The Dynamin-like Protein Vps1p of the Yeast Saccharomyces cerevisiae Associates with Peroxisomes in a Pex19p-dependent Manner*

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Organelle division is a dynamic process orchestrated by multicomponent protein complexes that drive the constriction and fission of organellar membranes (1, 2). Members of the Pex11p family of peroxins, which includes Pex25p (3) and Pex27p (4, 5) in the budding yeast Saccharomyces cerevisiae, have been shown to effect peroxisome division in different organisms (6–10). There is also evidence for a metabolic control of peroxisome division (11, 12), which may be mediated by signals derived from the β-oxidation of fatty acids (13–15). We recently showed that the peroxisomal integral membrane proteins Pex28p through Pex32p are also involved in controlling peroxisome number and size in S. cerevisiae (16, 17); however, their exact roles in peroxisome division have yet to be determined.

One family of proteins implicated in organelle division are the dynamins. Dynamin and dynamin-like proteins are a highly conserved family of large GTPases involved in a variety of cellular processes, including endocytosis, intracellular protein trafficking and organelle partitioning (18, 19). In S. cerevisiae, the dynamin-like protein Dnm1p has been shown to assemble in a multicomponent complex at the outer mitochondrial membrane and mediate the division of mitochondria (20), whereas the dynamin-like protein vacuolar protein sorting protein 1 (Vps1p) has been implicated in peroxisome fission (21). Cells lacking Vps1p have fewer and enlarged peroxisomes as compared with wild-type cells. Vps1p is related to mammalian DLP1. Peroxisome elongation and constriction can occur independently of DLP1, whereas peroxisome fission requires DLP1 (22). Pex1p has been suggested to recruit DLP1 to peroxisomes, although no direct interaction between these two proteins has been observed (23). We have reported that overexpression of Pex1p in pex1Δ cells results in the formation of peroxisomes that are similar in size and number to the peroxisomes of wild-type cells, suggesting that overproduction of Pex1p can overcome the requirement for Pex1p in controlling peroxisome fission (16). Vps1p has also been suggested to regulate the actin cytoskeleton through its interaction with proteins capable of promoting actin assembly (24–28). Interestingly, a vps1Δ rho1Δ double mutant of S. cerevisiae showed accumulation of actin on peroxisomes, suggesting that actin is reorganized/disassembled before peroxisome fission (29).

All of these observations support a direct role for Vps1p in peroxisome fission at the level of the peroxisome itself, but it remains to be determined how Vps1p could associate with peroxisomes. One protein that could recruit Vps1p to peroxisomes is Pex19p. Pex19p is a farnesylated peroxin involved in peroxisome membrane biogenesis (30, 31). Studies suggest that Pex19p functions as an import receptor for peroxisomal membrane proteins (PMPs)4 and/or as a chaperone acting in the assembly or stabilization of PMPs in the cytosol or at the peroxisomal membrane (32–36). Here we show that the association of Vps1p with peroxisomes is dependent on Pex19p and that the interaction of Vps1p with Pex19p is required for peroxisomal fission. Our findings are con-

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4 The abbreviations used are: PMP, peroxisomal membrane protein; NLS, nuclear localization signal; GFP, green fluorescent protein; CPY, carboxypeptidase Y; 20Kg5, 20,000 × g supernatant; 20KgP, 20,000 × g pellet.

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Role of Pex19p in Vps1p Association with Peroxisomes

### Experimental Procedures

**Strains and Culture Conditions**—The *S. cerevisiae* strains used in this study are listed in Table 1. Strains were cultured at 30 °C. Media components were as follows: YPD (1% yeast extract, 2% peptone, 2% glucose); YPBO (0.3% yeast extract, 0.5% peptone, 0.5% KH₂PO₄, 0.5% KH₂PO₄, 1% Brij 35, 1% oleic acid); synthetic minimal (SM) medium (0.67% yeast nitrogen base without amino acids, 2% glucose, 1% Complete Supplement Mixture (Bio 101) without histidine and/or leucine); and YNBD (0.67% yeast nitrogen base without amino acids, 2% glucose, 1% Complete Supplement Mixture (Bio 101) without histidine and/or leucine). Cells grown overnight at 30 °C in YPD medium were subcultured in YPD medium at 30 °C until they reached an A600 of 0.5. 1 μl of 8% FM4-64 (44) was added to 100 μl of cells, and the cells were incubated for 30 min at 30 °C. The cells were washed twice with water and then observed by fluorescence microscopy.

### Subcellular Fractionation and Isolation of Peroxisomes—Subcellular fractionation of oleic acid-incubated cells was done as described previously (3) and involved the isolation of a post-nuclear supernatant fraction and 20,000 × g supernatant (20KgS) and pellet (20KgP) fractions enriched for cytosol and for peroxisomes and mitochondria, respectively. Peroxisomes were purified from the 20KgP fraction by isopycnic density centrifugation on Nycozenz gradients (3).

### Antibodies—Antibodies to the carboxyl-terminal SKL tripeptide, peroxisomal thiolase, and to the mitochondrial enzyme Shdh2p (succinate dehydrogenase 2) have been described previously (5). Antibodies to Vps1p were raised in rabbit as described previously (45). Antibodies to CPY were raised in rabbit and were a kind gift of Dr. William Wickner (Dartmouth College). Rabbit antibodies to *S. cerevisiae* glucose-6-phosphate dehydrogenase were from Sigma-Aldrich. Fluorescein isothiocyanate-conjugated anti-rabbit IgG and rhodamine-conjugated anti-guinea pig IgG (The Jackson Laboratory) were used to detect primary antibodies in immunofluorescence microscopy.

### Analytical Procedures—Extraction of nucleic acid from yeast lysates and manipulation of DNA were performed as described previously (46). Immunoblotting was performed using a wet transfer system (46), and antigen-antibody complexes in immunoblots were detected by enhanced chemiluminescence (Amersham Biosciences). Protein concentration was determined spectrophotometrically, and manipulation of DNA were performed as described previously (46).
Role of Pex19p in Vps1p Association with Peroxisomes

**RESULTS**

*Pex19p Interacts with Vps1p*—*Vps1p* belongs to the dynamin family of proteins and is required for sorting proteins to the vacuolar compartment. *Vps1p* also has a role in peroxisome biogenesis, as cells deleted for the *VPS1* gene contain reduced numbers of enlarged peroxisomes (21). *Vps1p* has been suggested to cycle from the cytosol to the surface of the peroxisome (21). The peroxin Pex19p has been implicated in peroxisome (32–36). Recent work has defined a consensus sequence for recognition of a protein by *Pex19p* (47). *Vps1p* contains two stretches of amino acids that conform to this consensus sequence (Fig. 1A). yeast two-hybrid analysis showed an interaction between *Vps1p* and *Pex19p* (Fig. 1A).

**Deletion of the First Consensus Pex19p Recognition Sequence in Vps1p Yields the vps1Δ Peroxisomal Phenotype**—Cells carrying chromosomally integrated mutant forms of *VPS1* deleted for sequences encoding the first (vps1Δ509–523) or second (vps1Δ633–647) putative *Pex19p* recognition sequences were observed by immunofluorescence microscopy with antibodies to the PTS1 tripeptide Ser-Lys-Leu (*SKL*) or to the PTS2-containing protein thiolase. Rabbit antibodies (*SKL*) or guinea pig primary antibodies (thiolase) were detected with rhodamine-conjugated secondary antibodies. 

**Vacuolar morphology and vacuolar protein sorting are unaffected in cells deleted for the first Pex19p recognition sequence of Vps1p**—A. vacuolar morphology of cells of the wild-type BY4742 and *vps1Δ* strains and of strains deleted for either the first (vps1Δ509–523) or second (vps1Δ633–647) putative *Pex19p* recognition sequence of *Vps1p*. vacuoles were stained by the dye FM4-64 and observed by fluorescence microscopy. Scale bar, 1 μm. B. CPY secretion assay. Cells of the wild-type BY4742 and *vps1Δ* strains and of strains deleted for either the first (vps1Δ509–523) or second (vps1Δ633–647) putative *Pex19p* recognition sequence of *Vps1p* were spotted on YPD agar in 2-fold serial dilutions and overlaid with nitrocellulose. Secretion of CPY is detected by immunoblotting with anti-CPY antibody.

**Electron microscopy confirmed the presence of enlarged peroxisomes in vps1Δ cells** (see Fig. 5B), which were similar in size to peroxisomes of *vps1Δ* cells (see Fig. 5C) and much larger than those of wild-type cells (see Fig. 5A). Our findings show that the consensus *Pex19p* recognition sequence in *Vps1p* at amino acids 509–523 is required for *Vps1p* function in controlling peroxisome size and number.

**Cells Deleted for the First Pex19p Recognition Sequence of Vps1p Are Unaffected in Vacuolar Morphology and Protein Sorting**—Vacuolar biogenesis requires membrane fusion and fission events and the actin-dependent transport of vacuolar membranes. Mutations of genes involved in these processes often lead to vacuoles with aberrant morphology (48) and defects in vacuolar protein sorting (49). We stained wild-type and *vps1Δ* mutant cells with the fluorescent vacuolar vital stain FM4–64 to examine their vacuolar morphology (Fig. 3A). The majority of *vps1Δ* cells exhibited a fragmented vacuolar morphology, as has been observed previously (48, 50). Both vps1Δ509–523 and vps1Δ633–647 cells exhibited wild-type vacuolar morphology.
We also analyzed the sorting of the soluble vacuolar hydrolase carboxypeptidase Y (CPY) (Fig. 3B). Defects in vacuolar protein sorting lead to secretion of CPY (49). vps1Δ cells did not show evidence of enhanced secretion of CPY when compared with wild-type cells. This result suggests that the peroxisome and vacuolar biogenic roles of Vps1p are separate and separable. vps1Δ cells showed a slightly increased level of secreted CPY but much less than that observed for vps1Δ cells, suggesting that these amino acids of Vps1p may have some role in vacuolar protein sorting.

A Point Mutation in the First Pex19p Recognition Sequence of Vps1p Disrupts the Interaction of Vps1p with Pex19p—Substitution of a proline for any amino acid within the consensus Pex19p recognition sequence has been shown to disrupt the interaction of the PMPs Pex11p and Pex13p with Pex19p (47). Introduction of proline within the Pex19p binding region likely disrupts its α-helical conformation, which is thought to promote the association of a partner protein with Pex19p. Accordingly, we substituted proline for valine 516 (V516P) within the first putative Pex19p binding site of Vps1p (Vps1pV516P). Two-hybrid analysis showed that the V516P substitution greatly reduced the interaction of Vps1p with Pex19p (Fig. 4A). Immunofluorescence microscopy (Fig. 4B) and electron microscopy (Fig. 5D) showed that cells of a strain genomically expressing Vps1pV516P contained reduced numbers of enlarged peroxisomes, similar to cells of the vps1Δ strain.

The Subcellular Distribution of Vps1p Is Unaffected by Overproduction or Directed Mistargeting of Pex19p—Vps1p in glucose-grown cells has been shown to be primarily cytosolic with some localization in the trans-Golgi network (51). Localization of Vps1p to peroxisomes has been difficult to demonstrate (21). Because we have shown that Pex19p interacts with Vps1p, we wanted to determine whether overproduction of Pex19p would alter the subcellular location of Vps1p and possibly...
Role of Pex19p in Vps1p Association with Peroxisomes

FIGURE 7. Vps1p association with peroxisomes is dependent on its interaction with Pex19p. A, wild-type BY4742 cells and cells expressing the mutant Vps1p forms Vps1pΔsso9–523 or Vps1pV516P were incubated in oleic acid-containing medium, and their homogenates were subjected to differential centrifugation to yield a post-nuclear supernatant fraction, a 20,000 × g supernatant (20KgS) fraction enriched for cytosol, and a 20,000 × g pellet (20KgP) fraction enriched for peroxisomes and mitochondria. An equal percentage of each subcellular fraction was separated by SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting with antibodies to Vps1p, thiolase (peroxisomes), Sdh2p (mitochondria), or glucose-6-phosphate dehydrogenase (cytosol). B, the 20KgP fraction from oleic acid-incubated wild-type cells was subjected to isopycnic density centrifugation on a discontinuous Nycodenz gradient. The gradient was collected in 15 equal fractions from the bottom of the centrifuge tube. An equal percentage of each fraction was subjected to SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting with antibodies to Vps1p, thiolase, or Sdh2p.

lead to an increased, detectable amount of Vps1p associating with peroxisomes. In wild-type BY4742 cells, Vps1p-GFP exhibited both a diffuse cytosolic fluorescence and localization to punctate structures, which did not correspond to peroxisomes labeled with the fluorescent peroxisomal marker DsRed-PTS1 (Fig. 6A). Overexpression of the PEX19 gene carried on the multicopy plasmid YEp13 in BY4742 cells did not alter the subcellular distribution of Vps1p, and no preferential association of Vps1p with peroxisomes was observed (Fig. 6A).

In mammalian cells, attachment of a NLS to Pex19p has been shown to redirect Pex19p to the nucleus, which in its turn redistributes a Pex19p-associating protein from peroxisomes to the nucleus (35, 52). We therefore wanted to determine whether a nuclear-targeted chimera of Pex19p, NLS-Pex19p, containing the NLS of the spindle assembly checkpoint protein Mad1p (53, 54) could redirect the fluorescent chimera Vps1p-GFP to the nucleus. NLS-GFP-Pex19p localized to the nucleus, as expected (Fig. 6B). NLS-Pex19p was capable of directing a GFP chimera of the peroxisomal membrane protein Pex32p (17) to the outer surface of the nucleus, most probably the nuclear envelope (Fig. 6B). Complete import of Pex32p-GFP into the nucleus may have failed because of steric or conformational incompatibility of the Pex32p-GFP-NLS-Pex19p complex for nuclear import. In contrast, NLS-Pex19p did not redirect Vps1p-GFP to the nucleus from the cytosol or from punctate structures (Fig. 6B). The results of our experiments on the overproduction or directed mistargeting of Pex19p do not support a role for Pex19p in recruiting Vps1p to peroxisomes.

Vps1p Associates with Peroxisomes in a Pex19p-dependent Manner—Because our microscopic analyses provided no evidence of a peroxisomal association for some Vps1p and to determine what role the interaction between Vps1p and Pex19p might play in this association.

Subcellular fractionation followed by immunoblotting with specific antibodies showed that in wild-type BY4742 cells incubated in oleic acid-containing medium, most Vps1p was localized to a 20,000 × g supernatant (20KgS) fraction enriched for cytosol (Fig. 7A). However, a small but reproducible amount of Vps1p was localized to the 20,000 × g pellet (20KgP) fraction enriched for peroxisomes and mitochondria. The Vps1p present in the 20KgP fraction was not due to cytosolic contamination of the 20KgP fraction, as the cytosolic enzyme glucose-6-phosphate dehydrogenase was found exclusively in the 20KgS fraction. Isopycnic density gradient centrifugation of the 20KgP fraction from wild-type cells showed that some Vps1p was found in fractions enriched for peroxisomes, as marked by the presence of the peroxisomal matrix protein thiolase and the absence of the mitochondrial protein Sdh2p (Fig. 7B). In cells expressing Vps1pΔsso9–523 or Vps1pV516P, to Vps1p was found localized to the 20KgP fraction (Fig. 7A). As expected, thiolase and Sdh2p remained preferentially localized to the 20KgP fraction in cells expressing Vps1pΔsso9–523 or Vps1pV516P. These results show that some fraction of Vps1p is associated with peroxisomes and that this association is dependent on its interaction with Pex19p through its first Pex19p recognition sequence.

DISCUSSION

Organelles are highly dynamic structures that undergo fission and fusion to control their numbers, modify their morphology in response to intracellular and extracellular cues, and permit their proper segregation at cell division. Maintenance of the overall compartmental integrity of the eukaryotic cell requires the tight coordination of mechanisms controlling these events. The dynamics and dynamin-like proteins play key roles in this process.

In yeast, fission of mitochondria occurs by a multistep pathway that involves recruitment of the dynamin-like protein Dnm1p and accessory proteins to sites on mitochondrial tubules, constriction of the mitochondrial tubules at these sites, and coordinated division of the outer and inner mitochondrial membranes to generate new tubule ends (20). Another dynamin-like protein, Vps1p, is involved in regulating peroxisome fission. Deletion of the VPS1 gene leads to cells containing a few enlarged peroxisomes (21). Vps1p has been shown to have a role in regulating cytoskeletal dynamics (28), and cells mutant for both the VPS1 gene and for the RHO1 gene encoding a small GTPase have been found to accumulate actin on peroxisomes (29). How might Vps1p regulate fission at the level of the peroxisome? Because the peroxin Pex19p has been shown to function in assembly of the peroxisomal membrane, we proposed that Vps1p could associate with peroxisomes in a Pex19p-dependent manner so as to participate in peroxisome fission. Using the
Role of Pex19p in Vps1p Association with Peroxisomes

consensus sequence for Pex19p recognition (47), we found two putative Pex19p binding sites in Vps1p and showed by yeast two-hybrid analysis that Pex19p indeed interacts with Vps1p.

If the association of Vps1p with peroxisomes is dependent on Pex19p, then mutations in Vps1p that disrupt its interaction with Pex19p would be predicted to give rise to the peroxisomal phenotype exhibited by vpsiΔ cells, i.e. reduced numbers of enlarged peroxisomes. Cells expressing a mutant form of Vps1p lacking the first Pex19p binding site (Vps1pΔ509–523), but not the second site (Vps1pΔ633–647), did indeed exhibit reduced numbers of enlarged peroxisomes as such vpsiΔ cells. A single substitution mutation within the first Pex19p recognition sequence of Vps1p, V516P, greatly diminished the interaction of Pex19p with Vps1p, and cells expressing Vps1pV516P also contained fewer and enlarged peroxisomes, similar to the peroxisome phenotype of vpsiΔ cells.

Deletion of the first Pex19p recognition site of Vps1p did not affect nuclear protein targeting, whereas deletion of the second site led to only a modest increase in secretion of the nuclear hydrolase CPY. It is noteworthy that deletion of the second Pex19p recognition site spans the dynamin GTPase effector domain of Vps1p (50). This domain has been shown to target to the nucleus and to direct proteins with which it interacts both as a cytosolic/peroxisomal PMP chaperone and as a PMP assembly of the peroxisomal membrane, and that the association of Vps1p with peroxisomes is dependent on Pex19p. Disruption of the interaction between Vps1p and Pex19p results in the abnormal peroxisome phenotype of reduced numbers of enlarged peroxisomes observed in vpsiΔ cells but no change in vacuolar morphology or vacuolar protein sorting. Therefore, the peroxisome biogenic and vacuole biogenic functions of Vps1p are apparently both separate and separable.

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REFERENCES

1. Osteryoung, K. W. (2001) Curr. Opin. Microbiol. 4, 639–646
2. Shaw, J. M., and Nunnari, J. (2002) Trends Cell Biol. 12, 178–184
3. Smith, J. J., Marelli, M., Christmas, R. H., Vizeacoumar, F. J., Dilworth, D. J., Ideker, T., Galtioli, T., Dimmott, K., Rachubinski, R. A., and Aitchison, J. D. (2002) J. Cell Biol. 158, 259–271
4. Rottensteiner, H., Stein, K., Sonnenholz, E., and Erdmann, R. (2003) Mol. Biol. Cell 14, 4316–4328
5. Tam, Y. Y. C., Torres-Guzman, J. C., Vizeacoumar, F. J., Smith, J. J., Marelli, M., Aitchison, J. D., and Rachubinski, R. A. (2003) Mol. Biol. Cell 14, 4089–4102
6. Erdmann, R., and Blobel, G. (1995) J. Cell Biol. 128, 509–523
7. Marhall, P. A., Krenkerich, Y. I., Lark, R. H., Dyer, J. M., Veenhuys, M., and Gooden, J. M. (1995) J. Cell Biol. 129, 345–355
8. Sakai, Y., Marshall, P. A., Saiganji, A., Tabake, K., Saiki, K., Hato, N., and Goodman, J. M. (1995) J. Bacteriol. 177, 6773–6781
9. Li, X., and Gould, S. J. (2002) J. Cell Biol. 156, 643–651
10. Li, X., Baumgart, E., Dong, G. X., Morrell, J. C., Jimenez-Sanchez, G., Valle, D., Smith, K. D., and Gould, S. J. (2002) Mol. Biol. Cell 13, 8226–8240
11. Hoepfner, D., van den Berg, M., Lopes, F., Roels, B., Vanale, J., Schutgens, R. B. H., Wanders, R. J. A., van Roermund, C. W. T., van Wijland, M. J. A., Schram, A. W., Tager, J. M., and Saudubray, J. (1988) Am. J. Hum. Genet. 44, 422–434
12. Sacksteder, K. A., and Gould, S. J. (2000) Annu. Rev. Genet. 34, 623–652
13. Chang, C. C., South, S., Warren, D., Jones, J., Moser, A. B., Moser, H. W., and Gould, S. J. (1999) J. Cell Sci. 112, 1579–1590
14. van Roermund, C. W. T., Tabak, H. F., van den Berg, M., Wanders, R. J. A., and Hettema, E. H. (2000) J. Cell Biol. 150, 489–498
15. Smith, J. J., Brown, T. W., Eitzen, G. A., and Rachubinski, R. A. (2000) J. Biol. Chem. 275, 20168–20178
16. Vizeacoumar, F. J., Torres-Guzman, J. C., Tam, Y. Y. C., Aitchison, J. D., and Rachubinski, R. A. (2003) J. Cell Biol. 161, 321–332
17. Vizeacoumar, F. J., Torres-Guzman, J. C., Bouard, D., Aitchison, J. D., and Rachubinski, R. A. (2004) Mol. Cell. Biol. 15, 6835–6845
18. Tanger, K. E. (2000) Annu. Rev. Cell Dev. Biol. 16, 483–519
19. Danino, D., and Hindshaw, J. E. (2001)Curr. Opin. Cell Biol. 13, 454–460
20. Osteryoung, K. W., and Nunnari, J. (2003) Science 302, 1698–1704
21. Koch, A., Schneider, G., Luers, G. H., and Schrader, M. (2004) J. Cell Sci. 117, 3995–4006
22. Li, X., and Gould, S. J. (2003) J. Biol. Chem. 278, 17012–17020
23.越来越, P., Hutton, J. L., Olson, J. M., and Payne, G. S. (2002) J. Cell Biol. 157, 315–326
24. Warren, D. T., Andrews, P. D., Gourlay, C. W., and Aycsous, K. R. (2002) J. Cell Sci. 115, 1703–1715
25. Gourlay, C. W., Dewar, H., Warren, D. T., Costa, R., Satish, N., and Aycsous, K. R. (2003) J. Cell Biol. 161, 2551–2564
26. Yu, X., and Cai, M. (2004) J. Cell Sci. 117, 3839–3853
27. Marelli, M., Smith, J. J., Fang, Y., and Cai, M. (1997) Mol. Cell. Biol. 17, 4304–4309
28. Howard, J. P., Hutton, J. L., Olson, J. M., and Payne, G. S. (2002) J. Cell Biol. 157, 315–326
29. Smith, J. J., Marelli, M., Christmas, R. H., Vizeacoumar, F. J., Dilworth, D. J., Ideker, T., Galtioli, T., Dimmott, K., Rachubinko, R. A., and Aitchison, J. D. (2002) J. Cell Biol. 158, 259–271
30. Gorte, K., Girzalsky, W., Linkert, M., Bauman, G., Kammerer, S., Kunau, W. H., and Erdmann, R. (1998) Mol. Cell. Biol. 18, 616–628
31. Matsuzono, Y., Kinoshita, N., Tamura, S., Shimozawa, N., Hamasaki, M., Ghaedi, K., Wanders, R. A., Suzuki, Y., Kondo, N., and Fujiki, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2116–2121
32. Sacksteder, K. A., Jones, J. M., South, S. T., Li, X., Liu, Y., and Gould, S. J. (2000) J. Cell Sci.
Role of Pex19p in Vps1p Association with Peroxisomes

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12823