of today's efficient methods for sequencing genetic material existed. Instead, Woese relied on a tedious, labor-intensive technique known as oligonucleotide cataloging. In this method, an rRNA molecule, which is a long string of four nucleotides (adenine, cytosine, uracil, and guanine, or A, C, U, G), was broken into small fragments by cutting it at every G residue. Each of these fragments or oligonucleotides was then broken into subfragments with enzymes that sliced at different residues; this allowed Woese to reconstruct the sequence of the original rRNA fragment. These oligonucleotides were usually quite short by today's standards—from six to 20 nucleotides—but long enough that most occurred only once in an rRNA. So Woese could seek matching oligonucleotides in other microbes to determine how closely they were related.



Tree of life. The Woese family tree shows that most life is one-celled, and that the oldest cells were hyperthermophiles.

Physically, these RNA fragments appeared as fuzzy spots on film, and Woese stored thousands of such films in large, canary-yellow Kodak boxes. It took him a full year to make and read his first catalog. He was one of "only two or three people in the world" to learn this backbreaking technique, he says, and he labored alone. "Carl was the only one who could read the films," recalls William Whitman, a former graduate student of Woese's and now a microbiologist at the University of Georgia, Athens. Soon, every flat surface in Woese's office sported a light box with an oligonucleotide film clipped to its surface. Other films hung in front of Woese's "luminescent wall"—a sheet of translucent plastic with lights inside that blocked the windows and stretched the length of his room. "He stood there all day, every day, looking at these, searching for patterns," says Whitman. A stash of Dr Pepper and a chin-up bar kept him going. Nevertheless, the work was of such mind-numbing tedium that Woese himself says it left him "just completely dulled down."

He worked this way for a decade, completing the rRNA sequences of about 60 diverse bacteria and arranging them by genetic similarity. Slowly he began unraveling the tangle of microbial relationships, publishing phylogenies of chloroplasts and mitochondria—cellular organelles thought to have originated as symbiotic bacteria—and groups of bacteria. In some cases, he made surprising findings, for example that the anaerobic bacteroids and the aerobic flavobacteria are related. During this period, Woese received modest $50,000 grants from NASA and served the university by teaching molecular biology. But with each untangled group, he added another twig to his tree of life.

Then in 1976, he attached an entire limb. Ralph Wolfe, a close colleague at the University of Illinois, suggested that Woese try his technique on an odd group of bacteria that produced methane as a byproduct. "We knew of about eight different methanogens at that time, and nobody knew where they fit," recalls Wolfe. "They were diverse morphologically—rods, spirals, marblelike cells—but they all had the same kind of biochemistry. That's what intrigued Carl."

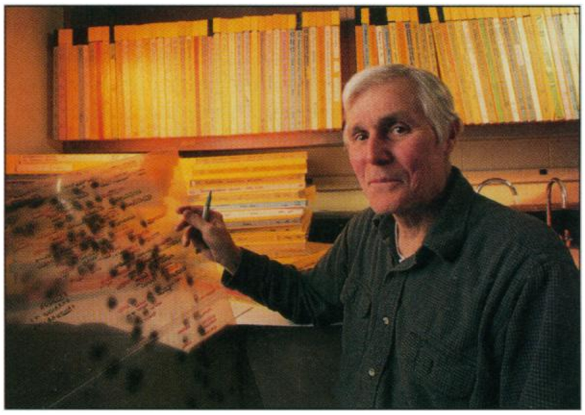
But when Woese studied their sequences, the methanogens did not register as bacteria. "They were completely missing the oligonucleotide sequences that I had come to recognize as characteristic of bacteria," he explains. Thinking the sample had somehow been contaminated, he ran a fresh one. "And that's when Carl came down the hall, shaking his head," says Wolfe. "He told me, 'Wolfe, these things aren't even bacteria.' And I said, 'Now, calm down, Carl; come out of orbit. Of course, they're bacteria; they look like bacteria.' " But, as Woese now knew, morphology in bacteria meant nothing. Only their molecules told the story. And the molecules proclaimed that the methanogens were not like any other prokaryote or eukaryote—they were something unto themselves, a third branch of life.

The silent treatment

That's what Woese and NASA put in their press release the next year, announcing what he then called the archaebacteria (he has since dropped the "bacteria"); the scientific paper appeared in the *Proceedings of the National Academy of Sciences* (*PNAS*), with Wolfe as one of the co-authors. But it was the newspaper accounts of the discovery that most scientists first read, and by and large, they were skeptical. Woese's tree, after all, overturned one of biology's most basic concepts—that life was divided into two large groups; it seemed outrageous to claim a third.

And Woese's solitary years at his light table had left him with a reputation as an odd person, "a crank, who was using a crazy technique to answer an impossible question," as one researcher put it. His tiny snippets of rRNAs were considered too fragmentary to be reliable indicators of evolutionary relationships, says Pace. Molecular biologist Alan Weiner of Yale University recalls that many leading biologists thought Woese was "crazy," and that his RNA tools couldn't possibly answer the question he was asking.

Few said anything to Woese directly, or even responded in journals. "The backlash was rarely if ever put into print," says Woese, "which saddens me because it would be helpful to have that record." Instead, many researchers directed comments to Wolfe, who was well established and highly regarded. Recalls Wolfe: "One Nobel Prize winner, Salvador Luria, called me and said, 'Ralph, you're going to ruin your career. You've got to disassociate yourself from this nonsense!' " Ernst Mayr of Harvard University scoffed to reporters that the notion of a third domain of life was nonsense, an opinion that he and a handful of other skeptics hold to this day. "I do give him credit for recognizing the archaebacteria as a very distinct group," says Mayr, who insists on keeping the word bacteria attached to the Archaea. "However, the difference between the two kinds of bacteria is not nearly as great as that between the prokaryotes and eukaryotes."



DEWEY HENTGES

Counting catalogs. Woese with one of the thousands of oligonucleotide films he analyzed.

Woese's retiring nature didn't help. He says he has "an almost visceral" dislike of meetings, feeling that they are largely political gatherings. He seldom even attended the annual meetings of the American Society for Microbiology (ASM) and so had few opportunities to argue in person on behalf of the Archaea. It was left to Wolfe to parry the criticisms and catch the gossip. At one ASM meeting in the early 1980s, for example, R.G.E. Murray, the editor of microbiology's bible, *Bergey's Manual*, passed Wolfe in the hallway. He didn't bother to stop, but "just waved his hand dismissively and muttered, 'The archaebacteria are only bacteria.' " (Murray finally put the Archaea in the manual in 1986, but kept them as a subgroup within the kingdom Prokaryotae.)

In Germany, however, Woese was hailed as a hero from the beginning, thanks largely to the influential microbiologist Otto Kandler, who had also realized from his analysis of methanogen cell walls that these microbes were different from other bacteria. Thus, he was prepared to accept Woese's work. "Kandler's word was not taken lightly," says Wächtershäuser. "When he said we should study three things [the three branches of life], as Woese was saying, then everyone did." German researchers such as Wolfram Zillig of the Max Planck Institute near Munich plunged into the chemical structure of the Archaea, ultimately coming up with more evidence of the group's uniqueness. It was Kandler who organized the world's first Archaea conference in 1981; the previous year, he held a special Archaea seminar at the Big Hall of the Botanic Institute at the University of Munich. Knowing that Woese "was a little bit depressed by all this resistance" in the United States, Kandler arranged for a church brass choir to "blow full power" in greeting as Woese stepped onto the podium.

But at home, Woese waited in vain for a response from the two microbial biologists he expected would recognize his work's importance: van Niel and Stanier (both now deceased). He typed their important papers into his computer and today can quickly summon quotations to show that his work solved the problem they had pondered throughout their careers. Worse, a few years later, a colleague used Woese's method to resolve another question about microbial relationships and received a warm letter from Stanier. "I felt hurt and jealous," Woese recalls, "because Stanier had never written to me praising what I was doing."

At times the silence left Woese fuming, says Pace: "Even now, he sometimes lashes out at people on whose shoulders he stood and who, he thinks, failed him because they didn't recognize the Archaea or the universal tree." However, says Weiner, Woese may have been expecting too much. "He was making a claim of extraordinary scope. He was saying that we had missed one-third of all living things. People don't like to be told that they're missing it big—and that what's missing is big—particularly when it didn't seem to make a difference" to the rest of the work in microbiology at the time.

More than a decade has passed since Woese was badly treated, and many of his friends and colleagues say that they wish he could put that time behind him and accept the accolades now streaming in. "We all know how important he is," says Weiner. But "it's as if he doesn't hear it. When someone suffers a rejection like that, how long does it take to undo all the damage, all the years when the field blasted him? We can say, 'Oh, that's just the way science works.' But it was a personal experience for him. He's the one who had to live through it."

Woese only shrugs and smiles wryly when asked about the snubs of the past. He says he drew strength from those such as Wolfe, Pace, and Kandler, who believed in him. "That's what happens when you break a paradigm; people scoff, they don't treat you seriously," he says. "But I'd read Thomas Kuhn [*The Structure of Scientific Revolutions*], so I knew exactly what was going on."

Yet even in the United States, the tide had begun to turn in Woese's favor by 1980. The previous year, he and Wolfe had published a review article of the methanogens, clearly establishing their differences from the bacteria. "That went a long way toward stopping the doubters," says Wolfe. "It made people stop and think." Other microbiologists began finding other ways in which the Archaea are unique and discovering other unusual organisms that fell into the group, such as the salt-loving halophiles and the "thermoacidophiles"—sulfur-metabolizing methanogens. And although grad students still didn't beat a path to Woese's door, his own output of landmark papers increased, with major studies on aspects of the three domains appearing in *Science* and *PNAS*. Invitations to give lectures and attend conferences poured in; true to form, Woese declined most—although he agreed to give Berkeley's Stanier lecture.

The new phylogeny is the basis for a range of new explorations in microbial biology, from the origin of life to its diversity. "It's the model to reckon with," says John Baross, an evolutionary microbiologist at the University of Washington, Seattle. "Even if you don't like its conclusions, [the Woesian tree] is the best we've ever had and have; it's here to stay." The final genetic seal of approval came with last summer's sequencing work. The complete sequence of *Methanococcus jannaschii* revealed a raft of genes unlike any others known, confirming that the Archaea are indeed a unique domain and showing that they have closer ties to eukaryotes than to bacteria (*Science*, 23 August 1996, pp. 1043 and 1058). Today, every major microbiology textbook carries the Woesian tree of life, although general biology texts are still catching up.

For his part, Woese continues to peruse the genetic differences among microbes—only now he summons sequences onto his finger-smudged computer screen, where a program scans for similarities. "Microbial diversity, like microbial evolution, has become a real science," says Woese, ever the physicist at heart. "The same [is true] with microbial ecology. And it's all because we can now interpret these things within the framework of the tree."

–Virginia Morell