DOI: 10.1534/genetics.107.083055

Multiple Pathways Influence Mitochondrial Inheritance in Budding Yeast

Rebecca L. Frederick,[1] Koji Okamoto[2] and Janet M. Shaw[3]

Department of Biochemistry, University of Utah, Salt Lake City, Utah 84112

Manuscript received October 6, 2007
Accepted for publication November 30, 2007

ABSTRACT

Yeast mitochondria form a branched tubular network. Mitochondrial inheritance is tightly coupled with bud emergence, ensuring that daughter cells receive mitochondria from mother cells during division. Proteins reported to influence mitochondrial inheritance include the mitochondrial rho (Miro) GTPase Gem1p, Mmr1p, and Ypt11p. A synthetic genetic array (SGA) screen revealed interactions between *gem1*Δ and deletions of genes that affect mitochondrial function or inheritance, including *mmr1*Δ. Synthetic sickness of *gem1*Δ *mmr1*Δ double mutants correlated with defective mitochondrial inheritance by large buds. Additional studies demonstrated that *GEM1*, *MMR1*, and *YPT11* each contribute to mitochondrial inheritance. Mitochondrial accumulation in buds caused by overexpression of either Mmr1p or Ypt11p did not depend on Gem1p, indicating these three proteins function independently. Physical linkage of mitochondria with the endoplasmic reticulum (ER) has led to speculation that distribution of these two organelles is coordinated. We show that yeast mitochondrial inheritance is not required for inheritance or spreading of cortical ER in the bud. Moreover, Ypt11p overexpression, but not Mmr1p overexpression, caused ER accumulation in the bud, revealing a potential role for Ypt11p in ER distribution. This study demonstrates that multiple pathways influence mitochondrial inheritance in yeast and that Miro GTPases have conserved roles in mitochondrial distribution.

MITOCHONDRIA contribute to many cellular processes, including calcium homeostasis, cell death, cellular respiration, and metabolism. Studies in yeast, flies, worms, and mammals have established that mitochondrial shape and distribution are important for organelle function and cell survival (recently reviewed by Karbowski and Youle 2003; Chan 2006; Frazier *et al.* 2006; Szabadkai *et al.* 2006). Moreover, mitochondrial function is particularly important in neurons, where synaptic mitochondria provide the necessary energy for neurotransmitter release and recycling (Hollenbeck 2005; Ly and Verstreken 2006; Rikhy *et al.* 2007). Studies in the budding yeast *Saccharomyces cerevisiae* have advanced understanding of processes that impinge on mitochondrial function, including regulation of mitochondrial shape and distribution (recently reviewed by Shaw and Nunnari 2002; Okamoto and Shaw 2005; Escobar-Henriques and Langer 2006; Griffin *et al.* 2006). Even in yeast, where mitochondrial respiration is dispensable, mitochondria are essential for cell survival (Altmann and Westermann 2005; Kispal *et al.* 2005). Mitochondria cannot be generated *de novo* and must arise from existing organelles in the mother cell. Therefore, transfer of mitochondria to the emerging daughter cell is an essential process (McConnell *et al.* 1990; Warren and Wickner 1996).

Inheritance of the tubular mitochondrial network is actively regulated in dividing yeast cells. Mitochondria are inherited by small buds soon after bud emergence and initially appear to be associated with the bud tip. In addition, anchoring in the mother cell is thought to ensure that the mother retains a subset of mitochondria. By the time the mother and daughter are separated by cytokinesis, approximately equal amounts of mitochondria are distributed into each cell (Simon *et al.* 1997; Boldogh *et al.* 2001).

Although many aspects of mitochondrial distribution remain unclear, efficient mitochondrial distribution and inheritance in yeast requires the actin cytoskeleton. Monomeric actin polymerizes to form actin filaments, which are either bundled to make actin cables or clustered into actin cortical patches (Young *et al.* 2004). Cables are oriented along the mother-bud axis to facilitate bud-directed movement of cellular materials to the growing bud. Cortical actin patches, which typically function as sites of endocytosis (Kaksonen *et al.* 2003; Huckaba *et al.* 2004), localize to the new bud tip. As the bud enlarges and shifts to isotropic growth, cortical patches are distributed in the bud cortex (Pruyne and Bretscher 2000a,b; Moseley and Goode 2006). Simultaneous live imaging of yeast mitochondria and actin cytoskeleton revealed that mitochondria move along

[1]*Present address:* Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21218.

[2]*Present address:* Division of Molecular Cell Biology, National Institute for Basic Biology, Myodaiji, Okazaki 444-8585, Japan.

[3]*Corresponding author:* Department of Biochemistry, University of Utah, 15 N. Medical Dr. E., Salt Lake City, UT 84112.
E-mail: shaw@biochem.utah.edu

actin cables (Fehrenbacher *et al.* 2004). In addition, mutations that affect the organization or stability of filamentous actin structures lead to defects in mitochondrial inheritance (Drubin *et al.* 1993; Singer *et al.* 2000; Altmann and Westermann 2005).

A common feature of mutants with defects in mitochondrial inheritance is that they often display a delay, rather than a block, in inheritance. Thus, in viable mutants, newly emerged small buds lack mitochondria, but upon further growth, larger buds contain mitochondria. This observation implies that mitochondrial inheritance is not restricted to small buds. Rather, mitochondria can be moved into larger buds as well.

Two potential mechanisms for mitochondrial movement have been suggested. First, the mitochore complex, which consists of Mdm10p, Mdm12p, and Mmm1p, has been implicated in mitochondrial-actin associations, perhaps by recruitment of the Arp2/3 complex (Boldogh *et al.* 1998, 2003). This interaction has been proposed to facilitate anterograde, bud-directed mitochondrial movement via actin polymerization by a mechanism similar to Listeria movement (Fehrenbacher *et al.* 2003a,b). However, Arp2/3 functions to nucleate actin and form cortical actin patches, which themselves move in a retrograde fashion along cables toward the mother pole (Kaksonen *et al.* 2003; Huckaba *et al.* 2004). In addition, Mdm10p and Mmm1p, components of the mitochore complex, have been directly implicated in mitochondrial import of β-barrel outer membrane proteins (Meisinger *et al.* 2004, 2007). Moreover, Mmm1p mutations affect mitochondrial DNA nucleoid organization (Hobbs *et al.* 2001; Hanekamp *et al.* 2002; Meeusen and Nunnari 2003). Thus, the primary function(s) of the mitochore complex and its role in mitochondrial movement remain unclear.

The second proposed mechanism for mitochondrial movement depends on association with a motor. In higher organisms, kinesins and dyneins facilitate microtubule-based mitochondrial movement (Hollenbeck 1996; Hollenbeck and Saxton 2005). In yeast, the myosin V isoforms, Myo2p and Myo4p, are involved in bud-directed transport of several organelle cargoes, including vacuoles, secretory vesicles, and Golgi membranes (Pruyne *et al.* 1998; Schott *et al.* 1999; Rossanese *et al.* 2001; Pashkova *et al.* 2005). Mutational analysis of the Myo2p tail has generated alleles that specifically affect binding of single adapter proteins and therefore disrupt inheritance of specific organelles (Catlett *et al.* 2000; Ishikawa *et al.* 2003; Pashkova *et al.* 2006). At least one allele, *myo2-573*, specifically impairs mitochondrial inheritance without affecting cell polarization or vacuolar inheritance, suggesting that mitochondrial movement into buds is motor based (Itoh *et al.* 2002; Altmann and Westermann 2005). Overexpression of Mmr1p, a peripheral outer mitochondrial membrane protein required for efficient mitochondrial inheritance, can specifically suppress *myo2-573*. Co-immunoprecipitation studies raised the possibility that Mmr1p functions as a mitochondrial adapter for Myo2p (Itoh *et al.* 2004). Ypt11p, a Rab GTPase, also affects mitochondrial inheritance in a Myo2p-dependent manner. Ypt11p overexpression drives mitochondria into the bud, similar to what has been observed for Mmr1p (Itoh *et al.* 2002, 2004). Genetic interactions suggest that Mmr1p and Ypt11p do not require each other to function (Itoh *et al.* 2004). Ypt11p has also been suggested to anchor mitochondria at the bud tip in a Myo2p-dependent manner (Boldogh *et al.* 2004). Thus, at least two proteins can function to promote mitochondrial inheritance via association with Myo2p.

The mitochondrial rho (Miro) family of GTPases is conserved between yeast and higher eukaryotes (Fransson *et al.* 2003). Miro proteins contain two GTPase domains that flank a pair of calcium-binding EF-hand motifs. The yeast Miro ortholog, Gem1p, is important for maintenance of tubular mitochondrial morphology. In the absence of *GEM1*, cells contain large, globular mitochondria and display an inheritance defect in small-budded but not large-budded cells (Frederick *et al.* 2004). In fly larvae lacking Miro, mitochondria accumulate in cell bodies and are depleted from axons and synaptic boutons (Guo *et al.* 2005). The fly and human Miro orthologs bind to an adapter protein, Milton, which itself forms a complex with kinesin heavy chain (Stowers *et al.* 2002; Gorska-Andrzejak *et al.* 2003; Fransson *et al.* 2006; Glater *et al.* 2006). Several studies suggest motor protein attachment to mitochondria is modulated by Miro and Milton to facilitate transport along microtubules (Cox and Spradling 2006; Glater *et al.* 2006; Rice and Gelfand 2006).

We sought to understand whether the link between Miro function and mitochondrial movement is conserved in yeast. In this study, we identified synthetic sickness interactions between *gem1*Δ and deletion alleles of genes annotated to function in mitochondrial or endoplasmic reticulum (ER) distribution and morphology. Further analysis of selected double mutants revealed that strain fitness correlated with the efficiency of mitochondrial transfer into large buds. Genetic interactions and overexpression studies demonstrated that Gem1p, Mmr1p, and Ypt11p independently contribute to mitochondrial inheritance. Finally, ER inheritance can occur independently from mitochondrial inheritance. However, Ypt11p may function to promote inheritance of both organelles.

MATERIALS AND METHODS

Strains and plasmid construction: Standard methods were used to manipulate yeast (Sherman *et al.* 1986; Guthrie and Fink 1991) and bacterial (Maniatis *et al.* 1982) strains. All mutations, disruptions, and constructs were confirmed by PCR and DNA sequencing as appropriate. Yeast strains used for the synthetic genetic array (SGA) screen were from the consortium knockout collection (Research Genetics, Birmingham,

TABLE 1

Yeast strains

Strain name	Mating type	Genotype	Source
JSY7000	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100*	Frederick *et al.* (2004)
JSY7002	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 gem1Δ::HIS3*	Frederick *et al.* (2004)
JSY8040	*MAT*α	*ura3Δ::NatMX4 cyh2 lyp1Δ can1Δ::STE2pr-Sp_his5 his3Δ1 leu2Δ0 met15Δ0 LYS2+*	Tong *et al.* (2005)
JSY8071	*MAT*α	*can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+ gem1Δ::NatMX4*	This study
JSY8409	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 gem1Δ::HIS3 mmr1Δ::HIS3*	This study
JSY8413	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 mmr1Δ::HIS3*	This study
JSY8546	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 gem1Δ::HIS3 ypt11Δ::NatMX4*	This study
JSY8563	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 ypt11Δ::NatMX4*	This study
JSY8571	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 mmr1Δ::HIS3 ypt11Δ::NatMX4*	This study
JSY8865	*MAT***a**/*MAT*α	*ade2-1/ade2-1 leu2-3/leu2-3 his3-11,15/his3-11,15 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100 ypt11Δ::NatMX4/ypt11Δ::NatMX4 gem1Δ::URA3/GEM1 mmr1Δ::HIS3/MMR1*	This study
JSY8869	*MAT***a**	*ade2-1 leu2-3 his3-11,15, trp1-1 ura3-1, can1-100 gem1Δ::URA3 mmr1Δ::HIS3 ypt11Δ::NatMX4*	This study
JSY8870	*MAT*α	*ade2-1 leu2-3 his3-11,15, trp1-1 ura3-1, can1-100 gem1Δ::URA3 mmr1Δ::HIS3 ypt11Δ::NatMX4*	This study
JSY8908	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100*	This study
JSY8913	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 ssh1::(SSH1-GFP, HIS3)*	This study
JSY8921	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100 gem1Δ::HIS3*	This study
JSY8962	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100 ptc1Δ::NatMX4*	This study
JSY8966	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100 ypt11Δ::NatMX4*	This study
JSY8968	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100 ypt11Δ::NatMX4 gem1Δ::HIS3*	This study
JSY8971	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100 gem1Δ::HIS3 mmr1Δ::HIS3*	This study
JSY8977	*MAT***a**	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1::(HMG1-eGFP, URA3) can1-100 mmr1Δ::HIS3*	This study

AL) and were of the BY4741 background. All other strains were newly generated in the W303 background by homologous recombination of a PCR-generated marker cassette. Proper integration was confirmed by PCR, and strains were backcrossed prior to use. Strains and plasmids depicted in this study are listed in Tables 1 and 2. p416-*GAL1-MMR1* was generated by PCR amplification of the *MMR1* open reading frame that was cloned into p416-p*GAL1* using the restriction enzymes *Xma*I and *Xho*I. p416-*MET25-YPT11* was generated by PCR amplification of the *YPT11* open reading frame which was cloned into p416-*MET25* using the restriction enzymes *Xma*I and *Xho*I. For visualization of ER, pRS406-*HMG1*-eGFP (Du *et al.* 2001) was linearized with *Stu*I for integration at the *URA3* locus. pRS303-*SSH1*-GFP (Du *et al.* 2006) was linearized with *Eco*RI for integration at the *SSH1* locus, such that *SSH1*-GFP was the sole copy of *SSH1*. Integrants were confirmed by PCR and backcrossed prior to analysis.

The *gem1*Δ *mmr1*Δ *ypt11*Δ triple mutant was generated by isolation of colonies directly from sporulation plates followed by low efficiency transformation with the mtGFP plasmid. Suppressors of the slow growth of this mutant often arose. During our manipulations and analysis, we eliminated cultures that grew faster than the initial doubling time of >12 hr. Multiple independently generated triple-mutant strains showed similar levels of mitochondrial inheritance.

Synthetic lethal screen and subsequent analysis: A synthetic genetic array approach was performed essentially as described (Tong *et al.* 2001; Tong and Boone 2006). The *gem1*Δ haploid query strain (JSY8071) or *ura3*Δ (JSY8040) control strain was mated to the *MAT***a** haploid deletion collection (*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*). Diploids were sporulated, and selections were used to generate a collection of colonies derived from spores that were *MAT***a** double mutants. Strain handling was achieved at 1536 colony spots per Nunc Omnitray using a Biomek 2000 robot and floating pin tool. Pins were sterilized by sonication for 20 sec in 10% bleach, rinsed for 10 sec in water and then 12 sec in 100% ethanol, and dried over a fan for 30 sec. Half of the colonies were mated to a control query strain (*ura3*Δ*::natMX4*) and half to the *gem1*Δ query strain such that each Omnitray contained both the query strain and control strain in duplicate for each of 384 deletion collection spots. Diploids were selected and sporulated followed by selection of *MAT***a** haploids, then *MAT***a** haploids containing the deletion collection allele, and finally *MAT***a** double mutants exactly as described (Tong and Boone 2006). Small or absent *gem1*Δ double-mutant colonies that were present in all previous steps

TABLE 2

Plasmids

Number	Plasmid name	Purpose	Reference
B494	p416-*MET25*	Control for *YPT11* overexpression	ATCC 87324; Mumberg *et al.* (1994)
B496	p416-*GAL1*	Control for *MMR1* overexpression	ATCC 87332; Mumberg *et al.* (1994)
B1220	pYX142-Su9(1-69)-GFP+	Mitochondrial-targeted GFP	Frederick *et al.* (2004)
B1642	p414-*GPD*-Su9(1-69)-RFPff	Mitochondrial-targeted RFP	Frederick *et al.* (2004)
B2159	p416-*GAL1-MMR1*	*MMR1* overexpression	This study
B2160	p416-*MET25-YPT11*	*YPT11* overexpression	This study
B2294	pRS406-*HMG1*-eGFP	ER-targeted GFP integrating vector	Du *et al.* (2001)
B2295	pRS303-*SSH1*-GFP	ER-targeted GFP integrating vector	Du *et al.* (2006)

$n \geq 300$ cells.

of the screen were scored as having synthetic sickness, provided no fitness defects were observed for control double mutants (*ura3*Δ).

Via repeated screens, each deletion in the knockout collection was scored four times for genetic interactions with *gem1*Δ. Genes with at least two hits were considered putative interactors. Genes that were linked on the left arm of chromosome 1 where *GEM1* resides were eliminated from further consideration. Of 453 putative interactions, 231 were chosen for retesting by random spore analysis (Tong and Boone 2006). Thirteen diploids did not sporulate well enough for random spore analysis to be conclusive.

Dissection of tetrads was used to confirm several interactions. Sporulation of heterozygous diploids was achieved in liquid media (1% potassium acetate plus amino acids) for 7 days at room temperature. Tetrads were digested with β-glucoronidase (Sigma, St. Louis), dissected, and allowed to germinate for 3 days at 30°. Analysis of auxotrophic markers was used to determine spore genotypes. In several cases, knockout strains were newly generated in the W303 background and used to analyze fitness defects of double mutants. For W303, diploids were sporulated for 2–3 days at 30° and allowed to germinate for 2–3 days. Images of dissection plates were acquired by a flatbed scanner and processed using Adobe Photoshop CS and Adobe Illustrator CS.

Observation of organelle morphology and inheritance: Mitochondria were labeled by transforming cells with pYX142-Su9 (aa 1–69)-GFP$^+$ or p414-*GPD*-Su9 (aa 1–69)-RFPff (Frederick *et al.* 2004), henceforth referred to as mitochondrial-targeted GFP (mito-GFP) and RFP (mito-RFP). The expressed fusion proteins contain the targeting sequence of subunit 9 of the F_0 ATPase and localize to the mitochondrial matrix. ER was visualized as described previously with Hmg1p-eGFP or Ssh1p-GFP (Du *et al.* 2001, 2006). Yeast strains were grown to log phase (OD_{600} 0.3–1.2) in appropriate synthetic dextrose medium for observation of mitochondria and ER. Organelle inheritance was scored as the presence of fluorescent signal in the bud of small- (bud diameter approximately one-third of mother cell) or large- (diameter one-half to two-thirds of mother cell) budded cells. Mitochondrial inheritance was scored by direct observation of cultures. ER inheritance and mitochondrial inheritance were simultaneously quantified by analysis of epifluorescent (mitochondria) and deconvolved epifluorescent (ER) images of random fields of cells. Each deconvolved *z*-section was analyzed for the presence of cortical ER in the bud periphery to prevent buds above or below the plane of the mother nucleus from being scored incorrectly. Gain settings were adjusted during scoring as needed to visualize signal. Quantification of phenotypes represents the average of three independent experiments with total $n \geq 300$ cells unless noted. Bars indicate standard deviation between experiments.

Microscopy and image analysis: Cells were observed using a Zeiss Axioplan 2 microscope with 100× oil immersion objective (NA = 1.4). Images were acquired, deconvolved, analyzed, and assembled using Zeiss Axiovision version 4.1, Adobe Photoshop CS, and Adobe Illustrator CS. Brightness and contrast were adjusted using only linear adjustments applied to the entire image. For mitochondria, each *z*-stack slice of 0.275 μm was deconvolved with a regularized inverse filter algorithm and all slices were projected on the transparency setting. For mitochondria and ER images, separate stacks of mito-RFP and ER-GFP were obtained with *z*-stack slices 0.2 μm apart and deconvolved with a regularized inverse filter algorithm. Mitochondrial projections were performed as described above. The ER depicted was a single *z*-stack slice after deconvolution. For each cell, a peripheral section was chosen that displayed a cortical network but no nuclear outline (Figure 4A, third section). Center sections were those in which the perinuclear ER and mother cortical ER were visible in typical rim staining patterns (Figure 4A, fourth section).

Overexpression of Mmr1p and Ypt11p: Strains containing either p416-*GAL1-MMR1* or p416-*GAL1* (as a control) were grown in synthetic (S) raffinose (2%), diluted to 0.2 OD_{600}/ml in SGalactose (2%) medium for induction, and observed 1 hr later. For overexpression of Ypt11p, cells containing either p416-*MET25-YPT11* or p416-*MET25* were grown to log phase in SDextrose medium, washed and induced at 0.2 OD_{600}/ml in SDextrose lacking methionine. Organelle distribution was observed 3 hr later.

Accumulation of mitochondria in the bud was scored by visually comparing the fluorescence intensity of mito-GFP in live cells. Buds were scored as having accumulated mitochondria if the bud (Figure 3B) or region near the bud neck (Figure 3C) had greater fluorescence intensity than the mother cell. Accumulation of ER in the bud of Ypt11p or Mmr1p-overexpressing cells was scored by analysis of average fluorescence intensity in deconvolved peripheral *z*-sections. Buds with greater intensity were scored as containing accumulated ER. Scoring of ER accumulation was spot checked by calculation of the average pixel value of the mother and bud using ImageJ (Rasband 2007). Accumulation of mitochondria in these dual-labeling experiments was scored as above from projections of deconvolved *z*-stacks.

RESULTS

Disruption of *GEM1* and genes that encode mitochondrial proteins causes synthetic growth defects: We screened for nonessential yeast genes that displayed synthetic sickness as double mutants with *gem1*Δ. This strategy

(Tong *et al.* 2001) often reveals alternate pathways that contribute to a single essential process. A *gem1*Δ query strain or *ura3*Δ control strain was mated to each of >4000 strains lacking a single nonessential gene. Diploids were sporulated and subjected to a series of selections to generate a double-mutant collection, which was then evaluated for fitness defects. Of 453 putative interactions, 231 were chosen for retesting because they displayed strong synthetic sickness and/or encoded proteins with annotated mitochondrial or cytoskeletal localization or cellular signaling function. Retesting by random spore analysis (Tong *et al.* 2001; Tong and Boone 2006) revealed 46 reproducible interactions (Table 3). Several of these were also verified by dissection (our unpublished data) or by independent confirmation in another strain background (as indicated, Table 3). This screen was quite sensitive as some confirmed interactors exhibited moderate synthetic growth defects in double mutants (our unpublished data). Many of the genes that interacted with *gem1*Δ encode mitochondrial proteins (Table 3, underlined gene names), including four of the five strongest interactors. At least some of the genetic interactions identified in the screen were not strain-background specific as we observed synthetic sickness in the W303 strain background of *gem1*Δ with *mmr1*Δ (Figure 1A), *tom70*Δ, *fmp13*Δ, and *ice2*Δ (our unpublished data). *gem1*Δ *mmr1*Δ double mutants, in particular, were significantly sicker than either single mutant (Figure 1A), confirming a strong genetic interaction between these two loci.

Six genes that produce synthetic growth defects in combination with *gem1*Δ had annotated defects in mitochondrial or ER distribution and/or morphology (Table 3, gene names in boldface type; *fmp13*Δ strains had mitochondrial morphology defects, our unpublished data). Because recent work in mammals and flies suggested that Miro interacts with a mitochondrial motor adapter complex required for mitochondrial distribution (Fransson *et al.* 2006; Glater *et al.* 2006), we focused on this class of genes. Within this class, *gem1*Δ *mmr1*Δ strains had the strongest synthetic growth defect, therefore this interaction was used to understand the contribution of Gem1p to mitochondrial inheritance. In addition, because Ypt11p, like Mmr1p, was previously implicated in Myo2p-dependent mitochondrial distribution and because *YPT11* and *MMR1* genetically interact, we included Ypt11p in our analysis.

Unlike *gem1*Δ *mmr1*Δ double mutants, *gem1*Δ *ypt11*Δ double mutants grew nearly as well as either single mutant (Figure 1B). This observation was consistent with results from the screen, in which *ypt11*Δ was represented but no synthetic interaction was found. Previous studies demonstrated that *mmr1*Δ *ypt11*Δ double mutants were synthetically lethal (Itoh *et al.* 2004). In our W303 strain background, *mmr1*Δ *ypt11*Δ double mutants displayed a severe growth defect, but germinated and could be propagated for further analysis (Figure 1C).

TABLE 3

Synthetic genetic interactors with *gem1*Δ

Strong synthetic interactions	Medium synthetic interactions	Weak synthetic interactions
FMP13[a]	*APP1*[b]	*AAH1*
MMR1[a]	*ATP1*	*AEP1*
PHB2[b]	*CHO2*	*ARO8*
TOM70[a]	*COQ5*	*ATP10*
YGR182C[c]	*CYT2*	*ATP12*
	ICE2[a]	*ATP7*
	OPI3	*BRE5*
	PLP1	*CCE1*
	PTC1	*COX19*
	SNF1	*EOS1*
	YER093C-A	*FMP24*
	YHM2	*FMP30*
	YLR253W	*GCN1*
		IMP1
		MDM38
		MRPL3
		MSS18
		MSS51
		NPT1
		PET122
		RMD11
		TIM13
		VTS1
		YDL162C
		YGL057C
		YGL226W
		YLR422W
		YPL183W-A

Most genes identified as synthetic genetic interactors with *gem1*Δ encode mitochondrial proteins. Interaction between *gem1*Δ and deletions of the listed genes were all confirmed by random spore analysis. Underlined gene names encode proteins with a mitochondrial localization annotated in the Saccharomyces Genome Database (SGD, http://www.yeastgenome.org). Gene names in boldface type have annotated affects on mitochondrial or ER morphology or inheritance. Strength of genetic interaction was based on relative double-mutant fitness and reproducibility.

[a] Interactions confirmed in the W303 strain background.

[b] Interactions confirmed by dissection in the strain background used for the screen.

[c] YGR182c is a dubious ORF and the deletion is a truncation allele of *TIM13*.

Synthetic growth defects in *gem1*Δ *mmr1*Δ and *mmr1*Δ *ypt11*Δ mutants correlate with defective mitochondrial inheritance: To test the hypothesis that the mitochondrial inheritance defects previously observed in *gem1*Δ, *mmr1*Δ, and *ypt11*Δ single mutants (Itoh *et al.* 2002, 2004; Frederick *et al.* 2004) contribute to synthetic sickness in double mutants, we visualized mitochondria using mito-GFP. The presence of any detectable mitochondria in the bud was scored as inheritance (Figure 2, A, B, D, G, and H). In wild-type cells, mitochondria were efficiently inherited by both small (91%) and large buds (100%; Figure 2, A, D, and I). In the *gem1*Δ or *mmr1*Δ

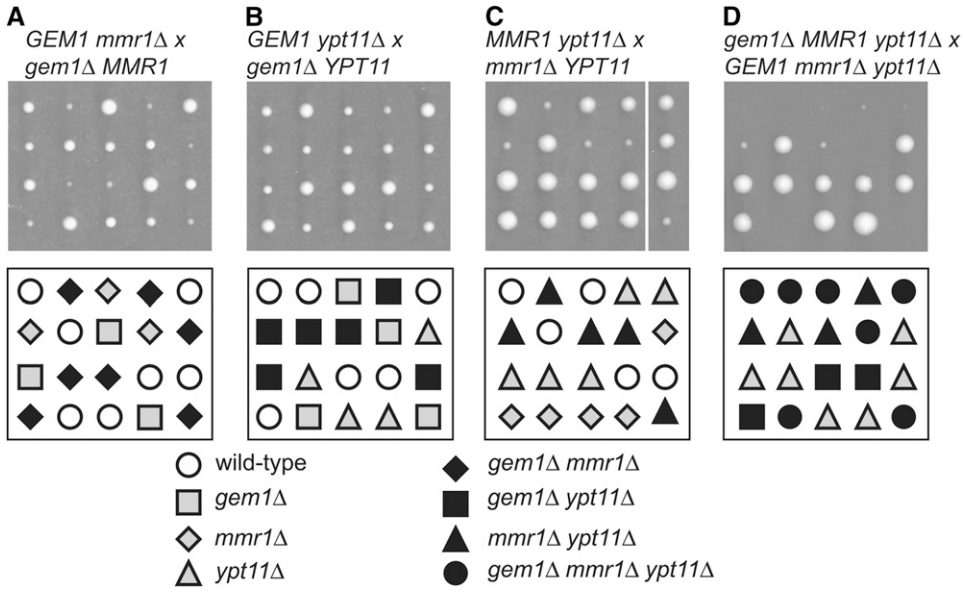


FIGURE 1.—Synthetic interactions among *gem1*Δ and genes that affect Myo2p-dependent mitochondrial inheritance (*MMR1* and *YPT11*) revealed that multiple pathways contribute to strain viability. *gem1*Δ *mmr1*Δ (A) and *mmr1*Δ *ypt11*Δ (C) double mutants displayed growth defects. *gem1*Δ *ypt11*Δ (B) double mutants grew as well as either single mutant. *gem1*Δ *mmr1*Δ *ypt11*Δ (D) triple mutants germinated poorly and grew very slowly (doubling time >12 hr). Diploid W303 strains with the partial genotypes indicated at the top were dissected, and the four progeny from a single tetrad were arranged in vertical columns. Each spore genotype is indicated by a symbol in the bottom sections corresponding to its position in the top.

single mutants, mitochondrial inheritance was defective in some small-budded cells (61% inheritance for both), but occurred later in the cell cycle such that faithful inheritance was observed in large buds (98–99%; Figure 2I). The *gem1*Δ *mmr1*Δ double mutant displayed a severe inheritance defect in small-budded cells (28% inheritance; Figure 2C). In contrast to the single mutants, this defect did not merely represent an inheritance delay as only 51% of large-budded cells inherited mitochondria (Figure 2, E and F). Of the large buds that displayed mitochondrial inheritance, about half contained only one or two mitochondrial pieces (our unpublished data, Figure 2, G and H). In addition, and as observed in other severe mitochondrial inheritance mutants (McConnell *et al.* 1990), *gem1*Δ *mmr1*Δ cells were often multibudded, contained large vacuoles, and appeared generally unhealthy (our unpublished data; Figure 2H). By contrast, the *gem1*Δ *ypt11*Δ mutant, which grew well, had efficient inheritance in large-budded cells (95%) and slightly impaired inheritance (44%) in small-budded cells compared to the single *gem1*Δ mutant (Figure 2I). In agreement with previous observations (Itoh *et al.* 2004), deletion of both *MMR1* and *YPT11* conferred severe mitochondrial inheritance defects in both small- and large-budded cells (5 and 28% inheritance, respectively; Figure 2I). As in the *gem1*Δ *mmr1*Δ mutant, large-budded *mmr1*Δ *ypt11*Δ cells that inherited mitochondria often had only one or two small mitochondrial pieces (our unpublished data). Thus, defective mitochondrial inheritance by large buds correlated with strain sickness (Figure 1). Importantly, mutant cells with normal mitochondrial morphology can still display severe mitochondrial inheritance defects (Table 4), indicating that these defects do not correlate with abnormal mitochondrial morphology.

Gem1p, Mmr1p, and Ypt11p each contribute to an essential process: In cases where mitochondrial inheritance is absolutely blocked, buds fail to separate, resulting in lethality (McConnell *et al.* 1990). To determine whether the Gem1p-, Mmr1p-, and Ypt11p-dependent pathways make individual contributions to mitochondrial inheritance, we constructed the *gem1*Δ *mmr1*Δ *ypt11*Δ triple mutant. As shown in Figure 1D, cells lacking all three genes only formed microcolonies. Thus, Gem1p, Mmr1p, and Ypt11p all make independent contributions to cell viability.

To confirm that the severe synthetic sickness of the triple mutant does not simply reflect a "sick and sicker" interaction, we grew several triple mutants and analyzed mitochondrial inheritance. *gem1*Δ *mmr1*Δ *ypt11*Δ strains used for this analysis grew very slowly (doubling time >12 hr) and were often multibudded. Fewer *gem1*Δ *mmr1*Δ *ypt11*Δ triple-mutant large buds inherited mitochondria (13 ± 1%) compared to *mmr1*Δ *ypt11*Δ double-mutant large buds (28 ± 4%). The Student's *t*-test ($P = 0.021$) indicates this is a statistically significant difference. Those buds that inherited mitochondria almost always had only one or two small mitochondrial pieces. It was not possible to score small buds because they were rarely found in these cultures. This residual level of mitochondrial inheritance could indicate that additional molecules or pathways mediate inheritance in the triple mutant. Alternatively, it could reflect the minimum level of mitochondrial inheritance that can be observed in large-budded cells due to stochastic events that allow single mitochondrial tubules to cross the bud neck and enter the bud.

Mmr1p- and Ypt11p-mediated mitochondrial accumulation phenotypes do not depend on Gem1p: To further test whether these proteins can function independently, we took advantage of previous observations that overexpression of *MMR1* or *YPT11* causes accumulation of mitochondria in buds with a concomitant decrease in the complement of mother cell mitochondria (Itoh *et al.* 2002, 2004). We constructed overexpression

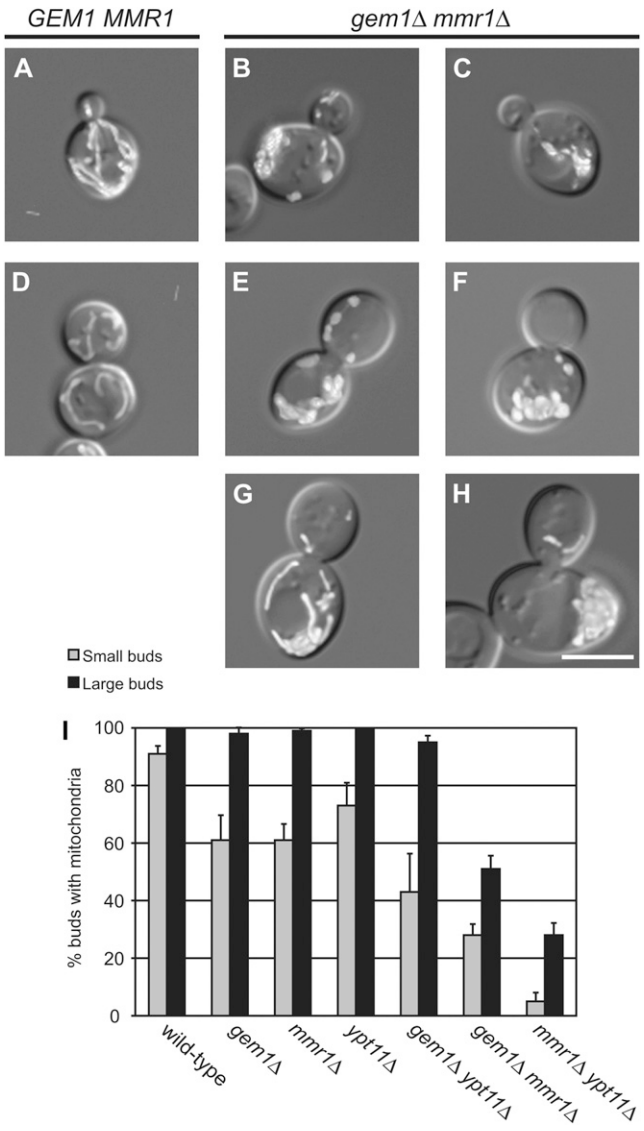


Figure 2.—Mitochondrial inheritance defects correlated with reduced viability in synthetic double mutants. (A, B, D, and E) Examples of successful mitochondrial inheritance. (C and F) Examples of defective mitochondrial inheritance. (G and H) Examples of severely reduced mitochondrial inheritance (≤2 mitochondrial pieces per bud). Representative small- (A–C) and large- (D–H) budded cells of wild type (A and D) and *gem1*Δ *mmr1*Δ (B, C, and E–H) cultures. (I) Quantification of small- (shaded bars) and large- (solid bars) budded inheritance in strains of the indicated genotype. Presence of any mitochondria in the bud was scored as successful mitochondrial inheritance. Signal derived from mito-GFP was overlaid on the DIC image in this and other figures. $N \geq 150$ for each genotype and bud size. Error bars indicate standard deviation between at least three independent experiments in this and other figures. Bar, 5 μm.

plasmids for Mmr1p and Ypt11p and characterized the mitochondrial distribution in wild-type and mutant strains.

During overexpression of *MMR1,* more mitochondria were observed in the bud than the mother cell, as reported previously (Figure 3B) (Itoh *et al.* 2004). In addition, mitochondria accumulated at the bud neck in large-budded cells (Figure 3C), a phenotype consistent with a Myo2p-dependent process (Myo2p participates in cytokinesis, Wagner *et al.* 2002). By contrast, control cells contained approximately equal amounts of well-distributed mitochondria in mother and daughter cells (vector only; Figure 3A). Mmr1p-mediated mitochondrial accumulation did not depend on Gem1p, as *gem1*Δ cells also exhibited accumulation in the bud during Mmr1p overexpression (Figure 3, E and F). Consistent with previous observations (Itoh *et al.* 2004), mitochondrial accumulation in *YPT11* and *ypt11*Δ cells overexpressing Mmr1p was similar (Figure 3G), indicating that Mmr1p can function independently of Ypt11p. We further tested independence of these pathways by overexpressing Mmr1p in the absence of both Gem1p and Ypt11p. *gem1*Δ *ypt11*Δ buds exhibited mitochondrial accumulation during Mmr1p overexpression (Figure 3G). Thus, Mmr1p does not require either Ypt11p or Gem1p for its function.

Overexpression of Ypt11p in wild-type cells caused mitochondrial accumulation in 73% of wild-type buds compared to 3% of buds in vector-only control populations (Figure 3, H–J and N), consistent with previous observations (Itoh *et al.* 2002). In the absence of *GEM1*, mitochondria still accumulated in the daughter cell during Ypt11p overexpression (Figure 3, K–M and N). Ypt11p overexpression caused mitochondrial accumulation even in the absence of Mmr1p, in agreement with previous observations (Figure 3N) (Itoh *et al.* 2004). Moreover, deletion of both Mmr1p and Gem1p did not affect accumulation phenotypes. Therefore, Ypt11p requires neither Mmr1p nor Gem1p for its function. Interestingly, despite the gross mitochondrial morphology defects in the absence of Gem1p, mitochondria could still be efficiently moved into the daughter bud during this overexpression experiment (globular mitochondria, Figure 3M), demonstrating that globular mitochondria are still capable of interacting with at least some of the transport machinery.

Mitochondrial inheritance is not a prerequisite for ER inheritance: Several observations raised the possibility that ER and mitochondrial inheritance are linked. First, physical interactions between mitochondria and peripheral ER in mammalian cells and yeast were discovered (Csordas *et al.* 2006; Perktold *et al.* 2007). Second, *ypt11*Δ, a mutant with established mitochondrial inheritance defects (Figure 2) (Boldogh *et al.* 2004; Itoh *et al.* 2004), was reported to reduce cortical ER abundance in small buds (Buvelot Frei *et al.* 2006). Third, several of the genes that interact genetically with *gem1*Δ (Table 3) promote cortical ER distribution, including *ICE2* (Estrada de Martin *et al.* 2005) and *PTC1* (Du *et al.* 2006). Despite these observations, careful analysis of ER distribution under conditions that impair mitochondrial inheritance had never been performed. Thus, we assessed mitochondrial inheritance and cortical ER distribution together in mutants that have mild or severe mitochondrial inheritance defects.

TABLE 4

Mitochondrial morphology does not correlate with severity of mitochondrial inheritance defects

Strain	Mitochondrial morphology				Mitochondrial inheritance in large buds (%)
	% highly connected tubular	% branched tubular	% irregular tubular	% globular and fragmented	
Wild type	5.34 ± 3	87.3 ± 2.4	6.4 ± 4.1	1.0 ± 1.0	100
*gem1*Δ	0.3 ± 0.6	3.0 ± 3.0	24.0 ± 4.4	72.6 ± 5.7	98
*mmr1*Δ	16.7 ± 2.1	63.0 ± 5.0	7.7 ± 3.1	12.7 ± 6.1	99
*ypt11*Δ	4.7 ± 1.5	85.3 ± 4.0	7.7 ± 3.2	2.3 ± 2.5	100
*gem1*Δ *mmr1*Δ	2.0 ± 1.7	2.7 ± 2.9	16.3 ± 6.5	79.0 ± 3.6	51
*gem1*Δ *ypt11*Δ	0.0 ± 0	1.5 ± 1.0	14 ± 1.8	84.5 ± 2.4	95
*mmr1*Δ *ypt11*Δ	25.0 ± 1.0	37.7 ± 4.7	13.7 ± 3	22.7 ± 7.6	28

Cortical ER inheritance occurs when an ER tubule moves into the bud tip. Soon afterwards, ER is distributed around the cortex of the small bud (Fehrenbacher *et al.* 2002; Du *et al.* 2004). This cortical distribution was visualized as a rim stain around the edge of the bud in *z*-slices through the center of the bud (Figure 4A, fourth section). Perinuclear ER, which surrounds the nucleus, was also visible in the mother cell. Cortical ER was also often visible as networks in peripheral *z*-sections closer to the top or bottom of the cell (Figure 4A, third section). We observed faithful ER inheritance and cortical distribution in 88% of small buds with our imaging conditions (Figure 4D), while only 12% of small-budded wild-type cells displayed a single tubule (Figure 4C) or completely lacked ER staining in the bud (not depicted; <3% of cells in all strains). ER distribution in small buds occurs in ~80% of *gem1*Δ, *mmr1*Δ, and *ypt11*Δ single mutants, in which mitochondrial inheritance is disrupted in up to 50% of small buds. Notably, even though mitochondrial inheritance was drastically reduced in *gem1*Δ *mmr1*Δ (Figure 4B) and *mmr1*Δ *ypt11*Δ double mutants, ER inheritance and distribution to the bud cortex occurred faithfully in at least 77% of small-budded cells (Figure 4D). The *ptc1*Δ mutation was previously shown

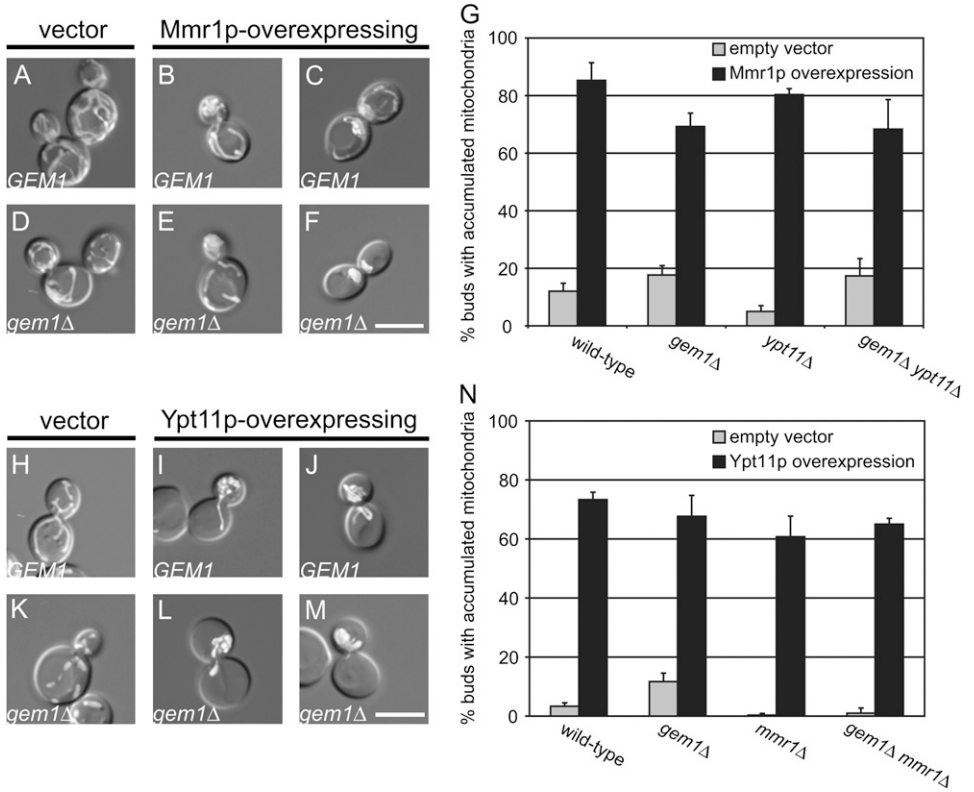
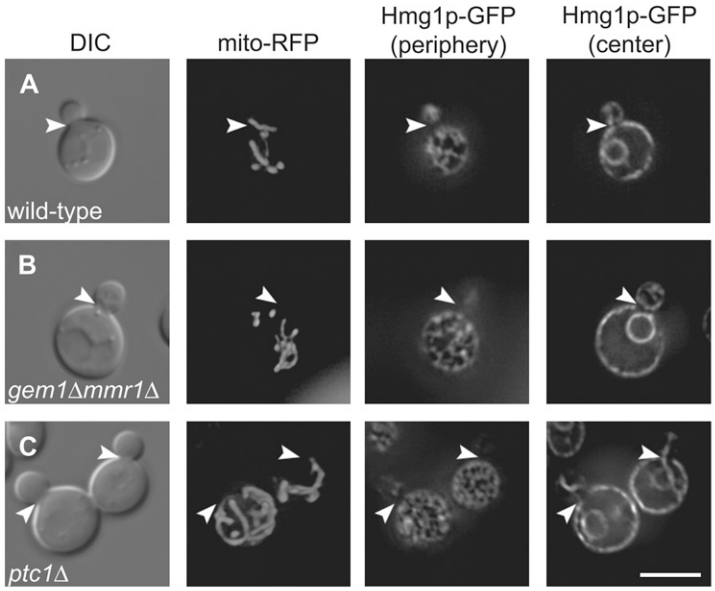


FIGURE 3.—Mmr1p or Ypt11p overexpression cause mitochondria to accumulate in buds independent of Gem1p function. (A–G) Mmr1p overexpression was achieved by galactose induction for 1 hr after preculture in raffinose. (A and D) *GEM1* and *gem1*Δ strains containing empty vector. (B and E) Mitochondria accumulated in the bud of wild-type and *gem1*Δ strains. (C and F) Mitochondria accumulated at the bud neck of wild-type and *gem1*Δ strains. (G) Quantification of accumulation at the bud or bud neck region in control cells (shaded bar) and cells overexpressing Mmr1p (solid bar) of the indicated genotype. $n \geq 300$ budded cells. (H–N) Ypt11p overexpression. (H and K) *GEM1* and *gem1*Δ cells containing empty vector. (I, J, L, and M) Representative images of *GEM1* and *gem1*Δ cells grown in medium lacking methionine for 3 hr to overexpress *MET25*-driven *YPT11*. (N) Quantification of mitochondrial accumulation in control experiments (shaded bars) or during Ypt11p overexpression (solid bars) in strains of the indicated genotypes. $n \geq 300$ medium and large-budded cells. Bar, 5 μm.



D

Mitochondrial inheritance		+	+	-	-	Total	n/a
Cortical ER distribution		+	-	+	-	n/a	Total
Wild-type	n = 179	80%	8%	7%	4%	88%	88%
gem1Δ	n = 112	46%	5%	37%	13%	51%	82%
mmr1Δ	n = 165	45%	6%	38%	12%	51%	82%
ypt11Δ	n = 137	54%	9%	26%	12%	63%	80%
gem1Δ mmr1Δ	n = 120	9%	1%	76%	14%	10%	85%
gem1Δ ypt11Δ	n = 100	28%	3%	59%	10%	31%	87%
mmr1Δ ypt11Δ	n = 104	2%	0%	75%	23%	2%	77%
ptc1Δ	n = 136	6%	4%	49%	41%	10%	55%

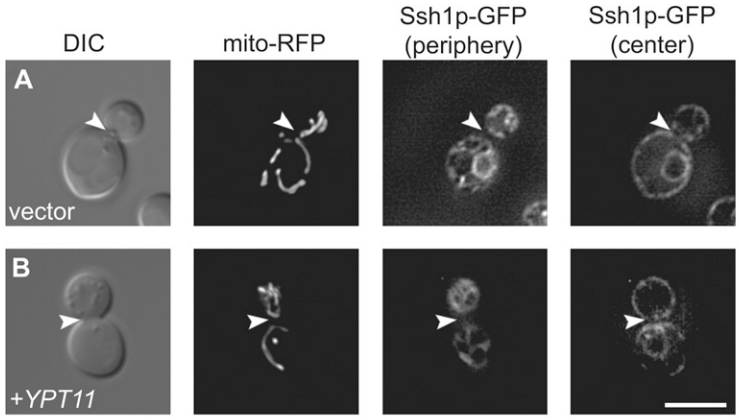
FIGURE 4.—Mitochondrial inheritance did not influence distribution of ER in the bud. Sections from left to right are DIC, mito-RFP deconvolved projection, Hmg1p-GFP-labeled ER deconvolved single *z*-sections at the periphery, and at the center of the cell. Inheritance and distribution of ER in wild type (A), and *gem1Δ mmr1Δ* (B) to the cortex of small buds is evident, regardless of mitochondrial inheritance status. (C) *ptc1Δ* mutant cells display inheritance of a single ER tubule but do not appropriately distribute ER to the cortex of small buds. Arrowheads mark the mother-bud neck. (D) Quantification reveals that mitochondrial inheritance is not a prerequisite for ER inheritance and cortical distribution. In the schematic, cortical ER is represented by shaded and mitochondria by solid lines. Bar, 5 μm.

to cause defects in both ER distribution (DU *et al.* 2006) and mitochondrial inheritance (ROEDER *et al.* 1998; DU *et al.* 2006). *ptc1Δ* mutants contained a single tubule of ER in ~45% of small buds but did not distribute ER to the cortex in those daughter cells and displayed significant defects in mitochondrial transfer to small buds (Figure 4). In Figure 4C, only dim ER signal rather than a bright cortical network was visible in the peripheral slice. In sections from the center of the cell, a lone ER tubule is visible (Figure 4C; fourth section), in stark contrast to the cortical ER distribution observed in wild-type cells (Figure 4A; fourth section). Slightly lower mitochondrial inheritance efficiency was observed in all strains during this experiment compared to efficiencies reported in Figure 2. This was due to reduced sensitivity when scoring from digital images rather than live cells.

Mitochondrial accumulation is not sufficient to cause alterations in ER distribution: To further address whether mitochondrial and ER inheritance are linked, we tested whether ER accumulates in the bud when mitochondrial accumulation is caused by Mmr1p or Ypt11p overexpression. Consistent with its ER localization (BUVELOT FREI *et al.* 2006), overexpression of Ypt11p was sufficient to cause a higher average fluorescence intensity of ER-localized Ssh1p-GFP in the bud than in the mother cell (Figure 5B, third section). This accumulation was evident in 75% of Ypt11p-overexpressing cells compared to 24% of control cells (Figure 5), and accumulation of both ER and mitochondria occurred more frequently than either alone (Figure 5C). By contrast, overexpression of Mmr1p, which has not previously been linked to ER inheritance or distribution, did not alter ER distribution (Figure 5C), even though mitochondria had accumulated in buds. Thus, mitochondrial accumulation in the bud was not sufficient to drive ectopic ER into the daughter cell. Ypt11p can therefore function in inheritance of both mitochondria and ER.

DISCUSSION

Mitochondria cannot be generated *de novo*, so their distribution and inheritance in budding yeast are crucial for cell viability. We identified genetic interactions between Myo2p-dependent mitochondrial inheritance pathways and the Miro GTPase, Gem1p (Figure 1



C

Wild-type expressing:	Normal mitochondrial distribution		Mitochondria accumulated in bud		ER bud > ER mother
	ER bud ≤ ER mother	ER bud > mother	ER bud ≤ ER mother	ER bud > ER mother	Total
Empty vector (for *YPT11*)	67%	19%	9%	5%	24% (31/129)
YPT11	18%	15%	7%	60%	75% (110/147)
Empty vector (for *MMR1*)	68%	22%	5%	5%	27% (32/118)
MMR1	19%	7%	49%	24%	31% (34/108)

Figure 5.—Overexpression of Ypt11p caused ER to accumulate in buds regardless of mitochondrial distribution. Sections from left to right are DIC, mito-RFP deconvolved projection, Ssh1p-GFP-labeled ER deconvolved single *z*-sections at the periphery, and at the center of the cell. (A) Wild-type cells expressing empty vector. (B) Overexpression of *YPT11* as in Figure 3. (C) Quantification revealed that ER accumulates in buds during Ypt11p overexpression but not Mmr1p overexpression. Arrowheads mark the mother-bud neck. Bar, 5 μm.

and Table 3). Sick double-mutant strains displayed more severe mitochondrial inheritance defects than the single mutants, particularly in large-budded cells (Figure 2). Overexpression experiments indicated that each of the proteins can function independently (Figure 3). Additional studies revealed that mitochondrial inheritance status does not affect ER inheritance or cortical distribution (Figures 4 and 5).

Multiple mitochondrial inheritance pathways: Several lines of evidence suggest that Gem1p, Mmr1p, and Ypt11p function in independent pathways to promote mitochondrial inheritance. First, the additive genetic interaction of the triple mutant compared to the single and double mutants reveals that each protein normally contributes to mitochondrial inheritance (Figure 1). Second, overexpression experiments indicate that none of the proteins are required for downstream effects of Mmr1p or Ypt11p. Because overexpression of Gem1p did not cause a substantial phenotype in wild-type cells (our unpublished data), we were unable to formally test whether Ypt11p and/or Mmr1p function downstream of Gem1p. Typically, however, additive effects such as those observed for mitochondrial inheritance and viability in the *gem1*Δ *mmr1*Δ and *gem1*Δ *mmr1*Δ *ypt11*Δ mutants indicate that parallel pathways have been compromised.

In other cases, additive phenotypes are due to disruption of the same step of the same pathway, often when members of the same complex are mutated (Youngman *et al.* 2004; Measday *et al.* 2005). This interpretation could apply to Mmr1p and Ypt11p because both form a complex with Myo2p. However, Mmr1p localizes to mitochondria (Itoh *et al.* 2004) while Ypt11p localizes to the ER (Buvelot Frei *et al.* 2006). Although both proteins are proposed to bind the Myo2p tail, allele specific interactions suggest they may form independent complexes with Myo2p (Itoh *et al.* 2002, 2004). In addition, Mmr1p and Ypt11p affect ER distribution differently when overexpressed (Figure 5), suggesting that they have distinct *in vivo* functions. Therefore, our data are most consistent with the interpretation that Gem1p, Mmr1p, and Ypt11p function independently to promote mitochondrial inheritance.

Our observations raise the question of why organisms would have multiple, independent mechanisms to coordinate organelle inheritance. For essential processes like mitochondrial inheritance, having a so-called "back-up" system would allow organism survival during failure of one mitochondrial inheritance mechanism. It seems more likely, however, that these pathways differentially contribute to inheritance during the yeast life cycle (Suda *et al.* 2007) or under other physiological conditions not replicated in the laboratory.

The role of physical associations between organelles in inheritance: Recently, physical associations between mitochondria and ER have been observed in mammalian cells and yeast (Csordas *et al.* 2006; Perktold *et al.* 2007). These physical contacts could, in principal, contribute to distribution of both organelles during division. However, our findings indicate that ER inheri-

tance and distribution are not affected by mitochondrial inheritance nor can mitochondrial accumulation drive ER accumulation in the bud. Thus, in yeast, the physical association between mitochondria and ER is not essential for ER inheritance. Previous studies showed that the *sec3*Δ mutant, which displays ER inheritance defects, does not have mitochondrial inheritance defects (Wiederkehr *et al.* 2003). Together, these observations demonstrate that mitochondrial inheritance and ER inheritance are not strictly coupled processes. In addition, we found no evidence for correlation between mitochondrial inheritance and nuclear inheritance in cells with defective mitochondrial inheritance (our unpublished data).

Conserved functions for Miro GTPases: We previously demonstrated that Gem1p functions to maintain mitochondrial morphology, retain mitochondrial DNA nucleoids, and promote mitochondrial inheritance in yeast (Frederick *et al.* 2004). More recent work has linked Miro GTPases to kinesin proteins required for microtubule-based mitochondrial movement in higher organisms (Guo *et al.* 2005; Cox and Spradling 2006; Fransson *et al.* 2006; Glater *et al.* 2006). We also observed both mitochondrial morphology and inheritance defects in *miro*Δ fission yeast (our unpublished data). The synthetic interactions we report here demonstrate that the contribution of Gem1p to mitochondrial inheritance in yeast is important for cell viability. Therefore, Gem1p/Miro has a conserved function in mitochondrial distribution. However, mitochondrial movement in yeast occurs on actin cables rather than microtubules, suggesting that the molecular details of Miro function must differ in different organisms.

Miro proteins have been implicated in kinesin-based mitochondrial movement in multicellular organisms, although a stable physical interaction between Miro and kinesin has not yet been captured. We considered the possibility that Gem1p coordinates with Myo2p to facilitate mitochondrial inheritance. However, to date we have been unable to find evidence for this hypothesis. Yeast two-hybrid and co-immunoprecipitation studies failed to detect interactions between Gem1p and Myo2p (our unpublished data). Identification of additional Miro protein-binding partners and characterization of hypomorphic alleles should help illuminate the molecular mechanisms by which Miro proteins contribute to mitochondrial distribution.

We thank members of the Shaw laboratory for helpful discussions. Charlie Boone (University of Toronto), Susan Ferro-Novick (Yale University, New Haven, CT), Yasushi Matsui (University of Tokyo), Mike Yaffe (University of California, San Diego), Lois Weisman (University of Michigan Life Sciences Institute), Brad Cairns (University of Utah), and Markus Babst (University of Utah) graciously provided strains or plasmids used in this work. Jim Metherall, Darren Warnick, and Casey Barnett assisted with robotics development and training. Support for this work was provided by the National Science Foundation Graduate Research Fellowship Program (to R.L.F.), the United Mitochondrial Disease Foundation (to K.O.), the American Heart Association (to K.O.), and the National Institutes of Health (to J.M.S., GM-53466). The University of Utah Health Sciences DNA/Peptide and Sequencing Facilities are supported by National Cancer Institute grant 5-P30CA42014.

LITERATURE CITED

Altmann, K., and B. Westermann, 2005 Role of essential genes in mitochondrial morphogenesis in Saccharomyces cerevisiae. Mol. Biol. Cell **16:** 5410–5417.

Boldogh, I., N. Vojtov, S. Karmon and L. A. Pon, 1998 Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. J. Cell Biol. **141:** 1371–1381.

Boldogh, I. R., H. C. Yang and L. A. Pon, 2001 Mitochondrial inheritance in budding yeast. Traffic **2:** 368–374.

Boldogh, I. R., D. W. Nowakowski, H. C. Yang, H. Chung, S. Karmon *et al.*, 2003 A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. Mol. Biol. Cell **14:** 4618–4627.

Boldogh, I. R., S. L. Ramcharan, H. C. Yang and L. A. Pon, 2004 A type V myosin (Myo2p) and a Rab-like G-protein (Ypt11p) are required for retention of newly inherited mitochondria in yeast cells during cell division. Mol. Biol. Cell **15:** 3994–4002.

Buvelot Frei, S., P. B. Rahl, M. Nussbaum, B. J. Briggs, M. Calero *et al.*, 2006 Bioinformatic and comparative localization of rab proteins reveals functional insights into the uncharacterized GTPases Ypt10p and Ypt11p. Mol. Cell. Biol. **26:** 7299–7317.

Catlett, N. L., J. E. Duex, F. Tang and L. S. Weisman, 2000 Two distinct regions in a yeast myosin-V tail domain are required for the movement of different cargoes. J. Cell Biol. **150:** 513–526.

Chan, D. C., 2006 Mitochondrial fusion and fission in mammals. Annu. Rev. Cell. Dev. Biol. **22:** 79–99.

Cox, R. T., and A. C. Spradling, 2006 Milton controls the early acquisition of mitochondria by Drosophila oocytes. Development **133:** 3371–3377.

Csordas, G., C. Renken, P. Varnai, L. Walter, D. Weaver *et al.*, 2006 Structural and functional features and significance of the physical linkage between ER and mitochondria. J. Cell Biol. **174:** 915–921.

Drubin, D. G., H. D. Jones and K. F. Wertman, 1993 Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. Mol. Biol. Cell **4:** 1277–1294.

Du, Y., M. Pypaert, P. Novick and S. Ferro-Novick, 2001 Aux1p/Swa2p is required for cortical endoplasmic reticulum inheritance in Saccharomyces cerevisiae. Mol. Biol. Cell **12:** 2614–2628.

Du, Y., S. Ferro-Novick and P. Novick, 2004 Dynamics and inheritance of the endoplasmic reticulum. J. Cell Sci. **117:** 2871–2878.

Du, Y., L. Walker, P. Novick and S. Ferro-Novick, 2006 Ptc1p regulates cortical ER inheritance via Slt2p. EMBO J. **25:** 4413–4422.

Escobar-Henriques, M., and T. Langer, 2006 Mitochondrial shaping cuts. Biochim. Biophys. Acta **1763:** 422–429.

Estrada de Martin, P., Y. Du, P. Novick and S. Ferro-Novick, 2005 Ice2p is important for the distribution and structure of the cortical ER network in Saccharomyces cerevisiae. J. Cell Sci. **118:** 65–77.

Fehrenbacher, K. L., D. Davis, M. Wu, I. Boldogh and L. A. Pon, 2002 Endoplasmic reticulum dynamics, inheritance, and cytoskeletal interactions in budding yeast. Mol. Biol. Cell **13:** 854–865.

Fehrenbacher, K., T. Huckaba, H. C. Yang, I. Boldogh and L. Pon, 2003a Actin comet tails, endosomes and endosymbionts. J. Exp. Biol. **206:** 1977–1984.

Fehrenbacher, K. L., I. R. Boldogh and L. A. Pon, 2003b Taking the A-train: actin-based force generators and organelle targeting. Trends Cell Biol. **13:** 472–477.

Fehrenbacher, K. L., H. C. Yang, A. C. Gay, T. M. Huckaba and L. A. Pon, 2004 Live cell imaging of mitochondrial movement along actin cables in budding yeast. Curr. Biol. **14:** 1996–2004.

Fransson, A., A. Ruusala and P. Aspenstrom, 2003 Atypical Rho GTPases have roles in mitochondrial homeostasis and apoptosis. J. Biol. Chem. **278:** 6495–6502.

Fransson, S., A. Ruusala and P. Aspenstrom, 2006 The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. Biochem. Biophys. Res. Commun. **344:** 500–510.

Frazier, A. E., C. Kiu, D. Stojanovski, N. J. Hoogenraad and M. T. Ryan, 2006 Mitochondrial morphology and distribution in mammalian cells. Biol. Chem. **387:** 1551–1558.

Frederick, R. L., J. M. McCaffery, K. W. Cunningham, K. Okamoto and J. M. Shaw, 2004 Yeast Miro GTPase, Gem1p, regulates mitochondrial morphology via a novel pathway. J. Cell Biol. **167:** 87–98.

Glater, E. E., L. J. Megeath, R. S. Stowers and T. L. Schwarz, 2006 Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. J. Cell Biol. **173:** 545–557.

Gorska-Andrzejak, J., R. S. Stowers, J. Borycz, R. Kostyleva, T. L. Schwarz *et al.*, 2003 Mitochondria are redistributed in Drosophila photoreceptors lacking milton, a kinesin-associated protein. J. Comp. Neurol. **463:** 372–388.

Griffin, E. E., S. A. Detmer and D. C. Chan, 2006 Molecular mechanism of mitochondrial membrane fusion. Biochim. Biophys. Acta **1763:** 482–489.

Guo, X., G. T. Macleod, A. Wellington, F. Hu, S. Panchumarthi *et al.*, 2005 The GTPase dMiro is required for axonal transport of mitochondria to Drosophila synapses. Neuron **47:** 379–393.

Guthrie, C., and G. R. Fink, 1991 Yeast genetics and molecular biology, pp. 1–933 in *Methods in Enzymology*, edited by J. N. Abelson and M. I. Simon. Academic Press, San Diego.

Hanekamp, T., M. K. Thorsness, I. Rebbapragada, E. M. Fisher, C. Seebart *et al.*, 2002 Maintenance of mitochondrial morphology is linked to maintenance of the mitochondrial genome in *Saccharomyces cerevisiae*. Genetics **162:** 1147–1156.

Hobbs, A. E., M. Srinivasan, J. M. McCaffery and R. E. Jensen, 2001 Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. J. Cell Biol. **152:** 401–410.

Hollenbeck, P. J., 1996 The pattern and mechanism of mitochondrial transport in axons. Front. Biosci. **1:** d91–d102.

Hollenbeck, P. J., 2005 Mitochondria and neurotransmission: evacuating the synapse. Neuron **47:** 331–333.

Hollenbeck, P. J., and W. M. Saxton, 2005 The axonal transport of mitochondria. J. Cell Sci. **118:** 5411–5419.

Huckaba, T. M., A. C. Gay, L. F. Pantalena, H. C. Yang and L. A. Pon, 2004 Live cell imaging of the assembly, disassembly, and actin cable-dependent movement of endosomes and actin patches in the budding yeast, Saccharomyces cerevisiae. J. Cell Biol. **167:** 519–530.

Ishikawa, K., N. L. Catlett, J. L. Novak, F. Tang, J. J. Nau *et al.*, 2003 Identification of an organelle-specific myosin V receptor. J. Cell Biol. **160:** 887–897.

Itoh, T., A. Watabe, E. A. Toh and Y. Matsui, 2002 Complex formation with Ypt11p, a rab-type small GTPase, is essential to facilitate the function of Myo2p, a class V myosin, in mitochondrial distribution in Saccharomyces cerevisiae. Mol. Cell. Biol. **22:** 7744–7757.

Itoh, T., E. A. Toh and Y. Matsui, 2004 Mmr1p is a mitochondrial factor for Myo2p-dependent inheritance of mitochondria in the budding yeast. EMBO J. **23:** 2520–2530.

Kaksonen, M., Y. Sun and D. G. Drubin, 2003 A pathway for association of receptors, adaptors, and actin during endocytic internalization. Cell **115:** 475–487.

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

Kispal, G., K. Sipos, H. Lange, Z. Fekete, T. Bedekovics *et al.*, 2005 Biogenesis of cytosolic ribosomes requires the essential iron-sulphur protein Rli1p and mitochondria. EMBO J. **24:** 589–598.

Ly, C. V., and P. Verstreken, 2006 Mitochondria at the synapse. Neuroscientist **12:** 291–299.

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

McConnell, S. J., L. C. Stewart, A. Talin and M. P. Yaffe, 1990 Temperature-sensitive yeast mutants defective in mitochondrial inheritance. J. Cell Biol. **111:** 967–976.

Measday, V., K. Baetz, J. Guzzo, K. Yuen, T. Kwok *et al.*, 2005 Systematic yeast synthetic lethal and synthetic dosage lethal screens identify genes required for chromosome segregation. Proc. Natl. Acad. Sci. USA **102:** 13956–13961.

Meeusen, S., and J. Nunnari, 2003 Evidence for a two membrane-spanning autonomous mitochondrial DNA replisome. J. Cell Biol. **163:** 503–510.

Meisinger, C., M. Rissler, A. Chacinska, L. K. Szklarz, D. Milenkovic *et al.*, 2004 The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. Dev. Cell **7:** 61–71.

Meisinger, C., S. Pfannschmidt, M. Rissler, D. Milenkovic, T. Becker *et al.*, 2007 The morphology proteins Mdm12/Mmm1 function in the major beta-barrel assembly pathway of mitochondria. EMBO J. **26:** 2229–2239.

Moseley, J. B., and B. L. Goode, 2006 The yeast actin cytoskeleton: from cellular function to biochemical mechanism. Microbiol. Mol. Biol. Rev. **70:** 605–645.

Okamoto, K., and J. M. Shaw, 2005 Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annu. Rev. Genet. **39:** 503–536.

Pashkova, N., N. L. Catlett, J. L. Novak and L. S. Weisman, 2005 A point mutation in the cargo-binding domain of myosin V affects its interaction with multiple cargoes. Eukaryot. Cell **4:** 787–798.

Pashkova, N., Y. Jin, S. Ramaswamy and L. S. Weisman, 2006 Structural basis for myosin V discrimination between distinct cargoes. EMBO J. **25:** 693–700.

Perktold, A., B. Zechmann, G. Daum and G. Zellnig, 2007 Organelle association visualized by three-dimensional ultrastructural imaging of the yeast cell. FEMS Yeast Res. **7:** 629–638.

Pruyne, D., and A. Bretscher, 2000a Polarization of cell growth in yeast. J. Cell Sci. **113**(Pt 4): 571–585.

Pruyne, D., and A. Bretscher, 2000b Polarization of cell growth in yeast. I. Establishment and maintenance of polarity states. J. Cell Sci. **113**(Pt 3): 365–375.

Pruyne, D. W., D. H. Schott and A. Bretscher, 1998 Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J. Cell Biol. **143:** 1931–1945.

Rasband, W. S., 2007 ImageJ: an open-source image-analysis program. http://rsb.info.nih.gov/ij/index.html. National Institutes of Health, Bethesda, MD.

Rice, S. E., and V. I. Gelfand, 2006 Paradigm lost: milton connects kinesin heavy chain to miro on mitochondria. J. Cell Biol. **173:** 459–461.

Rikhy, R., S. Kamat, S. Ramagiri, V. Sriram and K. S. Krishnan, 2007 Mutations in dynamin-related protein result in gross changes in mitochondrial morphology and affect synaptic vesicle recycling at the Drosophila neuromuscular junction. Genes Brain Behav. **6:** 42–53.

Roeder, A. D., G. J. Hermann, B. R. Keegan, S. A. Thatcher and J. M. Shaw, 1998 Mitochondrial inheritance is delayed in Saccharomyces cerevisiae cells lacking the serine/threonine phosphatase PTC1. Mol. Biol. Cell **9:** 917–930.

Rossanese, O. W., C. A. Reinke, B. J. Bevis, A. T. Hammond, I. B. Sears *et al.*, 2001 A role for actin, Cdc1p, and Myo2p in the inheritance of late Golgi elements in Saccharomyces cerevisiae. J. Cell Biol. **153:** 47–62.

Schott, D., J. Ho, D. Pruyne and A. Bretscher, 1999 The COOH-terminal domain of Myo2p, a yeast myosin V, has a direct role in secretory vesicle targeting. J. Cell Biol. **147:** 791–808.

Shaw, J. M., and J. Nunnari, 2002 Mitochondrial dynamics and division in budding yeast. Trends Cell Biol. **12:** 178–184.

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

Simon, V. R., S. L. Karmon and L. A. Pon, 1997 Mitochondrial inheritance: cell cycle and actin cable dependence of polarized mitochondrial movements in Saccharomyces cerevisiae. Cell Motil. Cytoskeleton **37:** 199–210.

Singer, J. M., G. J. Hermann and J. M. Shaw, 2000 Suppressors of mdm20 in yeast identify new alleles of ACT1 and TPM1 predicted to enhance actin-tropomyosin interactions. Genetics **156:** 523–534.

Stowers, R. S., L. J. Megeath, J. Gorska-Andrzejak, I. A. Meinertzhagen and T. L. Schwarz, 2002 Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. Neuron **36:** 1063–1077.

Suda, Y., H. Nakanishi, E. M. Mathieson and A. M. Neiman, 2007 Alternative modes of organellar segregation during sporulation in *Saccharomyces cerevisiae*. Eukaryot. Cell **6:** 2009–2017.

Szabadkai, G., A. M. Simoni, K. Bianchi, D. De Stefani, S. Leo *et al.*, 2006 Mitochondrial dynamics and Ca2+ signaling. Biochim. Biophys. Acta **1763:** 442–449.

Tong, A. H., and C. Boone, 2006 Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods Mol. Biol. **313:** 171–192.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:** 2364–2368.

Wagner, W., P. Bielli, S. Wacha and A. Ragnini-Wilson, 2002 Mlc1p promotes septum closure during cytokinesis via the IQ motifs of the vesicle motor Myo2p. EMBO J. **21:** 6397–6408.

Warren, G., and W. Wickner, 1996 Organelle inheritance. Cell **84:** 395–400.

Wiederkehr, A., Y. Du, M. Pypaert, S. Ferro-Novick and P. Novick, 2003 Sec3p is needed for the spatial regulation of secretion and for the inheritance of the cortical endoplasmic reticulum. Mol. Biol. Cell **14:** 4770–4782.

Young, M. E., J. A. Cooper and P. C. Bridgman, 2004 Yeast actin patches are networks of branched actin filaments. J. Cell Biol. **166:** 629–635.

Youngman, M. J., A. E. Hobbs, S. M. Burgess, M. Srinivasan and R. E. Jensen, 2004 Mmm2p, a mitochondrial outer membrane protein required for yeast mitochondrial shape and maintenance of mtDNA nucleoids. J. Cell Biol. **164:** 677–688.

Communicating editor: F. Winston