Mon2, a Relative of Large Arf Exchange Factors, Recruits Dop1 to the Golgi Apparatus*

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The protein Mon2 is distantly related to the guanine nucleotide exchange factors (GEFs) that activate Arf1 on Golgi membranes. However, unlike these “large” Arf GEFs, Mon2 lacks the Sec7 domain that catalyzes nucleotide exchange on Arf1. Here we report that yeast Mon2 shares extensive homology with the noncatalytic parts of both the BIG and Golgi brefeldin A resistance factor subfamilies of Arf GEFs and is located to the trans-Golgi. Moreover, we find that Mon2 forms a complex with Dop1, a large cytoplasmic protein conserved in evolution from humans to protozoa. Deletion of Mon2 results in mislocalization of Dop1 from the Golgi and defects in cycling between endosomes and the Golgi. However, unlike Mon2, Dop1 is essential for yeast viability. A conditional allele of Dop1 shows that loss of Dop1 activity not only affects endosome to Golgi transport but also causes a severe perturbation of the organization of the endoplasmic reticulum. Thus, it appears that Dop1 plays a widespread role in membrane organization, and Mon2 acts as a scaffold to recruit the Golgi-localized pool of Dop1.

Small GTP-binding proteins of the Arf and Rab family play a central role in membrane traffic in eukaryotic cells. In the GTP-bound form, they mediate the recruitment of a wide range of vesicle coats, motors, and tethering factors to specific membranes. One of the best characterized is Arf1, which is present throughout the Golgi stack and which binds directly to the COPI, AP1, and GGA1 vesicle coat proteins, as well as to Golgi-localized coiled-coil and BAR domain proteins of unknown function (1). The importance of GTPases like Arf1 has focused attention on the guanine nucleotide exchange factors (GEFs)2 that activate GTPases only on specific organelles (2). In the case of Arfs on the Golgi, this activation is mediated by a family of large peripheral membrane proteins. These “large” Arf GEFs can be further divided into two subfamilies, termed GBF and BIG after their human representatives (3, 4). The GBF GEFs (GBF1 in humans and Gea1/2p in *Saccharomyces cerevisiae*) are found on the cis side of the Golgi, whereas the BIG GEFs (Big1/2 in humans and Sec7 in *S. cerevisiae*) are on the trans-Golgi (5–8). The GBF and BIG subfamilies share a very well conserved 200-residue “Sec7” domain, which is necessary and sufficient for catalyzing nucleotide exchange on Arf1 in vitro. This domain is also the target of the fungal metabolite brefeldin A, which stabilizes a nonproductive complex of the Sec7 domain and Arf1 and so inhibits nucleotide exchange in vivo (9, 10). The Sec7 domain is also present in several smaller Arf GEFs, which activate Arf6 at the plasma membrane. However, in the GBF and BIG GEFs, the Sec7 domain accounts for only a small proportion of the total protein, which typically comprises 1500–2000 residues. Sequence analysis has revealed that the two subfamilies are conserved in evolution from humans to slime molds and moreover are distantly related to each other over much of their length (3, 4) (Fig. 1A). This raises the question of the biological role of the regions of the protein outside of the central Sec7 domain. It is likely that part of each protein mediates targeting of the GEF to the Golgi apparatus, and indeed in the case of human BIG1, the N-terminal 560 residues that do not include the Sec7 domain have been shown to be sufficient for Golgi targeting (11). However, given that other Sec7 domain-containing GEFs can be recruited to the plasma membrane by small pleckstrin homology domains (12), it seems unlikely that targeting is the sole function of the rest of these much larger proteins. Indeed, it has recently been reported for the yeast homologue Gea2 that mutations in the region N-terminal of the Sec7 domain result in defects in membrane traffic but not Gea2 targeting or activation of Arf1 (13).

In this paper, we report an examination of the yeast protein Mon2, which is distantly related to the GBF and BIG families over much of its length but lacks a Sec7 domain. MON2 was originally identified in yeast as a gene whose deletion results in sensitivity to the ionophore monensin (14) or a defect in viability when combined with a mutation in YPT51, a yeast homologue of the endosomal GTPase, Rab5 (and hence MON2 has also been termed YSL2) (15). It was also identified as a gene whose deletion is synthetically lethal with loss of the Rab6 homologue Ypt6 or its GEF, Ric1 (16, 17). Like the large Arf1 GEFs, Mon2 is a peripheral membrane protein that is well conserved in evolution with homologues from humans to *Dictostelium*. The relationship between Mon2 and the Arf1 GEFs has led to the suggestion that Mon2 might be an exchange factor for the Golgi-localized Arf-like GTPase Arl1 (15, 18). Here we report that Mon2 is not required for the membrane recruitment and activation of Arl1. However, we find that it is localized to the yeast trans-Golgi, where it is required for sorting between the Golgi and the endosomal system. Moreover, it binds directly to another large well conserved protein called Dop1. Unlike Mon2, Dop1 is essential for growth of yeast, and a temperature-sensitive form of Dop1 reveals that it is required not only for traffic between Golgi and endosomes but also for normal endoplasmic reticulum (ER) morphology. Thus, Dop1 appears to act in multiple compartments, and Mon2 acts as a scaffold to recruit it to Golgi membranes.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—Strains were based on the parental strains SEY6210, IAY11, or BY4741 (Table 1). The MON2 deletion strain was obtained from the EUROSCARF consortium in the BY4741 background. Full-length *DOP1*, including promoter and terminator regions, was cloned into pRS414 (*CEN, TRP1*) to create pJL, and into pRS416 (*CEN, URA3, ADE3*) to create pJL. A *DOP1* null strain carrying pJL was created in the IAY11 background3 (see below). Tagging of both

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2 The abbreviations used are: GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; HUS, homology upstream of Sec7; ER, endoplasmic reticulum.
3 J. Kilmartin, unpublished results.
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**TABLE 1** Yeast strains used in this study

| Strain          | Genotype                  |
|-----------------|---------------------------|
| SEY6210         | MATa leu2-3-112 ura3-52 his3Δ200 trp1Δ901 lys2-801 suc2Δ9 11 (4) |
| IAY11           | MATa ade2-1 trp1-1 can1-1 1-1000 leu2-3-112 his3Δ- 11,15 ura3-52 ade2-853 Ian Adams |
| BY4741          | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 (45) |
| c13ABY896       | MATa prb1-1 prb1-1 ppr1-1 scp1-3 ura3-2 his3 Δ4 (46) |
| JFY97           | SEY6210 GFP-MON2::Sp. his5+ |
| JFY106          | C13ABY886 MON2::ZZ::kanMX4 |
| JFY107          | C13ABY886 Dop1::ZZ::kanMX4 |
| JFY129          | SEY6210 GFP-Dop1::Sp. his5+ |
| BPy2            | IAY11 dop1::kanMX4 + p2C (dop1-2 plasmid) |
| AGY54           | BY4741 dop1-2 |
| AGY66           | BY4741 mon2Δ::kanMX4 GFP-DOP1::Sp. his5+ |
| AGY67           | BY4741 Suc2C::ZZ::Sp. his5+ |
| AGY68           | BY4741 mon2Δ::kanMX4 Suc2C::ZZ::Sp. his5+ |
| AGY69           | AGY54 Suc2C::ZZ::Sp. his5+ |
| AGY72           | BY4741 mon2Δ::Sp. his5+ |
| AGY73           | BY4741 dop1-2 mon2Δ::Sp. his5+ |

**RESULTS**

**Mon2 Is Related to Arf GEFs but Lacks a Sec7 Domain**—Mon2 was originally reported as an uncharacterized open reading frame whose deletion caused sensitivity to monensin and brefeldin A (14). This report noted that the N-terminal region was distantly related to the large Arf GEFs of the BIG subfamily. Recent sequence analysis has suggested a domain architecture for the large Arf GEFs (3, 4), with five distinct regions shared by the BIG and GFB subfamilies and showed significant homology to a part of Mon2 from many species and to no other protein family apart from the BIG and GFB GEFs (Fig. 1A). In addition, the homology upstream of Sec7 (HUS) domain contains a well conserved NYDCD motif, which in the Asn, Asp, and Cys residues are invariant in GFB and BIG members from all species so far analyzed. A core of this motif (Y/F/Y) is also invariant in the Mon2 family. At the N and C termini of Mon2 are regions that are conserved in Mon2 relatives from all other species but do not show significant homology by PSI-BLAST searches. Mon2 contains the structural domains that are shared between GFB and BIG Arf GEFs but lacks the Sec7 domain responsible for Arf GEF activity.

**Mon2 Is Not Required for the Activation of Arl1**—The relationship between Mon2 and the Arf GEFs has led to the suggestion that it might be required for recruiting the Arf-like GTPase Arl1 to Golgi membranes (15, 16). Thus, we examined the effect of deleting Mon2 on the localization of Arl1. Fig. 1B shows that the punctate distribution of Arl1-GFP is unaffected by deletion of MON2. In addition, the GTP-bound form of Arl1 is known to recruit the coiled-coil protein Imh1 to membranes by binding directly to its C-terminal GRIP domain. Loss of Arl1 or expression of a GDP-locked form of Arl1 results in a diffuse cytosolic distribution of Imh1p (22, 23). However, in the Δmon2 strain, the distribution of GFP-Imh1p appeared unaffected (Fig. 1B). Taken together, these
results suggest that Mon2 is not required for recruitment or activation of Arl1.

Mon2 Forms a Complex with Dop1—To learn more about Mon2 function, we looked for proteins that interact physically with Mon2. In order to facilitate immunoprecipitation, two copies of the IgG-binding Z domain of protein A were inserted at the C terminus of the MON2 gene in the haploid strain SEY6210. However, initial experiments showed that Mon2-ZZ was partially degraded in cell lysates and upon precipitation was barely apparent above the level of background bands (data not shown). To overcome this apparent susceptibility to proteolysis, MON2 was tagged in a strain that lacks the four major vacuolar proteases. Protein blotting showed no significant degradation of the fusion protein (data not shown), and immunoprecipitation gave a prominent doublet (Fig. 2A). The proteins in this doublet were identified by mass spectrometry. The band of larger apparent molecular weight was the Mon2-ZZ fusion protein. The other was an uncharacterized protein encoded by the gene YDR141c, recently named DOP1.

DOP1 is an essential gene, conserved among eukaryotes from humans to protozoa, whose function is unknown. It is predicted to encode a cytosolic protein of 195 kDa and is named for its homology to the DopA protein of the filamentous fungus Aspergillus nidulans, which was identified (as aco586ts) as a temperature-sensitive mutant defective in the early stages of the asexual reproductive cycle (24). The same study confirmed the findings of the Saccharomyces Gene Deletion Project that DOP1 is an essential gene in S. cerevisiae and showed that mutant spores from a heterozygous diploid are able to germinate and undergo 1–3 cellular divisions but are incapable of further growth.

Both Mon2 and Dop1 Are Present on Golgi Membranes—To examine the intracellular location of Mon2 and Dop1, GFP was inserted at the N terminus of the endogenous coding regions. Both proteins showed a punctate distribution (Fig. 3A), consistent with a similar distribution previously reported for Mon2-GFP (15). These punctate structures colocalized in living cells with the late Golgi proteins Arl1 or Sys1 tagged

FIGURE 1. Mon2 is related to Arf GEF5 but is not required for activation of the Arf-related GTPase Arl1. A, a schematic representation of large Arf GEFs and Mon2 from both humans and S. cerevisiae. Human BIG2 and yeast Gea2 are very similar to BIG1 and Gea1, respectively, and have been omitted. For each protein, the five putative domains defined by Mouratou et al. (4) for the large Arf GEFs are shown, along with the Sec7 domain. These are “dimerization and Cyp5 binding” (DCB), HUS, and “homology downstream of Sec7” (HDS1 to -3). Each domain from human BIG1 was used for a PSI-BLAST search using default settings (inclusion threshold E = 0.005), with iteration until no further hits were obtained (typically 6–9 iterations). For some domains, only human Mon2 (KIAA1040) gave a significant score, and the yeast domain was assigned by alignment with the human version. B, wide field fluorescent micrographs of live yeast expressing the indicated GFP fusions from a CEN plasmid (pRS416) under the control of a constitutive form of the PHO5 promoter. The yeasts were either BY4741 (wild type) or the same with MON2 deleted. The dop1-2 strain was incubated for 30 min at the indicated temperature prior to imaging.
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FIGURE 2. Mon2 is in a complex with Dop1. A, Coomassie-stained gel of immunoprecipitates from the protease-deficient strain c13ABY586 (−) and from the same strain in which genomic MON2 has been tagged at the C terminus with two copies of the Z domain of protein A (Mon2-ZZ). Tryptic peptides from the indicated proteins were identified by mass spectrometry. Several fainter bands migrating faster than Dop1 were found to correspond to abundant proteins that are frequent contaminants of such precipitations. These were heat shock proteins Ssa1/2, elongation factors Tef1/2, and enzymes Pdc1, Adh1, and Tdh3. Although these bands are present in the untagged control lane, they appear for unknown reasons to be less abundant, as repeatedly seen in experiments of this kind. B, reciprocal experiment to that shown in A. Immunoprecipitation of Dop1-ZZ gives a second prominent band, identified by mass spectrometry as Mon2.

FIGURE 3. Mon2 recruits Dop1 to the late Golgi. A, confocal micrographs of live wild type cells (SEY6210) with GFP attached by homologous recombination to the N terminus of the endogenous MON2 or DOP1 open reading frames, along with a constitutive form of the PHOS promoter. B, wide field fluorescent micrographs of live yeast as in A but also expressing from a CEN plasmid the indicated Golgi marker protein tagged with an internally dimeric form of DsRed (RFP). C, wide field fluorescent micrographs of live yeast as in A but with either the endogenous MON2 gene deleted or the same but covered with MON2 on a CEN plasmid under the control of its own promoter.

With an internally dimeric form of DsRed (RFP), Fig. 2B and data not shown).

The interaction between Dop1 and Mon2, we next asked if removal of Mon2 affected the distribution of Dop1. Fig. 3C shows that deletion of MON2 results in GFP-Dop1 being present in few puncta, with the majority of cells showing a diffuse cytoplasmic distribution. This delocalization was rescued by a plasmid-borne copy of MON2, indicating that it was not caused by the loss of the MON2 coding region perturbing expression of adjacent genes. Taken together, this indicates that Mon2 and Dop1 are found on the late Golgi and that Mon2 appears to be required to recruit Dop1 to this compartment.

Generation of a Temperature-sensitive Allele of DOP1—DOP1 is an essential gene, so to examine the role of the encoded protein, we generated a temperature-sensitive (ts) allele. The region of DOP1 encoding the highly conserved N-terminal region (residues 1–584) was mutagenized by error-prone PCR and cloned into a plasmid containing the rest of the gene. The resulting DOP1 genes were screened for the ability to confer growth to a Δdop1 strain at 25 but not 37 °C, and a robust ts allele was selected for further characterization. This allele (dop1-2) grew normally at 25 °C and 30 °C but growth was arrested at higher temperatures (Fig. 4A). Sequence analysis revealed four amino acid changes (Fig. 4B). As with the Δmon2 strain, Arl1-GFP and GFP-Lmh1 were not delocalized to the cytosol in the dop1-2 strain at the nonpermissive temperature (Fig. 1B).

The temperature sensitivity of the dop1-2 strain is not exacerbated by deletion of MON2 (Fig. 4A), suggesting that the Golgi-localized pool of Dop1 is not responsible for the essential function of the protein. When the dop1-2 allele was tagged at the N terminus with GFP as for the wild-type protein in Fig. 3, the protein appeared intact and stable as judged by protein blotting but was partially delocalized even at the permissive temperature (data not shown), again consistent with the Golgi pool of Dop1, like Mon2 itself, not being required for viability.

Both Dop1 and Mon2 Are Involved in Traffic in the Endocytic Pathway—Although Dop1 and Mon2 do not appear to be required for Arl1 activation, their Golgi localization suggests that they could still play a role in membrane traffic. Previous analysis of Mon2 has shown that deletion of the gene results in fragmentation of the vacuole (25, 26). Fig. 5A shows that a similar phenotype is seen in the dop1-2 strain when raised to the nonpermissive temperature. Such a fragmentation phenotype has previously been observed for other Golgi-localized proteins that participate in traffic between the Golgi and the endosomal system (25). Thus, we examined the distribution of the v-SNARE Snc1p that cycles between early endosomes and the trans-Golgi network (27). Fig. 5B shows that loss of Mon2 causes GFP-Snc1p to accumulate in internal membranes and be depleted from the plasma membrane. A similar effect was observed in the dop1-2 strain, but only at the nonpermissive temperature. In both cases, this change in distribution correlated with a marked reduction in the levels of a form of Snc1p that migrates more slowly upon electrophoresis. This form represents the addition of a phosphate that is present only on the plasma membrane-localized pool of the protein (28). We also examined another reporter for endocytic recycling, a form of the syntaxin Sso1 modified to contain an NPF endocytosis signal (29). This reporter also shifted from a plasma membrane pool to internal structures in both Δmon2 and dop1-2 strains, although in the latter case there appeared to be a partial effect even at the nonpermissive temperature (data not shown). A previous genome-wide screen for genes affecting vacuolar sorting found that MON2 is required for normal sorting of alkaline phosphatase, a membrane protein that requires the AP3 adaptor for targeting to the late endosome (25). Thus, we examined the activity of the AP3 pathway in these strains by using a
form of the SNARE Nyv1 (GFP-Nyv1(Snc1)) that has its transmembrane domain replaced with that of Snc1. Like Nyv1, this chimera is sorted to the vacuole in an AP3-dependent manner, but when missorted to the plasma membrane, it accumulates there, since it lacks the endocytosis signal present in the Nyv1 transmembrane domain (30). Fig. 5C shows that the sorting of GFP-Nyv1(Snc1) to the vacuole is unaffected in /H9004/ or /dop1-2/ at 37 °C, although, as previously reported, it is clearly relocalized to the plasma membrane in the absence of the AP3 subunit Apm3 (30). In addition, the punctate distribution of the AP3 subunit Apl6 is not affected by loss of Mon2 or Dop1 activity. Together, these results indicate that both Dop1 and Mon2 are required for normal traffic between endosomes and Golgi, but this does not reflect a loss of the AP3 pathway.

Dop1, but Not Mon2, Is Required for Normal ER Morphology—A role for Dop1 and Mon2 in endocytic recycling would be consistent with their localization but would not explain why Dop1 but not Mon2 is essential for growth. To search for other possible effects of loss of Dop1 activity, we examined the distribution of other organelle-specific markers by GFP tagging or immunofluorescence. A range of Golgi markers appeared unaffected in the dop1-2 strain at the nonpermissive temperature (Anp1, Arl1, Bet1, Gos1, Got1, Imh1, Rac1, Ruv3, and Sed5 (Fig. 1B and data not shown)). However, markers for the ER showed a marked
change in appearance in dop1-2 cells at elevated temperatures. Fig. 6A shows that an ER-localized GFP fusion loses its characteristic circumferential distribution under the plasma membrane and instead becomes fragmented. Moreover, in some cells, the ER marker also accumulated in larger clumps. These effects were also seen with immunofluorescence of an endogenous ER protein (Kar2) but not observed in the Δmon2 strain (Fig. 6A and data not shown). The reorganization of the ER does not apparently reflect a defect in protein translocation or ER exit, since the secreted protein invertase still received ER- and Golgi-specific glycosylation when induced in dop1-2 cells preincubated for 30 min at 37 °C (Fig. 6). Similar results were also obtained for the vacuolar protease CPY using pulse-chase analysis.

We also used electron microscopy to examine the intracellular membranes of the dop1-2 strain. Fig. 6D shows that in dop1-2 at the nonpermissive temperature, small membranous structures accumulated. In addition, some cells contained apparent elaborations of the cortical or nuclear ER that formed networks of membrane. These changes were not observed in Δmon2 or in dop1-2 at the permissive temperature. When dop1-2 cells were exposed to 37 °C for increasing times followed by incubation at permissive temperature, they did not show a substantial loss of viability for at least 4 h, indicating that the changes in dop1-2 at 37 °C are not a consequence of cell death (data not shown). These observations suggest that Dop1, but not Mon2, is required for the normal structure and organization of the ER.

DISCUSSION

In this paper, we have found that Mon2, a relative of the large Arf GEFs, acts to recruit the essential protein Dop1 to Golgi membranes. The relationship between Mon2 and the large Arf GEFs has led to the suggestion that Mon2 could be the GEF for Arl1 (15, 18). However, we find that neither Mon2 nor Dop1 is required for activation and targeting of Arl1. We originally came across MON2 as a mutant that was synthetically lethal with loss of RIC1, the gene encoding the exchange factor for the GTPase Ypt6 (16, 17). However, deletion of MON2 is also synthetically lethal with deletion of ARL1 (17). This implies that both Mon2 and Arl1 have activity in each other’s absence. Indeed, the fission yeast Schizosaccharomyces pombe lacks a homologue of Arl1 and yet has a clear homologue of Mon2 (31). Finally, Mon2 does not have a Sec7 domain, the part of the large Arf GEFs that mediates GTP exchange.

These results focus attention on the function of Mon2 and also raise the question of the role of the domains shared between Mon2 and the large Arf GEFs. It was originally suggested that the two families of proteins were only related in the N-terminal DBC domain (14, 15). However, our PSI-BLAST analysis found that the families are likely to be related over most of their length, with Mon2 having all five of the putative domains that have been proposed to be shared by the BIG and GBF subfamilies outside of the Sec7 domain. It is likely that at least some of these domains mediate membrane targeting, and in the case of human BIG1 an N-terminal section containing DBS and part of HUS1 was found to be necessary and sufficient for Golgi localization, although this was not subdivided further (11). However, this leaves the remainder of
the protein, and a number of binding partners have been reported for regions of the large Arf GEFs. Thus, yeast Gea1 and Gea2 are reported to bind to Gmh1, a conserved Golgi membrane protein of unknown function, through the region C-terminal to their Sec7 domain, and Gea2 also binds to a Golgi P-type ATPase through its Sec7 domain (32, 33). Neither of these interactions is required for Gea2 membrane targeting. In addition, mammalian GBF1 was found to bind to the Golgi coiled-coil protein p115 via a “poorly conserved” region at the C terminus, but removal of this region from GBF1 did not affect its Golgi localization (34). One possibility is that these large GEFs serve as scaffolds to recruit or retain a number of proteins in a particular part of the Golgi.

Our analysis of yeast Mon2 has shown that its major binding partner is Dop1, a well conserved protein of unknown function. Indeed, a recent study has independently found evidence for an association between Mon2 and Dop1, although the requirement for Mon2 in Dop1 localization and the phenotype of removing Dop1 were not examined (35). Dop1 was originally named as the yeast homologue of the DopA gene from Aspergillus, which was reported as corresponding to a temperature-sensitive mutant with severe defects in the asexual reproductive cycle that forms conidia (24). Deletion of DopA does not prevent vegetative growth, although hyphal morphology is aberrant. Humans contain two closely related homologues of Dop1, C21orf5/KIAA0933 and KIAA1117, and the former has been characterized as a human chromosome 21 gene with its mRNA being detected in all tissues examined (36). RNAi against the single Caenorhabditis elegans homologue caused embryonic lethality with severe defects in cell patterning, and hence the gene was termed pad-1 (for patterning-defective) (36).

Our results confirm that Dop1 is essential for normal growth of S. cerevisiae and indicate that loss of its activity affects multiple organelles. Both Mon2 and Dop1 appear to be required for recycling of Snc1, which travels from early endosomes to the Golgi (27). Such a role is consistent with the known synthetic lethality between deletion of MON2 and dele-
tion of either YPT6 or ARL1, two GTPases that appear to act directly in recycling from endosomes to Golgi (31, 37, 38). Interestingly, a homologue of Mon2 but not Dop1 can be found in the complete genomes of rice and Arabidopsis, suggesting that Mon2 has activities independent of Dop1. Mon2 may serve as a scaffold to recruit a subset of Dop1, and this role, like that of many proteins in this part of the secretory pathway, is nonessential. However, Dop1 must also have at least one other role elsewhere in the cell, and this role is Mon2-independent and essential for viability. Moreover, although both proteins are well conserved in evolution, only Dop1 has detectable homologues in protozoa, such as Plasmodium or Giardia, or in the compact genome of the microsporidium, Encephalitozoon cuniculi. We find that a temperature-sensitive allele of DOPI shows a marked perturbation of ER structure at the nonpermissive temperature, with fragmentation and the appearance of clumps of interconnected meshworks of membranes. This does not appear to reflect a block in either the translocation of proteins into the ER or ER to Golgi traffic. The meshwork structures of ER superficially resemble the “mitochondrial nets” seen in mutants where mitochondrial fission is blocked (42), suggesting that perhaps Dop1 is required for fission of ER tubules, a process that at present has no known mediators. A role for Dop1 in organelle morphology might explain its effects on multiple organelles, and in the case of the Golgi, this could result in defects in transport from endosomes. Although understanding the role of Mon2 and Dop1 at a molecular level will require further detailed investigation, it is already clear that such analysis will reveal more about the internal organization of intracellular membranes and also about the function of the enigmatic large Arf GEFs.

Acknowledgments—We thank Sew Peak-Chew and Farida Begum for mass spectrometric analysis of tryptic peptides and Hugh Pelham and Katja Röper for comments on the manuscript.

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