



Tansley review

Mitochondrial dynamics

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Received: 9 July 2003

Accepted: 19 August 2003

doi: 10.1046/j.1469-8137.2003.00918.x

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Contents

Summary	463	VI. Mitochondrial distribution	470
I. Introduction	464	VII. Plant specific proteins playing a role in mitochondrial dynamics	470
II. Mitochondrial evolution	464	VIII. Conclusions	471
III. Mitochondria and the cytoskeleton	465	Acknowledgements	475
IV. Mitochondrial morphology, biogenesis, proliferation and inheritance	466	References	475
V. Mitochondrial fission and fusion	468		

Summary

Key words: mitochondria, dynamics, morphology, development, division, fission, fusion.

Mitochondria cannot be created *de novo* but instead must arise from the fission (division) of a parental organelle. In addition to fission, mitochondria also fuse with one another and it is thought that a co-ordinated balance of these two processes controls mitochondrial shape, size and number. In the past 5–7 yr, molecular genetics coupled to state-of-the-art cell biology, in particular the use of mitochondrial-targeted green fluorescent protein (GFP), has enabled identification of proteins controlling mitochondrial shape, size and number in yeast and mammalian cells. Little is known about higher plant mitochondrial dynamics. Recently, however, several genes involved in the control of plant mitochondrial dynamics have been identified. The aim of this article is to bring together what is known about mitochondrial dynamics in any organisms and to relate this to our recent knowledge of the underlying processes in higher plants.

© *New Phytologist* (2003) **160**: 463–478

I. Introduction

Mitochondria are cytoplasmic organelles of eukaryotic cells. It is not possible to attribute the discovery of mitochondria to a single person since between the years 1850–1890 many cytologists observed granular bodies within cells, some of which may have been mitochondria (Lehninger, 1964). The first isolation of mitochondria has been attributed to Kolliker, who, in 1888 teased these granular bodies from insect muscle and demonstrated that they swelled in water and were contained by a membrane (Lehninger, 1964). In 1898, Benda first coined the name, mitochondrion, derived from the Greek *mitos*, a thread, and *chondros*, a grain (Tzagoloff, 1982) but it was another 50 yr till the mitochondrion was identified as the site of oxidative energy metabolism (Kennedy & Lehninger, 1949). The intricacies of the energy transducing reactions in mitochondria were elucidated over the second half of the last century. Aerobic respiration within mitochondria involves the controlled oxidation of reduced organic compounds via the Krebs cycle producing CO₂. Electrons released during the Krebs cycle are transferred through an electron transport chain comprising four respiratory complexes ultimately reducing O₂ to H₂O. As the electrons move along the transport chain the free energy released is used to generate a proton electrochemical gradient across the inner mitochondrial membrane. In the final step, the potential energy stored in the proton gradient is used by the ATP synthase, occasionally known as the fifth complex, to phosphorylate ADP. The basic aspects of mitochondrial respiration as described above are common to all mitochondria, however, the biochemistry of higher plant mitochondria is known to differ substantially from that of other eukaryotes in a number of respects. These include the existence of an alternative oxidase uncoupled to the generation of ATP, several NADH dehydrogenase isoforms (e.g. rotenone-insensitive bypass) and, in leaf mitochondria, a high capacity for glycine oxidation during photorespiration (Mackenzie & McIntosh, 1999). Plant mitochondria participate in many metabolic processes in addition to respiration including the supply of carbon skeletons for amino acid biosynthesis and the biosynthesis of sugars from lipids in some germinating seeds (Mackenzie & McIntosh, 1999; Bowsher & Tobin, 2001). In these processes and others, for example, the biogenesis of mitochondrial membranes, there is a requirement for co-ordination of function between mitochondria, the cytosol and other organelles. It is not clear, however, how mitochondria communicate with the rest of the cell.

Until recently, it was believed that mitochondria were simply passive organelles, receiving instruction from the rest of the cell. Now, however, this perspective has changed considerably and mitochondria have been recognised as active participants in signal transduction pathways (Rizzuto *et al.*, 2000; Brookes *et al.*, 2002). The central role of mitochondria over and above the provision of energy and metabolites can be exemplified by three recent discoveries. Firstly, mitochondria

have been shown to have a central role as regulators of redox cell signalling involving reactive oxygen and nitrogen species (Brookes *et al.*, 2002). Secondly, in addition to their primary role in the supply of energy to keep cells alive mitochondria are intimately involved in the control of cell death. The release of cytochrome c from mammalian mitochondria is a well-characterised response to apoptotic stimuli (Wang, 2001) and release of cytochrome c from plant mitochondria has been demonstrated in response to heat-induced programmed cell death (PCD) (Balk *et al.*, 1999) and during premature PCD of anther tissue in a cytoplasmic male sterile sunflower mutant (Balk & Leaver, 2001). Thirdly, mitochondria, which were until recently thought to have little role in intracellular calcium signalling, are now thought to be involved in the modulation of the cytoplasmic calcium signal (Duchen, 2000; Rizzuto *et al.*, 2000), at least in part via close association with the endoplasmic reticulum (Rutter & Rizzuto, 2000). These last two roles of mitochondria are closely interconnected and there is considerable interest in the role of mitochondrial calcium dynamics in apoptosis (Orrenius *et al.*, 2003). The role of mitochondrial calcium in plant cell signalling has received little attention because of the technical difficulties in measuring changes in the mitochondrial free calcium concentration ($[Ca^{2+}]_m$) *in vivo*. However, using stable Arabidopsis transformants expressing a green fluorescent protein (GFP)-aequorin chimera we were recently able to demonstrate that mitochondrial and cytosolic calcium dynamics are differentially regulated *in planta* (Logan & Knight, 2003). Whether $[Ca^{2+}]_m$ is a regulator of plant mitochondrial respiration and what role, if any, $[Ca^{2+}]_m$ plays in the control of PCD in higher plants are questions for future research.

II. Mitochondrial evolution

In a classic cytological study, Altman (1890) observed that mitochondria, which he termed bioblasts, were quite similar in shape and size to bacteria and postulated that it was a result of colonisation of the cell by mitochondria that the cell acquired the properties of life (Tzagoloff, 1982). It is now generally accepted that the first eukaryotic cell arose from a single endosymbiotic event around one to two billion years ago involving an anaerobic hydrogen-dependent autotrophic archaeobacterium ancestor (the host cell) and a eubacterium (the symbiont) (Martin & Muller, 1998; Gray *et al.*, 1999). Plastids also have a monophyletic prokaryotic origin. The first photosynthetic eukaryotic cell arose because of a single endosymbiotic event (Mereschkowsky, 1905; Martin & Kowallik, 1999) involving a cyanobacterial ancestor and a mitochondria-containing eukaryote that gave rise to all plastid-containing cells (Stoebe & Kowallik, 1999; Tomitani *et al.*, 1999; Moreira *et al.*, 2000; McFadden, 2001; Martin *et al.*, 2002). During evolution, the majority of mitochondrial and chloroplast genes have been lost to the nucleus (all but 57 in the case of *Arabidopsis* mitochondria, Unsel *et al.*

(1997)) such that mitochondrial and chloroplast biochemistry and dynamics are under nuclear control. For a detailed discussion of the origins of mitochondria and plastids, readers are directed to Allen & Raven (2003).

1. Homologues of bacterial division proteins are part of the plastid division apparatus

The evolutionary link to free-living cyanobacteria led to the suggestion that chloroplasts might share features of their division apparatus with modern free-living bacteria (Possingham & Lawrence, 1983). The first component of the chloroplast division apparatus to be identified was a homologue of the bacterial tubulin-like protein, FtsZ (Osteryoung & Vierling, 1995). FtsZ is found in nearly all prokaryotes and assembles into a large oligomeric structure forming a contractile ring around the interior surface of the cell membrane (Bi & Lutkenhaus, 1991). A precise role for this protein in bacterial cell division remains unknown. Plant protein homologues of FtsZ have been shown to be targeted to the chloroplast (Osteryoung & Vierling, 1995; Osteryoung *et al.*, 1998; Osteryoung & McAndrew, 2001), to form a ring around the chloroplast midpoint (Vitha *et al.*, 2001) and, in plants expressing antisense constructs of FtsZ genes, chloroplast are larger than wild-type and there are fewer per cell (Osteryoung *et al.*, 1998). Because of a shared prokaryotic origin, one can assume that at the times of their endosymbiosis the mechanisms of cell division in the eubacterial (alpha-proteobacterium) mitochondrial ancestor and the cyanobacteria plastid ancestor were similar. There are no potential mitochondrial FtsZ homologues in the genomes of *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, or *Arabidopsis thaliana* but homologues have been identified in the algae *Mallomonas splendens* (Beech *et al.*, 2000) and *Cyanidioschyzon merolae* (Takahara *et al.*, 2000).

2. A dynamin-related protein is involved in chloroplast division

Until recently, it was thought that chloroplasts divided by a purely prokaryotic mechanism using the machinery conserved since they were free-living cyanobacteria. Indeed, bacterial division served as a good model for chloroplast division and homologues of several key components of the bacterial division apparatus play vital roles in chloroplast division (Osteryoung & Vierling, 1995; Osteryoung *et al.*, 1998; Colletti *et al.*, 2000; Takahara *et al.*, 2000; Itoh *et al.*, 2001; McAndrew *et al.*, 2001). However, a dynamin-related protein has been implicated in chloroplast division in the red alga, *C. merolae* (CmDnm2, Miyagishima *et al.* 2003) and in *Arabidopsis* (ARC5; Gao *et al.*, 2003). It is postulated that these dynamin-like proteins act at the late stages of chloroplast division, when constriction of the organelle has already occurred, either to bring about further constriction or to act

in the severance of the outer membrane (Gao *et al.*, 2003; Miyagishima *et al.*, 2003). Phylogenetic analysis has shown that the *C. merolae* protein, CmDnm2 is more closely related to the *Arabidopsis* protein ARC5, than to the second dynamin-like protein in *C. merolae*, CmDnm1, that is involved in fission of mitochondria (Nishida *et al.*, 2003). This suggests that following the endosymbiotic acquisition of plastids it was not simply a matter of reassigning a mitochondrial-type dynamin to act in plastid division but instead a distinct dynamin-like protein subsumed this role. Before the discovery that chloroplast division also involves dynamin-like proteins, it was thought that during evolution the role of FtsZ in mitochondrial division had been replaced by a dynamin-like protein. It now appears that during evolution the role of FtsZ in mitochondrial division has been taken by a yet unidentified protein that works in concert with a dynamin-like protein(s). Presumably, the recruitment of eukaryotic dynamin-like proteins to play a role in organelle division was one step in the process whereby the cell nucleus acquired control over organelle division.

III. Mitochondria and the cytoskeleton

Eukaryotic cell dynamics are only possible by the presence of the cytoskeleton. The cytoskeleton is not only responsible for the movement of individual cells or the whole organism it is also vital for the active movement of organelles within the cytoplasm (Catlett & Weisman, 2000). Composed of three main protein filaments: actin filaments, intermediate filaments, and microtubules the cytoskeleton forms a complex network dispersed throughout the cytoplasm. Each filament type is composed of different protein monomers and together with various associated proteins can be assembled into a variety of structures. Some of these associated proteins are involved in the attachment of the different types of filaments to each other or to membranes (Svitkina *et al.*, 1996; Liao & Gundersen, 1998); others control the rate and direction of filament growth (Vantard & Blanchoin, 2002; dos Remedios *et al.*, 2003). A special class of associated proteins, the motor proteins, control movement of the filaments themselves or the movement of various forms of tethered cargo (for a comprehensive guide to the cytoskeletal elements and associated proteins see Kreis & Vale (1999)). Mitochondria have been shown to associate with microtubules and actin and there is evidence for the cooperative involvement of all three elements in the transport and dynamics of mitochondria and other organelles (Goode *et al.*, 2000; Rogers & Gelfand, 2000). In animal cells and some fungi (e.g. *Neurospora crassa* and *Shizosaccharomyces pombe*) mitochondrial movement is mainly associated with microtubules (Heggeness *et al.*, 1978; Yaffe *et al.*, 1996; Yaffe, 1999; Westermann & Prokisch, 2002) whilst in budding yeast (*S. cerevisiae*) and plants, movement is mainly associated with actin (Westermann & Prokisch, 2002; Olyslaegers & Verbelen, 1998; Van Gestel & Verbelen,

2002). It is likely, however, that mitochondria are able to switch from one type of filament to the other at specific times during cell development or to enable fine positioning of the organelle (Yaffe, 1999). For example, in yeast and plants, association with microtubules, via attachment to members of the kinesin motor protein family, probably controls the cortical positioning and/or tethering of mitochondria (Yaffe, 1999; Van Gestel & Verbelen, 2002) but during mitosis, when the cytoplasmic microtubules depolymerise, mitochondria may be passed to intermediate filaments or to actin microfilaments (Yaffe, 1999). Experiments with animal cells provide strong evidence for the role of kinesin-like proteins in the movement of mitochondria along microtubules. The motor protein KIF1B colocalises with mitochondria *in vivo* and can move mitochondria along microtubules *in vitro* (Nangaku *et al.*, 1994). Knocking out the mouse *kif5B* gene, encoding a conventional kinesin heavy chain, promoted clustering of mitochondria around the nucleus (Tanaka *et al.*, 1998). However, the clusters remained associated with microtubules suggesting that components other than KIF1B were involved in this association (Tanaka *et al.*, 1998). Kinesin light chains are thought to form the cargo-binding domain of the motor protein so it is of interest that these molecules have been found to associate with mitochondria in various cultured cells (Khodjakov *et al.*, 1998). There is evidence of a role for kinesin-like proteins in specific mitochondrial movements in *N. crassa* but confirmation of a more general role in mitochondrial transport in this species awaits future work (Westermann & Prokisch, 2002). Motor proteins involved in the transport of mitochondria along actin microfilaments have yet to be identified. One actin associated motor protein, myosin V, is responsible for the transport of unidentified organelles in neurons and in *Drosophila* (Mermall *et al.*, 1998) but there was no evidence of a specific association of mitochondria with myosin V in neuronal cells (Evans *et al.*, 1997) and none of the yeast myosins have been localised to mitochondria (Lillie & Brown, 1994; Goodson *et al.*, 1996; Jansen *et al.*, 1996). Mitochondria in *S. cerevisiae* use actin cables for segregation into daughter buds (Bretscher, 2003) although this does not show a dependence on myosin motors. Instead, segregation of mitochondria in budding yeast appears to be driven by Arp2/3-dependent actin polymerisation (Simon *et al.*, 1997; Loisel *et al.*, 1999; Boldogh *et al.*, 2001a). It has been proposed that mitochondrial actin binding protein(s) (Lazzarino *et al.*, 1994) mediates binding of mitochondria to actin and once bound the Arp2/3 complex nucleates actin filament assembly (Boldogh *et al.*, 2001a). Unidirectional movement of mitochondria along actin cables occurs following the cross-linking of the newly polymerised mitochondrial bound filament to the polarised actin cable (Boldogh *et al.*, 2001a,b). There are orthologues of the Arp2/3 complex subunits in *Arabidopsis* (McKinney *et al.*, 2002) but whether they are involved in actin-mediated mitochondrial motility is unknown.

IV. Mitochondrial morphology, biogenesis, proliferation and inheritance

1. Mitochondrial morphological heterogeneity

Using simple bright field microscopy Lewis & Lewis (1914) were the first to describe the movement of mitochondria in the cytoplasm, and the regular changes in organelle size and shape. The development of phase contrast microscopy provided new opportunities for cell biological studies and in the 1950s and 1960s numerous studies were published describing mitochondrial morphology and movement in a variety of cell types and the response of the mitochondria to environmental and physical perturbations (Tyler, 1992; Bereiter-Hahn & Voth, 1994). However, phase contrast does not allow the unambiguous identification of mitochondria. By the 1980s, the most powerful tool to visualise mitochondria was fluorescence microscopy using fluorochromes such as rhodamine 123. The development of green fluorescent protein as a non-invasive marker has revolutionised cell biology (Chalfie & Kain, 1998). Its unique property in forming a chromophore of three amino acids within its primary structure that requires no cofactors other than molecular oxygen makes it an ideal marker for *in vivo* studies.

Microscopy has shown that while the basic structural organisation of mitochondria is conserved: outer membrane, inner membrane and matrix, the external shape of mitochondria is highly variable (Lloyd, 1974; Munn, 1974; Tzagoloff, 1982; Bereiter-Hahn & Voth, 1994). There is considerable debate about how many mitochondria or populations of mitochondria are present in a cell, if multiple discrete organelles exist, or whether the mitochondrion is a single reticular network. However, it is clear from the literature that there is great heterogeneity in the dynamics and morphology of mitochondria in different organisms and even in different cell types of the same organism. Even within a single cell, there is heterogeneity of mitochondrial morphology. For example, in *Arabidopsis* leaf epidermal cells, the mitochondrial population can comprise organelles with different morphologies (spherical, kidney shape, vermiform) and switching between these forms can occur within seconds (unpublished observations). This heterogeneity effectively precludes discussion of generic mitochondrial morphology.

2. Biogenesis and proliferation

Mitochondrial biogenesis is the term given to a range of processes effecting the maintenance of functional mitochondria. These processes include co-ordinated expression of the nuclear and mitochondrial genomes, import of nuclear encoded, cytoplasmically synthesised polypeptides and the assembly of polypeptides into large multimeric protein complexes (Attardi & Schatz, 1988; Leon *et al.*, 1998). However, mitochondria cannot be created *de novo* but instead are formed from pre-existing organelles.

In the budding yeast, *S. cerevisiae*, proliferation occurs vegetatively. During vegetative growth of haploid or diploid cells, the yeast produces buds that are eventually released to become independent cells. During the budding process, the daughter buds receive a nuclear genome and a portion of the cytoplasm containing mitochondria and other organelles. Although *S. cerevisiae* can grow by fermentation, mitochondrial membranes and mitochondrial DNA (mtDNA) are required for normal *S. cerevisiae* cell function and the mechanisms of inheritance and proliferation of both components are highly regulated (Hermann & Shaw, 1998; Yaffe, 1999). Central to the inheritance and proliferation of mitochondria in yeast are the processes of mitochondrial fission and fusion (see below) and the involvement of the cytoskeleton (Catlett & Weisman, 2000).

Little is known about the mechanisms controlling mitochondrial inheritance and proliferation in multicellular organisms. During the human cell cycle, mitochondria switch between two predominant morphological states (Barni *et al.*, 1996; Margineantu *et al.*, 2002). During the G1 phase of the cell cycle mitochondria fuse to form a reticulum, bringing the number of individual organelles to half the number before M phase (Karbowski *et al.*, 2001). As cells proceed from G1 to S phase mitochondrial numbers increase as a result of the fragmentation (division) of the mitochondrial reticulum (Barni *et al.*, 1996; Karbowski *et al.*, 2001; Margineantu *et al.*, 2002). It is tempting to speculate that mitochondrial fragmentation during specific points in the cell cycle promotes the equal segregation of mitochondrial membranes and mtDNA molecules into the two daughter cells during mitosis.

3. Yeast mitochondrial inheritance and morphology mutants

The first yeast mitochondrial development mutants identified were defective in mitochondrial inheritance (McConnell *et al.*, 1990) and their discovery has led to the identification of some of the proteins involved in this process (Hermann & Shaw, 1998; Yaffe, 1999). Mitochondrial morphology and inheritance are closely interlinked and therefore, in addition to defects in mitochondrial inheritance, many of these mutants had aberrant mitochondrial morphologies and were named *mdm* mutants for mitochondrial distribution and morphology (Table 1). To date components of the mitochondrial inheritance machinery fall into one of two categories. Firstly, there are cytosolic proteins likely to interact with the cytoskeleton such as Mdm1p (McConnell *et al.*, 1990), Mdm14p (Hermann *et al.*, 1997; Shepard & Yaffe, 1997) and Mdm20p (Hermann *et al.*, 1997). Mdm1p is vital for mitochondrial and nuclear inheritance and mutations in *MDM1* that affect mitochondrial inheritance also cause the fragmentation of the mitochondrial reticulum producing numerous small round mitochondria (McConnell *et al.*, 1990). Mutation of Mdm14 or Mdm20 affect mitochondrial inheritance and the

corresponding proteins have been suggested to function cooperatively with Mdm1p (Yaffe, 1999). Additionally Mdm20p has been implicated in the control of actin organisation and stability suggesting that Mdm20p is involved in the assembly or function of actin-associated structures necessary for mitochondrial segregation (Hermann *et al.*, 1997).

Members of the second class of proteins involved in the segregation of mitochondria during budding were identified by analysis of further yeast mitochondrial inheritance mutants (Burgess *et al.*, 1994; Sogo & Yaffe, 1994; Berger *et al.*, 1997). All three proteins, Mmm1p, Mdm10p and Mdm12p, have been shown to be required for maintenance of the normal mitochondrial reticulum and mitochondrial inheritance. Cells containing mutations in any of these three genes contain large spherical mitochondria that although retaining the classical mitochondrial ultrastructure are defective in division and inheritance (Sogo & Yaffe, 1994; Burgess *et al.*, 1994; Berger *et al.*, 1997). All three proteins are outer membrane proteins, and Mmm1p and Mdm10p have large cytoplasmic domains, but their biochemical function is unknown. It has been suggested that, because cells with mutations in two or three of these genes retain the same giant-mitochondrial phenotype, all three proteins function at the same point (Yaffe, 1999). It is possible that these proteins function as attachment points to anchor the mitochondria to the cytoskeleton or are involved in the attachment of mitochondria to cytoskeleton-associated motor protein in a similar way to the integral membrane protein kinectin is postulated to act as an organelle-membrane receptor for kinesin binding (Ong *et al.*, 2000). A further mutation, *mdm17*, causes mitochondrial fragmentation, defective inheritance and loss of mtDNA (Shepard & Yaffe, 1999). Shepard & Yaffe (1999) mapped the mutant locus to a previously identified gene, *MGMI*, encoding a dynamin-like protein (Jones & Fangman, 1992; Guan *et al.*, 1993, see below). A further 10 yeast mutants have recently been identified by screening for aberrant mitochondrial morphology (Dimmer *et al.*, 2002) (Table 1). In each case, the mutant genes encode previously uncharacterised proteins. Five yeast genes, *MDM31*, *MDM32*, *MDM33*, *MDM37* and *MDM38* encode proteins of unknown function that are predicted to be targeted to the mitochondrial inner membrane (Dimmer *et al.*, 2002; Messerschmitt *et al.*, 2003). *MDM39* encodes a predicted membrane protein but lacks a mitochondrial presequence; *MDM30*, *MDM34*, *MDM35* and *MDM36* encode proteins lacking both presequences and transmembrane domains (Dimmer *et al.*, 2002). The mitochondrial morphology of these mutants ranges from fragmented tubules and/or aggregated mitochondria (*mdm30*, *mdm31*, *mdm32*, *mdm36*, *mdm37*, *mdm39*) through spherical (*mdm34*, *mdm35*), enlarged, lasso-like structures (*mdm38*), to giant ring-like structures (*mdm33*) (Dimmer *et al.*, 2002). *MDM33* has been suggested to play a distinct role in inner membrane fission (Messerschmitt *et al.*, 2003) but further research is required to delineate the precise mechanism involved.

4. Plant homologues of yeast mitochondrial inheritance genes

There are no *Arabidopsis* homologues to most identified yeast mitochondrial morphology or inheritance proteins (e.g. Mmm1p, Mdm10p, Mdm12p, Mdm14p and Mdm20p, Mdm30p, Mdm31p, Mdm32p, Mdm34p, Mdm36p, Mdm39p) (Table 1). *MDM38* encodes an evolutionarily conserved protein with homologues in the *Plasmodium*, *S. pombe*, *Arabidopsis*, *Caenorhabditis*, human, mouse, *Drosophila* and *Anopheles* genomes. Mdm38p has been shown to be a mitochondrial protein in *Drosophila* (Caggese *et al.*, 1999) and the *Arabidopsis* homologue is predicted with high probability to be targeted to mitochondria using the TargetP (Emanuelsson *et al.*, 2000) or MitoProtII (Claros & Vincens, 1996) algorithms. Mdm38p is a calcium binding protein containing two EF-hand domains but whether this protein plays a role in mitochondrial calcium dynamics that in turn modulates mitochondrial morphology remains to be determined. The *Arabidopsis* genome contains one *MDM37* homologue in which the homology is restricted to a conserved rhomboid domain. The rhomboid protein family are integral membrane proteins related to the *Drosophila* rhomboid protein (Rho-1): an intramembrane serine protease that is postulated to play a key role intercellular signalling. A bacterial homologue of *Drosophila* Rho-1 functions in density-dependent gene regulation (quorum sensing, Gallio *et al.*, 2002) but the role of the *Arabidopsis* homologue, predicted by TargetP to be targeted to mitochondria, is unknown. It is tempting to speculate that this protein is involved in intermitochondrial signalling and forms part of the machinery controlling mitochondrial number per cell, i.e. mitochondrial quorum sensing. *MDM33* shows weak homology to an *Arabidopsis* myosin homologue. Whilst the homology is not significant, it is interesting since Mdm33p is highly homologous to a *S. pombe* coiled-coil protein with similarity to myosin. However, since Mdm33p shows no homology to *S. cerevisiae* myosins it is not clear if these homologies have any functional significance.

V. Mitochondrial fission and fusion

1. Dynamin-related proteins are involved in fission

Mitochondria in various eukaryotic cells undergo continuous cycles of fission and fusion and these processes control the number, size and distribution of these organelles (Bereiter-Hahn & Voth, 1994; Shaw & Nunnari, 2002; Yaffe, 1999; Catlett & Weisman, 2000). Research using *S. cerevisiae*, in which there are typically 5–10 tubular mitochondria forming an extended reticular network beneath the cell cortex (Stevens, 1977), have led to the identification of genes encoding components involved in mitochondrial fission and fusion in this organism (Hermann & Shaw, 1998; Yaffe, 1999; Catlett & Weisman, 2000; Shaw & Nunnari, 2002).

In a screen to identify genes controlling yeast mitochondrial inheritance, Hermann *et al.* (1997) identified one mutant, *mdm29*, in which the mitochondrial reticulum collapses into a bundle of tubules along one side of the cell (Ostuga *et al.*, 1998). The *mdm29* mutation was mapped to *DNM1* (Ostuga *et al.*, 1998), which encodes a protein structurally related to the evolutionarily conserved dynamin-related proteins required for membrane scission during exocytosis (Herskovits *et al.*, 1993; van der Blik *et al.*, 1993; Damke *et al.*, 1994, 1995; Hinshaw & Schmid, 1995). Dynamin-related proteins have also been implicated in mitochondrial division in mammals (Drp1, Smirnova *et al.*, 1998), nematodes (DRP-1, Labrousse *et al.*, 1999), red algae (CmDnm1, Nishida *et al.*, 2003) and higher plants (ADL2b, Arimura & Tsutsumi, 2002).

2. *Arabidopsis* dynamin-like proteins and mitochondrial fission

The *Arabidopsis* genome contains 11 dynamin-like (and related phragmoplastin-like) homologues. The annotation of these genes in the databases is complex since identical genes have been given different names by different research groups. One dynamin-like protein originally named ADL2 (since renamed ADL2a; At4g33650) following the cloning of a related gene, *ADL2b*, shares 37–39% amino acid identity with Dnm1p and Drp1, respectively, and has been localised to plastids (Kang *et al.*, 1998). Kang *et al.* (1998) have suggested that ADL2a may be involved in vesicle formation within plastids and possibly in thylakoid membrane biogenesis. However, we recently analysed mitochondrial morphology in an *Arabidopsis* *ADL2a* T-DNA knockout following transformation with a mitochondrial-targeted GFP construct allowing observation of mitochondrial morphology *in vivo* (D. C. Logan *et al.*, unpublished). In the knockout the mitochondria formed long tubular organelles with many constrictions. A second aspect of the mutant phenotype was the presence of thin protuberances, up to many tens of micrometers in length, extending from the mitochondria. The significance of these protuberances, which we call matrixules, is unclear. The closely related protein, ADL2b (At2g14120), bears the highest similarity to Dnm1p/Drp1 (39–41% amino acid identity) of the *Arabidopsis* dynamins. In an elegant series of experiments, Arimura & Tsutsumi (2002) have shown that *Arabidopsis* plants expressing dominant-negative mutations in *ADL2b* have altered mitochondrial morphology. Mitochondria in the mutants were both longer and less numerous than wild-type suggesting a reduction in the efficiency or frequency of mitochondrial fission. Furthermore, using a GFP::ADL2b fusion protein Arimura & Tsutsumi (2002) were able to show localisation of ADL2b to the constriction sites and tips of mitochondria. These results suggest that ADL2b is the *Arabidopsis* analogue of Dnm1p/Drp1 and is a vital component of the mitochondrial division apparatus. The strong

similarity between ADL2a and ADL2b (70% amino acid identity) suggests there may be functional similarity and raises the possibility that both proteins are involved in chloroplast and mitochondrial development. It has been shown that the N-terminal 35 amino acids of ADL2a are sufficient to direct the protein to chloroplasts (Kang *et al.*, 1998) but ADL2b has no identifiable chloroplast-targeting signal. However, it is not yet known whether ADL2a, like ADL2b localises to mitochondria and whether it too is involved in division of mitochondria. A third *Arabidopsis* dynamin-like protein, ADL1Ap (originally called ADL1; At5g42080) has been localised to plastids and proposed to be involved in the biogenesis of thylakoid membranes (Park *et al.*, 1998). However, a previous study reported localisation of ADL1Ap to the cell plate (Lauber *et al.*, 1997). A third study confirmed the localisation of ADL1Ap to the cell plate and implicated this protein in various aspects of plant growth and development (Kang *et al.*, 2001). One possible explanation for the conflicting results is that while both earlier studies used the same antibody they identified different proteins. This antibody was raised to the conserved GTPase domain so it is possible that the protein identified by Park *et al.* (1998) was in fact ADL2a or another member of the *Arabidopsis* dynamin-like family.

3. Additional components of the yeast mitochondrial fission apparatus

The yeast dynamin, Dnm1p, has been shown to assemble on the outer mitochondrial membrane at sites where division occurs. However, little is known about the composition and assembly of these Dnm1p containing complexes or the proteins involved in their recruitment to the membrane. Two further proteins have been identified which may interact with Dnm1p to control yeast mitochondrial division. The first of these proteins, independently identified by four groups, is a mitochondria-associated cytosolic protein that, like Dnm1p, is localised to the site of mitochondrial division (Fekkes *et al.*, 2000; Mozdy *et al.*, 2000; Tieu & Nunnari, 2000; Cervený *et al.*, 2001). Yeast cells disrupted in this new gene showed aberrant mitochondrial morphology characterised by a net-like sheet of interconnected tubules – similar to the phenotype of *dmn1pΔ* mutants. The four groups: Fekkes *et al.* (2000); Mozdy *et al.* (2000); Tieu & Nunnari (2000) and Cervený *et al.* (2001) called this protein Mdv1p (mitochondrial division), Fis2p (fission), Gag3p (glycerol adapted growth) and Net2p (network), respectively. Database searches fail to identify an *Arabidopsis* homologue of yeast Mdv1p. The second gene, identified by two groups, encodes a yeast mitochondrial outer membrane protein. The two groups: Mozdy *et al.* (2000); Tieu & Nunnari (2000), called this protein Mdv2p or Fis1p, respectively. The phenotype of the *fis1Δ/mdv2pΔ* yeast mutant is very similar to the *mdv1pΔ/fis2pΔ/gag3pΔ/net2pΔ* mutant (and therefore the *dmn1pΔ* mutant). If Mdv1p is deleted from yeast cells, there is no obvious effect

on the localisation of Dnm1p but deletion of Dnm1p caused redistribution of Mdv1p along the surface of the mitochondria, suggesting that Dnm1p is needed for assembly of Mdv1p (Fekkes *et al.*, 2000; Tieu & Nunnari, 2000). In the case of Fis1p, this protein is evenly distributed along the surface of the yeast mitochondria unlike Dnm1p and Mdv1p, which are located in discrete spots where division occurs (Tieu & Nunnari, 2000; Tieu *et al.*, 2002). Mutations in Fis1p cause most of the Dnm1p and Mdv1p protein to remain cytosolic (Mozdy *et al.*, 2000) and there is biochemical and genetic evidence that Fis1p functions at two distinct steps during mitochondrial division (Tieu & Nunnari, 2000; Tieu *et al.*, 2002). In the prevailing model of yeast mitochondrial fission, Fis1p is first involved in the recruitment and assembly of Dnm1p at the mitochondrial membrane, and secondly at a later stage, Fis1p interacts with Mdv1p to induce conformational changes in a Dnm1p-containing structure to effect the scission of mitochondrial membranes (Tieu *et al.*, 2002). The yeast protein Fis1p/Mdv2p is represented in the *Arabidopsis* genome by one homologue displaying 25% identity and 50% similarity to Fis1p/Mdv2p, complete with C-terminal transmembrane domain (Mozdy *et al.*, 2000). It is not known if the *Arabidopsis* *FIS1* homologue is involved in mitochondrial fission.

4. Mitochondrial fusion

A large GTP-binding protein, Fzo, was shown to be required for normal mitochondrial development during spermatogenesis in *Drosophila* (Hales & Fuller, 1997). Analyses of the *fzo1* mutation in yeast confirmed the role of this protein in mitochondrial fusion (Hermann *et al.*, 1998; Rapaport *et al.*, 1998) and led to confirmation of the role of the dynamin-like protein, Dnm1p, in yeast mitochondrial fission (Bleazard *et al.*, 1999; Sesaki & Jensen, 1999). The yeast Fzo1p GTPase regulates mitochondrial fusion, and loss of Fzo1p function causes the mitochondrial network to rapidly fragment (Hermann *et al.*, 1998). By contrast, mutation of *DNM1* in yeast causes mitochondria to lose their normal structure and instead form a large network of interconnected tubules (Sesaki & Jensen, 1999). In *fzo1 dnm1* double mutants, the wild-type yeast mitochondrial phenotype is restored. This analysis elegantly demonstrated that a delicate balance of fission and fusion is required to maintain yeast mitochondrial shape, size and number. In addition to being defective in mitochondrial fusion, yeast *fzo1* mutants also lose mtDNA (Hermann *et al.*, 1998) and this loss of mtDNA can be suppressed by knocking out Dnm1p function (Bleazard *et al.*, 1999; Sesaki & Jensen, 1999). In order to identify novel mitochondrial fusion mutants, Sesaki & Jensen (2001) took advantage of this selective mtDNA loss by screening for yeast mutants that maintain mtDNA in the absence of Dnm1p activity but lose mtDNA in the presence of functional Dnm1p. In addition to identifying mutant alleles of Fzo1 and

Mgm1, both of which lose mtDNA in a Dnm1 dependent manner, two novel mutants were discovered and named *ugo1* and *ugo2* (Sesaki & Jensen, 2001). Both mutant phenotypes are characterised by the fragmentation of the mitochondrial tubular network. As in *fzo1* mutants, fragmentation of the mitochondria in *ugo1* mutants could be suppressed in the absence of Dnm1p (Sesaki & Jensen, 2001). Ugo1p therefore functions in mitochondrial fusion, like Fzo1p, with an activity antagonistic to that of Dnm1p. In both fusion mutants, the fragmentation of mitochondria results from a disruption in mitochondrial fusion in a normal mitochondrial fission background. Restoration of wild-type mitochondrial phenotype in *dnm1pΔ ugo1Δ* cells results from a lack of division and a lack of fusion rather than a restoration of fusion ability (Sesaki & Jensen, 2001). As previously mentioned, mutations in *MGM1*, a dynamin-related GTPase, were found to cause mitochondrial fragmentation and loss of mtDNA suggesting that Mgm1p might be involved in fusion (Shepard & Yaffe, 1999; Wong *et al.*, 2000). Mgm1p is present in the inter-membrane space and has been shown to associate with Ugo1p and Fzo1p (Wong *et al.*, 2003). Although the precise role of Mgm1p in yeast mitochondrial fusion is unclear, it has been suggested that the protein functions in inner membrane remodelling and may connect the outer and inner membranes via interactions with Ugo1p and Fzo1p (Wong *et al.*, 2003). As with Mgm1p, the exact roles of Fzo1p and Ugo1p in fusion remain to be determined. There are no *Arabidopsis* homologues of Mdv1p/Fis2/Gag3/Net2p or Fzo1p and nothing is known about the genes, proteins and mechanisms regulating mitochondrial fusion in any plant.

VI. Mitochondrial distribution

As a result of the morphology of yeast mitochondria, it is difficult to distinguish between changes in morphology and changes in distribution. Indeed, as the research identifying Dnm1p as a component of the mitochondrial fission apparatus has shown, morphology and distribution are interlinked. Yeast mitochondrial distribution is also affected by mutation of another conserved protein, Clu1p. In yeast cells in which the *CLU1* gene is deleted the mitochondrial network collapses to one side of the cell, a change in distribution similar to that seen in *dnm1* mutants (Fields *et al.*, 1998). *CLU1* is a functional homologue of the *cluA* gene, previously identified in the slime mould *Dictyostelium discoideum*, as having a role in mitochondrial distribution; disruption of the *cluA* gene caused mitochondria to aggregate near the cell centre (Zhu *et al.*, 1997). Whilst the molecular function of these proteins is unknown, it has been suggested, because of the similarity between the yeast *dnm1* mutant and *clu1* phenotypes, that they may be involved in a common step in mitochondrial development (Field *et al.*, 1998; Yaffe, 1999).

We have recently identified an *Arabidopsis* mitochondrial mutant, *fmt* (friendly mitochondria), with a mitochondrial

phenotype similar to the *D. discoideum cluA* mutant (Fig. 1k,l). The majority of mitochondria in the *fmt* mutant are aggregated into clumps of tens or hundreds of organelles and using a combination of forward and reverse genetics we were able to identify the mutant gene as the *Arabidopsis* homologue of *cluA* (Logan *et al.*, 2003). The *Arabidopsis* CluA protein homologue, FMT (locus: CAB41334) is 26% identical and 41% similar to the *Dictyostelium* protein and 20% identical, 34% similar to yeast Clu1p. There are *Clu*-type homologues present as open reading frames in all eukaryotic genomes sequenced to date but, apart from a short tetratricopeptide repeat (TPR) domain that is thought to function in protein–protein interactions, the *Clu*-type proteins have no homology to known proteins. TPR domains are also present in a cargo-binding region of the microtubule motor protein kinesin (Stenoien & Brady, 1997; Verhey *et al.*, 2001) and kinesin has been found to associate with mitochondria (Khodjakov *et al.*, 1998). It is tempting to speculate that the *Clu*-type proteins are involved in the interaction of mitochondria and microtubules.

VII. Plant specific proteins playing a role in mitochondrial dynamics

As detailed in the previous sections, there are few *Arabidopsis* homologues of animal or yeast proteins playing a direct role in mitochondrial dynamics. In some cases, this can be explained by the fundamental differences between yeast and plant mitochondrial morphology and reproductive biology. For example, the budding yeast, *S. cerevisiae*, proliferates by budding whereby the mother cell produces a daughter bud that grows and eventually becomes an independent cell. An essential part of this process is the transport of mitochondria and other organelles into the daughter bud. Mmm1p, mdm10p, mdm12p, mdm14p and mdm20p are all involved in the transmission of mitochondria to the daughter buds (Yaffe, 1999) and since cell proliferation in plants occurs by cell division, these proteins are not required. Equally, the differences between yeast and plant cells mean that there are likely to be many proteins involved in mitochondrial dynamics that are either specific to multicellular organisms or plant-specific. The corollary of this is that dogmatic adherence to the yeast paradigm is unlikely to advance our knowledge of plant mitochondrial dynamics.

We recently initiated a novel programme of research with the aim of identifying *Arabidopsis* mutants with aberrant mitochondrial dynamics. *Arabidopsis* plants expressing GFP targeted to the mitochondria (Logan & Leaver, 2000) were mutagenised using EMS and the M2 generation were then screened for altered mitochondrial shape, size, number and distribution using a fluorescence microscope. Six viable mutants with distinct mitochondrial phenotypes were identified from a population of approximately 9500 individuals and we are in the process of using positional cloning

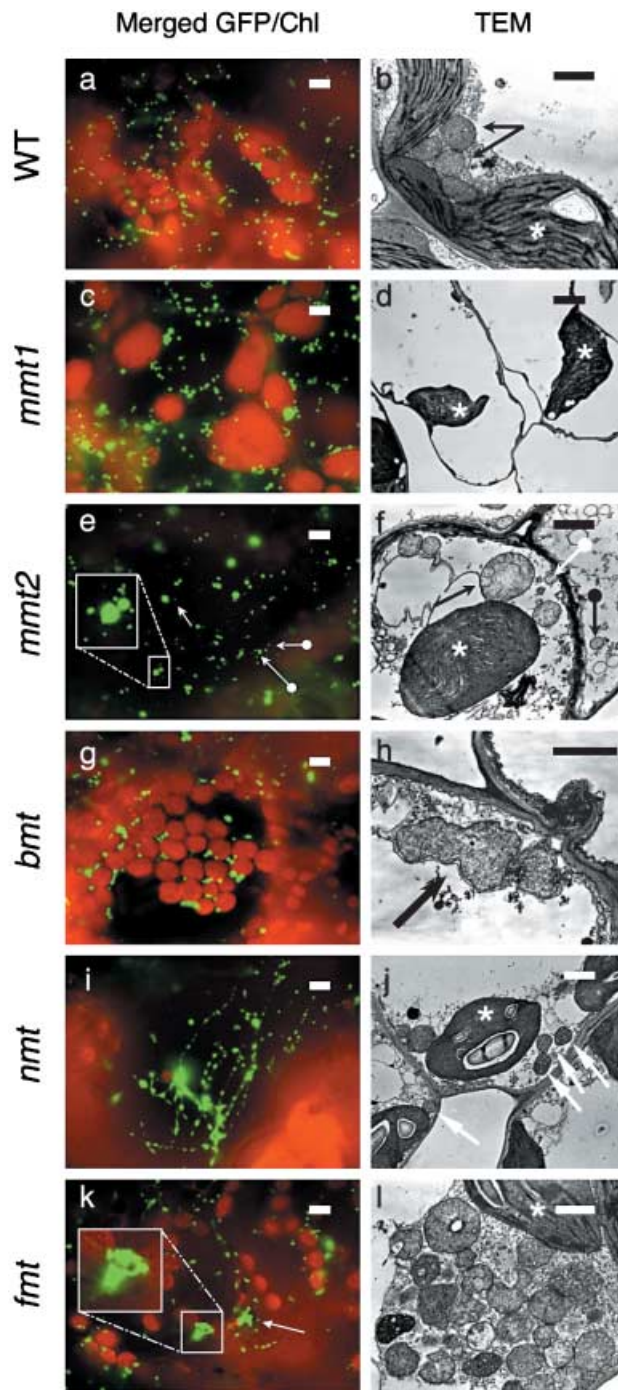


Fig. 1 Epifluorescent (left hand panels) and transmission electron microscopy (TEM) (right hand panels) micrographs of wild-type and mutant *Arabidopsis* leaf mitochondria. Epifluorescent micrographs are false-coloured for green fluorescent protein (GFP) (green) and chlorophyll (red). (a, b) Wild-type: arrows, mitochondria; asterisk, chloroplast. (c, d) *mnt1* mutant: asterisk, chloroplast. (e, f) *mnt2* mutant: plain arrows, large mitochondria; arrows with circle, small mitochondria, the boxes indicate an area magnified to highlight the heterogeneity of mitochondria size within a single cell; asterisk, chloroplast with dense mass of internal membranes. (g, h) *bmt* mutant: arrow, mitochondria. (i, j) *nmt* mutant: arrows indicate small

mitochondria; asterisk, chloroplast. (k, l) *fmt* mutant: arrow, large mitochondrial cluster, boxes indicate an area magnified to highlight a large cluster of mitochondria; asterisk, chloroplast. Scale bars in epifluorescent images, 5 μm ; in TEMs, 1 μm , except in d where bar, 5 μm . Figure reproduced with kind permission of *The Plant Journal* ©.

complemented by reverse genetics, where possible, to identify the mutant genes. In the first motley mitochondrial mutant (*mnt1*) the mitochondrial population is highly heterogeneous varying in size from one quarter to four times the average plan area of wild-type mitochondria (Fig. 1c,d). In addition, the size distribution of chloroplasts is affected in the mutant, chloroplast plan areas in the mutant range from 4 to 240 times the plan area in the wild-type (Logan *et al.*, 2003). The second motley mitochondrial mutant (*mnt2*) contains a highly heterogeneous mitochondrial population similar to *mnt1* although in this second mutant the gross chloroplast morphology remains normal (Fig. 1e,f). Transmission electron microscopy (TEM) of the *mnt2* mutant, in addition to confirming the mitochondrial heterogeneity, demonstrated that the internal structure of the chloroplasts is severely altered (Fig. 1f). Chloroplasts in the *mnt2* mutant contain a mass of densely packed membranes instead of the normal morphology of granal stacks connected by stromal lamellae and there are a large number of electron dense particles within the chloroplasts. Mitochondria in the big mitochondrial mutant (*bmt*) have plan areas approximately two to four times wild-type and there are approximately half as many per microscope field-of-view (Fig. 1g,h). The network mitochondrial mutant (*nmt*) is characterised by the presence of long interconnected mitochondrial tubules (Fig. 1i) extending to many tens of micrometers in length. Examination of leaf tissue of *nmt* plants under the TEM showed that the aberrant mitochondrial architecture was not maintained in the fixed tissue (Fig. 1j) instead the mitochondrial tubules fragmented to form organelles as small as 1/16th the plan area of those in wild-type cells. We have mapping data for the *mnt1*, *mnt2*, *bmt* and *nmt* mutants and no previous mitochondrial development genes/mutants have been mapped to the regions containing the mutant loci nor are any obvious candidate genes in these regions (Logan *et al.*, 2003). Our five mutants: *fmt*, *mnt1*, *mnt2*, *bmt* and *nmt*, are, to our knowledge, the first plant mitochondrial dynamics or morphology mutants and four of these represent novel loci involved in these processes.

Conclusions

Mitochondria are highly dynamic organelles able to change shape and position within a few seconds. Studies using yeast have shown that a delicate balance of mitochondrial fission and fusion controls mitochondrial shape, size, number and inheritance (Hermann & Shaw, 1998; Yaffe, 1999; Shaw & Nunnari, 2002). Analysis of the mitochondrial complement of higher plant cells highlights fundamental differences in the

mitochondria; asterisk, chloroplast. (k, l) *fmt* mutant: arrow, large mitochondrial cluster, boxes indicate an area magnified to highlight a large cluster of mitochondria; asterisk, chloroplast. Scale bars in epifluorescent images, 5 μm ; in TEMs, 1 μm , except in d where bar, 5 μm . Figure reproduced with kind permission of *The Plant Journal* ©.

Table 1 Genes playing a role in mitochondrial dynamics

Gene	Organism	Location	Mutant phenotype	Protein properties/Role	Reference	Arabidopsis homologue
<i>DNM1/Drp1/DRP-1</i>	<i>S. cerevisiae/</i> <i>H. sapiens/</i> <i>C. elegans</i>	Cytoplasm	Net-like sheet of interconnected tubules	Dynamin-related GTPase/membrane fission	Ostuga <i>et al.</i> (1998); Smirnova <i>et al.</i> (1998); Labrousse <i>et al.</i> (1999)	<i>Adl2b</i> analogue
<i>ADL2a</i>	<i>A. thaliana</i>	Cytoplasm	Mitochondria form long tubules with many constrictions and protuberances (matrixules)	Dynamin-related GTPase/membrane fission (?)	D. C. Logan <i>et al.</i> (unpublished)	–
<i>ADL2b</i>	<i>A. thaliana</i>	Cytoplasm	Mitochondria form long interconnected tubules	Dynamin-related GTPase/membrane fission (?)	Arimura & Tsutsumi (2002)	–
<i>cluA/CLU1</i>	<i>D. discoideum/</i> <i>S. cerevisiae</i>	Cytoplasm	Mitochondria aggregates. In yeast they collapse to side of cell	Kinesin-like domain/outer membrane fission	Fields <i>et al.</i> (1998); Zhu <i>et al.</i> (1997); Fields <i>et al.</i> (2003)	<i>FMT</i> , See below.
<i>MDM1</i>	<i>S. cerevisiae</i>	Cytoplasm	Fragmentation of tubules. Defective transmission to daughter buds	Intermediate filament-like	McConnell & Yaffe (1992)	At2g15900, 3.9e-08
<i>MDM10</i>	<i>S. cerevisiae/</i> <i>Podospira anserina</i>	Mitochondrial outer membrane	Large spherical mitochondria. Defective transmission to daughter buds	Integral membrane protein	Sogo & Yaffe (1994); Jamet-Vierny <i>et al.</i> (1997)	None
<i>MDM12</i>	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Large spherical mitochondria. Defective transmission to daughter buds	Integral membrane protein	Berger <i>et al.</i> (1997)	None
<i>MDM14</i>	<i>S. cerevisiae</i>	Cytoplasm	Mitochondria aggregate. Defective transmission to daughter buds	Coiled-coil domain	Shepard & Yaffe (1997).	None
<i>MDM20</i>	<i>S. cerevisiae</i>	Cytoplasm	Defective transmission to daughter buds	Coiled-coil domain/disrupts actin cables	Hermann <i>et al.</i> (1997)	None
<i>MDM30</i>	<i>S. cerevisiae</i>	Cytosolic?	Fragmented or aggregated; few short tubules	?	Dimmer <i>et al.</i> (2002)	None
<i>MDM31</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Compact mitochondrial aggregates	?	Dimmer <i>et al.</i> (2002)	None

Table 1 continued

Gene	Organism	Location	Mutant phenotype	Protein properties/Role	Reference	Arabidopsis homologue
<i>MDM32</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Compact mitochondrial aggregates		Dimmer <i>et al.</i> (2002)	None
<i>MDM33</i>	<i>S. cerevisiae</i>	Mitochondrial inner membrane protein	Giant ring-like mitochondria	Coiled-coil domains, part of high molecular weight complex/putatively involved in inner membrane fission	Dimmer <i>et al.</i> (2002); Messerschmitt <i>et al.</i> (2003).	Weak homology to At3g53350, 2.5e-4
<i>MDM34</i>	<i>S. cerevisiae</i>	Cytoplasmic	Spherical mitochondria	?	Dimmer <i>et al.</i> (2002)	None
<i>MDM35</i>	<i>S. cerevisiae</i>	Cytoplasmic	Spherical mitochondria	?	Dimmer <i>et al.</i> (2002)	At4g33100, 8.7e-09
<i>MDM36</i>	<i>S. cerevisiae</i>	Cytoplasmic	Mitochondrial tubules aggregate/collapse to one side of cell	?	Dimmer <i>et al.</i> (2002)	None
<i>MDM37</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Fragmented mitochondrial tubules	Rhomboid-like protein	Dimmer <i>et al.</i> (2002)	At1g18600, 9.0e-11.
<i>MDM38</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Lasso-like mitochondria	Calcium binding protein	Dimmer <i>et al.</i> (2002)	At3g59820, 8.9e-55.
<i>MDM39</i>	<i>S. cerevisiae</i>	Predicted integral membrane protein – no mitochondrial targeting presequence	Fragmented mitochondrial tubules	?	Dimmer <i>et al.</i> (2002)	None
<i>MMM1</i>	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Large spherical mitochondria Defective transmission to daughter buds	Integral membrane protein	Burgess <i>et al.</i> (1994)	None
<i>MGM1</i>	<i>S. cerevisiae</i>	Inner membrane space	Mitochondria fragmentation. Loss of mtDNA. Defective transmission to daughter buds.	Dynamain-related protein/Inner membrane modelling, May interact with Ugo1p and Fzo1p	Jones & Fangman (1992); Guan <i>et al.</i> (1993); Shepard & Yaffe (1997,1999); Wong <i>et al.</i> (2000, 2003)	Dynamain-like genes in Arabidopsis, closest is ADL2a, At4g33650,

Table 1 continued

Gene	Organism	Location	Mutant phenotype	Protein properties/Role	Reference	Arabidopsis homologue
<i>fzo/FZO1</i>	<i>Drosophila melanogaster</i> <i>S. cerevisiae</i>	Mitochondrial outer membrane	Aberrant mitochondrial fusion; fragmentation of tubules	GTPase/mitochondrial fusion	Hales & Fuller (1997); Rapaport <i>et al.</i> (1998); Hermann <i>et al.</i> (1998)	None
UGO1	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Fragmentation of mitochondrial tubules	Involved in mitochondrial fusion	Sesaki & Jensen (2001)	None
<i>MDV1/FIS2/GAG3/NET2</i>	<i>S. cerevisiae</i>	Cytoplasm/associates with mitochondrial outer membrane	Net-like sheet of interconnected tubules	Predicted coiled-coil and seven WD-40 repeats/interacts with Dnm1p & Fis1p in fission process	Fekkes <i>et al.</i> (2000); Mozdy <i>et al.</i> (2000); Tieu & Nunnari (2000); Cervený <i>et al.</i> (2001); Tieu <i>et al.</i> (2002)	Homology to WD-40-repeat containing proteins
<i>FIS1/MDV2</i>	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Net-like sheet of interconnected tubules	Integral membrane protein. Interacts with Dnm1p & Mdv1p in fission process	Tieu & Nunnari (2000); Mozdy <i>et al.</i> (2000); Tieu <i>et al.</i> (2002)	At3g57090, 1.1e-08 & At5g12390, 1.4e-08.
<i>FMT</i>	<i>A. thaliana</i>	?	Clusters of mitochondria	TPR-domain; homologue of <i>cluA</i> and <i>CLU1</i>	Logan <i>et al.</i> (2003)	–
<i>MMT1</i>	<i>A. thaliana</i>	?	Much larger and smaller mitochondria in same cell. Altered thylakoid morphology	?	Logan <i>et al.</i> (2003)	–
<i>MMT2</i>	<i>A. thaliana</i>	?	Much larger and smaller mitochondria in same cell. Giant chloroplasts	?	Logan <i>et al.</i> (2003)	–
<i>BMT1</i>	<i>A. thaliana</i>	?	Fewer but larger mitochondria per cell	?	Logan <i>et al.</i> (2003)	–
<i>BMT2</i>	<i>A. thaliana</i>	?	Fewer but larger mitochondria per cell	?	D. C. Logan & A. K. Tobin (unpublished)	–
<i>NMT</i>	<i>A. thaliana</i>	?	Mitochondria form extensive interconnected network	?	Logan <i>et al.</i> (2003)	–

gross morphology of plant and yeast mitochondria and it is therefore not surprising that homology searching has failed to identify plant homologues of some important yeast mitochondrial morphology genes. To date only two types of proteins have been shown to be involved in plant mitochondrial dynamics: Firstly, dynamin-like proteins have been identified that localise to mitochondrial constriction sites (Arimura & Tsutsumi, 2002; Nishida *et al.*, 2003) and one has been shown to be involved in the maintenance of normal mitochondrial morphology (Arimura & Tsutsumi, 2002); secondly, a protein of unknown function has been shown to be required for maintenance of correct mitochondrial distribution (Logan *et al.*, 2003). A recent review, concentrating on yeast mitochondrial dynamics (Shaw & Nunnari, 2002), concluded that since five proteins had been identified as playing a role in mitochondrial fission and fusion (Dnm1p, Fis1p, Fzo1p, Mdv1p and Mgm1p), work could be done to delineate the biochemical roles of the proteins and the mechanisms co-ordinating their activity. Clearly, we are not at the same stage with plant mitochondrial dynamics but the suite of plant mitochondrial mutants we recently identified together with advances in plant molecular genetics and functional genomics will hopefully provide us with the tools to quickly uncover more about the fundamental cell biology of this fascinating organelle.

Acknowledgements

The author, in collaboration with Dr Alyson K Tobin (University of St Andrews) is funded by the UK Biotechnology and Biological Sciences Research Council.

Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/NPH/NPH918/NPH918sm.htm>

Fig. S1 This movie shows mitochondria in Arabidopsis leaf epidermal cells (magnification 200X). The movie consists of 40 images captured at one second intervals and is presented at 6 frames per second (i.e. 6 X real-time).

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