The role of Fis1p–Mdv1p interactions in mitochondrial fission complex assembly

Mary Anne Karren, Emily M. Coonrod, Teresa K. Anderson, and Janet M. Shaw

Mitochondrial division requires coordinated interactions among Fis1p, Mdv1p, and the Dnm1p GTPase, which assemble into fission complexes on the outer mitochondrial membrane. The integral outer membrane protein Fis1p contains a cytoplasmic domain consisting of a tetratricopeptide repeat (TPR)–like fold and a short NH$_2$-terminal helix. Although it is known that the cytoplasmic domain is necessary for assembly of Mdv1p and Dnm1p into fission complexes, the molecular details of this assembly are not clear. In this study, we provide new evidence that the Fis1p–Mdv1p interaction is direct. Furthermore, we show that conditional mutations in the Fis1p TPR-like domain cause fission complex assembly defects that are suppressed by mutations in the Mdv1p-predicted coiled coil. We also define separable functions for the Fis1p NH$_2$-terminal arm and TPR-like fold. These studies suggest that the concave binding surface of the Fis1p TPR-like fold interacts with Mdv1p during mitochondrial fission and that Mdv1p facilitates Dnm1p recruitment into functional fission complexes.

Introduction

In most eukaryotic cells, mitochondria form dynamic tubular networks that undergo frequent fusion and fission events. Studies in fungi (Hermann and Shaw, 1998; Yaffe, 1999; Jensen et al., 2000; Shaw and Nunnari, 2002; Westermann, 2002, 2003; Westermann and Prokisch, 2002; Mozdy and Shaw, 2003; Oстерюng and Nunnari, 2003), worms (Labrousse et al., 1999; van der Bieke, 2000), flies (Hales and Fuller, 1997), and mammals (Bossy-Wetzel et al., 2003; Chen and Chan, 2004; Okamoto and Shaw, 2005) indicate that these processes play a critical role in maintaining normal mitochondrial function. In cases where mitochondrial fission is compromised, a variety of defects have been reported. For example, fission is essential for mitochondrial fragmentation and cytochrome $c$ release during apoptosis in both worms (Jagasia et al., 2005) and mammalian cell culture (Frank et al., 2001; James et al., 2003; Karbowski and Youle, 2003; Lee et al., 2004). Mitochondrial fission events affect mitochondria–ER interactions important for cellular Ca$^{2+}$ homeostasis and Ca$^{2+}$-regulated steps in apoptosis (Scorrano, 2003; Szabadkai et al., 2004). A recent study demonstrated that blocking mitochondrial fission limits the maintenance and formation of synapses in cultured neurons, which are processes critical for learning and memory (Li et al., 2004). The latter study raises the possibility that mitochondrial fission defects contribute to a variety of neurodegenerative diseases linked to mitochondrial dysfunction, including Parkinson’s disease, Alzheimer’s disease, and Huntington’s chorea.

Mitochondrial fission has been studied extensively in the budding yeast Saccharomyces cerevisiae. Yeast mitochondrial fission requires coordinated interactions between at least three proteins—Fis1p, Dnm1p, and Mdv1p. Fis1p is a tail-anchored outer membrane protein with a tetratricopeptide repeat (TPR)–like domain facing the cytoplasm (Mozdy et al., 2000; Suzuki et al., 2005). Dnm1p is a large GTPase related to mammalian dynamin (Otsuga et al., 1998; Bleazard et al., 1999; Sesaki and Jensen, 1999; Fukushima et al., 2001), and Mdv1p is a WD-repeat protein with an NH$_2$-terminal arm and TPR-like fold. These studies suggest that the concave binding surface of the Fis1p TPR-like fold interacts with Mdv1p during mitochondrial fission and that Mdv1p facilitates Dnm1p recruitment into functional fission complexes.
These structures, referred to as fission complexes, catalyze mitochondrial division. Only a subset of these complexes are associated with sites that subsequently divide, indicating that assembly and fission are temporally distinct events (Shaw and Nunnari, 2002; Legesse-Miller et al., 2003). Early events include recruitment and assembly of fission complexes, and late events presumably activate processes culminating in membrane scission.

Mdv1p, Dnm1p, and Fis1p interact with each other in vivo, and the domains required for some of these interactions have been mapped (Tieu et al., 2002; Cerveny and Jensen, 2003). However, the order of protein addition into fission complexes and the direct physical contacts required for assembly are unclear. One model suggests that Dnm1p initially assembles onto mitochondria to mark the site of fission and subsequently recruits both Mdv1p and Fis1p to form active fission complexes (Cerveny and Jensen, 2003). A variation of this model postulates that Fis1p-dependent recruitment of Dnm1p occurs first and that addition of Mdv1p remodels and activates the fission complex (Shaw and Nunnari, 2002; Tieu et al., 2002; Osteryoung and Nunnari, 2003). A third possibility is that Fis1p–Mdv1p interactions are a prerequisite for Dnm1p recruitment and assembly. Regardless of the accuracy of these models, Fis1p clearly plays a critical role in assembly because neither Dnm1p nor Mdv1p is recruited to mitochondria in its absence (Shaw and Nunnari, 2002).

The Fis1p cytoplasmic domain contains two distinct structural elements. Structures of mouse (Holm and Sander, 1993), human (Suzuki et al., 2003; Dohm et al., 2004), and yeast Fis1 (Suzuki et al., 2005) proteins reveal that the bulk of the cytoplasmic domain forms a highly conserved TPR-like fold with a concave surface predicted to form a binding interface (D’Andrea and Regan, 2003). The second structural element of the Fis1p cytoplasmic domain is a stretch of residues NH₂-terminal to the TPR-like domain (Suzuki et al., 2003, 2005; Dohm et al., 2004), hereafter referred to as the NH₂-terminal arm. In yeast, the first six residues of the NH₂-terminal arm are flexible, followed by a short helix that binds the concave surface of the TPR-like domain (Suzuki et al., 2005). Multiple functions for this arm have been proposed. One model suggests that the arm facilitates Mdv1p recruitment into fission complexes, and a variation of this model proposes that the arm regulates access to the concave binding surface (Dohm et al., 2004; Suzuki et al., 2005). Both scenarios predict that the Fis1p NH₂-terminal arm and TPR-like domain function independently, although this idea has not been rigorously tested.

To understand the mechanism of Fis1p function, we analyzed Fis1 proteins containing mutations in the TPR-like binding pocket or lacking the NH₂-terminal arm. Our analyses show that Mdv1p binds to the concave surface of the Fis1p TPR-like domain. In support of this idea, mutations exposed at this concave surface are suppressed by mutations in Mdv1p. We provide new evidence that the Fis1p–Mdv1p interaction is direct and define separable functions for the Fis1p NH₂-terminal arm and TPR-like domain at early and late steps during fission. In addition, our results support a revised model in which Dnm1p recruitment into functional fission complexes depends on Fis1p–Mdv1p interactions.

Results

The temperature-sensitive fis1-3 mutant disrupts mitochondrial fission at 37°C

We hypothesized that the concave TPR-like binding pocket of Fis1p is important for interaction with either Mdv1p or Dnm1p. To test this idea, we isolated fis1 temperature-sensitive mutations (fis1-ts; see Materials and methods) and chose one allele with mutations in the concave surface of the TPR-like domain for further study. This allele (fis1-3) encodes three amino acid substitutions (E78D, I85T, and Y88H; Fig. 1 A) that alter semiconserved or conserved residues in helix 4 of the TPR-like domain. Fis1-3p expression and targeting to mitochondria is similar to wild type at both permissive (25°C) and nonpermissive (37°C) temperatures (unpublished data). Individual and pair-wise analyses of each mutation indicate that all three contribute to the temperature-sensitive phenotype of the fis1-3 allele (unpublished data).

The ability of Fis1-3p to mediate mitochondrial fission was tested by plate phenotype in an fzo1Δ fis1Δ strain background. FZO1 is a gene required for mitochondrial fusion. In fzo1Δ strains with a functional copy of Fis1p, mitochondrial fission causes fragmentation, loss of mitochondrial genomes, and a respiratory defect (Hermann et al., 1998; Rapaport et al., 1998). This respiratory defect prevents growth on media containing nonfermentable carbon sources, such as glycerol (Fig. 1 B, top). Blocking fission in fzo1Δ cells by mutating fis1 prevents mitochondrial fragmentation and genome loss, allowing the fzo1Δ fis1Δ mutant strains to grow on glycerol (Fig. 1 B, middle). fzo1Δ fis1Δ cells expressing Fis1-3p from a plasmid fail to grow on glycerol at 25°C, demonstrating that Fis1-3p mediates fission at this temperature. Growth on glycerol is

Figure 1.  Mutations in the TPR-like domain of Fis1-3p cause a temperature-sensitive fission defect. [A] Schematic representations of the yeast Fis1-3p cytoplasmic domain with mutated residues indicated in red and the NH₂-terminal 15 residues in blue. Ribbon diagram shows mutated residues in α-helix 4 of the TPR-like domain (left). Space-fill representations of Fis1-3p showing the position of mutated residues in the concave TPR-like binding pocket are shown with (middle) or without (right) the NH₂-terminal 15 residues. [B] Growth phenotypes of wild-type and fission mutant strains (4 × 10³ cells per spot) grown on 5% glycerol selective medium at 25 or 37°C. [C] Quantification of mitochondrial morphology in strains grown at 25 or 37°C (n ≥ 300; SDs are indicated).
partially restored at 37°C, indicating that Fis1-3p is temperature sensitive for mitochondrial fission (Fig. 1 B, bottom).

The fis1-3 allele was integrated at the FIS1 locus, and mitochondrial morphology was quantified in log phase cells grown at 25°C or after shifting to 37°C for 2 h. Previous studies showed that fission mutants contain a range of defective mitochondrial morphologies, including a single mitochondrion collapsed to one side of the cell, mitochondrial nets, or mitochondrial tubules lacking branches (Otsuga et al., 1998; Bleazard et al., 1999; Sesaki and Jensen, 1999). By contrast, fission-competent cells contain multiple or branched mitochondrial networks distributed at the cell cortex. In this study, we categorize the latter phenotypic group as “fission competent.” When grown at 25 or 37°C, 96% of wild-type cells and 0% of fis1Δ cells contain fission-competent mitochondria (Fig. 1 C). fis1-3 cells, however, displayed 73% fission-competent mitochondria at 25°C versus 2% at 37°C, demonstrating that this allele causes temperature-sensitive mitochondrial morphology defects.

**Fis1-3p disrupts mitochondrial fission complex assembly**

Fis1-3p could affect assembly of fission complexes or later events that activate fission. To distinguish between these possibilities, we evaluated GFP-tagged Dnm1p or Mdv1p localization in fis1-3 cells grown at 25°C or shifted to 37°C for 2 h.

In wild-type cells, Dnm1-GFP in fission complexes appears as punctate structures distributed evenly along mitochondrial tubules (Fig. 2 A). At 25°C, ~95% of wild-type and fis1-3 cells contain Dnm1-GFP puncta that colocalize with mitochondrial tubules. This localization pattern is maintained in FIS1 wild-type cells shifted to 37°C. Conversely, all visible Dnm1-GFP localizes to the cytoplasm in fis1-3 cells shifted to 37°C, either in rapidly moving dots or in several randomly localized structures, similar to those in fis1Δ cells.

Localization of functional GFP-Mdv1p to mitochondria is also significantly reduced in fis1-3 mdv1Δ cells at both temperatures (Fig. 3 A). In wild-type cells, GFP-Mdv1p localizes in punctate fission complexes (Tieu and Nunnari, 2000; Cerveny et al., 2001) and is sometimes also uniformly distributed on mitochondrial tubules (unpublished data). Both localization patterns were scored as punctate GFP-Mdv1p localization in this study. In the majority of FIS1 mdv1Δ cells, punctate GFP-Mdv1p colocalizes with mitochondria at both temperatures. However, GFP-Mdv1p puncta on mitochondria are only observed in 49% of fis1-3 mdv1Δ cells at 25°C. These puncta are often nearly obscured by cytosolic fluorescence not present in FIS1 control cells expressing equivalent amounts of GFP-Mdv1p (unpublished data). At 37°C, GFP-Mdv1p localizes almost entirely to the cytoplasm in fis1-3 mdv1Δ cells, similar to the GFP-Mdv1p localization pattern in fis1Δ mdv1Δ cells. These studies demonstrate that Dnm1-GFP and GFP-Mdv1p do not assemble into mitochondrial fission complexes in fis1-3 cells at 37°C.

**Mdv1E250Gp suppresses mitochondrial fission defects in fis1-3 cells**

We screened for suppressor mutations in either Dnm1p or Mdv1p that rescued mitochondrial fission defects at 37°C (see Materials and methods). At the time of submission, we had
identified no _dnm1_ alleles that suppressed _fis1-3_ phenotypes. However, we identified several robust suppressor alleles that altered residues in _MDV1_. These suppressor mutations are located in or immediately upstream of the _MDV1_ predicted coiled-coil–forming domain. One of these, _mdv1^{E250G}_p (Fig. 4 A), was characterized in detail.

A glycerol growth assay with the _fzo1-1_ strain indicates that _Mdv1^{E250G}_p restores mitochondrial fission in _fis1-3_ cells at 37°C. In temperature-sensitive _fzo1-1_ cells, ongoing mitochondrial fission causes fragmentation, mitochondrial genome loss, and inability to grow on glycerol medium at 37°C (Fig. 4 B, top; Hermann et al., 1998). Disrupting fission in this strain by introducing _fis1-3_ mutations prevents mitochondrial fragmentation and genome loss, allowing _fzo1-1 fis1-3_ strains to grow on glycerol at the elevated temperature. Expression of wild-type _Mdv1p_ from a plasmid does not restore the temperature-sensitive glycerol growth defect in this strain (Fig. 4 B, middle). By contrast, _fzo1-1 fis1-3_ cells expressing _Mdv1^{E250G}_p from a plasmid fail to grow on glycerol at 37°C (Fig. 4 B, bottom), indicating that _Mdv1^{E250G}_p restores mitochondrial fission.

The effect of _Mdv1^{E250G}_p on _fis1-3_ mitochondrial morphology was compared in cells expressing either wild-type _Mdv1p_ or _Mdv1^{E250G}_p from the inducible _MET25_ promoter. Although the suppressors were isolated by plate phenotype without induction of the _MET25_ promoter, suppression of mitochondrial morphology defects was optimal in log phase cultures after induction for 2 h in medium lacking methionine. Under these conditions, mitochondria in 70% of _fis1-3_ _mdv1Δ_ cells incubated at 37°C and expressing _Mdv1^{E250G}_p are tubular and branched, with multiple mitochondria per cell (Fig. 4 C). By contrast, <10% of _fis1-3_ _mdv1Δ_ cells expressing equivalent amounts of wild-type _Mdv1p_ have fission-competent mitochondria.

_Mdv1^{E250G}_p rescues fission phenotypes in _mdv1Δ_ cells and behaves like wild-type _Mdv1p_ under all other conditions tested (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200506158/DC1). Thus, the effects of the _Mdv1^{E250G}_ suppressor protein are only apparent in _fis1_ mutant cells.

**_Mdv1^{E250G}_p suppresses mitochondrial fission complex assembly defects in _fis1-3_ cells**

Localization experiments confirm that fission complex assembly is restored in _fis1-3_ cells expressing _Mdv1^{E250G}_p. After induction of _Mdv1^{E250G}_p from the _MET25_ promoter in the _fis1-3 mdv1Δ_ strain at 37°C, Dnm1-GFP assembles into fission complexes on mitochondria in 75% of cells (Fig. 2 B, bottom). Interestingly, overexpression of wild-type _Mdv1p_ also allows Dnm1-GFP to assemble into punctate structures in 30% of the population at 37°C (Fig. 2 B, top). However, mitochondrial fission is not significantly rescued in these cells (Fig. 4 C). Thus, although Dnm1p can be recruited to Fis1-3p–containing mitochondria by overexpressed _Mdv1p_, this Dnm1p localization is not sufficient to mediate mitochondrial fission. Instead, restoration of mitochondrial fission in _fis1-3_ cells requires the _Mdv1^{E250G}_ suppressor protein.

Analysis of GFP-Mdv1^{E250G}_p localization in _fis1-3_ _mdv1Δ_ cells demonstrates that the _E250G_ substitution enhances _Mdv1p_ recruitment to _Fis1-3p_–containing mitochondria. At 25°C, GFP-Mdv1^{E250G}_p localizes to punctate structures on mitochondria in 91% of cells, a 40% increase in localization compared with wild-type GFP-Mdv1p. After shifting to 37°C for 2 h, GFP-Mdv1^{E250G}_p continues to localize to mitochondria in 26% of the population as compared with 3% of GFP-Mdv1p–expressing cells. These results demonstrate that GFP-Mdv1^{E250G}_p localizes more efficiently to _Fis1-3p_–containing mitochondria than GFP-Mdv1p.
Fis1-3p interaction defects are suppressed by Mdv1E250Gp

In vivo localization experiments show that Fis1-3p abolishes recruitment of both Mdv1p and Dnm1p to mitochondria at 37°C and that Mdv1E250Gp suppresses these defects. As a complementary approach, we used coimmunoprecipitation (coIP) assays to analyze the physical interaction between wild-type or mutant Fis1 proteins with either Dnm1p or Mdv1p. We also tested the effect of the Mdv1E250Gp suppressor on these interactions.

Previous attempts to coimmunoprecipitate Fis1p and Dnm1p from yeast have been unsuccessful, possibly because Dnm1p-containing structures are easily dissociated from Fis1p and mitochondria during cell solubilization. To prevent this dissociation, we spheroplasted cells expressing wild-type 9Myc–Fis1p or mutant 9Myc–Fis1-3p and treated with a cross-linking reagent, dithiobis(sulfosuccinimidylpropionate) (DSP), before homogenization. After immunoprecipitation with anti-Myc–conjugated beads, the eluted protein fractions were assayed by immunoblotting with Dnm1p-specific antibodies.

9Myc-Fis1p and 9Myc–Fis1-3p are efficiently immunoprecipitated from DSP-treated cells (Fig. 5 A, anti-Myc, lanes 2, 4, and 6), and a fraction of cellular Dnm1p reproducibly coimmunoprecipitates with 9Myc-Fis1p (Fig. 5 A, anti-Dnm1p, lane 2). Neither Fis1p nor Dnm1p immunoprecipitates when Fis1p lacks the 9Myc tag (Fig. 5 A, anti-Dnm1p and anti-Myc, lane 8), indicating that the precipitation is antibody dependent. Significantly, Dnm1p does not coimmunoprecipitate with 9Myc–Fis1-3p when wild-type Mdv1p is expressed (Fig. 5 A, anti-Dnm1p, lane 4), confirming that the Fis1-3p mutations disrupt Dnm1p–Fis1p complex formation. Expression of Mdv1E250Gp rather than Mdv1p increases the amount of Dnm1p recovered with 9Myc–Fis1-3p (Fig. 5 A, anti-Dnm1p, lane 6), although not to wild-type levels. Together, the in vivo localization and coIP experiments demonstrate that Mdv1E250Gp partially restores Dnm1p association with Fis1-3p in mitochondrial fission complexes.

CoIP assays to test the Fis1-3p physical interaction with Mdv1p were performed from solubilized cells (without cross-linking) expressing 9Myc-Fis1p or 9Myc–Fis1-3p. Proteins were immunoprecipitated with anti-Myc–conjugated beads, and eluted proteins were analyzed by Western blotting with Mdv1p-specific antibodies. Both 9Myc-Fis1p and 9Myc–Fis1-3p are efficiently immunoprecipitated from cell lysates (Fig. 5 B, anti-Myc, lanes 2, 4, 6, and 8). As reported previously, Mdv1p coimmunoprecipitates with 9Myc-Fis1p (Fig. 5 B, anti-Mdv1p, lane 2; Tieu et al., 2002) in an antibody-dependent manner (Fig. 5 B, anti-Myc, lanes 2, 4, 6, and 8). As reported previously, Mdv1p coimmunoprecipitates with 9Myc-Fis1p (Fig. 5 B, anti-Mdv1p, lane 2; Tieu et al., 2002) in an antibody-dependent manner (Fig. 5 B, anti-Myc, lanes 2, 4, 6, and 8). As reported previously, Mdv1p coimmunoprecipitates with 9Myc-Fis1p (Fig. 5 B, anti-Myc, lanes 2, 4, 6, and 8). As reported previously, Mdv1p coimmunoprecipitates with 9Myc-Fis1p (Fig. 5 B, anti-Mdm1p and anti-Myc, lanes 10 and 12). However, Mdv1p does not coimmunoprecipitate appreciably with 9Myc–Fis1-3p (Fig. 5 B, anti-Mdv1p and anti-Myc, lanes 10 and 12). This result confirms that the Fis1-3p mutations disrupt physical interactions with Mdv1p. When Mdv1E250Gp rather than Mdv1p is expressed, significantly more Mdv1E250Gp coimmunoprecipitates with both 9Myc-Fis1p and 9Myc–Fis1-3p (Fig. 5 B, anti-Mdv1p, lanes 4 and 8). This result demonstrates that cellular Mdv1E250Gp interacts with Fis1 proteins more efficiently than wild-type Mdv1p.
The Mdv1 E250G substitution primarily affects interactions between Mdv1p and Fis1p

Mdv1p can interact with Fis1p, with Dnm1p, and with itself (Tieu et al., 2002; Cerveny and Jensen, 2003). The E250G substitution might stabilize fission complex assembly and suppress fis1-3 phenotypes by affecting one or more of these binding partner interactions. We were unable to purify soluble forms of Mdv1p or Mdv1E250Gp to quantitatively measure relative binding affinities in vitro. Instead, we used a two-hybrid approach to determine qualitatively which Mdv1 protein interactions are most affected by the E250G substitution.

As published previously (Tieu et al., 2002; Cerveny and Jensen, 2003), Mdv1p fused to the Gal4 activating domain (AD-Mdv1p) interacts with the Fis1p cytoplasmic domain fused to the Gal4 DNA-binding domain (BD-Fis11–127p) to stimulate transcription of GAL4 reporter genes (Fig. 6 A). Although AD-Mdv1p fails to interact with the mutant BD–Fis1-31–127p, the suppressor AD-Mdv1 E250Gp interacts robustly with BD–Fis1-31–127p. Introducing the E250G mutation does not affect interaction of Mdv1p with Dnm1p (Fig. 6 B) or with itself (Fig. 6 C) in this assay. In control experiments, constructs paired with empty vectors did not activate transcription. These results are consistent with the idea that the E250G mutation primarily affects heterotypic interactions of Mdv1p with Fis1p, rather than with Dnm1p or with itself. In combination with our coIP experiments, these studies suggest that suppression of Fis1-3p by Mdv1E250Gp is primarily the result of increased interaction between Mdv1 and Fis1 proteins.

Fis1p interacts directly with Mdv1p

A mechanistic understanding of fis1-3 suppression by Mdv1E250Gp requires precise knowledge of how the wild-type proteins interact. To date, all experiments evaluating Fis1p–Mdv1p interactions have been performed under conditions where other yeast proteins are present (Tieu et al., 2002; Cerveny and Jensen, 2003). Therefore, these experiments do not determine whether Fis1p–Mdv1p interactions are direct or bridged by other yeast proteins. To address this issue, we performed a two-hybrid assay in bacterial cells (Fig. 7). The fis1 cytoplasmic domain (amino acids 1–127) and full-length MDV1 were cloned into bait plasmids (pBTs) and target plasmids (pTRGs), respectively, and expressed in bacteria. A positive interaction in this assay stimulates transcription of bacterial HIS3. This expression allows cells to grow in histidine-limiting conditions in the presence of 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product. In serial dilution experiments, cells cotransformed with pBT-fis11–127 (encoding amino acids 1–127) and pTRG-MDV1 grow significantly better on medium lacking histidine and containing 3AT than cells containing either construct alone. This experiment establishes for the first time that the Fis1p–Mdv1p interaction requires no additional yeast proteins.

Purified Fis1-3p is more susceptible to proteolytic cleavage than wild-type Fis1p

Fis1-3p mutations lie on the concave surface of the TPR-like fold. These substitutions could affect a specific binding surface without compromising the overall protein fold, or the mutations could cause global folding changes. To distinguish between these possibilities, limited proteolysis was performed on the purified cytoplasmic domains of Fis1p and Fis1-3p (amino acids 1–127 fused to 10His at the COOH terminus).

Western blot analysis shows that wild-type Fis11–12710His is extremely resistant to trypsin digestion, with uncut protein persisting at high protease concentrations (Fig. 8, top panel, top band). Digestion with increasing trypsin concentration converts this full-length protein to a stable, lower molecular weight band (Fig. 8, top panel, bottom band). Mass spectrometry of digested samples reveals a mixture of several proteolytic fragments generated predominantly by cleavage at the NH2 and COOH termini (unpublished data). NH2-terminal cleavage sites occur in the first five residues, consistent with nuclear magnetic resonance studies showing that the NH2-terminal five residues of yeast Fis1p are unstructured (Suzuki et al., 2005). COOH-terminal cleavage sites are located throughout the 10His tag used for purification. Importantly, fragments generated by cleavage at sites internal to the protein are rarely observed. Thus, the TPR-like domain and NH2-terminal helix form a highly stable fold, with the most labile portion at the extreme NH2 terminus.
By contrast, trypsin sites in the mutant Fis1-31–12710His are more accessible to protease. As observed with wild type, the higher molecular weight band (consisting of uncut protein) persists at high trypsin concentrations (Fig. 8, bottom panel, top band). However, digestion of the mutant protein yields multiple lower molecular weight products that are rapidly degraded as trypsin concentration increases (Fig. 8, bottom panel, bracket). Mass spectrometry confirms that trypsin digestion of the mutant protein does not produce the same stable lower molecular weight species as digestion of wild type (unpublished data). Instead, minor species contain cut sites at both the NH$_2$ and COOH termini and at random sites within the TPR-like fold. The presence of uncut protein in digested samples suggests that the mutant protein folds in vitro. However, the structure is clearly more labile in solution than the wild-type protein.

Mitochondrial fission complex assembly and fission can occur without the Fis1p NH$_2$-terminal arm

To further define the functions of the Fis1p NH$_2$-terminal arm and TPR-like domain, we evaluated mitochondrial morphology and fission complex assembly in cells expressing a Fis1 mutant lacking the NH$_2$-terminal arm (Fis115–155p). Fis115–155p alone or Fis115–155p and either Mdv1p or Mdv1E250Gp were expressed under control of the inducible MET25 promoter using a 30-min induction period, and mitochondrial morphology was quantified. Consistent with a previous study (Suzuki et al., 2005), <10% of fis1Δ cells expressing Fis115–155p and endogenous Mdv1p have fission-competent mitochondria. (Fig. 9 A). However, when both Fis115–155p and Mdv1p are simultaneously induced from MET25 promoters in fis1Δ mdv1Δ cells, 45% of cells contain fission-competent mitochondria. In control experiments, 58% of fis1Δ mdv1Δ cells expressing wild-type Fis1p and Mdv1p contain fission-competent mitochondria. Interestingly, Mdv1E250Gp and Mdv1p rescue mitochondrial fission defects to the same extent in this experiment (unpublished data).

We also evaluated the effect of the NH$_2$-terminal arm truncation on localization of GFP-tagged Dnm1p and Mdv1p in cells expressing Fis115–155p. Dnm1-GFP localizes to mitochondria in 44% of fis1Δ cells (44.1% ± 15.0 localized, n = 600) expressing high levels of Fis115–155p and endogenous Mdv1p but localizes to mitochondria in 75% of cells after a 30-min induction of both Fis115–155p and Mdv1p (75.2 ± 6.14 localized, n = 500). Thus, Dnm1-GFP localization is reduced in fis1Δ cells, and this defect is rescued by Mdv1p overexpression.

When low levels of Fis115–155p and GFP-Mdv1p are expressed in fis1Δ mdv1Δ cells, GFP-Mdv1p colocalizes with mitochondria in <10% of cells (Fig. 9 B). However, GFP-Mdv1p colocalizes with mitochondria in ~55% of these cells after induction of both Fis115–155p and GFP-Mdv1p. Interestingly, GFP-Mdv1E250Gp localizes to mitochondria significantly better than GFP-Mdv1p in these cells, both under low (49% localized) and high (76% localized) expression conditions. This result indicates that interactions between Mdv1p and the TPR-like domain of Fis1p are enhanced by the Mdv1 E250G substitution.

These combined observations suggest that the NH$_2$-terminal arm facilitates, but is not strictly required for, Mdv1p recruitment or stabilization of fission complexes. Moreover, the Fis1p TPR-like domain lacking the NH$_2$-terminal arm is sufficient to mediate fission complex assembly and fission under specific conditions.

**Discussion**

The experiments described here make three main advances. First, we define early and late functions for the Fis1p NH$_2$ terminus and TPR-like domain. Second, our data demonstrate that Fis1p and Mdv1p interact directly and strongly suggest that...
this interaction occurs via the concave surface of the TPR-like domain. Third, our experiments support a revision of current models for fission complex assembly. In the following sections, we discuss each of these issues in detail.

Separable functions of the Fis1p NH2-terminal arm and TPR-like domain

In this study, we sought to relate the structure of Fis1p to its molecular role in fission. Fis1p contains three distinctive features: a COOH-terminal transmembrane domain, an NH2-terminal arm, and a six-helix TPR-like domain with a hydrophobic pocket on its concave surface. In yeast, a short helical portion of the NH2-terminal arm occupies the hydrophobic pocket. The structure of the TPR-like fold, and presumably its function, is highly conserved among orthologues (Suzuki et al., 2003, 2005; Dohm et al., 2004). However, the length, conservation, and placement of the NH2-terminal arms differ (Mozdy et al., 2000; James et al., 2003; Yoon et al., 2003; Dohm et al., 2004; Stojanovski et al., 2004), suggesting that this region has acquired a function distinct from that of the TPR-like fold.

We provide evidence that the NH2-terminal arm acts early, during complex assembly. Consistent with a previous study (Suzuki et al., 2005), removal of the Fis1p NH2-terminal arm disrupts Mdv1p and Dnm1p recruitment into fission complexes. Thus, the NH2-terminal arm is important for an early assembly step. However, in the absence of the NH2-terminal arm, fission complex assembly and fission are rescued by over-expression of Mdv1p. Therefore, the NH2-terminal arm is not essential after assembly, and the TPR-like domain is sufficient for fission events once assembly has occurred.

Characterization of the Fis1p–Mdv1p interaction

To date, all experiments evaluating Fis1p–Mdv1p interactions have been performed in live cells (yeast two-hybrid assay and genetic studies) or extracts (coIP) containing other yeast proteins. Here we use an Escherichia coli two-hybrid assay to demonstrate that Fis1p and Mdv1p interact directly in the absence of other yeast proteins.

Is the Mdv1p-binding site on yeast Fis1p formed by the NH2-terminal arm, the TPR-like domain, or both? It has been hypothesized that the TPR-like hydrophobic pocket is a binding surface for other fission complex proteins (Dohm et al., 2004; Suzuki et al., 2005). Three lines of evidence that are presented in this study support the idea that Mdv1p occupies the Fis1p TPR-like binding pocket, possibly by displacing the NH2-terminal arm (Fig. 10). First, mutations in the Fis1p TPR-like binding pocket are suppressed by mutations in Mdv1p. Second, over-expressed Mdv1p interacts with Fis1p, which lacks the NH2-terminal arm. Third, the Mdv1p suppressor interacts with Fis1p, even when expressed at low levels. Thus, the TPR-like domain alone interacts with Mdv1p, and this interaction is augmented by the Mdv1p E250G substitution. Although these studies establish that Mdv1p interacts with the TPR-like domain, the Fis1p NH2-terminal arm is clearly important. We propose that the Fis1p NH2-terminal arm facilitates recruitment and/or stabilization of Mdv1p during fission complex assembly.

Mechanism of Fis1-3p suppression by Mdv1p

What molecular changes are caused by fis1-3 mutations, and how are these overcome by Mdv1p? We show that the Fis1p–binding pocket mutations increase the overall flexibility and protease sensitivity of the protein fold. However, the mutant protein can fold in vitro, is expressed and targeted to mitochondria at levels similar to wild type in vivo, and is rescued by the Mdv1p suppressor protein. We propose that the mutant protein fold resembles wild type but is more flexible at elevated temperatures. This increased flexibility weakens the interaction between the Fis1p TPR-like pocket and its binding partners, abolishing stable fission complex assembly. The E250G suppressor mutation stabilizes Fis1–Mdv1p interactions, and these stabilized complexes efficiently recruit Dnm1p to form functional fission complexes.

Three different assays indicate that fission complex stabilization results from increased affinity of Mdv1p for Fis1p. First, GFP-Mdv1p localizes to Fis1p-containing mitochondria more efficiently than wild-type GFP-Mdv1p. Second, significantly more Mdv1p coimmunoprecipitates with Fis1p and Fis1-3p compared with wild-type Mdv1p. Third, Mdv1p interaction with Fis1-3p is restored by the Mdv1p E250G mutation in yeast two-hybrid assays. Importantly, the Mdv1p suppressor protein primarily affects Mdv1p–Fis1p interactions; in two-hybrid assays, Mdv1p–Mdv1p and Mdv1p–Dnm1p interactions are unaffected by this suppressor mutation.

Despite its increased interaction with both wild-type Fis1p and Fis1-3p (Fig. 5 B), Mdv1p does not cause fission defects in cells expressing wild-type Fis1p. Thus, enhanced binding between Mdv1p and Fis1p does not appear to interfere with downstream events in fission. One possible interpretation of this result is that dissociation of Mdv1p from Fis1p is not required for late steps in fission.

Although the Mdv1p suppressor increases interactions with Fis1-3p, the suppression is not allele specific. Mdv1p also rescues other conditional fis1 alleles contain-
ing TPR domain mutations (unpublished data). Moreover, two additional MDV1 alleles containing mutations in or near the predicted coiled-coil suppress fission defects in fis1Δ-3 (unpublished data). We propose that different TPR-like binding pocket mutations in Fis1p cause similar structural changes and that these changes are suppressed by a novel class of MDV1 alleles with mutations in the predicted coiled coil.

Previous work showed that amino acids NH2-terminal to the Mdv1p coiled coil are sufficient for Fis1p interactions (Tieu et al., 2002). This study also suggested that the coiled-coil region mediates Mdv1p self-interactions. Our studies suggest that Mdv1p suppressor mutations in the predicted coiled-coil region affect Fis1p, but not Mdv1p, interactions. Clearly, the mechanisms regulating Mdv1p–Fis1p interactions in vivo are more complicated than previously appreciated. One possibility is that the Mdv1p coiled coil regulates binding of the Mdv1p NH2-terminal region to the Fis1p TPR-like domain.

A revised model for assembly of mitochondrial fission complexes

Current models propose that Dnm1p assembles into mitochondrial fission complexes, and Mdv1p recruitment into these complexes activates a rate-limiting step in the fission pathway (Shaw and Nunnari, 1999; Cerveny and Jensen, 2003; Oster-young and Nunnari, 2003). In our study, Dnm1p assembly defects in fis1Δ mutants are rescued by restoring Fis1p interactions with either Mdv1p or Mdv1E250Gp. Based on these findings, we favor the idea that direct association of Mdv1p with Fis1p precedes and facilitates Dnm1p recruitment (Fig. 10). According to this revised model, Mdv1p recruitment into fission complexes cannot be rate limiting.

Several other observations are consistent with the idea that Dnm1p assembles onto preexisting Fis1p–Mdv1p complexes. GFP-Mdv1p is constitutively and uniformly bound to Fis1p on the outer membrane as well as in fission complexes with Dnm1p (unpublished data). Thus, it is likely that Dnm1p assembles from the cytoplasm onto Fis1p–Mdv1p complexes. Although Dnm1p assembles into nonfunctional fission complexes in mdv1Δ-null cells (Fekkes et al., 2000; Mozdy et al., 2000; Tieu and Nunnari, 2000; Cerveny et al., 2001), we observed that the number, distribution, and size of Dnm1p complexes differ slightly from those in MDV1 wild-type cells (unpublished data). The latter observation is explained by a recent study showing that Mdv1p and its coiled coil are sufficient for Fis1p interactions (unpublished data). Moreover, two pocket mutations in Fis1p cause similar structural changes and that these changes are suppressed by a novel class of MDV1 alleles with mutations in the predicted coiled coil.

Materials and methods

Strain and plasmid construction

Strains used in this study were derived from the FY genetic background (Winston et al., 1995). Standard methods were used for growth, transformation, and genetic manipulation of S. cerevisiae (Sherman et al., 1986; Guthrie and Fink, 1991) and E. coli (Maniatis et al., 1982). All mutations, disruptions, tag insertions, and replacements were confirmed by PCR, DNA sequencing, and, where appropriate, Western blotting.
moter were induced for either 2 h or 30 min. For 2-h inductions, strains were diluted to 0.5 OD600 in medium lacking methionine. Strains induced for 30 min were grown for 1.5 h after dilution, resuspended in medium lacking methionine, and grown another 30 min into log phase. In log phase cultures under repressed conditions, the MET25 promoter is leaky and produces two-to-fivefold more Mdv1p or Mdv1ΔMDV1p than the endoge- nous MDV1 promoter. After induction for 30 min to 2 h, production of Mdv1p or Mdv1ΔMDV1p by the MET25 promoter increased to ~50-fold endogenous levels.

Cells were visualized using a microscope (Axioplan 2 Imaging; Carl Zeiss Microimaging, Inc.) equipped with differential interference con- trast (DIC) optics, epifluorescence capabilities, and a Plan-Apochromat ×100 objective (NA 1.4; Carl Zeiss Microimaging, Inc.). Filter sets (excita- tion/beam splitter emission) for GFP and red fluorescent protein fluores- cence were BP 470 ± 20 FT 495/5P 525 ± 25 and BP 546 ± 6 FT 560/6P 575–640, respectively. Images were captured and deconvolved using a monochrome digital camera (AxioCam; Carl Zeiss Microimaging, Inc.) and appropriate software (AxioVision 3.1; Carl Zeiss Microimaging, Inc.) and were assembled using Photoshop (Adobe Systems).

**Protein purification and limited proteolysis**

BL21(DE3) E. coli cells (Novagen) containing either pET24a + fis1Δ12710HIS or pET24a + fis1Δ12710HIS/ps in pET24a were grown in LB medium plus 30 μg/mL of kanamycin at 37°C to mid-log phase and induced with 1 mM IPTG for 2–4 h.

**CoIP**

Myc-Fis1p, or 9Myc–Fis1-3p from the genomic locus and either pRS416- MET25 or pRS416-mdv1E250G–Myc, and cells expressing Fis1-3 1–12710Hisp were induced at RT. Wild-type and mutant Fis1 fusion proteins were purified from cleared cell lysates on Ni2+ affinity column chromatography as described by the manu- facturer (Novagen).

**Limited proteolysis of purified Fis1 Δ12710Hisp and Fis1 Δ312710Hisp protein**

was performed by incubating 1 or 1.8 μg, respectively, in buffer (100 mM NaCl, 20 mM sodium phosphate, pH 7.0, 1 mM DTT, and 100 μM EDTA) with either 0, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, or 5.0 μg of trypsin (Sigma-Alrider) in a 10 μL reaction volume. Reactions were prepared on ice, incubated for 2 min at 37°C, and stopped by boiling in 5× SDS-PAGE sample buffer. Samples were analyzed by SDS-PAGE and Western blotting using Anti-Fis1p antibodies (Mozdy et al., 2000). For mass spectrometry analysis, purified proteins were analyzed by ESI-MS. Protein mixtures were digested with 4 μg of trypsin for 2 min at 37°C. Reactions were stopped with 1% trifluoroacetic acid at RT and then frozen at −80°C until analysis. Samples were analyzed by electrospray ionization at the University of Utah Mass Spectrometry Facility.

References

Arimura, S., and N. Tsutsumi. 2002. A dynamin-like protein (ADL2b), rather than FisZ, is involved in Arabidopsis mitochondrial division. Proc. Natl. Acad. Sci. USA. 99:5727–5731.

Bevis, B.J., and B.S. Glick. 2002. Rapidly mutating variants of the Discosoma [D. pulchra] red fluorescent protein (DsRed). Curr. Biol. 12:R73–R77.

Bleazard, W., J.M. McCaffery, E.J. King, S. Bale, A. Mozdy, Q. Tieu, J. Nunnari, and J.M. Shaw. 1999. The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast. Nat. Cell Biol. 1:298–304.

Boeke, J.D., J. Trueheart, G. Natsoulis, and G.R. Fink. 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. 154:164–175.

Bossy-Wetzel, E., M.J. Barsoum, A. Godzik, R. Schwarzenbacher, and S.A. Lipton. 2003. Mitochondrial fission in apoptosis, neurodegeneration and aging. Curr. Opin. Cell Biol. 15:706–716.

Cerveny, K.L., and R.E. Jensen. 2003. The HD-repeat of Net2p interacts with Dnm1p and Fis1p to regulate division of mitochondria. Mol. Biol. Cell. 14:4406–4419.

Cerveny, K.L., J.M. McCaffery, and R.E. Jensen. 2001. Division of mitochon- dria requires a novel DNM1-interacting protein, Net2p. Mol. Biol. Cell. 12:309–321.

Chen, H., and D.C. Chan. 2004. Mitochondrial dynamics in mammals. Curr. Top. Dev. Biol. 59:119–144.

D’Andrea, L.D., and L. Regan. 2003. TPR proteins: the versatile helix. Trends Biochem. Sci. 28:655–662.

Dohm, J.A., S.J. Lee, J.M. Hardwick, R.B. Hill, and A.G. Gittis. 2004. Cyto- solic domain of the human mitochondrial fusion protein fis1 adopts a TPR fold. Proteins. 54:153–156.

Fekkes, P., K.A. Shepard, and M.P. Yaffe. 2000. Gag3p, an outer membrane protein required for fission of mitochondrial tubules. J. Cell Biol. 151:333–340.

Frank, S., B. Gaume, E.S. Bergmann-Leitner, W.W. Leitner, E.G. Roberts, F. Catez, C.L. Smith, and R.J. Youle. 2001. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev. Cell. 1:515–525.

Fukushima, N.H., L. Brisch, B.R. Keegan, W. Bleazard, and J.M. Shaw. 2001. The GTPase effector domain sequence of the Dnm1p GTPase regulates self-assembly and controls a rate-limiting step in mitochondrial division. Mol. Biol. Cell. 12:2756–2766.

Griffin, E.E., J. Graumann, and D.C. Chan. 2005. The WD40 protein Caf4p is a component of the mitochondrial fission machinery and recruits Dnm1p to mitochondria. J. Cell Biol. 170:257–268.

Guthrie, C., and G. Fink. 1991. Guide to yeast genetics and molecular biology. Methods in Enzymology. Vol. 194. San Diego: Academic Press, Inc.
Guthrie, C., and G. Fink. 2002. Guide to yeast genetics and molecular biology. Methods in Enzymology. Vol. 350. San Diego: Academic Press, Inc.

Hales, K.G., and M.T. Fuller. 1997. Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase. Cell. 90:121–129.

Hermann, G.J., and J.M. Shaw. 1998. Mitochondrial dynamics in yeast. Annu. Rev. Cell Dev. Biol. 14:265–303.

Hermann, G.J., J.W. Thatcher, J.P. Mills, K.G. Hales, M.T. Fuller, J. Nunmari, and J.M. Shaw. 1998. Mitochondrial fusion in yeast requires the transmembrane GTGase Fzo1p. J. Cell Biol. 143:359–373.

Holm, L., and C. Sander. 1993. Protein structure comparison by alignment of distance matrices. J. Mol. Biol. 233:123–138.

Jagasia, R., P. Grote, B. Westermann, and B. Conradt. 2005. DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in C. elegans. Nature. 433:754–760.

Karbowski, M., and R.J. Youle. 2003. Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death Differ. 10:870–880.

Kondo-Okamoto, N., J.M. Shaw, and K. Okamoto. 2003. Mmm1p spans both the outer and inner mitochondrial membranes and contains distinct domains for targeting and foci formation. J. Biol. Chem. 278:36373–36379.

Lee, Y.J., S.Y. Jeong, M. Karbowski, C.L. Smith, and R.J. Youle. 2004. Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol. Biol. Cell. 15:5001–5011.

Legesse-Miller, A., R.H. Massol, and T. Kirchhausen. 2003. Constriction and Dnm1p recruitment are distinct processes in mitochondrial fission. Mol. Biol. Cell. 14:1953–1963.

Li, Z., K. Okamoto, Y. Hayashi, and M. Sheng. 2004. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell. 119:873–887.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.

Mozdy, A.D., and J.M. Shaw. 2003. A fuzzy mitochondrial fusion apparatus comes into focus. Nat. Rev. Mol. Cell Biol. 4:468–478.

Mozdy, A.D., J.M. McCaffery, and J.M. Shaw. 2000. Dnm1p GTGase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. J. Cell Biol. 151:367–380.

Mumberg, D., R. Muller, and M. Funk. 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Methods. Mol. Enzymol. 101:228–245.

Ostryoung, K.W., and J. Nunmari. 2003. The division of endosymbiotic organelles. Science. 302:1698–1704.

Otsuga, D., B.R. Keegan, E. Brisch, J.W. Thatcher, G.J. Hermann, W. Bleazard, and J.M. Shaw. 1998. The dynamin-related GTGase Dnm1p controls mitochondrial morphology in yeast. J. Cell Biol. 143:333–349.

Rapaport, D., M. Brunner, W. Neupert, and B. Westermann. 1998. Fzo1p is a mitochondrial outer membrane protein essential for the biogenesis of functional mitochondria in Saccharomyces cerevisiae. J. Biol. Chem. 273:20150–20155.

Rothstein, R. 1991. Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. 194:281–301.

Scorrano, L. 2003. Divide et impera: Ca2+ signals, mitochondrial fission and sensitization to apoptosis. Cell Death Differ. 10:1287–1289.

Sesaki, H., and R.E. Jensen. 1999. Division versus fusion: Dnm1p and Fzo1p antagonistically regulate mitochondrial shape. J. Cell Biol. 147:699–706.

Sherman, F., G.R. Fink, and J.B. Hicks. 1986. Methods in Yeast Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 186 pp.

Smirnova, E., D.L. Shurland, S.N. Ryazantsev, and A.M. van der Bliek. 1998. A human dynamin-related protein controls the distribution of mitochondria. J. Cell Biol. 143:351–358.

Stojanovski, D., O.S. Koutouposoulos, K. Okamoto, and M.T. Ryan. 2004. Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. J. Cell Sci. 117:1201–1210.

Suzuki, M., S.Y. Jeong, M. Karbowski, R.J. Youle, and N. Tjandra. 2003. The solution structure of human mitochondria fission protein Fis1 reveals a novel TPR-like helix bundle. J. Mol. Biol. 334:445–458.

Suzuki, M., A. Neutzner, N. Tjandra, and R.J. Youle. 2005. Novel structure of the N terminus in yeast Fis1 correlates with a specialized function in mitochondrial fission. J. Biol. Chem. 280:21444–21452.

Szabadkai, G., A.M. Simoni, M. Chami, M.R. Wieckowski, R.J. Youle, and R. Rizzuto. 2004. Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+-mediated apoptosis. Mol. Cell. 16:59–68.

Tieu, Q., and J. Nunmari. 2000. Mdv1p is a WD repeat protein that interacts with the dynamin-related GTGase Dnm1p, to trigger mitochondrial division. J. Cell Biol. 151:353–366.

Tieu, Q., V. Okreglak, K. Naylor, and J. Nunmari. 2002. The WD repeat protein, Mdv1p, functions as a molecular adaptor by interacting with Dnm1p and Fis1p during mitochondrial fission. J. Cell Biol. 158:445–452.

van der Bliek, A.M. 2000. A mitochondrial division apparatus takes shape. J. Cell Biol. 151:F1–F4.

Westermann, B. 2002. Merging mitochondria matters: cellular role and molecular machinery of mitochondrial fusion. EMBO Rep. 3:527–531.

Westermann, B. 2003. Mitochondrial membrane fusion. Biochim. Biophys. Acta. 1641:195–202.

Westermann, B., and H. Proskisch. 2002. Mitochondrial dynamics in filamentous fungi. Fungal Genet. Biol. 36:91–97.

Yoon, Y., E.W. Krueger, B.J. Oswald, and M.A. McNiven. 2003. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. Mol. Cell. Biol. 23:5409–5420.