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The *Saccharomyces cerevisiae MVP1* Gene Interacts with *VPS1* and Is Required for Vacuolar Protein Sorting

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The *VPS1* gene of *Saccharomyces cerevisiae* encodes an 80-kDa GTPase that associates with Golgi membranes and is required for the sorting of proteins to the yeast vacuole. Vps1p is a member of a growing family of high-molecular-weight GTPases that are found in a number of organisms and are involved in a variety of cellular processes. Vps1p is most similar to mammalian dynamin and the *Drosophila Shibire* protein, both of which have been shown to play a role in an early step of endocytosis. To identify proteins that interact with Vps1p, a genetic screen was designed to isolate multicopy suppressors of dominant-negative *vps1* mutations. One such suppressor, *MVP1*, that exhibits genetic interaction with *VPS1* and is itself required for vacuolar protein sorting has been isolated. Overproduction of Mvp1p will suppress several dominant alleles of *VPS1*, and suppression is dependent on the presence of wild-type Vps1p. *MVP1* encodes a 59-kDa hydrophilic protein, Mvp1p, which appears to colocalize with Vps1p in *vps1*^{d} and *vps27*Δ yeast cells. We therefore propose that Mvp1p and Vps1p act in concert to promote membrane traffic to the vacuole.

In the biogenesis of the *Saccharomyces cerevisiae* vacuole, proteins that are destined to reside in the vacuole must be recognized and sorted away from proteins that are to be delivered to the cell surface. Both classes of proteins enter the secretory pathway at the endoplasmic reticulum and from there are transported to the *cis*-Golgi and through the Golgi compartments via transport vesicles (12, 17, 18, 26, 34, 50). It is in a late Golgi compartment that soluble vacuolar proteins are recognized by a sorting signal present in the propeptide regions of their primary amino acid sequences, are sorted away from secreted proteins, and are packaged into vesicles to be targeted to the vacuole (8, 14, 17, 52, 53). Nearly 50 genes that are required to efficiently carry out the process of sorting and targeting soluble vacuolar proteins have been identified in *S. cerevisiae* (19, 31, 32, 36, 37, 40). Mutation in any one of these genes results in the mislocalization of vacuolar proteases, such as carboxypeptidase Y (CPY), to the cell surface, where they are secreted.

Data on a growing family of high-molecular-weight GTPases that have been shown to play a role in vesicular transport, although their function is not yet well understood, are now emerging. Members of this family include mammalian dynamin (29, 47) and its *Drosophila melanogaster* equivalent, *Shibire* protein (4, 54), both of which have been shown to be required for the early stages of endocytosis (9, 55), and the *S. cerevisiae VPS1*-encoded protein, mutations in which lead to the mislocalization of soluble vacuolar proteins (39, 40). These proteins share a high degree of sequence similarity (66% identity) over their amino-terminal GTP-binding regions, but exhibit less similarity (28% identity) in their carboxyl-terminal halves.

Paralysis of *shi*^{ts} mutant flies at the nonpermissive temperature correlates with a decrease in the number of synaptic vesicles at neuromuscular junctions (30). In addition, further work has shown that *shi*^{ts} mutant cells exhibit a general defect in the early stages of endocytosis and are unable to form either clathrin-coated or noncoated vesicles (16, 20, 24). Similarly, recent work with dynamin has shown that it plays a role in endocytosis as well. The expression of dominant alleles of rat or human dynamin in COS-7 or HeLa cells results in the inability of these cells to form clathrin-coated vesicles (9, 55). In both instances, no defect in exocytosis was caused by the mutant dynamin.

VPS1 is a nonessential gene in yeast cells that encodes an 80-kDa hydrophilic protein (39). Localization of Vps1p by indirect immunofluorescence (39) indicates that it normally associates with Golgi membranes (7, 33, 44). Its role in vacuolar protein sorting and its Golgi membrane localization suggest that Vps1p might function at a late Golgi compartment in a manner analogous to that of dynamin at the plasma membrane of mammalian cells. *vps1* mutant cells have been found to divert vacuole-bound membrane traffic to the plasma membrane (27a), suggesting that Vps1p may function together with clathrin in the formation of Golgi apparatus-derived vacuole-targeted vesicles.

Mutational analysis of *VPS1* has led to the hypothesis that Vps1p is composed of two functionally distinct domains (56). The amino-terminal half of the protein possesses a GTP-binding–hydrolysis activity and will bind to GTP in the absence of the carboxyl-terminal half. Numerous dominant point mutations that cause wild-type cells to mislocalize vacuolar proteins have been isolated in this region (56). Expression of just the carboxyl-terminal half of Vps1p in wild-type cells also disrupts vacuolar protein sorting. In our working model for Vps1p function, the amino-terminal domain is proposed to provide an essential GTP-hydrolysis activity that may regulate the interactions of the carboxyl-terminal domain. In the absence of this functional GTP-hydrolysis activity, such as with the dominant point mutations, the carboxyl-terminal half of Vps1p is proposed to associate with and titrate out a limiting factor that normally interacts with Vps1p in promoting protein sorting. In doing so, this factor is made unavailable to engage in a functional interaction with wild-type Vps1p. Interestingly, similar dominant point mutations have been isolated in dynamin (9, 55). Deletion of the GTP-binding domain of dynamin also results in a mutant protein that interferes with the function of the wild-type protein (10).

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TABLE 1. *S. cerevisiae* strains used in this study

Strain[a]	Genotype	Reference
SF838-1D	*MAT*α *ade6 leu2-3,112 ura3-52 his4-519 gal2 pep4-3*	
KEY2	*MAT*α *ade6 leu2-3,112 ura3-52 his4-519 gal2 pep4-3 VPS1*::*LEU2*::*vps1-922-4*	This study
KEY4	*MAT*α *ade6 leu2-3,112 ura3-52 his4-519 gal2 VPS1*::*LEU2*::*vps1-922-4*	This study
JHRY20-2C	*MAT***a** *his3-*Δ*200 ura3-52 leu2-3,112*	38
TSY105	*MAT***a** *his3-*Δ*200 ura3-52 leu2-3,112 vps1-*Δ*2*::*LEU2*	56
TSY106	*MAT***a** *his3-*Δ*200 ura3-52 leu2-3,112 vps27*Δ::*LEU2*	31
KEY10	*MAT***a** *his3-*Δ*200 ura3-52 leu2-3,112 mvp1*Δ::*LEU2*	This study

[a] Strains SF838-1D, KEY2, and KEY4 are isogenic, except at the *PEP4* or *VPS1* loci. Strains JHRY20, TSY105, TSY106, and KEY10 are isogenic, except at the *VPS1*, *VPS27*, or *MVP1* loci.

We have used the dominant mutations in *VPS1* to search for yeast proteins that normally associate with Vps1p. A gene encoding a potential Vps1p-interacting protein was isolated by its ability to suppress a *vps1*^{d} allele when the gene is provided in multiple copies. This gene, *MVP1*, exhibits genetic interaction with *VPS1* and is itself required for the efficient delivery of soluble proteins to the vacuole. We show that the product of the *MVP1* gene, Mvp1p, colocalizes with Vps1p in certain yeast mutants and discuss these findings in relation to the possible role of these two proteins in membrane traffic to the yeast vacuole.

MATERIALS AND METHODS

Materials. The following materials were obtained from the indicated sources: enzymes were from New England Biolabs (Beverly, Mass.); IgGSorb was from The Enzyme Center (Malden, Mass.); oxalyticase was from Enzogenetics (Corvallis, Oreg.); acrylamide was from Boehringer Mannheim Biochemical Corp. (Indianapolis, Ind.); Zymolyase was from ICN Biochemical, Inc. (Costa Mesa, Calif.); all secondary and fluorochrome-conjugated antibodies for immunofluorescence were from Jackson Immunoresearch Laboratories, Inc. (West Grove, Pa.); nitrocellulose filters were from Millipore (Bedford, Mass.); and alkaline phosphatase-conjugated goat anti-rabbit and goat anti-mouse immunoglobulin G were from Promega Corp. (Madison, Wis.). All other reagents were from Sigma Chemical Co. (St. Louis, Mo.).

Yeast strains and culture conditions. The yeast strains used in this study are listed in Table 1. Strain KEY2 was constructed by integrating the plasmid pKE6 cut with *Bsu*36I, which cuts within the *VPS1* open reading frame, into SF838-1D cells. Correct integration into the *VPS1* locus was confirmed by Southern blot analysis as previously described (51). Yeast genomic DNA (27) was cut with *Xba*I and probed with [^{32}P]dCTP-labeled *VPS1* DNA (Multiprime DNA labeling kit; Amersham Corp., Arlington Heights, Ill.). The *PEP4* version of this strain, KEY4, was constructed by transforming these cells with a linearized *PEP4*::*URA3* plasmid and then by selecting for excision of the plasmid on medium containing 5-fluoro-orotic acid (5-FOA) (2). Retention of the wild-type *PEP4* allele was confirmed by APNE (*N*-acetyl-phenylalanine-β-naphthyl-ester) analysis (57). Strains disrupted for the *MVP1* gene were constructed by integration of the *mvp1*::*LEU2* *Pst*I-*Hin*dIII fragment from pKE16. Correct replacement of the *MVP1* open reading frame was confirmed by Southern blot analysis.

All yeast strains were grown in rich medium (YEPD [1% yeast extract, 2% Bacto Peptone, 2% glucose]) or in standard minimal medium with appropriate supplements (45). Transformations of yeast cells were done with lithium acetate (46) or by electroporation with a Bio-Rad electroporator according to the manufacturer's recommendations.

DNA manipulations and plasmid constructions. DNA manipulations and transformation of *Escherichia coli* were performed as previously described (41). The plasmids used in this study are listed in Table 2. For the sequencing of the *MVP1* gene, nested deletions were created in plasmids pKE9 and pKE10 with the Erase-a-Base kit from Promega Corp. Single-stranded DNA sequencing from the M13 universal primer was performed with Sequenase version 2.0 (U.S. Biochemical Corp., Cleveland, Ohio).

Plasmid pKE1 was the library plasmid isolated in the suppressor screen. Amino acids 125 to 250 (*Xba*I-*Eco*RI) of *MVP1* are replaced with the entire *LEU2* gene in pKE16. The *Bgl*II site was engineered into *MVP1* by oligo-directed mutagenesis with the oligonucleotide 5′-GATTTCTCGTGAAGATCTATA AATTTTACGGAG-3′ (21).

CPY colony immunoblot and immunoprecipitation. CPY colony blots were performed as previously described (35, 38). For the screening of the library (3) for suppressors, colonies from transformation plates (at 500 to 900 colonies per plate) were replica-plated to fresh selective plates, covered with nitrocellulose, and incubated for 14 to 18 h at 30°C. Blots were blocked for several hours and incubated with primary polyclonal or monoclonal anti-CPY antibodies overnight, rinsed, and incubated with secondary antibodies overnight. For other blots, cells were grown in liquid culture to log phase and identical numbers of cells were spotted onto selective plates and immediately covered with nitrocellulose.

CPY immunoprecipitations were performed as previously described (31). Briefly, cells grown to an optical density at 600 nm of 1.0 in selective synthetic medium lacking methionine (SD-Met) were centrifuged and resuspended at an optical density at 600 nm of 1.0 in SD-Met–50 mM potassium phosphate (pH 5.7)–2 mg of bovine serum albumin (BSA) per ml. The cells were then pulse-labeled for 10 min at 30°C with 200 μCi of ^{35}S-Express label (New England Nuclear) per A_{600} unit and chased with the addition of cold methionine and cysteine to a final concentration of 80 μg/ml. The chase was terminated by the addition of sodium azide to 10 mM. The cells were separated from the medium by centrifugation and spheroplasted. CPY was then immunoprecipitated from the resulting intracellular and extracellular fractions and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (23) and fluorography. Radioactivity was quantified with the AMBIS Radioanalytic Imaging System (Ambis Systems Inc., San Diego, Calif.).

Anti-Mvp1p antibodies and Western blotting (immunoblotting). Rabbit polyclonal antiserum against Mvp1p was raised as previously described (35). Antigen was prepared by gel purifying a recombinant Mvp1p fragment (amino acids 218 to 511) expressed from plasmid pKE23 in *E. coli*. Antibodies were affinity purified against the same expressed Mvp1p fragment and used at a 1:500 dilution. Yeast whole-cell extracts were run on SDS–10% PAGE gels (23) and electroblotted onto nitrocellulose. The blots were probed with anti-Mvp1p antibody followed by alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G. Immune complexes were visualized according to the manufacturer's recommendations.

TABLE 2. Plasmids used in this study

Plasmid	Description
pCKR19d-N	*vps1* allele *N2*, *N3*, *N4*, *N5*, or *N14* in pRS316
pCKR50	*VPS1* gene in YEp24
pKE1	6.5-kb yeast genomic DNA insert in YEp24
pKE6	*vps1-922-4* allele in pRS305
pKE9	4.5-kb *Hin*dIII fragment from pKE1 in pBluescriptKS^{+}
pKE10	4.5-kb *Hin*dIII fragment from pKE1 in pBluescriptKS^{+}
pSP65nx	pSP65 cut with *Xba*I and *Nhe*I and religated
pKE7	*Pst*I-*Hin*dIII *MVP1* fragment in pSP65nx
pKE16	*Hpa*I *LEU2*-containing fragment into *Xba*I-*Eco*RI sites of pKE7
pKE17	*Xba*I-*Spe*I fragment containing the *vps1*^{d} allele *N5* in pRS313
pKE18	*Xba*I-*Spe*I fragment containing the *vps1*^{d} allele *922-4* in pRS313
pKE23	*Xba*I-*Ssp*I *MVP1* fragment in pCWori^{+}H
pKE41	*Ssp*I fragment containing *MVP1* from pKE1 in pRS316
pKE42	*Ssp*I fragment containing *MVP1* from pKE1 in YEp351
pKE61	*Ssp*I fragment containing *MVP1* from pKE1 in pBluescriptKS^{+}
pKE65	pKE61 with *Bgl*II site introduced by mutagenesis
pKE66	120-bp *HA* fragment in *Bgl*II site of pKE65
pKE70	*MVP1*:*HA* allele from pKE66 in pRS316
pKE82	*vps1*-Δ*N* allele in pRS316

Fluorescence microscopy. Indirect immunofluorescence was performed as described previously (31) with the following exceptions. The cells were spheroplasted in 100 mM potassium phosphate (pH 7.5)–0.2% β-mercaptoethanol–20 μg of Zymolyase per ml, treated with 2.5% SDS for 1 min, and incubated on slides with phosphate-buffered saline–BSA for 1 h before the first antibody was added. Rabbit anti-Vps1p antibodies were affinity purified and used at a 1:10 dilution (39, 56). Mouse monoclonal anti-60-kDa antibodies (Ab 13D11; Molecular Probes, Eugene, Oreg.) were used at a dilution of 1:10. Mouse anti-hemagglutinin (HA) (Ab 12CA5; Babco Inc.) antibody was used at a 1:250 dilution. All secondary antibodies were used at dilutions of 1:500, except for Texas Red-conjugated antibodies, which were used at a 1:2,500 dilution.

Nucleotide sequence accession number. The *MVP1* sequence has been deposited in GenBank under accession number U16137.

RESULTS

Isolation of a multicopy suppressor of a dominant *VPS1* allele. Previously, a number of dominant point mutations were generated in *VPS1* by hydroxylamine mutagenesis (56). All of the loss of function mutations that mapped to the first half of the protein conferred a dominant-negative phenotype for vacuolar protein sorting, that is, cells carrying a *vps1*^{d} allele and a wild-type *VPS1* gene secreted newly synthesized CPY. In contrast, mutations in the second half of *VPS1* were recessive, and these *vps1* alleles encoded truncated or unstable Vps1 proteins (56). Interestingly, dominant interference in vacuolar protein sorting by *vps1*^{d} was relieved by deletion of the 287 carboxyl-terminal codons (this truncated allele produces a stable 45-kDa Vps1p), indicating that dominance is mediated through the carboxyl-terminal domain (56). Finally, the CPY secretion phenotype of *vps1*^{d} cells could be suppressed by overexpression of wild-type *VPS1*, suggesting a model in which the mutant Vps1p-d protein encoded by the *vps1*^{d} gene titrates out some limiting factor that normally interacts with Vps1p in promoting vacuolar protein sorting. In this study, these dominant *vps1*^{d} alleles were exploited in a search designed to identify proteins that interact with Vps1p.

Quantitation of the CPY sorting defects indicated that whereas wild-type yeast cells secreted less than 5% of the newly synthesized CPY, integration of the *vps1*^{d} allele adjacent to a wild-type copy of the *VPS1* gene (producing a *VPS1*/*vps1*^{d} strain, KEY2, with a tandem duplication at the *VPS1* locus) resulted in the secretion of 55% of the CPY (Fig. 1A, lanes 1 to 4). This level of CPY secretion was intermediate between the level of mislocalization found for *vps1*^{d} cells (>90% CPY secretion) (56) and that for wild-type cells (~5% CPY secretion). Therefore, the KEY2 yeast strain (carrying two genomic copies of the *VPS1* gene, one a wild type and the other *vps1*^{d}) was used to isolate multicopy suppressors of the *vps1*^{d} mutation.

KEY2 cells were transformed with a 2μm-based (YEp24), multicopy yeast genomic DNA library (3), and Ura^{+} colonies were selected. Colonies from the transformation were tested for CPY secretion by the CPY colony immunoblot procedure (35, 38). In order to determine if suppression was plasmid linked, cells were cured of plasmids by growth on medium containing 5-FOA and screened for loss of suppression of CPY secretion. Finally, plasmids were rescued from the transformants, amplified in *E. coli*, and transformed into KEY2 cells. Those plasmids that continued to suppress CPY secretion upon retransformation were then restriction mapped to identify overlapping or known restriction patterns. In this way, three different genes, *PEP4* (1), *VPS1* (39), and a novel gene, were identified. *PEP4* was isolated because expression of this gene at a high level leads to the degradation of external CPY, thus reducing the amount of CPY seen on the colony blot without reducing the actual level of CPY secretion. *VPS1* was expected to have been isolated in this screen since previous work had demonstrated that additional copies of wild-type *VPS1* would suppress CPY secretion in cells carrying *vps1*^{d} alleles (56). A third suppressor, called *MVP1* for multicopy suppressor of *vps1*, was identified and chosen for further characterization. *PEP4* was isolated twice, and *VPS1* and *MVP1* were each isolated three times from the 37,000 transformants screened.

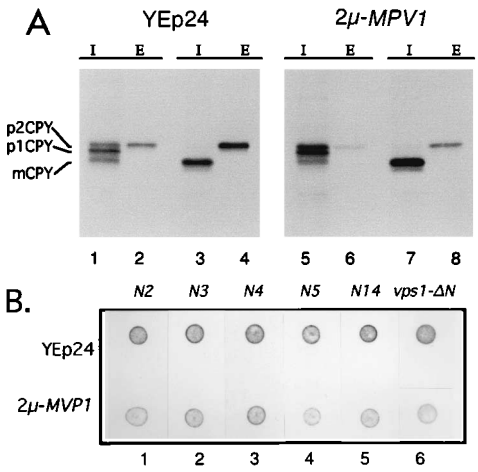

FIG. 1. Increased expression of the *MVP1* gene suppresses the CPY mislocalization phenotype of *VPS1*/*vps1*^{d} cells. (A) KEY4 cells were transformed with either a vector alone (YEp24, lanes 1 to 4) or with a multicopy plasmid containing the *MVP1* gene (pKE1, lanes 5 to 8). Cells were labeled for 10 min at 30°C with ^{35}S-Express label and chased for either 0 min (lanes 1, 2, 5, and 6) or 30 min (lanes 3, 4, 7, and 8). CPY was then immunoprecipitated from intracellular (I) and extracellular (E) fractions and analyzed by SDS-PAGE and fluorography. (B) SF838-1D cells were transformed with low-copy-number (CEN) plasmids containing dominant-negative *vps1* alleles *N2* (pCKR19d-N2), *N3* (pCKR19d-N3), *N4* (pCKR19d-N4), *N5* (pCKR19d-N5), *N14* (pCKR19d-N14), and *vps1*-Δ*N* (pKE82) as well as either YEp24 or 2μm-*MVP1* (pKE42). Cultures were grown in selective medium, and identical numbers of cells were spotted onto plates lacking uracil and leucine for CPY colony immunoblots as described in Materials and Methods. (*vps1*^{d} is the dominant-negative allele of the *VPS1* gene.) p1CPY and p2CPY refer to the 67-kDa endoplasmic reticulum- and 69-kDa Golgi apparatus-modified forms of the CPY precursor, respectively.

To test whether high-level expression of *MVP1* resulted in more efficient CPY sorting, pulse-chase immunoprecipitations, after the cells were radiolabeled with [^{35}S]methionine, were carried out to determine the level of intracellular (vacuolar) and extracellular (secreted) CPY. For this experiment, a *PEP4* strain, KEY4, that was otherwise isogenic to KEY2 was used so that delivery of CPY to the vacuole could be monitored by its cleavage to the mature form (15, 25, 58). When not overexpressing *MVP1*, KEY4 cells secreted ~55% of their newly synthesized CPY (Fig. 1A, lanes 1 to 4). When these cells harbored the 2μm-*MVP1* plasmid (pKE1), CPY secretion was reduced to ~25% (Fig. 1A, lanes 5 to 8). A comparison of the 0-min chase and the 30-min chase times showed that no loss of CPY occurred. Furthermore, intracellular CPY was present in the mature form, indicating that it had been correctly targeted to the vacuole. Therefore, suppression of CPY secretion was due to an increase in CPY sorting efficiency and was not a result of CPY degradation or retention within the cell in a location other than the vacuole.

***MVP1* will suppress several alleles of *VPS1*.** Multiple dominant mutations that map throughout the amino-terminal GTP-binding domain have been isolated in *VPS1* (56). To determine if suppression by *MVP1* was specific to the *vps1*

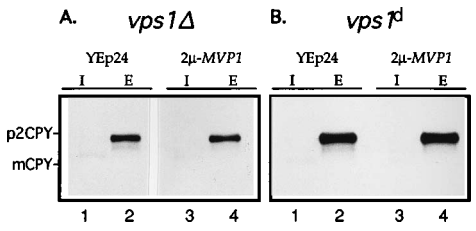

FIG. 2. Overproduction of Mvp1p does not bypass Vps1p function or enable mutant Vps1p to function. (A) TSY105 cells (*vps1*Δ) carried a vector alone (YEp24, lanes 1 and 2) or a multicopy plasmid containing the *MVP1* gene (pKE1, lanes 3 and 4). (B) TSY105 cells carried *vps1*^{d} on a low-copy-number plasmid (CEN-*vps1-922-4*, pKE18) and either YEp24 (lanes 1 and 2) or pKE1 (lanes 3 and 4). Cells were labeled for 10 min at 30°C with ^{35}S-Express label and chased for 30 min. CPY was then immunoprecipitated from intracellular (I) and extracellular (E) fractions and analyzed by SDS-PAGE and fluorography.

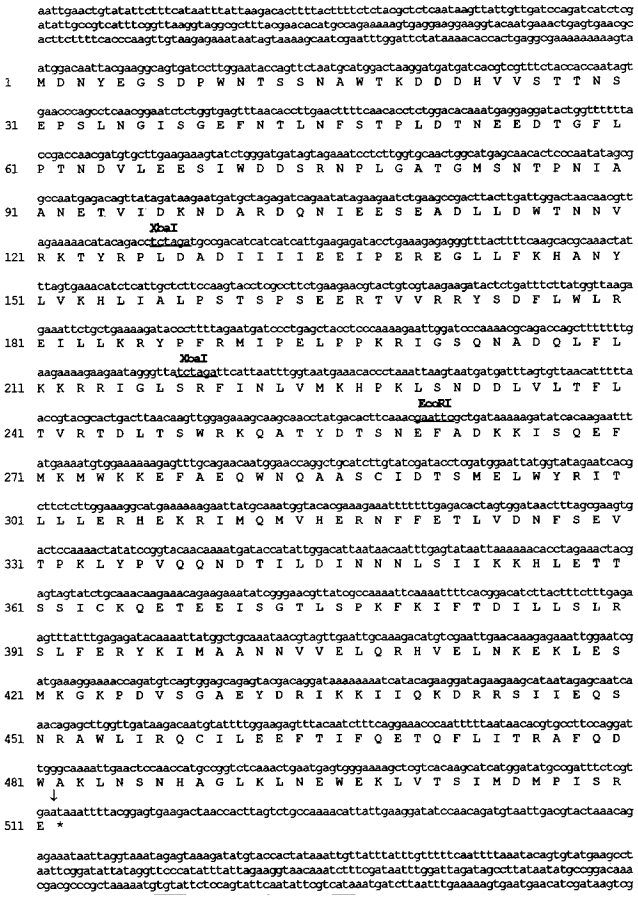

FIG. 3. Nucleotide and amino acid sequence of the *MVP1* gene. The predicted amino acid sequence of Mvp1p is depicted in the one-letter code, and the position of the insertion of the HA epitope is noted with an arrow.

allele used in the original screen, the ability of *MVP1* to suppress other dominant *vps1* alleles was examined.

The wild-type strain, SF838-1D, was transformed with each of six different *vps1*^{d} alleles on low-copy-number, centromere-based plasmids and with either the 2μm control plasmid, YEp351, or with YEp351 containing the *MVP1* gene, pKE42. The transformants were then grown in liquid culture, spotted onto minimal plates, and covered with nitrocellulose for CPY colony immunoblot assays. As can be seen from Fig. 1B, the expression of the *vps1*^{d} alleles in wild-type cells resulted in the mislocalization and secretion of CPY as expected. However, secretion was reduced when these cells simultaneously overexpressed the wild-type *MVP1* gene. Suppression by 2μm levels of *MVP1* was seen for each of the dominant alleles of *VPS1* tested and was not specific to the one allele used in the original screen. Included in the alleles suppressed were not only point mutations in *VPS1* (Fig. 1B, lanes 1 to 5) but also a dominant allele of *VPS1*, *vps1*-Δ*N* (Fig. 1B, lane 6), in which the entire GTP-binding domain (amino acids 19 to 356) had been deleted (56). In fact, no dominant *vps1* alleles that were tested were not suppressed by *MVP1*.

Suppression by *MVP1* requires the presence of wild-type *VPS1*. It is possible that the overproduction of Mvp1p creates an alternate pathway to the vacuole that is independent of the *VPS1* gene product. To address this possibility, the ability of *MVP1* to suppress CPY secretion in cells carrying a *vps1*Δ null mutation was tested. CPY was immunoprecipitated from intracellular and extracellular fractions of radiolabeled *vps1*Δ cells harboring either 2μm-*MVP1* (pKE1) or the control plasmid YEp24. In either the presence or absence of pKE1, >95% of the newly synthesized CPY was secreted (Fig. 2A). Overproduction of Mvp1p did not reduce the level of CPY secretion in *vps1*Δ cells, indicating that the Vps1p-dependent step in vacuolar protein sorting was not bypassed.

To test the effect of high-level expression of *MVP1* on cells that expressed only a dominant mutant *vps1* allele, *vps1*Δ cells (TSY105) were transformed with pKE17, a centromere-based (CEN) plasmid containing a *vps1*^{d} gene, and either YEp24 or pKE1. CPY was then immunoprecipitated from the intracellular and extracellular fractions of radiolabeled cells. As can be seen from Fig. 2B, cells that overexpressed *MVP1* secreted as much CPY (>95%) as did cells that carried the control plasmid. Therefore, increased production of Mvp1p did not enable the mutant Vps1p to function in the sorting of CPY to the vacuole.

Molecular characterization of *MVP1*. The isolated genomic plasmid carrying the *MVP1* gene contained a 6.5-kb insert. Restriction analysis and Tn*10*::*LacZ* insertions (13) narrowed the suppressing region to 4 kb and indicated the direction of transcription. The nucleotide sequence (GenBank U16137) was then determined by single-stranded sequencing of nested deletions created in the *MVP1* gene (see Materials and Methods). A single large open reading frame, in the direction indicated by the Tn*10* insertion mutagenesis and able to code for a protein of 511 amino acids with a molecular mass of 59 kDa, was revealed (Fig. 3). The protein is predicted to be highly hydrophilic, with no hydrophobic sequences of sufficient length to serve as a transmembrane domain or a signal sequence (22). A search for related sequences revealed no proteins with a significant similarity to *MVP1*, indicating that *MVP1* is a novel gene.

Rabbit polyclonal antibodies were raised against a recombinantly expressed fragment (amino acids 218 to 511) of the Mvp1 protein (Fig. 4A). Figure 4B (lanes 1 and 2) shows that these antibodies detected a single protein with a molecular mass of ~60 kDa on Western blots of whole-cell extracts from wild-type but not *mvp1*Δ cells. The level of this protein increased dramatically in cells carrying the 2μm-*MVP1* plasmid pKE1 (Fig. 4B, lane 3), demonstrating that this plasmid does result in overproduction of Mvp1p.

***MVP1* is required for vacuolar protein sorting.** To investigate the role of *MVP1* in vacuolar protein sorting, a deletion construct was used to delete the *MVP1* gene in diploid and haploid cells, replacing the *MVP1* sequence with that of the *LEU2* gene (Fig. 4A). Tetrad analysis of *MVP1*/*mvp1*Δ::*LEU2*

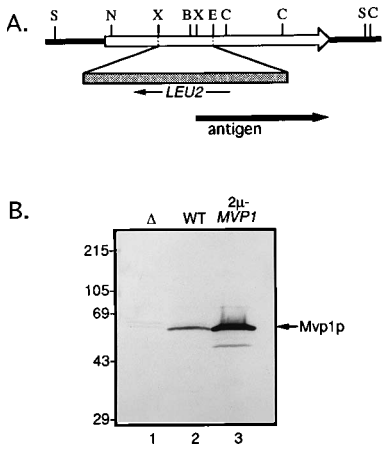

FIG. 4. The 2μm-*MVP1* plasmid, pKE1, results in overproduction of the 60-kDa Mvp1 protein. (A) Diagram of the physical map of *MVP1*. The smallest subclone that complements the *mvp1*Δ mutation is the 2.6-kb *Ssp*I-*Ssp*I fragment. The *MVP1* open reading frame is represented by the open arrow. The region of *MVP1* (*Xba*I to *Eco*RI) replaced by the *LEU2* gene to generate the *mvp1* disruption cassette is indicated. The region of Mvp1p expressed in *E. coli* to produce antigen for the production of antiserum is shown by the large solid arrow (amino acids 218 to 511). B, *Bam*HI; C, *Cla*I; E, *Eco*RI; N, *Nsi*I; S, *Ssp*I; X, *Xba*I. (B) Western blot of whole-cell extracts from *mvp1*Δ cells (lane 1), wild-type cells (lane 2), and wild-type cells carrying the 2μm-*MVP1* plasmid pKE1 (lane 3). Anti-Mvp1p affinity-purified polyclonal antibodies were used at a 1:500 dilution to detect Mvp1p. The numbers at the left are kilodaltons.

diploid cells demonstrated that from 24 tetrads, all spores were viable and segregated 2+:2− for CPY secretion as determined by colony immunoblot. CPY secretion was linked to leucine prototrophy and therefore is a result of the *mvp1*Δ mutation. Complementation analysis revealed that *mvp1* complemented all of the *vps* mutants (31), and therefore *mvp1* represents a new gene required for the sorting of proteins to the yeast vacuole (6).

The quantitation of the CPY mislocalization phenotype in *mvp1*Δ cells was determined by CPY pulse-chase immunoprecipitation. As can be seen from Fig. 5 (lanes 1 and 2), the *mvp1*Δ mutation resulted in the secretion of ~65% of the newly synthesized CPY and this mutation was fully complemented by low- or high-level expression of *MVP1* from a plasmid (Fig. 5, lanes 3 to 6). Colony immunoblot assays revealed that *mvp1*Δ cells also secreted another soluble vacuolar protease, PrA, in addition to CPY, which is consistent with Mvp1p having a role in the general sorting of proteins to the vacuole (6).

Mutations in some of the *VPS* genes, of which *VPS1* is one,

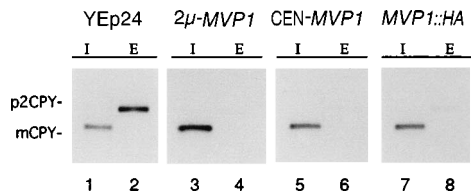

FIG. 5. The *MVP1* gene is required for the efficient sorting of CPY to the vacuole. KEY10 cells were transformed with a vector alone (YEp24, lanes 1 and 2), a multicopy plasmid containing the *MVP1* gene (pKE1, lanes 3 and 4), a low-copy-number plasmid containing the *MVP1* gene (CEN-*MVP1*, lanes 5 and 6), and a low-copy-number plasmid containing an HA-tagged *MVP1* allele (*MVP1*::*HA*, lanes 7 and 8). The cells were labeled for 10 min at 30°C with ^{35}S-Express label and chased for 30 min. CPY was then immunoprecipitated from intracellular (I) and extracellular (E) fractions and analyzed by SDS-PAGE and fluorography.

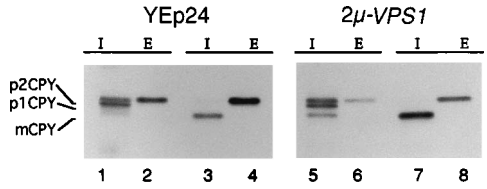

FIG. 6. Overexpression of wild-type *VPS1* suppresses the CPY mislocalization defect of *mvp1*Δ cells. KEY10 cells were transformed with either a vector alone (YEp24, lanes 1 to 4) or with a multicopy plasmid containing the wild-type *VPS1* gene (2μm-*VPS1*, lanes 5 to 8). The cells were labeled for 10 min at 30°C with ^{35}S-Express label and chased for either 0 min (lanes 1, 2, 5, and 6) or 30 min (lanes 3, 4, 7, and 8). CPY was then immunoprecipitated from intracellular (I) and extracellular (E) fractions and analyzed by SDS-PAGE and fluorography.

result in a temperature-sensitive growth defect (39, 40). For this reason, the effect of an *mvp1* null mutation on yeast cell growth at elevated temperatures was investigated. *mvp1*Δ cells were able to grow on minimal or rich medium at 30°C or 37°C at a rate identical to that of wild-type cells (data not shown). *vps1*Δ cells, on the other hand, grew more slowly at 30°C than wild-type cells and failed to grow at 37°C.

The *vps* collection can be divided into six classes on the basis of vacuolar morphology (31) as determined by the immunolocalization of two known vacuolar proteins: the integral membrane protein alkaline phosphatase and the 60-kDa subunit of the vacuolar H^+-ATPase. On the basis of these criteria, vacuolar morphology in *mvp1*Δ cells was indistinguishable from that in wild-type cells; both had a class A phenotype (data not shown). Interestingly, *vps1*Δ cells contain vacuoles that are more fragmented than those of wild-type cells; *vps1*Δ cells have been designated class F (31).

Increased expression of *VPS1* will partially suppress an *mvp1* null mutation. To further investigate the relationship between *MVP1* and *VPS1*, we determined whether increased expression of *VPS1* could bypass the need for *MVP1* in vacuolar protein sorting. In CPY pulse-chase immunoprecipitations with *mvp1*Δ cells carrying the control plasmid YEp24, a 65% secretion of CPY was observed (Fig. 6, lanes 1 to 4). However, when these cells overexpressed wild-type *VPS1*, CPY secretion was reduced to only 30% (Fig. 6, lanes 5 to 8). The kinetics of delivery of CPY to the vacuole indicated that suppression was not the result of the slowing of the movement of CPY through the secretory pathway. Suppression was also not a result of degradation of CPY, since there was no net loss of radiolabeled CPY during the chase.

Vps1p and Mvp1p colocalize in *vps1*^{d} and *vps27*Δ mutants. Previous experiments have shown that Vps1p staining in immunofluorescence experiments is consistent with the classification of Vps1p as a Golgi membrane-associated protein (39). In order to visualize Mvp1p by immunofluorescence, the protein was tagged with the HA epitope at its carboxyl terminus. The tagged gene (*MVP1*::*HA*) fully complemented an *mvp1*Δ mutation (Fig. 5, lanes 7 to 8). Indirect immunofluorescence with anti-HA antibodies in cells expressing the HA-tagged protein showed a very faint, punctate cytoplasmic staining pattern (Fig. 7C). Unfortunately, this staining was too faint to test for colocalization with Vps1p, and increasing the expression of Mvp1p-HA resulted in a diffuse cytoplasmic staining pattern (6).

A more discrete staining pattern was obtained when Mvp1p-HA was produced in *vps1*^{d} cells. Figure 7E shows that mutant Vps1p primarily localized to one or two concentrated patches that were always present adjacent to the vacuole. This pattern is in contrast to that of the multiple spots observed for

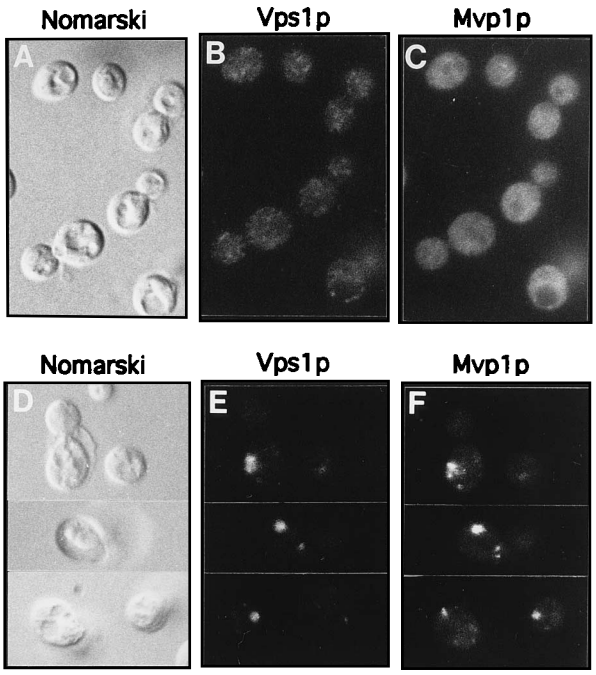

FIG. 7. Mvp1p and Vps1p colocalize in *vps1*^{d} cells. JHRY20-2C (wild-type) cells carried a low-copy-number plasmid containing the *MVP1*::*HA* gene (pKE70; A to C). TSY105 cells (*vps1*Δ) carried a low-copy-number plasmid containing the *vps1-N5* allele (pKE17) and pKE70 (D to F). Vps1p staining is shown in panels B and E, Mvp1p staining is shown in panels C and F, and the corresponding Nomarski images of cells are shown in panels A and D. Anti-HA monoclonal antibodies were used to localize Mvp1p, and anti-Vps1p affinity-purified polyclonal antibodies were used to localize Vps1p.

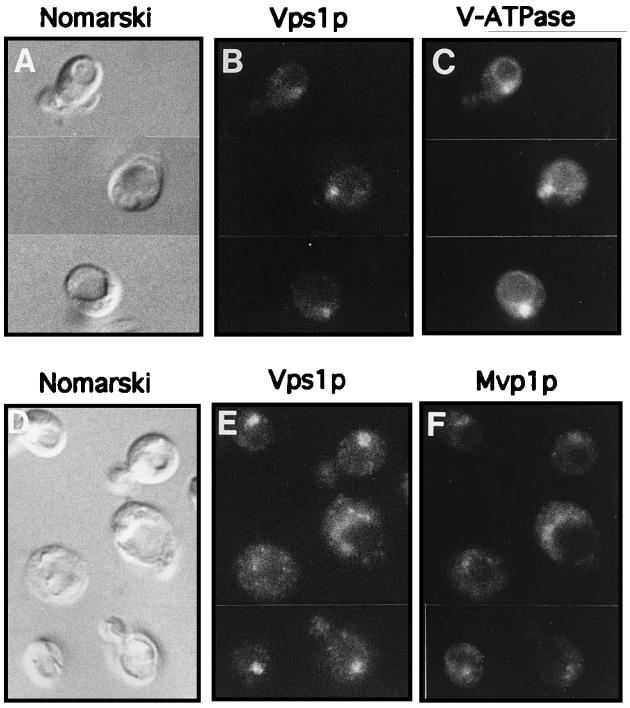

FIG. 8. Mvp1p and Vps1p colocalize in *vps27*Δ cells. *vps27*Δ cells (TSY106) carried a low-copy-number plasmid containing the *MVP1*::*HA* gene (pKE70). Vps1p staining is shown in panels B and E, vacuolar H^+-ATPase staining is shown in panel C, Mvp1p staining is shown in panel F, and the corresponding Nomarski images of cells are shown in panels A and D. Vps1p and Mvp1p were visualized as described in the legend to Fig. 7, and anti-60-kDa subunit monoclonal antibodies were used to localize the vacuolar H^+-ATPase.

wild-type Vps1p (Fig. 7B) (39). In experiments in which antibodies to both HA and Vps1p were used, Mvp1p was found to colocalize with the concentrated patches of Vps1p-d staining (Fig. 7D to F).

We also tested for colocalization of Mvp1p and Vps1p in *vps27* cells, since these mutants accumulate a novel prevacuolar, late-endosomal organelle (5, 31). It has recently been found that in *vps27*^{ts} cells, membrane traffic from this organelle both to the vacuole and back to the late Golgi compartment is blocked (29a), leading to the accumulation of some late Golgi membrane proteins associated with this structure. A large fraction of Vps1p was found to colocalize with the 60-kDa subunit of the vacuolar H^+-ATPase (Fig. 8A to C), a marker for the prevacuolar compartment in *vps27* cells (31). When anti-HA antibodies were used to immunolocalize HA-tagged Mvp1p, it was observed that Vps1p and Mvp1p were both present on the accumulated prevacuolar compartment (Fig. 8D to F). The colocalization of Mvp1p and Vps1p in *vps1*^{d} and *vps27*Δ cells supports the notion that these two proteins may functionally interact to promote vacuolar protein sorting in yeast cells.

DISCUSSION

In this paper, we report that the yeast *VPS1* gene interacts with a novel gene, *MVP1*. Like *VPS1*, *MVP1* is a nonessential gene required for the efficient sorting of CPY and other soluble vacuolar proteins. *MVP1* encodes a 60-kDa hydrophilic protein with no sequence similarity to other proteins in the database.

The expression of a dominant-negative *vps1*^{d} allele in wild-type cells leads to the secretion of soluble vacuolar proteins such as CPY (56). When wild-type Mvp1p was overproduced in these cells, their ability to sort and deliver CPY to the vacuole was restored. In suppressed cells, all internal CPY was present in the mature form (15, 25, 58) and the kinetics of maturation was indistinguishable from that of wild-type cells. Thus, suppression of the *vps1*^{d} phenotype by overproduction of Mvp1p did not slow or halt the progression of vacuolar proteases through the secretory pathway. Instead, increased *MVP1* expression overcame the interference effects of the mutant Vps1p to restore proper sorting and delivery of CPY to the vacuole.

The model of *vps1*^{d} interference predicts that dominance is mediated through the carboxyl-terminal domain and that all of the *vps1*^{d} alleles disrupt sorting via the same mechanism (56). That is, in each case, the C terminus of the mutant Vps1p interacts nonproductively with a limiting but necessary component of the sorting machinery. *MVP1* was able to suppress all of the dominant *vps1* alleles for which suppression was tested, which is consistent with the notion of a common mechanism of disruption of vacuolar protein sorting. It is important to note that *MVP1* also suppressed the *vps1*-Δ*N* allele, which completely lacks the GTP-binding domain (amino acids 19 to 356). This observation suggests that Mvp1p may interact with the carboxyl-terminal domain of Vps1p.

The model also predicts that Mvp1p, when overexpressed in *VPS1*/*vps1*^{d} cells, overcomes the titration effect of the mutant Vps1p and associates in a productive interaction with wild-type Vps1p. Consistent with this prediction, *MVP1* overexpression did not create a sorting pathway for CPY that was independent of Vps1p or enable the mutant *vps1*^{d}-encoded proteins to regain function. *vps1*Δ cells secreted >95% CPY whether or not

Mvp1p was overproduced. Similarly, elevated levels of *MVP1* had no effect on the level of CPY secretion in cells that expressed only mutant *vps1*^{d}. Suppression, therefore, was absolutely dependent upon the presence of the wild-type *VPS1* gene product.

The association of Vps1p with Golgi membranes is likely to require a protein-protein interaction (39, 56). Mvp1p by itself is unlikely to serve as a membrane anchor for Vps1p. It possesses no hydrophobic domain or carboxyl-terminal motifs known to attach proteins to membranes via prenylation (43). Also, disruption of the *MVP1* gene had no effect on the cellular location of Vps1p (6). While no dependence on Mvp1p was observed for Vps1p localization, mutant Vps1p could affect the subcellular distribution of Mvp1p. In *vps1*^{d} cells, both Vps1p and Mvp1p were localized to one or two discrete patches adjacent to the vacuole. These patches were not seen for either protein in wild-type cells (39) (Fig. 7). Colocalization of Mvp1p and Vps1p in *vps1*^{d} cells suggests that these proteins normally interact and that mutant Vps1p stably associates with Mvp1p to form a nonproductive complex. However, the exact nature of the compartment or protein complex in which Mvp1p and mutant Vps1p are found is not known, and their colocalization in these structures may be indirect.

It was not possible to test for colocalization of Vps1p and Mvp1p in wild-type cells by indirect immunofluorescence because of the low Mvp1p signal. However, in class E *vps* mutants, which accumulate a prevacuolar, late-endosomal organelle (29a, 31), both Mvp1p and Vps1p were found to associate with this compartment. In the absence of definite markers, however, it is not possible at this time to determine whether either or both of these proteins are normally associated with the prevacuole in wild-type yeast cells. Nevertheless, their presence on this structure in *vps27*Δ cells suggests that these proteins do associate with organelles involved in the delivery of proteins to the vacuole (31) and that under certain conditions they associate with the same organelle. Previous indirect immunofluorescence experiments have shown that Vps1p is associated with Golgi membranes in *sec7*^{ts} cells (39). However, Golgi membrane association in *sec7*^{ts} cells does not preclude the possibility that Vps1p may also associate with a prevacuolar compartment. In wild-type yeast cells, Vps1p may associate with a late Golgi compartment or a prevacuolar compartment or it may cycle between the two.

There are several possible models for the role of Mvp1p in relation to Vps1p and vacuolar protein sorting. Genetic and immunolocalization data are consistent with the notion of Mvp1p and Vps1p engaging in a physical interaction, although the exact nature of that interaction is not known. Chemical cross-linking and coimmunoprecipitation studies have failed to reveal a direct interaction between Mvp1p and Vps1p. However, these negative results could be attributable to the very low abundance of Mvp1p or mean that Mvp1p and Vps1p interact only transiently or that they do not interact directly at all. Because overexpression of *VPS1* can partially bypass the need for *MVP1*, Mvp1p may not be strictly required for the sorting event but may be necessary for sorting to occur with high efficiency. In this role, Mvp1p may affect the role of Vps1p in GTP hydrolysis, GDP dissociation, or GDP-GTP exchange (28). GTP hydrolysis by the Vps1p homolog dynamin has been shown to be stimulated by microtubules and proteins containing SH3 domains (48) and is dependent on the extreme C-terminal, proline-rich region of dynamin where these proteins are predicted to bind (10).

Defining whether Mvp1p functions as a GTPase effector protein for Vps1p or whether it serves a different role awaits further study. It also remains to be seen if Mvp1p-like proteins that interact with dynamin in initiating endocytosis in mammalian cells will be found. Current efforts are under way to determine whether Mvp1p influences the GTPase cycle of Vps1p and to identify additional Vps1p-interacting proteins.

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