Life's Last Domain

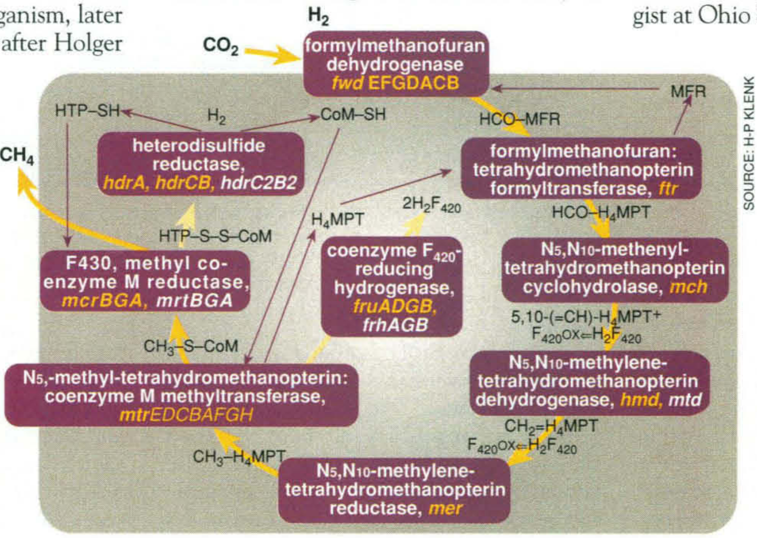
With the genome of the archaeon microbe *Methanococcus jannaschii* sequenced, researchers now have genomes for life's three domains. And only 44% of the archaeon's genes are familiar

In 1982, a pilot guided the research submarine *Alvin* to a hot spot about 3 kilometers beneath the Pacific Ocean. There, from a deep-sea hydrothermal chimney, grayish-white fluid spiraled out like smoke from Earth's interior, partly obscuring colonies of plumed worms and mats of bacteria hugging the vents of this "white smoker." *Alvin*'s grasping arm reached into these bacterial mats and pulled out a microbe never seen before: a methane-producing organism, later named *Methanococcus jannaschii* after Holger Jannasch, the expedition leader. It was recognized as a member of the archaea, a primitive form of life, but other than that, little was known about *M. jannaschii*.

There's a lot known now. In what many scientists are calling a tour de force, a team of researchers headed by Carol J. Bult from The Institute for Genomic Research (TIGR)* in Rockville, Maryland, report on page 1058 that they have sequenced this microbe's entire genomic blueprint. It is only the fourth organism for which researchers have teased out the full set of genes. But it is the first archaeon, and with it researchers have their first chance to compare the genomes of organisms from what now seem to be the three major branches of life: the archaea, bacteria, and eukarya (which includes multicellular organisms such as plants and animals).

"This completes that basic set, and so it will certainly have a major impact," says W. Ford Doolittle, an evolutionary geneticist in Nova Scotia with the Canadian Institute for Advanced Research. Adds Douglas W. Smith, a molecular biologist at the University of California, San Diego, "We've now entered a new age: the age of complete genomic comparisons. It's a fundamental breakthrough for molecular biology and biology in general."

Just how much of a breakthrough is clear from the genome team's initial comparison of *M. jannaschii*'s DNA with that of other organisms: A startling 56% of the archaeon's 1738 genes are entirely new to science, unlike any found in the two other branches of life. Says Smith, "That's about half of the organism! It shows just how little we know about life." The dramatic genetic distinctions have also vindicated the three-domain view of life, which had been introduced in 1977 as a challenge to the conventional view that divided life into two primary groups, prokaryotes (cells without nuclei) and eukaryotes (which have nuclei). The "old paradigm has been shattered," says Norman R. Pace, a microbiologist at the University of California, Berkeley. "It's time to rewrite the textbooks" using the three domains instead.



SOURCE: H-P KLENK

Making methane. Although most of *M. jannaschii*'s genes aren't known, the genes and enzymes in the energy pathway (red boxes) are similar to those of other methanogens.

As if that weren't enough, researchers predict that *M. jannaschii*'s genome holds clues to everything from better methods of producing methane for energy (which is one reason the Department of Energy helped fund this project as part of its Microbial Genome Initiative) to the study of the origins of life—and not only on Earth, but possibly elsewhere in the universe. A few researchers have already speculated about connections between the unusual organism and the putative microfossils reported last week from a Martian meteorite (*Science*, 16 August, pp. 864 and 924). "This organism is as good a candidate as you could want for something arriving on a Martian meteorite," notes John N. Reeve, a microbiologist at Ohio State University in Columbus.

Microbe hunt. J. Craig Venter goes to the bottom of the sea for genomes.

Going for a triple

M. jannaschii's very weirdness was one of the things that encouraged J. Craig Venter, genome scientist and TIGR's president, to study its genome. It lives at temperatures ranging from 48 to 94 degrees Celsius and at pressures of more than 200 atmospheres; oxygen kills it, and it's an autotroph, existing on carbon dioxide, nitrogen, and hydrogen. Says Venter, "It's like something out of science fiction. Not so long ago, no one would have believed you if you'd told them such organisms existed on Earth." It's also a methane-producer, and consequently along with other methanogens has "a huge, and little understood, effect on the respiratory cycle of the planet," he says.

But a bigger factor was *M. jannaschii*'s classification as an archaeon. Two decades ago, Carl R. Woese, an evolutionist at the University of Illinois and an author on the paper, characterized RNA from many microbes and saw that some were not prokaryotes (bacteria and blue-green algae) as had long been thought. "Boom!" says Woese, recalling that moment of recognition. "The archaea all fell into a single group, one that was completely unrelated to the other bacteria." His research indicated that such organisms, which included certain sulfur-loving thermophiles and the methanogens, were more closely allied—although not identical—to eukaryotes. Woese proposed that instead of being divided into prokaryotes and eukaryotes, life was more logically separated into three domains. But for many years, Woese's view of life was dismissed. "His ideas were blasphemous to a lot of people," says Venter, "so I thought

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*http://www.tigr.org/

sequencing *M. jannaschii* would be a good test.”

The researchers began work on the project in 1995. Venter's team had already successfully deciphered the genomes of two bacteria, *Haemophilus influenzae* and *Mycoplasma genitalium* (*Science*, 28 July 1995, pp. 468, 496, and 538; and 20 October 1995, p. 397). Another group had sequenced the first eukaryote, *Saccharomyces cerevisiae*, a yeast.

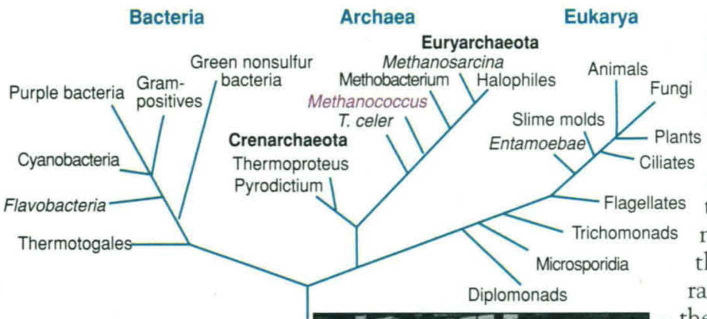
To sequence *M. jannaschii*'s genome, the team relied on Venter's once-controversial and now highly praised “whole-genome random sequencing” method. To reduce an organism's genome to a string of A's, T's, C's, and G's—the nucleotides adenine, thymine, guanine, and cytosine—many geneticists first make a map of small overlapping clones from the DNA. But this is a tricky process, because some pieces of DNA don't clone very well, leaving gaps in the map. It's also time-consuming because the clones must be sequenced one at a time.

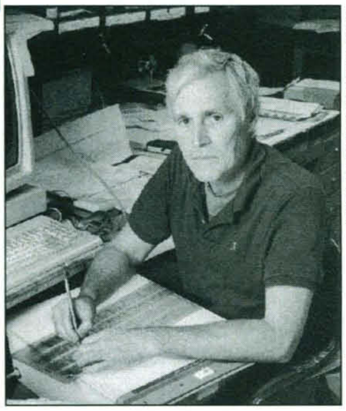
So TIGR's crew skips the mapping step. They sequence the DNA before they know how the clones fit together, then rely on computers to assemble the full sequence. They begin by building two libraries—one small, one large—of the organism's cloned DNA. In the case of *M. jannaschii*, Bult's team divided the genome into 36,000 fragments of DNA, each no more than 2.5 kilobases (or 2500 letters) in length. They sequenced both ends of 17,000 clones and ran the results through the TIGR Assembler software program, which looked for segments with overlapping sequences. Two fragments that end with 50 identical letters were a match. The program joins these pieces together at the overlap, forming lengthier sections called contigs. “That gave us 14 large contigs that we then had to fit together,” explains Bult.

That's where the second, larger clone library comes in. It has only 400 to 500 clones, but these are much longer, at 15 kilobases each. Again, the team sequences only the ends of each of these clones. “That creates a scaffold,” says Bult, which can be placed over the small library segments, thus showing the overall shape of the genome and identifying any gaps. Adds Venter, “It shows us that we've got the DNA, and all that we've got to do is reclone the missing segments.” Getting all the pieces still takes time, but a year after starting the project, the TIGR team snapped the last two contigs together, revealing a genome that was as strange as the microbe itself.

Extraordinary evolutionary insight

That genome resides on a single large chromosome and two smaller extrachromosomal elements (ECEs). The chromosome contains 1682 genes, while there are 44 genes on the larger ECE and only 12 on the smaller ECE. “We really don't know what these ECEs do,” says Bult. “It's possible that they're physically integrated with the main chromosome, so that there's some exchange of genetic material between them, but we don't know if that happens.” Venter suggests they may even be primitive viruses.





BILL WIEGAND/UI NEWS BUREAU

Three's the charm. The new archaeon sequence vindicates Carl Woese's theory that life is divided into three domains.

The researchers identified hypothetical genes from these sequences by looking for start and stop codons, segments that are known to bracket genes. Then, with the sequences of these “likely” genes in hand, the team searched existing databases of bacterial and eukaryotic genomes for homologs—known genes coding for similar proteins. “But the majority of these genes don't match any of those that have already been published, which is just a phenomenal result,” says Bult. For example, the team found very few homologies between *M. jannaschii*'s genes and those of the two previously sequenced bacteria (11% for *H. influenzae* and 17% for *M. genitalium*). Nor could they identify a single gene on the small ECE, adding to its mystery.

Altogether, the team found matches for only 44% of *M. jannaschii*'s genes. “We knew it was different, but this shows us just how different,” says Nobel Prize–winning biologist Richard J. Roberts of New England Biolabs in Beverly, Massachusetts.

Even that small number has given scientists some insights into the archaea's evolutionary relationships to the bacteria and eukarya, however. “There are genes here that are totally bacterialike, and others that distinctly resemble eukaryotes,” says Venter. “There are even genes from this hot organism that, instead of matching some bizarre bacteria, are most similar to genes from humans and yeast.”

The most striking similarity to yeast and other eukaryotes, note Woese and others, lies in *M. jannaschii*'s information-processing systems: the genes that control the translation and transcription of DNA, and the genome's replication. The latter was especially “a big surprise: that it has nothing in common with the bacterial method of genome replication,” says Woese, “but instead is like the eukaryotes. The difference [between the two methods] is like night and day.” Some of the translation genes raised eyebrows, too, for these had never been seen in any archaea before, although they are well known in eukaryotes. Bacteria, on the other hand, do not have these genes. Says Doolittle, “The transcription and translation are very eukaryote-like, as if the archaea were preparing to become eukaryotes.” (*M. jannaschii* does actually have genes homologous to those in both eukaryotes and bacteria for the initiation part of the translation process. “So which is doing the actual function?” queries Hans-Peter Klenk, a biochemist and member of the TIGR team. “And what is the other doing there?”)

Another striking similarity between *M. jannaschii* and the eukaryotes can be seen in their histone genes, says Ohio State's Reeve. In eukaryotes, these help form the DNA into particular compact structures; bacterial cells, however, don't appear to organize their DNA this way. But it seems that *M. jannaschii* does: Three such genes are found on the main chromosome and two on the larger ECE.

Together, these strong similarities spell vindication for Woese's hypothesis about the close relationship between archaea and eukarya, say many researchers. “This gives him the hard evidence he's needed for years and years,” says Bult.

Yet at the metabolic level, *M. jannaschii* is more like the bacteria, implying that both the archaea and bacteria derived central biochemical pathways from a common ancestor, says Reeve. For example, *M. jannaschii*'s genes that control the transport across the cell of inorganic ions, such as potassium and sodium, are more bacterialike than not. *M. jannaschii* also has homologs with some bacterial genes that help construct the microbes' surface structures, again indicating a common heritage.

The organism's overall energy cycle, however, is identical to that of other methane-producing archaea: It reduces carbon dioxide and hydrogen, making methane. It also fixes nitrogen, like other methanogens. Researchers will be closely studying these genes, says Bult, for they hold the metabolic code to "a renewable, nonpolluting source of energy: natural gas."

Startling omissions

M. *jannaschii* is also surprising in what it doesn't have. For example, it apparently gets by with just a single DNA polymerase enzyme for replicating the genome. "Even in *Escherichia coli*, you need three DNA polymerases for this task," notes Roberts. "So if it's really getting away with one, this tells us that either one polymerase can do everything—or there's a whole new way to do this work that we know nothing about."

The archaeon also appears to be missing several transfer RNA (tRNA) charging enzymes, used to produce proteins in all other organisms. "These are very basic elements of life; they lie at the very heart of how an organism makes proteins," says Roberts. "So, again, either [archaea] have a very novel method for doing this, or the genes are so different that we just aren't able to recognize them." He and others were further surprised by M. *jannaschii*'s numerous inteins (18 in all), little understood elements that are inserted into proteins and then removed after the proteins are completed. "That's an astonishingly high number for something that most biologists don't even know exists," Roberts says.

That knowledge gap—and the number of unknown M. *jannaschii* genes—will shrink as other archaea genomes are sequenced. Reeve anticipates decoding the sequence of *Methanobacterium thermoautotrophicum* (the methane microbe in everyone's compost pile) before the end of the year, and more are in the works at other labs, including TIGR's. Those sequences as well as others from more primitive forms of bacteria and eukaryotes should eventually lead scientists back to the root of the tree of life. "When we have enough sequences, we'll find out the things that are universal to all life and how others diverged from this core," says Woese.

Ultimately, that search for the core may lead beyond Earth. Indeed, the recent announcement of possible fossils in a Martian meteorite had the archaea genome sequencing community all abuzz. Perhaps, suggests Venter, the original archaea arrived from space, aboard such a meteorite. Says Reeve, "Twenty years ago, no one would have thought that M. *jannaschii* could live and survive in the conditions it does—and on Earth. So it wouldn't be unexpected to find something like this elsewhere in the universe." Once on Earth, says Venter, the ancestral archaea might have "salted" the Earth's seas with the ingredients for life: "If something that was a complete autotroph, like M. *jannaschii*, splashed down from Mars, it could have created the nutrient-rich seas that many theorists regard as essential for life to have evolved."

Already, NASA is laying plans to begin searching for similar life forms on Mars (see story on p. 1040) and Jupiter's moon, Europa—and any number of labs are revving up their sequencing machines in anticipation. They may yet prove that in the beginning was the microbe.

–Virginia Morell

ECOLOGY

Exploring Biodiversity's Benefits

PROVIDENCE, RHODE ISLAND—Seeking to shed their image as ivory-tower academics, ecologists recently met here with conservation biologists* under the theme "Ecologists as Problem Solvers." Living up to that slogan, one team reported new results clarifying a problem as old as Darwin and as urgent as extinction: How does biodiversity affect an ecosystem's health?

Some scientists have favored the notion, put forward by Darwin himself, that ecosystems with many species are more productive; others have proposed the added benefit of greater stability. But the data haven't yielded clear support for either idea, as there are plenty of simple ecosystems that appear both stable and productive. In the past few years, however, a handful of studies has boosted Darwin's view. In February, for example, G. David Tilman of the University of Minnesota and colleagues reported field trials showing that species-rich ecosystems are more productive and retain more nutrients than species-poor ones (*Science*, 15 March, p. 1497). Now Tilman has new results that expand and may help explain these effects.

He told attendees at a standing-room-only session that experiments completed just this summer show that the effects of plant diversity echo throughout the food web to herbivores and pathogens, suggesting that diversity loss may destabilize ecosystems. At the same time, his group has developed new mathematical models exploring how biodiversity boosts productivity. It all adds up to an important practical message, he says: The loss of biodiversity can threaten natural ecosystems, and boosting it could improve stability and productivity in forests, grazing lands, and other managed ecosystems. The new studies "have implications for human concerns as well as the stability of ecosystems over the long term," agrees Syracuse University ecologist Sam McNaughton, who did pioneering work on ecosystem diversity in the 1970s.


D. TILMAN

Weed warriors. Students hand-weed prairie plots as part of diversity experiment.

Not all ecologists agree about these implications, but there is broad admiration for Tilman's painstaking experiments. Working with colleagues Jim Groth, Johannes Knops, Charles Mitchell, Peter Reich, Mark Ritchie, and David Wedin at the Cedar Creek Long-Term Ecology Research site in Minnesota, he planted 500 plots with varying numbers of 24 prairie species. The plots have been hand-weeded and measured by an army of students for two growing seasons thus far, in a mighty labor that Tilman calls his "25-acre heartache experiment." But the results were worth it: As reported this spring, plots with more species had more biomass, retained more soil nitrogen, and fared better during drought.

In this year's data, gathered only a few weeks ago, the team found that high-diversity plots were also more resistant to disease. For example, in low-diversity plots, aster plants were hit harder by a fungal pathogen, and prairie bunch clover plants were stricken by a different disease, bent stem syndrome. Low-diversity plots also suffered more predation by plant-eating insects (probably grasshoppers) and had higher biomasses of weedy invaders. Theory and mixed-crop agricultural experiments had suggested that diversity might affect such properties, but these controlled experiments offer important documentation, says Stanford University ecologist Peter Vitousek.

Tilman and colleagues are still exploring the mechanisms behind these effects, but they are also using their data to generate new models. With Minnesota's Clarence Lehman and Kendall Thompson, Tilman is developing models to explain the correlation between diversity and productivity. These models assume that in any group of species in a given environment, some will be more productive and therefore better competitors. If diversity is high, these species are more likely to be present, and will outcompete and even-

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* Ecological Society of America and Society for Conservation Biology meetings, 11–14 August.