

# Chapter 2

## Mitochondrial Dynamics

Iain Scott and David C. Logan

**Abstract** Mitochondria cannot be created de novo, meaning that any new mitochondrion must be formed from the division of an existing organelle. In addition to division, mitochondria also undergo fusion where two or more individual organelles join to produce a single mitochondrion. Mitochondrial division and fusion are the primary processes controlling mitochondrial form and together control mitochondrial size and number. Traditionally, the mitochondrion has been portrayed as an immobile, oval-shaped body. In reality, mitochondria are very dynamic organelles, capable of changing size and shape in a matter of seconds. Additionally, they undergo short- and long-distance vectorial transport mediated by association with the cytoskeleton. Advances in bioimaging have allowed scientists to reevaluate the behavior of mitochondria in vivo, stimulating a surge of interest in determining the genes, proteins, and mechanisms that control mitochondrial shape, size, number, and distribution (collectively termed *mitochondrial dynamics*).

**Keywords** Dynamics • Cytoskeleton • Actin • Microtubules • Fission • Fusion • Motility • Inheritance • Cell death • Morphology

### 2.1 Introduction

Mitochondrial dynamics is the study of the dynamic behavior of mitochondria in living cells. This behavior includes mitochondrial division and fusion: the two events controlling mitochondrial shape, size, and number, in addition to changes in the morphology of individual organelles, their motility in the cytosol, and their inheritance during cell division. The dynamic nature of mitochondria was evident in some of the earliest published studies of mitochondria in living cells (Cavers 1914) and in

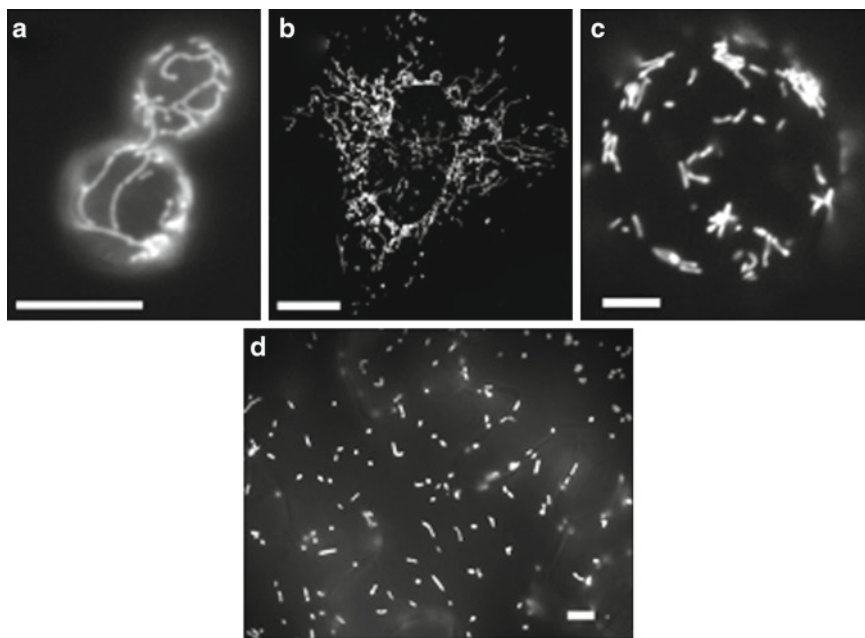
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recent years, mainly thanks to the development of green fluorescent protein (GFP) as an *in vivo* reporter (Chalfie et al. 1994), we have been able to study these dynamic processes in more detail (Rizzuto et al. 1995; Kohler et al. 1997; Sesaki and Jensen 1999; Logan and Leaver 2000).

Visualization of mitochondria in living cells has highlighted some fundamental steady-state differences in chondriome<sup>1</sup> organization and structure in different organisms, and cell types within the same organism. The chondriome of *Saccharomyces cerevisiae* is typically organized as a network of interconnected tubules (Fig. 2.1a), although the structure is partly dependent on growth medium: ramification of the chondriome increases when cells are grown on nonfermentable media, and the volume of the mitochondrial compartment increases (Egner et al. 2002). In animal



**Fig. 2.1** Chondriome structure in yeast, human HeLa cell line, and plants. (a) *S. cerevisiae* chondriome labeled with mitochondria-targeted GFP expressed from plasmid pYES-mtGFP (Westermann and Neupert 2000). The cell has been grown in rich medium containing galactose as a carbon source (image by Bastian Seubert and reproduced courtesy of Benedikt Westermann, Universitaet Bayreuth, Germany). (b) HeLa cell chondriome labeled with anti-mouse cytochrome c and Invitrogen Alexa 594 goat anti-mouse antibody (image courtesy of Megan Cleland from the laboratory of Richard J. Youle, NINDS, NIH). (c) Arabidopsis mesophyll protoplast expressing mitochondria-targeted GFP (Logan and Leaver 2000; image by Sanjaya Ekanayake, Logan Lab., University of Saskatchewan). (d) Leaf epidermis of *Arabidopsis* plant expressing mitochondrial-targeted GFP (Logan and Leaver 2000; image by DC Logan). Scale bar in all images = 5  $\mu$ m

<sup>1</sup>Here, the term chondriome refers to all the mitochondria in a cell, collectively. However, the term is sometimes used to describe the mitochondrial genome. In this book the mitochondrial genome is referred to as the *chondrome*.

cells, although chondriome structure is highly variable depending on animal and cell type, mitochondria are generally more tubular and ramified (Fig. 2.1b) than is typical in higher plants where the chondriome is typically organized as a population of several hundred physically discrete organelles (Logan 2006). Discovery of the genetic and physiological reasons for differences in chondriome structure between the different eukaryotic lineages, and between different cell types of the same organism, are key aims of researchers working on mitochondrial dynamics. Molecular cell biological studies, taking advantage of the tractability of model organisms and their sequenced genomes, have enabled the identification of numerous genes involved in mitochondrial dynamics (Table 2.1). In turn, gene identification has enabled studies aimed at identifying the mechanisms controlling mitochondrial dynamics, and we are now increasing our understanding of how such fundamental cell biological processes are involved in cell and organism physiology, and how mitochondrial dynamics underpins organism health and development. The aim of this chapter is to summarize our knowledge of mitochondrial dynamics in eukaryotes in general, and to relate this to the situation in plants in particular.

## 2.2 Division

### 2.2.1 *Animal and Yeast Mitochondrial Division*

Mitochondrial division is a highly coordinated process that involves the formation of two or more daughter organelles by scission of a single mitochondrion. To an extent the mechanisms that carry out mitochondrial fission reflect the evolutionary history of the organelle. Mitochondria are descended from  $\alpha$ -proteobacteria (Zimmer 2009; see also Chap. 1), and studies of extant species, such as *Escherichia coli*, show that cytokinesis is carried out by proteins such as filamentous temperature-sensitive Z (FtsZ), a bacterial tubulin homologue (Bi and Lutkenhaus 1991). FtsZ localizes to the inner surface of the cell membrane at division sites, where it forms a ring structure (Z ring) that enables constriction and scission of the parent into two daughter cells. FtsZ is a GTPase, which can hydrolyze guanosine triphosphate to provide a source of energy. However, it is thought that FtsZ may act as a scaffold for other proteins to effect the final scission, rather than providing the mechanical forces required (Dajkovic et al. 2008). As mitochondria are direct descendents of proteobacteria, it would be logical to assume that they may have retained this system for their own division. Indeed lower eukaryotes, such as the algae *Mallomonas splendens* and *Cyanidioschyzon merolae*, and the slime mould *Dictyostelium discoideum*, use FtsZ for this purpose (Beech et al. 2000; Takahara et al. 2000; Gilson et al. 2003). However, FtsZ has been lost from the mitochondrial division apparatus of most eukaryotes, and in higher plants the protein is primarily involved in plastid division (Osteryoung and Vierling 1995; Stokes et al. 2000). Instead, the eukaryotic cell appears to have evolved a mitochondrial fission system that shares many features with the scission of membrane vesicles during endocytosis. Scission of the plasma

**Table 2.1** Genes involved in mitochondrial dynamics

Gene	Organism	Location	Mutant phenotype	Protein properties/role	Reference	Arabidopsis homologue
<i>BIGYIN1</i> ( <i>BGY1</i> )/ <i>FISSION1A</i>	<i>A. thaliana</i>	Mitochondrial outer membrane (?); peroxisome (?)	Reduced number of large, spherical mitochondria, peroxisomes	Interacts with dynamin in division (?).	Scott et al. (2006), Lingard et al. (2008), Zhang and Hu (2008), and Fujimoto et al. (2009)	–
<i>BIGYIN2</i> ( <i>BGY2</i> )/ <i>FISSION1B</i>	<i>A. thaliana</i>	Mitochondrial outer membrane (?); peroxisome (?)	Reduced number of large, spherical mitochondria, peroxisomes	Interacts with dynamin in division (?).	Scott et al. (2006), Lingard et al. (2008), Zhang and Hu (2008), and Fujimoto et al. (2009)	–
<i>BMT1</i>	<i>A. thaliana</i>	?	Fewer but larger mitochondria per cell.	?	Logan et al. (2003)	–
<i>BMT2</i>	<i>A. thaliana</i>	?	Fewer but larger mitochondria per cell.	?	Logan (unpublished)	–
<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.	Zhu et al. (1997) and Fields et al. (1998, 2002)	<i>FMT</i> , see below.
<i>DNM1/Drp1</i> / <i>DRP-1</i>	<i>S. cerevisiae</i> / <i>H. sapiens</i>	Cytoplasm	Net-like sheet of interconnected tubules	Dynamin-related GTPase/membrane fission	Otsuga et al. (1998) and Smirnova et al. (2001)	<i>DRP3A</i> & <i>DRP3B</i> orthologues.

<i>DRP3A</i> (formerly <i>ADL2a</i> )	<i>A. thaliana</i>	Cytoplasm	Mitochondria form long tubules with many constrictions and protuberances (matrixules)	Dynamin-related GTPase/membrane fission (?)	Arimura et al. (2004) and Logan et al. (2004)	–
<i>DRP3B</i> (formerly <i>ADL2b</i> )	<i>A. thaliana</i>	Cytoplasm	Mitochondria form long interconnected tubules	Dynamin-related GTPase/membrane fission (?)	Arimura and Tsutsumi (2002)	–
<i>FIS1/MDV2/ hFIS1</i>	<i>S. cerevisiae/ H. sapiens</i>	Mitochondrial outer membrane	Net-like sheet of interconnected tubules	Integral membrane protein. Interacts with dynamin & Mdv1p ( <i>S. cerevisiae</i> only) in fission process.	Mozdy et al. (2000), Tieu and Nunnari (2000), Tieu et al. (2002), Suzuki et al. (2003, 2005), and Yoon et al. (2003)	At3g57090 ( <i>BIGYIN1</i> ), & At5g12390 ( <i>BIGYIN2</i> ) orthologues.
<i>fzo/FZO1/Mfn1/ Mfn2</i>	<i>Drosophila melanogaster/ S. cerevisiae/ H. sapiens</i>	Mitochondrial outer membrane	Aberrant mitochondrial fusion; fragmentation of tubules.	GTPase/mitochondrial fusion.	Hales and Fuller (1997), Hermann et al. (1998), Rapaport et al. (1998), Santel and Fuller (2001), and Rojo et al. (2002)	None
<i>KIF5B</i>	<i>Mus musculus</i>	Cytoplasm, mitochondrial associated	Mitochondria collapse around nucleus	Kinesin heavy chain protein, attachment to cytoskeleton	Tanaka et al. (1998)	Similarity to At3g63480.
<i>MDM1</i>	<i>S. cerevisiae</i>	Cytoplasm	Fragmentation of tubules. Defective transmission to daughter buds.	Intermediate filament- like	McConnell and Yaffe (1992)	Similarity to At2g15900.

(continued)

**Table 2.1** (continued)

Gene	Organism	Location	Mutant phenotype	Protein properties/role	Reference	Arabidopsis homologue
<i>MDM10</i>	<i>S. cerevisiae</i> / <i>Podospora anserina</i>	Mitochondrial outer membrane	Large spherical mitochondria. Defective transmission to daughter buds.	Integral membrane protein	Sogo and Yaffe (1994)	None
<i>MDM12</i>	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Large spherical mitochondria. Defective transmission to daughter buds.	Integral membrane protein	Berger et al. (1997)	None
<i>MDM14</i>	<i>S. cerevisiae</i>	Cytoplasm	Mitochondria aggregate. Defective transmission to daughter buds.	Coiled-coil domain	Shepard and Yaffe (1997)	None
<i>MDM20</i>	<i>S. cerevisiae</i>	Cytoplasm	Defective transmission to daughter buds.	Coiled-coil domain/ disrupts actin cables.	Hermann et al. (1997)	None
<i>MDM30</i>	<i>S. cerevisiae</i>	Cytosolic?	Fragmented or aggregated; few short tubules.	?	Dimmer et al. (2002)	None
<i>MDM31</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Compact mitochondrial aggregates	?	Dimmer et al. (2002)	None
<i>MDM32</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Compact mitochondrial aggregates	?	Dimmer et al. (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 et al. (2002), Messerschmitt et al. (2003)	Similarity to At3g53350.
<i>MDM34</i>	<i>S. cerevisiae</i>	Cytoplasmic?	Spherical mitochondria	?	Dimmer et al. (2002)	None
<i>MDM35</i>	<i>S. cerevisiae</i>	Cytoplasmic?	Spherical mitochondria	?	Dimmer et al. (2002)	Similarity to At4g33100.
<i>MDM36</i>	<i>S. cerevisiae</i>	Cytoplasmic?	Mitochondrial tubules aggregate/collapse to one side of cell	?	Dimmer et al. (2002)	None
<i>MDM37</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Fragmented mitochondrial tubules	Rhomboid-like protein	Dimmer et al. (2002)	Similarity to At1g18600.
<i>MDM38</i>	<i>S. cerevisiae</i>	Predicted mitochondrial inner membrane protein	Lasso-like mitochondria	Calcium-binding protein	Dimmer et al. (2002)	Similarity to At3g59820.
<i>MDM39</i>	<i>S. cerevisiae</i>	Predicted integral membrane protein – no mitochondrial targeting presequence	Fragmented mitochondrial tubules	?	Dimmer et al. (2002)	None
<i>MDV1/FIS2/GAG3 NET2</i>	<i>S. cerevisiae</i>	Cytoplasmic/associates with mitochondrial outer membrane	Netlike sheet of interconnected tubules	Predicted coiled-coil and seven WD-40 repeats/interacts with Dnm1p and Fis1p in fission process.	Fekkes et al. (2000), Mozdy et al. (2000), Tieu and Numari (2000), Cervený et al. (2001), Tieu et al. (2002)	Similarity to WD-40-repeat containing proteins.

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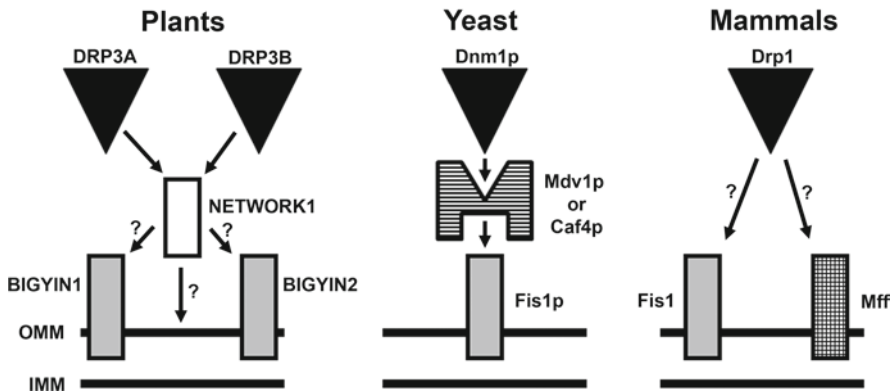
Table 2.1 (continued)

Gene	Organism	Location	Mutant phenotype	Protein properties/role	Reference	Arabidopsis homologue
<i>Mff</i>	<i>H. sapiens</i>	Mitochondrial outer membrane, C-terminal anchor	Elongated mitochondrial tubules	?	Gandre-Babbe and van der Blek (2008)	None
<i>MGM1/Opal</i>	<i>S. cerevisiae/H. sapiens</i>	Inner membrane space	Mitochondria fragmentation, Loss of mtDNA, Defective transmission to daughter buds.	Dynamamin-related protein/inner membrane modeling, may interact with Ugo1p and Fzo1p.	Jones and Fangman (1992), Guan et al. (1993), Shepard and Yaffe (1999), Alexander et al. (2000), Wong et al. (2000), Delettre et al. (2001), Misaka et al. (2002), and Wong et al. (2003)	Dynamamin-like genes in Arabidopsis. Most similar is DRP3A, At4g33650.
<i>MMM1</i>	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Large spherical mitochondria. Defective transmission to daughter buds.	Integral membrane protein	Burgess et al. (1994)	None
<i>MMT1</i>	<i>A. thaliana</i>	?	Much larger and smaller mitochondria in same cell. Altered thylakoid morphology.	?	Logan et al. (2003)	–
<i>MMT2</i>	<i>A. thaliana</i>	?	Much larger and smaller mitochondria in same cell. Giant chloroplasts.	?	Logan et al. (2003)	–



<i>NETWORK1</i> ( <i>ELM1</i> )	<i>A. thaliana</i>	Mitochondrial outer membrane	Mitochondria form long tubules and sometimes an interconnected network.	?	Logan et al. (2003) and Arimura et al. (2008)	–
<i>PCP1/RBD1/hPARL</i>	<i>S. cerevisiae/H. sapiens</i>	Mitochondrial inner membrane	Fragmented mitochondria, aggregated.	Rhomboid-like protein, processing of Mgm1p	Herlan et al. (2003) McQuibban et al. (2003)	Rhomboid domain proteins. Most similar is At1g18600.
<i>UGO1</i>	<i>S. cerevisiae</i>	Mitochondrial outer membrane	Fragmentation of mitochondrial tubules.	Involved in mitochondrial fusion.	Sesaki and Jensen (2001)	None
<i>CAF4</i>	<i>S. cerevisiae</i>	Cytosolic, associates with mitochondrial outer membrane	No change, partially rescues <i>mdv1p</i> mutants	WD-40 repeat protein, mediates Fis1p/Dnm1p interactions	Griffin et al. (2005)	Similarity to WD-40 repeat proteins
<i>FMT</i>	<i>A. thaliana</i>	?	Clusters of mitochondria	TPR-domain; homologue of <i>cltA</i> and <i>CLU1</i>	Logan et al. (2003)	–
<i>MsfsZ-mi/CnfsZ1-1/CnfsZ1-2/fszAfszB</i>	<i>Mallomonas splendens/Cyanidioschyzon Merolae/D. discoideum</i>	Mitochondrial division sites and tips.	Elongated tubular mitochondria ( <i>D. discoideum</i> )	Tubulin-like, ring-forming protein, organelle constriction.	Beech et al. (2000), Takahara et al. (2000), and Gilson et al. (2003)	Chloroplast FisZ division genes; At5g55280 and At2g36250
<i>YME1L1/YME1</i>	<i>H. sapiens/S. cerevisiae</i>	Mitochondrial inner membrane space	Fragmented mitochondria	AAA-type protease; processing of OPA1	Gripapic et al. (2007) and Song et al. (2007)	FisH proteases, e.g. At2g26140
<i>Paraplegin</i>	<i>H. sapiens</i>	Mitochondrial inner membrane	Fragmented mitochondria	AAA-type protease; processing of OPA1	Cipolat et al. (2004, 2006)	FisH proteases, e.g. At1g07510

membrane during endocytosis is carried out by dynamin, a mechanical enzyme that forms tight spirals around the neck of vesicles to constrict and then cleave them off (Doherty and McMahon 2009). Many of the proteins involved in mitochondrial fission and fusion are members of the dynamin GTPase protein family (see Table 2.1), and they exhibit a similar mode of action to the endocytic form. The dynamin homologues involved in mitochondrial fission, called Dnm1p in yeast (*S. cerevisiae*) and Drp1 in mammals (Fig. 2.2) are predominantly found in the cytosol. During fission events they cycle to the outer mitochondrial membrane at scission site (Labrousse et al. 1999; Smirnova et al. 2001), where they form large homomultimeric complexes that encircle the mitochondrion in spirals, which finally constrict in an energy-dependent manner until membrane fission occurs. Studies of Dnm1p in vitro show that the spirals match the minimal diameter of constricted mitochondria in yeast during fission, indicating that Dnm1p is tailored to the process of mitochondrial division (Ingeman et al. 2005). Mutations in both Dnm1p and Drp1 block mitochondrial fission (Otsuga et al. 1998; Smirnova et al. 2001),



**Fig. 2.2** Mitochondrial division machinery. The mitochondrial division machineries of plants, yeast, and animals are composed of multiple proteins, many of which are common to the three groups. The main constriction and scission forces are provided by homologues of dynamin GTPases, called Dnm1p in yeast and Drp1 in mammals, while plants have two homologues, DRP3A and DRP3B. These proteins are directed to the mitochondrial membrane at scission sites by a number of adapters. In yeast, Dnm1p interacts with Fis1p, an outer mitochondrial membrane protein anchored to the organelle by a C-terminal transmembrane domain. This interaction is mediated by a second set of yeast-specific cytosolic adapters, called Mdv1p and Caf4p, which function semi-redundantly. In mammals, Drp1 may interact directly with the homologous hFis1 protein on the outer mitochondrial membrane, or Drp1 may be directed to mitochondria by a mammalian-specific adapter, such as Mff. In plants, the two DRP proteins may, like in mammals, interact directly with the Fis1-type proteins BIGYIN1 (also known as FISSION1A) or BIGYIN2 (FISSION1B), or they may use the plant-specific adapter protein NETWORK1 (ELM1) in a manner analogous to the situation in yeast. Alternatively, DRP3A or DRP3B may be recruited directly to the outer mitochondrial membrane by the mitochondrial-localized NETWORK1. Once localized to the outer mitochondrial membrane by these adapters, the dynamin homologues encircle the organelle, and proceed to constrict and sever the mitochondrion

upsetting the balance between fission and fusion that controls overall mitochondrial morphology. This leads to the formation of networks of highly interconnected mitochondrial tubules, as mitochondrial fusion continues as normal.

Dynamin is recruited to the outer mitochondrial membrane by a series of adapter proteins, which were initially identified by genetic screens in yeast. Fis1p is an outer mitochondrial membrane protein, which is anchored to the organelle by a C-terminal transmembrane domain (Mozdy et al. 2000). The remainder of the protein faces the cytosol, where it interacts with dynamin via a tetratricopeptide repeat (TPR) domain to initiate fission. In yeast, this interaction is mediated by a second set of partially redundant adapters, Mdv1p and Caf4p, which can bind both Fis1p and Dnm1p to allow their association (Tieu and Nunnari 2000; Cervený et al. 2001; Griffin et al. 2005). Loss of either Fis1p, or Mdv1p, and to a lesser extent Caf4p, prevents Dnm1p from associating with mitochondria, and hence blocks mitochondrial division. In mammals there are no homologues of Mdv1p or Caf4p, therefore the association between Fis1p and Drp1 at the outer membrane may be more direct (Yoon et al. 2003). Knockdown of Fis1 protein levels in mammalian cells leads to an increase in the interconnectivity of mitochondrial tubules, similar to those seen in Drp1 mutants, whereas overexpression of Fis1 has been shown to increase mitochondrial fragmentation (Yoon et al. 2003). Recently a second outer membrane protein involved in division, Mff, has been described (Gandre-Babbe and van der Bliek 2008). Like Fis1, it is anchored by a C-terminal transmembrane domain and faces the cytosol. Knockdown of Mff leads to an increase in elongated mitochondria, indicating that it may function in a similar manner to Fis1. However no direct interaction between Mff and Drp1 has been shown, and Mff and Fis1 appear to reside in different protein complexes, leaving its actual role in mitochondrial division open to speculation (Gandre-Babbe and van der Bliek 2008). In addition to their role in mitochondrial fission, all three mammalian proteins (Drp1, Fis1, and Mff) localize to peroxisomes and regulate their size (Koch et al. 2003a, b; Koch et al. 2005; Gandre-Babbe and van der Bliek 2008). How these proteins are dual-targeted to different organelles, and how this process is regulated, are not currently understood.

### 2.2.2 *Plant Mitochondrial Division*

The first proteins shown to be involved in plant mitochondrial division were identified through *in silico* homology searches, using mammalian and yeast fission proteins as bait. There are roughly 16 dynamin homologues in the Arabidopsis genome; and two of these, DRP3A and DRP3B, have been implicated in mitochondrial division (Arimura and Tsutsumi 2002; Arimura et al. 2004a, b, c; Logan et al. 2004; Mano et al. 2004). Both DRP3A and DRP3B share 37–41% similarity at the amino acid level with the dynamins involved in animal and yeast mitochondrial division and, like these proteins, localize to constriction sites of dividing mitochondria (Arimura and Tsutsumi 2002; Arimura et al. 2004a, b, c). Disrupting the function of DRP3A or DRP3B by genetic knockout, or overexpression of constructs with a dominant-negative

mutation in the GTPase domain, leads to an increase in the number of large, elongated mitochondria, indicating that these proteins are part of the division apparatus (Arimura and Tsutsumi 2002; Arimura et al. 2004a, b, c; Logan et al. 2004). The increased complexity of the plant fission system, in terms of number of homologous proteins involved, is replicated when looking at Fis1-type proteins. In *Arabidopsis*, there are two orthologues of Fis1p, named BIGYIN1 (also known as FISSION1A) and BIGYIN2 (FISSION1B) (Scott et al. 2006). Both of these proteins localize to the outer mitochondrial membrane (among other locations), and disruption of either leads to a decrease in mitochondrial division, as evidenced by an increase in organelle size and decrease in organelle number per cell (Scott et al. 2006; Fujimoto et al. 2009). As has been found in animals, the proteins involved in mitochondrial division appear to also have a role in peroxisome division. All four proteins (DRP3A, DRP3B, BIGYIN1, and BIGYIN2) have been shown to localize to peroxisomes and, to a greater or lesser extent, affect the size and number of these organelles (Lingard et al. 2008; Fujimoto et al. 2009). Unlike yeast, but in common with animals, there are no sequence homologues of either of the adapter proteins Mdv1p, or Caf4p, in the *Arabidopsis* genome. As such, it remains unclear whether there is any direct, or indirect, interaction between Drp1- and Fis1-type proteins in the plant division apparatus. However, recent research has identified another plant-specific putative adapter protein involved in mitochondrial fission (Box 2.1).

### **Box 2.1** The NETWORK protein

Initially identified as NETWORK (now named NETWORK1 – a paralogue was evident following identification of the *NETWORK* gene) in a screen for novel plant mitochondrial morphology proteins (Logan et al. 2003), ELM1 was shown to localize to the outer mitochondrial membrane (Arimura et al. 2008). Plants with mutations in NETWORK1/ELM1 exhibit an elongated mitochondrial phenotype, suggesting a fault in the division process. To further examine the role of NETWORK1/ELM1, Arimura et al. (2008) investigated whether NETWORK1/ELM1 interacts with DRP3A, DRP3B, or BIGYIN1 in yeast two-hybrid assays. These experiments failed to show interaction between BIGYIN1 and NETWORK1, although weak interaction between DRP3A/B and NETWORK1 was reported (interaction between DRP3B and NETWORK1 was greater than between DRP3A and NETWORK1; Arimura et al. 2008). These results suggest that NETWORK1 may act to direct dynamin to mitochondrial fission sites – a hypothesis that was strengthened by the observation that in NETWORK1/ELM1 mutants, DRP3A does not localize to mitochondria (Arimura et al. 2008). Further work is required to see if NETWORK1/ELM1 acts as a functional homologue of Mdv1p/Caf4p, i.e., linking DRP3A and DRP3B to BIGYIN1 (and perhaps BIGYIN2); or whether it acts independently of Fis1-type proteins. Importantly, peroxisome morphology in NETWORK1/ELM1 mutants appears to be unchanged (Arimura et al. 2008), which suggests that this protein may provide selectivity of function for the dual-targeted DRP3A/B division proteins.

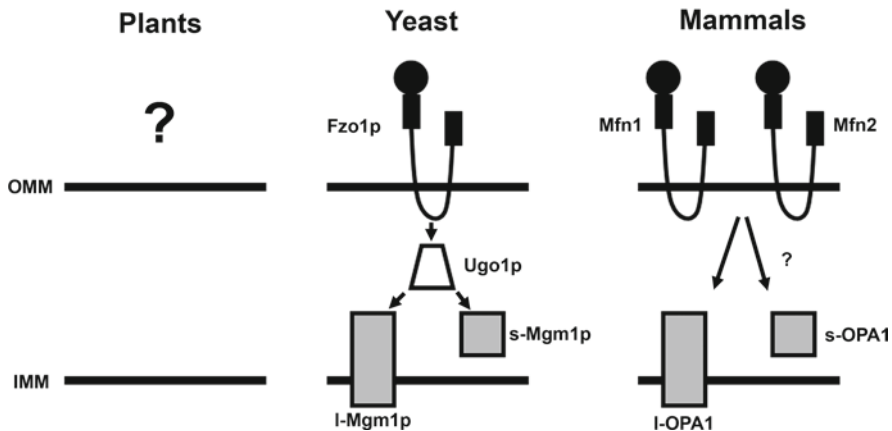
## 2.3 Fusion

### 2.3.1 Animal and Yeast Mitochondrial Fusion

The counterpoint to mitochondrial division is fusion (Fig. 2.3), where two or more individual organelles join together to create a single, larger mitochondrion. Fusion is a complex process, requiring the tethering of adjacent organelles and the coordinated joining of two independent membranes (the inner and outer mitochondrial membranes), without the significant loss of any mitochondrial proteins (e.g., cytochrome *c*) that could damage or kill the cell. The first genetic component of the mitochondrial fusion machinery was discovered in studies of *Drosophila* spermatogenesis where, following meiosis, the entire mitochondrial population of the cell fuses into two organelles called a Nebenkern. In flies harboring a mutation in an outer mitochondrial membrane GTPase, dubbed *fuzzy onions* (*Fzo*), the Nebenkern structure is altered due to a lack of organelle fusion (Hales and Fuller 1997). Fuzzy onions, so called because the Nebenkern normally resembles an onion slice in cross-section, has homologues in mammals (*Mfn1* and *Mfn2*) and yeast (*Fzo1p*), which perform a similar function in controlling outer membrane fusion (Hermann et al. 1998; Santel and Fuller 2001). *Fzo*-like proteins are anchored to the outer mitochondrial membrane by two transmembrane domains, and have a GTPase domain and twin coiled-coil regions facing the cytosol. The coiled-coil domains mediate homotypic binding between *Fzo*-like proteins on neighboring mitochondria, and the tethering of organelles in this fashion is required to initiate outer membrane fusion (Koshiba et al. 2004). Loss of *Fzo*-like proteins, or mutations in either the interaction or GTPase domains, leads to an increase in the number of small organelles caused by a lack of mitochondrial fusion (Hermann et al. 1998; Santel and Fuller 2001).

Inner membrane fusion is controlled by a final dynamin-like GTPase, called *Mgm1p* in yeast, and *OPA1* in mammals, which is found in the intermembrane space, or associated with the inner membrane (Wong et al. 2000; Olichon et al. 2002). There are eight variants of *OPA1* in human cells, formed as the result of differential transcript splicing from a single gene, and further processing by mitochondrial proteases (Delettre et al. 2001). A similar process occurs in the yeast homologue *Mgm1p*, and a combination of both the long and short isoforms of either *OPA1* or *Mgm1p* is required to allow inner membrane fusion. A number of proteins have been implicated in mammalian *OPA1* processing, including *Yme1L1*, a AAA-metalloprotease found in the intermembrane space (Gripapic et al. 2007; Song et al. 2007); paraplegin, another AAA protease located in the inner mitochondrial membrane (Ishihara et al. 2006); and *PARL*, a rhomboid protease, whose yeast homologue *Rbd1p/Pcp1p* performs the same function on *Mgm1p* (Herlan et al. 2003; McQuibban et al. 2003; Sesaki et al. 2003; Cipolat et al. 2006).

Analysis of the yeast components *in vitro* have shown that the fusion of both the inner and outer membranes are mechanically distinct (Meeusen et al. 2004).



**Fig. 2.3** Mitochondrial fusion machinery. The proteins involved in mitochondrial fusion in yeast and mammals are closely related, and appear to act in a similar manner. The main protagonists are dynamin-like GTPases, which provide the energy required for this process. On the outer mitochondrial membrane, homologues of the *Drosophila* *FUZZY ONIONS* (*FZO*) protein, called Fzo1p in yeast and Mitofusin1 (Mfn1) and Mitofusin2 (Mfn2) in mammals, tether neighboring organelles through homotypic coiled-coil binding domains on opposing proteins (e.g., a Fzo1p molecule on one mitochondrion will bind with a Fzo1p molecule on a second mitochondrion, allowing them to become tethered in the first stage of fusion). On the inner mitochondrial membrane a second GTPase, called Mgm1p in yeast and OPA1 in mammals, provides the energy for inner membrane fusion. These proteins are found in two isoforms (long and short), and the presence of both is required to allow fusion. While the interaction between membranes may be direct in mammals, particularly between Mfn1 and OPA1, this interaction is mediated in yeast by an adaptor protein, Ugo1p. While it is known that mitochondrial fusion exists in plants, the proteins involved in this process are currently unknown

However there is an interaction between the outer membrane GTPase Fzo1p and the inner membrane Mgm1p, which is mediated through an adaptor protein, Ugo1p (Sesaki and Jensen 2001). While there may be a direct interaction in human cells with the homologous proteins, particularly Mfn1 and OPA1, there is no human homologue of Ugo1p. The fusion of both membranes requires GTP hydrolysis to provide energy, however, the inner membrane also requires the maintenance of the electrochemical membrane potential (Meeusen et al. 2004). This may be because the homologous human inner membrane GTPase, OPA1, undergoes increased proteolytic processing when membrane potential is lost (Ishihara et al. 2006), leaving only short isoforms that are incapable of mediating fusion alone.

### 2.3.2 Plant Mitochondrial Fusion

As noted above, homology searches led to the discovery of several plant mitochondrial division proteins. However, the same method has failed to yield any genes

involved in plant mitochondrial fusion. There appears to be only weak sequence similarity between Arabidopsis proteins and either OPA1/Mgm1p, or Ugo1p (see Table 2.1), while the closest Fzo/Mitofusin homologue, FZL, is involved in the regulation of chloroplast thylakoid morphology (Gao et al. 2006). Despite a lack of genetic evidence, observations of mitochondrial dynamics in plants have confirmed the existence of this process. Gross observations of plant mitochondria at different stages of the cell cycle show that mitochondria undergo massive fusion events prior to cytokinesis (discussed below). In addition, time-lapse microscopy of plant mitochondria has clearly shown fusion events in a number of cell types (see [www.plantcellbiologyondvd.com](http://www.plantcellbiologyondvd.com)), indicating that this process also occurs in nondividing cells. To quantify mitochondrial fusion in plant cells (Arimura et al. 2004) transiently expressed, in onion epidermal cells, a matrix-targeted fluorescent protein called Kaede, which can be induced to change its emission spectrum irreversibly from green to red upon by illumination with UV light. By inducing half of the organelles in a cell to fluoresce red, Arimura et al. were able to observe mitochondrial fusion through the appearance of yellow mitochondria as a result of mixing of the matrix-localized fluorescent proteins. All the mitochondria in the cell were fluorescing yellow after 1–2 h (Arimura et al. 2004), indicating that fusion is a relatively rapid and constitutive event. In summary, while plant mitochondria clearly fuse under normal conditions, the mechanisms behind the process remain an enigma.

## 2.4 Regulation of Chondriome Structure

### 2.4.1 Temporal Regulation

Changes in mitochondrial ultrastructure during mitochondrial maturation in developing tissues have been recorded in a number of diverse organisms including rat conceptus tissues (Alcolea et al. 2002), rat brown adipose tissue (Justo et al. 2005), and maize embryos (Logan et al. 2001). Such changes in ultrastructure are often accompanied by changes in the gross morphology of mitochondria. Similarly, changes in mitochondrial gross morphology have been reported to occur in various animal tissues during normal differentiation (Chen and Chan 2004). However, most reports of changes in mitochondrial morphology arise from pathological defects in fusion, or division (Zuchner et al. 2005; Yang et al. 2008), or from studies where the normal function of proteins involved in mitochondrial fusion, or division, have been altered in order to determine the role of normal fusion, or division, in the development of a healthy organism (e.g., Chen et al. 2003; Wakabayashi et al. 2009).

Changes observed during the cell cycle provide one of the clearest examples of developmentally related temporal modifications to chondriome structure. During the human cell cycle, mitochondria alternate between two morphologic states (Barni et al. 1996; Karbowski et al. 2001; Margineantu et al. 2002). In the G1 phase



mitochondria fuse to form reticula, halving the number of individual organelles relative to the number prior to M phase (Karbowski et al. 2001). As cells proceed from G1 to S phase there is an increase in mitochondrial number as a result of fragmentation (division) of the mitochondrial reticula (Barni et al. 1996; Karbowski et al. 2001; Margineantu et al. 2002). A similar situation has recently been shown to occur in plants. Segui-Simarro and colleagues demonstrated, by means of an analysis of electron micrographs of serial thin-sections prepared from Arabidopsis apical meristems at various developmental stages that, in addition to smaller physically discrete organelles, there existed a large sheet-like mitochondrion that underwent characteristic morphological and architectural changes during the cell cycle (Segui-Simarro et al. 2008). During G1 to S phase this large mitochondrion was described as having a tentaculate morphology and enveloped one pole of the nucleus. In the G2 phase the large and small mitochondria double in volume and the large mitochondrion forms a cage around the nucleus. During M phase ca. 60% of the small mitochondria fuse with the large mitochondrion, thereby increasing its volume to ca. 80% of the total mitochondrial volume. During cytokinesis the cage-like mitochondrion divides to form two physically discrete tentacular mitochondria which then each undergo further division. Segui-Simarro et al. hypothesized that the tentaculate/cage-like mitochondria provide an efficient means to deliver ATP for cell cycle and cytokinesis activities (Segui-Simarro et al. 2008). However, since mitochondria with these morphologies are not seen in root apical meristems, Segui-Simarro et al. proposed that the major role of the tentaculate/cage-like mitochondria is to provide a structure to enable efficient mixing and recombination of mtDNA (Segui-Simarro et al. 2008) which is inherent in the discontinuous whole hypothesis governing plant mitochondrial dynamics (Logan 2006).

### 2.4.2 *Physical Regulation*

The proteins involved in mitochondrial fission and fusion are highly regulated, both at the transcriptional and post-transcriptional levels. As changes to mitochondrial morphology can occur over the course of a few seconds, the ability to quickly switch proteins between active and inactive states relies heavily on coordinated post-translational modifications. In mammals, Drp1 has been identified as a substrate for multiple modifications. Phosphorylation of human Drp1 by different kinases has been shown to both promote (CDK/cyclinB; Taguchi et al. 2007) and repress (cAMP-dependent protein kinase A; Chang and Blackstone 2007; Cribbs and Strack 2007) its function, depending on which serine on the protein is modified. Drp1 has also been shown to be a substrate for reversible SUMOylation by SUMO1 (SUMOylation; Harder et al. 2004) and SENP5 (deSUMOylation; Zunino et al. 2007), which promote, or repress Drp1 function, respectively. As such, Drp1 appears to be controlled by a number of rapidly-reversible post-translational processes, indicating that changes in mitochondrial dynamics require a high degree of specific regulation.



Protein turnover is another method used to regulate the state of the mitochondrial population. While addition of SUMO acts as an on-off switch for dynamin, its close homologue ubiquitin is more often used to mark proteins for proteasomal degradation. Steady-state levels of the yeast fusion protein Fzo1p, and possibly mammalian Mfn1 and Mfn2, appear to be regulated by polyubiquitination, as inhibition of the proteasome, either genetically, or chemically, leads to increased expression of these proteins (Neutzner and Youle 2005; Escobar-Henriques et al. 2006; Karbowski et al. 2007). Recently, the first ubiquitin ligase to regulate mitochondrial morphology proteins was identified by three independent groups (Nakamura et al. 2006; Yonashiro et al. 2006; Karbowski et al. 2007). MARCH5, a RING-domain E3 ubiquitin ligase, is localized to the outer mitochondrial membrane and interacts with Drp1. Inhibition of MARCH5 leads to mitochondrial elongation and clustering of an inactive form of Drp1 at the mitochondria (Karbowski et al. 2007), suggesting the MARCH5 either acts as a molecular switch to aid mitochondrial division, or that it ubiquitinates an unknown regulator of Drp1 that inhibits its function.

Presently, there have been no reports of plant mitochondrial dynamics proteins being regulated by post-translational modifications. However, given the ubiquity of this process in animals and yeast, it would appear that this will be a fruitful area for further research.

## 2.5 Death

The best described link between changes in plant mitochondrial dynamics and cell function occurs during cell death (Logan 2008; see also Chaps. 17–19, and Box 2.2). The documented changes in mitochondrial dynamics are all likely due to changes in the mitochondrial inner membrane permeability (the mitochondrial permeability transition, mPT), although changes in mitochondrial-cytoskeleton interactions are also likely important since cell death induction leads to a cessation of mitochondrial movement, at least in *Arabidopsis* (Gao et al. 2008; Zhang et al. 2009; Scott and Logan, unpublished). Early evidence for a mPT in plants was obtained from *in vitro* experiments on *Arabidopsis* (Tiwari et al. 2002), oat (Curtis and Wolpert 2002), wheat (Virolainen et al. 2002), and potato (Fortes et al. 2001; Arpagaus et al. 2002) mitochondria. Swelling of isolated potato mitochondria was induced by  $\text{Ca}^{2+}$ , but not  $\text{Mg}^{2+}$ , while  $\text{Ca}^{2+}$ -induced swelling could be inhibited by cyclosporin A (CsA), but only in the presence of a reducing agent (Arpagaus et al. 2002). Oxidative stress-induced swelling of isolated *Arabidopsis* mitochondria was also sensitive to CsA (Tiwari et al. 2002). In other studies swelling was reported to be insensitive to CsA (Curtis and Wolpert 2002; Virolainen et al. 2002), although this may be due to the reported requirement for the addition of CsA under reduced conditions (Arpagaus et al. 2002), a situation that was not tested in the other studies. Swelling led to disruption of the outer, but not inner, mitochondrial membrane and release of cytochrome *c* (Arpagaus et al. 2002). Virolainen et al. (2002) and Arpagaus et al. (2002) both provided evidence for the release of cytochrome *c* as a

**Box 2.2** Cell death and mitochondrial morphology

We recently published the results of an investigation into the effects of the induction of cell death on mitochondrial morphology in *Arabidopsis* protoplasts expressing mitochondrial-targeted GFP (Scott and Logan 2008). Treatment with strong oxidants, or a heat shock, resulted in a rapid and consistent change in mitochondrial morphology, characterized as an increase in the size of individual organelles (termed the *mitochondrial morphology transition*), that preceded by many hours any measureable effect on cell death. Preincubation of protoplasts with the calcium channel-blocker lanthanum chloride, or the permeability transition pore inhibitor cyclosporin A, inhibited both the mitochondrial morphology transition and subsequent cell death (Scott and Logan 2008). In addition, pretreatment of protoplasts with a cell permeable superoxide dismutase analogue, TEMPOL, also blocked the morphology transition and subsequent cell death. The mitochondrial morphology transition was observed much earlier than similar changes in morphology that were detected in previous studies using *in vivo* assays (Yoshinaga et al. 2005; Zottini et al. 2006). We concluded that the mitochondrial morphology transition that we measured directly in intact protoplasts, or leaf cells, is synonymous with the mPT, as measured *in vitro* by others, and that perturbation of the permeability of the inner mitochondrial membrane is at least one important mechanism promoting cell death in plants (Scott and Logan 2008).

consequence of mitochondrial swelling. While cytochrome *c* release has a clear role in apoptosis through its role in the formation of the caspase-activating apoptosome, it is not clear if cytochrome *c* release has a specific role to play in the execution of a programmed cell death (PCD) pathway in plant cells, nor is it known if there is a link between mechanisms underlying the mPT and cytochrome-*c* release in plants; no links have been established *in vivo* between these two events.

The role of a mPT in plant PCD has been demonstrated in a number of studies using a variety of death inducers. Yu and colleagues showed that CsA could inhibit PCD of tracheary elements in *Zinnia elegans*, and that there were subtle changes in mitochondrial ultrastructure, but no report of mitochondrial swelling, prior to loss of tonoplast integrity and cellular autolysis (Yu et al. 2002). Yu et al. commented that the changes in mitochondrial ultrastructure were different from those that occur during apoptosis of animal cells, but in fact an increased density of the matrix and swelling of the intracrystal spaces, as reported, are the same as those reported to occur during apoptosis (Zhuang et al. 1998; Martinou et al. 1999). Using a hypersensitive response elicitor, ceramide, or protoporphyrin IX (PPIX), Yao et al. demonstrated that *Arabidopsis* leaf cell death was accompanied by a loss of mitochondrial membrane potential and nuclear DNA cleavage, and that these

effects could be partially prevented by CsA, as could death itself (Yao et al. 2004). The study by Yao et al. is one of the few plant cell death studies in which death was measured rather than inferred. Subsequent work by Yao and Greenberg demonstrated that PPIX caused mitochondria to become swollen, and more round in shape (Yao and Greenberg 2006).

Swelling of mitochondria has also been reported in vivo during the self-incompatibility response in poppy pollen (Geitmann et al. 2004) and during abortion of microspores in CMS sunflower (Laveau et al. 1989). However, although the changes to mitochondrial morphology can be postulated (and indeed are likely) to be linked to the PCD that occurs in these systems, further studies are required to show direct links.

## 2.6 Motility, Distribution, and Inheritance

One of the most striking phenomena of mitochondria when visualized in living plant cells is their dynamism. Since the plant chondriome is organized as a discontinuous whole (Logan 2006), correct mitochondrial function will require precise control over mitochondrial motility and cellular distribution. In fact it is inherent in the discontinuous whole hypothesis that there is a “need to meet” to enable the exchange and/or complementation of mtDNA, and clearly there is a requirement for movement to drive the meeting of physically discrete organelles. In growing *Arabidopsis* root hairs, mitochondria have been recorded moving through the cytosol at speeds up to  $10 \mu\text{m s}^{-1}$ , although average speeds range between  $0.6$  and  $3.4 \mu\text{m s}^{-1}$  depending on location within the hair (Zheng et al. 2009). Variability of the speed of mitochondrial movement has also been reported in maize BY-2 cells in which a single mitochondrion was recorded travelling at speeds between  $0.1$  and  $0.5 \mu\text{m s}^{-1}$  depending on location within the cell (Watanabe et al. 2007).

### 2.6.1 Mitochondrial Movement and the Cytoskeleton

There is considerable diversity in the mechanisms underpinning mitochondrial movement in different cell types in different organisms. Movement of mitochondria in mammals and most yeast is microtubule based (for review, see Boldogh and Pon 2007), whereas in *S. cerevisiae*, *Aspergillus*, and plants, mitochondria move predominantly on the actin cytoskeleton (Simon et al. 1995; Olyslaegers and Verbelen 1998; Van Gestel et al. 2002; Koch et al. 2003a, b; Sheahan et al. 2004). There is very little evidence for the involvement of microtubules in the distribution of mitochondria in plants beyond their effects on the geometry of the actin cytoskeleton (Zheng et al. 2009). However, circumstantial evidence from one study suggested that microtubules may play a role in organizing mitochondria into transverse arrays in the cortical cytoplasm in the absence of actin filaments (Van Gestel et al. 2002), but importantly these mitochondria appeared immobilized and so there

is no published evidence that microtubules serve as tracts for mitochondrial movement in plants. Recent studies, predominantly using yeast, have shown that there are several mechanisms in operation in addition to the classic motor-protein mediated means of organelle transport on the cytoskeleton (for a review, see Boldogh and Pon (2007)).

### 2.6.1.1 Mitochondrial Movement and Microtubules

Tubulin is a highly conserved dimeric protein present in all eukaryotes. Tubulin self-assembles into microtubules which are polar dynamic structures regulated by assembly and disassembly mediated by GTP hydrolysis (Kreis and Vale 1998). Two motor proteins are responsible for movement on microtubules: dynein drives mitochondrial movement to the minus end of microtubules (attached to the microtubule organizing center), whereas kinesin drives movement to the plus end (toward the cell periphery). A number of mitochondrial-associated proteins have been implicated in mitochondrial movement on microtubules and the most-studied of these adaptors are Milton and Miro.

Milton was identified in a genetic screen based on defective photoreceptor function. Mutant *milton* protein failed to deliver mitochondria to *Drosophila* nerve terminals with the result that mutant flies were viable but blind (Stowers et al. 2002). Milton is an essential adaptor protein that recruits kinesin heavy chain to mitochondria and interacts with a second adaptor named Miro (Glater et al. 2006). Although Milton localizes to mitochondria it has neither a mitochondrial targeting signal nor a transmembrane domain and therefore must interact with another protein(s) that is more tightly associated with mitochondria: Miro.

Miro is a Rho-type GTPase that localizes to the mitochondrial outer membrane by means of a C-terminal transmembrane domain and interacts with Milton via two GTPase domains separated by two EF-hands that have the potential to bind calcium (Fransson et al. 2003, 2006). Miro orthologues are present in all eukaryotes for which genome sequences are available, therefore including *S. cerevisiae*, *Aspergillus*, and *Arabidopsis*: organisms in which mitochondrial movement is predominantly actin based. As a result it has been suggested that the function of Miro, at least in *S. cerevisiae*, is different than in *Drosophila* and other animals and that Miro might function as “a more general mitochondrial adaptor that binds to other motile complexes” (Rice and Gelfand 2006). The *Arabidopsis* genome contains three Miro orthologues, two of which are transcribed ubiquitously (Yamaoka and Leaver 2008). Mutation of *MIRO2* had no apparent effect on plant development but mutation of *MIRO1* led to arrest of embryogenesis at an early stage and an impairment in pollen germination and tube growth (Yamaoka and Leaver 2008). Mitochondria in pollen tubes of *miro1* mutant plants exhibited abnormal morphology, being larger and more tubular than wild type, and they exhibited a disruption in their normal streaming movement within the pollen tube, movement that was dependent on the actin cytoskeleton (Yamaoka and Leaver 2008). Given that mitochondrial movement in pollen tubes is actin dependent (Yamaoka and Leaver 2008), and the

apparent absence of Milton homologues from the *Arabidopsis* genome, MIRO clearly has a different role in plants just as its orthologue, Gem1P, does in *S. cerevisiae* (Frederick et al. 2004). Future research will hopefully uncover the exact role MIRO/Gem1p plays in mitochondrial dynamics.

### 2.6.1.2 Mitochondrial Movement and Actin

Actin is a highly conserved protein that is a major component of microfilaments in eukaryotes. Actin self-assembles under physiological conditions into long polymers and polymerization, depolymerization, and higher order assembly (e.g., bundling) of filaments results in the production of a variety of transient, or more stable, structures. Actin is important for many types of intracellular motility that are driven by actin polymerization, or by the movement of the motor protein myosin along actin filaments (Kreis and Vale 1998).

#### Myosin

Myosins are a large superfamily of actin-activated ATPases that act as motors to propel cargo along actin filaments, or propel actin filaments along immobile cell structures. Myosins have been implicated in the movement of other organelles on actin filaments (e.g., peroxisomes, Jedd and Chua 2002); therefore, it seems likely that myosin is involved in some mitochondrial movements. Several recent studies have identified myosin family members that likely serve as motor proteins driving movement along actin filaments. The *Arabidopsis* myosin gene family comprises 17 members: the majority (13) are in group XI, and the remaining four are in group VIII (Reddy and Day 2001). By means of extensive analyses of myosin-tail fluorescent-protein fusions, four *Arabidopsis* group XI myosins have been reported to be either directly or indirectly involved in the movement of mitochondria in *Nicotiana benthamia*: XI-C, XI-E, XI-I, and XI-K (Avisar et al. 2008, Avisar et al. 2009; Sparkes et al. 2008). In a colocalization study, again using *Arabidopsis* proteins but this time transiently expressed in *N. tabacum*, only myosin XI-J (specifically a XI-J tail fusion lacking the motor domain) was found to sometimes colocalize with mitochondria (Reisen and Hanson 2007).

#### Mechanisms Other Than Myosin-Based

Evidence for mechanisms moving mitochondria on the actin cytoskeleton other than those involving myosin was recently obtained in a study using a pharmaceutical approach (Zheng et al. 2009). Tracking and analysis of mitochondria in *Arabidopsis* root hairs using a combination of evanescent wave microscopy and spinning disc confocal microscopy following treatment with various drugs that perturb the

cytoskeleton has suggested that, as in other organisms, mitochondria move using myosin-motor dependent and myosin-motor independent mechanisms (Zheng et al. 2009). Zheng et al. (2009) concluded that the mechanisms controlling mitochondrial speed, positioning, and direction of movement were the result of the coordinated activity of myosin and the rate of actin turnover. In addition, supporting the conclusions of early work on mitochondrial movement (Van Gestel et al. 2002), microtubule dynamics had a role to play, in this instance by influencing the arrangement of actin filaments (Zheng et al. 2009).

The Arp2/3 complex is a multisubunit ubiquitous regulator of actin nucleation in eukaryotes responsible for the actin-based motility manifest by comet-tail formation (Fehrenbacher et al. 2003). The Arp2/3 complex binds newly polymerized actin, and promotes actin nucleation (Pollard and Beltzner 2002) thereby generating forces to facilitate mitochondrial movement into the developing bud in *S. cerevisiae* (Boldogh et al. 2001, 2005). Disruption of either *ARP2* or *ARP15*, two subunits of the complex, leads to a decrease in mitochondrial motility, and changes to mitochondrial morphology (Boldogh et al. 2001). There is no published evidence of the involvement of the plant Arp2/3 complex in driving mitochondrial motility. Although myosin was initially thought not to be of importance for Arp2/3 mediated mitochondrial movement in *S. cerevisiae*, recent evidence suggests that myosin functions directly and significantly in this process (Altmann et al. 2008). It has been suggested that multiple mechanisms for mitochondrial transport exist to ensure that mitochondria, vital to all mitochondria eukaryotes, are accurately inherited during cytokinesis (Valiathan and Weisman 2008).

### ***2.6.2 Mitochondrial Motility Delivers the Organelle to the Right Places***

Whilst it is generally accepted that mitochondrial motility, which enables their close association with other motile structures within the cell, is necessary for mitochondrial or cellular function, there are very few studies linking location to function in plants. Association of mitochondria with energy-consuming structures or organelles has been described in a range of non-plant organisms (see Munn 1974; Tyler 1992; Bereiter-Hahn and Voth 1994). One classic example is the formation of the Nebenkern, a collar around the sperm axoneme, formed during spermatogenesis and comprising two giant mitochondria formed by repeated fusion events (Hales and Fuller 1996, 1997). In living plant tissues containing chloroplasts, visualization of mitochondria has shown the frequent close proximity of these two organelles (Stickens and Verbelen 1996; Logan and Leaver 2000). It is assumed that this facilitates exchange of respiratory gases and possibly metabolites, although direct evidence for this is lacking. In characean internode cells, it has been suggested that the spatiotemporal distribution of mitochondria within the cell promotes their

association with chloroplasts (Foissner 2004). However, care needs to be taken in evaluating the experimental evidence required to support the hypothesis that mitochondrial and cellular function are dependent on the juxtaposition of mitochondria with other cell structures when this evidence is simply qualitative, e.g., micrographs showing apparent close association. This is particularly true in highly vacuolated mesophyll cells (Evert and Esau 2006), where the volume of cortical cytoplasm dictates that all cellular organelles are in close association.

A recent paper presented qualitative and semiquantitative evidence that chloroplast movements under different lighting regimes were associated with changes in the location of mitochondria (Islam et al. 2009). In the dark, mitochondria were distributed randomly in palisade mesophyll cells. However, under low-intensity blue light illumination mitochondria moved with chloroplasts to the periclinal walls of the cell, while under high-intensity blue light mitochondria adopted an anticlinal location similar to the light-avoidance response of chloroplasts (Islam et al. 2009). It is not known whether the response of the mitochondria is independent of the chloroplasts, either from a signaling or physical perspective. For example, whether or not a subpopulation of mitochondria becomes physically associated with a chloroplast through cytoskeletal interactions, and then moves with the chloroplast as it reacts to changes in illumination.

### 2.6.3 Inheritance and Cellular Distribution

Mitochondrial inheritance has been extensively studied during budding in *S. cerevisiae* and is dependent on the cytoskeleton, mitochondrial division, and mitochondrial fusion (Hermann and Shaw 1998; Catlett and Weisman 2000). During the budding process the mitochondrial reticulum moves towards the bud site and a single tubule moves into the newly formed bud. This movement continues until mother and daughter cells have an equal mitochondrial complement, at which point cytokinesis can occur (Catlett and Weisman 2000; Boldogh et al. 2005).

Research into mitochondrial morphology mutants has identified many genes involved in inheritance. The first mutants defective in mitochondrial inheritance were isolated during a screen of temperature-sensitive yeast cell lines for individuals that failed to pass mitochondria to the daughter bud prior to cytokinesis (McConnell et al. 1990). Many of these mutant lines exhibited altered mitochondrial morphology, and were named *mdm*, for mitochondrial distribution and morphology (see Table 2.1). The mutant genes fell into two broad categories: genes encoding integral outer mitochondrial membrane proteins, and genes encoding cytosolic proteins (Yaffe 1999).

Little is known about the mechanisms regulating the cellular distribution of mitochondria in higher plants. However, the actin cytoskeleton has been implicated since disruption of actin polymerization affected the dispersal of mitochondria and



resulted in a biased distribution of mitochondria in the daughter cells (Sheahan et al. 2004).

One plant mitochondrial dynamics mutant has been identified with a grossly altered cellular distribution of mitochondria (Logan et al. 2003). Disruption of the *FRIENDLY* gene (*FMT*) results in the formation of large mitochondrial clusters of ten or hundreds of organelles, although some mitochondria remain apparently normally distributed as singletons throughout the cytoplasm (Logan et al. 2003). *FMT* is a conserved eukaryotic gene and disruption of orthologues in *Dictyostelium discoideum* (*cluA*) and *S. cerevisiae* (*CLU1*) also cause aberrant mitochondrial phenotypes (Zhu et al. 1997; Fields et al. 1998). In the *cluA*<sup>-</sup> mutant of *D. discoideum* the mitochondria cluster near the cell center (Zhu et al. 1997), while in the *S. cerevisiae* *clu1* mutant the mitochondrial tubules collapse to one side of the cell (Fields et al. 1998). It is not known how *FRIENDLY* or its orthologues are involved in the maintenance of normal mitochondrial cellular distribution.

## 2.7 Conclusions

Advances in light microscopy and genetic techniques over the last two decades have allowed us to gain an unprecedented insight into mitochondrial dynamics. Over this time, we have uncovered many proteins that control mitochondrial shape, size, and position in the cell, allowing us to formulate models of the mechanisms involved. However, as each year passes, another new fission or fusion protein seems to be discovered, indicating that we are far from having the complete picture of what controls mitochondrial dynamics. This is particularly true in plants, where the combination of a more complex wild-type chondriome structure, and a smaller research community, has limited the advances made into understanding mitochondrial dynamics relative to animals and yeast. However, rather being a negative, this should be seen as a future opportunity to discover a uniquely plant-based cell biology system. The discovery of *NETWORK1/ELMI* as a likely mediator of mitochondrial fission, along with the lack of plant fusion protein homologues, suggests that plants have evolved a mitochondrial dynamics system that is distinct from that found in any other kingdom. The quest for the future, therefore, is threefold: (1) to identify the other plant-specific members of the mitochondrial dynamics machinery; (2) to discover how these proteins interact to control mitochondrial form and function; and (3) to uncover why plants evolved such a different dynamics system from the other extant chondriate eukaryotes. As such, it is clear that we still have much to learn about these crucial organelles.

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## Glossary

**Chondriome:** All the mitochondria in a cell, collectively.

**Chondrome:** The mitochondrial genome.

**Mitochondrial dynamics:** Study of the shape, size, number, motility, cellular distribution and cellular inheritance of mitochondria.

**Mitochondrial division:** Division of parental organelle to form two or more daughter organelles.

**Mitochondrial fusion:** Fusion of physically discrete mitochondria to form single organelle.

**Dynamin:** Mechanoenzyme that uses the energy from the hydrolysis of GTP to sever membranes.

**Green fluorescent protein:** One of two photoproteins (the other being aequorin) that together are responsible for the characteristic luminescence emitted from the light organs in the rim of the bell of the jelly fish *Aequorea victoria*.

**Cytoskeleton:** Proteinaceous scaffold within the cytoplasm of cells.

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