

Ring out the Truth – How Do Eukaryotes Divide their Mitochondria?

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Mitochondria are semi-autonomous, energy-producing organelles within eukaryotic cells. These organelles evolved from a bacterial endosymbiont that was acquired more than a billion years ago and have been faithfully inherited from one cell generation to the next during this time. New mitochondria cannot be made de novo but must be produced by the division of pre-existing organelles. Cells require new mitochondria, not only as they divide and grow, but also to replace damaged or ageing organelles. In recent years, several proteins involved in the division of mitochondria have been discovered and their functions are being characterised (1-4). These proteins form patches and/or rings around the outside and the inside of dividing organelles. Outer rings may have been developed by eukaryotes from the earliest times, when the bacterial endosymbiont that later became a mitochondrion lost its cell wall. Protein components of the inner ring are not as well characterised, and may be much more variable in composition across the eukaryotic spectrum than those of the outer ring. In animals and fungi the inner ring appears to share similar proteins with the outer ring but, in the ancient protistan eukaryotes, mitochondrial inner ring proteins are often direct descendants of the proteins that bacteria use to divide (Fig. 1).

Bacterial cell division

An elaborate system of proteins forms a ring on the inside of the bacterial cell. The ring not only marks out the cell's division plane, but is likely to also pull the cell membrane inwards and hence bring about division. A major component of the ring is the FtsZ protein and, of all the proteins that the majority of prokaryotes use for division, FtsZ is the most conserved and widespread. FtsZ is a GTPase that bears significant structural similarity to the eukaryotic cytoskeletal protein, tubulin (5); both proteins are capable of polymerising into similar structures *in vitro* and it is believed that tubulin may have evolved from FtsZ (6). Prior to division, FtsZ polymerises into a ring (called the Z ring) that is attached to the inner membrane of the bacterial cell by linker proteins. As the cell constricts, the Z ring decreases in diameter. Although FtsZ polymers can change conformation upon hydrolysis of GTP, it is not known if FtsZ actually provides the contractile force

necessary to pinch the cell in two, or whether other proteins participate as well. A more detailed discussion of bacterial division and placement of the Z ring is given in the article by Migocki and Harry in this *Showcase on Research*. The first observation of FtsZ in a eukaryote was reported several years ago – in chloroplasts of the model plant, *Arabidopsis thaliana* (7).

FtsZ is used to divide all chloroplasts and some mitochondria

The *Arabidopsis* nucleus encodes three FtsZs that fall into two family groups, and they are all targeted to the inside (stroma) of the chloroplast. Chloroplasts are descended from cyanobacteria and, as one would expect, chloroplast FtsZs are most like their counterparts in these bacteria. Antisense inhibition of the expression of any two of these FtsZs (e.g.

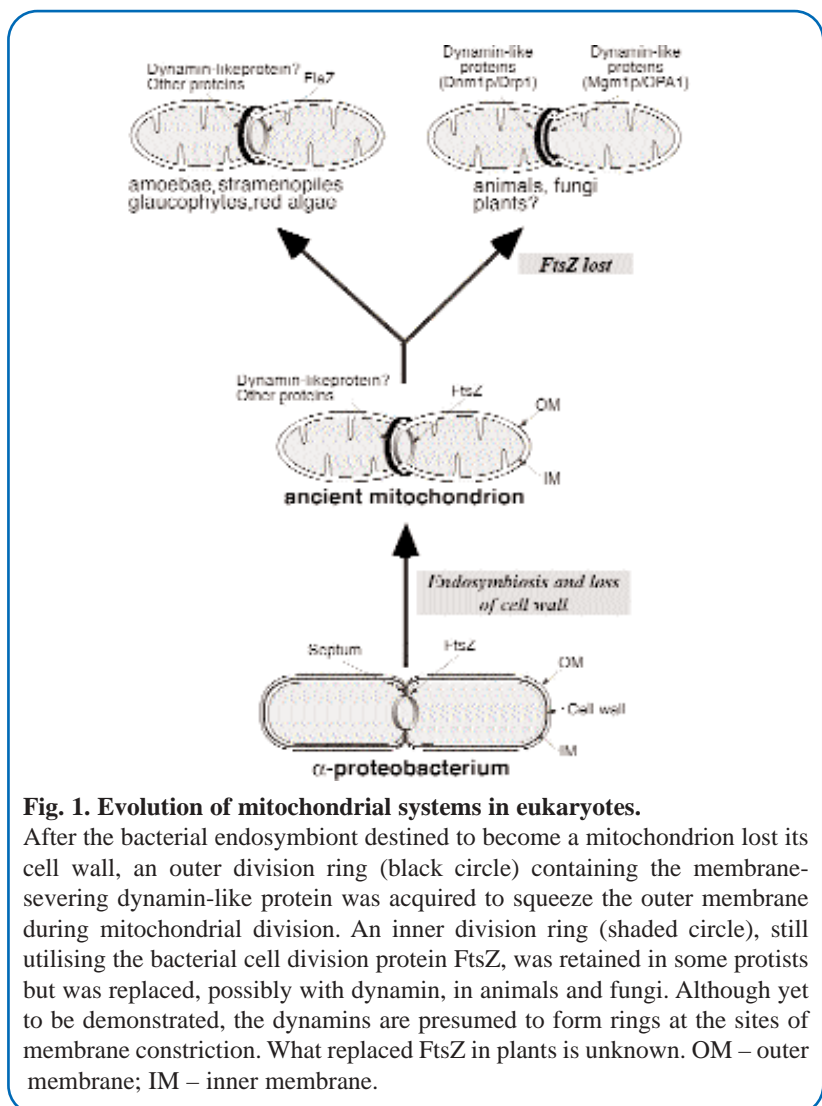


Fig. 1. Evolution of mitochondrial systems in eukaryotes.

After the bacterial endosymbiont destined to become a mitochondrion lost its cell wall, an outer division ring (black circle) containing the membrane-severing dynamin-like protein was acquired to squeeze the outer membrane during mitochondrial division. An inner division ring (shaded circle), still utilising the bacterial cell division protein FtsZ, was retained in some protists but was replaced, possibly with dynamin, in animals and fungi. Although yet to be demonstrated, the dynamins are presumed to form rings at the sites of membrane constriction. What replaced FtsZ in plants is unknown. OM – outer membrane; IM – inner membrane.

AtFtsZ1-1 or AtFtsZ2-1) prevented chloroplast division, indicating that the proteins function together to effect division (8). Immunolocalisation experiments have shown that AtFtsZ1-1 and AtFtsZ2-1 form co-aligned rings at the dividing regions of chloroplasts (9). Chloroplast FtsZs have now been isolated from most groups of land plants and many kinds of algae and are probably used by all chloroplasts for division; however, the same cannot be said for mitochondria.

Searches of gene databases for mitochondrial FtsZs failed to identify any candidates in the complete genomes of *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, humans and *A. thaliana*. However, animals, fungi and plants occupy only a few branches of the tree of life, and there are many other ancient and simple eukaryotic groups that might still use FtsZ to divide their mitochondria. These other eukaryotes, collectively known as the protists, are the wellspring of eukaryotic life and include the amoebae, ciliates, protozoal parasites and all the algae. In the stramenopile alga, *Mallomonas splendens* (diatoms and brown seaweeds are better-known stramenopiles), two FtsZs have been found. The first, MsFtsZ-cp, was a chloroplast protein and the second, MsFtsZ-mt, was the first mitochondrial FtsZ characterised from any eukaryote (2). MsFtsZ-mt was most similar to FtsZs of the α -proteobacteria, the group from which mitochondria were derived, and a MsFtsZ-mt/green fluorescent protein fusion could be imported into yeast mitochondria. Furthermore, MsFtsZ-mt was localised to regions of *Mallomonas* mitochondria that appeared about to divide, or had recently done so. Although much of the labelling was punctate, we occasionally observed belts of MsFtsZ-mt around the equators of mitochondria, and these were possibly Z rings (2).

Since discovering a mitochondrial FtsZ in *Mallomonas*, other examples have been found in several eukaryotic groups: namely *Dictyostelium*, several different groups of phytoplankton (diatoms, haptophyte algae and glaucophytes; our unpublished data) and the red alga, *Cyanidioschyzon merolae* (10). It is now apparent that ancient FtsZ-based mitochondrial division systems may still be used by many eukaryotic groups to divide their organelles from the inside, and that in a few advanced groups, specifically animals, fungi and plants, it has been abandoned (**Fig. 1**) (11). Since *Dictyostelium* is a genetically tractable protist, we began to investigate the function of mitochondrial FtsZ in this organism.

Dictyostelium is soil dwelling, social amoeba. When its bacterial food supply runs out, the normally solitary amoebae aggregate to form a multi-cellular slug that migrates to the soil surface. The slug then metamorphoses into a stalked, spore-producing, fruiting body. *Dictyostelium* contains two mitochondrially-targeted FtsZ proteins, called FszA and FszB. Microscopy has revealed that FszA appears to form tiny belts that transect the mitochondrion and are probably components of an inner ring (our unpublished observations). The mitochondria in most cells are normally spherical to rod-shaped but, when the *fsz* genes were disrupted by insertional mutagenesis, the mitochondria elongated into tubules (our unpublished

observations). The block in division was probably not complete, since the mitochondria, though clearly elongated, still formed many, separate tubules. Other proteins, which probably normally operate in concert with Fsz proteins but which can work in their absence, are likely to also be involved in dividing the mitochondria, albeit less efficiently. Evidence is now emerging that mitochondrial division in FtsZ-containing organisms may not be so different from their animal and fungal counterparts and that all eukaryotes might have similar outer rings that can still function in the absence of the inner ring.

Outer mitochondrial division rings may be conserved in all eukaryotes

Recent breakthroughs in our understanding of mitochondrial fission have been achieved by using clever mutational screens for fission (division) proteins in budding yeast (1). In *S. cerevisiae*, mitochondria form elongated, branched tubules around the periphery of the cell. This network is maintained by a balance of tubule fission and fusion (12). Briefly, when the fusion mutant *fzo1-1* (fuzzy onions) is grown at the non-permissive temperature (37°C), the mitochondria fragment due to an imbalance between fission and fusion events. Without the ability to fuse at the non-permissive temperature, the mitochondria of *fzo1-1* become smaller and smaller due to ongoing fission events and eventually lose their DNA and become non-functional. On normal growth media, *fzo1-1* cells can survive without functioning mitochondria because they can use fermentation to produce energy. If, however, they are grown on a non-fermentable carbon source, such as glycerol, they will die unless they develop a suppressor mutation in a gene of the mitochondrial fission system that prevents the mitochondria from fragmenting. Several groups have successfully used these screens to recover three fission proteins: Dnm1p, Fis1p and Mdv1p (Table 1) (1,3,4,13).

Dnm1p is a dynamin-like protein that probably forms a ring or collar around the outer mitochondrial membrane and helps to squeeze the mitochondrion during fission (**Fig. 1**). Homologues of this dynamin exist in animals (Drp1) (14,15), plants (16) and *Dictyostelium* (17). Null mutations of the *Dictyostelium* dynamin-like protein, DymA, are known to influence mitochondrial morphology and dynamin-like proteins may therefore be an important component of the outer division ring in all eukaryotes (**Fig. 1**) (17). In animals, Drp1 localises to mitochondria at regions that appear about to or have recently divided (14). Knockouts of Drp1 in animals cause the mitochondria to form large masses of highly interconnected tubules (14,15).

Fis1p is predicted to anchor the fission complex to the outer mitochondrial membrane and has homologues in animals and plants. In animals, the small GTPase, Rab32, is also implicated in organelle fission, since introducing non-functional versions of the protein into cells causes the tubular mitochondria to become highly interconnected, indicating a reduction in organelle fission (**Table 1**). Interestingly, *Dictyostelium* also has a Rab32 homologue whose localisation and function we are presently investigating.

It is curious that, despite considerable effort, yeast genetic screens have only uncovered proteins involved in dividing the outer, and not the inner, mitochondrial membranes (1). We know that an inner membrane fission system exists, at least in *C. elegans*, since inhibition of Drp1 expression prevents the outer mitochondrial membrane from dividing, but not the inner membrane (14). It is likely, therefore, that knockouts of internal division proteins in fungi might not effectively inhibit the outer ring from continuing to divide the mitochondria, as also appears to be the case with FtsZ in *Dictyostelium*. If FtsZ is a component of the inner ring in some protists, what has replaced it in animals and fungi?

Dynamin-like proteins may have replaced FtsZ in animals and fungi

The mitochondrially-targeted yeast protein, Mgm1p, influences both mitochondrial fusion and fission events (18). Mgm1p is a dynamin-like protein of the inter-membrane space that is peripherally associated with the inner membrane (Fig. 1, Table 1). The mitochondria of Mgm1p mutants undergo a complex series of morphological changes at non-permissive temperatures, suggestive of a role for Mgm1p in membrane fission and membrane remodelling to make mitochondria fusion-competent (18). Because Mgm1p

is a dynamin-like protein, it could form a constrictive collar around the outside of the inner membrane and could possibly squeeze the inner membrane from the outside. It is unknown if Mgm1p interacts directly with the outer membrane Dnm1p/Mdv1p/Fis1p fission complex to form a coordinated bimembrane fission system. In animals, mutations in the homologue of Mgm1p, OPA1, cause a genetic disease of the eye. When OPA1 was over-expressed the normally tubular mitochondria fragment, indicating that excessive numbers of fission events were occurring (19). Mgm1p and OPA1 possess N-terminal mitochondrial targeting sequences to direct them into the inter-membrane space. Although plants contain many dynamin-like proteins, none appears to be targeted into this region of the mitochondrion, and so it is unknown how plants divide their inner mitochondrial membrane (Table 1).

Conclusions

The inner mitochondrial division ring of eukaryotes has undergone significant changes over time, unlike the dynamin-based outer ring, which is possibly used by all mitochondria-bearing eukaryotes. In many protists, the inner ring likely still contains the ancient cell division protein, FtsZ, but this appears to have been replaced by a dynamin-

Table 1. Mitochondrial division proteins in eukaryotes.

Protein	Function	Organism					
		<i>S. cerevisiae</i>	<i>H. sapiens</i> , <i>C. elegans</i>	<i>A. thaliana</i>	<i>D. discoideum</i>	<i>M. splendens</i>	<i>C. merolae</i>
Outer ring							
Outer dynamin ring	Constriction	Dnm1p	Drp1, DRP1	ADL2b	DymA	?	?
Fis1p	Membrane anchor	Fis1p	Yes	Yes	?	?	?
Mdv1p	WD repeat protein that interacts with Dnm1 and Fis1p	Mdv1p	No	No	?	?	?
Rab GTPase	A-kinase anchoring protein	No ¹	Rab32	Yes ²	RabE	?	?
Inner ring							
FtsZ	Forms inner ring, may constrict	No	No	No	FszA FszB	MsFtsZ-mt	Yes
Dynamin of intermembrane space	Constrict inner membrane	Mgm1p	OPA1	No ³	?	?	?

Yes indicates that proteins homologous to those functionally characterised are present in the gene database.

? indicates that no homologous proteins have been discovered so far in the incompletely sequenced genomes of these organisms.

¹ A protein with high sequence identity to Rab32 is present in yeast but it appears to be specific for endosomes.

² *Arabidopsis* contains a protein very similar to Rab32 but it lacks a key alanine residue characteristic of this group.

³ *Arabidopsis* contains several dynamin-like proteins but none appears to possess a mitochondrial targeting sequence characteristic of Mgm1/OPA1.



like protein in animals and yeast. Since dynamin can only squeeze from the outside and cannot pull from the inside like FtsZ, dynamins of the inner-ring reside in the intermembrane space and not the mitochondrial matrix. Why FtsZ was possibly replaced with dynamins in animals and fungi is not known, but perhaps the answer can be found in morphology and dynamics of mitochondria. In *Mallomonas*, *Dictyostelium* and *Cyanidioschyzon*, three protists known to use FtsZ, the mitochondria typically form small, separate, bacterium-shaped organelles, whereas in fungi and animals mitochondria tend to form larger, elongated, branching tubules that appear to undergo fission and fusion frequently. Perhaps this morphological plasticity could only be achieved by replacing the rigid bacterial-like FtsZ system with a more flexible one based on dynamin.

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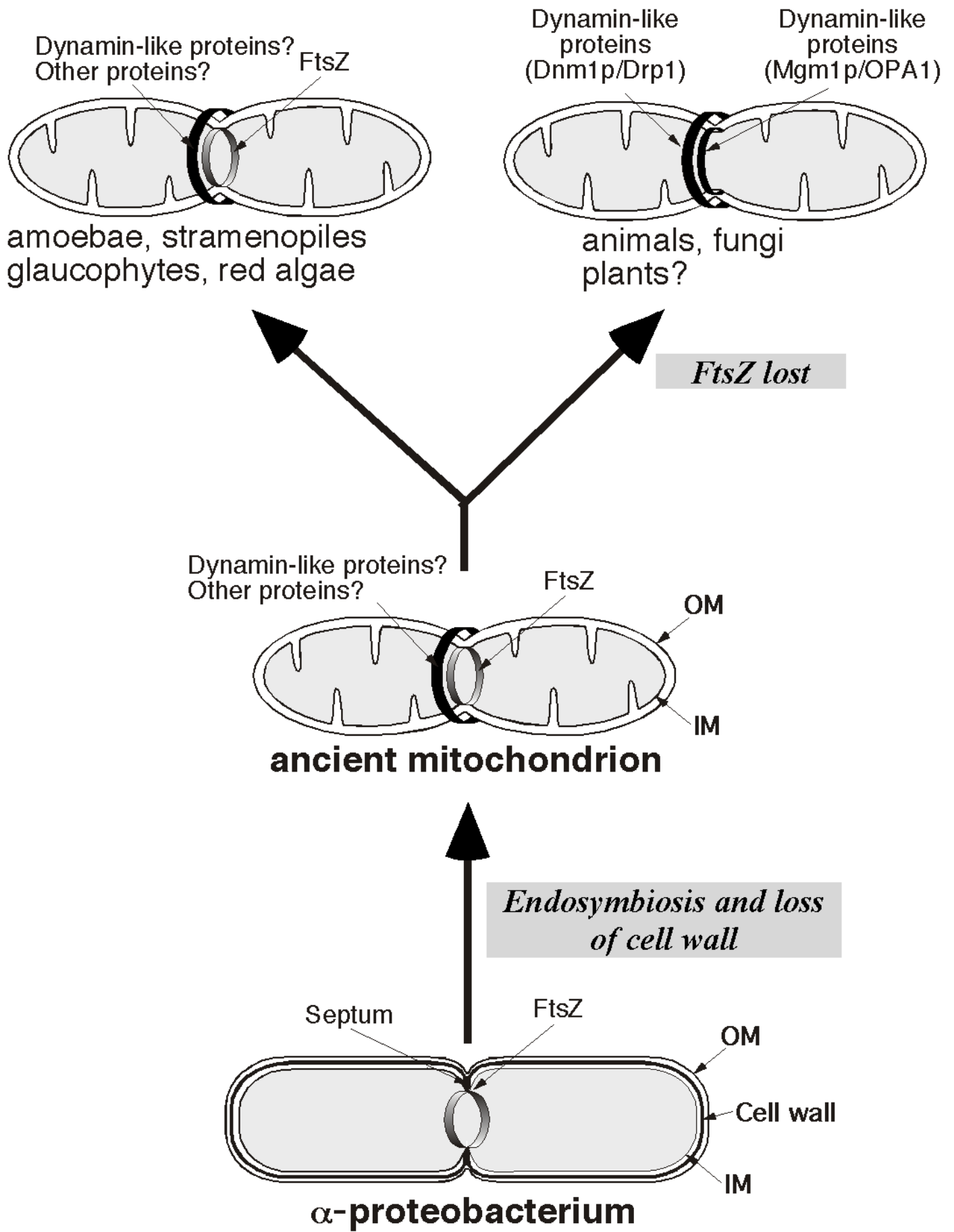


Fig. 1