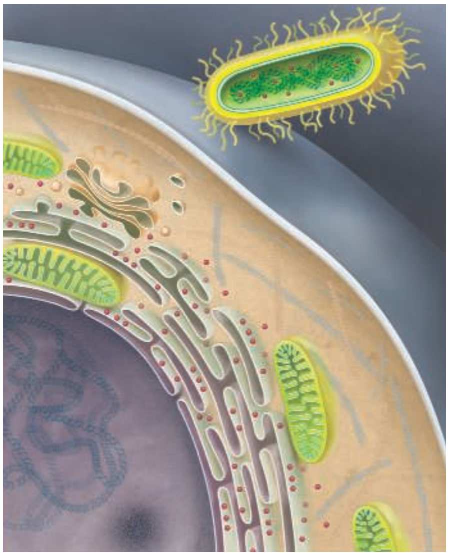
On the Origin of Eukaryotes



YOU MAY NOT FEEL AS THOUGH YOU HAVE much in common with a toadstool, but its cells and ours are strikingly similar. Animals and fungi both keep their DNA coiled up in a nucleus. Their genes are interspersed with chunks of DNA that cells have to edit out to make proteins. Those proteins are shuttled through a maze of membranes before they can float out into the cell. A cell in a toadstool, like your own cells, manufactures fuel in compartments called mitochondria. Both species' cells contain the same molecular skeleton, which they can break down and reassemble in order to crawl.

This same kind of cell is found in plants and algae; single-celled protozoans have the same layout as well. Other microbes, such as the gut bacterium *Escherichia coli*, lack it. All species with our arrangement are known as eukaryotes. The word is Greek for "true kernel," referring to the nucleus. All other living things that lack a nucleus, mitochondria, and the eukaryote LEGO-like skeleton are known as prokaryotes. "It's the deepest divide in the living world," says William Martin of the University of Düsseldorf in Germany.

The evolution of the eukaryote cell is one of the most important transitions in the history of life. "Without the origin of eukaryotes, we wouldn't be here to discuss the question," says T. Martin Embley of Newcastle University in the United Kingdom. Along with animals, eukaryotes gave rise to every other multicellular form of life. Indeed, when you look at the natural world, most of what you see are these "true kernel" organisms.

The fossil record doesn't tell us much about their origin. Paleontologists have found fossils of prokaryotes dating back 3.45 billion years. The earliest fossils that have been proposed to be eukaryotes—based on their larger size and eukaryotelike features on their surfaces—are only about 2 billion years old. Paleontologists have not yet discovered any transitional forms in the intervening 1.45 billion years, as they have for other groups, such as birds or whales. "One gets a bit of fossil envy," says Anthony Poole of Stockholm University. Fortunately, living eukaryotes and prokaryotes still retain some clues to the transition, both in their cell biology and in their genomes.

By studying both, researchers have made tremendous advances in the past 20 years in understanding how eukaryotes first emerged. A key step in their evolution, for example, was the acquisition of bacterial passengers, which eventually became the mitochondria of eukaryote cells. But some scientists now argue that the genes of these bacteria also helped give rise to other important features of the eukaryote cell, including the nucleus. "It's been a really cool journey," says Embley.

THE YEAR OF DARWIN

This essay is the eighth in a monthly series. For more on evolutionary topics online, see the Origins blog at **blogs.sciencemag.org/origins**. For more on eukaryotes, listen to a podcast by author Carl Zimmer at **www.sciencemag.org/multimedia/podcast.**

Unexpected ancestry

Scientists first divided life into prokaryotes and eukaryotes in the mid-1900s, using increasingly powerful microscopes to see the fine details of cells. But they couldn't say much about how prokaryotes and eukaryotes were related. Did the two groups branch off from a common ancestor? Or did eukaryotes evolve from a particular lineage of prokaryotes long after the evolution of the first prokaryotes?

An important step toward an answer to these questions was taken in the 1970s. Carl Woese of the University of Illinois, Urbana-Champaign, and his colleagues compared versions of an RNA molecule called 16*S* rRNA in a wide range of prokaryotes and eukaryotes. They reasoned that species with similar sequences were closely related and used that reasoning to draw a tree of life. Eukaryotes were all more closely related to one another than any were to prokaryotes, they found, which suggests that eukaryotes all belong to a single lineage and that the eukaryote cell evolved only once in the history of life.

But Woese and his colleagues got a surprise when they looked at the prokaryotes. The prokaryotes formed two major branches in their analysis. One branch included familiar bacteria such as *E. coli*. The other branch included a motley crew of obscure microbes—methane-producing organisms that can survive on hydrogen in oxygen-free swamps, for example, and others that live in boiling water around deep-sea hydrothermal vents. Woese and his colleagues argued that there were three major groups of living things: eukaryotes, bacteria, and a group they dubbed archaea. And most surprising of all, Woese and his colleagues found that archaea were more closely related to eukaryotes than they were to bacteria.

Although archaea and bacteria may seem indistinguishable to the nonexpert, Woese's discovery prompted microbiologists to take a closer look. They found some important differences, such as in the kinds of molecules archaea and bacteria use to build their outer membranes. A number of scientists began to study archaea to get some clues to the origins of their close relatives, the eukaryotes.

Many scientists assumed that after the ancestors of eukaryotes and archaea split apart, eukaryotes evolved all of their unique traits through the familiar process of small mutations accumulating through natural selection. But Lynn Margulis, a microbiologist now at the University of Massachusetts, Amherst, argued that a number of parts of the eukaryote cell were acquired in a radically different way: by the fusion of separate species.

Reviving an idea first championed in the early 1900s, Margulis pointed to many traits that mitochondria share with bacteria. Both are surrounded by a pair of membranes, for example. Mitochondria and some bacteria can also use oxygen to generate fuel, in the form of adenosine triphosphate (ATP) molecules. And mitochondria

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have their own DNA, which they duplicate when they divide into new mitochondria. Margulis argued that mitochondria arose after bacteria entered host cells and, instead of being degraded, became so-called endosymbionts.

> "As soon as you've got one prokaryote inside another prokaryote, you've completely transformed the cell and what it can do."
>
> —Nick Lane, University College London

Many studies have bolstered this once-controversial hypothesis. The genes in mitochondria closely resemble genes in bacteria, not those in any eukaryote. In fact, a number of mitochondrial genes point to the same lineage of bacteria, part of the alpha proteobacteria.

Additional evidence for the endosymbiont hypothesis comes from the genes in the eukaryote nucleus. Some of the proteins that carry out reactions in mitochondria are encoded in nuclear DNA. When scientists have searched for the closest relatives of these genes, they find them among bacterial genes, not eukaryote genes. It seems that after the ancestors of mitochondria entered the ancestors of today's eukaryotes, some of their genes got moved into the eukaryote's genome.

Mitochondria everywhere

Although most eukaryotes have mitochondria, a few don't—or so it once seemed. In 1983, Thomas Cavalier-Smith of the University of Oxford in the United Kingdom proposed that these eukaryotes branched off before bacteria entered the eukaryote cell and became mitochondria. According to his so-called archezoa hypothesis, mitochondria first evolved only after eukaryotes had already evolved a nucleus, a cellular skeleton, and many other distinctively eukaryotic features.

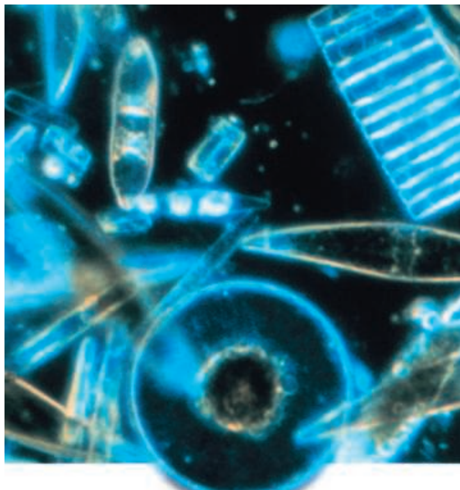
But a closer look at mitochondria-free eukaryotes raised doubts about the archezoa hypothesis. In the 1970s, Miklós Müller of the Rockefeller University in New York City and his colleagues discovered that some protozoans and fungi make ATP without mitochondria using structures called hydrogenosomes. (They named it for the hydrogen it produces as waste.) In 1995, scientists discovered mitochondrialike genes in eukaryotes that only had hydrogenosomes. Further research has now confirmed that hydrogenosomes and mitochondria descend from the same endosymbiont.

By 1998, Müller and Martin of the University of Düsseldorf were arguing that it was time to throw out the archezoa hypothesis. They maintained that the common ancestor of all living eukaryotes already carried an endosymbiont. They predicted that further study would reveal mitochondrialike structures in eukaryotes that seemed to be missing mitochondria at the time.

Based on the biochemistry of mitochondria and hydrogenosomes, Martin and Müller sketched out a scenario for how the original merging of two cells occurred. They pointed out that it is very common for bacteria and archaea to depend on each other, with one species producing waste that another species can use as food. "That sort of stuff is all over the bottom of the ocean," says Martin. Martin and Müller proposed that mitochondria descend from bacteria that fed on organic carbon and released hydrogen atoms. Their partner was an archaea that used the hydrogen to make ATP, as many archaea do today. Over time, the archaea engulfed the bacteria and evolved the ability to feed their newly acquired endosymbionts organic carbon.

In the 11 years since Martin and Müller proposed their "hydrogen hypothesis," scientists have come to agree that the common ancestor of living eukaryotes had an endosymbiont. "It is certain," says Eugene Koonin of the National Center for Biotechnology Information in Bethesda, Maryland.

One by one, exceptions have fallen away. Along with making ATP, mitochondria also make clusters of iron and sulfur atoms. While studying *Giardia*, a "mitochondria-free" eukaryote, Jorge Tovar of Royal Holloway, University of London, and his colleagues discovered proteins very similar to the proteins that build iron-sulfur clusters in mitochondria. The scientists manipulated the proteins so that they would light up inside *Giardia*. It turned out that the proteins all clumped together in a tiny sac that had, until then, gone unnoticed. In 2003, Tovar and his colleagues dubbed this sac a



Eukaryotes all! Tarsiers (*left*), toadstools (*top right*), and diatoms (*lower right*) are all made up of complex cells.

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mitosome. Scientists now agree that mitosomes are vestigial mitochondria.

Mosaic genomes

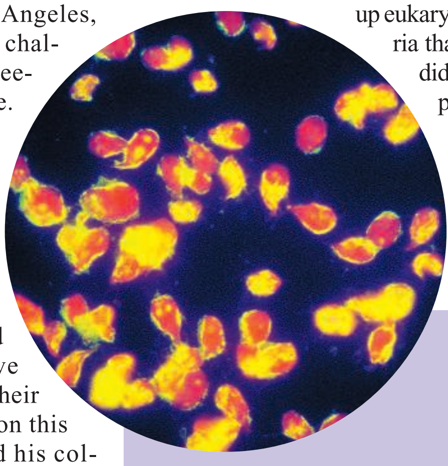
In 1984, James Lake of the University of California, Los Angeles, and his colleagues challenged Woese's three-domain view of life. Lake and his colleagues took a close look at ribosomes, the protein-building factories found in all living things. They classified species based on the distinctive lobes and gaps in their ribosomes. Based on this analysis, Lake and his colleagues found that eukaryotes do not form a distinct group on their own. Instead, they share a close ancestry with some lineages of archaea and not others. In effect, they found that there are only two major branches of life—bacteria and archaea. Eukaryotes are just a peculiar kind of archaea. Lake dubbed the archaeal ancestors of eukaryotes eocytes (dawn cells).

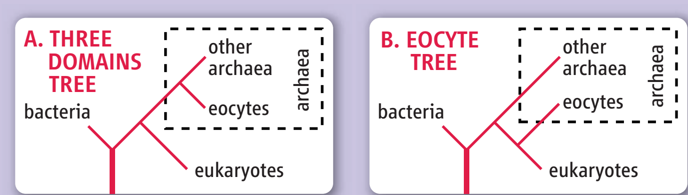
Since then, a number of scientists have tried to choose among the three-domain hypothesis, the eocyte hypothesis, and several others. They've analyzed more genes in more species, using more sophisticated statistical methods. In the 12 August issue of the *Philosophical Transactions of the Royal Society*, Embley and his colleagues present the latest of these studies, comparing 41 proteins in 35 species. "It is the eocyte tree that is favoured and not the three-domains tree," they concluded.

Embley and his colleagues selected proteins that preserved the clearest signal of the deep ancestry of life. They have been carried down faithfully from ancestor to descendant for billions of years. But eukaryote genomes also include genes that have been imported from other species through a process called horizontal gene transfer. About 75% of all eukaryote genes are more closely related to genes found in bacteria than ones in archaea.

Scientists have tried to make sense of this genetic mélange by cataloging the kinds of jobs archaeal and bacterial genes do in our cells. Archaeal genes tend to be involved in information processing. Bacterial genes tend to be associated with metabolism and the structure of our cells. But the line is not always easy to draw between archaeal and bacterial genes. Koonin and his colleagues have found that the proteins that make up the walls of the nucleus are made up of both archaeal and bacterial genes.

One possible explanation for the mixed-up eukaryote genome is that the bacteria that gave rise to mitochondria didn't just shrivel up into ATP-producing factories. Instead, many of their genes were transferred to the nucleus of their archaeal host. Those genes then helped produce the eukaryote membranes, nucleus, and metabolism. Most of our genes, in other words, were transferred from an endosymbiont.





Unsettled origins. The two trees depict different views on the ancestry of eukaryotes, which include *Giardia* (*inset*), once thought to lack mitochondria.

Having a second genome in such close quarters, Koonin and Martin have argued, may have posed a hazard to the survival of early eukaryotes. Along with protein-coding genes and other useful pieces of DNA, the genomes of many species also carry viruslike stretches of genetic material called mobile elements. Mobile elements can, on rare occasion, jump from one host genome to another. And once in their new host genome, they can make copies of themselves that are reinserted back in the genome. As mobile elements bombard a genome, they can disrupt the proper working of its genes.

Koonin and Martin suspect that with an endosymbiont in their midst, early eukaryotes would have been particularly vulnerable to attacks from mobile elements. They propose that the nucleus—the structure that gives eukaryotes their name—evolved as a defense against this attack. After mobile elements are transcribed into single-stranded RNA, they are copied back into the genome. With the invention of a nucleus, RNA molecules were moved across a barrier out of the nucleus in order to be translated into proteins. That wall reduced the chances of mobile elements being reinserted back into the genome.

Despite all the new insights into the origin of eukaryotes, scientists are far from agreed on all the details. In the July issue of *Bioessays*, for example, Yaacov Davidov and Edouard Jurkevitch of the Hebrew University of Jerusalem propose that the ancestors of mitochondria were not mutualists with archaea but predators that pushed their way into other prokaryotes and devoured them. Instead of killing their prey, Davidov and Jurkevitch argue, some predators took up residence there.

Scientists are also still debating how many bacterial genes eukaryotes got from the original endosymbiont. Prokaryotes sometimes pass DNA between distantly related species with the result that their genomes have become mosaics of genes. It's possible, some researchers argue, that many genes were transferred this way into the eukaryote genome from a variety of bacteria.

Testing these ideas will demand a better knowledge of the diversity of both prokaryotes and eukaryotes. It may also require new methods for reconstructing events that happened 2 billion years ago. "These are some of the hardest problems in biology," says Embley.

Whatever the exact series of events turns out to be, eukaryotes triggered a biological revolution. Prokaryotes can generate energy only by pumping charged atoms across their membranes. That constraint helps limit their size. As prokaryotes grow in size, their volume increases much faster than their surface area. They end up with too little energy to power their cells. Eukaryotes, on the other hand, can pack hundreds of energy-generating mitochondria into a single cell. And so they could get big, evolving into an entirely new ecological niche. "You don't have to compete for the same nutrients," says Nick Lane of University College London, author of *Life Ascending: The Ten Great Inventions of Evolution*. "You simply eat the opposition."

The eukaryote also opened the way to more complex species. Single-celled eukaryotes could evolve into multicellular animals, plants, and fungi. Individual cells in those organisms could evolve into specialized forms, such as muscles and neurons. "As soon as you've got one prokaryote inside another prokaryote," says Lane, "you've completely transformed the cell and what it can do."

–CARL ZIMMER

Carl Zimmer is the author of *Microcosm: E. coli and the New Science of Life*.

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