11. P. D. Boyer, *Biochim. Biophys. Acta* **1140**, 215 (1993); *Annu. Rev. Biochem.* **66**, 717 (1997).
12. D. B. Knaff, *Photosynth. Res.* **35**, 117 (1993).
13. M. Saraste, *Q. Rev. Biophys.* **23**, 331 (1990).
14. J. E. Walker, *ibid.* **25**, 253 (1992).
15. J. M. Skehel, I. M. Fearley, J. E. Walker, *FEBS Lett.* **438**, 301 (1998).
16. T. Friedrich, K. Steinmüller, H. Weiss, *ibid.* **367**, 107 (1995).
17. U. Brandt, *Biochim. Biophys. Acta* **1318**, 79 (1997).
18. V. Guenebaut, A. Schlitt, H. Weiss, K. Leonard, T. Friedrich, *J. Mol. Biol.* **276**, 105 (1998).
19. N. Grigorieff, *ibid.* **277**, 1033 (1998).
20. P. L. Dutton, C. C. Moser, V. D. Sled, F. Daldal, T. Ohnishi, *Biochim. Biophys. Acta* **1364**, 245 (1998).
21. C. Hägerhäll, *ibid.* **1320**, 107 (1997).
22. H.-P. Braun and U. K. Schmitz, *Trends Biochem. Sci.* **20**, 171 (1995).
23. U. Brandt, *Biochim. Biophys. Acta* **1275**, 41 (1996).
24. A. R. Crofts and E. Berry, *Curr. Opin. Struct. Biol.* **8**, 501 (1998).
25. J. L. Smith, *Science* **281**, 58 (1998).
26. P. Lappalainen, R. Aasa, G. G. Malmström, M. Saraste, *J. Biol. Chem.* **268**, 26416 (1993).
27. B. G. Malmström and R. Aasa, *FEBS Lett.* **325**, 49 (1993).
28. M. Wikström, *Curr. Opin. Struct. Biol.* **8**, 480 (1998).
29. M. L. Verhovskaya *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10128 (1997).
30. A. A. Konstantinov, S. Siletsky, D. Mitchell, A. Kaulen, R. B. Gennis, *ibid.*, p. 9085.
31. P. R. Rich, *Aust. J. Plant Physiol.* **22**, 479 (1995).
32. M. Wikström *et al.*, *Biochim. Biophys. Acta* **1187**, 106 (1996).
33. G. T. Babcock and M. Wikström, *Nature* **356**, 301 (1992).
34. M. Wikström, *ibid.* **338**, 776 (1989).
35. P. Hellwig *et al.*, *Biochemistry* **37**, 7390 (1998).
36. J. Behr, P. Hellwig, W. Mäntele, H. Michel, *ibid.* **37**, 7400 (1998).
37. R. Lutter *et al.*, *Biochem. J.* **295**, 799 (1993).
38. B. Böttcher, L. Schwarz, P. Gräber, *J. Mol. Biol.* **281**, 757 (1998).
39. T. Elston, H. Wang, G. Oster, *Nature* **391**, 510 (1998).
40. Y. Shirakihara *et al.*, *Structure* **5**, 825 (1997).
41. M. A. Bianchet, J. Hullihen, P. L. Petersen, L. M. Amzel, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11065 (1998).
42. D. Sabbert, S. Engelbrecht, W. Junge, *Nature* **381**, 623 (1996).
43. ———, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4401 (1997).
44. Y. Zhou, T. M. Duncan, R. L. Cross, *ibid.*, p. 10583.
45. H. Noji, R. Yasuda, M. Yoshida, K. Kinosita, *Nature* **386**, 299 (1997).
46. R. Yasuda, H. Noji, K. Kinosita, M. Yoshida, *Cell* **93**, 1117 (1998).
47. H. Noji, *Science* **282**, 1844 (1998).
48. H. Wang and G. Oster, *Nature* **396**, 279 (1998).
49. P. C. Jones and R. H. Fillingame, *J. Biol. Chem.* **273**, 29701 (1998).
50. R. Jenkyns, *N.Y. Review Books* **XLV** (no. 9), 4 (1998).

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REVIEW

The Machinery of Mitochondrial Inheritance and Behavior

Michael P. Yaffe

The distribution of mitochondria to daughter cells during cell division is an essential feature of cell proliferation. Until recently, it was commonly believed that inheritance of mitochondria and other organelles was a passive process, a consequence of their random diffusion throughout the cytoplasm. A growing recognition of the reticular morphology of mitochondria in many living cells, the association of mitochondria with the cytoskeleton, and the coordinated movements of mitochondria during cellular division and differentiation has illuminated the necessity for a cellular machinery that mediates mitochondrial behavior. Characterization of the underlying molecular components of this machinery is providing insight into mechanisms regulating mitochondrial morphology and distribution.

Mitochondria have long been recognized as prominent and vital residents of the cytoplasm of eukaryotic cells. These ubiquitous organelles were identified 50 years ago as the site of oxidative energy metabolism (*1*). Subsequent studies have uncovered myriad mitochondrial proteins that catalyze numerous biosynthetic and degradative reactions fundamental to cell function (*2*). These activities depend on a distinctive mitochondrial structure, with different enzymes and reactions localized in discrete membranes and aqueous compartments. The characteristic mitochondrial structural organization is the product of both local synthesis of macromolecules within the mitochondria and the import of proteins and lipids synthesized outside the organelle (*3*). Synthesis and import of mitochondrial components are required for mitochondrial proliferation, but rather than producing new organelles, these processes facilitate the growth of preexisting mitochondria. Because the mitochondrial membranes and the mitochondrial DNA must serve as essential templates for the growth of the organelle, mitochondrial continuity requires the transmission of mitochondria to daughter cells before every cell division.

Mitochondria display an amazing plasticity of form and distribution. Although their internal structural organization is highly conserved, the external shape of mitochondria is variable. In addition to the classic kidney bean–shaped organelles observed in electron micrographs, mitochondria are frequently found as extended reticular networks (*4*) (Fig. 1). These networks are extremely dynamic in growing cells, with tubular sections dividing in half, branching, and fusing to create a fluid tubular web (*5*). In differentiated cells, such as those found in cardiac muscle or kidney tubules, mitochondria are often localized to specific cytoplasmic regions rather than randomly distributed (*6*). Some alterations in mitochondrial shape and distribution are developmentally programmed, with characteristic mitochondrial migrations or morphological changes occurring at key stages in cellular differentiation (*7*, *8*). Additionally, alterations in mitochondrial distribution and morphology are associated with a variety of pathological conditions, including liver disease (*9*), muscular dystrophy (*10*), cardiomyopathy (*11*), and cancer (*12*).

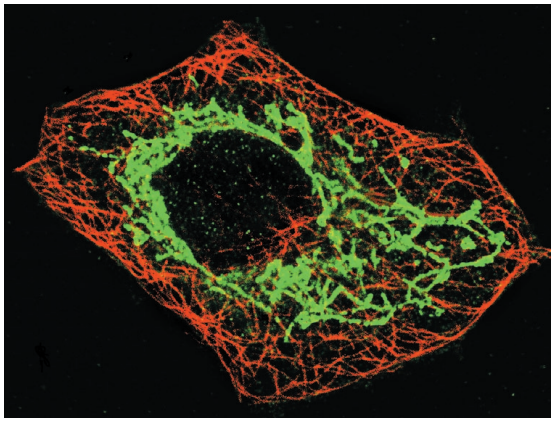

Fig. 1. Mitochondrial network in a mammalian fibroblast. A COS-7 cell labeled to visualize mitochondria (green) and microtubules (red) was analyzed by indirect immunofluorescence confocal microscopy. Mitochondria were labeled with antibodies to the β subunit of the F_1-ATPase and a rhodamine-conjugated secondary antibody. Microtubules were labeled with antibody to tubulin and a fluorescein-conjugated secondary antibody. Pseudocolor was added to the digitized image. Scale: 1 cm = 10 μm.

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In considering the dynamics of mitochondrial shape and behavior, three questions motivate much ongoing research. What structures and molecular components mediate changes in mitochondrial distribution and morphology? What mechanisms ensure the faithful inheritance of mitochondria in every cell cycle? Finally, how is mitochondrial behavior coordinated with other central events in cell proliferation and development?

Insights into the molecular basis of mitochondrial behavior are emerging from a combination of microscopic, genetic, and biochemical approaches. The first molecules mediating mitochondrial inheritance were discovered through isolation and analysis of mutant yeast cells that displayed conditional defects in mitochondrial distribution and morphology (*13*). More recently, proteins facilitating mitochondrial distribution have been identified through studies of the cytoskeleton and analysis of proteins that play fundamental roles in the movement of other organelles and membranes (*14*–*16*). In addition, studies of cellular differentiation during sperm formation have uncovered a novel component involved in mitochondrial fusion (*17*). Some of the components mediating mitochondrial behavior are highly conserved proteins that are likely to function in a broad array of eukaryotic cells (*18*–*20*).

Tracking Mitochondrial Movement

Microscopic studies performed more than 80 years ago revealed vigorous movement of mitochondria in the cytoplasm (*21*). These observations supported early suggestions that mitochondria were related to bacteria (*22*), foreshadowing widespread acceptance of the endosymbiotic theory of mitochondrial descent from prokaryotic cells that were symbiotically established in the cytoplasm of a eukaryotic progenitor (*23*). However, mitochondria lack flagella, cilia, or other structures associated with bacterial motility, and the mechanisms of mitochondrial movement remained obscure for many years.

The first clues to the mechanisms of mitochondrial distribution and movement emerged from studies of the cytoskeleton. Microscopic analysis revealed colocalization of mitochondria with certain cytoskeletal components. In particular, many studies documented colocalization of mitochondria with microtubules in diverse cell types including mammalian neurons, sperm cells, and cultured fibroblasts as well as in the fission yeast *Schizosaccharomyces pombe* and the protozoan *Acanthamoeba castellanii* (*18*, *24*–*26*). Involvement of microtubules was further supported by the observation that mitochondria redistribute in cultured mammalian cells treated with agents that disassemble microtubule networks (*24*, *27*). Fluorescence and video microscopy of *Acanthamoeba* (*26*) and the fungus *Neurospora crassa* (*28*) revealed mitochondria tracking along microtubules in live cells. Microscopic analysis of transport in chick neuronal axons revealed that microtubule inhibitors reduced mitochondrial movement and that actin microfilaments might participate in axonal mitochondrial transport (as discussed below) (*29*). Furthermore, disruption of microtubules by certain conditional mutations in genes encoding tubulins (the building blocks of microtubules) caused aberrant mitochondrial distribution in *S. pombe*, providing genetic evidence that microtubules position mitochondria in this organism (*18*).

A pivotal advance in identifying the molecular basis of organellar movement on microtubules was the discovery of the microtubule-based motor proteins, kinesin and cytoplasmic dynein (*30*). These proteins bind microtubules and transduce chemical energy into mechanical work as they hydrolyze adenosine triphosphate (ATP) to power polarized movement along microtubules (*31*). Both proteins can bind and transport "cargo" in the form of vesicles, organelles, or other proteins, and mitochondria appear to be among the favored cargoes. In particular, several different members of the kinesin superfamily have been localized preferentially to mitochondria in animal cells. One of these proteins, KIF1B, was localized to mitochondria in vivo and copurified with mitochondria isolated from mouse cells (*32*) (Table 1). Purified mitochondria with bound kinesin displayed motility on microtubules in vitro, and this translocation activity was blocked by antibodies inhibiting KIF1B function. Mitochondrial localization was also found for KLP67A, a kinesin-like protein identified in proliferating embryonic cells of the fruit fly *Drosophila melanogaster* (*33*). Intriguingly, KLP67A was localized to mitochondria near or on the astral microtubules in mitotic cells, perhaps indicating a function for the protein in mitochondrial localization during cell division.

Analysis of another kinesin family member, KIF5B, has recently provided direct evidence of a role for kinesin in mitochondrial distribution (*34*). Disruption of the corresponding gene in mice resulted in embryonic death, so the in vivo effects of KIF5B deficiency could not be assessed. However, cells derived from the visceral yolk sac of mutant embryos displayed dramatically altered mitochondrial distribution: mitochondria were clustered near the nucleus rather than spread throughout the cytoplasm and toward the cell periphery. Interestingly, the clustered mitochondria remained associated with microtubules, indicating that additional proteins may mediate binding to microtubules.

Intermediate filaments (IFs) also appear to play a role in mitochondrial positioning. Indirect immunofluorescence microscopy has shown that mitochondria and cytoplasmic IFs colocalize in certain types of animal cells, and mitochondrial distribution is altered by treatments that collapse the IF network (*25*, *35*). IF-based molecular motors have not been identified thus far, so IFs might play a more passive or structural role by anchoring mitochondria at particular cytoplasmic locations. It is also possible that microtubules and IFs both mediate mitochondrial distribution but function at different times in the cell cycle. For example, mitochondria might be transported toward the cell periphery along microtubules during interphase and then passed to IFs as mitosis begins (and cytoplasmic microtubules are disassembled). Electron micrographs of frog axons have revealed molecular cross-bridges between single mitochondria and both microtubules and neuronal IFs (*36*).

The role of actin in mitochondrial movement is less clear. In characean algal cells, mitochondria and other organelles move by cytoplasmic streaming (*37*), a process dependent on actin filaments and type-I myosin motors (*38*). Mitochondrial movement in locust photoreceptor cells and Malpighian tubule cells of the insect *Rhodnius prolixus* was blocked by inhibitors of actin polymerization (*39*). Additionally, in the presence of anti-microtubule drugs, mitochondrial movement in neuronal axons became dependent on actin filaments (*29*). In the yeast *Saccharomyces cerevisiae*, a fraction of the mitochondrial tubules appears to colocalize with actin cables (*40*), and specific mutations in the single actin gene cause abnormal mitochondrial distribution and morphology (*40*). Additionally, isolated yeast mitochondria bound to actin filaments and exhibited actin-based motility in an in vitro assay (*41*, *42*). These observations suggest that mitochondria might be transported along actin cables that extend between the mother portion of the cell and the developing daughter bud. However, certain mutations cause total loss of these actin cables, but mitochondrial transport remains normal (*40*, *43*). Furthermore, mutations in the five yeast myosin genes have no effect on mitochondrial inheritance (*42*, *44*). Further clarification of actin's role in mitochondrial movement may require the identification of specific actin-binding proteins that mediate interactions with mitochondria.

Dynamin-Related Proteins and Mitochondrial Dynamics

Members of a second class of protein—the dynamin superfamily of large guanosine triphosphate (GTP)–binding proteins—were recently found to play important roles in mitochondrial distribution. This function in distribution appears to be intimately related to the role of these dynamin homologs in determining normal mitochondrial morphology. Indeed, it is difficult to distinguish changes in morphology from changes in distribution, particularly in cells where mitochondria are normally found in extended reticular networks (*45*).

Dynamin was originally identified as a protein essential for endocytosis in animal cells, where it facilitates the internalization and scission step by which clathrin-coated pits become coated vesicles (*46*). Eukaryotic cells contain several dynamin-related proteins, and the function of one of these, Drp1, was recently investigated by examining the expression of mutant versions of the human protein in cultured cells (*15*). Expression of Drp1 containing alterations in the conserved GTP-binding site caused striking changes in mitochondrial distribution, with the normally extended tubular projections collapsing into large perinuclear aggregates of tubules. Effects of mutant Drp1 were specific for mitochondria. Wild-type Drp1 was localized throughout the cytoplasm and did not appear to associate with mitochondria.

Independently, another role for a dynamin family member was uncovered through analysis of a *S. cerevisiae* mutant, *mdm29* (*16*). In cells with this mutation, mitochondria collapse from their normal peripheral distribution around the cell cortex (the cytoplasmic zone underlying the plasma membrane) into a linear bundle of tubules aligned along one side of the cell. The *mdm29* mutation was mapped to *DNM1*, a gene encoding a dynamin-like protein most closely related to Drp1 (*47*). Yeast cells expressing Dnm1p mutated in the conserved GTP-binding site also display collapsed mitochondria. Wild-type Dnm1p was predominantly distributed in the cytosol, but, intriguingly, a fraction of the protein in wild-type cells was found in punctate structures localized along mitochondrial tubules at points of apparent association with the cell cortex.

Mutation of another conserved protein, Clu1, also produces changes in mitochondrial distribution similar to those caused by mutations in the dynamin-related proteins. In *S. cerevisiae* cells in which *CLU1* has been deleted, the mitochondria collapsed along one side of the cell (*48*), and disruption of the homologous *cluA* gene in the slime mold *Dictyostelium discoideum* caused mitochondrial aggregation and clustering near the center of the cell (*49*). Clu1p is not a dynamin homolog, and its protein sequence provides few hints as to its molecular function, but the similarity of the yeast mutant phenotype to that caused by mutations in *dnm1* suggests that these two proteins may facilitate a common step in the lateral distribution and arborization of mitochondrial tubules. Dnm1p and Clu1p may act in concert with molecular motors to pull mitochondria out toward the cell periphery, or they may modify local morphology of a mitochondrial tubule to promote branching and network extension.

Mitochondrial Fusion Factor

Mitochondrial fusion is a key aspect of mitochondrial dynamics. As mitochondria undergo morphological changes or alterations in distribution during cellular growth and differentiation, individual mitochondria or separate tubules of reticular mitochondrial networks frequently fuse into larger or more highly branched structures (*5*, *50*). Similar membrane fusion events involving other organelles depend on a well-characterized set of proteins including *N*-ethylmaleimide sensitive factor (NSF), soluble NSF attachment protein receptors (SNAREs), and guanosine triphosphatases of the rab family (rab GTPases) (*51*), but most of these components do not appear to function in mitochondrial dynamics.

One of the molecular components mediating mitochondrial fusion was recently identified through the analysis of a *Drosophila* mutant defective in sperm development (*17*). At a critical stage in *Drosophila* spermatogenesis, mitochondria aggregate and fuse into two giant structures that are incorporated into the midpiece of the developing spermatid and later produce ATP to power the sperm's flagellar motor (*8*). In one *Drosophila* mutant, mitochondria aggregate but do not fuse; instead, they form a structure resembling a "fuzzy onion." The *fuzzy onions* gene en-

Table 1. Proteins that appear to play a primary or direct role in mitochondrial dynamics.

Protein	Organism	Location	Mutant phenotype	Protein properties	References
KIF5B, KIF1B; KLP67A	Mouse; fly	Microtubules/ mitochondrial surface	Mitochondrial aggregation near nucleus	Kinesin homolog	(*32–34*)
Drp1; Dnm1p	Human; yeast	Cytoplasm	Mitochondrial aggregation, reduced lateral distribution and/or branching	Dynamin-related protein	(*15*, *16*)
CluA; Clu1p	Slime mold; yeast	Cytoplasm	Mitochondrial aggregation, reduced lateral distribution and/or branching	Coiled-coil domain containing protein	(*48*, *49*)
Mdm1p	Yeast	Cytoplasm	Defective mitochondrial transmission to buds; fragmentation of tubules	Intermediate filament-like protein	(*59*, *60*)
Mdm20p	Yeast	Cytoplasm	Defective mitochondrial transmission to buds	Coiled-coil domain containing protein	(*62*)
Mdm14p	Yeast	Cytoplasm	Defective mitochondrial transmission to buds; mitochondrial aggregation	Coiled-coil domain containing protein	(*61*)
Rsp5p	Yeast	Cytoplasm	Defective mitochondrial transmission to buds; mitochondrial aggregation	Ubiquitin-protein ligase	(*69*)
Mdm10p	Yeast; fungus	Mitochondrial outer membrane	Defective mitochondrial transmission to buds; giant spherical mitochondria	Integral membrane protein	(*20*, *63*)
Mmm1p	Yeast	Mitochondrial outer membrane	Defective mitochondrial transmission to buds; giant spherical mitochondria	Integral membrane protein	(*64*)
Mdm12p	Yeast	Mitochondrial outer membrane	Defective mitochondrial transmission to buds; giant spherical mitochondria	Integral membrane protein	(*19*)
Mgm1p	Yeast	Mitochondrial outer membrane	Defective mitochondrial transmission to buds; mitochondrial aggregation	Integral membrane protein; dynamin-related protein	(*61*, *66*, *67*)
Fuzzy onions, Fzo1p	Fly; yeast	Mitochondrial outer membrane	Aberrant mitochondrial fusion; fragmentation of tubules	Integral membrane protein; GTPase	(*17*, *52*, *53*)

codes a large membrane-bound GTP-binding protein that localizes to mitochondria and is expressed in the developing spermatids specifically at the onset of mitochondrial fusion. The *fuzzy onions* gene is highly conserved, and homologs are expressed in a variety of mammalian tissues, suggesting a general role for the protein in mitochondrial fusion.

New insights into the function of the *fuzzy onions* protein have emerged from studies of the yeast homolog, Fzo1p (*52*, *53*). Mutations in Fzo1p caused fragmentation of mitochondrial tubules and eventual loss of mitochondrial DNA. Mitochondrial fusion, which normally follows the mating of two yeast cells, did not occur when Fzo1p was defective or absent, providing direct evidence of a role for the protein in the fusion process (*53*). Consistent with such a role, Fzo1p was localized to the mitochondrial outer membrane, with the bulk of its structure (including the conserved GTP-binding site) exposed to the cytosol. Fzo1p was further shown to be part of a high–molecular weight complex that may comprise a multiprotein fusion machinery localized on the mitochondrial surface (*52*).

The biochemical events underlying mitochondrial fusion remain to be uncovered. This process may well be more complex than vesicular fusion in the endocytotic or secretory pathways because mitochondrial fusion requires joining of both outer and inner membranes in a regulated manner that maintains the integrity of the distinct mitochondrial compartments. The *fuzzy onions* protein might initiate interaction between outer membranes of adjacent mitochondrial tubules, facilitate changes in the membrane lipid bilayers, or regulate additional components of the fusion machinery. One candidate component of this machinery is the *Don Juan* protein, another *Drosophila* factor required for spermatogenesis and associated with mitochondria (*54*).

Mitochondrial Inheritance Mutants

In contrast to animal cells, the yeast *S. cerevisiae* proliferates by a budding process in which a mother cell produces a daughter bud that grows larger and eventually becomes an independent cell. An essential feature of this mode of growth is the vectorial transport of mitochondria and other organelles into the developing bud (*55*) (Fig. 2). The analysis of yeast mutants defective in this transport process has led to the identification of proteins that facilitate mitochondrial inheritance; in at least some cases, homologous proteins appear likely to mediate mitochondrial behavior in diverse eukaryotic cells.

The first mutants affecting mitochondrial inheritance were isolated by microscopically screening collections of temperature-sensitive strains for cells that failed to transport mitochondria into daughter buds (*13*). In addition to conditional defects in mitochondrial inheritance, many of the mutant cells displayed aberrant mitochondrial shape, and the mutants were named *mdm* for mitochondrial distribution and morphology. Although other yeast mutants that lose mitochondrial DNA and become respiration-deficient still retain mitochondrial compartments and remain viable (*56*), buds of *mdm* mutants devoid of mitochondria never became viable cells, reflecting the essential requirement for mitochondria even in a facultative anaerobe like *S. cerevisiae*.

The mitochondrial inheritance components identified to date (*45*, *57*) fall into two broad categories: cytosolic proteins likely to be associated with the cytoskeleton, and integral proteins of the mitochondrial outer membrane. Mdm1p, which belongs to the cytosolic class, is essential for both mitochondrial and nuclear inheritance (*13*), but it appears to play distinct roles in these two processes, because mutant forms of Mdm1p have been isolated that affect only one process (*58*). Mutations in *mdm1* affecting mitochondrial inheritance also cause fragmentation of mitochondrial tubules, resulting in small, round mitochondria that otherwise resemble wild type. Other cellular processes including secretion, vacuole distribution, mitochondrial protein import, and functions of the actin and tubulin cytoskeletons are unaffected by *mdm1* mutations. Mdm1p assembles in vitro into 10-nm-diameter filaments closely resembling IFs in animal cells (*59*). In yeast cells, Mdm1p localizes to punctate structures throughout the cytoplasm, and under conditions leading to the disassembly of these structures, mitochondrial transmission to buds is defective (*60*). Mdm1p appears to function as a component of a novel cytoskeleton-like system that mediates mitochondrial and also nuclear inheritance.

Mdm14p and Mdm20p are two additional cytoplasmic components that may function in concert with Mdm1p. Mutations in the corresponding genes affect mitochondrial inheritance, and the *mdm14* mutant also displays defects in nuclear transmission (*61*, *62*). Mdm20p is also important for the stability or organization of the actin cytoskeleton (*62*), although it is unclear how this function is related to mitochondrial distribution. Neither Mdm14p nor Mdm20p contains obvious functional motifs or active sites, but both have putative coiled-coil domains that are essential for their activity in mitochondrial inheritance.

Proteins belonging to the second class of inheritance components have been identified through analysis of *mdm10*, *mmm1*, and *mdm12* mutants (*19*, *63*, *64*). Cells bearing any of these three distinct mutations have giant, round mitochondria that retain classical ultrastructural features including double membranes and inner membrane cristae and remain partially competent for respiration, but are defective for division and inheritance by daughter buds. Mdm10p, Mmm1p, and Mdm12p are integral proteins of the mitochondrial outer membrane; Mdm10p and Mmm1p both have large domains that project into the cytoplasm. Depletion of these proteins converts the tubular mitochondrial reticulum into giant spherical organelles in a reversible manner. Mdm10p, Mmm1p, and Mdm12p are likely to act at the same step, because cells harboring mutations in two or three of the genes display phenotypes identical to those of the single mutants. Additionally, mutations in *MDM10*, *MMM1*, or *MDM12* (or any combination) are suppressed by a mutant form of a cytoplasmic protein, Sot1p (*19*, *65*). Although the underlying molecular details remain to be described, this suppression illustrates the critical interplay of membrane and cytosolic components in determining mitochondrial morphology and distribution.

Analysis of another mutant, *mdm17*, has uncovered a role for a second dynamin family protein present on the mitochondrial surface (*61*, *66*). In *mdm17* cells, mitochondria aggregate and are not transported to buds. The mutation resides in *MGM1*, a gene encoding a dynamin-like protein previously shown to be required for maintenance of mitochondrial DNA and normal mitochondrial morphology (*67*). Mgm1p is an integral protein of the mitochondrial outer membrane, the bulk of which, including the essential GTP-binding site, is exposed to the cytosol. Mgm1p, together with the outer membrane proteins Mdm10p, Mmm1p, and Mdm12p, may function as an anchor point or "handle" for attachment of mitochondria to the cytoskeleton or to as-yet-unidentified molecular motors. Alternatively, these proteins may catalyze alterations in physical properties of the outer membrane that underlie changes in morphology and distribution.

The four outer membrane proteins associated with mitochondrial inheritance are evolutionarily conserved. Homologs have been

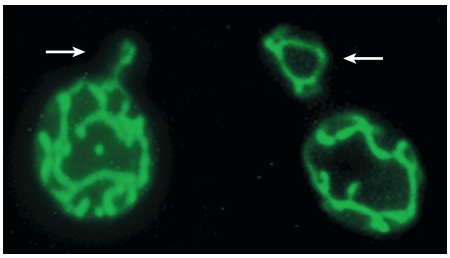

Fig. 2. Mitochondrial network in the budding yeast *S. cerevisiae*. Wild-type yeast cells were engineered to express green fluorescent protein fused to a mitochondrial targeting sequence (the NH_2-terminal 22 residues of cytochrome oxidase subunit 4) and were visualized by fluorescence microscopy. Pseudocolor was added to the digitized image. Arrows show the budding daughter cells. The neck connecting the mother cell on the right with its bud is not apparent in this optical section. Scale: 1 cm = 3 μm.

identified in *S. pombe* (*45*, *68*), and a homolog of Mdm10p was found in the filamentous fungus *Podospora anserina* (*20*). Mdm10p in *P. anserina* and Mdm12p in *S. pombe* have been linked to mitochondrial distribution and morphology (*19*, *20*), and it is likely that each of the four proteins plays a conserved role in a variety of organisms.

Mitochondrial behavior is likely to be highly regulated. A potential regulatory mechanism was revealed by analysis of a genetic suppressor that corrects inheritance defects caused by a specific mutation in *mdm1* (*69*). The suppressor gene encodes Rsp5p, a ubiquitin-protein ligase that modifies target proteins, leading to their degradation or altered function (*70*). Cells harboring only the suppressor (mutant) form of *RSP5* have striking defects in mitochondrial distribution including empty daughter buds. In addition, expression of a mutant form of ubiquitin causes aberrant mitochondrial distribution and morphology. Protein phosphorylation may also regulate mitochondrial inheritance, as mitochondrial transmission to yeast buds is delayed by mutations in the gene encoding serine-threonine phosphatase Ptc1p (*71*).

Microtubules play no role in mitochondrial distribution in *S. cerevisiae* (*72*). However, in *S. Pombe*, mitochondria colocalize with cytoplasmic microtubules, and conditional mutations in tubulin genes cause dramatic alterations in mitochondrial distribution (*18*). This distinction contrasts with the conservation of outer membrane inheritance components (Mdm10p, Mmm1p, Mdm12p, and Mgm1p) and suggests that conserved structures on the mitochondrial surface might be adapted for interaction with different cytoskeletal networks. Alternatively, these outer membrane proteins might maintain mitochondrial morphology by mechanisms other than interaction with cytoplasmic or cytoskeletal components.

Prospects

The mitochondrial outer membrane is the frontier between the mitochondrion and the rest of the cell, and molecular activities at this boundary largely determine mitochondrial behavior. Studies in both animal cells and unicellular eukaryotes support a model in which mitochondrial morphology and distribution depend on the regulated interaction of the mitochondrial outer membrane with the cytosol and, in particular, with cytoskeletal components. Such interactions could serve to direct mitochondrial movement, transport mitochondria to regions of cell growth or areas of greatest metabolic need, pull mitochondria into tubular morphologies, and maintain mitochondrial position in the cytoplasm during cellular movement and function. These functions may depend on the activity of molecular motors that move membranes along cytoskeletal tracks. Alternatively, mitochondrial dynamics may involve a sequential binding and release between mitochondrial surface components and a cytoskeletal scaffolding, leading to a "crawling" movement of a mitochondrial tubule. Both of these mechanisms would depend on a cadre of outer membrane proteins that regulate physical properties of the outer membrane and mediate an association of the mitochondrial surface with molecular motors or the cytoskeleton. Some of the molecular components that facilitate mitochondrial inheritance and determine mitochondrial morphology have now been characterized (Table 1), but additional components remain to be identified. An even greater challenge for future investigations is to determine how the machinery of mitochondrial behavior is regulated during growth and development to respond to cellular metabolic needs and to ensure mitochondrial continuity for future generations of eukaryotic cells.

References and Notes

1. E. P. Kennedy and A. L. Lehninger, *J. Biol. Chem.* **179**, 957 (1949).
2. A. Tzagoloff, *Mitochondria* (Plenum, New York, 1982).
3. G. Attardi and G. Schatz, *Annu. Rev. Cell Biol.* **4**, 289 (1988); W. Neupert, *Annu. Rev. Biochem.* **66**, 863 (1997).
4. L. B. Chen, *Annu. Rev. Cell Biol.* **4**, 155 (1988); J. Bereiter-Hahn, *Int. Rev. Cytol.* **122**, 1 (1990).
5. J. Bereiter-Hahn and M. Voth, *Microsc. Res. Tech.* **27**, 198 (1994).
6. K. R. Porter and M. A. Bonneville, *Fine Structure of Cells and Tissues* (Lea & Febiger, Philadelphia, 1973).
7. F. Mignotte, M. Tourte, J.-C. Mounolou, *Biol. Cell* **60**, 97 (1989); D. K. Barnett, J. Kimura, B. D. Bavister, *Dev. Dyn.* **205**, 64 (1996).
8. M. T. Fuller, in *The Development of* Drosophila melanogaster, M. Bate and A. M. Arias, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993), pp. 71–147.
9. B. Tandler and C. L. Hoppel, *Ann. N.Y. Acad. Sci.* **488**, 65 (1986); T. Inagaki *et al.*, *Hepatology* **15**, 46 (1992).
10. I. Nishino *et al.*, *Muscle Nerve* **21**, 40 (1998).
11. E. Arbustini *et al.*, *Am. J. Pathol.* **153**, 1501 (1998).
12. M. Djaldetti, *Acta Haematol.* **68**, 241 (1982); C. E. Eapen *et al.*, *Scand. J. Gastroenterol.* **33**, 975 (1998).
13. S. J. McConnell, L. C. Stewart, A. Talin, M. P. Yaffe, *J. Cell Biol.* **111**, 967 (1990).
14. N. Hirokawa, *Science* **279**, 519 (1998).
15. E. Smirnova, D. L. Shurland, S. N. Ryazantsev, A. M. van der Bliek, *J. Cell Biol.* **143**, 351 (1998).
16. D. Otsuga *et al.*, *ibid.*, p. 333.
17. K. G. Hales and M. T. Fuller, *Cell* **90**, 121 (1997).
18. M. P. Yaffe *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11664 (1996).
19. K. H. Berger, L. F. Sogo, M. P. Yaffe, *J. Cell Biol.* **136**, 545 (1997).
20. C. Jamet-Vierny, V. Contamine, J. Boulay, D. Zickler, M. Picard, *Mol. Cell. Biol.* **17**, 6359 (1997).
21. M. R. Lewis and W. H. Lewis, *Am. J. Anat.* **17**, 339 (1915).
22. R. Altmann, *Die Elementarorganismen und ihre Beziehungen zu den Zellen* (Veit, Leipzig, 1890); I. E. Wallin, *Am. J. Anat.* **36**, 131 (1925).
23. L. Margulis, *Origin of Eukaryotic Cells* (Yale Univ. Press, New Haven, CT, 1970).
24. M. H. Heggeness, M. Simon, S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3863 (1978).
25. J. R. Couchman and D. A. Rees, *Eur. J. Cell Biol.* **27**, 47 (1982); I. C. Summerhayes, D. Wong, L. B. Chen, *J. Cell Sci.* **61**, 87 (1983).
26. O. Baumann and D. B. Murphy, *Cell Motil. Cytoskelet.* **32**, 305 (1995).
27. E. H. Ball and S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 123 (1982).
28. G. Steinberg and M. Schliwa, *J. Cell Sci.* **106**, 555 (1993).
29. R. L. Morris and P. J. Hollenbeck, *J. Cell Biol.* **131**, 1315 (1995).
30. R. D. Vale, *Annu. Rev. Cell Biol.* **3**, 347 (1987).
31. _____ and R. J. Fletterick, *Annu. Rev. Cell Dev. Biol.* **13**, 745 (1997).
32. M. Nangaku *et al.*, *Cell* **79**, 1209 (1994).
33. A. J. Pereira, B. Dalby, R. J. Stewart, S. J. Doxsey, L. S. B. Goldstein, *J. Cell Biol.* **136**, 1081 (1997).
34. Y. Tanaka *et al.*, *Cell* **93**, 1147 (1998).
35. K. L. David-Ferreira and J. F. David-Ferreira, *Int. Cell Biol. Rep.* **4**, 655 (1980).
36. N. Hirokawa, *J. Cell Biol.* **94**, 129 (1982).
37. B. Kachar and T. S. Reese, *ibid.* **106**, 1545 (1988).
38. R. J. Adams and T. D. Pollard, *Nature* **322**, 754 (1986).
39. K. Stürmer, O. Baumann, B. Walz, *J. Cell Sci.* **108**, 2273 (1995); T. J. Bradley and P. Satir, *J. Supramol. Struct.* **12**, 165 (1979).
40. D. G. Drubin, H. D. Jones, K. F. Wertman, *Mol. Biol. Cell* **4**, 1277 (1993).
41. D. A. Lazzarino, I. Boldogh, M. G. Smith, J. Rosand, L. A. Pon, *ibid.* **5**, 807 (1994).
42. V. R. Simon, T. C. Swayne, L. A. Pon, *J. Cell Biol.* **130**, 345 (1995).
43. H. Liu and A. Bretscher, *Cell* **57**, 233 (1989).
44. H. V. Goodson, B. L. Anderson, H. M. Warrick, L. A. Pon, J. A. Spudich, *J. Cell Biol.* **133**, 1277 (1996).
45. K. H. Berger and M. P. Yaffe, *Experientia* **52**, 1111 (1996).
46. D. E. Warnock and S. L. Schmid, *Bioessays* **18**, 885 (1996).
47. A. E. Gammie, L. J. Kurihara, R. B. Vallee, M. D. Rose, *J. Cell Biol.* **130**, 553 (1995).
48. S. D. Fields, M. N. Conrad, M. Clarke, *J. Cell Sci.* **111**, 1717 (1998).
49. Q. L. Zhu, D. Hulen, T. Y. Liu, M. Clarke, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7308 (1997).
50. B. J. Stevens, *Biol. Cell* **28**, 37 (1977); R. Azpiroz and R. A. Butow, *Mol. Biol. Cell* **4**, 21 (1993); J. Nunnari *et al.*, *Mol. Biol. Cell* **8**, 1233 (1997).
51. Y. Goda, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 769 (1997).
52. D. Rapaport, M. Brunner, W. Neupert, B. Westermann, *J. Biol. Chem.* **273**, 20150 (1998).
53. G. J. Hermann *et al.*, *J. Cell Biol.* **143**, 359 (1998).
54. A. Santel, N. Blumer, M. Kampfer, R. Renkawitz-Pohl, *J. Cell Sci.* **111**, 3299 (1998).
55. B. Stevens, in *The Molecular Biology of the Yeast* Saccharomyces cerevisiae, J. N. Strathern, E. W. Jones, J. R. Broach, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1981), vol. 1, pp. 471–504.
56. A. Tzagoloff and C. L. Dieckmann, *Microbiol. Rev.* **54**, 211 (1990).
57. G. J. Hermann and J. M. Shaw, *Annu. Rev. Cell Biol.* **14**, 265 (1998).
58. H. A. Fisk and M. P. Yaffe, *J. Cell Biol.* **138**, 485 (1997).
59. S. J. McConnell and M. P. Yaffe, *Science* **260**, 687 (1993).
60. _____, *J. Cell Biol.* **118**, 385 (1992).
61. K. A. Shepard and M. P. Yaffe, *Mol. Biol. Cell* **8**, 444a (1997).
62. G. J. Hermann, E. J. King, J. M. Shaw, *J. Cell Biol.* **137**, 141 (1997).
63. L. F. Sogo and M. P. Yaffe, *ibid.* **126**, 1361 (1994).
64. S. M. Burgess, M. Delannoy, R. E. Jensen, *ibid.*, p. 1375.
65. K. H. Berger and M. P. Yaffe, *Mol. Biol. Cell* **9**, 102a (1998).
66. K. A. Shepard and M. P. Yaffe, *J. Cell Biol.*, in press.
67. B. A. Jones and W. L. Fangman, *Genes Dev.* **6**, 380 (1992); K. Guan, L. Farh, T. K. Marshall, R. J. Deschenes, *Curr. Genet.* **24**, 141 (1993).
68. L. Pelloquin, P. Belenguer, Y. Menon, B. Ducommun, *Biochem. Biophys. Res. Commun.* **251**, 720 (1998).
69. H. A. Fisk, thesis, University of California, San Diego (1998); _____ and M. P. Yaffe, in preparation.
70. J. Huibregtse, M. Scheffner, S. Beaudenon, P. M. Howley, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2563 (1995); M. Hochstrasser, *Cell* **84**, 813 (1996).
71. A. D. Roeder, G. J. Hermann, B. R. Keegan, S. A. Thatcher, J. M. Shaw, *Mol. Biol. Cell* **9**, 917 (1998).
72. T. C. Huffaker, J. H. Thomas, D. Botstein, *J. Cell Biol.* **106**, 1997 (1988); C. W. Jacobs, A. E. M. Adams, P. J. Szaniszlo, J. R. Pringle, *ibid.* **107**, 1409 (1988).

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