Not so special, after all?

The parasite *Giardia* was thought to represent a throwback to the earliest days of advanced cellular life. But biologists are now arguing over its true status. Jonathan Knight reports.

A taste of the past: the gut parasite *Giardia intestinalis* (green) has for years been an evolutionary icon.

P. M. MOTTA/F. M. MAGLIOCCA/SPL

Intestinal cramps rarely inspire deep thoughts about evolution. But anyone who has suffered an infection of the gut parasite *Giardia intestinalis* can boast of having an intimate, if painful, familiarity with an icon of evolutionary theory. Textbooks have for years held up this single-celled parasite as a living relic of the very early days in the evolution of plants, animals and other higher life forms.

But this may have to change. A new discovery about *Giardia*'s own innards, which makes the parasite look rather more run-of-the-mill, has caused a rift among evolutionary biologists. Some argue that its role in the history of life will have to be reconsidered. But others are fighting to retain *Giardia*'s iconic status.

At the heart of this debate lie mitochondria, sacs of enzymes that use oxygen to generate energy for cells. Organisms from people, through house flies and elm trees, to single-celled yeast and algae, all have them. These are the eukaryotes, whose cells contain many types of other useful sacs, or 'organelles', as well as a nucleus, all packaged in the same sort of fatty membrane that surrounds the cell.

The rest of life lacks this equipment. Prokaryotes, which include bacteria and archaea — ancient, single-celled creatures found in extreme environments such as volcanic springs — are devoid of mitochondria and other specialized organelles.

Biologists now believe that eukaryotes gained their mitochondria sometime between 1.5 billion and 2.2 billion years ago[1]. At the time, rising oxygen levels in Earth's atmosphere were causing problems for organisms adapted to life without the gas. The theory goes that the ancestor of mitochondria was a prokaryotic cellular parasite that could process oxygen. It took up residence inside an ancestral eukaryote and helped it by soaking up toxic dissolved oxygen. Over time, this parasite became an indispensable source of chemical energy for the host, using oxygen to 'burn' sugars. Most of its genes relocated to the host's nucleus, sealing the partnership.

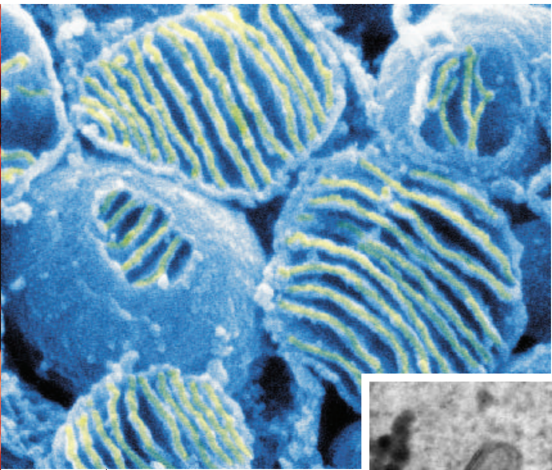
Primitive appearance

Until a few months ago, *Giardia* was thought to represent a throwback to the time before this union. Mitochondria are relatively easy to see under the microscope because they are large and have a characteristic appearance. But none were ever seen in *Giardia*, so it was thought to be a direct descendent of pre-mitochondrial eukaryotes. Partly as a result, resources have been ploughed into *Giardia* research, including a project to sequence its genome. "A lot of people's efforts were invested in the idea that *Giardia* was the most primitive eukaryote," says Andrew Roger, who studies molecular evolution at Dalhousie University in Nova Scotia, Canada.

Doubts about *Giardia*'s evolutionary position first began to emerge in 1998, when Roger and his colleagues found a strong similarity between a *Giardia* gene, called *cpn60*, and a gene from the α-proteobacteria[2]. These bacteria, which include the pathogen that causes typhus, are thought to be the closest living relatives to the prokaryotes that gave rise to mitochondria. Given the genetic similarity, Roger and his colleagues concluded that *Giardia* once had mitochondria but had lost them.

But the gene might also have been acquired from another invader that took up residence in eukaryotic cells and did not evolve into mitochondria. This is not as improbable as it might sound — hundreds of examples of such 'endosymbiosis' are known[3]. So far as we know, in billions of years, endosymbiosis has led to the formation of only two cellular organelles: mitochondria and chloroplasts, the site of photosynthesis in plants and algae, which are believed to be related to modern cyanobacteria.

But the real bombshell came last November, thanks to an international team led by molecular parasitologist Jorge Tovar of Royal Holloway, a college of the University of London in Egham, Surrey. His team found in *Giardia* a number of proteins related to those associated with mitochondria in other eukaryotes. More significantly, these



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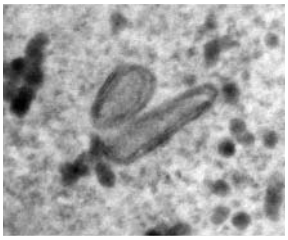

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Body of evidence: it was thought that *Giardia* had never evolved mitochondria (above), but electron micrographs (inset) have now revealed it contains sacs that resemble diminished versions of this cellular equipment.

proteins were clustered together at discrete locations in the organism's cells. Using electron microscopy, the researchers saw tiny sacs at those spots, which they identified as mitochondrial relics called mitosomes[4].

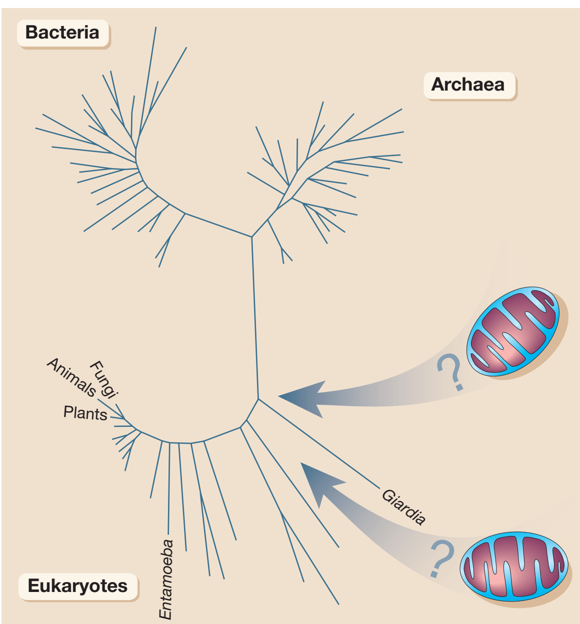
Gut feeling

Tovar first identified mitosomes in 1999 in an unrelated organism called *Entamoeba histolytica*, a deadly intestinal parasite[5]. Like *Giardia*, *Entamoeba* was once considered to have branched off the trunk of the eukaryotic evolutionary tree before mitochondria came on the scene, and the two were lumped together in a class called Archezoa[6]. But in the 1980s, DNA sequence evidence showed that *Entamoeba* branched much later than other organisms with fully fledged mitochondria[7]. To many researchers, this suggested that it had lost its mitochondria at some point. Tovar's discovery of mitosomes indicated that they were not entirely gone, only much reduced.

For Tovar and many others, the evidence that *Giardia* once had mitochondria is solid. For example, the electron micrographs of its mitosomes revealed a double membrane surrounding the sacs. This is a property of only three known cellular structures: chloroplasts, the nucleus and mitochondria. Furthermore, contained within those membranes are two proteins that process iron–sulphur clusters, complex structures that are essential to the mitochondrial job of energy production. "We now know that *Giardia* diverged after the evolution of mitochondria," Tovar asserts.

But Patricia Johnson, an evolutionary biologist at the University of California, Los Angeles, is among those who are not convinced. Johnson studies the evolution of hydrogenosomes, another cellular sac that may similarly be the product of mitochondrial reduction. One problem, she says, is that the protein produced by *cpn60* does not migrate to Tovar's mitosomes. Yet in fully fledged mitochondria and in other microorganisms with mitosomes or hydrogenosomes, it does. "The data make it a toss-up, but it's not being treated with that kind of caution," she says. To strengthen his case, Tovar is trying to identify all the proteins in *Giardia* mitosomes, to see if more of those normally associated with mitochondria are present.

Mitchell Sogin of the Marine Biological Laboratory in Woods Hole, Massachusetts, who heads the *Giardia* genome project, also wants to see more evidence before changing his mind about the parasite's evolutionary status. He points out that sequence analysis shows *Giardia*'s iron–sulphur cluster genes to be only weakly related to mitochondrial genes[8]. "Evolutionary trees must be strong if you are making strong statements about evolutionary history," he says.



SOURCE: N. R. PACE *PROC. NATL ACAD. SCI. USA* **98**, 805–808 (2001)

A question of timing: did advanced cellular life gain mitochondria after *Giardia* branched off from the evolutionary tree or before?

This attitude frustrates people such as William Martin, who studies molecular evolution at Heinrich Heine University in Düsseldorf, Germany. He is convinced that the best and simplest explanation for the data is that *Giardia* once had mitochondria[9]. Some people, he argues, refuse to accept this because they have spent too many years working on the opposite assumption. "They don't want it to have mitochondria because it spoils their soup," he says. "This thinking is deeply ingrained."

The thinking has its roots in the concept of the Archezoa, Martin argues, the group that was conceived to bring together a range of single-celled eukaryotes thought to lack mitochondria. *Giardia* was the grandaddy, having branched off on its own before any other eukaryote, according to evolutionary trees built using sequences of RNA from ribosomes, the organelles in which proteins are made (see Figure, below left).

Family ties

But one by one, the Archezoa all proved to have either a set of mitochondrial genes in their nuclei, or relics of mitochondria such as mitosomes or hydrogenosomes. This isn't entirely surprising, because many of these organisms are parasites, which tend over evolutionary time to become stripped-down versions of their former selves as they become increasingly dependent on their hosts.

Giardia's status as the earliest branching eukaryote has also been questioned. Some recent evolutionary trees that take into account the variable rates at which different DNA bases mutate paint a much muddier picture of the early branches[10].

If Tovar, Roger and Martin are correct, how bad is the news for *Giardia* research? Sogin contends that even if *Giardia* is shown definitively to have once had mitochondria, it would have no adverse impact on his genome project. Apart from the organism's importance to human health, its sequence should still reveal much about evolution because it branched off the eukaryotic tree very early, he says.

But *Giardia*'s status as a uniquely important throwback to the earliest days of the eukrayotes surely would be usurped if someone discovers a new member of the Archezoa, sans mitochondria or mitosomes, lurking in the oxygen-starved muck at the bottom of a lake. This possibility hasn't escaped the notice of Roger and others, who are still collecting samples in the hope of finding something new. "There is a lot more out there, and it's possible that such organisms still exist," Roger says. ■

Jonathan Knight writes for *Nature* from San Francisco.

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