The Hydrogen Hypothesis

How did complex cells get their power stations?

By JOHN TRAVIS

William Martin just couldn't shake the picture from his mind.

He first saw the image in 1993 during a talk by Miklós Müller of the Rockefeller University in New York. Among Müller's slides was an electron micrograph showing the interior of a eukaryotic cell, the kind of cell that makes up complex animals, including people, and many single-celled creatures.

Like all eukaryotic cells, but unlike bacteria, this cell had a nucleus, the membrane-surrounded compartment that holds an organism's DNA. The cell also harbored hydrogenosomes, odd energy-generating machines that Müller has studied for more than 2 decades. While organelles called mitochondria power most eukaryotic cells, hydrogenosomes replace them in a few instances.

Most intriguing to Martin, Müller's slide showed bacteria nestled against the hydrogenosomes inside the single-celled eukaryote. The bacteria were methanogens, which feed on hydrogen, carbon dioxide, and acetate and give off methane gas as a waste product. Since hydrogenosomes, as they generate energy, emit lots of hydrogen, as well as some carbon dioxide and acetate, the methanogens had stumbled upon a feast inside the cell.

This image "just fascinated me. I could not let go of it," says Martin.

Last year, Müller again visited Martin, who works at the Braunschweig Technical University in Germany, and again showed the slide. Over the next few days, the two investigators chatted at length. While working late one night shortly afterward, recalls Martin, his exhausted mind experienced a flash of inspiration.

Martin imagined what could result if the hydrogenosome-methanogen relationship that he had seen in Müller's slide had developed billions of years ago between two free-living microorganisms.

The conjecture that resulted, dubbed the hydrogen hypothesis by Martin and Müller in the March 5 NATURE, offers a novel explanation for the origin of both hydrogenosomes and mitochondria and may also shake up beliefs about the birth of eukaryotic cells.

"Whether true or false, it is the first new hypothesis about eukaryotic origins in 30 years to have been really thoroughly articulated at the biochemical, molecular, and cellular levels," W. Ford Doolittle of Dalhousie University in Halifax, Nova Scotia, writes in an accompanying commentary.


Bland Finley

In a single-celled eukaryote, methane-producing bacteria (light green) feed off the waste products made by energy-generating hydrogenosomes (dark green).

At its most basic level, the hydrogen hypothesis contends that a host cell formed a symbiotic relationship with, and eventually engulfed, a bacterium that in some of the host's descendants gave rise to hydrogenosomes and in others to mitochondria.

While Martin and Müller are suggesting that a novel pact underlies this particular symbiosis, the general notion that features of eukaryotic cells evolved from one symbiont living inside another—a so-called endosymbiosis—dates back to the turn of the century.

Endosymbiosis fell out of fashion for many decades, but in the early 1970s, Lynn Margulis of the University of Massachusetts in Amherst breathed new life into the idea. Among other things, she theorized that mitochondria arose from a parasitic bacterium that invaded a larger bacterium and made itself at home. Over time, said Margulis, the parasite transferred most of its genes to its host.

This gradual shift would have created the modern mitochondrion, essentially a stripped-down microbe that employs oxygen to convert food into the energy-storage molecule adenosine triphosphate (ATP), used by all cells. Supporting this theory, the rod-shaped organelles look like bacteria, have their own small number of genes, and replicate by splitting in two in a manner closely resembling bacterial reproduction.

Tom Cavalier-Smith, an evolutionary biologist at the University of British Columbia in Vancouver, later modified this endosymbiotic model. Noting that several lines of single-celled eukaryotes harbor no mitochondria, he reasoned that the organelle must have arisen after cells with nuclei had evolved from bacteria. Cavalier-Smith hypothesized that an ancestral eukaryote, rather than a bacterium, took in the microbe that ultimately evolved into mitochondria.

The contract behind the endosymbiosis that produced mitochondria was considered obvious: an energy boost to the host cell in exchange for food and protection for the guest. The host, standard theory goes, was anaerobic, generating ATP from sugar molecules without using oxygen. In contrast, the guest bacterium was an aerobic creature, using oxygen to break down even more complex organic molecules and synthesize ATP.

Since aerobic respiration generates ATP many times more efficiently than the alternative anaerobic pathway, there's a huge incentive for an anaerobic organism to form a symbiosis with an aerobic partner, explains Cavalier-Smith.

Nice story, but Martin and Müller don't buy it. They argue that the endosymbiosis that resulted in mitochondria occurred before nuclei arose in cells or near the same time.

Their thinking stems in large part from recently gathered information about the ancestry of the hydrogenosome. Observed for decades in cells as rodlike objects resembling mitochondria but lacking their own DNA, "they were looked at as a curiosity, an oddball organelle. Their evolutionary significance was not recognized by the scientific community," says Martin.

In the 1970s, Müller and a coworker became the first to separate hydrogenosomes from other cellular contents, a step that enabled them to identify many of their biochemical properties. Hydrogenosomes, it turned out, anaerobically break down the carbohydrate pyruvate, producing ATP, hydrogen, and carbon dioxide in the process.

While hydrogenosomes and mitochondria were both thought to stem from endosymbiosis, many investigators assumed they arose independently. Eukaryotic cells with mitochondria had once taken in an aerobic bacterium; those with hydrogenosomes had instead forged a bond with an anaerobic bacterium that could generate additional energy for the host cell.

Yet scientists have now gathered compelling evidence that mitochondria and hydrogenosomes are two sides of the same ancient coin. "I think the most likely interpretation of the data is that they indeed evolved from a common precursor organelle," says Patricia Johnson of the University of California, Los Angeles. "The perception among many scientists is that it's all but proven."

This perception comes largely from work on some unusual eukaryotes. In late 1996, four research groups, including one led by Johnson, showed that trichonomads—anaerobic, single-celled eukaryotes with hydrogenosomes but no mitochondria—possess genes resembling those used by the mitochondria of other eukaryotes. Moreover, the proteins resulting from these trichonomad genes end up in the hydrogenosomes.

Even eukaryotes that lack both mitochondria and hydrogenosomes have begun to reveal remnants of an organelle that might have been their common precursor. Microsporidia and diplomonads, single-celled eukaryotes that live as parasites inside other cells, seem to carry genes resembling those that encode mitochondrial proteins.

In the December 1997 Current Biology, T. Martin Embley of the Natural History Museum in London and his colleagues report that the microsporidian *Vairimorpha necatrix* has DNA resembling a gene for a key mitochondrial protein. In the Jan. 6 Proceedings of the National Academy of Sciences, researchers led by Mitchell L. Sogin of the Marine Biological Laboratory in Woods Hole, Mass., describe a gene in the diplomonad *Giardia lamblia* that appears to be related to a gene for another mitochondrial protein.

The most likely explanation for the presence of mitochondrial genes in eukaryotes that bear neither mitochondria nor hydrogenosomes, investigators now conclude, is that the organisms originally had the precursor to both but shed the energy-producing organelles as they became parasites in other eukaryotic cells. In essence, they decided it was easier to rob than to work for a living.

The newly recognized relationship between mitochondria and hydrogenosomes, as well as the research on microsporidia and diplomonads, pushes the origin of these two organelles back to the earliest eukaryotes, if not earlier. This scenario inspired Martin and Müller to rethink why a host cell would ever establish a symbiotic relationship with the precursor of these organelles. In doing so, the scientists have redefined the nature of the host, suggesting it was neither a bacterium nor an early eukaryotic cell.

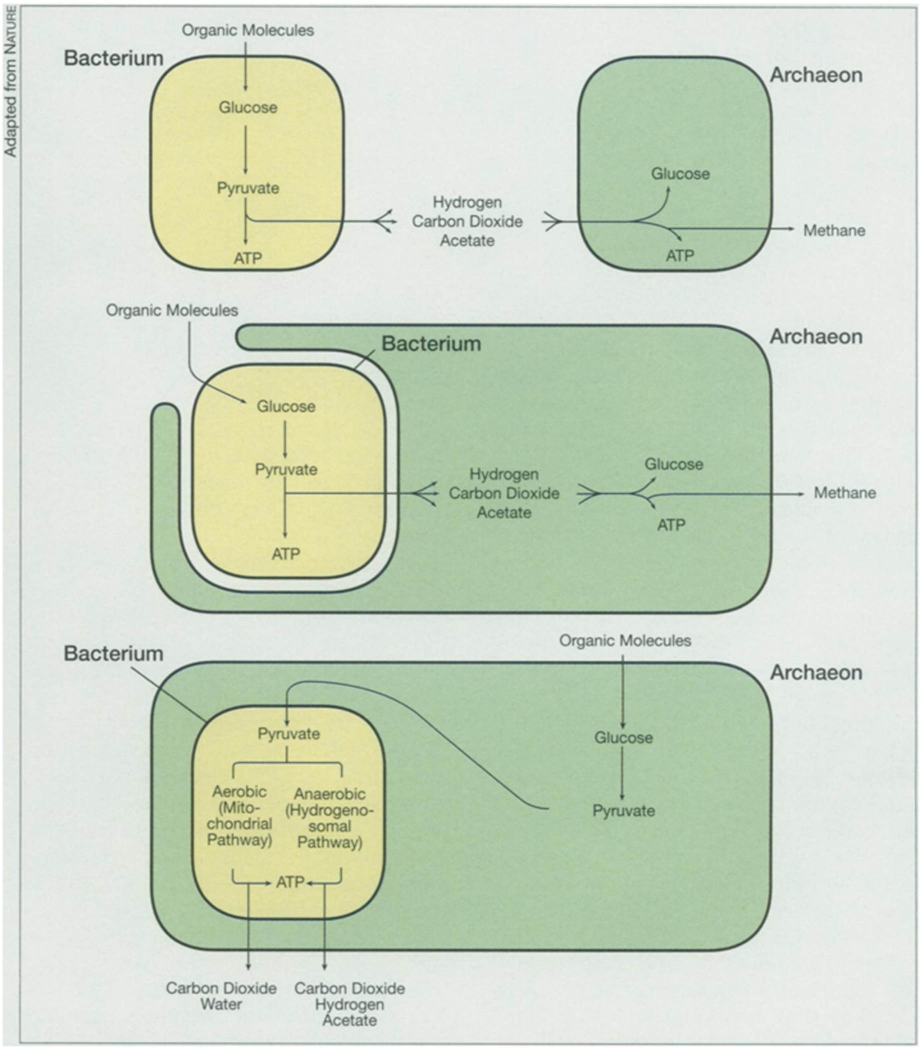
In recent years, notes Martin, scientists have grudgingly accepted that there are life forms other than bacteria and eukaryotes. Starting in the late 1970s, Carl R. Woese of the University of Illinois at Urbana-Champaign and other investigators found that a number of microorganisms usually classified as bacteria because they lack a nucleus differ dramatically from bacteria in crucial ways.

Woese argued that these single-celled creatures were a new branch of life, and recent genetic evidence has bolstered this view (SN: 8/24/96, p. 116).

As a result, scientists often divide life into bacteria or eubacteria, archaea or archaebacteria, and eukarya. Since some data suggest that archaea are more closely related to plants and animals than to bacteria, many investigators also believe that eukaryotic cells arose from archaea rather than from bacteria.

While previous theories on mitochondrial origins argued that an anaerobic, sugar-metabolizing bacterial or eukaryotic host cell captured an aerobic bacterium to provide a better way of making ATP, Martin and Müller counter that the host was an archaeon whose metabolism resembled that of the methanogens crowding around the hydrogenosomes in the slide that so fascinated Martin.

Unable to metabolize organic mole-



Adapted from Nature

In the hydrogen hypothesis, a bacterium becomes a permanent resident of an archaeon, which feeds off its guest's waste products. This archaeon later steals bacterial genes, allowing it to metabolize organic molecules. In return for food—pyruvate—the bacterium then provides extra energy—ATP—for its host. If it makes the ATP by using oxygen, the bacterium evolves into a mitochondrion; if it proceeds anaerobically, it can become a hydrogenosome.

cules, the free-living microbe would have survived solely on nonbiological sources of hydrogen, carbon dioxide, and acetate. Consequently, joining forces with a bacterium that gives off all three as waste products of its metabolism would have given such an archaeon a major advantage—another food supply.

Yet once it distanced itself from geologic sources of nourishment, the archaeon would become fully dependent on its bacterial partner. That reliance would encourage it to hold onto, if not engulf, the bacterium, says Martin.

Internalizing the bacterium, however, would cut off the supply of organic molecules that the bacterium feeds upon. The archaeon, now a true host, "has to learn to feed the symbiont," says Martin.

To do so, he and Müller speculate, the organism stole many of the genes normally used by the bacterium, such as those encoding proteins that take in organic molecules and convert them to pyruvate, a carbohydrate that is directly metabolized by the bacterium. Through this theft, the endosymbiont would have lost its independence and come to depend on the host cell for its food.

The final result would resemble a modern eukaryotic cell, which takes in organic molecules and creates pyruvate from them so that mitochondria or hydrogenosomes can make ATP.

Martin and Müller's hypothesis assumes that the bacterium taken in by the archaeon could generate ATP by metabolizing organic molecules aerobically or anaerobically. Their original symbiosis would have the engulfed bacterium metabolizing along the anaerobic pathway.

As oxygen became more pervasive in the atmosphere, or at least in the host's environment, the endosymbiont could have switched over to the more efficient, aerobic means of ATP production. That shift would have set the endosymbiont on the road to becoming a mitochondrion and enabled its previously anaerobic host to become an aerobic creature.

In contrast, host cells remaining in an anaerobic environment provided the opportunity for the endosymbiont to shed its aerobic metabolism and become a hydrogenosome. The few known eukaryotes that have hydrogenosomes live in oxygen-poor environments.

Finally, the eukaryotes that became intracellular parasites, with little need of making ATP of their own, would ultimately have shed the endosymbiont almost completely. Microsporidia and diplomonads would have been their descendants.

Martin and Müller note that their postulated host cell doesn't have to be a methanogen, merely an archaeon dependent on hydrogen. Nor does their hypothesis require that the host already have a nucleus or other eukaryotic characteristics.

Indeed, Martin speculates that the merger of a bacterium with the archaeal host cell prompted the development of the nucleus and many of the other features that distinguish eukaryotic cells from bacteria and archaea, such as the internal framework called the cytoskeleton.

"Under our hypothesis, the cytoskeleton results, like many other features, from the genetic complexity conferred by the forced integration of eubacterial genes into archaebacterial chromosomes," he says. "I would say that the nucleus is a relatively late invention on the way to becoming a real eukaryote."

That's a provocative statement, considering that the nucleus is the unifying feature of eukaryotic cells and is the source of their name. Not surprisingly, then, Martin and Müller's hypothesis has stimulated lots of discussion among evolutionary biologists and other researchers.

"This is a fun idea. It might even be true," says John C. Samuelson of the Harvard School of Public Health in Boston, who has been looking for mitochondrial-like genes in an amoebic eukaryote lacking both mitochondria and hydrogenosomes.

"I think the hydrogen hypothesis is very clever. It's incredibly useful," adds Johnson.

Even Cavalier-Smith acknowledges that the hydrogen hypothesis raises an alternative to the standard theory. Still, while there may be little evidence that clearly favors one idea over the other, Cavalier-Smith holds fast to tradition.

"Their hypothesis, in my view, suffers from the weakness that it requires a huge changeover in the metabolism of the host from an autotroph [which makes ATP from nonorganic fuel] to a heterotroph [which generates energy from organic molecules]," he says. "The conventional view doesn't require such a dramatic change and is therefore more plausible."

Martin, not surprisingly, disagrees. He notes that a similarly dramatic metabolic shift occurred—in the other direction—when photosynthesis developed in plants. Moreover, he argues, there is already solid evidence that the sugar metabolism of eukaryotic cells depends on genes more closely related to bacteria than to archaea, presumably a legacy of when the archaeal host stole the genes from its endosymbiont.

In fact, genes may prove the deciding factor between the hydrogen hypothesis and more traditional views of mitochondrial origins. Martin and Müller confidently predict that as more and more eukaryotic genes come to light, many should closely resemble those of modern hydrogen-dependent archaea rather than those of other microorganisms.

That's a fair test, agrees Cavalier-Smith. "I think there might even be enough data to do the analysis now," he says.

Don't be surprised if the answers that emerge remain ambiguous, warn some scientists.

"We're talking about ancient happenings here, and reconstructing such past events is extremely difficult," notes evolutionary biologist Jeffrey D. Palmer of Indiana University. "These are very tough problems to solve, and so we have to go into them with a great deal of caution and humility. This may be a situation where we can put forward a quite reasonable hypothesis, and it's difficult to satisfactorily test it." ☐