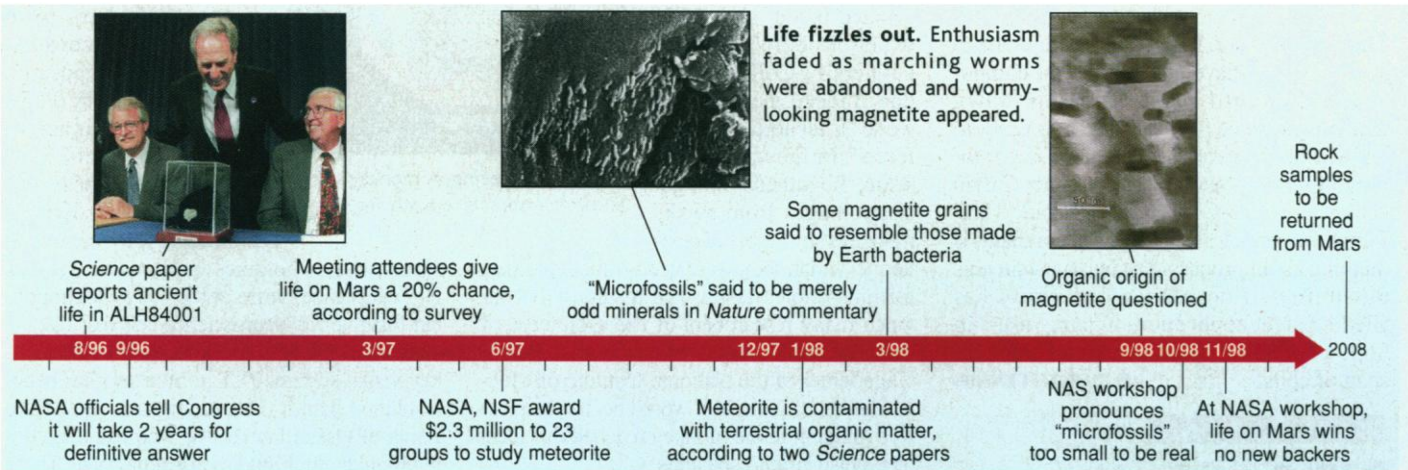
Signs of ancient life in a martian meteorite startled the world, but after 2 years of research the evidence has dwindled, and few scientists now believe the claim

# Requiem for Life on Mars? Support for Microbes Fades

**HOUSTON**—Just over 2 years ago, NASA Administrator Dan Goldin rushed to the White House to brief the president and vice president on a discovery that was about to rock the world: signs of ancient life on Mars. "We are not talking about 'little green men,'" said Goldin, but even so, little gray worms from a meteorite that was once a chunk of Mars made the front page day after day. The microscopic features helped jump-start planetary exploration as well as the field of exobiology and left everyone from priests to pundits wrestling with the implications of learning that life on Earth is not unique.

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Since then, ALH84001, a martian meteorite scooped from the Antarctic ice cap, has become the most intensively studied 2 kilograms of rock in history. With $2.3 million in funds from NASA and the National Science Foundation, scientists have sectioned it, imaged it, identified its minerals, measured its isotopes, and analyzed its organic matter. All this effort was aimed at testing and, if possible, extending each of the original four lines of evidence for life: mineral shapes that look like fossilized bacteria, traces of organic matter, rosettes of minerals perhaps formed through bacterial action, and grains of a magnetic mineral resembling those produced by bacteria. But at a NASA workshop here early this month,* scientists concluded that all the effort has not strengthened the claims. Indeed, key parts of the original case have been scaled back. Most researchers agree that the case for life is shakier than ever.

"The case has weakened dramatically," says meteoriticist Horton Newsom of the University of New Mexico, Albuquerque. "It was plausible and a good piece of work" when published in *Science* in August 1996 (16 August 1996, p. 924), but after intensive study, "a number of lines of evidence have gone away." Paleontologist Andrew Knoll of Harvard University agrees that the hypothesis "has not fared well. You would have a hard time finding even a small number of people who are enthused by the idea of life being recorded in this meteorite." If there ever was life on Mars, ALH84001 offers no persuasive evidence of it, these researchers say.

But the originators of the life-on-Mars hypothesis are not ready to call it quits. "This hypothesis is still alive and kicking," three of them wrote in a position paper prepared for the workshop. The three—geologist David McKay of the Johnson Space Center in Houston, who was the lead author on the *Science* paper, geochemist Everett Gibson of JSC, and microscopist Kathie L. Thomas-Keprta of Lockheed Martin Space Mission Systems and Services in Houston—say they "are more confident than ever that these meteorites likely contain traces of ancient life on Mars." They point to new data highlighting the similarity between some magnetite grains in the meteorite and those produced by earthly bacteria. Still, "we haven't solved the question," admits McKay. "These rocks are a lot more complicated than anyone could have imagined." Firmly identifying reliable biomarkers—anything that can be taken as a sign of past life—is "our work for the next 5 to 10 years," he says.

McKay's team has already withdrawn much of its most dramatic evidence. In the *Science* paper, they had suggested that spheroidal and tubular objects found in fractures within the meteorite could be fossilized extraterrestrial microbes. At the NASA press conference on the finds, they presented more possible martian bacteria, including a striking example dubbed "The Worm" as well as swarms of smaller structures that looked like armies of wormlike creatures marching in formation.

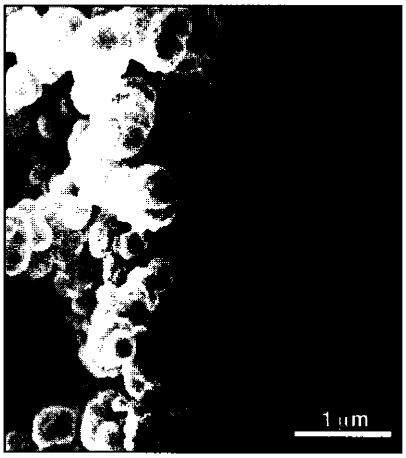
Just tens of nanometers across, most of these objects are much smaller than the smallest earthly bacteria. Indeed, they're well below the size cutoff embraced at a National Academy of Sciences (NAS) workshop last month, where participants concluded that even the most basic molecular machinery of life would take up a volume equal to a 200-nanometer sphere (see side-

* Martian Meteorites: Where Do We Stand and Where Are We Going?, 2–4 November, Lunar and Planetary Institute, Houston, Texas; see cass.jsc.nasa.gov/meetings/meetings.html. Summaries of ALH84001 studies can be found at cass.jsc.nasa.gov/lpi/meteorites/mars_meteorite.html

## Finding Life's Limits

**WASHINGTON, D.C.**—Everything in life is getting smaller, it seems—computers, telephones, camcorders. Even cells seem to be shrinking, with reports of tiny, ancient microbes on Mars (see main text) and claims of so-called "nannobacteria" on Earth—putative cells occupying only 0.01% of the volume of a typical *Escherichia coli* bacterium. But unlike computer chips that shrink as they are reinvented with new materials, all life on Earth seems to use the same standard parts. Those components—DNA, RNA, and the ribosomes that help translate the genetic code into proteins—have a fixed size.

That puts a limit on how small a self-replicating cell can be, according to a group of experts from physics, biochemistry, ecology, and microbiology who gathered at the National Academy of Sciences last month* to discuss the limits of life at the tiniest level. Assuming that a cell needs DNA and ribosomes to make its proteins, a spherical cell much smaller than about 200 nanometers (nm) in diameter—about one-tenth the diameter of an *E. coli*—"is not compatible with life as we know it," says cell biologist Christian de Duve of the Christian de Duve Institute of Cellular Pathology in Brussels and The Rockefeller University in New York City. Only radical new biology could relax these size limits, he and his colleagues concluded.




**Beyond the limit?** Kajander sees bacteria as small as 50 nm in images like this one.

One of the claims prompting this exploration of the limits of small came from geologist Robert Folk of the University of Texas, Austin. He reported finding tiny bacteria-like objects as small as 30 nm across—which he calls "nannobacteria"—in everything from tapwater to tooth enamel (*Science*, 20 June 1997, p. 1777). Then there were the putative martian microbes, as small as 20 nm by 100 nm, and a Finnish report of bacteria smaller than 100 nm—their term is "nanobacteria"—cultured from human and cattle blood.

Such dimensions do not leave enough room for the basic set of genes needed for life, said workshop participants. By identifying the genes shared by the simplest organisms, researchers have recently concluded that at least 250 or so are required for survival as a self-replicating cell. That's about half the number present in the smallest known bacterial genome. (Viruses, which can't replicate on their own, can be smaller.) The DNA needed for 250 genes would just about fill a sphere 100 nm in diameter; add enough room for ribosomes (each of which is 20 nm in diameter), for the DNA to unwind for replication, and for chemical reactions of the cell, and 200 nm is needed. At 50 nm in diameter, biochemist Michael Adams of the University of Georgia, Athens, calculated, a spherical cell would have room for two ribosomes, 260 proteins, and only eight genes' worth of DNA.

Even with 250 genes, cells would have to be parasites, relying on ready-made nutrients from their hosts. "By the time you get to an organism of this size," said biochemist Peter Moore of Yale University, "you are either an obligate parasite or you have a standing order at [the biological supply house] SIGMA." If the putative fossils from Mars were free-living, they "would have to reflect a biochemistry unlike any we know," he said.

But biochemist and physician Olavi Kajander of the University of Kuopio in Finland says he has organisms that prove the theoretical limits wrong. He says that although the nanobacteria he has cultured from blood and urine are mostly between 200 and 500 nm, he can get viable cultures after passing them through a 100-nm filter. He also claims to see spheres as small as 50 nm in diameter under the electron microscope.

Kajander argues that his smallest particles, if not viable alone, might join together to make a reproducing organism. He adds that perhaps they can get along with less, growing so slowly that they need few ribosomes, for example. As for Folk, he is still finding 40-nm, bean-shaped "cells" in electron micrographs that he believes are biological, although they may be "some sort of new life-form."

Other scientists at the meeting were doubtful. "It's really easy to get fooled," says microbiologist Don Button of the University of Alaska, Fairbanks. Cells larger than 100 nm might have squeezed through Kajander's filter, he says, and the preparation process for electron microscopy sometimes shrinks cells. Paleontologist Andrew Knoll of Harvard University agrees. "I think everyone pretty much agreed that ... nothing much smaller than 200 nm is likely to be viable."

Still, researchers admit that unknown kinds of life-forms might not face the same limits. Before DNA, ribosomes, and proteins, there must have been simpler life-forms. With a single molecule capable of both replicating itself and catalyzing reactions—such as RNA, for example—a cell would need far less space, several scientists told the meeting. A sphere 50 nm across could comfortably contain the 50 or so catalytic "genes" necessary for self-replication and basic metabolism, with plenty of room left over for chemical reactions, chemist Steve Benner of the University of Florida, Gainesville, said.

If such primitive life-forms once existed here, they were outcompeted by the more complex forms now populating Earth, but they might exist elsewhere. "We really have no assurance that our biology exhausts the possibilities for life in the universe," says Knoll. But until a persuasive sample of such life is discovered, earthly and extraterrestrial nanocandidates will face tough scientific scrutiny.

**—Gretchen Vogel**

* Workshop on Size Limits of Very Small Microorganisms, 22–23 October.

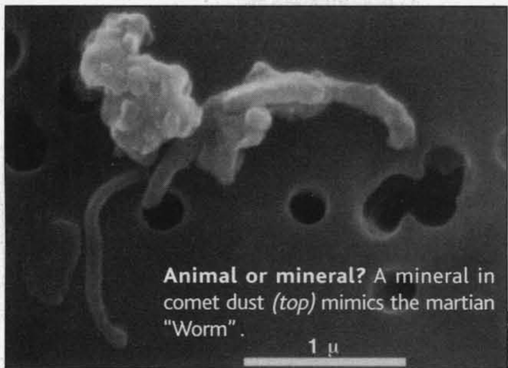
CREDIT: O. KAJANDER/UNIVERSITY OF KUOPIO

bar). And at the Houston workshop, McKay went part of the way toward accepting that limit. Anything smaller in volume than a 100-nanometer sphere "we simply don't believe is indicative of bacteria," he said. That criterion eliminates the objects in the *Science* paper as well as "The Worm," which is 250 nanometers long but too slender to make the cut. McKay also ruled out the phalanxes of worms, agreeing that, as another group argued last year, they are merely the jutting edges of mineral crystals reshaped by a coating used to prepare the sample for the scanning electron microscope (*Science*, 5 December 1997, p. 1706).

But McKay wouldn't write off the bacteria-like forms completely, saying that they "may very well be parts of bacteria from Mars." And as for intact bacteria, "we think there are large objects that are still candidates," he said, although he declined to offer any examples. "Until we get our data straightened out, that's all I want to say."

Even if McKay's team does come up with larger examples, few researchers are likely to be persuaded by simple bacteria-like shapes. Inorganic deposition can take such lifelike forms that shape alone proves little, say paleontologists and mineralogists. "Unfortunately, nature has a perverse sense of humor," explained microscopist John Bradley of MVA Inc. in Norcross, Georgia, as he showed the NAS workshop examples of lifelike micrometer-scale minerals grown inorganically and a picture of a wormlike structure found in comet dust. "It's easy to be fooled by shapes," agrees mineralogist Allan Treiman of the Lunar and Planetary Institute in Houston. That's especially true at the nanometer scale. "When you get to this size range, there are a lot of things we don't understand," says Treiman.

Attendees at the Houston workshop also gave short shrift to a second line of evidence—the presence of a distinctive type of organic matter. McKay *et al.*'s *Science* paper had argued that polycyclic aromatic hydrocarbons, or PAHs, found at parts-per-million concentrations in the meteorite's fractures, could be the decay products of ancient martian life. Then in January 1998, other researchers reported finding considerable amounts of the short-lived isotope carbon-14 in ALH84001 organic matter. Because carbon-14 would have long since vanished from the original sample, that indicated heavy contamination with terrestrial organics during the thousands of years the meteorite lay on the Antarctic ice. But in a July paper in *Faraday Discussions*, chemist Simon Clemett of MVA, one of the *Science* authors, reported that other Antarctic meteorites exposed for much longer than ALH84001 did not contain the same kind of PAHs. The finding implied that although much of the organic material in the fractures was terrestrial, the traces of PAHs were probably from Mars.




**Animal or mineral?** A mineral in comet dust *(top)* mimics the martian "Worm".

Even so, no one considers the existence of PAHs to be credible evidence for life. As meteoriticist John Kerridge of the University of California, Los Angeles, pointed out, the PAHs could just as easily be from Mars's own "primordial soup" that never achieved life. "That is why I don't think for a moment PAHs will ever figure in our list of biomarkers," he said. No one at the workshop, including Clemett, disagreed.

A third line of evidence—50-micrometer "rosettes" of carbonate in the meteorite's fractures—sparked sharp debate over the past 2 years, but like PAHs they now seem unlikely to yield persuasive evidence of life. The *Science* authors suggested that the rosettes may have formed under the chemical influence of bacteria. But rosettes are not a persuasive sign of life and might be inorganically produced, noted Henry Chafetz of the University of Houston.

Still, the carbonates of ALH84001 have provoked other lively arguments, focused on whether they were deposited from mineral-laden fluids at high or low temperatures. High temperatures, clearly above the 113°C known limit of life on Earth, would suggest that no martian life was around when they formed (*Science*, 4 April 1997, p. 30). The first temperature estimate, based on the suite of minerals present, gave temperatures on the order of 700°C. Next, another team suggested a low temperature—perhaps less than 100°C—because the mineral-forming process had not disturbed the rock's ancient magnetization. Then, different isotopic analyses pointed to both low and moderately high temperatures, depending on the minerals analyzed.

After bouncing around in this fashion for 2 years, at the workshop estimates seemed to be staying under 300°C. But the possible range still runs from 0° to 300°C or so, with little prospect of an imminent resolution. And even a low-temperature carbonate formation wouldn't prove that martian life existed; it would only mean that life cannot be ruled out.

**Magnetite mysteries remain**

The one line of evidence that still holds some promise of support for martian life is the tiny grains of the iron oxide mineral called magnetite. These grains are found throughout fractures in the meteorite, especially in the fine-grained rims of the carbonate rosettes. In the *Science* paper, McKay and his colleagues noted that in size—typically 50 nanometers in length—and shape, the magnetite resembles that produced by terrestrial bacteria. Bacteria use internally produced magnetite as magnetic compasses and externally induced, more irregular grains as a dump for their excess iron. But critics soon noted that not all the magnetite in ALH84001 looked lifelike.

Thomas-Keprta and her colleagues now offer a new take on the magnetite. They concede that 75% of it could have been produced inorganically, but they have found that about 25% of the grains in the rims have an elongated shape that is hexagonal in cross section, which is just the shape produced internally by certain terrestrial strains of bacteria. "We don't know of any inorganic source that will produce these," said Thomas-Keprta. "Their presence strongly suggests previous biogenic activity. We believe these may indeed be martian biomarkers."

Many other researchers have reservations. "We haven't been able to grow [this kind of crystal] inorganically," concedes rock magnetist Bruce Moskowitz of the University of Minnesota, Twin Cities. "But we haven't looked in detail at lava flows" and other geologic settings where such magnetite might grow. "I was not persuaded," says microscopist Peter Buseck of Arizona State University in Tempe, who has studied both biogenic and inorganic magnetite. It's the same problem as with the carbonate rosettes, he says: "At this point, we don't know how to tell whether a given [magnetite] crystal has been formed organically or inorganically."

Although most of the evidence appears to be fading away after 2 years and millions of dollars of research, McKay and colleagues still say that it adds up to a good case. As they concluded in *Science*: "None of these observations is in itself conclusive for the existence of past life. Although there are alternative explanations for each of these phenomena taken individually, when considered collectively ... we conclude that they are evidence for primitive life on early Mars."

Even 2 years ago, many researchers were unimpressed with that holistic argument. "I never bought the reasoning that the compounding of inconclusive arguments is conclusive," says petrologist Edward Stolper of the California Institute of Technology in Pasadena. And it was clear at the workshop that now, as pieces of the argument weaken, it is losing its grip over the rest of the community.

Indeed, workshop participants doubted whether the martian life issue will ever be resolved by studying ALH84001. The McKay hypothesis is "very hard to disprove," says Stolper. If living Martians pop out of the first sample return from Mars, scheduled for 2008, the issue will be settled. Otherwise the life claims for ALH84001, not proven but never conclusively refuted, may just fade away.

–RICHARD A. KERR

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