Annu. Rev. Cell Dev. Biol. 1998. 14:265–303

MITOCHONDRIAL DYNAMICS IN YEAST

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KEY WORDS: organelle inheritance, membrane morphology, fusion, cytoskeleton, GTPase

ABSTRACT

Proteins that control mitochondrial dynamics in yeast are being identified at a rapid pace. These proteins include cytoskeletal elements that regulate organelle distribution and inheritance and several outer membrane proteins that are required to maintain the branched, mitochondrial reticulum. Interestingly, three of the high molecular weight GTPases encoded by the yeast genome are required for mitochondrial integrity and are potential regulators of mitochondrial branching, distribution, and membrane fusion. The recent finding that mtDNA mixing is restricted in the mitochondrial matrix has stimulated the hunt for the molecular machinery that anchors mitochondrial nucleoids in the organelle. Considering that many aspects of mitochondrial structure and behavior are strikingly similar in different cell types, the functional analyses of these yeast proteins should provide general insights into the mechanisms governing mitochondrial dynamics in all eukaryotes.

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1081-0706/98/1115-0265$08.00

INTRODUCTION

The mitochondrion is a complex organelle with a double membrane, its own genome, and a separate protein synthetic machinery. A variety of important cellular functions are carried out by the mitochondrial compartment including reactions of the tricarboxylic acid (TCA) cycle, oxidative phosphorylation and ATP production, and the biosynthesis of cellular metabolites including some amino acids and lipids (Attardi & Schatz 1988). Because these organelles cannot be synthesized de novo, pre-existing mitochondria must grow continuously throughout the cell cycle and be inherited by daughter cells during division (Birky 1983, Palade 1983, Yaffe 1991, Berger & Yaffe 1996, Warren & Wickner 1996). Several decades of research have established that mitochondrial growth requires the import of nuclear-encoded proteins from the cytoplasm and the synthesis of polypeptides encoded by the organelle genome. The incorporation of lipids and the replication of mtDNA are also essential features of mitochondrial biogenesis. Detailed information on the ultrastructure and biogenesis of mitochondria can be found in a number of excellent reviews (Tandler & Hoppel 1972, Stevens 1981, Wolstenholme & Fauron 1995, Lill & Neupert 1996, Nunnari & Walter 1996, Schatz 1996, Neupert 1997), and chapters on this topic are a standard feature of most cell biology textbooks. In contrast, investigators have only recently begun to analyze the molecular basis of mitochondrial dynamics in cells, specifically, mitochondrial shape and cellular

distribution, mitochondrial division and fusion, and mitochondrial inheritance during cell division.

The morphology and distribution of mitochondria are usually tailored to meet the specialized energy needs of a cell. In textbooks, mitochondria are commonly depicted as static, sausage-shaped structures scattered throughout the cytoplasm. However, in living cells the organelles often form a tubular network that changes shape and size frequently due to fission and fusion events (Bereiter-Hahn 1990, Bereiter-Hahn & Voth 1994). In some organisms, these fusion events are developmentally regulated and serve to remodel the mitochondrial compartment as cells differentiate. One of the most dramatic examples occurs during spermatogenesis in insects where mitochondrial fusion generates two giant organelles that are tightly wrapped around the base of the rapidly beating flagellum (Fuller 1993). Mitochondria in other cell types are also localized near structures that consume large amounts of ATP. In muscle cells, for example, mitochondria stacked in columns between actin-myosin bundles generate the energy required for muscle contraction (Bakeeva et al 1978). Similarly, nerve cell mitochondria are found clustered at the presynaptic membrane where they provide the ATP required for neurotransmitter release and neurosecretory vesicle recycling and reloading (Landau & Kwanbunbumpen 1969). The cytoskeleton plays a key role in determining these different mitochondrial morphologies and distributions and is also required for polarized mitochondrial movements and mitochondrial inheritance in most cell types (Bereiter-Hahn & Voth 1994, Hollenbeck 1996, Simon & Pon 1996).

Due to its many experimental advantages, the budding yeast *Saccharomyces cerevisiae* has emerged as a favorite model system to study mitochondrial dynamics. Interest has been fueled by the growing awareness that many aspects of mitochondrial behavior in yeast are similar to those observed in other cell types. Genetic screens carried out by a number of groups have identified a set of proteins required for mitochondrial morphology maintenance and mitochondrial inheritance during mitotic division. In addition, in vitro assays that reconstitute aspects of mitochondrial motility have been developed, setting the stage for a biochemical dissection of this process. This review summarizes what has been learned recently about the molecular components that play a role in yeast mitochondrial dynamics.

MITOCHONDRIAL MORPHOLOGY AND DISTRIBUTION THROUGHOUT THE YEAST LIFE CYCLE

Detailed descriptions of the *S. cerevisiae* life cycle and mitochondrial ultrastructure have been published elsewhere (Stevens 1981, Pon & Schatz 1991). Below

we review the relevant features of mitochondrial morphology and behavior in yeast during mitosis, mating and meiosis, and sporulation.

Mitosis

The electron microscope was used in early studies to examine yeast mitochondrial ultrastructure and morphology after fixation and thin sectioning (Stevens 1977, 1981). More recently, investigators have begun to use a number of vital dyes that preferentially accumulate in actively respiring organelles as well as targeted forms of the green fluorescent protein (GFP) (Bereiter-Hahn 1976, 1990, Johnson et al 1980, Chen 1989, Koning et al 1993, Nunnari et al 1997). In mitotically dividing cells, actively respiring mitochondria appear as a highly branched, tubular network located near the cell periphery (Hoffman & Avers 1973, Stevens 1981). Within this network, mitochondrial DNA (mtDNA nucleoids) stained with the DNA-specific dye DAPI (Williamson & Fennell 1979), are visualized as bright spots distributed at widely spaced intervals. Although it sometimes appears as if there is a single, continuous mitochondrial compartment in yeast cells, the actual number of mitochondria can range from one to ten because the organelles frequently fuse and divide (Stevens 1981, Koning et al 1993, Nunnari et al 1997).

Yeast cells can survive without their mtDNA, which encodes gene products required for mitochondrial protein synthesis, electron transport, and oxidative phosphorylation. However, other metabolic functions that occur in the mitochondrial compartment such as reactions of the TCA cycle and amino acid and lipid biosynthesis are essential (Kovacova et al 1968, Gbelska et al 1983, Yaffe & Schatz 1984). As a consequence, yeast buds can only survive if they inherit part of the mitochondrial network from the mother cell during division. Mitochondrial inheritance begins early in the cell cycle (late G1/early S phase) when a portion of the network extends into the developing daughter cell or bud (Figure 1*A*) (Stevens 1981, McConnell et al 1990, Simon et al 1997). As the bud grows (S/G2 phase), additional mitochondrial membranes are transferred in from the mother cell. Mitochondria are reported to move in a linear and polarized fashion during this period (Simon et al 1995, 1997). A transient clustering of mitochondria at the bud tip is also observed (Simon et al 1997), suggesting that mitochondria can be captured and immobilized immediately after transfer to prevent their accidental return to the mother cell. Prior to cytokinesis, these immobilized mitochondria are redistributed throughout the bud.

Mating

Haploid yeast cells exposed to mating pheromone develop mating projections, adhere to one another, and ultimately fuse to form a dumbbell-shaped zygote (Figure 1*B*) (Sprague & Thorner 1994). Prior to (or concomitant with) nuclear

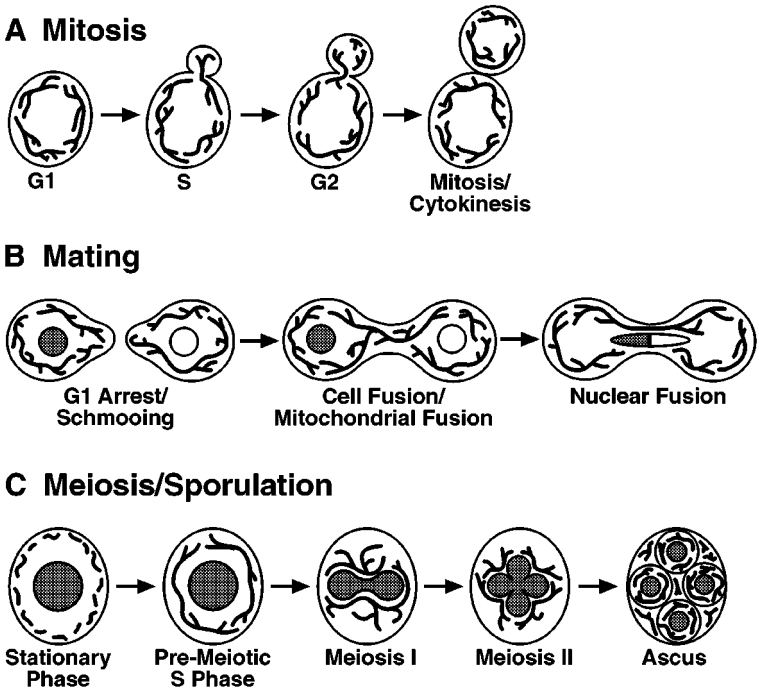

Figure 1 Mitochondrial dynamics during the life cycle of *S. cerevisiae.* (*A*) Mitochondrial morphology and inheritance during mitotic cell division. Mitochondria are located near the cortex of the cell as a branched tubular network. They are partitioned continuously from the mother cell into the bud from early in S phase until cytokinesis. (*B*) Mitochondrial fusion during mating. Haploid yeast cells of different mating types form a diploid zygote by cellular and nuclear fusion. Prior to nuclear fusion, the parental mitochondrial networks in the zygote fuse to form one interconnected organelle. (*C*) Mitochondrial morphology and inheritance during meiosis and sporulation. Meiosis and sporulation in diploid yeast produces four haploid daughter cells enclosed within the mother cell (an ascus). Individual mitochondrial compartments in pre-meiotic stationary phase are dispersed at the cell cortex. In pre-meiotic S phase, these individual mitochondria fuse to form one large branched network. During meiotic nuclear divisions, the mitochondrial membrane remains closely associated with the nucleus. This association leads to the incorporation of part of the mitochondrial network into newly formed spores.

fusion, mitochondrial compartments derived from each parent rapidly fuse in the zygote, creating a single, continuous network (Azpiroz & Butow 1993, Nunnari et al 1997). This mitochondrial fusion is accompanied by the complete mixing and redistribution of most mitochondrial components throughout the network. As a consequence of this mitochondrial fusion and compartment mixing, buds produced from any position on the zygote inherit mitochondrial networks of fairly uniform membrane and protein composition.

Meiosis and Sporulation

The most dramatic changes in mitochondrial distribution and morphology occur during meiosis and sporulation in *S. cerevisiae* (Figure 1*C*). Mitochondria in pre-meiotic cells appear as punctate structures dispersed at the cell cortex (Miyakawa et al 1984, Smith et al 1995). By early prophase, these discrete units have fused to form a tubular reticulum. The mtDNA nucleoids within this reticulum are highly condensed and resemble a series of beads on a string when stained with DAPI (Miyakawa et al 1984). The mitochondrial reticulum next migrates to the cell center where it remains associated with the nuclear membrane during the first and second meiotic divisions. At the end of meiosis II, four discrete mitochondrial tubules remain, each one located near an individual nuclear lobe. This intimate association of the mitochondrial and nuclear membranes ensures that mitochondria are included when each nuclear lobe is enclosed by the prospore cell membrane.

APPROACHES TO THE STUDY OF MITOCHONDRIAL DYNAMICS

Morphological screens are being used very successfully to identify genes required for Mitochondrial Distribution and Morphology (MDM) and Mitochondrial Morphology Maintenance (MMM) (McConnell et al 1990, Burgess et al 1994, Hermann et al 1997). By shifting temperature-sensitive yeast strains to 37°C and staining with fluorescent dyes to visualize the mitochondrial compartment, mutations have been isolated that fall into several different classes including (*a*) mutations that block mitochondrial inheritance but do not affect mitochondrial morphology, (*b*) mutations that alter mitochondrial morphology and block mitochondrial inheritance, and (*c*) mutations that alter mitochondrial morphology but do not block mitochondrial inheritance. The defects in most of the mutants appear to be specific for mitochondria and do not affect the morphology or inheritance of other cytoplasmic organelles that have been examined (e.g. nuclei and vacuoles). Independent selections for genes affecting Mitochondrial Genome Maintenance (MGM) (Jones & Fangman 1992) and Yeast Mitochondrial Escape (YME) (Thorsness & Fox 1993) have also yielded genes required for the maintenance of mitochondrial morphology and inheritance. Table 1 lists the published genes identified by the approaches cited above that are discussed in this review.

Cell-free assays that recapitulate mitochondrial behaviors in vivo are also being used to understand the mechanisms regulating yeast mitochondrial dynamics. To date, these assays focus exclusively on the interactions of yeast mitochondria with actin filaments and actin-based mitochondrial motility (see below).

ROLE OF THE CYTOSKELETON IN MITOCHONDRIAL DISTRIBUTION AND INHERITANCE

Studies in a wide variety of cell types indicate that mitochondria colocalize with microtubules (Heggeness et al 1978, Ball & Singer 1982, Summerhayes et al 1983), actin microfilaments (Drubin et al 1993), and intermediate filaments (Hirokawa 1982, Mose-Larsen et al 1982, Summerhayes et al 1983, Stromer & Bendayan 1990) and are transported through the cytoplasm using both microtubule-based (Nangaku et al 1994, Morris & Hollenbeck 1995, Pereira et al 1997, Hirokawa 1998) and actin-based (Kuznetsov et al 1992, Morris & Hollenbeck 1995, Simon et al 1995, Bearer et al 1996, Hollenbeck 1996) motor activities. To date, only the actin and intermediate filament cytoskeletons have been implicated in mitochondrial distribution and inheritance in *S. cerevisiae.* Current work focuses on understanding how these two cytoskeletal systems function at the molecular level to control different mitochondrial behaviors.

Microtubules

Although microtubules play a direct role in organelle motility and distribution in many eukaryotic cell types, they do not appear to participate in mitochondrial dynamics in *S. cerevisiae.* Disruption of the yeast microtubule network with mutations in β tubulin (Huffaker et al 1987) or with microtubule destabilizing drugs (Jacobs et al 1988) has no effect on mitochondrial morphology or mitochondrial transmission to daughter cells. These results are not surprising given that *S. cerevisiae* lacks an extensive cytoplasmic microtubule array and that mitochondria do not colocalize with microtubules in budding yeast (Adams & Pringle 1984, Kilmartin & Adams 1984). Microtubules also appear to be dispensable for mitochondrial movements in the filamentous fungi *Aspergillus nidulans* (Oakley & Reinhart 1985).

Cytoplasmic microtubules are required, however, for mitochondrial distribution in the fission yeast *Schizosaccharomyces pombe* (Yaffe et al 1996). Fission yeast contain a cortical network of tubular mitochondria similar to that observed in budding yeast (Davison & Garland 1977). Disruption of the *S. pombe* microtubule cytoskeleton with mutations in α or β tubulin, or with drugs that destabilize microtubules, results in clumped and unequally distributed mitochondria (Yaffe et al 1996). This effect on mitochondrial morphology is likely to be direct, since mitochondria partially colocalize with cytoplasmic microtubules throughout interphase in *S. pombe* cells. Kinesin-like motors that transport mitochondria on microtubules and/or regulate mitochondrial distribution in vivo have been identified in mammals and flies (Nangaku et al 1994, Pereira et al 1997). Thus the molecular mechanisms controlling mitochondrial distribution

Table 1 Genes involved in mitochondrial inheritance and morphology in yeast

Gene	Amino acids	Mutant phenotype	Properties	Reference
ACT1	375 aa (42 kDa)	Some mutant alleles have clumped mitochondria and exhibit defects in mitochondrial transfer to buds	Conventional actin	(Drubin et al 1993) (Lazzarino et al 1994)
CLU1	1277 aa (145 kDa)	Partial collapse of mitochondrial reticulum	Homologous to *cluA* in *Dictyostelium*	(Zhu et al 1997) (Fields et al 1998)
DNM1/ MDM29	757 aa (85 kDa)	Mitochondria collapse into one large elongated structure	Dynamin-like GTPase; localized to punctate structures along mitochondria	(Gammie et al 1995) (D Otsuga et al, submitted)
FZO1	855 aa (98 kDa)	Collapsed mitochondria; loss of mtDNA	Predicted GTPase; outer mitochondrial membrane protein required for mitochondrial fusion	(Hales & Fuller 1997) (GJ Hermann et al, submitted)
MDM1	443 aa (51 kDa)	Fragmented mitochondrion; defective transfer of mitochondria and/or nuclei into buds	Vimentin-like protein; forms 10-nm filaments in vitro; protein is localized to punctate spots in the cytoplasm	(McConnell et al 1990) (McConnell & Yaffe 1992) (McConnell & Yaffe 1993) (Fisk & Yaffe 1997)
MDM10	493 aa (56 kDa)	Large spherical mitochondria; defective transfer of mitochondria into buds; increased loss of mtDNA	Novel outer mitochondrial membrane protein	(Sogo & Yaffe 1994)
MDM12	271 aa (31 kDa)	Large spherical mitochondria; defective transfer of mitochondria into buds; increased loss of mtDNA	Novel outer mitochondrial membrane protein	(Berger et al 1997)

MDM20	796 aa (93 kDa)	Normal mitochondrial morphology; defective transfer of mitochondria into buds; loss of actin cables	Novel protein; potential heptad repeats	(Hermann et al 1997)
MGM1/ MDM17	912 aa (101 kDa)	Aggregated mitochondria; defective transfer of mitochondria into buds; loss of mtDNA	Dynamin-like GTPase; outer mitochondrial membrane protein	(Jones & Fangman 1992) (Guan et al 1993) (KA Shephard & MP Yaffe, unpublished data) (SW Gorsich & JS Shaw, unpublished data)
MMM1/ YME6	426 aa (49 kDa)	Large spherical mitochondria; defective transfer of mitochondria into buds; loss of mtDNA	Novel outer mitochondrial membrane protein	(Burgess et al 1994) (Thorsness & Weber 1996)
OLE1/ MDM2	510 aa (58 kDa)	Aggregated mitochondria; defective transfer of mitochondria into buds	Δ9 fatty acid desaturase	(Stukey et al 1990) (Stewart & Yaffe 1991) (Kohlwein et al 1997)
PTC1/ MDM28	281 aa (32 kDa)	Delayed transfer of mitochondria into buds	Serine/threonine phosphatase	(van Zyl et al 1989) (Maeda et al 1993) (Roeder et al 1998)
YME1	747 aa (82 kDa)	Clumped and swollen mitochondria; increased escape of mtDNA to the nucleus	ATP and zinc-dependent protease; inner mitochondrial membrane protein	(Thorsness et al 1993) (Campbell et al 1994) (Weber et al 1996)

in *S. pombe* may be more similar to those found in mammals and flies than in other fungi. To resolve this issue, it will be important to determine whether the actin and intermediate filament cytoskeletons also contribute to mitochondrial positioning, morphology, and motility in *S. pombe* as they do in *S. cerevisiae*.

Intermediate Filaments

MDM1 Genetic evidence that intermediate filaments are required for mitochondrial morphology and inheritance comes from studies of the *S. cerevisiae MDM1* gene (McConnell et al 1990). Mutations in *MDM1* cause temperature-sensitive growth defects, abnormal mitochondrial morphology (fragmentation and clumping), and block the transfer of both mitochondria and nuclei into growing buds. The *mdm1* effect on nuclear migration (the result of a misoriented mitotic spindle) is interesting because other mutations that prevent nuclear migration and division do not disrupt mitochondrial partitioning (Thomas & Botstein 1986, Huffaker et al 1988).

MDM1 encodes a protein with limited similarity to two mammalian intermediate filament proteins, keratin and vimentin (McConnell & Yaffe 1992). Like other intermediate filament proteins, the purified Mdm1 protein (Mdm1p) will self-assemble into 10-nm filaments in vitro (McConnell & Yaffe 1993). However, Mdm1p does not form a recognizable network of cytoplasmic filaments in vivo. Instead, Mdm1p is found in abundant punctate structures distributed throughout the mother and bud cytoplasms (McConnell & Yaffe 1992). These structures are distinct from actin microfilaments and microtubules and do not exclusively colocalize with mitochondria, nuclei, or any other cytoplasmic organelle in wild-type cells. Nevertheless, defects in mitochondrial and nuclear inheritance are correlated with the loss of these Mdm1p structures at the restrictive temperature in the conditional *mdm1-1* mutant. Although the organization of these Mdm1p structures looks substantially different from intermediate filament networks found in other cell types, all the available evidence suggests that Mdm1p is a bona fide intermediate filament protein. For the purposes of this discussion, we refer to these yeast Mdm1p structures as the Mdm1p cytoskeleton.

Fisk & Yaffe recently isolated additional mutant alleles of *mdm1* that retain cytoplasmic Mdm1p cytoskeletons at all temperatures (Fisk & Yaffe 1997). These *mdm1* alleles can be grouped into different phenotypic classes, which demonstrate that the Mdm1p cytoskeleton performs separate functions in mitochondrial dynamics and nuclear segregation. Class I mutations disrupt both mitochondrial inheritance and nuclear segregation, class II mutations block only mitochondrial inheritance, and class III mutations block only nuclear segregation. These data are consistent with the idea that Mdm1p forms an intermediate filament cytoskeleton that binds multiple cytoplasmic structures. All the

mutants defective in mitochondrial inheritance contain small, round, clumped mitochondria instead of the tubular membrane reticulum found in wild-type cells. Thus it is unclear whether Mdm1p's primary role is in maintaining mitochondrial morphology, directing mitochondrial inheritance, or both. The mutations in *MDM1* affecting nuclear segregation fall into two categories: (*a*) those that do not segregate nuclei into daughter cells and (*b*) those that partition all of the nuclear DNA into the bud. The nuclear defects in these *mdm1* alleles resemble those reported for mutations that affect mitotic spindle positioning and may reveal a role for the Mdm1p network in binding and orienting spindle pole bodies (SPBs). Finally, when the different *mdm1* mutant alleles are combined, complex genetic interactions including synthetic effects, intragenic complementation, and intragenic suppression are observed. These results support the notion that Mdm1p functions in vivo in an oligomeric state.

Based on the similarity of the Mdm1p amino acid sequence to intermediate filament proteins, the ability of Mdm1p to form 10-nm filaments in vitro, and the effects of *mdm1* mutations on the distribution of two different cellular organelles, it seems likely that the Mdm1p spots visualized by indirect immunofluorescence experiments form a cytoskeletal framework distributed throughout the yeast cytoplasm. SPB binding to this Mdm1p cytoskeleton (directly or indirectly) could act to orient the mitotic spindle during cell division. Similarly, mitochondrial morphology and positioning in cells could be regulated by anchoring mitochondrial membranes or membrane-associated proteins to this Mdm1p cytoskeleton. It seems unlikely that the transport of mitochondria into daughter cells is mediated by a conventional motor protein that moves along Mdm1p filaments because intermediate filaments contain no intrinsic polarity, a feature required for directed motor movements along cytoskeletal structures (Fuchs & Weber 1994). In fact, intermediate filament-dependent motor proteins have not been identified in any cell type. Yaffe and colleagues have proposed an alternative model for Mdm1p-mediated mitochondrial motility that involves the sequential binding and release of mitochondria to generate a crawling or amoeboid-like movement along Mdm1p filament tracks (Berger & Yaffe 1996). A better understanding of Mdm1p network architecture in cells and of the nature of Mdm1p-organelle interactions should help to determine whether Mdm1p functions as a scaffold for mitochondrial binding or as a cytoskeletal track for mitochondrial movement.

Actin

The actin cytoskeleton has been shown to control mitochondrial positioning in a variety of cell types (Drubin et al 1993, Bereiter-Hahn & Voth 1994, Hollenbeck 1996), and it is now clear that mitochondria are transported along filamentous actin tracks in neuronal axons (Kuznetsov et al 1992, Morris & Hollenbeck

1995) and insect cells (Bradley & Satir 1979, Sturmer et al 1995). In yeast, genetic and biochemical studies suggest that the actin cytoskeleton mediates both mitochondrial positioning and mitochondrial transport.

ACT1 *S. cerevisiae* contains a single essential actin gene, *ACT1*, that participates in a wide variety of cellular processes (Shortle et al 1982, Botstein et al 1997, Winsor & Schiebel 1997). In vivo, filamentous actin (F-actin) is organized into cables (bundles of F-actin) and cortical patches (associated with the plasma membrane) that undergo cell cycle–regulated changes in distribution (Adams & Pringle 1984, Kilmartin & Adams 1984, Welch et al 1994). In mitotically dividing cells, actin cables are oriented parallel to the mother-bud axis and are well positioned to deliver membrane-bound organelles, such as mitochondria, to the daughter cell. In fact, indirect immunofluorescence studies have revealed a striking colocalization of elongated mitochondrial tubules along these actin cables throughout the mitotic cell cycle (Drubin et al 1993, Lazzarino et al 1994, Simon et al 1997, Roeder et al 1998).

Although mutations in the *ACT1* gene cause a variety of cellular phenotypes (Botstein et al 1997), only a subset of *act1* mutants display defects in mitochondrial morphology and inheritance. Drubin and coworkers first showed that certain *act1* mutants contain clumped and disorganized mitochondrial networks (Drubin et al 1993). Similar mitochondrial morphology defects, as well as mitochondrial inheritance defects, are observed in *act1-3* mutant cells (Lazzarino et al 1994). The mitochondrial inheritance defect in *act1-3* has been correlated with a lack of normal mitochondrial motility in time-lapse video microscopy studies (Simon et al 1995). *act1* mutants also alter mitochondrial morphology and motility during meiotic division in yeast (Smith et al 1995). Finally, defects in mitochondrial behavior have also been reported in wild-type yeast cells treated with the actin depolymerizing drug latrunculin A (Lat-A). Within minutes of Lat-A treatment, wild-type mitochondrial networks fragment and long-distance mitochondrial movements slow down and become less polarized (Boldogh et al 1998). When combined with the observation that yeast mitochondria align along actin cables in vivo, these results strongly suggest that actin cables provide a scaffold for the attachment and movement of mitochondrial membranes in yeast cells.

MITOCHONDRIAL MOTILITY AND ACTIN-DEPENDENT MOTORS Drubin and colleagues noted that the majority of *act1* mutants with amino acid substitutions under or near the myosin foot print exhibited defects in mitochondrial organization (Drubin et al 1993). This result was very exciting because it suggested that a myosin-like motor activity bound to the outer mitochondrial membrane might be responsible for transporting the organelle along actin filaments or

cables. Two assays developed by Pon and coworkers indicate that such actin-mitochondrial interactions can occur in vitro. The first is a sedimentation assay that reconstitutes the binding of isolated yeast mitochondria to phalloidin stabilized yeast F-actin (Lazzarino et al 1994). Mitochondrial binding to F-actin is saturable, ATP dependent, and reversible and requires at least two mitochondrial components: (*a*) a peripheral mitochondrial membrane protein(s) with ATP-sensitive actin-binding activity (called mitochondrial actin binding protein or mABP) and (*b*) an integral membrane protein component required for docking of mABP (Lazzarino et al 1994, Boldogh et al 1998). Two integral outer membrane proteins shown to control mitochondrial morphology in vivo, Mmm1p and Mdm10p (Burgess et al 1994, Sogo & Yaffe 1994), are required to dock mABP on the outer mitochondrial membrane (Boldogh et al 1998). These results have led to the suggestion that Mdm10p and Mmm1p act as mitochondrial receptors for mABP, which can, in turn, act as an adaptor to indirectly attach mitochondria to F-actin.

The second assay reconstitutes ATP-dependent, actin-filament sliding on the surface of immobilized yeast mitochondria (Simon et al 1995). In this assay, both the concentration of ATP required and the rate of actin filament sliding are consistent with the presence of a myosin-like motor activity on the outer mitochondrial surface. The *S. cerevisiae* genome encodes five genes, *MYO1–MYO5*, that exhibit sequence similarity to the myosin superfamily in the motor domain. Surprisingly, this myosin-like motor activity does not appear to be encoded by any of the five *S. cerevisiae* myosin genes; mitochondrial motility is unaffected in the single *myo1*, *myo2*, *myo3*, *myo4*, and *myo5* mutants and in double *myo2-myo4* and *myo3-myo5* mutants (Simon et al 1995, Goodson et al 1996). Although all combinations of myosin mutations have not been tested, these results raise the possibility that a novel type of actin-dependent motor mediates mitochondrial motility in yeast. It will be interesting to see whether the mABP activity identified in the mitochondrial sedimentation assay is responsible for this motor activity or if it contributes to the motor activity in some fashion.

MDM20 Additional genetic evidence that actin is required for mitochondrial inheritance in yeast comes from studies of the *mdm20* mutant (Hermann et al 1997). Cells lacking *MDM20* exhibit severe defects in mitochondrial inheritance but retain normal mitochondrial morphology. Although cortical actin patches are still present in *mdm20* cells, actin cables aligned along the mother bud axis cannot be detected in rhodamine-phalloidin staining experiments. Significantly, both the actin organization defects and the mitochondrial inheritance defects in *mdm20* can be suppressed by increasing the dose of *TPM1* or *TPM2*. *TPM1* and *TPM2* encode two different yeast tropomyosins that bind

directly to F-actin and promote the assembly and/or stabilization of actin filaments (Liu & Bretscher 1989, 1992, Drees et al 1995). In the *mdm20* mutant, extra copies of *TPM1* and *TPM2* suppress mitochondrial inheritance defects by partially restoring actin cables in the cytoplasm (Hermann et al 1997). When combined with the observations that mitochondria bind to actin filaments and move along actin filaments in vitro, these genetic studies strongly suggest that mitochondria are transported into yeast buds along actin filaments or cables in vivo.

Mutations in the *TPM1* gene also disrupt actin cables but not cortical actin patches (Liu & Bretscher 1989). Surprisingly, mitochondrial inheritance is not blocked when *TPM1* is disrupted in the strain background used for the *MDM20* studies (Hermann et al 1997). It is important to note that cells lacking actin cables may still contain individual actin filaments that are not detected by conventional staining methods. Thus cells lacking the Tpm1 protein might still contain unbundled actin filaments that are capable of supporting mitochondrial movement. In fact, Pon and coworkers have shown that the velocity of mitochondrial movement is normal in the *tpm1* mutant strain (Simon et al 1997). However, long-distance mitochondrial movement in *tpm1* cells is less linear and polarized and, as a consequence, the organelles appear to move more randomly and sometimes enter buds late. The nonlinear mitochondrial movement observed in these cells suggests that actin filaments are present in the *tpm1* mutant but are not completely polarized along the mother-bud axis.

If disorganized actin arrays can support mitochondrial inheritance in the *tpm1* mutant strain, why don't similar actin arrays support mitochondrial inheritance in the *mdm20* mutant? One attractive model is that actin filaments required for mitochondrial inheritance may be present in the *tpm1* mutant but absent in the *mdm20* mutant. According to this scenario, the *MDM20* gene product somehow acts in vivo to define actin-containing structures that participate in mitochondrial transport. Although the Mdm20 protein localizes to the cytoplasm, it does not contain any functional domains or structural motifs implicated in actin assembly or regulation (Hermann et al 1997). Additional studies are required to determine the biochemical activity of this novel protein and its role in regulating actin organization and mitochondrial transport.

COORDINATING MITOCHONDRIAL INHERITANCE WITH BUD EMERGENCE

PTC1/MDM28

Transfer of the mitochondrial network in wild-type yeast always begins immediately after bud emergence, suggesting that mitochondrial inheritance is tightly

linked to the cell cycle (Stevens et al 1981, McConnell et al 1990, Simon et al 1997). This cell cycle coordination is disrupted in the *mdm28* mutant resulting in a striking delay in mitochondrial inheritance (Roeder et al 1998). When mitochondrial distribution is examined in synchronized cultures after α-factor release, networks are not detected in new *mdm28* buds until they are greater than half the diameter of the mother cell. In contrast, mitochondrial networks are always detected in wild-type buds regardless of bud size. Surprisingly, the mitochondrial inheritance delay in *mdm28* does not cause a significant change in growth rate relative to wild-type on rich medium. This last observation suggests that the timing of mitochondrial inheritance in yeast is not critical as long as buds receive mitochondria prior to cytokinesis.

MDM28 is identical to *PTC1*, a gene encoding a serine/threonine phosphatase in the High Osmolarity Glycerol response (HOG) pathway (van Zyl et al 1989, Maeda et al 1993, 1994). The HOG pathway plays an important role in the yeast osmostress response and is composed of a signal transducer and a MAP kinase cascade that terminates with the Pbs2p and Hog1p kinases (Morgan et al 1995, Schultz et al 1995, Posas et al 1996). An increase in extracellular osmolarity results in the activation of the HOG kinase cascade and induces yeast to accumulate glycerol (Brewster et al 1993, Maeda et al 1994). Genetic evidence suggests that Ptc1p acts as a negative regulator of the Pbs2p and Hog1p kinases in this pathway (Maeda et al 1993, 1994). Consequently, cells lacking *PTC1* contain hyperactive Pbs2p and Hog1p kinases and accumulate elevated levels of intracellular glycerol (Jiang et al 1995).

Increasing the cytoplasmic glycerol concentration in wild-type cells does not cause a mitochondrial inheritance delay and thus is not responsible for the mitochondrial phenotype observed in the *ptc1* mutant (Roeder et al 1998). Although an increase in intracellular glycerol concentration can be accompanied by the transient disassembly and reassembly of the yeast actin cytoskeleton (Chowdhury et al 1992), actin organization appears wild-type in the *ptc1* mutant and is not responsible for the mitochondrial inheritance delay observed in these cells (Roeder et al 1998). In addition, epistasis experiments with *ptc1* and mutations in the HOG pathway kinases suggest that *PTC1* is not acting through the HOG pathway to control the timing of mitochondrial inheritance. These observations have led to the suggestion that *PTC1* acts either directly or through a different signaling pathway to affect the mitochondrial segregation machinery in the cell. Identification of the downstream targets of Ptc1p may reveal how the timing of mitochondrial inheritance is regulated.

Yeast cells may rely on more than one mechanism to coordinate mitochondrial inheritance with bud emergence. First, the molecular machinery that transports mitochondrial membranes into buds could be activated at (or immediately prior to) the onset of bud formation. According to this model, late activation of

the transport machinery would account for the mitochondrial inheritance delay observed in the *ptc1* mutant. Second, a portion of the mitochondrial network could attach to the incipient bud site in a cell cycle–regulated manner and be passively pulled into the expanding bud. In support of this model, mitochondria are reported to converge on the ring of actin patches that mark the incipient bud site (Simon et al 1997), although a physical attachment to this site has not been demonstrated. The observation that mitochondria can move into *ptc1* buds long after they first emerge (Roeder et al 1998) suggests that mitochondrial inheritance does not strictly require a physical link between the organelle and the incipient bud site.

Although *PTC1* is the first gene demonstrated to control the timing of mitochondrial transport to buds, a similar phenotype is reported for a strain carrying mutations in two different genes (*BRO1* and *CAF1*) (Nickas & Yaffe 1996). *BRO1* also encodes a component of a yeast signal transduction pathway (the PKC pathway), although its exact function in this pathway is not understood. A detailed analysis of mitochondrial behavior in the *BRO1* and *CAF1* single and double mutants should provide useful information regarding the link between the PKC signaling pathway and the temporal control of mitochondrial inheritance.

Mitochondrial Inheritance and Cell Cycle Checkpoint Controls

In all the mitochondrial inheritance and morphology mutants described to date, daughter cells that fail to receive a mitochondrial compartment do not separate from the mother cell (McConnell et al 1990, Burgess et al 1994, Sogo & Yaffe 1994, Berger et al 1997, Hermann et al 1997). This is somewhat surprising because in many cases a complete septum appears to form between the mother and bud, and buds can be released from the mother cell by digesting with enzymes that break down the cell wall (MP Yaffe, personal communication). This defect in bud separation does not block progression through the cell cycle, and mitochondrial inheritance mutants often accumulate multiple attached daughter cells, all lacking the organelle. Although it has been suggested that these cell separation defects represent a cytokinesis checkpoint that prevents bud release in the absence of mitchondrial inheritance, this model has not been tested by showing that the cytokinesis block is absent in mutants that abolish checkpoint control (Hartwell & Weinert 1989).

GENES REQUIRED FOR MITOCHONDRIAL MORPHOLOGY MAINTENANCE

The formation of the yeast mitochondrial reticulum is an amazing architectural feat. The compartment must be shaped into a series of elongated tubules that

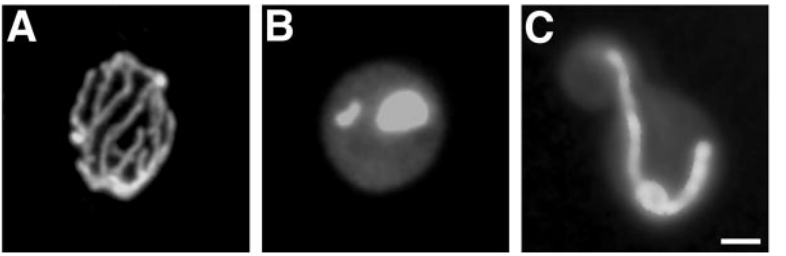

Figure 2 Mitochondrial morphology in wild-type and mutant *S. cerevisiae* cells. Mitochondrial morphology in (*A*) wild-type, (*B*) *mdm12*, and (*C*) *dnm1* cells was visualized using a matrix-targeted form of the green fluorescent protein. The cells shown in (*A*) and (*B*) are unbudded. In (*C*), the bud is in the upper left. Bar = 2 μm.

have the capacity to divide and fuse with one another. In addition, the entire structure must be localized to the peripheral cytoplasm and must be distributed evenly around the circumference of the cell (see Figure 2*A*). As described below, the analysis of mutants with defective mitochondrial morphology has begun to identify molecules required to maintain this extended reticulum. These studies indicate that changes in wild-type mitochondrial morphology can have dramatic affects on the motility and inheritance of the organelle and on mitochondrial genome maintenance.

MMM1

Jensen and coworkers identified the *MMM1* gene in a morphological screen for temperature-sensitive mutations affecting mitochondrial morphology (Burgess et al 1994). In strains harboring the *mmm1-1* allele, a shift to the nonpermissive temperature rapidly (and reversibly) transforms the mitochondrial network into one or a few large, spherical organelles (similar to those shown in Figure 2*B*). Despite their abnormal morphology, the *mmm1* mitochondrial spheres retain typical features of organelle ultrastructure including an outer membrane and an inner membrane, with deep invaginations or cristae. Defects in *mmm1* mitochondrial function occur only after prolonged incubation at the nonpermissive temperature and appear to be a secondary consequence of the change in mitochondrial morphology.

MMM1 encodes a mitochondrial outer membrane protein with a single transmembrane domain and a large, C-terminal domain facing the cytoplasm (Burgess et al 1994). Cells lacking this protein contain large, spherical mitochondria at all temperatures, grow slowly on the fermentable carbon source glucose, and are inviable on nonfermentable carbon sources such as glycerol. These growth phenotypes appear to result from a severe defect in mitochondrial inheritance. Jensen and coworkers suggested that this mitochondrial inheritance

defect results from the inability of giant *mmm1* mitochondria to fit through the mother-bud neck, effectively preventing their delivery to daughter cells (Burgess et al 1994). Although the inheritance of these large mitochondria is also blocked in glucose-grown *mmm1* cells, these cells survive because they also contain a number of small mitochondrial compartments that are efficiently transmitted to buds.

MDM10

MDM10 was identified by Yaffe and coworkers in a screen for mitochondrial distribution and morphology mutants (Sogo & Yaffe 1994). The novel protein encoded by this gene is also anchored in the mitochondrial outer membrane. Null mutations in *MDM10* result in temperature-sensitive growth on glucose, collapse of the mitochondrial network to form giant spheres, and defects in mitochondrial inheritance at all temperatures. In addition, *mdm10* null strains exhibit an increased frequency of petite generation, which is usually associated with deletions in mtDNA or its loss (Berger et al 1997). Unlike *mmm1* mutant strains, *mdm10* null cells do grow, albeit extremely slowly, on nonfermentable carbon sources (Sogo & Yaffe 1994). The ability of *mdm10* cells to proliferate under some conditions is presumably due to the inheritance of smaller mitochondrial compartments, which are also observed in these cells.

A *MDM10* homologue was recently identified in the filamentous fungi *Podospora anserina* (*PaMDM10*, 35.9% identity) (Jamet-Vierny et al 1997). *P. anserina* has captured the interest of scientists studying cellular aging because it undergoes a programmed senescence correlated with changes in the structure of the mitochondrial genome. Like the *mdm10* mutation in budding yeast, a mutation in *PaMDM10* converts most of the normal tubular mitochondria into giant spherical, oval, or tadpole-shaped organelles (Jamet-Vierny et al 1997). The change in mitochondrial morphology is associated with increased thermosensitivity and a mating type-specific decrease in lifespan. In addition, two deleted forms of the *P. anserina* mtDNA reproducibly accumulate during the senescence program of the *PaMDM10* mutant strain. The similar phenotypes observed for *mdm10* mutations in budding yeast and filamentous fungi underscore the importance of mitochondrial compartment integrity to mitochondrial genome stability.

MDM12

MDM12 encodes a third mitochondrial outer membrane protein required for mitochondrial morphology maintenance and mitochondrial inheritance (Berger et al 1997). Cells lacking this protein are temperature-sensitive for growth and contain giant mitochondrial spheres similar to those observed in the *mdm10* and *mmm1* mutant strains (Figure 2*B*).

The fission yeast *S. pombe* contains a *MDM12* homologue (32% identity) whose function in mitochondrial dynamics is at least partially conserved (Berger et al 1997). Although the *S. pombe MDM12* does not rescue mitochondrial phenotypes in the *S. cerevisiae mdm12* mutant, it does induce dominant mitochondrial morphology and inheritance defects in wild-type *S. cerevisiae* cells that are similar to those observed in the *S. cerevisiae mdm12* mutant. Thus proteins required for mitochondrial morphology and inheritance may be conserved between fission and budding yeast even though different cytoskeletal structures may control mitochondrial behavior in the two species.

As described above, the *mmm1*, *mdm10*, and *mdm12* strains all survive under certain growth conditions, suggesting that some mitochondrial inheritance is occurring in these strains. This inheritance is restricted to very small mitochondrial compartments that appear to bud from the giant organelles found in these cells (Burgess et al 1994). The simplest explanation for these results is that it is the size, and not the molecular composition, of giant mitochondria that prevents their transfer to daughter cells. A mutation in a suppressor designated *SOT1* (suppressor of *mdm10/mdm12*; *SOT1* is dominant) restores wild-type mitochondrial morphology and mitochondrial inheritance in *mdm10* and *mdm12* mutant cells (Berger et al 1997). Because *SOT1* does not exhibit any mitochondrial phenotype on its own, the mechanism by which it suppresses *mdm10* and *mdm12* phenotypes is uncertain. *SOT1* may act solely to restore a mitochondrial shape that is competent to interact with the inheritance machinery and thin enough to fit through the mother-bud neck. Alternatively, *SOT1* could activate or be part of a redundant cellular pathway that also transmits mitochondria to buds. Why such a bypass mechanism would also restore mitochondrial morphology is not clear.

Models for the functions of Mdm10p, Mdm12p, and Mmm1p are still highly speculative. It is possible that these proteins act within the organelle to maintain mitochondrial integrity and establish mitochondrial shape. In this case, defects in mitochondrial movement and inheritance would be an indirect consequence of changes in mitochondrial morphology. Alternatively, the cytoplasmic domains of Mdm10p, Mdm12p, and Mmm1p could act individually or in concert to mediate mitochondrial attachment to cytoplasmic structures including the cytoskeletal components Mdm1p and actin. Such interactions could be responsible for generating the elongated mitochondrial tubules and the directed mitochondrial movements observed in wild-type cells. Although an interaction between these outer membrane proteins and Mdm1p has not been demonstrated, a recent study indicates that a peripheral membrane component required for F-actin binding to mitochondria fails to assemble onto mitochondria isolated from *mmm1* and *mdm10* mutant cells (Boldogh et al 1998). Identification of protein-binding partners and/or the specific biochemical activities of

Mdm10p, Mdm12p, and Mmm1p should lead to a better understanding of their functions.

DNM1/MDM29

The mitochondrial morphology defect caused by the *mdm29* mutation is strikingly different from that observed in *mmm1*, *mdm10*, and *mdm12*. In the *mdm29* strain, the mitochondrial reticulum collapses to one side of the cell and forms an elongated structure that appears to function normally and is inherited by daughter cells during division (D Otsuga, BR Keegan, GJ Hermann, E Brisch, W Bleazard, JM Shaw, submitted) (Figure 2*C*). Unlike other mitochondrial distribution and morphology mutants that affect cell growth, *mdm29* null cells grow as well as wild-type at all temperatures and on all carbon sources.

MDM29 is identical to *DNM1* (Gammie et al 1995) and encodes one of three dynamin-related proteins in yeast. The dynamins are a family of high molecular weight GTPases implicated in membrane transport and remodeling events at distinct cellular locations (De Camilli et al 1995, Warnock & Schmid 1996, Schmid 1997, Urrutia et al 1997; A van der Bliek, submitted). Mammalian dynamin is the best characterized of the family members and has been shown to assemble into collars around the base of clathrin-coated pits (Hinshaw & Schmid 1995, Takei et al 1995). Subsequent GTP hydrolysis by dynamin in these collars stimulates a "pinchase" that releases endocytic vesicles (Sweitzer & Hinshaw 1998). Previous studies showed that mutations in *dnm1* do not block endocytosis in yeast (Gammie et al 1995). More recently, Shaw and coworkers showed that the controlled depletion of Dnm1p causes the gradual collapse of the mitochondrial network to one side of the cell, while re-expression of Dnm1p quickly restores wild-type mitochondrial distribution and morphology (D Otsuga, BR Keegan, GJ Hermann, E Brisch, W Bleazard, JM Shaw, submitted). These data strongly suggest that Dnm1p functions to spread the mitochondrial reticulum evenly around the cell periphery. Consistent with this model, the Dnm1 protein is found in punctate structures at the cell cortex that colocalize with the tips and sides of mitochondrial tubules and branchpoints in the mitochondrial network. Dnm1p appears to be bound either directly or indirectly to the mitochondrial compartment and remains associated with the collapsed, spherical mitochondria found in the *mdm10* and *mdm12* mutants.

Like other members of the dynamin family, the N-terminal domain of Dnm1p contains a conserved, tripartite GTP-binding motif (Gammie et al 1995). GTP binding and/or hydrolysis appears to be required for Dnm1p function in vivo because Dnm1 proteins containing mutations in this region fail to rescue mitochondrial distribution and morphology defects in the *dnm1* mutant (D Otsuga, BR Keegan, GJ Hermann, E Brisch, W Bleazard, JM Shaw, submitted). These mutant Dnm1 proteins also induce dominant mitochondrial distribution and

morphology defects in wild-type cells that can be rescued by overexpressing the wild-type Dnm1 protein. This last observation suggests that, like mammalian dynamin, Dnm1p associates with itself and/or with additional cellular components that help to establish mitochondrial distribution and morphology.

Exactly how the Dnm1 GTPase acts to control mitochondrial morphology and distribution is unclear. Dnm1p assembly on the mitochondrial membrane might regulate the formation of tubules or branches that are required to elaborate the network at the cell surface. Alternatively, Dnm1p could simply be part of a structure that anchors portions of the mitochondrial reticulum at the cell cortex.

MGM1/MDM17

MGM1 encodes a second dynamin-like GTPase implicated in mitochondrial dynamics (Jones & Fangman 1992, Guan et al 1993). Although this gene was first identified in a screen for mutations affecting mitochondrial genome maintenance (Jones & Fangman 1992), it was recently re-isolated as *MDM17* by Shepard & Yaffe (KA Shepard, MP Yaffe, submitted). Mitochondrial membranes in an *mgm1* disruption strain aggregate (Guan et al 1993) and are not efficiently transferred to buds (KA Shepard, MP Yaffe, submitted). The loss of mtDNA observed in this mutant strain appears to be a secondary consequence of the change in mitochondrial morphology (KA Shepard, MP Yaffe, submitted). In wild-type cells, the Mgm1 protein localizes to the mitochondrial compartment where it uniformly associates with the cytoplasmic face of the outer membrane (KA Shepard, MP Yaffe, submitted; SW Gorsich, JM Shaw, unpublished observations). Antibodies specific for Mgm1p recognize two forms of the protein present in equimolar amounts: a 100-kDa species that behaves as an integral membrane protein and a 90-kDa form that peripherally associates with the organelle (KA Shepard, MP Yaffe, submitted). Pulse-chase studies suggest that there is not a precursor/product relationship between these two Mgm1 polypeptides. The ability of the larger and smaller Mgm1p species to complement mitochondrial morphology and mtDNA-loss phenotypes will have to be assessed by independently expressing the two forms of the protein in *mgm1* cells. Studies are under way to determine whether the Mgm1p GTPase domain is required for function in vivo and whether Mgm1 assembles into rings on or near the mitochondrial outer membrane. Given how little we currently know about the biochemical activity of Mgm1p, roles for this protein in mitochondrial positioning, inheritance, branching, fission, and fusion are equally feasible.

CLU1

The story of *Saccharomyces CLU1* begins with the isolation of a novel, 150-kDa protein from *Dictyostelium* called CluA (Zhu et al 1997). CluA was initially identified based on its cross-reactivity with antibodies generated against an

unconventional myosin. However, analysis of the purified CluA protein and the polypeptide sequence predicted from the CluA gene revealed no myosin-like activities or structural features. Further analysis showed that CluA was required for mitochondrial positioning. Mitochondria in *Dictyostelium* cells are normally found dispersed uniformly throughout the cytoplasm. In *cluA*$^-$ cells, all the mitochondria were found clustered in the cell center. An increased frequency of multinucleate cells was also observed, suggesting that cytokinesis was defective in the *cluA*$^-$ mutant. Despite its obvious role in mitochondrial distribution, CluA behaved like a soluble protein and was not enriched in mitochondrial fractions.

The yeast *CLU1* gene encodes a functional homologue of the *Dictyostelium* CluA gene and can restore mitochondrial distribution in the *cluA*$^-$ mutant (Fields et al 1998). Genetic studies indicate that *CLU1* is also required for wild-type mitochondrial distribution in yeast. In *clu1* null cells, the mitochondrial reticulum appears less branched and is sometimes confined to one half of the cell cortex. Despite this change in distribution, *clu1* mitochondria appear to function normally and are inherited by buds during division. Although the partially collapsed mitochondrial morphology in *clu1* cells is not as severe as that observed in the *dnm1* mutant, the *clu1* and *dnm1* mitochondrial phenotypes are similar, suggesting that these two genes may act in a common pathway to control mitochondrial distribution and shape. The ability of Clu1p to complement mitochondrial distribution defects in the *Dictyostelium cluA*$^-$ mutant and the similarity of the yeast *clu1* and *Dictyostelium cluA*$^-$ mitochondrial phenotypes provide a striking demonstration of the degree to which cellular mechanisms governing mitochondrial dynamics have been conserved between species.

Maintenance of a Branched Mitochondrial Network

There is never a time during mitotic growth when mitochondrial morphology has to be established de novo. Nevertheless, the mutant mitochondrial morphologies described above suggest that a number of independent events are required to generate and/or maintain the yeast mitochondrial reticulum. A simple and highly speculative diagram of these events is shown in Figure 3. Mitochondria that have lost important cellular interactions (e.g. cytoskeletal attachment) collapse into spherical organelles, which can be viewed as the ground state (*mdm10, mdm12*, and *mmm1* strains). Attachment of these spherical organelles to cytoplasmic structures serves to stretch out or elongate the membranes and anchor them to the cell cortex. Candidates for these cytoplasmic attachment sites include cytoskeletal filaments composed of actin and the intermediate filament-like protein Mdm1. At some point before, during, or after these events, the mitochondrial double membrane must be shaped into long tubules that are joined to form a branched network or reticulum. Although our current understanding of this

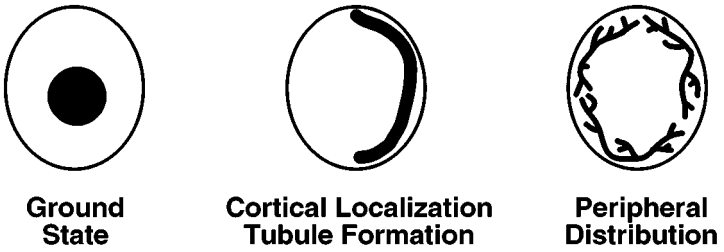

Figure 3 A highly speculative model for the maintenance of a branched mitochondrial network in *S. cerevisiae*. From mutant mitochondrial morphologies that have been observed, a number of independent events required to generate the mitochondrial reticulum can be envisioned. (*Left*) Mitochondria that have lost important cellular attachments collapse into giant spheres (ground state). (*Center*) Attachment of these organelles to cytoplasmic structures anchors them to the cortex and elongates the membranes. (*Right*) The branched reticulum (formed by an unknown mechanism) is distributed evenly around the cell periphery.

process is murky at best, it probably requires regulated membrane fission and fusion, as well as the activities of membrane-remodeling proteins (for example, Mgm1p and Dnm1p). Once the mitochondrial reticulum is formed, it must be distributed evenly in the peripheral cytoplasm. This could be accomplished by attaching the reticulum to Dnm1p-containing complexes, which appear to have the correct cellular distribution for this function. It is also possible that mitochondrial reticulum formation and peripheral distribution are obligatorily coupled. Although aspects of this model may prove to be incorrect, it includes a number of testable features and should provide a useful framework for the molecular, genetic, and biochemical analysis of mitochondrial architecture.

ROLE OF THE MITOCHONDRIAL MEMBRANE

OLE1/MDM2

Although it seems obvious that the lipid composition of the mitochondrial membrane is critical for organelle integrity and behavior, only one mutant, *mdm2*, reveals an essential role for lipids in mitochondrial dynamics. The *mdm2* mutant causes abnormal mitochondrial aggregation and defective mitochondrial inheritance (Stewart & Yaffe 1991). *MDM2* is allelic to *OLE1*, a gene previously shown to encode a $\Delta 9$ fatty acid desaturase (Stukey et al 1989, 1990). Mutations in *OLE1/MDM2* are thought to cause defects in mitochondrial morphology and inheritance by altering the level of unsaturated fatty acids in, and thus the physical properties of, mitochondrial membranes (Stewart & Yaffe 1991). Shifting the *mdm2-1* strain to the nonpermissive temperature results in a 2.5-fold decrease in unsaturated fatty acid levels and the fragmentation of the

mitochondrial network into smaller vesicles that aggregate within the mother cell (Kohlwein et al 1997). These mitochondrial fragments retain normal organellar ultrastructure and can still be labeled with potential-dependent dyes, suggesting that the integrity of the inner mitochondrial membrane is not grossly compromised (Stewart & Yaffe 1991). The mitochondrial phenotypes and the conditional growth defect in *ole1/mdm2* can be rescued by adding oleic acid to the growth medium, suggesting that it is the change in fatty acid levels and not some additional activity of the desaturase that is responsible for these phenotypes (Stewart & Yaffe 1991). Although fatty acids are important for membrane integrity throughout the cell, the membrane defect caused by *ole1/mdm2* has not been reported to affect the morphology or segregation of other cytoplasmic organelles.

Because cardiolipin is an abundant inner membrane component rich in unsaturated fatty acids (Hoch 1992, Kent 1995), it initially seemed possible that cardiolipin was the major lipid affected in *ole1/mdm2*. However, several groups have now reported the surprising finding that yeast cells lacking cardiolipin are viable on both fermentable and nonfermentable carbon sources (Jiang et al 1997, Tuller et al 1998). Based on these results, it seems likely that defects in a different mitochondrial lipid, or multiple classes of mitochondrial lipids, are responsible for the phenotypes observed in the *ole1/mdm2* mutant.

What is the role of unsaturated fatty acids in mitochondrial dynamics? One possibility is that changes in unsaturated fatty acid levels affect the activities of integral membrane proteins, which depend on specific lipid modifications or a specific bilayer composition. An alternative model proposed by Schneiter & Kohlwein is that changes in membrane composition lead to alterations in membrane curvature that are required to regulate fission and fusion (Schneiter & Kohlwein 1997). Tests of these models will require further in vivo analyses as well as in vitro assays that reconstitute various mitochondrial membrane-dependent behaviors.

MAINTENANCE OF MITOCHONDRIAL DNA

The *S. cerevisiae* mitochondrial genome encodes RNAs and proteins essential for mitochondrial translation, electron transport, and ATP synthesis (Dujon 1981, Pon & Schatz 1991). Yeast cells contain 50–100 copies of mtDNA, which are packaged together with proteins into structures referred to as nucleoids (Williamson & Fennell 1979). Although yeast buds inherit mtDNA along with the mitochondrial compartment during mitosis, mtDNA segregation does not occur randomly. Instead, it is hypothesized that a segregation apparatus controls the movement of mtDNA nucleoids relative to other mitochondrial constituents (Azpiroz & Butow 1993, Nunnari et al 1997).

Early studies of zygotic first buds (pedigree analysis) indicated that mtDNA movement was restricted in yeast. When haploid cells containing genetically marked mtDNAs were mated, recombinant mitochondrial genomes were found exclusively in buds produced from the zygote neck (heteroplasmic buds) (Birky et al 1978, Strausberg & Perlman 1978, Zinn et al 1987). In contrast, buds produced from the ends of the zygote usually contained only one type of mtDNA derived from the closest parent (homoplasmic buds). These results suggested that mitochondrial fusion and genome mixing occurred during mating, but that mtDNA mixing was limited to the medial portion of the zygote. This nonrandom mtDNA segregation pattern was also apparent under conditions where other mitochondrial matrix components, specifically the soluble matrix enzyme citrate synthase 1 (CS1), redistributed freely in the zygote (Azpiroz & Butow 1993). A demonstration of restricted mtDNA movement was recently provided by Nunnari and coworkers who devised a method of labeling the mtDNA of one haploid parent with 5-bromodeoxyuridine (Nunnari et al 1997). After mating, these labeled mitochondrial nucleoids remained in one half of the zygote even after mitochondrial fusion and matrix component mixing had occurred. This result provided a direct explanation for the nonrandom mtDNA inheritance patterns observed in yeast zygotes and suggested that mtDNA nucleoids are (*a*) anchored in the mitochondrial matrix (perhaps to the inner membrane) and (*b*) actively segregated during division. Interestingly, mtDNA movement in zygotes does not appear to be restricted under all conditions. When haploid parents with (rho^+) and without (rho^o) mtDNA are mated, 100% of the progeny inherit mtDNA (Dujon 1981). Azpiroz & Butow showed that this mtDNA inheritance results because mitochondrial nucleoids can equilibrate freely throughout zygotes formed by mating rho^+ and rho^o cells ($rho^+ \times rho^o$) (Azpiroz & Butow 1993). Although the mechanisms responsible for mtDNA segregation in wild-type and non-wild-type matings are not understood, the search for the molecular components that mediate this process is now under way.

Studies of altered mtDNA segregation patterns suggest that specific nucleotide sequences in mtDNA can promote mitochondrial genome inheritance. In rho^- strains, the majority of the mtDNA is deleted and the remaining mtDNA (usually a single short mtDNA sequence) is amplified, resulting in a mass of DNA equivalent to that observed in a rho^+ strain (Dujon 1981). Some of these amplified rho^- mtDNAs have been termed hypersuppressive because they are preferentially inherited (95% of the time) by zygotic buds in matings with rho^+ strains (Dujon 1981, Piskur 1994). Hypersuppressive rho^- mtDNAs usually contain tandem repeats of one of several 300-bp stretches called *rep* sequences (Blanc & Dujon 1980, De Zamaroczy et al 1981). The enhanced inheritance of rho^- mtDNAs in crosses indicates that these *rep* sequences may be preferentially replicated or segregated into daughter cells.

A number of nuclear genes required for the stable maintenance of mtDNA have been identified. Mutations in some of these genes lead to defects in mitochondrial morphology (*FZO1, MDM10, MDM12, MGM1*) and, in some cases, it has been shown that the loss of mtDNA nucleoids in these strains is a secondary consequence of changes in mitochondrial shape (GJ Hermann, JW Thatcher, JP Mills, KG Hales, MT Fuller, J Nunnari, JM Shaw, submitted; KA Shepard, MP Yaffe, submitted). Other genes are required for mtDNA replication, repair, and recombination, or for mitochondrial genome expression (Costanzo & Fox 1990, Schmitt & Clayton 1993, Fox 1996, Shadel & Clayton 1997). Two genes, *MGT1* and *ABF2*, are of particular interest because their roles in mtDNA segregation have been explored in some detail.

MGT1

The *MGT1* (Mitochondrial Genome Transmission) gene was identified in a screen for mutations that restored normal mtDNA segregation patterns in crosses between rho$^+$ and hypersuppressive rho$^-$ strains (Zweifel & Fangman 1991). *MGT1* encodes a mitochondrial endonuclease that specifically cleaves Holliday junctions formed during recombination (Kleff et al 1992, Lockshon et al 1995). Although these Holliday junctions are not easily detected in the mtDNA of wild-type strains, cells lacking *MGT1* have elevated levels of these unresolved recombination intermediates (Lockshon et al 1995). The increased physical linkage between mitochondrial genomes in *mgt1* mutants causes the mtDNA to aggregate into fewer and larger nucleoids that, because of their decreased number, are less likely to be transmitted to daughter cells. In *mgt1*Δ hypersuppressive strains, rho$^-$ mtDNAs with tandemly repeated *rep* sequences undergo homologous recombination more frequently than rho$^+$ mtDNAs and, as a result, suffer a greater degree of genome crosslinking and aggregation. Thus the ability of *mgt1* mutations to restore normal mtDNA segregation patterns in crosses between rho$^+$ and hypersuppressive rho$^-$ strains is due to the fact that these aggregated rho$^-$ mtDNAs are inherited less efficiently. These results indicate that the nucleoid is the segregating unit in mitochondria and support the idea that recombination among mtDNAs plays an important role in the segregation of mitochondrial genomes.

ABF2

The abundant Abf2 protein is a member of the high mobility group (HMG) family of DNA-binding proteins and is associated with mtDNA nucleoids in vivo (Caron et al 1979; Diffley & Stillman 1991, 1992; Megraw & Chae 1993). Studies indicate that changes in Abf2p levels affect a variety of mtDNA phenotypes including mtDNA organization, maintenance, copy number, and recombination.

In *abf2* null cells, mtDNA nucleoids have altered protein profiles and increased DNase I sensitivities, indicating that Abf2p is important for mtDNA organization (Newman et al 1996). The level of Abf2p expression in cells has also been shown to be important for mtDNA stability. High levels of Abf2p expression result in mitochondrial genome loss (Megraw & Chae 1993, Zelenaya-Troitskaya et al 1998), and cells lacking Abf2p exhibit an increased frequency of mtDNA nucleoid loss when grown on the fermentable carbon source glucose at 30°C (Diffley & Stillman 1991, Megraw & Chae 1993). In contrast, *abf2* null cells can maintain mitochondrial genomes on the nonfermentable carbon source glycerol at 30°C, indicating that Abf2p is not essential for mtDNA replication, gene expression, or transmission to buds. However, when cells are grown on glycerol at elevated temperatures (37°C), Abf2p is essential for viability (Megraw & Chae 1993). Alterations in Abf2p levels also appear to affect mtDNA copy number; the mtDNA content is reduced by approximately 50% in *abf2* null cells maintained on glycerol and increased by 50 to 150% (relative to wild type) in cells containing several extra copies of the *ABF2* gene (Zelenaya-Troitskaya et al 1998). Finally, both the distribution of mtDNA nucleoids and the efficiency of mtDNA recombination are severely affected in crosses between parental strains lacking Abf2p (Zelenaya-Troitskaya et al 1998).

How does Abf2p affect mtDNA maintenance, copy number, and recombination in vivo? One possibility is that Abf2p serves a histone-like function in cells, and the loss of this protein causes pleiotropic defects in mtDNA-related behaviors. The abundance of Abf2p in mitochondria (approximately 1 molecule of Abf2p per 30 base pairs of mtDNA) (Diffley & Stillman 1992) and the observation that Abf2p has supercoiling activity (Caron et al 1979, Diffley & Stillman 1992) are consistent with this model. An alternative explanation is suggested by the recent reports that mtDNA replication may be a recombination-dependent process in yeast (Lockshon et al 1995, Bendich 1996). It is possible that altered Abf2p levels cause defects in mtDNA recombination that lead to secondary defects in mtDNA replication and genome maintenance. Several observations suggest a role for Abf2p in recombination. First, Abf2p is a HMG family member, and other HMG proteins have been shown to bind preferentially to cruciform DNA, which resembles Holliday junction intermediates formed during replication (Teo et al 1995). Second, a recent study showed that Abf2p promotes the formation or stabilization of Holliday recombination junctions in wild-type mtDNA (MacAlpine et al 1998). The level of these recombination intermediates appears to decrease in cells lacking Abf2p and to increase in cells overproducing Abf2p. Thus mitochondrial genome copy number and maintenance may depend on the formation of recombination structures that prime mtDNA replication.

ESCAPE OF MITOCHONDRIAL DNA TO THE NUCLEUS

Thorsness & Fox have isolated a number of mutations that increase the rate at which DNA is lost from the mitochondrion and transferred to the nucleus in yeast (Thorsness & Fox 1993). Three of these *YME* genes encode mitochondrial proteins and appear to be important for mitochondrial genome maintenance and/or mitochondrial integrity.

Mutations in *YME1* increase the rate of mtDNA escape and cause temperature-sensitive growth defects on both fermentable and nonfermentable carbon sources (Thorsness et al 1993). *YME1* encodes an ATP and zinc-dependent protease that is tightly associated with the inner mitochondrial membrane (Thorsness et al 1993, Weber et al 1996). The proteolytic domain of Yme1p is located in the intermembrane space and is required for the turnover of mitochondrial inner membrane proteins (Nakai et al 1995, Pearce & Sherman 1995, Leonhard et al 1996, Weber et al 1996). *yme1* mutant cells also contain fragmented, clumped, and swollen mitochondria that appear to be associated with proteolytic vacuoles (Campbell et al 1994). A second gene, *YME2*, also encodes a mitochondrial inner membrane protein exposed to the intermembrane space (Hanekamp & Thorsness 1996). Mutations in *YME2* can suppress the cold-sensitive growth defect of the *yme1* mutant and exhibit synthetic growth defects with *yme1* on nonfermentable carbon sources. A third gene, *YME6* (Thorsness & Weber 1996), is identical to the previously characterized mitochondrial morphology gene *MMM1* (see above) (Burgess et al 1994).

All three *YME* genes described above encode proteins localized to the mitochondrial compartment, and mutations in two of these genes appear to cause severe defects in mitochondrial morphology. Thus the increased rate of mtDNA loss/escape observed in these *yme* mutants may result from breaches in the mitochondrial compartment introduced by these morphology changes or by degradative processes that turn over defective mitochondria in these cells (Thorsness & Weber 1996). Regardless of the mechanism of DNA escape, the studies of yeast mitochondrial morphology mutants and these *yme* mutants indicate that mitochondrial genome maintenance depends heavily on the integrity of the mitochondrial compartment.

MITOCHONDRIAL FISSION AND FUSION

Over the past two decades, researchers have identified many of the molecules that regulate heterotypic fusion reactions (between unlike membranes) and homotypic fusion reactions (between like membranes) in yeast, mammalian cells, and neurons. These include the NSF (NEM sensitive factor) ATPase

and its related family members, SNAPs (soluble NSF attachment proteins); the cognate membrane-docking proteins called v-SNAREs and t-SNAREs (SNAP receptors); proteins such as Sec1p that regulate SNARE availability; and the Rab family of small GTPases, which contribute to the specificity and efficiency of the fusion reaction (Pfeffer 1996, Rothman 1996, Hay & Scheller 1997, Novick & Zerial 1997). In addition, there is now evidence that the dynamin GTPase regulates membrane division or fission at the cell surface (Sweitzer & Hinshaw 1998). Surprisingly, none of these molecules has been shown to control the fission or fusion of mitochondria in any cell type. Mitochondria are one of the few cellular organelles that have two distinct membranes. The dispensability of these molecules for mitochondrial membrane dynamics may indicate that a different type of molecular machinery is required to coordinate the behavior of these two membranes during division and fusion. Although we are far from understanding the molecular basis of these events, a potential regulator of mitochondrial fusion in flies and yeast was recently described, and two of the proteins implicated in yeast mitochondrial morphology maintenance share molecular features with the membrane fission protein dynamin.

Fission

In *S. cerevisiae*, mitochondrial fission events at branchpoints or within tubules generate new ends in the reticular network (Nunnari et al 1997). Membrane division or fission is also required to separate the mother and daughter mitochondrial networks during cytokinesis. Although division might be accomplished by simply pulling on opposite ends of a mitochondrial tubule, an alternative possibility is that a protein (or proteins) assembled on the cytoplasmic face of the mitochondrion constricts and pinches the compartment into two pieces. This is the mechanism by which the dynamin GTPase catalyzes the release of clathrin-coated vesicles from the plasma membrane (Sweitzer & Hinshaw 1998). The yeast *MGM1* and *DNM1* genes (described above) encode predicted GTPases that are structurally related to dynamin. Although mutations in these genes cause dramatic changes in mitochondrial morphology, it is not known if these changes result from defects in membrane fission or division. More work is required to determine the activities and topologies of the Dnm1 and Mgm1 proteins. In addition, other unidentified molecules may act from inside or outside the organelle to control mitochondrial division.

Fusion

Time-lapse analyses of fluorescently labeled mitochondrial networks suggest that membrane fission events are balanced by frequent fusion events (Nunnari et al 1997). As in other cell types, mitochondrial fusion in yeast appears to occur when a free mitochondrial end or tip encounters another mitochondrial

end or a tubule side (Bereiter-Hahn & Voth 1994, Nunnari et al 1997). These observations have led to the idea that components of the fusion machinery are either concentrated at, or specifically activated at, mitochondrial tips (Nunnari et al 1997). In both yeast and mammalian cells, fusion has been proposed to initiate at stable contact sites between the inner and outer mitochondrial membranes (Bereiter-Hahn & Voth 1994).

Studies of mitochondrial dynamics during yeast mating provide a clear demonstration of mitochondrial fusion. As described above, early genetic studies indicated that marked mtDNAs derived from different haploid parents recombined in yeast zygotes (Thomas & Wilkie 1968, Dujon 1981). This recombination could occur only if mitochondria from each parent fused in the zygote to allow mitochondrial genome mixing. Using indirect immunofluorescence techniques, Azpirov & Butow (1993) demonstrated that a matrix component present in one parent quickly redistributed throughout the diploid zygote. This result indicated that mitochondria derived from each parent were mixing in the zygote, but did not confirm that the two populations of mitochondria were actually fusing with one another. In a later study, Nunnari and colleagues (1997) used fluorescent tags of different colors to visualize the mitochondria of both haploid parents in living cells. After mating, these two mitochondrial markers rapidly redistributed and colocalized throughout zygotes, indicating that mitochondrial fusion had occurred. Because one of the fluorescent markers used in this study was localized to the mitochondrial matrix, mixing could only result if both the inner and outer mitochondrial membranes fused.

In crosses between wild-type yeast cells, the rate at which different mitochondrial constituents mix after fusion can vary depending on the type of molecule and its location in the organelle. As described above, soluble matrix proteins equilibrate rapidly throughout the zygote after mitochondrial fusion (Azpiroz & Butow 1993, Nunnari et al 1997). Butow and coworkers have examined the redistribution of GFP-tagged inner and outer mitochondrial membrane proteins in zygotes and find that these proteins equilibrate at a slower rate than matrix proteins throughout the fused mitochondrial network (Okamoto et al 1997). In contrast, mtDNA genomes behave as if they are anchored within the organelle and do not mix extensively in the zygote (Nunnari et al 1997). Interestingly, the sorting patterns of mitochondrial nucleoids and proteins can change dramatically when matings are performed with rho$^-$ or rho^o cells (Azpiroz & Butow 1993). The molecular basis for these changes in sorting patterns is not understood.

FZO1 The cloning by Hales & Fuller (1997) of the *Drosophila fuzzy onions* (*fzo*) gene represents a major leap forward for the mitochondrial fusion field. This gene encodes the first protein known to play a role in mitochondrial fusion

in any system. The Fzo protein defines a family of related molecules, one of which is clearly required for mitochondrial dynamics in *S. cerevisiae*.

During *Drosophila* spermatogenesis, mitochondria aggregate and fuse to form two giant organelles that are tightly coiled around one another, like the layers of an onion (Fuller 1993). In *fzo* mutant males, the individual mitochondria aggregate normally but fail to fuse, giving rise to structures that look more like fuzzy onions when viewed in cross section with the electron microscope (Hales & Fuller 1997). The *fzo* gene encodes an 81.5-kDa protein with a predicted GTPase domain near the N terminus, two closely spaced potential membrane spanning domains near the C terminus, and three predicted heptad repeats. The Fzo protein appears on spermatid mitochondria just as fusion begins and disappears soon after fusion is complete. The correlation between the timing of mitochondrial fusion and Fzo expression is striking and suggests that this protein could be the actual mediator of membrane fusion. The fly Fzo protein has homologues in yeast and nematodes and is ubiquitously expressed in mammalian tissues. Thus Fzo defines a new family of GTPases that may play a general role in regulating mitochondrial fusion, and/or other mitochondrial behaviors in cells. Recent studies of the yeast *fzo* homologue suggest that this is indeed the case.

The *S. cerevisiae fzo* gene, called yeast *FZO1* (*yFZO1*; 19% identity to *fzo*), also performs an essential role in mitochondrial fusion. Yeast cells lacking the Fzo1 protein contain multiple spherical or slightly elongated mitochondrial compartments that are inherited normally by buds but lose their mtDNA nucleoids (GJ Hermann, JW Thatcher, JP Mills, KG Hales, MT Fuller, J Nunnari, JM Shaw, submitted). Fzo1p depletion studies indicate that this defect in mitochondrial genome maintenance is a secondary consequence of changes in mitochondrial morphology. A conditional mutation in *FZO1* causes fragmentation of the mitochondrial network and blocks mitochondrial fusion during mating. These results indicate that the mitochondrial morphology changes in *fzo1* null cells result from defects in mitochondrial fusion. Like the fly Fzo protein, yeast Fzo1p is distributed over the length of the mitochondrion and behaves like an integral membrane protein. However, unlike *Drosophila* Fzo, which is expressed in a very narrow developmental window when mitochondrial fusion is occurring (Hales & Fuller 1997), yeast Fzo1p is present on mitochondria at relatively constant levels throughout the yeast life cycle (GJ Hermann, JW Thatcher, JP Mills, KG Hales, MT Fuller, J Nunnari, JM Shaw, submitted). This may explain why mitochondrial fusion events appear to occur continuously during mitotic growth. Protease protection studies indicate that the N-terminal GTPase domain of yeast Fzo1p is exposed to the cytoplasm where it is available to interact with other proteins and cellular structures that could regulate its activity and the behavior of the mitochondrial compartment.

The similarities between the fly and yeast Fzo proteins leave no doubt that the Fzo family of transmembrane GTPases plays an essential and conserved role in regulating mitochondrial dynamics.

Although the nucleotide hydrolyzing activities and binding preferences of fly and yeast Fzo have not been tested, mutations expected to alter these properties have been generated in the predicted GTPase domains of both proteins. These mutant proteins localize to mitochondrial membranes in their respective organisms, but fail to support mitochondrial fusion in *Drosophila* or rescue mitochondrial morphology defects in yeast (Hales & Fuller 1997; GJ Hermann, JW Thatcher, JP Mills, KG Hales, MT Fuller, J Nunnari, JM Shaw, submitted). Unlike the equivalent mutations in proteins of the ras and dynamin superfamilies, the yeast and fly *fzo* mutations do not produce dominant interfering phenotypes in vivo (Hales & Fuller 1997; GJ Hermann, JW Thatcher, JP Mills, KG Hales, MT Fuller, J Nunnari, JM Shaw, submitted). The latter result suggests that the mechanism of Fzo function may differ from that of other characterized GTPases.

The behavior of yeast mitochondria in vivo suggests that components of the fusion machinery are localized or activated at mitochondrial ends (Nunnari et al 1997). Yet, fusion of free ends often occurs with the sides of mitochondrial tubules, indicating that molecules mediating fusion are distributed throughout the organelle. Yeast Fzo1p covers the mitochondrial outer membrane with its predicted GTPase domain facing the cytoplasm (GJ Hermann, JW Thatcher, JP Mills, KG Hales, MT Fuller, J Nunnari, JM Shaw, submitted) and, as proposed by Hales & Fuller (1997), could perform a SNARE-like function to control mitochondrial-mitochondrial docking prior to fusion. In this context, GTP binding and/or hydrolysis by yeast Fzo1p might act to regulate mitochondrial docking just as Rab GTPase family members facilitate SNARE complex formation in other intracellular transport reactions (Pfeffer 1996, Novick & Zerial 1997). Alternatively, Fzo1p might be required for the fusion of mitochondrial membranes after docking has already occurred. Other roles for Fzo1p have not been excluded. For example, Fzo1p might regulate mitochondrial morphology in yeast by interacting with cytoskeletal components in the cytoplasm, or other mitochondrial membrane proteins. Both in vivo and in vitro studies are now under way to distinguish between these models for Fzo1 protein function.

PERSPECTIVES AND FUTURE DIRECTIONS

It is clear that mitochondrial morphology and inheritance are regulated in most cell types. In the past eight years, *S. cerevisiae* has emerged as a powerful experimental system to study different mitochondrial behaviors. Although we know

the identities of several genes that control mitochondrial shape and inheritance, additional genes and proteins will almost certainly be involved. Now that the sequence of the yeast genome is complete, the identification of these additional components should proceed quickly.

As is usually the case, what we have learned about mitochondrial dynamics has raised many new questions. What is the relationship between mitochondrial morphology and mitochondrial inheritance? What are the exact roles of the actin- and Mdm1-based cytoskeletons in maintaining mitochondrial morphology and directing mitochondrial partitioning? What is the identity of the myosin-like motor on the mitochondrial surface and is this the molecule that provides the force for directed mitochondrial movements during cell division? How are the distribution and copy number of mtDNA nucleoids maintained, and how are their movements restricted in the mitochondrial matrix? What are the molecular mechanisms that control mitochondrial membrane fission and fusion, and how are these processes regulated? Identifying the protein players involved in these events is a necessary first step but cannot provide all the answers. We will also need to develop in vitro assays that reconstitute different mitochondrial behaviors (similar to the assays for mitochondrial-actin interactions) if we hope to understand the precise molecular mechanisms by which these molecules work. There are many excellent opportunities for geneticists, cell biologists, and biochemists in this field, and we can look forward to new developments and insights in the next few years.

ACKNOWLEDGMENTS

We thank the past and present members of the Shaw laboratory who contributed to this review including William Bleazard, Ellen Brisch, Noelle Fukushima, Steve Gorsich, Brian Keegan, Denichiro Otsuga, Amy Roeder, and John Thatcher. Denichiro Otsuga and Brian Keegan contributed the confocal images in Figure 2. We also thank Ronald Butow, Margaret Clarke, Margaret Fuller, Rob Jensen, Sepp Kohlwein, Jodi Nunnari, Philip Perlman, Liza Pon, Peter Thorsness, and Michael Yaffe for sending reprints and for communicating results prior to publication. We are grateful to Jodi Nunnari and Joe Dickinson for many helpful discussions and critical reading of the manuscript. Work in the Shaw laboratory is supported by grants from the National Institutes of Health (GM53466), the American Cancer Society (RPG 940 3504 CSM), and the University of Utah Huntsman Cancer Institute. G Hermann was supported by a National Institutes of Health Predoctoral Genetics Training Grant (5 T32 GM07464) and a University of Utah Graduate Research Fellowship.

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