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Mitochondrial distribution and inheritance

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Abstract. Mechanisms mediating the inheritance of mitochondria are poorly understood, but recent studies with the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have begun to identify components that facilitate this essential process. These components have been identified through the analysis of conditional yeast mutants that display aberrant mitochondrial distribution at restrictive conditions. The analysis of these mutants has uncovered several novel proteins that are localized either to cytoskeletal structures or to the mitochondria themselves. Many mitochondrial inheritance mutants also show altered mitochondrial morphology and defects in maintenance of the mitochondrial genome. Although some inheritance components and mechanisms appear to function specifically in certain types of cells, other conserved proteins are likely to mediate mitochondrial behavior in all eukaryotic cells.

Key words. Mitochondria; mitochondrial inheritance; cytoskeleton; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; membrane proteins; organelle movement; mitochondrial morphology.

Cell proliferation requires the biogenesis and inheritance of subcellular organelles [1, 2]. In the case of mitochondria, which arise only from pre-existing mitochondria, biogenesis depends on the coordinate expression of both nuclear and mitochondrial genetic systems, and on elaborate mechanisms that target, transport and assemble polypeptide subunits into functional enzyme complexes. Remarkable progress in describing this biogenesis has been accomplished over the past 20 years [3, 4]; yet, an essential corollary process of mitochondrial proliferation, the transmission of mitochondria to daughter cells prior to cell division, remains poorly understood. Recently, the identity and characteristics of some of the components that mediate mitochondrial inheritance are beginning to emerge from studies of the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

Microscopic observations have documented changes in the distribution and form of mitochondria in a diverse variety of eukaryotic cells [5–7]. Electron microscopy of serial cell sections [8, 9] and fluorescence microscopy of live cells stained with mitochondria-specific dyes [10, 11] have revealed that mitochondria generally comprise a tubular network or reticulum. Time-lapse video microscopy has indicated that this mitochondrial reticulum displays a dynamic character, with tubules frequently dividing in two, fusing with one another or branching into extended, arborated structures [12]. In addition, mitochondrial tubules or individual mitochondria move about the cell, redistributing within interphase cells (e.g. traversing between the cell center and periphery [13]) as well as displaying movements specialized to mitotic cell proliferation and the inheritance of mitochondria [12, 14]. This review will focus on recent studies of mechanisms mediating the positioning and movement of mitochondria and will consider, in particular, mitochondrial distribution in budding and fission yeast. Other reviews have addressed mitochondrial movement in a wider range of cell types and the variety of broader mitochondrial behaviors [15–18].

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Role of the cytoskeleton

A large number of observations have indicated a role for the cytoskeleton in the positioning and movement of mitochondria. Microscopic analysis has revealed the colocalization of mitochondria with various cytoskeletal structures [11, 13, 19–21], and chemical agents or treatments that perturbed cytoskeletal networks also altered mitochondrial distribution [20, 22]. Association of mitochondria with and movement along microtubules was recently observed in live *Acanthamoeba* cells, and colchicine treatment which depolymerized microtubules inhibited this mitochondrial motility [23]. Similar observations, documenting microtubule-associated mitochondrial movements, were made in studies of the filamentous fungus *Neurospora crassa* [24]. In addition, in vitro studies have demonstrated the transport of isolated organelles, including mitochondria, along reconstituted cytoskeletal filaments [25]. To expand upon these largely correlative microscopic and in vitro studies, yeast has offered the opportunity for genetic analysis of mitochondrial inheritance.

In the budding yeast, *S. cerevisiae*, a likely candidate for a cytoskeletal element that mediates mitochondrial distribution is Mdm1p. This protein was originally iden-

tified through the analysis of *mdm1*, a mutant displaying defective transfer of mitochondria and nuclei into growing daughter buds [26]. The *mdm1-1* mutation showed no effect on the distribution of other subcellular organelles or on the apparent structure or function of the actin- and tubulin-based cytoskeletons. *MDM1* was found to encode an essential protein (Mdm1p) with modest sequence similarity to the intermediate filament proteins of animal cells [27]. Antibodies specific to Mdm1p recognized punctate structures distributed throughout the cytoplasm by indirect immunofluorescence microscopy, and these structures were absent from temperature-sensitive *mdm1-1* cells incubated at the nonpermissive temperature [27]. In vitro, purified Mdm1p formed 10-nm filaments very similar to those formed by established intermediate filament proteins [28]. Additionally, purified mutant Mdm1p (encoded by *mdm1-1*) was temperature-sensitive for filament formation in the in vitro assembly assay.

Additional temperature-sensitive *mdm1* alleles were identified in which cytoplasmic Mdm1p structures remained stably assembled even at the nonpermissive temperature (H. Fisk and M. Yaffe, unpublished observations). Furthermore, certain of these new *mdm1* mutations produced defects specific for either mitochondrial or nuclear distribution, thereby suggesting that different regions or sites on Mdm1p may mediate these processes. For those *mdm1* alleles causing a mitochondrial inheritance defect, alterations of mitochondrial morphology were also apparent, with mitochondria typically small, round and frequently clustered, rather than the elongated tubules found in wild-type cells. Genetic interactions between various *mdm1* alleles further supported a model in which Mdm1p assembles and functions as a multimeric complex. Thus, Mdm1p structures may comprise a scaffolding or cytoskeleton that extends throughout the cytoplasm and mediates mitochondrial distribution. Although the relationship between the Mdm1p structures in *S. cerevisiae* and the intermediate filament networks of higher eukaryotic cells is unclear, it is intriguing that mitochondria often colocalize with intermediate filaments in animal cells [11, 21, 29].

The recent analysis of a second mutant, *mdm14*, has identified a novel protein that may function as a partner with Mdm1p. The temperature-sensitive *mdm14* mutant was identified in a screen for mutants that showed genetic interaction with *mdm1*, and cells harbouring the *mdm14* mutation displayed defects in mitochondrial and nuclear inheritance similar to those found in *mdm1* cells (K. Shepard and M. Yaffe, unpublished data). Gene cloning and analysis revealed the *MDM14* gene encodes a novel, 35-kDa protein. Furthermore, an epitope-tagged version of this protein (which could complement the *mdm14* mutant) was localized by indirect immunofluorescence to cytoplasmic punctate structures similar to those identified by antibodies to Mdm1p. Details of the possible interaction of Mdm14p with Mdm1p and the molecular activity of this potential partner protein remain to be uncovered.

Several studies have provided evidence to suggest a role for actin-based structures in the movement and distribution of mitochondria in *S. cerevisiae*; however, it is unclear whether this is a direct function of actin. Identifying and characterizing a role for actin is complicated by the essential character of the single actin gene [30] and the multiple phenotypes displayed by conditional actin mutants [31]. In cells harbouring certain actin mutant alleles, mitochondria in some cells appeared aggregated and abnormally distributed [32]. Such cells also displayed a variety of other defects including aberrant cell morphogenesis, abnormal nuclear distribution and osmotic sensitivity. Particular temperature-sensitive alleles of actin also appeared to affect both mitochondrial shape and movement during meiotic division [33]. In a microscopic analysis of fixed *S. cerevisiae* cells, a fraction of the cells contained some mitochondria colocalized with actin cables [32]. However, certain mutants that completely lacked actin cables (e.g. *act1-113* or *tpm1*) nonetheless displayed normal mitochondrial distribution [32, 34]. Therefore, a significant obstacle to assigning an unequivocal role for actin in mitochondrial inheritance remains the lack of an actin mutant whose defect is specific to mitochondria and substantially defective for some aspect of mitochondrial behaviour.

Many of the actin mutations that resulted in altered mitochondrial organization affected amino acid residues implicated in actin's interaction with myosin (the 'myosin footprint' region of the actin molecule) [32]. This suggests that a myosin-like motor activity localized to the cytoplasmic face of the mitochondrial outer membrane might facilitate transport of mitochondria along actin filaments. In vitro, yeast mitochondria exhibited an ATP-dependent actin-binding activity, and a protease-sensitive actin-based motor activity requiring ATP hydrolysis was identified on the mitochondrial surface [35, 36]. Although these findings strongly suggest involvement of a myosin family member, single mutations in each of the known *S. cerevisiae* myosin genes, *MYO1*, *MYO2*, *MYO3* and *MYO4*, as well as the double *myo2*, *myo4* mutation, failed to affect mitochondrial movement [33]. The clarification of a role for the actin cytoskeleton in mitochondrial distribution may require the identification and mutagenesis of a novel myosin or myosin-like protein on the mitochondrial surface.

The third major cytoskeletal network, that composed of microtubules, has been shown to mediate mitochondrial movement in neuronal axons [37–39], and has been implicated in the positioning of mitochondria in a variety of animal cells [11, 13, 22]. Despite their clear involvement in these other systems, microtubules ap-

pear completely dispensable for mitochondrial inheritance in *S. cerevisiae*. Specifically, mitochondrial transmission into buds occurred normally in the presence of either mutations or drugs that caused depolymerization of microtubules [40, 41]. In striking contrast, recent data demonstrated that mitochondrial distribution in the fission yeast, *S. pombe*, is mediated by microtubules. This role of microtubules emerged from studies of a temperature-sensitive *S. pombe* mutant, *ban5*, that displayed aggregated and asymmetrically distributed mitochondria after incubation at the restrictive temperature [42]. Genetic analysis revealed that *ban5* was an allele of *atb2*$^+$, the gene encoding α2-tubulin. The cold-sensitive *nda3* mutation in β-tubulin also caused aberrant mitochondrial distribution. Consistent with a central role for microtubules in mitochondrial distribution, microscopic analysis revealed that mitochondria largely co-aligned with microtubules during interphase in *S. pombe* cells [42]. Fission yeast should now provide a sophisticated genetic system for the analysis of microtubule-based mitochondrial transport.

Mitochondrial surface components

Cytoskeletal factors comprise one broad category of likely candidates for components mediating mitochondrial distribution. The second frontier for identification of potential mitochondrial inheritance components is the mitochondrial outer membrane [43]. To date, three different outer membrane proteins, Mdm10p, Mmm1p and Mdm12p, have been identified as important factors for mitochondrial inheritance [44, 45] (K. Berger et al., unpublished observations). Loss of function of any of these three proteins produced a mitochondrial inheritance defect as well as a dramatic alteration of mitochondrial morphology in which the normal tubular structures were converted to one or a few giant spheres. Mitochondrial metabolism was also affected by loss of these proteins, and cells lacking Mdm10p or Mdm12p grew extremely slowly on nonfermentable carbon sources, while cells lacking Mmm1p were completely unable to grow on this medium.

Mdm10p, Mmm1p and Mdm12p are all proteins of the mitochondrial outer membrane. Mdm10p encodes a 56.2-kDa integral membrane protein which is partially exposed to the cytoplasm [44]. Controlled expression studies demonstrated that depletion of Mdm10p caused tubular mitochondria to collapse into thickened loglike structures and spheres, and re-expression of *MDM10* restored wild-type mitochondrial morphology [44]. Mmm1p was identified by a mutation that conferred temperature-sensitive growth on glycerol but not on glucose and which showed a temperature-sensitive, reversible defect in mitochondrial morphology, with enlarged spherical mitochondria specifically at restrictive temperature [45].

The *mmm1*-null mutant exhibited abnormal mitochondrial morphology at all temperatures, and was unable to grow on glycerol medium. *MMM1* encodes a 48.7-kDa protein, which, like Mdm10p, is an integral membrane protein with a carboxyl terminus that is exposed to the cytoplasm [45]. The third mitochondrial outer membrane protein required for normal mitochondrial inheritance and morphology is Mdm12p, a 30.8-kDa protein. Mdm12p is the first mitochondrial inheritance component for which a homologue has been identified in fission yeast (K. Berger et al., unpublished observations).

Although a null mutation in *MDM10*, *MMM1* or *MDM12* results in a temperature-sensitive growth defect, none of these genes is essential for growth at lower temperatures on glucose (although the mutant cells grow substantially slower than wild-type cells). Therefore, at 23 °C, some mitochondrial transmission must take place even in the absence of these outer membrane proteins. This residual mitochondrial inheritance may depend on a bypass or backup pathway that operates at low but adequate levels at permissive temperatures. Although the identities of proteins that facilitate such a bypass are unknown, a recently identified mutation, *SOT1*, which can partially suppress the defects of *mdm10*-null or *mdm12*-null mutants (K. Berger et al., unpublished observations), may define such a component.

The specific biochemical activities or molecular roles of the three inheritance components of the outer membrane are unknown; however, two broad models could explain the function of these proteins. One possibility is that Mdm10p, Mmm1p and Mdm12p comprise a structure that serves as an attachment site mediating the interaction of the mitochondrial outer membrane with cytoskeletal components. This interaction may facilitate the movement and positioning of mitochondria as well as the maintenance of a tubular, reticulated morphology. Alternatively, the primary function of the three outer membrane proteins may be to regulate the shape of the organelle through some direct interaction with other mitochondrial components. In this model, defects in mitochondrial inheritance may be a secondary consequence of a morphology that is not permissive for transport.

Mitochondrial distribution and mitochondrial integrity

Cells with *mdm10*, *mmm1* or *mdm12* mutations lose mitochondrial DNA and generate respiration-deficient cells at elevated rates [17] (K. Berger et al., unpublished data). This characteristic is shared with a number of other mutations affecting mitochondrial function [46], and the properties of several of these other mutants suggest relationships between mechanisms mediating mitochondrial distribution and the behavior of mitochondrial DNA. One such mutant, *mgm1*, was identified by its defect in mitochondrial genome mainte-

nance [47, 48]. Cells defective for Mgm1p also exhibit altered mitochondrial morphology and distribution, with marked mitochondrial aggregations [48]. *MGM1* encodes an 89.5-kDa protein homologous to dynamin [47, 48], a nucleotide-sensitive microtubule-binding protein [49]. While this identification of a dynamin family member with a function in mitochondrial distribution might suggest a role for microtubules in this process, the relationship between dynamin and microtubule-based motility in vivo remains unclear. Although proteins of the dynamin family exhibit microtubule binding and GTPase activity stimulated by microtubules, they have not been demonstrated to possess motor activity [49, 50]. In addition (as described above), there is no evidence for a role of microtubules in mitochondrial distribution in *S. cerevisiae*. The function of Mgm1p in both facilitating mitochondrial distribution and maintenance of the mitochondrial genome remains to be elucidated.

A second class of mutants that appear defective for maintenance of mitochondrial DNA as well as mitochondrial structural integrity was identified in a screen to detect cells with increased escape of mitochondrial DNA to the nucleus (*yme* mutants, for yeast mitochondrial DNA escape [51]). One such mutant, *yme1*, exhibited temperature-sensitive growth specifically on nonfermentable carbon sources, and extremely slow growth in the absence of mitochondrial DNA [52]. The *yme1* mutant cells also contained enlarged and misshapen mitochondria [53]. Yme1p encodes an adenosine triphosphate (ATP)-dependent zinc metalloprotease localized to the mitochondrial inner membrane [54]. Yme1p proteolytic activity, which was shown to be required for efficient turnover of an unassembled cytochrome oxidase subunit, may play a role in regulating levels of components affecting diverse aspects of mitochondrial function [54]. Alterations in mitochondrial morphology were observed also in several other *yme* mutants (P. Thorsness, personal communication). Among these mutants, *yme6* proved to be a novel allele of *MMM1* [17]. Although the molecular activity of the product of this gene is unknown (as discussed above), this identity and the properties of other *yme* mutants support the concept of the interdependence of mitochondrial morphology, mitochondrial structural integrity, maintenance of the mitochondrial genome, and distribution and transmission of mitochondria.

One additional yeast mutant, *mdm2*, indicates the underlying importance of membrane fluidity for normal mitochondrial behavior. The *mdm2* mutant exhibited defects in both mitochondrial morphology and inheritance, with abnormally clumped or clustered, rounded mitochondria which were defective for transmission to buds as well as distribution within mating projections [55]. The *mdm2* mutation was found to be a mutant allele of the *OLE1* gene, encoding fatty acid desaturase, and the *mdm2* mutant phenotypes could be complemented by the addition of oleic acid to the medium [55]. The *mdm2* mutant thereby uncovered a requirement for unsaturated fatty acids in mitochondrial transmission to buds during mitotic growth, and also for mitochondrial distribution into mating projections preceding conjugation.

Mitochondrial dynamics during mating and meiosis

Most investigations of mitochondrial distribution and inheritance have focused on cells engaged in mitotic growth. For *S. cerevisiae*, specialized movements of mitochondria also occur during mating and meiosis. An analysis of mitochondrial behaviour during yeast cell conjugation provided evidence indicating that different mitochondrial constituents can move within the cell with different kinetics. In this study, distinct markers were used to follow segregation of mitochondrial DNA and a mitochondrial matrix protein, citrate synthase, during conjugation [56]. Subsequent to mating, the rate and progression of mixing of the parental mitochondrial genomes lagged significantly behind those of the matrix protein. An implication of this finding is that transmission of mitochondrial DNA may occur by a mechanism distinct from that mediating distribution of protein constituents of the mitochondrial matrix. Furthermore, these findings suggest that a mitochondrion may not necessarily behave as a single, unitary structure, and that components of different mitochondrial subcompartments (e.g. the inner membrane and the matrix) may possess different mobility properties.

Mitochondria also display characteristic dynamics during meiosis. Microscopic analysis of yeast cells undergoing meiotic sporulation revealed that mitochondria condensed from elongated, threadlike organelles into a large, multibranched structure [57]. Later in meiosis, this giant mitochondrion fragmented into small spheres which were distributed among the haploid meiotic progeny [14, 57]. Mutants specifically defective for meiotic mitochondrial inheritance have not yet been identified, but at least some mitotic mitochondrial inheritance mutants also appear to be defective in this process. The enlarged mitochondria of *mdm10* and *mdm12* mutant cells may be defective for some aspect of meiotic rearrangement and/or distribution, because diploid cells lacking Mdm10p or Mdm12p failed to undergo sporulation (K. Berger et al., unpublished observations). Additionally, specific combinations of certain *mdm* mutations produced reduced spore viability (K. H. Berger, K. A. Shepard, and M. P. Yaffe, unpublished results). Meiotic inheritance and distribution of mitochondria is likely to require many of the same components as mitotic distribution, and other factors specific to meiotic processes may yet be discovered.

Mitochondrial distribution in fission yeast

In the characterization of mitochondrial inheritance in yeast, the majority of studies have focused on *S. cerevisiae*. Recent investigations using *S. pombe* have uncovered distinct differences in mitochondrial behavior between fission and budding yeast. In particular, the central role of microtubules in *S. pombe* but not in *S. cerevisiae* (as discussed above) indicates fundamental mechanistic variation. Despite this difference, mitochondrial inheritance in these two species as well as in other eukaryotic cells is likely to retain some common elements, particularly considering the highly conserved nature of mitochondrial activities, enzymes and basic structures among disparate species.

At present, studies have identified a single mitochondrial inheritance component, Mdm12p, that is conserved between budding and fission yeast (K. Berger et al., unpublished data). As described above, loss of Mdm12p function in *S. cerevisiae* results in giant, spherical mitochondria which are defective for inheritance. A search of protein sequence databases identified a potential Mdm12p homologue in *S. pombe*. The corresponding *S. pombe* gene was cloned and expressed in *S. cerevisiae*. In otherwise wild-type cells, expression of the *S. pombe* Mdm12p homologue caused mitochondria to display abnormally enlarged and rounded morphology, resembling the *mdm12* mutant phenotype. The ability of the foreign gene to confer a dominant negative phenotype suggests partial functional as well as structural conservation. The cellular requirement for the *S. pombe* Mdm12p homologue in fission yeast is currently under investigation.

Models of mitochondrial movement

Recent studies have begun to identify proteins that facilitate the movement and inheritance of mitochondria in yeast. These proteins may be grouped into two broad categories: cytoskeletal components (such as Mdm1p) and mitochondrial proteins (such as Mdm10p and Mmm1p). While the specific biochemical activities and interactions of most of these components have yet to be described, their distributions within the cell suggest two general models for mechanisms mediating mitochondrial movement and inheritance. One possibility is that molecular motors bind to the mitochondrial surface and pull mitochondria along cytoskeletal tracks. This appears to be a principal mode of mitochondrial transport in neuronal axons [25, 38, 39], and a similar mechanism may operate in yeast, perhaps utilizing microtubules in *S. pombe* and a different cytoskeletal system in *S. cerevisiae*. A second model for mitochondrial motility is that mitochondria move via internal changes in morphology and the sequential binding and release of surface structures along a cytoskeletal scaffold. This model envisions a 'crawling' or amoeboid-like mechanism in which movement is catalysed from within the organelle, and the cytoskeleton serves either to channel movement or as a passive substratum. The identification of additional proteins that mediate mitochondrial inheritance both in fission and budding yeast and the characterization of interactions between these various components should lead to an elucidation of mechanisms mediating mitochondrial movement and inheritance.

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