Importance of Mitochondrial Dynamics During Meiosis and Sporulation

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Opposing fission and fusion events maintain the yeast mitochondrial network. Six proteins regulate these membrane dynamics during mitotic growth—Dnm1p, Mdv1p, and Fis1p mediate fission; Fzo1p, Mgm1p, and Ugo1p mediate fusion. Previous studies established that mitochondria fragment and rejoin at distinct stages during meiosis and sporulation, suggesting that mitochondrial fission and fusion are required during this process. Here we report that strains defective for mitochondrial fission alone, or both fission and fusion, complete meiosis and sporulation. However, visualization of mitochondria in sporulating cultures reveals morphological defects associated with the loss of fusion and/or fission proteins. Specifically, mitochondria collapse to one side of the cell and fail to fragment during presporulation. In addition, mitochondria are not inherited equally by newly formed spores, and mitochondrial DNA nucleoid segregation defects give rise to spores lacking nucleoids. This nucleoid inheritance defect is correlated with an increase in petite spore colonies. Unexpectedly, mitochondria fragment in mature tetrads lacking fission proteins. The latter finding suggests either that novel fission machinery operates during sporulation or that mechanical forces generate the mitochondrial fragments observed in mature spores. These results provide evidence of fitness defects caused by fission mutations and reveal new phenotypes associated with fission and fusion mutations.

INTRODUCTION

Mitochondria contribute to a variety of cellular processes, including carbon metabolism, fatty acid metabolism, amino acid and nucleic acid biosynthesis, and programmed cell death (Attardi and Schatz, 1988; Pon and Schatz, 1991; Green and Reed, 1998). However, these organelles are best known for their ability to generate ATP through oxidative phosphorylation reactions that occur on enzyme complexes embedded in the inner mitochondrial membrane. A few of the protein subunits of these respiratory complexes are encoded by mitochondrial DNA (mtDNA) and are synthesized on mitochondrial ribosomes. As a consequence, newly formed daughter cells must inherit both the mitochondrial compartment and (in some cases) mtDNA to survive. The mechanism of mitochondrial partitioning into daughter cells during mitotic division has been studied in a variety of organisms and cell types and is usually mediated by a molecular motor that transports the organelle along polarized cytoskeletal elements (Birky, 1983, 2001; Hollenbeck, 1996; Boldogh et al., 2001).

Mitochondrial partitioning during mitosis has been studied extensively in the budding yeast, *Saccharomyces cerevisiae*. Yeast mitochondria form a network of interconnected tubules that coalign with actin filaments and cables at the cell cortex (Stevens, 1981; Simon et al., 1995, 1997; Simon and

Pon, 1996; Hermann et al., 1997; Hermann and Shaw, 1998). The morphology of this network is determined by opposing mitochondrial fission and fusion events that occur approximately every 2 min (Nunnari et al., 1997; Bleazard et al., 1999; Sesaki and Jensen, 1999; Shaw and Nunnari, 2002; Mozdy and Shaw, 2003). Mitochondrial fission occurs within a mitochondrial tubule or at a branch point in the network, whereas mitochondrial fusion occurs between two tubule tips or the tip and the side of a tubule. Approximately 10–50 structures called nucleoids are evenly distributed throughout the mitochondrial network (Williamson and Fennell, 1979). Each nucleoid consists of 5–10 copies of mtDNA packaged together with proteins (Williamson and Fennell, 1979). During mitotic division, a mitochondrial tubule with its resident nucleoids is transported along actin filaments and cables into the growing daughter cell (bud), where it appears to cluster at the bud tip (Yang et al., 1999). Subsequently, a new mitochondrial network is established in the bud before cytokinesis. Additional studies indicate that mtDNA nucleoids are also actively partitioned into daughter cells formed after mating and zygote formation (Azpiroz and Butow, 1993; Nunnari et al., 1997; Okamoto et al., 1997).

Mitochondrial partitioning during meiotic division appears to be more complex. During meiosis, the diploid yeast genome undergoes one round of replication and two rounds of segregation, yielding four haploid nuclei (Esposito and Klapholz, 1981; Kupiec et al., 1997) (Figure 1, nuclear stage). Because the nuclear envelope of *S. cerevisiae* does not break down during meiosis I or II, the result is a four-lobed, single nuclear compartment containing four haploid genomes (Figure 1, nuclear stage, meiosis II). Each haploid genome is ultimately enclosed by a prospore membrane and a cell wall, to form an individual spore (Figure 1, nuclear stage, four-spored ascus). Before completion of the prospore membrane and wall, additional cellular organelles fill the developing spore sac, and division of each nuclear lobe is completed
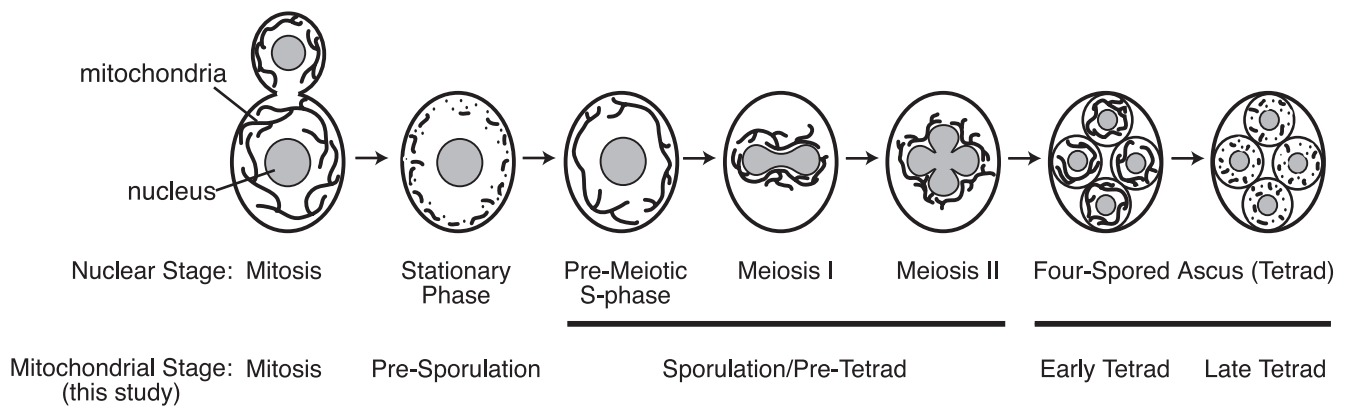
Figure 1. Mitochondrial dynamics during meiosis and sporulation in budding yeast. Nuclei (gray) and mitochondria (black) are depicted during mitosis, meiosis, and spore formation in *S. cerevisiae* (Esposito and Klapholz, 1981). The corresponding nuclear stage or mitochondrial stage (Miyakawa *et al.,* 1984; and this study) is indicated below each diagram. Mitotically dividing cells contain tubular mitochondrial networks (mitosis). Mitochondrial tubules fragment after cells arrest in G_1 and prepare for meiosis and sporulation (stationary phase, presporulation). mtDNA and respiration are essential throughout this stage. During premeiotic S-phase (beginning of sporulation/pretetrad stage), mitochondrial fragments rejoin to form tubules. Throughout meiosis I and II (middle and end of sporulation/pretetrad stage), mitochondrial tubules associate with the lobes of the dividing nucleus. The organelles remain tubular through the end of meiosis II and during early tetrad formation (early tetrad). After tetrads mature, mitochondrial tubules fragment once again (late tetrad; day 12 of sporulation in this study).

(Esposito and Klapholz, 1981; Kupiec *et al.,* 1997). The resulting four spores (a tetrad) are contained within the remains of the diploid mother cell, a protective structure called the ascus.

A number of stage-specific changes in mitochondrial morphology and distribution occur during yeast meiosis and sporulation (Miyakawa *et al.,* 1984) (Figure 1, mitochondrial stage). In the premeiotic stationary phase, mitochondria fragment and form puncta at the cell periphery (Figure 1, mitochondrial stage, presporulation). Each mitochondrial fragment contains one mtDNA nucleoid (Miyakawa *et al.,* 1984). During this period, functional mitochondrial genomes, respiration, and protein synthesis are essential for expression of the meiosis regulatory gene *IME1* (Treinin and Simchen, 1993) and progression through the meiotic program (Kuenzi *et al.,* 1974; Jayachandran *et al.,* 1977; Marmiroli *et al.,* 1983). In contrast, functional mitochondrial genomes and mitochondrial protein synthesis are dispensable during the remainder of meiosis, sporulation, and germination, although respiration does continue (Kuenzi *et al.,* 1974; Tingle *et al.,* 1974; Jayachandran *et al.,* 1977). Soon after transfer to sporulation medium, mitochondrial fragments generated during the stationary phase rejoin to form a tubular network (Miyakawa *et al.,* 1984) (Figure 1, nuclear stage, premeiotic S-phase, and mitochondrial stage, beginning of sporulation/pretetrad). Within this network, the mtDNA nucleoids coalesce and form a continuous thread that resembles "beads-on-a-string" (Miyakawa *et al.,* 1984). Before meiosis II, the mitochondrial network moves toward the cell center and becomes associated with the nuclear membrane, where it remains throughout meiosis II (Miyakawa *et al.,* 1984) (Figure 1, mitochondrial stage, middle and end of sporulation/pretetrad). These changes in organelle distribution and morphology ensure that portions of the mitochondrial network and its resident mtDNA nucleoids are enclosed within the developing spores. The mitochondria remain tubular and well distributed throughout prospore membrane formation, prospore wall formation, and early tetrad development (Figure 1, mitochondrial stage, early tetrad). In mature or late tetrads, however, mitochondria again fragment and remain fragmented until

spores begin to germinate (Miyakawa *et al.,* 1984) (Figure 1, mitochondrial stage, late tetrad). Once tetrads form, discrete mtDNA nucleoids are visible within each spore, although some nucleoids are excluded from spores and remain in the residual cytoplasm of the ascus (Miyakawa *et al.,* 1984).

Maintenance of yeast mitochondrial morphology during mitosis is known to require conserved proteins that regulate either mitochondrial fission or fusion. Key components of the outer membrane fission machinery include the cytoplasmic GTPase Dnm1p (Otsuga *et al.,* 1998; Bleazard *et al.,* 1999; Fukushima *et al.,* 2001), its binding partner Mdv1p/Fis2p/Gag3p/Net2p (Fekkes *et al.,* 2000; Mozdy *et al.,* 2000; Tieu and Nunnari, 2000; Cerveny *et al.,* 2001), and the integral outer membrane protein Fis1p/Mdv2p (Mozdy *et al.,* 2000; Tieu and Nunnari, 2000). These three proteins form a punctate complex on the outer mitochondrial membrane that mediates mitochondrial constriction and fission through an unknown mechanism (Tieu *et al.,* 2002; Cerveny and Jensen, 2003). Proteins that regulate mitochondrial fusion include the outer membrane GTPase Fzo1p (Hermann *et al.,* 1998; Rapaport *et al.,* 1998; Fritz *et al.,* 2001), the intermembrane space-localized GTPase Mgm1p (Shepard and Yaffe, 1999; Wong *et al.,* 2000), and the outer membrane protein Ugo1p (Sesaki and Jensen, 2001). All three fusion proteins form a complex (Sesaki *et al.,* 2003; Wong *et al.,* 2003) that is required for mitochondrial fusion in mitotically growing cells and in zygotes formed during haploid yeast mating. In mitotic cells lacking one or more fission proteins, mitochondrial tip fusion continues, generating elaborate net-like structures (Bleazard *et al.,* 1999; Sesaki and Jensen, 1999; Fekkes *et al.,* 2000; Mozdy *et al.,* 2000; Tieu and Nunnari, 2000; Cerveny *et al.,* 2001; Shaw and Nunnari, 2002). These fission mutants partition net-like mitochondria into buds as efficiently as wild-type cells but are reported to mutate or lose mtDNA at elevated rates under some growth conditions (Hanekamp *et al.,* 2002). In contrast, mitochondria fragment in cells lacking one or more fusion proteins, because of unopposed fission (Hermann *et al.,* 1998; Rapaport *et al.,* 1998; Fekkes *et al.,* 2000; Wong *et al.,* 2000; Sesaki and Jensen, 2001; Mozdy and Shaw, 2003). As a consequence of this fragmentation, mtDNA is lost through an unknown mech-

anism, and fusion mutants become respiratory incompetent (Scott *et al.*, 2003). Once again, these defects do not interfere with mitochondrial partitioning during division, inasmuch as fragments lacking mtDNA nucleoids are inherited normally by buds of yeast fusion mutants (Hermann *et al.*, 1998; Wong *et al.*, 2000; Sesaki and Jensen, 2001). Therefore, whereas fission and fusion are important for maintenance of network morphology and retention of mtDNA nucleoids, neither fission nor fusion is essential for organelle inheritance during mitosis. Moreover, when both fission and fusion are blocked in mitotically dividing yeast, tubular mitochondrial networks are restored, mtDNA loss is suppressed to some degree, and mitochondrial inheritance is unaffected (Bleazard *et al.*, 1999; Sesaki and Jensen, 1999), leading some to wonder why fission and fusion have been conserved during evolution.

In this study, we tested the hypothesis that yeast mitochondrial fission and fusion are important for efficient sporulation and subsequent spore fitness. Here we report that mutant strains defective for mitochondrial fission alone or both fission and fusion are able to form spores. Meiosis and sporulation progress despite the fact that mitochondrial networks in mutant cells fail to fragment during presporulation and subsequently collapse to one side of the cell. These defects in mitochondrial dynamics and distribution ultimately have consequences for spore viability and fitness. In mutant sporulation/pretetrad cells (Figure 1) containing collapsed organelles, some spores fail to inherit mitochondria, resulting in decreased spore viability. Fluorescent labeling studies reveal additional mtDNA nucleoid distribution and segregation defects that give rise to spores lacking detectable nucleoids. These nucleoid-deficient spores are able to germinate but cannot respire, resulting in "petite" colonies that are unable to grow on glycerol. Unexpectedly, when fission proteins are absent during sporulation, mitochondria still fragment in spores of late tetrads. The latter finding suggests that 1) novel mitochondrial fission machinery operates during yeast sporulation or 2) mechanical forces generate the mitochondrial fragments observed in late spores. These combined results provide conclusive evidence of fitness defects caused by mitochondrial fission mutations and reveal new phenotypes associated with mitochondrial fission and fusion defects.

MATERIALS AND METHODS

Yeast Strains and Plasmids

All strains used in this study were derived from FY10 (Winston *et al.*, 1995), including JSY4776, *MATa/α ura3-52/ura3-52 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 TRP1/TRP1 mgm1::HIS3/mgm1::HIS3 dnm1::HIS3/dnm1::HIS3*; JSY4806, *MATa/α ura3-52/ura3-52 leu2Δ1/leu2Δ1 his3Δ200/his3Δ200 TRP1/TRP1 dnm1::HIS3/dnm1::HIS3*; JSY4807, *MATa/α ura3-52/ura3-52 leu2Δ1/leu2Δ1 his3Δ200/his3Δ200 TRP1/TRP1 fis1::HIS3/fis1::HIS3*; JSY4812, *MATa/α ura3-52/ura3-52 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 lys2Δ202/lys2Δ202 mdv1::HIS3/mdv1::HIS3*; JSY4790, *MATa/α leu2Δ1/leu2Δ1 his3Δ200/his3Δ200 ura3-52/ura3-52 trp1Δ63/trp1Δ63 fzo1::HIS3/fzo1::HIS3 dnm1::HIS3/dnm1::HIS3*; JSY4609, *MATa/α ura3-52/ura3-52 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 lys2Δ202/lys2Δ202*. Standard methods were used to grow, transform, and manipulate yeast strains (Sherman *et al.*, 1986; Guthrie and Fink, 1991). The strains were transformed with pVT100U-mtGFP (mito-green fluorescent protein [GFP]) (Westermann and Neupert, 2000; Fritz *et al.*, 2001) or pRS424ADH-mtRFP (Mozdy and Shaw, unpublished data) to allow visualization of mitochondrial compartments during sporulation or pRS416ABF2-GFP (Abf2p is a mitochondrial nucleoid protein) (Zelenaya-Troitskaya *et al.*, 1998) to allow visualization of mtDNA nucleoids during sporulation.

Sporulation and Quantification of Sporulation Phenotypes

Wild-type and mitochondrial fission and fusion mutant strains expressing mito-GFP or ABF2-GFP were grown for 12–18 h at 25°C in liquid synthetic

dextrose lacking uracil or tryptophan. Cells were pelleted and resuspended at a final concentration of 0.5 OD_{600} U/ml in liquid YPG medium, to select for cells containing mtDNA. After 18–36 h at 30°C, <3% budded forms were present in the population. Cells (2×10^7) were harvested, washed twice in water, resuspended in 5 ml of sporulation medium (1% potassium acetate supplemented with 10 μg/ml adenine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, uracil, and valine) in a 50-ml glass flask, and shaken (225 rpm, gyratory G2 tabletop shaker; New Brunswick Scientific, Edison, NJ) at 25°C for 12 d. Aliquots of sporulating cells were examined daily with fluorescence and differential interference contrast (DIC) microscopy. Cultures were scored for sporulation efficiency, mitochondrial morphology and inheritance, and nucleoid distribution and inheritance as described below. Fluorescence microscopy and DIC microscopy were performed with an Axioplan Zeiss microscope, as described previously (Hermann *et al.*, 1997). DIC and fluorescence microscopic images of sporulating cells were obtained with a Zeiss confocal microscope, as described previously (Mozdy *et al.*, 2000).

Quantification of Spore Viability and Petite Spore Colony Formation

Wild-type and mutant tetrads were dissected onto YPD plates and incubated at 30°C for 5 d. Spores that formed visible colonies were designated viable. The viable spore colonies were replica-plated onto YPD and YPG media. Colonies that were able to grow on YPD medium but were unable to grow on YPG medium were designated petite.
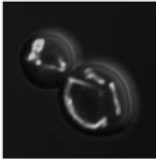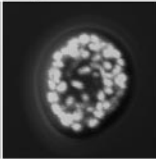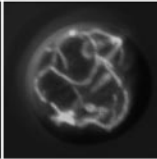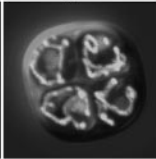
RESULTS
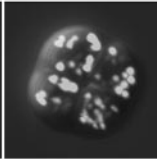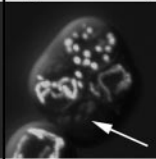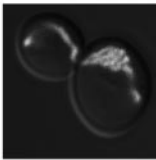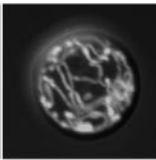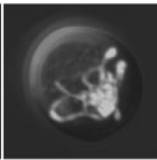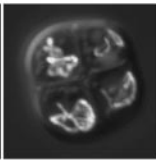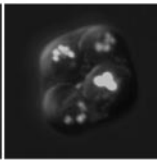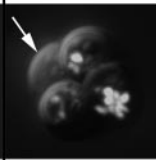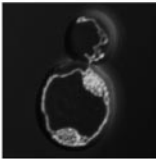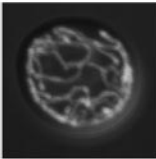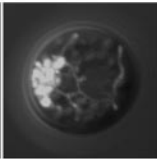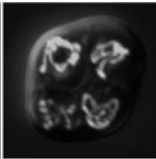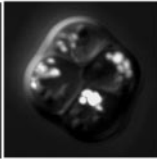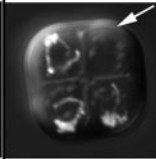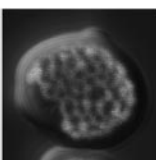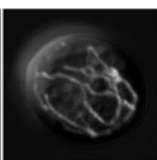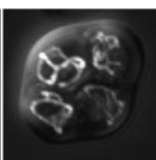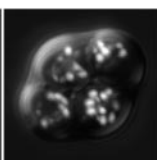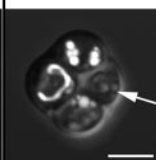
Mitochondrial Fission and Fusion Proteins are Not Essential for Yeast Sporulation

The morphology of GFP-labeled mitochondrial compartments during sporulation was monitored with fluorescence microscopy. The presence/absence of prospore walls was simultaneously monitored with DIC microscopy. With this approach, mitochondria in sporulating diploid cells were observed to progress through the stages depicted in Figure 1, including mitosis, presporulation (equivalent to nuclear stationary phase), sporulation/pretetrad (includes nuclear premeiotic S-phase and meiosis I and II), early tetrad (ear-
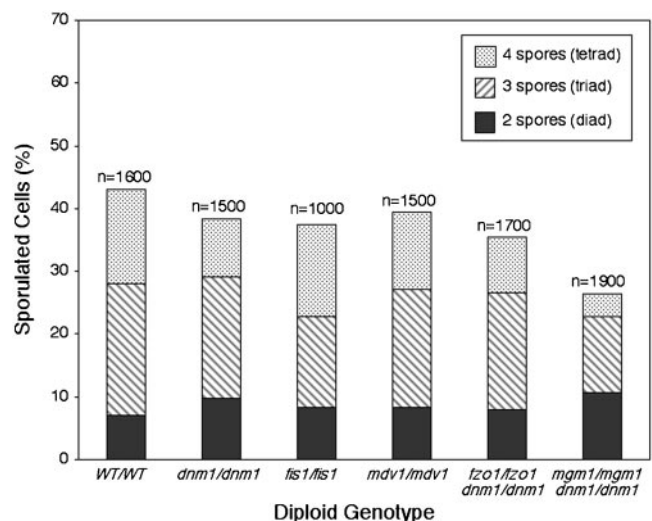
Figure 2. Yeast diploids form spores in the absence of mitochondrial fusion and/or fission proteins. Diploid fission or *dnm1*/fusion mutant strains were constructed in the FY background. The strains were grown for 18–36 h in YPG medium to maintain mtDNA nucleoids, shifted to sporulation medium, and incubated at 30°C for 12 d. The percentage of sporulated cells (diads, triads, and tetrads) was scored daily for each strain. The bar graph shows combined peak sporulation day results for five to eight experiments (n = total number scored).

Figure 3. Mitochondrial distribution and morphology in sporulating wild-type and fission mutant yeast cells. Wild-type and homozygous mutant diploids expressing mito-GFP were sporulated. Mitochondrial morphology was scored daily for 12 d after transfer to sporulation medium. Representative images of cells in mitosis and presporulation, sporulation/pretetrad, early tetrad, and late tetrad stages are shown. The percentages of cells containing mitochondria with different morphological features (tubule/net, aggregated, or fragmented) are indicated below each image. Examples of tetrads that failed to distribute GFP-labeled mitochondria into one or more spores are shown at the far right (% inheritance defect, white arrows). Bar, 5 μm.

liest point at which spore walls are visible), and late tetrad (12 d in sporulation medium).

To determine whether mitochondrial fission was essential during sporulation, homozygous fission mutants (*dnm1/dnm1*, *mdv1/mdv1*, and *fis1/fis1*) were examined during a sporulation time course. We were unable to examine homozygous fusion mutants in isolation because mtDNA loss and respiratory defects in these mutants prevent progres-

sion beyond the respiration-dependent presporulation stage (Olempska-Beer, 1987; Treinin and Simchen, 1993; Hermann *et al.*, 1998; Rapaport *et al.*, 1998; Shepard and Yaffe, 1999; Wong *et al.*, 2000). However, mitochondrial tubules and respiration can be maintained in *fzo1* and *mgm1* fusion mutants with deletion of the *DNM1* gene, which abolishes mitochondrial fission (Bleazard *et al.*, 1999; Sesaki and Jensen, 1999; Fekkes *et al.*, 2000; Mozdy *et al.*, 2000; Wong *et*

	Mitosis	Pre-Sporulation	Sporulation Pre-Tetrad	Early Tetrad	Late Tetrad	% Inheritance Defect (n=tetrad)
WT/WT						
Tubule/Net (%):	90	31	85	90	9	
Aggregated (%):	0	1	2	0	0	5
Fragmented (%):	10	68	13	10	91	
n (c=cells; s=spores):	100 c	700 c	2340 c	1068 s	1420 s	(388)
fzo1/fzo1 dnm1/dnm1						
Tubule/Nets (%):	90	95	46	89	20	
Aggregated (%):	10	4	53	6	6	20
Fragmented (%):	0	1	1	5	74	
n (c=cells; s=spores):	100 c	700 c	2928 c	654 s	545 s	(232)
mgm1/mgm1 dnm1/dnm1						
Tubule/Nets (%):	92	75	26	84	7	
Aggregated (%):	8	24	73	14	49	34
Fragmented (%):	0	1	1	2	44	
n (c=cells; s=spores):	100 c	700 c	2350 c	87 s	517 s	(125)

Figure 4. Mitochondrial distribution and morphology in sporulating wild-type and *dnm1*/fusion mutant yeast cells. Wild-type and *dnm1*/fusion mutant diploids expressing mito-GFP were sporulated. Mitochondrial morphology was scored daily for 12 d after transfer to sporulation medium. Representative images of cells in mitosis and presporulation, sporulation/pretetrad, early tetrad, and late tetrad stages are shown. The percentages of cells containing mitochondria with different morphological features (tubule/net, aggregated, or fragmented) are indicated below each image. Examples of tetrads that failed to distribute GFP-labeled mitochondria into one or more spores are shown at the far right (% inheritance defect, white arrows). Bar, 5 μm.

al., 2000). For this reason, we monitored sporulation in fusion mutants that also contained *dnm1* fission mutations (*fzo1/fzo1 dnm1/dnm1* and *mgm1/mgm1 dnm1/dnm1*). Because the *ugo1/ugo1 dnm1/dnm1* strain grew poorly and did not sporulate well, this strain was not analyzed in this study. As shown in Figure 2, both the homozygous fission mutant and *dnm1*/fusion mutant strains sporulated to some extent. Moreover, almost all of the strains formed similar percentages of two-spore diads, three-spored triads, and four-spored tetrads. Staining of nuclear DNA with the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) confirmed that meiosis I and II and formation of four haploid nuclei had occurred, suggesting that spore walls failed to enclose all four nuclei in diads and triads (Gorsich and Shaw, unpublished data). In the case of the *mgm1/mgm1 dnm1/dnm1* strain, overall sporulation efficiency decreased to 26.4%, compared with 43% in wild-type diploids. These data indicate that the three fission proteins, Dnm1p, Mdv1p, and Fis1p, and two fusion proteins, Fzo1p and Mgm1p, are not essential for spore formation.

Fission and Fusion Defects Disrupt Mitochondrial Morphology, Distribution, and Inheritance During Sporulation

We next determined whether fission and fusion were important for mitochondrial morphology, distribution, and inheritance during the stages of sporulation depicted in Figure 1 (mitochondrial stages). All mutant and wild-type strains exhibited predominantly tubular mitochondrial morphology during mitotic growth (Figures 3 and 4, mitosis, images at far left in each row). Tubular mitochondria in fission mutant strains also contained nets, as expected. As described previously (Miyakawa *et al.,* 1984), when wild-type cells were grown in presporulation medium (YPG), mitochondrial tubules in 68% of the cells were converted to fragments that remained well distributed (Figures 3 and 4, *WT/WT*, presporulation). In contrast, mitochondria remained predominantly tubular or net-like in the fission and *dnm1*/fusion mutants during presporulation (Figures 3 and 4, mutant strains, presporulation). These results suggest that

Dnm1p, Mdv1p, and Fis1p fission proteins mediate the mitochondrial fragmentation that occurs during presporulation. After transfer to liquid sporulation medium, mitochondria again appeared tubular and well distributed in 85% of wild-type cells (Figures 3 and 4, *WT/WT*, sporulation/pretetrad). Although mitochondria in the fission and *dnm1*/fusion mutant cells remained tubular in the sporulation/pretetrad stage, the tubules began migrating toward one end of the cell, forming large aggregates (Figures 3 and 4, mutant strains, sporulation/pretetrad). Formation of these asymmetric mitochondrial aggregates was least severe in cells lacking Mdv1p (20% vs. 53–73% in other mutant strains) (Figures 3 and 4). Although mitochondrial tubules extended from the mitochondrial aggregates of most strains at this stage, mitochondria in cells lacking Mgm1p appeared globular or fragmented and contained fewer discernable tubules (Figure 4, *dnm1/dnm1 mgm1/mgm1*, sporulation/pretetrad).

Mitochondria were largely tubular in the early tetrads of both wild-type cells and cells lacking fission and fusion proteins. However, unlike spores formed from wild-type diploids, all mutant strains generated some spores with aggregated mitochondria. As wild-type tetrads matured (Figures 3 and 4, *WT/WT*, late tetrad), mitochondrial tubules fragmented in the majority (91%) of spores. Unexpectedly, mitochondrial compartments also fragmented in 44–91% of spores formed by fission and *dnm1*/fusion mutant strains (Figure 3, mutant strains, late tetrad). This result was surprising, inasmuch as mitochondria cannot fragment in haploid strains lacking Dnm1p, Mdv1p, and Fis1p, because of the defect in fission.

We reasoned that sporulation/pretetrad-stage cells with defects in mitochondrial distribution might fail to incorporate mitochondrial compartments into newly formed spores. As shown in Figures 3 and 4, one or more spores of a tetrad often failed to inherit detectable levels of mitochondria when fission or *dnm1*/fusion proteins were absent (Figures 3 and 4, images at far right). Although an inheritance defect was observed in a small percentage of wild-type tetrads (5%), the number of tetrads lacking mitochondria in one or more spores increased when fission proteins were absent (*dnm1/dnm1*, 17%; *fis1/fis1*, 19%; *mdv1/mdv1*, 15%) or when both fusion and Dnm1p fission proteins were absent (*fzo1/fzo1 dnm1/dnm1*, 20%; *mgm1/mgm1 dnm1/dnm1*, 34%).

In Figure 5, the results indicate the percentages of tetrads lacking mitochondria in one, two, or three of the four spores. Among the wild-type and mutant tetrads with an inheritance defect, usually only one of the four spores in a tetrad lacked detectable mitochondria. However, a small percentage of tetrads lacked mitochondria in two or three of the spores. Cells lacking the Mgm1p fusion protein produced a larger percentage of defective tetrads in all three categories, consistent with our observation that *mgm1* mutant strains exhibited the most severe defects in mitochondrial aggregation and distribution (Figure 4, *mgm1/mgm1 dnm1/dnm1*).

Spore Viability and Respiratory Competence are Reduced When Mitochondrial Fission or dnm1/Fusion Proteins are Absent

To determine whether reduced mitochondrial inheritance affected spore viability, wild-type (n = 157), *dnm1/dnm1* (n = 155), *fis1/fis1* (n = 133), *mdv1/mdv1* (n = 122), *fzo1/fzo1 dnm1/dnm1* (n = 153), and *mgm1/mgm1 dnm1/dnm1* (n = 151) tetrads were dissected onto YPD plates, and colony formation was monitored. Spores that were unable to form a visible colony were scored as inviable. Although 22% of wild-type tetrads contained at least one inviable spore, the percentage increased in all strains lacking fission and *dnm1*/

fusion proteins (Figure 6A). The number of tetrads with one, two, three, or four inviable spores also increased in the mutant strains. When total spore inviability was calculated for each strain (no. of inviable spores/no. of spores dissected × 100), the differences between strains were similar, with the most severe defects occurring in *dnm1* and *mgm1 dnm1* spores (Figure 6B). In fact, the percentage of *dnm1* mutant tetrads containing inviable spores (Figure 6A) was larger than expected if this inviability was attributable solely to mitochondrial inheritance defects in these tetrads (Figure 5). This discrepancy suggests that Dnm1p plays a role in spore germination and/or viability independent of its role in mitochondrial division and inheritance.

In tetrads from all sporulation experiments, there were more inviable spores than spores that failed to inherit mitochondria. Therefore, some spores scored as containing mitochondria must not have been able to germinate and/or form colonies. This phenomenon is likely attributable to the fact that mitochondrial inheritance by spores was scored very conservatively. A spore was characterized as containing mitochondria regardless of how little mito-GFP fluorescence was detected. Many of the inviable spores might have inherited insufficient mitochondria to sustain subsequent germination and/or colony growth. To determine whether inviable wild-type and mutant spores were able to germinate, spores that did not form visible colonies were examined microscopically. For each strain, the percentage of inviable spores that germinated and divided at least once was as follows: wild-type, 93%; *dnm1*, 85%; *fis1*, 86%; *mdv1*, 92%; *fzo1 dnm1*, 64%; *mgm1 dnm1*, 93%. Although large percentages of *mgm1 dnm1* (20%) and *dnm1* (27%) spores were inviable, the ability of spores from these two strains to germinate was identical or only slightly decreased, relative to wild-type spores. These findings suggest that some spores inherit sufficient mitochondria to support germination and one or more cell divisions but not growth of an entire colony.
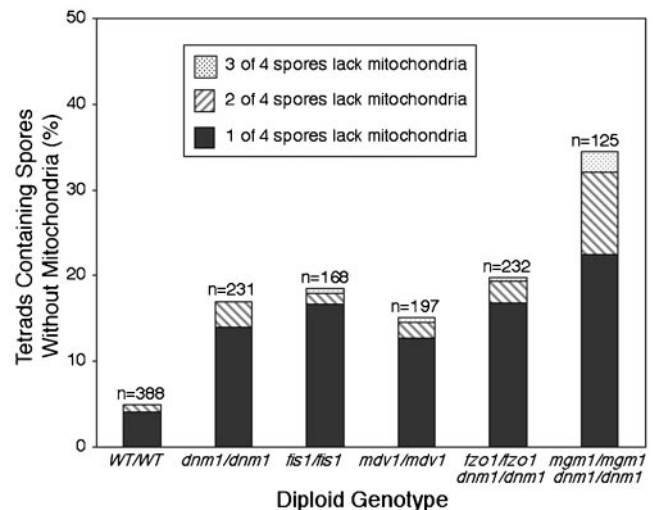
Figure 5. Mitochondrial partitioning to spores is defective in diploid fission and *dnm1*/fusion mutants. Wild-type and homozygous mutant diploids expressing mito-GFP were sporulated for 12 d. Wild-type and fission or *dnm1*/fusion mutant tetrads were scored for the presence or absence of GFP-labeled mitochondria in one (solid bars), two (hatched bars), or three (stippled bars) spores of a tetrad. Tetrads lacking GFP-labeled mitochondria in all four spores were not scored, because these cells might have lost the mito-GFP-expressing plasmid (n = total number scored).
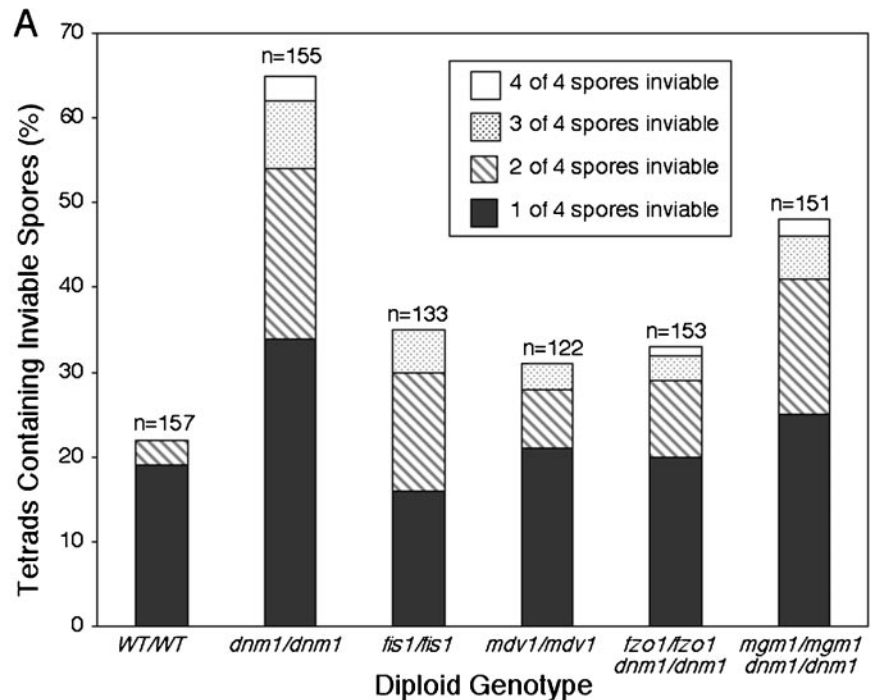
Figure 6. Viability (colony formation) is reduced in diploid fission and *dnm1*/fusion mutants. (A) Wild-type, fission mutant, and *dnm1*/fusion mutant tetrads were dissected onto YPD medium, and the ability of each spore to form a colony was assessed. For each strain, the number of tetrads with one (solid bars), two (hatched bars), three (stippled bars), or four (white bars) inviable spores is shown (n = total number scored). (B) The percentages of total inviable spores produced by wild-type and mutant tetrads dissected in A are shown (n = total number scored).

Spore Genotype	% Total Inviable Spores	n
WT	6	628
dnm1	27	620
fis1	15	532
mdv1	11	488
fzo1 dnm1	13	612
mgm1 dnm1	20	604

Viable spore colonies indicated in Figure 6 were analyzed for their ability to respire and grow on the nonfermentable carbon source glycerol. As shown in Figure 7, viable spores derived from cells lacking mitochondrial fission or fusion proteins gave rise to glycerol growth-defective (petite) colonies more often than did spores derived from wild-type cells (wild-type, 0.2%; *dnm1*, 13%; *fis1*, 16%; *mdv1*, 1%; *fzo1 dnm1*, 11%; *mgm1 dnm1*, 18%). DAPI staining of spore colonies from a *dnm1*/*dnm1* sporulation experiment revealed that 64% of the petite spore colonies contained rho⁰ cells (lacking DAPI-stained mtDNA nucleoids), whereas 36% of the petite spore colonies contained rho⁻ cells (containing DAPI-stained nucleoids; we presume that these cells are rho⁻ because nucleoids are present but respiration is compromised). These data suggest that petite spore colonies arising in these experiments result both from a failure to inherit nucleoids and from increased occurrence of nonfunctional (rho⁻) mtDNA in nucleoids. Very few viable spores derived from the *mdv1*/*mdv1* strain were petite, suggesting that Mdv1p is less critical for formation of respiration-competent spores during meiotic division.

To determine whether the results reported above were specific for the FY strain background, we repeated these studies with *WT*/*WT* and *dnm1*/*dnm1* diploids generated in the W303 strain background. Compared with *WT*/*WT* and *dnm1*/*dnm1* diploids in the FY background, W303 strains produced a slightly higher percentage of inviable spores and

a similar percentage of respiration-incompetent spores. On the basis of this comparison, it seems unlikely that the FY strain contains background mutations responsible for the defects observed in our fission and fusion mutant studies.

Inheritance of mtDNA Nucleoids is Disrupted When Mitochondrial Fission or dnm1/Fusion Proteins are Absent

The increase in petite colony formation described above suggested that partitioning of mtDNA nucleoids might be disrupted in sporulating cells lacking fission and *dnm1*/fusion proteins. To test this idea, mitochondrial nucleoids were visualized in wild-type and mutant diploid cells with a GFP-tagged form of the Abf2 nucleoid-binding protein (Zelenaya-Troitskaya *et al.,* 1998). Although costaining of cells with DAPI confirmed that Abf2-GFP puncta contained mtDNA (data not show), Abf2-GFP proved to be a more reliable marker of mtDNA nucleoid distribution during sporulation, because DAPI-labeled mtDNA was often obscured by brightly labeled nuclear DNA in mutant cells containing mitochondrial aggregates.

Abf2-GFP distribution was quantified in wild-type and mutant cells in mitosis or in presporulation and sporulation/pretetrad stages (Figure 8A). Abf2-GFP distribution was considered wild-type if five or more green fluorescent puncta were present and evenly distributed on mitochon-
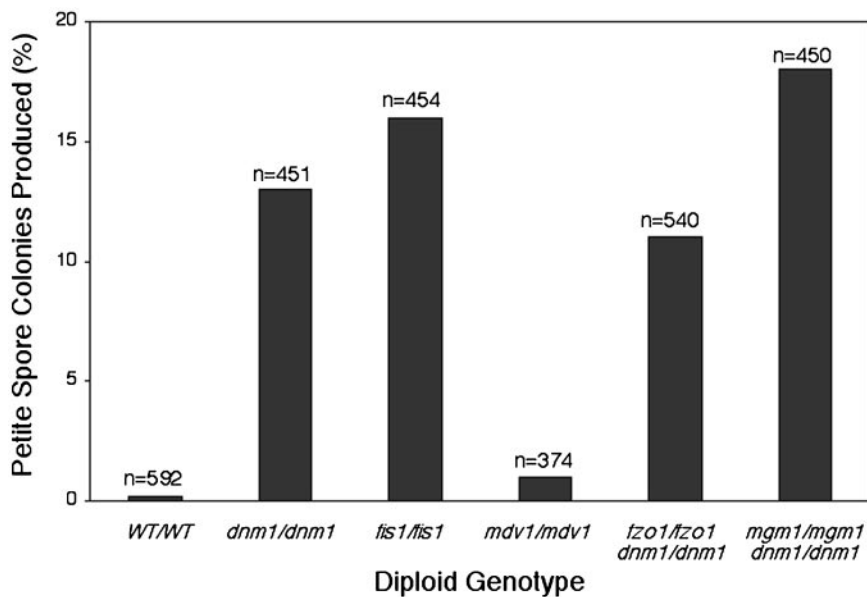
Figure 7. Fission and *dnm1*/fusion mutant strains produce more petite spore colonies than do wild-type cells. Wild-type, fission mutant, and *dnm1*/fusion mutant tetrads were dissected onto YPD medium. Respiration-deficient (petite) spore colonies were identified by their inability to grow after replica plating onto YPG medium (n = total number scored).

dria or if nucleoid staining resembled the beads-on-a-string pattern reported previously (Miyakawa *et al.,* 1984). Representative images of Abf2-GFP-labeled nucleoids in wild-type (*WT/WT*) cells during mitosis and presporulation and sporulation/pretetrad stages are shown in Figure 9. When wild-type cells were in mitosis or in presporulation and sporulation/pretetrad stages, only 2% or 15% of the population, respectively, exhibited abnormal Abf2-GFP distribution (Figure 8A, *WT/WT*). The percentage of cells with abnormal Abf2-GFP distribution during mitosis increased in the fission and *dnm1*/fusion mutants, although the defects were moderate (9–22%) (Figure 8A). More severe mtDNA nucleoid distribution defects occurred in many mutant strains in presporulation and sporulation/pretetrad stages (22–52%) (Figure 8A). In cells with abnormal distribution, Abf2-GFP-labeled nucleoids appeared as two or more large aggregates that were often asymmetrically localized. Representative images of abnormal Abf2-GFP-labeled nucleoid distribution in *dnm1/dnm1* and *dnm1/dnm1 fzo1/fzo1* mutant strains during mitosis and presporulation and sporulation/pretetrad stages are shown in Figure 9.

To determine whether this abnormal nucleoid appearance and distribution affected nucleoid inheritance by spores, wild-type and mutant tetrads were examined for spores lacking Abf2-GFP labeling (only asci containing four spores were used for this calculation) (Figure 8B). Although very few wild-type spores lacked detectable Abf2-GFP (1%), the nucleoid inheritance defect increased in spores derived from all fission and *dnm1*/fusion mutant strains (*dnm1*, 12%; *fis1*, 12%; *mdv1*, 8%; *fzo1 dnm1*, 12%; *mgm1 dnm1*, 20%). The observation that mtDNA nucleoid inheritance by spores is reduced in fission and *dnm1*/fusion mutants is consistent with our finding that these mutant strains give rise to glycerol growth-defective (petite) colonies more often than the wild-type strain (Figure 7). Moreover, this observation is consistent with our DAPI staining studies showing that 64% of petite colonies contain cells with no visible mtDNA nucleoids. Together, these results indicate that mitochondrial fission and fusion proteins are important for proper mtDNA nucleoid distribution and inheritance during meiosis and sporulation.

DISCUSSION

In the wild, yeast cells are found as mitotically dividing diploids (Mortimer *et al.,* 1994; Mortimer, 2000). However, their ultimate survival depends on their ability to undergo meiosis and sporulation when exposed to adverse environmental conditions. In this study, we demonstrate that loss of fission proteins alone or both fission and fusion proteins causes significant defects in mitochondrial distribution and inheritance during meiosis and sporulation. These defects also result in decreased mtDNA nucleoid inheritance, germination, and viability, all of which are important for yeast survival and fitness in the wild.

As reported by Miyakawa *et al.* (1984) and observed in this study, mitochondria undergo what appear to be temporally regulated fission and fusion events during meiosis and sporulation. Although fission and fusion are balanced to maintain mitochondrial tubules during mitotic growth, they are shifted toward mitochondrial fragmentation during the presporulation stage of the meiosis and sporulation program. This shift toward fragmentation could be achieved by increasing the rate of fission or decreasing the rate of fusion. Although we were unable to evaluate mitochondrial morphology in strains lacking fusion proteins alone, we were able to monitor mitochondrial fragmentation during presporulation in cells lacking fission proteins. Our finding that mitochondria remained tubular during presporulation in cells lacking Dnm1p, Mdv1p, or Fis1p indicates that these proteins contribute to mitochondrial fragmentation at this stage of the meiotic program.

When wild-type cells are sporulated, tubular mitochondria also fragment during the late tetrad stage. We anticipated that this fragmentation would be blocked in cells lacking the fission proteins Dnm1p, Fis1p, or Mdv1p. Unexpectedly, mitochondria were still able to fragment in late tetrads formed by cells lacking any one of these fission proteins or all three of these proteins (Gorsich and Shaw, unpublished data). We further explored the role of Dnm1p at this stage by examining the localization of Dnm1p-GFP during a wild-type sporulation time course. Studies of mitotic wild-type cells showed that Dnm1p-GFP localized in many discrete puncta (fission complexes) along mitochon-
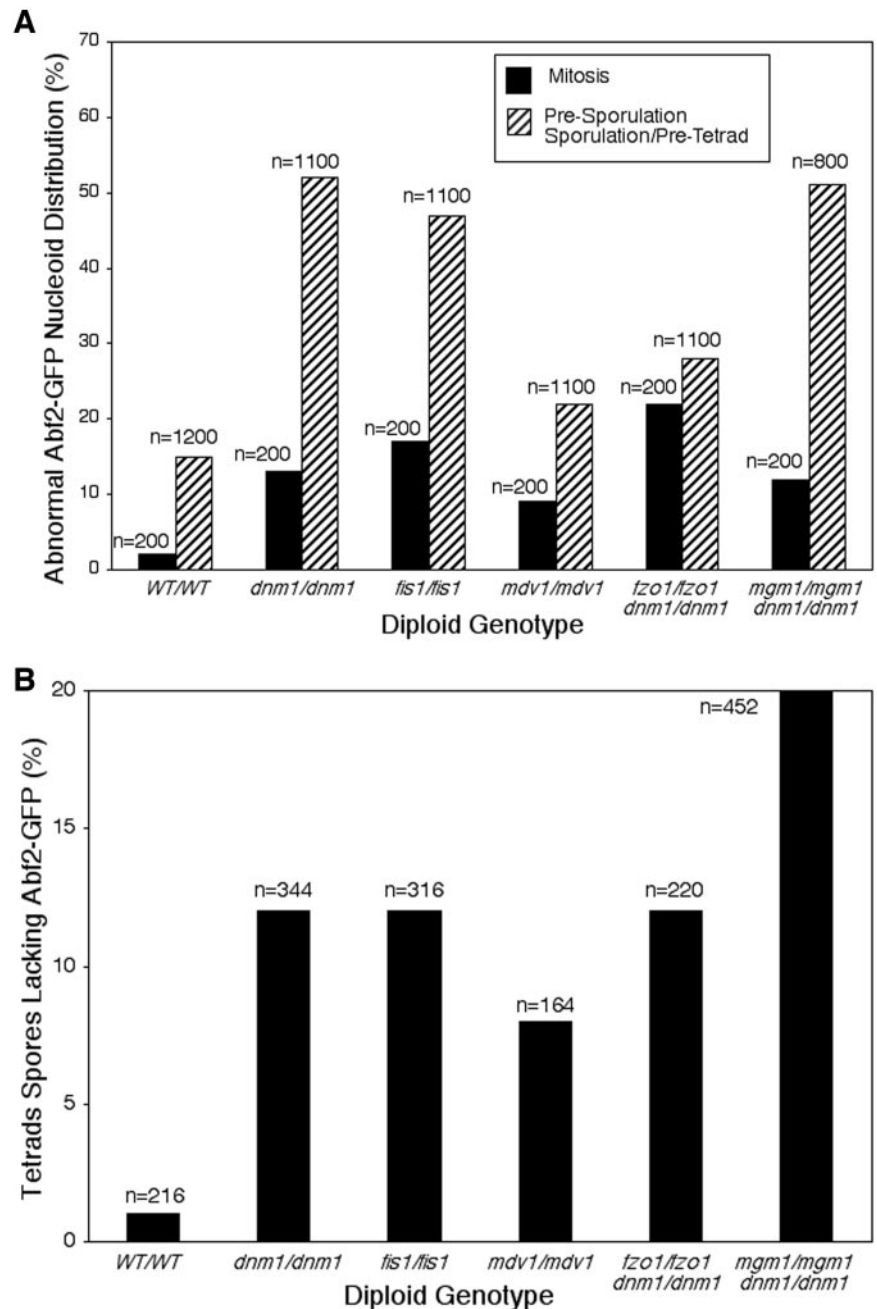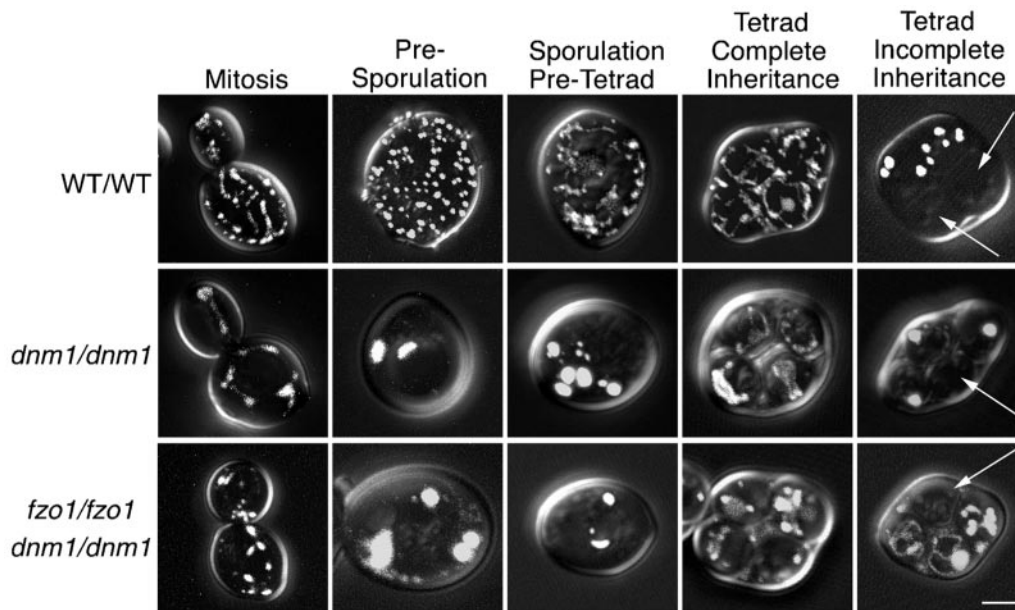
Figure 8. mtDNA nucleoid distribution and inheritance are disrupted in sporulating fission and *dnm1*/fusion mutants. (A) The distribution of Abf2-GFP–labeled mtDNA nucleoids was examined in wild-type and mutant strains during mitosis (black bars) and presporulation and sporulation/pretetrad stages (gray bars). Mitochondrial nucleoid appearance and distribution in single cells were scored as wild-type (five or more dispersed nucleoids or beads-on-a-string appearance) or abnormal (less than four nucleoids or aggregates). Abnormal Abf2-GFP nucleoid distribution is shown (n = total number scored). (B) Wild-type and mutant tetrad spores were scored for the presence or absence of Abf2-GFP–labeled mitochondrial nucleoids in each spore (n = total number scored).

drial tubules (Otsuga *et al.,* 1998; Bleazard *et al.,* 1999). In our sporulating wild-type culture, we conservatively scored cells with five or more evenly distributed Dnm1p-GFP puncta as wild-type and cells with zero to four puncta as mutant. As shown in Table 1, we observed a wild-type distribution of Dnm1p-GFP puncta (>90% of cells) when cells were in mitosis or presporulation or pretetrad stages. However, visible Dnm1p-GFP puncta redistributed into one or a few large aggregates in ~50% of the cells in early and late tetrad stages. With the assumption that such aggregates are not functional, these results suggest that Dnm1p may be less active during late stages of sporulation. Alternatively, active Dnm1p-GFP puncta may be less visible at these late stages. If Dnm1p activity is reduced or absent during late stages of sporulation, then two alternative explanations

could account for the mitochondrial fragmentation observed in late tetrads. First, there may be additional genes specifically required for mitochondrial morphology maintenance during the later stages of sporulation, particularly during tetrad maturation. If mutations in such genes failed to produce mitotic phenotypes, then they would have escaped detection in mitochondrial morphology mutant screens of the viable gene disruption collection (Dimmer *et al.,* 2002). Second, some type of mechanical force could cause mitochondrial fragmentation during the late tetrad stage of sporulation, independent of Dnm1p, Fis1p, or Mdv1p. This type of mechanical fragmentation could result from changes in actin-mitochondria interactions. In support of this model, mitochondria are reported to aggregate, fragment, and become immobile during sporulation in various actin (*act1*)

Figure 9. Distribution of Abf2-GFP-labeled mtDNA nucleoids is abnormal in sporulating fission and *dnm1*/fusion mutants. Wild-type (*WT/WT*), fission mutant (*dnm1/dnm1*), and *dnm1/dnm1 fzo1/fzo1* mutant strains expressing Abf2-GFP were examined during a sporulation time course. Representative confocal microscopic images of cells in mitosis and presporulation, sporulation/pretetrad, and tetrad stages are shown. In each row, the fourth panel from the left shows an image of a tetrad containing Abf2-GFP labeling in all four spores. The far right panels show images of tetrads in which one or more spores lack detectable Abf2-GFP labeling (white arrows). Bar, 5 μm.

mutants (Smith *et al.,* 1995) and in haploid cells treated with the actin filament- and cable-disrupting drug latrunculin A (Boldogh *et al.,* 1998). Two additional observations argue against this model. First, *dnm1Δ* net-like mitochondria do not fragment when actin cables are disrupted by a mutation in a gene called *MDM20* (Bleazard *et al.,* 1999). Second, mitochondrial nets in *dnm1Δ* or *mdv1Δ* cells are not disrupted when these cells are treated with latrunculin A (Cerveny *et al.,* 2001; Jensen *et al.,* 2001). Additional studies will be necessary to determine the basis of mitochondrial fragmentation in mature spores of fission and *dnm1*/fusion mutants.

We observed that homozygous fission mutants (*dnm1/dnm1*, *fis1/fis1*, and *mdv1/mdv1*) and *dnm1*/fusion mutants (*fzo1/fzo1 dnm1/dnm1* and *mgm1/mgm1 dnm1/dnm1*) were able to form spores. Clearly, mitochondrial fission and fusion are not essential for prospore membrane and cell wall formation. Moreover, DAPI staining of sporulated cultures revealed no binucleate or multinucleate spores, suggesting that nuclear DNA replication and meiotic divisions were occurring normally (Gorsich and Shaw, unpublished data).

Instead, strains lacking mitochondrial fission and fusion proteins exhibited defects in mitochondrial morphology, the most striking of which was the aggregation and collapse of mitochondrial membranes during the sporulation/pretetrad stage. The fission and fusion mutants with asymmetrically distributed mitochondria produced spores lacking this organelle, providing direct evidence that mitochondria must be evenly distributed at or near the nuclear lobes to be included in all four spores. Finally, we showed that cells lacking fission proteins alone or both fission and fusion proteins produced more inviable spores than did wild-type cells. This viability defect may be a direct consequence of the mitochondrial inheritance defect, because mitochondria cannot be generated de novo. In contrast, spores that fail to inherit wild-type amounts of vacuoles/lysosomes do not exhibit viability defects (Roeder and Shaw, 1996). Instead, such spores regenerate new vacuoles before germination, either de novo or from an undetected pool of inherited membranes. Two additional observations suggest that production of colony-forming spores may require the inheritance of a critical amount of mitochondria. First, for most

Table 1. Dnm1p-GFP localization during sporulation

Stage	0 Dnm1p-GFP puncta (%)	1–4 Dnm1p-GFP puncta (%)	>5 Dnm1p-GFP puncta (%)	n
Mitosis	0.0	8.0	92.0	100(c)
Presporulation	0.0	6.7	93.3	150(c)
Pretetrad	0.0	6.0	94.0	400(c)
Early tetrad	4.6	50.4	45.0	480(s)
Late tetrad	2.6	48.7	48.7	880(s)

Data from sporulation time course of a wild-type diploid in the FY background. (c), cells; (s), spores.

mutant strains, the number of tetrads with inviable spores was greater than the number of tetrads with mitochondria-deficient spores (lacking detectable mitochondria). Therefore, some spores that receive detectable amounts of mitochondria may receive too little for effective germination. Second, in many strains, a significant fraction of spores that did not form colonies were able to germinate and divide at least once. Therefore, it is possible that some spores inherit insufficient mitochondria to support germination and/or colony formation.

In this study, sporulating wild-type cells produced respiration-competent spores with high efficiency (1 of 589 dissected spores was petite), indicating that a high-fidelity mechanism exists to faithfully partition mtDNA during sporulation. This partitioning mechanism is disrupted in cells lacking fission proteins alone or both fission and fusion proteins, inasmuch as spores from these strains sometimes failed to inherit mtDNA nucleoids and formed respiration-incompetent spore colonies more frequently than did wild-type spores. Therefore, mitochondrial fission and fusion indirectly contribute to yeast survival and fitness by enhancing the fidelity of mtDNA nucleoid inheritance during meiotic division and sporulation. Together, our observations suggest that mitochondrial fission and fusion have been conserved during evolution in part because of their importance during meiosis and sporulation. These processes are also important for survival and fitness in mitotically dividing yeast, inasmuch as mitochondrial fragmentation in fusion mutants causes mtDNA loss and respiratory defects (Hermann *et al.,* 1998; Rapaport *et al.,* 1998; Shepard and Yaffe, 1999; Sesaki and Jensen, 2001) and *dnm1* fission mutants exhibit defects in mtDNA stability (Hanekamp *et al.,* 2002).

Because studies in mitotically growing cells demonstrated that the fission proteins Dnm1p, Fis1p, and Mdv1p function in the same pathway, we expected that diploid strains lacking these proteins would display similar sporulation phenotypes. However, the sporulation phenotypes of cells lacking individual fission proteins differed in severity. Specifically, *dnm1/dnm1* sporulation defects were the most severe, whereas *mdv1/mdv1* defects were the least severe. These different phenotypes suggest that the need for each protein during fission may vary. During mitotic growth, the Dnm1p GTPase assembles to form punctate structures on the sides of mitochondrial tubules (Otsuga *et al.,* 1998). These structures (mitochondrial fission complexes) are associated with sites of inner and outer membrane constriction in immunogold labeling studies (Bleazard *et al.,* 1999) and often occur at sites of mitochondrial fission in time-lapse imaging studies (Sesaki and Jensen, 1999; Legesse-Miller *et al.,* 2003). The Mdv1p protein binds to Dnm1p and is also found in these mitochondrial fission complexes (Fekkes *et al.,* 2000; Tieu and Nunnari, 2000; Tieu *et al.,* 2002; Cerveny *et al.,* 2001; Cerveny and Jensen, 2003). Previous studies showed that, when Mdv1p is absent during mitotic growth, Dnm1p is able to form punctate complexes on the outer mitochondrial membrane (Fekkes *et al.,* 2000; Mozdy *et al.,* 2000; Tieu and Nunnari, 2000; Cerveny *et al.,* 2001). However, fission is still reduced or blocked, indicating that Mdv1p plays an important role in fission. Because less severe mitochondrial morphology defects are observed in *mdv1/mdv1* sporulating cells, the function of Mdv1p may be less important during sporulation.

Mitochondria also undergo dramatic morphological changes during human spermatogenesis (Seitz *et al.,* 1995; Meinhardt *et al.,* 1999) and oogenesis (Pozo *et al.,* 1990; Motta *et al.,* 2000; Sathananthan and Trounson, 2000), and a number of human fertility disorders have been linked to defects in mitochondrial distribution and function (Mundy *et al.,* 1995; Piasecka and Kawiak, 2003). Moreover, defects in mitochondrial function appear to contribute to chromosomal nondisjunction and age-related trisomies such as Down's syndrome (Schon *et al.,* 2000; Arbuzova *et al.,* 2001, 2002). Other studies have demonstrated that postmeiotic fertility and developmental defects can be caused by abnormal mitochondrial morphology in mammals (Van Blerkom *et al.,* 2000; Chen *et al.,* 2003). The studies presented here raise the possibility that some inherited human diseases, fertility disorders, and developmental defects result from or are compounded by meiotic defects in mitochondrial fission and fusion. Now that mammalian homologues of some mitochondrial fission and fusion proteins have been identified, the importance of these processes during gametogenesis, fertilization, and early development in mammals can be investigated.

ACKNOWLEDGMENTS

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