Essence of mitochondria

Katrin Henze and William Martin

For years, a unicellular creature called *Giardia* has occupied a special place in biology because it was thought to lack mitochondria. But it does have them — though tiny, they pack a surprising anaerobic punch.

All known life-forms are either prokaryotes or eukaryotes; there is nothing in between. Eukaryotic cells have a nucleus and compartments that are surrounded by two membranes, but prokaryotic cells never do. The prokaryotes came first; eukaryotes (all plants, animals, fungi and protists) evolved from them, and to this day biologists hotly debate how this transition took place, with about 20 different theories on the go[1]. In most textbook accounts, an ancient prokaryotic lineage supposedly evolved a nucleus, giving rise to a eukaryote, which then acquired mitochondria — the double-membrane-bounded powerhouses of eukaryotic cells. A cornerstone of this view has been the single-celled eukaryote *Giardia intestinalis*, which many consider to be a primitive intermediate[2,3], a 'living fossil' from the time of the prokaryote-to-eukaryote transition, because it possesses a nucleus but lacks mitochondria. Or so we thought.

The paper on page 172 of this issue will surprise many people. There, Jorge Tovar and colleagues[4] show that *Giardia* (Fig. 1) does have mitochondria after all. They are highly reduced, and are called mitosomes. Unlike the mitochondria familiar to most biologists, *Giardia*'s mitosomes do not generate ATP (energy). Rather, they are factories for the assembly of iron–sulphur (Fe–S) clusters, which *Giardia* requires to make ATP. These findings mark a turning point for views of early eukaryotic and mitochondrial evolution: *Giardia*'s place as an intermediate stage in standard schemes of eukaryotic evolutionary history[2,3] is no longer tenable.

Giardia intestinalis (formerly *G. lamblia*) is a pathogen that shuns oxygenated environments and causes diarrhoea when it infects the human intestine. Like all cells, it requires a constant supply of ATP. Treatments for *Giardia* infection exploit the fact that its ATP-synthesizing biochemistry differs from ours. Whereas human cells make their ATP in mitochondria and require oxygen to do so, *Giardia* makes all of its ATP in the cytosol with the help of simple anaerobic pathways[5,6]. Several enzymes of *Giardia*'s ATP-generating machinery contain Fe–S clusters[5–9], which are ubiquitous cofactors in the electron-transfer reactions involved in ATP production. They are essential for the parasite's survival. Among *Giardia*'s proteins that contain Fe–S clusters are hydrogenases[7–9], ferredoxins and

Figure 1 **Mitochondria where least expected. *Giardia* has been the textbook example of a single-celled eukaryote that lacks mitochondria. Now, with the paper by Tovar and colleagues[4], it becomes a prime example of an anaerobic eukaryote that possesses mitosomes — highly reduced mitochondria that do not function in core ATP synthesis, but are essential for the assembly of iron–sulphur clusters. The image is about 100,000 times life size.**

pyruvate:ferredoxin oxidoreductase[5,8,9] (PFO, which incidentally is the target for anti-*Giardia* drugs).

In other eukaryotes — yeast and humans, for example — the synthesis of Fe–S clusters always begins in mitochondria[10]. But if, as almost everyone believes, *Giardia* lacks mitochondria yet has Fe–S clusters, where are the clusters synthesized? Tovar *et al.*[4] have investigated this question, and find the answer to be that they are made in curious little membrane-bounded structures, several dozen of which are present in each *Giardia* cell. These organelles are surrounded by two membranes, a decisive observation that leaves only two reasonable possibilities as to what the organelles might be. This is because, in a century of delving into cells, biologists have discovered only two kinds of organelle that are surrounded by a double membrane: chloroplasts and mitochondria.

Chloroplasts are the organelles of photosynthesis in plants, and are descendants of cyanobacteria (photosynthetic prokaryotes) that, deep in evolutionary history, took up residence inside a eukaryotic host, forming a symbiotic partnership. But the *Giardia* organelles are almost certainly not chloroplasts, because no trace of chloroplast-specific biochemistry has ever been found in this protist. So are they mitochondria? Indeed they are.

Mitochondria are hallmarks of eukaryotic cells, and can be traced to another prokaryote — an α-proteobacterium[11] — that took up residence in its host long before chloroplasts arose. Mitochondria comprise a diverse family of organelles, including many anaerobic forms[12]. Most notable among these are hydrogenosomes, hydrogen-producing forms of mitochondria discovered 30 years ago by Miklós Müller. As Tovar *et al.*[4] explain, mitosomes are the most recent addition to the mitochondrial family of organelles. They are small, functionally reduced forms that were first found in the

amoeboid parasite *Entamoeba histolytica*[13] and the pathogen *Trachipleistophora hominis*[14] (which belongs to a group of fungi called microsporidia). Before those reports, *Entamoeba* and microsporidia were thought to lack mitochondria altogether. Now mitosomes appear in *Giardia*, too, and we have the first direct glimpse of their function.

The biochemical role of the mitosome can hardly be core ATP synthesis. That occurs in the cytosol in both *Entamoeba*[5] and *Giardia*[5]. And it is uncertain that microsporidia even make their own ATP, because they can steal it from the cells that they infect[15]. Tovar *et al.*[4] show that, instead, the *Giardia* mitosomes harbour critical enzymes of Fe–S cluster assembly. Furthermore, cell fractions enriched for the organelle assemble Fe–S clusters *in vitro*. This pins a function to the mitosome in *Giardia* and is a major advance in understanding.

Possibly, *Giardia* assembles Fe–S clusters in mitosomes for the same reason that yeast and humans assemble them in mitochondria: the process is very oxygen-sensitive. The mitochondrial matrix (the space inside the organelle) is the most oxygen-poor compartment in oxygen-respiring cells because oxygen is consumed in the surrounding membrane. Lloyd *et al.*[16] have shown that *Giardia* possesses organelles that accumulate mitochondrion-specific dyes and that can transfer electrons from donors to acceptors, which could easily include oxygen. But it remains to be seen whether the organelles observed by Lloyd *et al.*[16] and Tovar *et al.*[4] are identical.

Although mitochondria are usually considered to be oxygen-dependent, *Giardia*'s tiny mitochondria have an anaerobic function (Fe–S cluster assembly) in the synthesis of oxygen-sensitive proteins such as hydrogenase and PFO, which are normally found in hydrogenosomes[1,4–9,12]. Eukaryotes diversified while the oceans were largely anoxic[17,18], so these anaerobic functions are most easily seen as biochemical relics of the mitochondrion's anaerobic past. Such anaerobic relics are abundantly preserved in diverse eukaryotic lineages today[9,12].

We know that mitochondria arose as intracellular symbionts in the evolutionary past[11]. But in what sort of host? That question still has biologists dumbfounded[1]. In the most popular theories, *Giardia* is seen as a direct descendant of a hypothetical eukaryotic host lineage that existed before mitochondria did[2,3]. But Tovar and colleagues' findings[4] show that *Giardia* cannot have descended directly from such a host, because *Giardia* has mitosomes. So our understanding of the original mitochondrial host is not improved by these new findings, but our understanding of mitochondria certainly is. In its role as a living fossil from the time of prokaryote-to-eukaryote transition, *Giardia* is now retired. But it assumes a new place in the textbooks as an exemplary eukaryote with tiny mitochondria that have a tenacious grip on an essential — and anaerobic — biochemical pathway. ∎

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Chemistry

Dendrimers set to self-destruct

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The versatility of the branched macromolecules known as dendrimers is being exploited in various ways — explosively so, in the context of their application as potential drug-delivery systems.

If a good idea for scientific innovation emerges, you can be sure that several teams of researchers will be quickly on the case. An example comes in the form of three reports[1–3] that explore the prospects of using dendrimers for drug release inside diseased cells.

Dendrimers are artificial macromolecules, constructed in step-by-step fashion using repetitive chemistry. The macromolecule constituents radiate in branching form from a central core, creating a sphere of chemical groups that can be tailored according to requirements. The results of the process are not only aesthetically appealing but offer chemists wonderful opportunities for exploring new ideas[4]. Dendrimers are large, but can be synthesized and characterized with a precision similar to that possible with smaller organic molecules. They do not suffer from the problem of 'polydispersity' that dogs linear macromolecules: that is, constituents of a given set of dendrimers have exactly the same molecular weight, rather than being a mixture of chains with a distribution of molecular weights. And the large number of identical chemical units in the branching units, as well as those at the periphery, confers great versatility. The end groups can be designed for various purposes, including sensing, catalysis or biochemical activity.

In this last instance, one of the potential virtues of dendrimers comes under the heading of 'multivalency': the enhanced effect that stems from lots of identical molecules being present at the same time and place. The combination of multivalency with precision architectures has made dendrimers of increasing interest for biomedical applications, not least for drug delivery[5]. Dendrimers can enter cells remarkably easily, a property that means they have been investigated as potential gene-transfection agents. The chemical groups that bristle from the ends of the branches allow for tuning of biological properties, and can anchor one or more target groups onto the dendrimer. The compound that constitutes the drug itself can be physically encapsulated in the dendrimer or bound to it. There have been attempts to achieve total and simultaneous release of active agents through changing pH conditions. But generally the traditional route has been that of getting one chemical trigger to release one drug molecule.

Independently of one another, teams led by de Groot[1], Shabat[2] and McGrath[3] have explored a much more advanced concept — simultaneous release of all of a dendrimer's functional groups by a single chemical trigger. All three exploit the fact that the dendrimer skeleton can be constructed in such a way that it can be made to disintegrate into known molecular fragments once the disintegration process has been initiated. Variously termed "cascade-release dendrimers"[1], "dendrimer disassembly"[3] and — colourfully — "self-immolative dendrimers"[2], these systems in effect perform a chemical amplification reaction. Triggered by a specific chemical signal, the dendrimer scaffold falls apart in several steps in a chain reaction, releasing all of the constituent molecules.

Two of the teams[2,3] demonstrate the process in systems in which relatively simple