Appendix

Identification of candidate mitochondrial fusion and fission factors

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In collaboration with Johannes Graumann in Ray Deshaies' lab, we have taken a proteomics-based approach to identify new regulators of mitochondrial fusion and fission. In the past, a number of genetic screens have identified genes required for mitochondrial dynamics in *S. cerevisiae*. For example, temperature-sensitive screens identified mutants defective for mitochondrial distribution and maintenance (mdm mutants) (Hermann et al., 1997; McConnell et al., 1990) and maintenance of mitochondrial morphology (mmm mutants) (Berger and Yaffe, 1998), and suppressor screens identified Mdv1p and Fis1p as components of the mitochondrial fission pathway (Fekkes et al., 2000; Mozdy et al., 2000; Tieu and Nunnari, 2000). Recently, a visual screen of yeast with transcriptionally repressed essential genes (Altmann and Westermann, 2005) and deletion of non-essential genes (Dimmer et al., 2002) has systematically tested the majority yeast genome for mitochondrial morphology mutants.

Identification of interacting proteins provides an important complement to genetic screens for two primary reasons. First, biochemical strategies may identify proteins that are missed in forward genetic screens because they are essential, redundant, or have weak or conditional phenotypes. Second, identification binding partners can provide important mechanistic clues about a protein. Here, we present results from TAP-MudPIT experiments designed to identify binding partners of mitochondrial fusion and fission machinery. Purifications were performed from rich dextrose cultures as described in Chapter 2 unless otherwise noted. Candidate binding partners are classified for their presence in highly purified mitochondrial preparations (Sickmann et al., 2003), their annotated localization in the *Saccharomyces* Genome Database, and their localization as

GFP-fusion proteins (Huh et al., 2003). In addition, we note the number of unique peptides identified for each protein and whether mitochondrial morphology defects were detected in visual screens of yeast with deletions in non-essential genes (Dimmer et al., 2002) or essential genes transcriptionally repressed with the TetO promoter repression system (Altmann and Westermann, 2005). Finally, we highlight particularly interesting candidate binding partners that may warrant further investigation.

Abbreviations

TAP, tandem affinity purification; MudPIT, multi-dimensional protein identification technology; ACP, actin cortical patches; cyt, cytosol; NPC, nuclear pore complex; nuc, nucleus; mito, mitochondria; OM, outer membrane; IM, inner membrane; 19S, 19S subunit of the proteasome; 26S, 26S subunit of the proteasome; punc, punctate; vac, vacuole; ER, endoplasmic reticulum; endo, endosome; memb, membrane; Δ, deletion mutant; tetO, transcriptionally repressed tetO promoter; frags, fragments; GFP, green fluorescent protein; III, genes important but not required for mitochondrial morphology; I, genes required for mitochondrial morphology.

Common contaminants

We observed a large number of proteins that were identified in a high proportion of experiments. For example, ribosomal proteins frequently comprised more than 50% of the peptides identified in a single experiment. Because ribosomal proteins likely contaminate TAP purifications due to their high abundance in the cell, we excluded them from further consideration. There were also several non-ribosomal proteins that

frequently contaminated our TAP purifications. Table 1 lists proteins found in greater than half of the experiments. We reasoned these proteins are either high abundance proteins or have a strong tendency to copurify with the TAP purification procedure. For example, Ssa1p, Ssa2p, Ssa3p, Ssa4p, Ssz1p, Ssb1p, Ssb2p, and Zuo1p are chaperones that were present in more than half the experiments performed. Chaperones likely contaminate these experiments because they can bind exposed hydrophobic regions on a wide range of proteins. Elimination of common contaminants from consideration in current or future TAP-MudPIT experiments can save significant effort by reducing the likelihood of following false leads.

Table 1. Common Contaminants in TAP-MudPIT experiments.

ORF	Gene	Frequency	ORF	Gene	Frequency
YAL005c	SSA1	92.3%	YHR020w	YHR020w	76.9%
YAL035w	FUN12	76.9%	YHR064c	SSZ1	76.9%
YAL038c	CDC19	100%	YHR174w	ENO2	53.8%
YAR073w	IMD1	84.6%	YHR216w	IMD2	84.6%
YBL075c	SSA3	92.3%	YIL078w	THS1	53.8%
YBR030w	YBR030w	61.5%	YJL034w	KAR2	53.8%
YBR079c	RPG1	61.5%	YJL052w	TDH1	76.9%
YBR118w	TEF2	100%	YJL130c	URA2	92.3%
YBR127c	VMA2	76.9%	YJR009c	TDH2	100
YBR221c	PDB1	100%	YLL024c	SSA2	92.3%
YBR263w	SHM1	61.5%	YLR044c	PDC1	76.9%
YCL028w	RNQ1	53.8%	YLR150w	STM1	100
YCL037c	SRO9	84.6%	YLR153c	ACS2	53.8%
YDL014w	NOP1	69.2%	YLR175w	CBF5	61.5%
YDL055c	PSA1	53.8%	YLR197w	SIK1	84.6%
YDL229w	SSB1	92.3%	YLR249w	YEF3	76.9%
YDR371w	CTS2	69.2%	YLR342w	FKS1	76.9%
YDR432w	NPL3	84.6%	YLR432w	IMD3	92.3%
YEL026w	SNU13	53.8%	YML056c	IMD4	92.3%
YEL034w	HYP2	53.8%	YML085c	TUB1	53.8%
YER103w	SSA4	92.3%	YML124c	TUB3	53.8%
YER165w	PAB1	84.6%	YMR012w	CLU1	53.8%
YER178w	PDA1	92.3%	YMR108w	ILV2	84.6%
YFL037w	TUB2	53.8%	YNL071w	LAT1	92.3%
YFL039c	ACT1	61.5%	YNL112w	DBP2	53.8%
YGL008c	PMA1	76.9%	YNL138w	SRV2	61.5%
YGL049c	TIF4632	53.8%	YNL209w	SSB2	92.3%
YGL105w	ARC1	61.5%	YOL086c	ADH1	69.2%
YGL245w	YGL245w	61.5%	YOR198c	BFR1	61.5%

YGR032w	GSC2	61.5%
YGR054w	YGR054w	84.6%
YGR086c	PIL1	69.2%
YGR159c	NSR1	53.8%
YGR162w	TIF4631	84.6%
YGR192c	TDH3	100%
YGR285c	ZUO1	69.2%
YHL034c	SBP1	53.8%

YOR204w	DED1	92.3%
YOR310c	NOP58	69.2%
YPL004c	LSP1	53.8%
YPL036w	PMA2	69.2%
YPL119c	DBP1	53.8%
YPR080w	TEF1	100%
YPR171w	BSP1	92.3%

Fission pathway proteomics

Mdv1p and Caf4p

Three classes of proteins reproducibly co-purified with Mdv1p-His_8/TEV/9XMyc: septins, endocytosis proteins, and components of the chaperonin CCT-Ring. Septins are GTPases that assemble a ring structure at the bud neck and are required for cytokinesis (Longtine and Bi, 2003). Interestingly, it has been noted that at least one Mdv1p-GFP puncta is always found at the bud neck (Cerveny et al., 2001), suggesting that they may associate at the bud neck. In the single Caf4p-His_8/TEV/9XMyc experiment, Cdc3p and Cdc10p were identified (Table 3) raising the possibility that Caf4p and Mdv1p both interact with septins.

Twelve endocytosis proteins specifically co-purified with Mdv1p. Crn1p, End3p, Sla2p, Sac6p, Bzz1p, Prk1p, and Ysc84p were uniquely identified in Mdv1p experiments. Five other proteins (Pan1p, Sla1p, Bbc1p, Myo3p, and Myo5p) were identified it least two Mdv1p experiments and only one of ten other TAP-MudPIT experiments. All 12 proteins localize to actin cortical patches where they regulate the ARP2/3 complex (Engqvist-Goldstein and Drubin, 2003). ARP2/3 activity contributes to the movement of mitochondria in *S. cerevisiae* (Boldogh et al., 2001). Thus, Mdv1p could provide a link between mitochondrial dynamics and the cytoskeleton. Several endocytosis mutants

(*end3*, *sla2*, *pan1*, *vps4*, and *vps18*) have normal mitochondrial morphology, indicating they are not required for mitochondrial fission (Otsuga et al., 1998).

The TRiC/CCT complex is a chaperonin required for the folding cytoskeletal components such as actin and tubulin (Dunn et al., 2001). As discussed above, chaperones and chaperonins are expected contaminants in biochemical purifications. However, the components of the CCT complex were almost exclusively identified with Mdv1p and Caf4p (Cct4p was found one time with Fis1p and Cct8p was identified once with both Dnm1p and Fis1p), suggesting their interaction with Mdv1p and Caf4p may be specific.

Table 2. Results from Mdv1p TAP-MudPIT.

ORF	**Gene**	**Run**			**Localization**			**Mitochondrial Morphology**	
		1	**2**	**3**	**Mito Prep**	**Annotated**	**GFP**	**Δ**	**tetO**
Bait									
YJL112w	MDV1	27	36	41	Yes	mito OM	mito	Nets	
Septins									
YCR002c	CDC10	3	4	4	no	septin ring	bud neck	wt	
YLR314c	CDC3	4	5	6	no	septin ring	not tested		wt
YHR107c	CDC12	2	8	12	no	septin, cyt	bud neck		wt
YDL225w	SHS1		3	5	no	septin ring	bud neck	wt	
YJR076c	CDC11		2	5	no	septin ring	bud neck		wt
Endocytosis									
YIR006c	PAN1	3	8	16	no	ACP	ACP		wt
YBL007c	SLA1		13	19	no	ACP	ACP, cyt	wt	
YJL020c	BBC1		4	11	no	ACP	not tested	wt	
YKL129c	MYO3		3	4	no	ACP	ACP, cyt	wt	
YLR429w	CRN1	12	2	52	no	ACP	ACP	wt	
YMR109w	MYO5		3	3	no	ACP	ACP	wt	
YNL084c	END3	2	3	12	no	ACP	ACP	wt	
YNL243w	SLA2	4	16	18	no	ACP	ACP	not tested	
YDR129c	SAC6		2	2	no	ACP	ACP	wt	
YHR114w	BZZ1		3	4	no	ACP, cyt	ACP	wt	
YIL095w	PRK1		3	12	no	ACP	ACP	wt	
YHR016c	YSC84		2	4	no	ACP	not tested	wt	
TRiC/CCT complex									
YJL008c	CCT8	2	7	5	no	cytosol	not tested	not tested	
YDL143w	CCT4	6	6	7	no	cytosol	not tested		defect
YDR212w	TCP1	2	3	10	no	cytosol	not tested	not tested	

YDR188w	CCT6	2		3	no	cytosol	not tested		defect
YDR171w	HSP42	3		4	no	cytosol	cytosol	wt	
Other proteins									
YDR299w	BFR2		2	2	no	nucleolus	nucleolus		defect
YKL152c	GPM1		2	3	no	cytosol	cytosol	not tested	
YNL061w	NOP2		2	2	no	nucleolus	nucleolus		defect
YBR130c	SHE3		4	7	no	cytosol	cytosol	wt	
YCL018w	LEU2	2		2	no	cytosol	cytosol	not tested	
YFR016c	YFR016c		2	2	no	cytosol	cytosol	wt	
YOR341w	RPA190	2		3	no	RNA PolI	nucleolus	not tested	

Table 3. Results from Caf4p TAP-MudPIT.

ORF	**Gene**	**Run**	**Localization**			**Mitochondrial Morphology**	
		1	**Mito Prep**	**Annotated**	**GFP**	**Δ**	**tetO**
Bait							
YKR036c	CAF4	21	Yes	nuc, mito	mito	wt	
TRiC/CCT complex							
YJR064w	CCT5	5	no	cytosol	not tested	not tested	
YJL008c	CCT8	4	no	cytosol	not tested	not tested	
YDL143w	CCT4	7	no	cytosol	not tested		defect
YDR212w	TCP1	5	no	cytosol	not tested	not tested	
YDR188w	CCT6	5	no	cytosol	not tested		defect
YIL142w	CCT2	9	no	cytosol	nucleus	not tested	
YJL014w	CCT3	5	no	cytosol	not tested	not tested	
YJL111w	CCT7	4	no	cytosol	not tested	wt	
Septins							
YLR314c	CDC3	2	no	septin ring	not tested		wt
YCR002c	CDC10	2	no	septin ring	bud neck	wt	
Proteasome Pathway							
YDR394w	RPT3	2	no	19S	ambiguous	not tested	
YOR117w	RPT5	2	no	19S	nucleus	not tested	
YGL048c	RPT6	2	no	19S, nuc	nucleus		wt
YIL075c	RPN2	5	no	26S	cyt, nuc	not tested	
YDL132w	CDC53	2	no	nucleus	nuc, cytosol		defect
YLL039c	UBI4	1	no	cytosol	cytosol	wt	
mRNA Biogenesis							
YPR144c	NOC4	3	no	nucleus	nucleus		wt
YDL148c	NOP14	2	Yes	nuc, mito	not tested		wt
YPR137w	RRP9	4	no	nucleolus	nucleolus		wt
YLL011w	SOF1	3	no	nucleolus	nucleolus	not tested	
YJR002w	MPP10	2	no	nucleolus	not tested		wt
YPL169c	MEX67	2	no	NPC, cyt	nucleus		wt
YLR129w	DIP2	2	no	nucleolus	cyt, nuc	wt	
YOR078w	BUD21	2	no	nucleolus	nucleolus	wt	
YKL059c	MPE1	2	no	nucleus	nucleus		wt
YBR247c	ENP1	3	no	nucleolus	nuc, cyt	not tested	
YNL061w	NOP2	7	no	nucleolus	nucleolus		defect
YKL099c	UTP11	2	no	nucleolus	not tested		wt

YLR222c	UTP13	7	no	nucleolus	nucleolus	not tested	
YML093w	UTP14	4	no	nucleolus	nucleolus	not tested	
YMR093w	UTP15	2	no	nucleolus	nucleolus		wt
YJL069c	UTP18	2	no	nucleolus	nucleolus		wt
YLR409c	UTP21	2	no	nucleolus	nucleus	not tested	
YDR324c	UTP4	2	no	nucleolus	nucleolus		wt
YDR398w	UTP5	2	no	nucleolus	nucleolus		wt
YER082c	UTP7	2	no	nucleolus	not tested		wt
YGR128c	UTP8	5	no	nucleolus	nucleolus		wt
YCR077c	PAT1	2	no	cytosol	cytosol	wt	
YGR178c	PBP1	3	Yes	cyt nuc, mito	cyt, nuc	wt	
YDL053c	PBP4	2	no	cyt, nuc	cyt, nuc	wt	
YDR153c	ENT5	2	no	cyt, endo	punctate	wt	
YCL011c	GBP2	4	no	nucleus	nucleus	wt	
YDR211w	GCD6	2	no	cytosol	cytosol		wt
YHR108w	GGA2	2	no	golgi	punctate	wt	
YDL171c	GLT1	2	Yes	mitochondria	ambiguous	wt	
YGL245w	GUS1	7	no	cytosol	cytosol		wt
YJR075w	HOC1	2	no	golgi	golgi	wt	
YDR138w	HPR1	2	no	nucleus	nucleus	wt	
YNL004w	HRB1	3	no	cyt, nuc	nucleus	wt	
YPL204w	HRR25	2	no	nuc, memb	nuc, cyt		defect
YBR072w	HSP26	5	no	nuc, cyt	ambiguous	wt	
YNL037c	IDH1	2	Yes	mitochondria	mito	wt	
YCL009c	ILV6	2	Yes	mitochondria	mito	wt	
YLR309c	IMH1	3	no	golgi, cyt	cyt	wt	
YPR133c	IWS1	3	Yes	nucleus	nucleus		wt
YDL051w	LHP1	4	no	nucleus	nucleus	wt	
YFL018c	LPD1	2	Yes	mitochondria	mito		wt
YDL182w	LYS20	3	Yes	mito, nuc	nucleus	wt	
YDL131w	LYS21	3	no	nucleus	nucleus	wt	
YNL173c	MDG1	3	no	membrane	punctate	wt	
YLR106c	MDN1	2	Yes	mito, nuc	cyt, nuc		wt
YBR249c	ARO4	2	no	cyt, nuc	cyt, nuc	wt	
YBR039w	ATP3	2	Yes	mito IM	mito	not tested	
YJL020c	BBC1	3	no	ACP	not tested	wt	
YER155c	BEM2	2	Yes	mitochondria	cyt, neck		
YDR299w	BFR2	2	no	nucleolus	nucleolus		wt
YPL217c	BMS1	4	Yes	cyt, nuc, mito	periphery		wt
YNR051c	BRE5	2	no	cytosol	cytosol	wt	
YLR418c	CDC73	2	no	nucleus	nucleus	wt	
YLR330w	CHS5	2	no	cytosol	punctate	wt	
YDL145c	COP1	2	no	ER, golgi	punctate		wt
YOR065w	CYT1	3	Yes	mitochondria	mito	wt	
YJR144w	MGM101	3	Yes	mitochondria	not tested	III	
YNL085w	MKT1	2	no	cytosol	cyt, punctate	wt	
YDR245w	MNN10	2	no	golgi	golgi	wt	
YGL068w	MNP1	2	Yes	mitocondria	mito		wt
YNL252c	MRPL17	2	Yes	mitocondria	mito	wt	
YDR194c	MSS116	3	Yes	mitocondria	mito	wt	
YMR145c	NDE1	2	Yes	mitocondria	mito	wt	
YJL076w	NET1	3	no	nucleolus	nucleolus		wt
YGR240c	PFK1	4	no	cytosol	cytosol	wt	

YBL022c	PIM1	3	Yes	mito matrix	mito	wt	
YEL060c	PRB1	6	no	vacuole	cyt, punctate	wt	
YML017w	PSP2	2	no	cytosol	cytosol	wt	
YPR191w	QCR2	2	Yes	mitocondria	mito	wt	
YNL216w	RAP1	2	no	nucleus	nucleus		wt
YAR007c	RFA1	2	no	cyt, nuc	nuc	not tested	
YCR028c-a	RIM1	2	Yes	mitochondria	mito	wt	
YDR150w	NUM1	2	Yes	periphery, mit	punctate	I	
YMR131c	RRB1	2	no	nucleolus	nucleolus	not tested	
YFR037c	RSC8	2	no	nucleus	nucleus		wt
YER125w	RSP5	4	Yes	cyt, golgi, mit	nucleus	not tested	
YGR275w	RTT102	2	no	nucleus	nucleus	wt	
YPL085w	SEC16	4	no	ER	ER	not tested	
YIL109c	SEC24	2	no	ER	ER	not tested	
YDL195w	SEC31	5	no	ER	ER		defect
YLR398c	SKI2	2	no	cyt, nucleolus	cytosol	wt	
YBL007c	SLA1	3	no	ACP	ACP, cyt	wt	
YDR515w	SLF1	2	no	cytosol	cytosol	wt	
YIL105c	SLM1	3	Yes	cyt, mito	punctate	wt	
YGR074w	SMD1	2	no	nucleus	nucleus		wt
YLL021w	SPA2	5	no	bud	not tested	wt	
YDR293c	SSD1	2	no	cytosol	cytosol	wt	
YFL026w	STE2	2	no	periphery	vacuole	wt	
YML072c	TCB3	2	Yes	bud, mito	periphery	wt	
YGR186w	TFG1	2	no	nucleus	nucleus		wt
YKL140w	TGL1	2	no	membrane	punctate	wt	
YHR167w	THP2	2	no	nucleus	cyt, nucleus	wt	
YBR126c	TPS1	2	no	cytosol	cytosol	wt	
YML100w	TSL1	5	no	cytosol	cytosol	wt	
YGR094w	VAS1	6	Yes	cyt, mito	cyt		wt
YKL069w	YKL069w	1	no	cyt, nucleus	cyt, nuc	wt	
YKL214c	YRA2	2	no	nucleus	cyt, nuc	wt	

Dnm1p

The Dnm1p-His_8/TEV/9XMyc TAP-MUDPIT did not identify any of the proteins identified with Fis1p, Mdv1p, or Caf4p TAP-MudPIT experiments (Table 4). This is likely because mitochondrial association of Dnm1p is labile: Dnm1p does not immunoprecipitate with Fis1p, Mdv1p or Caf4p, and the majority of Dnm1p does not co-fractionate with mitochondria. Future experiments could take advantage of a recent observation that NaN_3/NaF treatment to reduce intracellular GTP levels stabilizes

Dnm1p/Fis1p complexes and activates Dnm1p-dependent mitochondrial fission (Bhar et al., 2006).

Table 4. Results from Dnm1p TAP-MudPIT.

ORF	Gene	Run	Localization			Mitochondrial Morphology	
		1	Mito	Annotated	GFP	Δ	tetO
Bait							
YLL001w	DNM1	152	Yes	Mitochondrial OM	Mito, punctate	Nets	
YBR033w	EDS1	2	no	unknown	not tested	wt	
YDL195w	SEC31	2	no	ER	ER		defect
YHR121w	YHR121w	2	no	cytosol, nucleus	cytosol, nucleus	wt	
YJL008c	CCT8	2	no	cytosol	not tested	not tested	
YML054c	CYB2	2	Yes	mito IM	not tested	wt	
YMR246w	FAA4	2	no	cytosol	lipid particle punc	wt	
YNL064c	YDJ1	2	no	cytosol	cytosol, nucleus	not tested	
YNR039c	ZRG17	2	no	ER	ER	wt	
YNR051c	BRE5	2	no	cytosol	cytosol	wt	
YOR317w	FAA1	2	Yes	lipid part, mito OM	ER	wt	

Fusion pathway proteomics

Mdm30p

Mdm30p is an F-box protein that regulates Fzo1p levels (Fritz et al., 2003). As has been previously reported, we identified Cdc53p in both of our Mdm30p-His_8/TEV/9XMyc experiments (Table 5) (Uetz and Hughes, 2000). However, other SCF components (Skp1p, Cdc34p, and Hrt1p) were not identified, suggesting Mdm30p may not be part of an SCF complex. In additional, none of the other proteins had a reported mitochondrial morphology defect except for two inner membrane proteins: Atp2p, a component of the ATP synthase and Pet9p, an ADP/ATP translocator.

Table 5. Results from Mdm30p TAP-MudPIT

ORF	Gene	Run		Localization			Mitochondrial Morphology	
		1	2	Mito	Annotated	GFP	Δ	tetO

Bait								
YLR368w	MDM30	21	19	Yes	mitochondria	not tested	frags	
YDL132w	CDC53	11	9	no	nucleus	nuc, cytosol		defect
YAL042w	ERV46	2		no	ER, golgi	not tested	wt	
YBL030c	PET9	2		Yes	mito IM	not tested		defect
YBL037w	APL3		2	no	clathrin coat	periphery	wt	
YBR078w	ECM33	2		Yes	mito, membrane	not tested	wt	
YBR086c	IST2	2		no	cell membrane	periphery		wt
YCR067c	SED4	2		no	ER	ER	wt	
YDL171c	GLT1	2		Yes	mitochondria	ambiguous	wt	
YDR023w	SES1	2		no	cytosol	cytosol		wt
YDR345c	HXT3	3		no	cell membrane	vac, periphery	wt	
YDR406w	PDR15	2		no	membrane	not tested	wt	
YDR429c	TIF35	2		no	cytosol	cytosol		wt
YER006w	NUG1	3		no	nuc, nucleolus	nuc, nucleolus		wt
YER086w	ILV1	2		Yes	mitochondria	mitochondria	wt	
YGL253w	HXK2	2		no	nuc, cytosol	cytosol	wt	
YJR121w	ATP2	2		Yes	mito IM	mitochondria	III	
YKL152c	GPM1	2		no	cytosol	cytosol	not tested	
YKR092c	SRP40	2		no	nucleolus	nucleolus	wt	
YLR293c	GSP1	2		no	nuc, cytosol	not tested	not tested	
YLR304c	ACO1	2		Yes	cytosol, mito	cytosol, mito	not tested	
YML072c	YML072c	3		Yes	bud, mito	periphery	wt	
YML074c	FPR3	2		no	nucleolus	nucleolus	wt	
YMR109w	MYO5	2		no	ACP	ACP	wt	
YMR120c	ADE17	2		no	cytosol	cytosol	wt	
YMR315w	YMR315w	2		no	nuc, cytosol	nuc, cytosol	wt	
YNL037c	IDH1	2		Yes	mitochondria	mitochondria	wt	
YNL087w	YNL087w	3		no	bud	periphery	wt	
YNL098c	Ras2	2		no	cell membrane	nuc, cytosol	wt	
YNR021w	YNR021w	2		no	ER	ER	wt	
YOR185c	GSP2	2		no	nucleus	nuc, cytosol	wt	
YPL160w	CDC60	2		no	cytosol	cytosol	not tested	
YPR052c	NHP6A	2		no	nucleus	nucleus	wt	
YPR149w	NCE102	2		Yes	cyt, ER, mito	punctate	wt	

Fzo1p

The results from the TAP purification of 9XMyc/TEV/His_8-Fzo1p from the whole cell lysate of two dextrose cultures are presented in Table 6. Because these experiments did not identify any particularly strong candidate Fzo1p binding partners, a third purification was performed that included the isolation of a mitochondrial fraction by differential centrifugation (Table 7). Interestingly, Cdc48p was identified in this experiment. Cdc48p is a AAA-ATPase that binds ubiquitinated substrates and

participates in protein degradation and homotypic ER fusion (Latterich et al., 1995). Preliminary experiments with a temperature-sensitive *cdc48* strain (*cdc48-3*) showed fragmentation of the mitochondrial reticulum at the non-permissive temperature. However, the *cdc48-3* mitochondrial fragmentation phenotype was not recessive and, given the importance of Cdc48p to protein degradation pathways, there is a concern that Cdc48p affects mitochondrial morphology indirectly. Specifically, Cdc48p regulates oleic acid synthesis directly through the transcription factor Spt23p and the fatty acid desaturase Ole1p (Braun et al., 2002; Rape et al., 2001) and *ole1* deficient yeast have severe mitochondrial morphology defects (Stewart and Yaffe, 1991). Future experiments should test the suppression of *cdc48-3* fragmentation by exogenous oleic acid which suppresses the morphology defect in *ole1* cells (Stewart and Yaffe, 1991).

A single peptide was identified with a potential ubiquitin moiety at Fzo1p residue K658. However, mutation of this lysine (*fzo1 K658R*) did not affect Fzo1p expression levels or the mitochondrial fusion, indicating that ubiquitination of K658 is not important for mitochondrial fusion (data not shown).

Table 6. Results from Fzo1p TAP-MudPIT (Dextrose cultures).

ORF	**Gene**	**Run**		**Localization**			**Mitochondrial Morphology**	
		1	**2**	**Mito Prep**	**Annotated**	**GFP**	**Δ**	**tetO**
Bait								
YBR179c	FZO1	26	13	Yes	mito OM	mito	frags	
YBR140c	IRA1	2	2	Yes	mitochondria	cytosol		wt
YBR245c	ISW1	2	2	no	nucleus	nucleus	wt	
YDR270w	CCC2	1	1	no	golgi	punctate	wt	
YLR454w	YLR454w	2	1	Yes	mito	not tested	wt	
YCR039c	MATα2	1	1	no	nucleus	not tested	not tested	
YLL039c	UBI4	1	2	no	cytoplasm	cytoplasm	wt	
YBL086c	YBL086c	2		no	unknown	periphery	wt	
YCL061c	MRC1		2	no	nucleus	nucleus	wt	
YDL116w	NUP84	2		no	NPC	nucleus	wt	
YDR038c	ENA5	3		no	periphery	periphery	not tested	

YDR039c	ENA2	3		Yes	mito, periphery	periphery	not tested	
YDR040c	ENA1	3		no	periphery	periphery	not tested	
YDR444w	YDR444w	2		no	cytoplasm	cytoplasm	wt	
YDR514c	YDR514c		2	no	mito, nucleus	mito, nuc	wt	
YER082c	UTP7		3	no	nucleolus	not tested		wt
YGR163w	GTR2		2	no	cyt, nuc, vac	vacuole	wt	
YIL073c	SPO22	2		no	unknown	not tested	wt	
YIL109c	SEC24	2		no	COPII	ER, golgi	not tested	
YIL148w	RPL40A		2	no	cytosol	cyt, nuc	wt	
YJL176c	SWI3	2		no	nucleus	nucleus	not tested	
YJR015w	YJR015w	2		no	cytosol, ER	cyt, ER	wt	
YJR144w	MGM101	2		Yes	mitochondria	not tested	III	
YKR094c	RPL40B		2	Yes	mito, cytosol	cytosol	wt	
YKR103w	NFT1	2		no	membrane	cyt, nuc	wt	
YLR087c	CSF1	2		Yes	mitochondria	not tested	wt	
YLR409c	UTP21	2		no	nucleus	nucleus	not tested	
YML103c	NUP188	2		no	NPC	nucleus	wt	
YOL021c	DIS3	2		Yes	cyt, nuc, mito	cyt, nuc		wt
YOL089c	HAL9	2		Yes	mito, nucleus	cyt, nuc	wt	
YOL139c	CDC33		2	no	cytosol, nucleus	cytosol		wt
YOR116c	RPO31	3		no	nucleus	nucleus		wt
YOR337w	TEA1	2		no	nucleus	cyt, nuc	wt	
YOR355w	GDS1	2		Yes	cyt, nuc, mito	cyt, nuc	wt	
YPL020c	ULP1	2		no	nucleus	nucleus		wt
YPR189w	SKI3	2		no	cytosol, nucleus	cytosol	wt	

Table 7. Results from Fzo1p TAP-MudPIT (dextrose cultures and mitochondrial isolation).

ORF	**Gene**	**Run**	**Localization**			**Mitochondrial Morphology**	
		1	**Purified Mito**	**Annotated**	**GFP**	**Δ**	**tetO**
Bait							
YBR179c	FZO1	42	Yes	mito OM	mito	frags	
YDL126c	CDC48	10	no	cyt, ER, nuc	cytosol, nuc		wt
YGL068w	YGL068w	3	Yes	mitochondria	mitochondria		wt
YLL039c	UBI4	2	no	cytosol	cytosol	wt	
YBL076c	ILS1	2	no	cytosol	cytosol		wt
YPR056w	TFB4	2	no	nucleus	cytosol, nuc	not tested	

Mitochondria isolated from yeast grown in glycerol media are much more active in an *in vitro* mitochondrial fusion assay than mitochondria isolated from yeast grown in dextrose (Meeusen et al., 2004). This observation suggests mitochondrial fusion complexes may be upregulated or more stable in glycerol cultures. When

9XMyc/TEV/His_8-Fzo1p and Ugo1p-His_8/TEV/9XMyc were TAP purified from glycerol cultures, several interesting candidate binding partners were identified including prohibitins (Phb1p and Phb2p) and the m-AAA protease (Afg3p and Yta12p) (Tables 8 and 9). In yeast lacking mtDNA, *phb1* and *phb2* mutants display fragmented mitochondria (Berger and Yaffe, 1998), suggesting a role in mitochondrial fusion. We focused on yBR230cp because it interacted robustly with Ugo1p and Fzo1p in co-immunoprecipitations and because it had not been characterized. Our work on *yBR230c* is presented in Chapter 4.

Table 8. Results from Fzo1p TAP-MudPIT (glycerol cultures).

ORF	**Gene**	**Run**	**Localization**			**Mitochondrial Morphology**	
		1	**Mito**	**Annotated**	**GFP**	**Δ**	**tetO**
Bait							
YBR179c	FZO1	28	Yes	mito OM	mitochondria	frags	
Mitochondrial proteins							
YBL099w	ATP1	7	Yes	mitochondrial IM	mitochondria	wt	
YJR121w	ATP2	6	Yes	mitochondria	mitochondria	III	
YBR039w	ATP3	4	Yes	mitochondrial IM	mitochondria	wt	
YPL078c	ATP4	3	Yes	mitochondria	mitochondria	III	
YDR298c	ATP5	3	Yes	mitochondria	mitochondria	III	
YDL126c	CDC48	4	no	cytosol, nuc, ER	cytosol, nucleus		wt
YBR230c	YBR230C	2	Yes	mitochondria	mitochondria	wt	
YKL212w	SAC1	4	Yes	golgi, ER, mito	ER, vacuole	III	
YCL017c	NFS1	5	Yes	mitochondria, nuc	mitochondria		wt
YCL009c	ILV6	3	Yes	mitochondria	mito	wt	
YCR028c-a	RIM1	3	Yes	mitochondria	mito	wt	
YGR231c	PHB2	2	Yes	mitochondria IM	mitochondria	frags	
YMR089c	YTA12	3	Yes	cytosol, mito IM	mitochondria	wt	
YER017c	AFG3	5	Yes	mitochondria IM	mitochondria	III	
YJR144w	MGM101	3	Yes	mitochondria	not tested	III	
YIL125w	KGD1	14	Yes	mito matrix	mitochondria	wt	
YDR148c	KGD2	7	Yes	mito matrix	mitochondria	wt	
YML008c	ERG6	3	Yes	ER, mito, lipid	lipid, punctate	III	
YAL054c	ACS1	9	Yes	cytosol, mito	cytosol, nucleus	wt	
YDL171c	GLT1	2	Yes	mitochondria	ambiguous	wt	
YDR322w	MRPL35	2	Yes	mito ribosome	mitochondria	wt	
YDR347w	MRP1	3	Yes	mitochondria	mitochondria	wt	
YER086w	ILV1	5	Yes	mitochondria	mitochondria	wt	
YDR296w	MHR1	2	Yes	mitochondria, nuc	mitochondria	III	
YDL182w	LYS20	4	Yes	mito, nuc	nucleus	wt	
YOR374w	ALD4	7	Yes	mitochondria	mitochondria	wt	

YFL018c	LPD1	8	Yes	mitochondria	mito		wt
YLL041c	SDH2	3	Yes	mitochondrial IM	mitochondria	wt	
YDR394w	RPT3	2	no	19S	ambiguous	not tested	
YEL060c	PRB1	8	no	vacuole	cyt, punctate	wt	
YER015w	FAA2	3	Yes	mito, peroxisome	not tested	not tested	
YDL060w	TSR1	2	no	cytosol, nucleus	cytosol, nucleus		wt
YGL068w	MNP1	3	no	mitochondria	mitochondria		wt
YBL022c	PIM1	3	Yes	mito matrix	mitochondria	wt	
YGR193c	PDX1	6	Yes	mitochondria	mitochondria	wt	
YOR187w	TUF1	2	Yes	mitochondria	mitochondria	III	
YOR317w	FAA1	2	Yes	lipid part, mito OM	ER	wt	
YGR215w	RSM27	2	Yes	mitochondria	mitochondria	wt	
YGR155w	CYS4	3	Yes	cytosol, mito	cytosol	wt	
YJL200c	YJL200C	2	Yes	mitochondria	mitochondria	wt	
YJR045c	SSC1	5	Yes	mitochondria	not tested	not tested	
YKL029c	MAE1	4	Yes	mitochondria	mitochondria	wt	
YJL045w	YJL045W	2	Yes	mitochondria	not tested	wt	
YKL081w	TEF4	6	Yes	cytosol, mito	cytosol	wt	
YGR234w	YHB1	3	Yes	cytosol	cytosol, mito	wt	
YKL148c	SDH1	3	Yes	mitochondrial IM	not tested	wt	
YKL182w	FAS1	2	no	cytosol, mito	cytosol	not tested	
YLR089c	ALT1	2	no	mitochondria	not tested	wt	
YMR186w	HSC82	5	Yes	cytosol, mito	cytosol	wt	
YNL009w	IDP3	3	Yes	cyt, mito, perox	not tested	wt	
YNL037c	IDH1	2	Yes	mitochondria	mitochondria	wt	
YNL104c	LEU4	2	Yes	cytosol, mito	cytosol, mito	wt	
YNL121c	TOM70	3	Yes	mitochondria	mitochondria	wt	
YKR001c	VPS1	3	no	cyt, mito OM	cyt, punctate	wt	
YNL252c	MRPL17	2	Yes	mitocondria	mito	wt	
YNR016c	ACC1	8	Yes	cyt, ER, mito	cyt, punctate	not tested	
YOR086c	TCB1	2	no	mitochondria	periphery	wt	
YCL050c	APA1	2	no	cytosol, nucleus	cytosol, nucleus	wt	

Non-mitochondrial proteins

YJR148w	BAT2	6	no	cytosol, nucleus	cytosol, nucleus	wt	
YHR089c	GAR1	3	no	nucleolus	nucleolus		wt
YKL060c	FBA1	6	no	cytosol	cytosol	not tested	
YDL145c	COP1	3	no	ER, golgi	punctate		wt
YKL104c	GFA1	7	no	unknown	not tested	not tested	
YER090w	TRP2	2	no	cytosol	cytosol	wt	
YIL033c	BCY1	2	no	cytosol, nucleus	cytosol, nucleus	not tested	
YKR009c	FOX2	17	no	peroxisome	not tested	wt	
YKR059w	TIF1	5	no	cytosol	cytosol	wt	
YKR067w	GPT2	3	no	cytosol, ER	ER	wt	
YLL039c	UBI4	1	no	cytoplasm	cytoplasm	wt	
YLR058c	SHM2	4	no	cytosol	cytosol, nucleus	wt	
YDL185w	TFP1	3	no	vacuole	vacuole	not tested	
YLR167w	RPS31	2	no	cytosol	not tested		wt
YLR192c	HCR1	2	no	cytosol	cytosol	wt	
YLR303w	MET17	10	no	cytosol	not tested	wt	
YLR438w	CAR2	3	no	cytosol, nucleus	cytosol, nucleus	wt	
YFL045c	SEC53	3	no	cytosol	cytosol, nucleus		defect
YER036c	ARB1	4	no	cytosol, nucleus	cytosol	not tested	
YJL020c	BBC1	2	no	ACP	not tested	wt	

YMR246w	FAA4	4	no	cytosol	lipid particle punc	wt	
YMR303c	ADH2	6	no	cytosol	cytosol	wt	
YGR240c	PFK1	3	no	cytosol	cytosol	wt	
YJL138c	TIF2	5	no	cytosol	cytosol	wt	
YNL014w	HEF3	2	no	cytosol	ambiguous	wt	
YBR072w	HSP26	7	no	cytosol, nucleus	ambiguous	wt	
YBR080c	SEC18	2	no	cytosol	golgi, punctate		defect
YNL248c	RPA49	2	no	nucleus	nucleus	wt	
YPL240c	HSP82	4	no	cytosol	cytosol	wt	
YDR172w	SUP35	2	no	cytosol	cytosol	not tested	
YDL131w	LYS21	3	no	nucleus	nucleus	wt	
YOR133w	EFT1	5	no	cytosol	cytosol	wt	
YPL210c	SRP72	2	no	ER	ER		defect
YPL218w	SAR1	3	no	ER	not tested		wt
YOR361c	PRT1	2	no	cytosol	cytosol		wt
YDR394w	RPT3	2	no	19S	ambiguous	not tested	
YBR025c	YBR025C	9	no	cytosol	cytosol	wt	
YDR380w	ARO10	3	no	cytosol	cytosol	wt	
YDR385w	EFT2	5	no	cytosol	cytosol	wt	

Table 9. Results from Ugo1p TAP-MudPIT (glycerol cultures).

ORF	**Gene**	**Run**	**Localization**			**Mitochondrial Morphology**	
		1	**Mito**	**Annotated**	**GFP**	**Δ**	**tetO**
Bait							
YDR470c	UGO1	23	Yes	mitochondrial OM	not tested	frags	
Mitochondrial proteins							
YOR211c	MGM1	2	Yes	mitochondria IMS	not tested	frags	
YER017c	AFG3	2	Yes	mitochondria IM	mitochondria	III	
YGR132c	PHB1	2	Yes	mitochondria IM	mitochondria	frags	
YGR231c	PHB2	4	Yes	mitochondria IM	mitochondria	frags	
YBL099w	ATP1	5	Yes	mitochondrial IM	mitochondria	wt	
YJR121w	ATP2	3	Yes	mitochondria	mitochondria	III	
YBR039w	ATP3	2	Yes	mitochondrial IM	mitochondria	wt	
YIL125w	KGD1	11	Yes	mito matrix	mitochondria	wt	
YDR148c	KGD2	4	Yes	mito matrix	mitochondria	wt	
YDR322w	MRPL35	4	Yes	mito ribosome	mitochondria	wt	
YGR193c	PDX1	4	Yes	mitochondria	mitochondria	wt	
YOR374w	ALD4	4	Yes	mitochondria	mitochondria	wt	
YJL045w	YJL045w	3	Yes	mitochondrial	not tested	wt	
YKL148c	SDH1	5	Yes	mitochondrial IM	not tested	wt	
YLL041c	SDH2	3	Yes	mitochondrial IM	mitochondria	wt	
YBL022c	PIM1	2	Yes	mito matrix	mitochondria	wt	
YBR230c	YBR230c	2	Yes	mitochondria	mitochondria	wt	
YDR347w	MRP1	2	Yes	mitochondria	mitochondria	wt	
YGL068w	MNP1	2	no	mitochondria	mitochondria		wt
YGR234w	YHB1	2	Yes	cytosol	cytosol, mito	wt	
YJR144w	MGM101	2	Yes	mitochondria	not tested	III	
YKL212w	SAC1	2	Yes	golgi, ER, mito	ER, vacuole	III	
Non-mitochondrial proteins							
YBR072w	HSP26	3	no	cytosol, nucleus	ambiguous	wt	
YBR025c	YBR025c	2	no	cytosol	cytosol	wt	

YHR089c	GAR1	2	no	nucleolus	nucleolus		wt
YIL148w	RPL40A	2	no	cytosol	cyt, nuc	wt	
YJR148w	BAT2	2	no	cytosol, nucleus	cytosol, nucleus	wt	
YKR094c	RPL40B	2	Yes	mito, cytosol	cytosol	wt	
YLL039c	UBI4	2	no	cytoplasm	cytoplasm	wt	
YLR167w	RPS31	2	no	cytosol	not tested		wt
YML123c	PHO84	2	no	cell membrane	ER	wt	
YKR009c	FOX2	5	no	peroxisome	not tested	wt	
YEL060c	PRB1	5	no	vacuole	cyt, punctate	wt	

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Future Directions

Mitochondrial Fission

Work by us and others has focused on understanding the recruitment of Dnm1p to mitochondria by Fis1p/Mdv1p or Fis1p/Caf4p (Cerveny and Jensen, 2003; Griffin et al., 2005; Mozdy et al., 2000; Tieu et al., 2002). Other steps in the fission pathway are poorly understood. As discussed in Chapter 1, only a minority of mitochondrial Dnm1p puncta mark sites of fission, and the Dnm1p/Fis1p/Caf4p complex supports much lower levels of fission than the Dnm1p/Fis1p/Mdv1p complex (Griffin et al., 2005; Legesse-Miller et al., 2003). These observations imply there are important steps following Dnm1p recruitment necessary for fission. Understanding the differences between Mdv1p and Caf4p could reveal those steps. One approach will be to identify key residues in Mdv1p responsible for activating fission. Chimeric Mdv1p/Caf4p molecules and a screen for gain-of-function mutations if *CAF4* are two promising strategies. The structure of the Mdv1p/Fis1p and Caf4p/Fis1p complexes may provide important clues about mechanistic differences between Mdv1p and Caf4p.

Mitochondrial fusion

A key priority for the future is to understand the basis for Om14p inhibition of mitochondrial fusion. Om14p interacts with both Ugo1p and Fzo1p, making them likely regulatory targets. We are currently testing whether Om14p regulates Fzo1p interactions (Chapter 3), and will test for regulation of Fzo1p/Ugo1p and Ugo1p/Mgm1p interactions (Sesaki and Jensen, 2004; Wong et al., 2003). Additionally, morphological analysis indicates *om14Δ* cells may have less overall mitochondrial mass than wild-type cells in

glycerol cultures. This indicates *OM14* could regulate mitochondrial biogenesis. We will attempt to quantitate mitochondrial mass using flow cytometry of DiOC labeled wild-type and *om14Δ* cells. Further work will address whether *OM14*'s role in mitochondrial biogenesis is related to its regulation of mitochondrial fusion.

A mechanistic understanding of mitochondrial fusion will require *in vitro* characterization of Fzo1p. Intermolecular Fzo1p interactions are critical for fusion, and we would like to characterize these interactions in more detail. For example, the interaction between Fzo1p-HRN/GTPase and Fzo1p-HR1/HR2 requires a functional GTPase domain, and it is attractive to speculate that GTPase activity and Fzo1p interactions are tightly coordinated. *In vitro* characterization of Fzo1p GTPase activity and domain interactions could address whether Fzo1p displays oligomerization dependent GTPase activity, like dynamin. Ultimately, the most compelling models for mitochondrial fusion will require structural information on the Fzo1p oligomer.

Mammalian mitochondrial dynamics

Yeast genetics and biochemistry have driven research into the mechanisms of mitochondrial fusion and fission and are the basis for the study of mammalian mitochondrial dynamics. Fzo1p (Mfn1 and Mfn2), Mgm1p (OPA1), Dnm1p (Drp1) and Fis1p (Fis1) play similar roles in yeast and mammalian mitochondrial fusion and fission. However, there likely many mammalian components of these pathways that have not been identified. For example, based on the yeast work (Chapter 2), we would predict the existence of a Fis1/Drp1 adaptor and a mitofusin/OPA1 binding partner, yet there are no clear *MDV1*/*CAF4* or *UGO1* homologs in mammals. Identification of mitofusin and Fis1

binding partners through TAP-MudPIT experiments is one strategy currently being pursued to identify new proteins important for mammalian mitochondrial dynamics.

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