The yeast dynamin-related GTPase Vps1p functions in the organization of the actin cytoskeleton via interaction with Sla1p

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Abstract

Recent studies have suggested that the function of the large GTPase dynamin in endocytosis in mammalian cells may comprise a modulation of actin cytoskeleton. The role of dynamin in actin cytoskeleton organization in the yeast *Saccharomyces cerevisiae* has remained undefined. In this report, we found that one of the yeast dynamin-related proteins, Vps1p, is required for normal actin cytoskeleton organization. At both permissive and non-permissive temperatures, the vps1Δ mutants exhibited various degrees of phenotypes commonly associated with actin cytoskeleton defects: depolarized and aggregated actin structures, hypersensitivity to the actin cytoskeleton toxin latrunculin-A, randomized bud site selection and chitin deposition, and impaired efficiency in the internalization of membrane receptors. Over-expression of the GTPase mutants of *vps1Δ* also led to actin abnormalities. Consistent with these actin-related defects, Vps1p was found to interact physically, and partially co-localize, with the actin-regulatory protein Sla1p. The normal cellular localization of Sla1p required Vps1p and could be altered by over-expression of a region of Vps1p that was involved in the interaction with Sla1p. The same region also promoted mis-sorting of the vacuolar protein carboxypeptidase Y upon over-expression. These findings suggest that the functions of the dynamin-related protein Vps1p in actin cytoskeleton dynamics and vacuolar protein sorting are probably related to each other.

Key words: GTPase, Dynamin, Actin, Vps1p, Sla1p

Introduction

Dynamin and dynamin-related proteins are an evolutionally conserved family of large GTPases engaged in a diversity of cellular processes, including endocytosis, intracellular protein trafficking, and organelle partitioning (Hinshaw, 2000; Danino and Hinshaw, 2001). The role of dynamin in clathrin-mediated endocytosis is first suggested in the analysis of a temperature-sensitive mutant of dynamin in *Drosophila*. At the non-permissive temperature, the mutant, shibire, exhibits a paralytic phenotype due to a block in endocytosis at the presynaptic terminals (Kosaka and Ikeda, 1983). The usual accumulation of long invaginations at these membranes indicates a failure in vesicle detachment (Kosaka et al., 1983; Koenig and Ikeda, 1989). One conspicuous feature of dynamin is its ability of self-assemble into spiral-like structures around lipid tubules, which has led to the proposal that dynamin acts as a mechano-enzyme to release the clathrin-coated vesicles using its GTPase-dependent conformational changes (Sweitzer and Hinshaw, 1998; Stowell et al., 1999; Marks et al., 2001; McGavin et al., 2001; Song and Schmid, 2003).

Recent studies reveal that the function of dynamins in endocytosis might depend on their roles as actin cytoskeleton regulators. Actin cytoskeleton has been known for quite some time to be important for endocytosis in the yeast *Saccharomyces cerevisiae*. Yeast mutants with an abnormal or perturbed cortical actin cytoskeleton are often found to be defective in endocytosis (Munn, 2001). The evidence for actin cytoskeleton participating in endocytosis in mammalian cells has also been accumulating in recent years. Numerous studies have established the ability of dynamin to interact with various actin regulatory factors including profilin (Witke et al., 1998), and the actin-binding protein Abp1 (Kessels et al., 2001), as well as syndapin, intersectin, and cortactin, which link dynamin to the Wiskott Aldrich Syndrome protein (WASP) and the Arp2/3 complex, the major actin assembly promoters (Qualmann et al., 1999; McGavin et al., 2001; Schafer et al., 2002). Despite these findings, however, the exact function of dynamin in endocytosis remains unresolved.

In addition to conventional dynamins, there are other proteins from the dynamin family that share high homology with dynamins in their N-terminal GTPase domain but show less or no sequence conservation in other regions. These dynamin-related proteins are generally also found to have functions distinct from dynamins. For example, one of the better-studied dynamin-like proteins, DLP1, is known to be required for organelle morphology in mammalian cells (Shin et al., 1997; Yoon et al., 1998; Imoto et al., 1998; Kamimoto et al., 1998; Smirnova et al., 1998; Sever et al., 1999). So far, there is no documentation yet to suggest that these dynamin-like proteins have an actin-related function similar to the conventional dynamins.

There are three dynamin-like proteins in yeast that are structurally more related to DLP1 than to conventional...
dynamins. Among them, Dnm1p and Mgm1p are known to be involved in the mitochondria morphology and inheritance, a function similar to that of DLP1, whereas Vps1p is required for multiple cellular processes including intracellular protein trafficking and peroxisome inheritance (Rothman et al., 1990; Vater et al., 1992; Guan et al., 1993; Gammie et al., 1995; Bleazard et al., 1999; Shepard and Yaffe, 1999; Bensen et al., 2000; Hoepfner et al., 2001). It has been suggested that Vps1p participates in Golgi-derived membrane trafficking in a clathrin-mediated manner, and the clathrin heavy-chain subunit Chc1p may function cooperatively with Vps1p in the retention of some Golgi membrane proteins and the biogenesis of some Golgi-derived secretory vesicles (Payne and Schekman, 1989; Seeger and Payne, 1992; Wilsbach and Payne, 1993; Tan et al., 1993; Bensen et al., 2000; Gurunathan et al., 2002). Although none of the three yeast dynamin-related proteins is known to have any role in actin cytoskeleton, the chc1 mutant has been reported to be defective in cortical actin structures (Henry et al., 2002).

In this report, we demonstrate that the yeast dynamin-related GTPase Vps1p is required for normal organization of cortical actin cytoskeleton. Vps1p is also found to be able to interact with the actin regulatory protein Slalp. These findings reveal another role for Vps1p in the modulation of the actin cytoskeleton in yeast.

**Materials and Methods**

**Strains, constructs, media, and general methods**

The yeast strains used in this study are listed in Table 1. All gene disruptions and integrations were confirmed by PCR. The GTPase domain mutations of vps1 were generated by site-directed mutagenesis and confirmed by DNA sequencing. The plasmid constructs are listed in Table 2. The oligonucleotides used in this study were generated by site-directed mutagenesis and confirmed by DNA sequencing. The plasmid constructs are listed in Table 2. The oligonucleotides used in this study are listed in Table 3. General methods, such as medium preparation, cell culture, protein extraction, immunoprecipitation and immunoblotting of the epitope-tagged proteins, were performed according to the procedures described previously (Tang et al., 1997; Tang et al., 2000). The yeast two-hybrid interaction assays followed the instructions of the manufacture (Clontech, CA). Testing fragments were cloned into pGADT7 containing the HA tag or pGBK7 containing the Myc tag. The plasmids were then co-transformed into the strain SFY526 and expression of each fusion protein was verified by western blotting using either anti-HA or anti-Myc antibodies. CFP and YFP tags were amplified from the template plasmid pDH3 and pDH5 from Yeast Resource Center, University of Washington.

**Fluorescence microscopy procedures**

Yeast cells were cultured in appropriate media as mentioned in figure legends (see Table 1 for yeast strains). For actin and chitin staining, cells were fixed with 3.7% formaldehyde, and stained with rhodamine phalloidin (Molecular Probes), or calcofluor (Sigma), respectively, as described elsewhere (Pringle et al., 1989). The samples were examined under a Leica DMAXA microscope equipped with a Hamamatsu C4742 digital camera. The analyses of actin organization and the budding scar patterns followed previous studies (Drubin et al., 1993; Chant and Pringle, 1995). Actin depolarization was scored only in budded cells with small- and medium-sized buds. In cases where quantification was required, at least 200 cells were counted for each sample.

**Endocytosis assays and measurement of the Ste3p half-life**

The assay for the uptake of uracil was carried out as described elsewhere (Zeng et al., 2001). Data were compiled from at least two independent experiments. The turnover of Ste3p was examined as follows. For strains containing the GAL1-STE3-EGFP, cells were cultured to mid-log phase at 25°C in a dropout medium supplemented with 2% raffinose. The expression of Ste3-GFP was induced by addition of 2% galactose. After 90 minutes of induction at 25°C, aliquots of cultures were shifted to 30°C or 37°C while glucose was added to the medium (to 3%) to repress the expression of Ste3p. At indicated time intervals after glucose addition, about 25 OD$_{600}$ units of cells were harvested, washed, and resuspended in 400 μl of ice-cold extraction buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, 5 mM EDTA) and lysed by vortexing with glass beads. The suspension was centrifuged briefly to remove cell debris and centrifuged again at a high speed (14,000 g) for 45 minutes. The pellet was resuspended in

**Table 1. Yeast strains**

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Relevant genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303-1-A</td>
<td>MATα ade2-1 trpl-1 can1-100 leu2-3,112 his3-11,15 ura3-52</td>
</tr>
<tr>
<td>W303-1-B</td>
<td>MATα ade2-1 trpl-1 can1-100 leu2-3,112 his3-11,15 ura3-52</td>
</tr>
<tr>
<td>YMC450</td>
<td>MATα sla1Δ::HIS3</td>
</tr>
<tr>
<td>YMC451</td>
<td>MATα vps1Δ::HIS3</td>
</tr>
<tr>
<td>YMC452</td>
<td>MATα dnm1Δ::LEU2</td>
</tr>
<tr>
<td>YMC453</td>
<td>MATα mgm1Δ::TRP1</td>
</tr>
<tr>
<td>YMC454</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC455</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC456</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC457</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC458</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC459</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC460</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC461</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC462</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC463</td>
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<tr>
<td>YMC464</td>
<td>MATα vps26Δ::HIS3</td>
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<tr>
<td>YMC465</td>
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</tr>
<tr>
<td>YMC466</td>
<td>MATα vps26Δ::HIS3</td>
</tr>
<tr>
<td>YMC467</td>
<td>MATα sla1Δ::HIS3</td>
</tr>
<tr>
<td>YMC468</td>
<td>MATα sla1Δ::HIS3</td>
</tr>
<tr>
<td>YMC469</td>
<td>MATα sla1Δ::HIS3</td>
</tr>
<tr>
<td>YMC470</td>
<td>MATα sla1Δ::HIS3</td>
</tr>
<tr>
<td>SFY526</td>
<td>MATα ura3-52 his3-200 ade2-101 lys2-801 trpl-901 leu23,112</td>
</tr>
</tbody>
</table>
100 µl of loading buffer and heated at 37°C for 10 minutes before loading onto an SDS gel. Ste3-EGFP was detected by a monoclonal anti-GFP antibody (Clontech, CA).

Halo assays for latrunculin-A sensitivity

The latrunculin-A (LAT-A) sensitivity was measured by the halo assay following the published procedures (Ayscough et al., 1997) with minor modifications. Cells were cultured overnight in appropriate media. The amounts of the cells from each culture were normalized by OD readings. We took 10 µl of these cells and added them to 2 ml of 2x relevant medium, followed by mixing with 2 ml of molten agar (1%, pre-warmed to 55°C). The cell suspension was quickly poured onto the surface of a plate containing the same medium. LAT-A (Molecular Probes) was diluted with H2O, and 5 µl of each dilution of the drug was pipetted onto the center of a sterile 6 mm filter disk. The disks were then placed on top of the agar. The plates were inverted and left at 30°C until halos were clearly visible. Relative apparent sensitivities were calculated as described before (Reneke et al., 1988).

Table 2. Plasmid constructs

<table>
<thead>
<tr>
<th>Name of the plasmid</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMC386</td>
<td>FUR4 in URA3, 2µ vector (pYEp352).</td>
</tr>
<tr>
<td>pTHY918</td>
<td>Sla1 in URA3, CEN vector (pRS316).</td>
</tr>
<tr>
<td>pTHY1089</td>
<td>Sla1 in TRP1, CEN vector (pRS314).</td>
</tr>
<tr>
<td>pTHY1108</td>
<td>HA-SLA1 in TRP1, CEN vector (pRS314).</td>
</tr>
<tr>
<td>pTHY1114</td>
<td>Sla1Δ140-355 (slalΔ1-410) in pRS314; slal deleted of three SH3 domains and expressed from its native promoter; generated by PCR.</td>
</tr>
<tr>
<td>pTHY1115</td>
<td>Sla1Δ140 (slalΔ1-855-1244) in pRS314; slal without the C-terminal Sla1p repeats expressed from its native promoter; generated by PCR.</td>
</tr>
<tr>
<td>pYGS203</td>
<td>Sla1Δ147-510 in pRS314; the sequence between the two EcoRI sites in pTHY1089 containing the third SH3 of Sla1p was removed.</td>
</tr>
<tr>
<td>pSLA1C-GFP</td>
<td>The DNA coding region for Sla1p (amino acids 1105-1244) generated by PCR and cloned inframe with a C-terminal GFP epitope, followed by the ADH1 terminator in pRS304.</td>
</tr>
<tr>
<td>pSLA1C-YFP</td>
<td>The GFP tag within pSLA1C-GFP was replaced with YFP epitope and the resulting construct was shifted into vector pRS305 again by cutting with PswI enzyme.</td>
</tr>
<tr>
<td>pGAL1-STE3-EGFP</td>
<td>STE3 coding region generated by PCR, cloned inframe with a C-terminal EGFP epitope followed by the ADH1 terminator, and placed under GAL1 promoter control in pRS316.</td>
</tr>
<tr>
<td>pGAL1-myc-VPS1</td>
<td>The VPS1 coding region isolated by PCR, fused inframe with the N-terminal Myc epitope, and placed under the GAL1 promoter in vector pRS306; the construct can be linearized by SstI in the URA3 coding region for integration.</td>
</tr>
<tr>
<td>pGAL1-myc-VPS1K42E</td>
<td>VPS1 with K42E mutation generated by PCR, replacing the wild-type sequence in pGAL1-myc-VPS1.</td>
</tr>
<tr>
<td>pGAL1-myc-VPS1S43N</td>
<td>VPS1 with S43N mutation generated by PCR, replacing the wild-type sequence in pGAL1-myc-VPS1.</td>
</tr>
<tr>
<td>pGAL1-myc-VPS1G315D</td>
<td>VPS1 with G315D mutation generated by PCR, replacing the wild-type sequence in pGAL1-myc-VPS1.</td>
</tr>
<tr>
<td>pVPS1-3myc</td>
<td>VPS1 coding region together with its 1000 bp promoter and 500 bp terminator amplified by PCR against yeast genomic DNA; the region from the stop codon of VPS1 to the end of its terminator was eliminated by replacing with an inframe fragment containing three repeats of the Myc epitope followed by the ADH1 terminator; the construct was cloned in vector pRS303 and could be linearized by NsiI digestion in the HIS3 coding region for integration.</td>
</tr>
<tr>
<td>pVPS1K42E-3myc</td>
<td>VPS1 with K42E mutation, replacing the corresponding sequence in pVPS1-3myc.</td>
</tr>
<tr>
<td>pVPS1S43N-3myc</td>
<td>VPS1 with S43N mutation, replacing the corresponding sequence in pVPS1-3myc.</td>
</tr>
<tr>
<td>pVPS1G315D-3myc</td>
<td>VPS1 with G315D mutation, replacing the corresponding sequence in pVPS1-3myc.</td>
</tr>
<tr>
<td>pGAL1-VPS1-C1</td>
<td>Gal-CFP-Vps1p (amino acids 276-704); the C-terminus of VPS1 made with primers VPS1-Y10 and VPS1-Y12 (names of oligonucleotides, see Table 3), fused inframe with the N-terminal CFP epitope, and placed under the GAL1 promoter in vector pRS306.</td>
</tr>
<tr>
<td>pGAL1-VPS1-C2</td>
<td>Gal-CFP-Vps1p (amino acids 276-616); the central domain of VPS1 was made with primers VPS1-Y10 and VPS1-Y15 (Table 3), and cloned into the same vector as that of pGAL1-VPS1-C1.</td>
</tr>
<tr>
<td>pGAL1-VPS1-C3</td>
<td>Gal-CFP-Vps1p (amino acids 357-704); another shorter C-terminus of VPS1 was made with primers VPS1-Y17 and VPS1-Y12 (Table 3) and fused into the same vector as that of pGAL1-VPS1-C1.</td>
</tr>
<tr>
<td>pGAL1-VPS1-C4</td>
<td>Gal-CFP-Vps1p (amino acids 614-704); the GED domain of VPS1 was made with primers VPS1-Y14 and VPS1-Y12 (Table 3), and ligated into the same vector as that of pGAL1-VPS1-C1.</td>
</tr>
<tr>
<td>pGAL1-VPS1-N1</td>
<td>Gal-CFP-Vps1p (amino acids 1-355); the N-terminal GTPase domain of VPS1 was made with primers VPS1-Y9 and VPS1-Y13 (Table 3), and cloned into the same vector as that of pGAL1-VPS1-C1.</td>
</tr>
<tr>
<td>pGAL1-VPS1</td>
<td>Gal-CFP-Vps1p (amino acids 1-704); full length VPS1 amplified by primers VPS1-Y9 and VPS1-Y12 (Table 3), and cloned into the same vector as that of pGAL1-VPS1-C1.</td>
</tr>
<tr>
<td>pGBK7-VPS1-N1</td>
<td>Asf1 (blunted) and PstI digested fragment from pGAL1-VPS1 (amino acids 1-704) cloned into pGBK7 between sites Smal and PstI.</td>
</tr>
<tr>
<td>pGBK7-VPS1-C2</td>
<td>pGBK7-VPS1-N1 (amino acids 1-355); derived from pGAL1-VPS1-N1 in the same way as pGBK7-VPS1-N1.</td>
</tr>
<tr>
<td>pGBK7-VPS1-C3</td>
<td>pGBK7-VPS1-N1 (amino acids 276-616); derived from pGAL1-VPS1-C2 in the same way as pGBK7-VPS1-C2.</td>
</tr>
<tr>
<td>pGBK7-VPS1-C4</td>
<td>pGBK7-VPS1-N1 (amino acids 357-704); derived from pGAL1-VPS1-C3 in the same way as pGBK7-VPS1-C3.</td>
</tr>
<tr>
<td>pGBK7-VPS1-C4</td>
<td>pGBK7-VPS1-N1 (amino acids 614-704); derived from pGAL1-VPS1-C4 in the same way as pGBK7-VPS1-C4.</td>
</tr>
<tr>
<td>pGBK7-VPS1</td>
<td>pGBK7-VPS1-C4 (amino acids 516-704); NarI and PstI digested fragment from pGBK7-VPS1 cloned into pGBK7 between sites Smal and PstI.</td>
</tr>
<tr>
<td>pGADT7-SLA1</td>
<td>The DNA coding region of Sla1p (amino acids 1-1244) generated by PCR and cloned into pGADT7.</td>
</tr>
</tbody>
</table>

Colony overlay immunoblot

Secretion of the CPY was studied using a colony overlay blot assay as described (Wilsbach et al., 1993). The vps1 and wild-type strains carrying a plasmid containing various domains of VPS1 were grown in standard yeast extract-peptone-dextrose (YPD) or appropriate selective media to saturation at 24°C. Cells were diluted to 1x10⁶ cells/ml, and 5 µl of diluted cells were spotted onto appropriate plates, overlaid with a nitrocellulose filter, and incubated overnight at 30°C. The filters were removed and rinsed with distilled water and probed with a monoclonal antibody against carboxypeptidase Y (CPY) (Molecular Probes).

Results

The yeast dynamin Vps1p is required for normal actin cytoskeleton organization and chitin deposition

We were attracted to the idea that Vps1p might have an actin-related function by the recent findings that the clathrin heavy
chain (Chc1p), the presumptive functional partner of Vps1p in vesicle transport, is required for normal actin organization in yeast (Henry et al., 2002), and that Scd5p, a high copy suppressor of the chc1 deletion mutant (chc1Δ), localizes to cortical actin patches and is essential for actin organization and endocytosis (Henry et al., 2002). To find out whether Vps1p is required for normal actin structures, a vps1Δ mutant was generated and analyzed with phalloidin staining. Deletion of the VPS1 gene caused a temperature-sensitive phenotype, as had been reported before (Ekenda and Stevens, 1995). In the vps1Δ cells growing at 30°C, the actin cytoskeleton appeared to be largely normal in morphology but was evidently

![Figure 1](3843.png)

*Fig. 1.* Vps1p is required for normal actin cytoskeleton organization. (A) Actin cytoskeleton in several yeast mutants at different temperatures. Strains (YMC451) vps1Δ (YMC452) dnm1Δ (YMC453) mgm1Δ (YMC454) vps26Δ and wild-type W303-1-B (W303) cultured to mid-log phase in YEPD at 24°C were shifted to 30°C or 37°C for 3 hours before being subjected to actin staining (bar, 5 μm). (B) Quantitative illustration of the populations of vps1Δ and W303 with actin abnormalities. Actin depolarization shown in the left panel was calculated only in budded cells with small- and medium-sized buds, and the actin aggregation shown in the right panel was based on the total cell population. (C) The abnormal budding pattern and chitin deposition in vps1Δ cells. Strains YMC456 (vps1Δ, diploid) and the wild type YMC455 (W303, diploid) were grown in YEPD to log phase at 24°C. Aliquots of the cultures were then shifted to either 30°C or 37°C for 3 hours before being subjected to Calcofluor staining. The budding patterns and chitin distributions of cells incubated at different temperatures were scored and shown in Table 4 (bar, 5 μm). (D) Prolonged half-life of Ste3p in vps1Δ cells. YMC451 (vps1Δ, diploid) and the wild-type YMC455 (W303, diploid) were grown in YEPD to log phase at 24°C. Aliquots of the cultures were then shifted to either 30°C or 37°C for 3 hours before being subjected to Calcofluor staining. The budding patterns and chitin distributions of cells incubated at different temperatures were scored and shown in Table 4 (bar, 5 μm). (D) Prolonged half-life of Ste3p in vps1Δ cells. YMC451 (vps1Δ, diploid) and the wild-type YMC455 (W303, diploid) were grown in YEPD to log phase at 24°C. Aliquots of the cultures were then shifted to either 30°C or 37°C for 3 hours before being subjected to Calcofluor staining. The budding patterns and chitin distributions of cells incubated at different temperatures were scored and shown in Table 4 (bar, 5 μm). (D) Prolonged half-life of Ste3p in vps1Δ cells. YMC451 (vps1Δ, diploid) and the wild-type YMC455 (W303, diploid) were grown in YEPD to log phase at 24°C. Aliquots of the cultures were then shifted to either 30°C or 37°C for 3 hours before being subjected to Calcofluor staining. The budding patterns and chitin distributions of cells incubated at different temperatures were scored and shown in Table 4 (bar, 5 μm).
forms a bud, a ring of chitin is deposited in the cell wall at the select bud site according to genetically determined spatial patterns: axial mainly in haploid cells and bipolar mainly in diploid cells. Wild-type yeast cells organization are often accompanied by abnormal budding sorting. Cells could be attributed to deficiencies of the mutant in protein is therefore unlikely that the actin abnormalities in the aberrant cellular organelles, rather than from a loss of a direct role of Vps1p in actin cytoskeleton organization. It may perhaps be argued that the abnormal actin cytoskeleton organization in vps1 cells was probably resulted from other defects of the mutant, such as protein sorting or aberrant cellular organelles, rather than from a loss of a direct role of Vps1p in actin cytoskeleton organization. One way to investigate this possibility is to examine other mutants of other two dynamin-related genes, dnm1Δ and mgm1Δ, as well as the wild-type cells, generally maintained their normal cortical actin morphology and distribution patterns under the same condition (Fig. 1A and B). These results suggest that Vps1p is indeed required for normal organization of actin cytoskeleton in yeast as we speculated. The turn-over of the membrane receptor protein Ste3p is impaired in vps1Δ As the integrity of actin cytoskeleton is required for endocytosis in yeast, we tested the vps1Δ mutant for its ability to carry out membrane receptor endocytosis. It is known that the a factor receptor, Ste3p, is constantly internalized in the absence of its ligand through the endocytic pathway and delivered to the vacuole for degradation without being recycled back to the cell membrane (Davis et al., 1993; Chen and Davis, 2000). The stability of the full-length Ste3p is indicative of the efficiency by which Ste3p is being endocytosed. At 37°C, most if not all of Ste3-GFP in wild-type cells was degraded within 60 minutes after the Ste3p expression was turned off (Fig. 1D). However, in vps1Δ cells, Ste3-GFP was turned over more slowly, and a substantial amount of the protein (about 70%) remained visible. This suggests that the actin cytoskeleton organization in vps1Δ cells was probably resulted from other defects of the mutant, such as protein sorting or aberrant cellular organelles, rather than from a loss of a direct role of Vps1p in actin cytoskeleton organization. One way to investigate this possibility is to examine other mutants of other two dynamin-related genes, dnm1Δ and mgm1Δ, as well as the wild-type cells, generally maintained their normal cortical actin morphology and distribution patterns under the same condition (Fig. 1A and B). 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remained after 90 minutes at 37°C, suggesting that the internalization of Ste3-GFP from the cell surface to the vacuole was impaired in the vps1 deletion strain. This result is in agreement with the above finding that the vps1 mutant suffered from severe actin defects at the non-permissive temperature.

The putative GTPase mutants of VPS1 exhibit similar defects as the null mutant
Among the three yeast dynamin-related proteins, Vps1p and Dnm1p are more related to mammalian dynamin than Mgm1p (Fig. 2A). The sequence similarity resides mainly in the GTPase domain, and, to a lesser degree, the GED domain (Fig. 2A). In mammalian cells, it has been shown that dynamin mutants deficient in GTP hydrolysis, such as K44E, S45N and G273D, are unable to internalize transferrin (Herskovits et al., 1993; Damke et al., 1994; Damke et al., 1995; Marks et al., 2001). As these key amino acid residues are also conserved in Vps1p, mutations with same alterations were generated in VPS1 and the resulting mutants, vps1K42E, vps1S43N and vps1G315D, were tagged with the Myc-epitope, expressed under its native promoter, and introduced into vps1Δ by integration. Western blot analysis confirmed that they were all expressed to a level similar to their wild-type counterpart (data not shown). All three mutants were temperature-sensitive when grown at 37°C (Fig. 2B).

We first examined the sensitivity of these mutants to the LAT-A, a drug that inhibits actin assembly by binding and

![Fig. 2.](image)

Fig. 2. The putative GTPase mutants of vps1 affect actin cytoskeleton. (A) Alignment of the GTPase domain and the GED domain from mammalian dynamin-1 with three yeast dynamin-related proteins. Numbers on the top indicate the amino acid positions of Vps1p. Underlined in the GTPase domain are the residues that were mutated. (B) Temperature sensitivities of the vps1Δ cells containing various GTPase mutants of vps1. Strains YMC457 (vector/vps1Δ), YMC458 (vps1WT/vps1Δ), YMC459 (vps1K42E/vps1Δ), YMC460 (vps1S43N/vps1Δ) and YMC461 (vps1G315D/vps1Δ) cultured at 24°C were first diluted to similar density from which further serial dilutions were made. We spotted 5 μl of each dilution onto the YEPD plates and incubated at indicated temperature for one day. (C) LAT-A sensitivity of the vps1Δ and GTPase mutants. Cells shown in (B) were cultured in YEPD at 30°C and subjected to LAT-A halo assay as described in Materials and Methods. The bar chart in the right shows the quantified result. (D) The uracil-uptake assays in the YMC458 (VPS1) (●), YMC457 (vps1Δ) (■) and YMC459-461 (vps1K42E, vps1S43N and vps1G315D) (▲, ■, ◆) at both 30°C (left) and 37°C (right).
Vps1p in actin cytoskeleton organization

sequestering actin monomers (Coue et al., 1987). The sensitivity to LAT-A has been regarded as an indicator of the actin cytoskeleton integrity (Ayscough et al., 1997). Consistent with its actin structure abnormalities, the \textit{vps1} null mutant was about twofold more sensitive to LAT-A than the wild-type cell (the same cell containing \textit{VPS1}), as shown in Fig. 2C. The three GTPase mutants of \textit{VPS1} all exhibited a similar level of sensitivity as the \textit{vps1A} mutant in this assay (Fig. 2C). This result suggests that the loss of \textit{VPS1} led to the instability of filamentous actin structures in vivo, and the GTPase activity of Vps1p is probably essential for its role in actin cytoskeleton organization.

These GTPase mutants were examined for their proficiency in endocytosis using the uracil uptake assay. The uracil permease, encoded by the \textit{FUR4} gene, is responsible for uptake of uracil in yeast. When protein synthesis is inhibited, Fur4p localized on the plasma membrane is rapidly internalized through the endocytotic machinery, resulting in impaired uracil uptake (Volland et al., 1994). The \textit{vps1A} mutant, as well as the three GTPase mutants, exhibited essentially a same kinetics of uracil uptake as the wild type at 30°C (Fig. 2D, left). At 37°C, conversely, the uracil transport activity in all four mutants persisted and remained at about 40% at a time when no activity was detected in wild-type cells (Fig. 2D, right). These results showed that the GTPase mutants and the deletion mutant of \textit{VPS1} were similarly deficient in the internalization of membrane proteins at the non-permissive temperature.

Over-expression of the GTPase mutants of \textit{VPS1} leads to actin defects and cell death at 37°C

The GTPase mutants of dynamin have been reported to exert a dominant-negative effect on endocytosis and actin dynamics when over-expressed in mammalian cells. For example, the dynamin 2 mutant K44A, defective in GTP binding and hydrolysis, inhibits actin dynamics upon over-expression (Schafer et al., 2002; Orth et al., 2002). To test whether over-expression of the putative GTPase mutants of \textit{VPS1} could similarly exert a negative effect on actin cytoskeleton in yeast, the Mhc-tagged \textit{VPS1}, \textit{vps1K42E}, \textit{vps1S43N} and \textit{vps1G315D} were each placed under the control of the \textit{GAL1} promoter and integrated into wild-type cells. Western blotting confirmed that all four proteins were expressed to similar levels upon induction (data not shown). These cells were first examined for their actin structures. After 4 hours of induction in galactose at 30°C, all three GTPase mutants, \textit{vps1K42E}, \textit{vps1S43N} and \textit{vps1G315D}, incited obvious actin depolarization and aggregation (Fig. 3A). Over-expression of the wild-type \textit{VPS1}, conversely, had little or no effect (Fig. 3A). Quantitative analysis showed that only about 20% of cells

![Fig. 3. The actin defects caused by over-expression of the GTPase domain mutants of vpsl.](image-url)
were able to maintain a normal actin-patch morphology and distribution in each of the three strains over-producing the GTPase mutants. Over-expression of the three GTPase mutants also caused lethality to the host cells at 37°C, as shown in Fig. 3B. Consistent with the abnormal actin phenotypes, over-expression of these GTPase mutants also resulted in hypersensitivity to LAT-A (Fig. 3C). These findings suggest that, similar to the dynamin mutants of mammalian cells, the GTPase mutants of vps1 also affect stability of filamentous actin structures upon over-expression in yeast cells.

Vps1p interacts with the actin regulatory protein Sla1p
A number of Src-homology-3 (SH3) domain-containing proteins, such as amphiphysin, endophilin, syndapin and intersectin, are known to interact with dynamin in mammalian cells (David et al., 1996). Intersectin is particularly interesting as it contains five SH3 domains and two Eps15 homology (EH) domains, structurally reminiscent of the yeast Pan1p-Sla1p complex identified in our laboratory to be required for actin cytoskeleton organization and endocytosis (Tang et al., 1997). It was thus speculated that the Pan1p-Sla1p complex could be a linkage between Vps1p and the actin regulatory machinery and the possible interaction between Vps1p and Sla1p, an SH3 domain-containing protein, was investigated. We first tested if there was any genetic interaction between sla1 and vps1 mutations. As shown in Fig. 4A, the sla1Δ vps1Δ double disruption was lethal, whereas sla1Δ dnm1Δ and sla1Δ mgm1Δ were not. Further analysis revealed that the third SH3 domain and the C-terminal repeats of Sla1p, two regions in Sla1p that are known to be required for its function in actin cytoskeleton organization (Ayscough et al., 1999), were essential for supporting the viability of the vps1 mutant (Fig. 4B and C). We next tested whether the Vps1p and Sla1p could physically interact with each other using the co-immunoprecipitation assay. Protein extracts from wild-type cells containing pGal1-myc-VPS1 and/or single copy of HA-tagged SLA1 on a separate vector were subjected to immunoprecipitation by the anti-HA antibody. HA-Sla1p migrated on the SDS-PAGE as a band with a molecular mass of 150 kDa. When the anti-HA immunoprecipitates were probed with the anti-Myc antibody, the 80 kDa Myc-Vps1p was readily detected (Fig. 5A, lane 5). The Myc-Vps1p was not found in the control sample made from the cell lysate containing no HA-SLA1 (Fig. 5A, lane 6). This showed that Vps1p and Sla1p could indeed associate with each other in vivo.

The Vps1p-Sla1p complex, however, was only weakly detectable if Vps1p was not over-produced from the GAL1 promoter (Fig. 5B, lane 10). We wondered whether this was partly because the interaction between Vps1p and Sla1p was a highly transient event in vivo. If so, it might be possible to increase the probability of the complex formation using the putative GTPase mutants described above, as the rate of dynamic exchange between different forms of Vps1p had to be decreased by these mutations. To test this hypothesis, the co-immunoprecipitation experiments were performed again using the HA-tagged Sla1p and different Myc-tagged GTPase mutants of Vps1p, each expressed from its native promoter. The expression levels of these proteins were similar to their wild-type counterpart (Fig. 5B). Indeed, the various Vps1p mutant proteins now became more readily detectable in the HA-Sla1p immuno-precipitates without having to be over-expressed. Notably, the Vps1pG315D mutant, which corresponded to the original mutation in shibire (Baba et al., 1999), interacted with Sla1p more strongly than the other two mutants (Fig. 5B, lane 16).

Alteration of cellular localization of Sla1p by vps1 mutations
Sla1p interacts with several actin regulatory proteins and co-localizes with cortical actin patches in wild-type cells (Tang et al., 1997; Tang et al., 2000; Zeng et al., 2001; Danino et al., 2001; Howard et al., 2002; Warren et al., 2002; Gourlay et al., 2003). The localization at the cortical actin patches is important for the function of Sla1p in actin organization and

Fig. 4. Synthetic lethality between vps1Δ and alleles of sla1 with actin defects. (A) The viabilities of various mutants after their SLA1-containing plasmid pTHY918 was lost on a 5-FOA plate: strains YMC467 (sla1Δ), YMC468 (sla1Δ mgm1Δ), YMC469 (sla1Δ dnm1Δ) and YMC470 (sla1Δ vps1Δ) grown on SC-Ura plate (left) at 24°C were replica-plated onto a 5-FOA plate (right) and incubated at 24°C for 2 days. (B) The schematic structures of Sla1p and its deletion constructs to be used in the following experiments. (C) The test of the strain YMC470 (sla1Δ vps1Δ) to lose the SLA1-containing plasmid pTHY918 in the presence of various sla1 deletion constructs: pRS514 (vector, lane 1), pYGS203 (sla1Δ dnm1SH3, lane 2), pTHY1114 (sla1Δ vps1Δ, lane 3), pTHY1115 (sla1Δ mgm1Δ, lane 4) and pTHY1089 (sla1Δ, lane 5).
endocytosis (Warren et al., 2002; Gourlay et al., 2003). In light of our new finding that Sla1p and Vps1p could form a complex in vivo, we investigated whether Vps1p could affect the normal subcellular localization of Sla1p. The SLA1 gene in vps1Δ and the three GTPase mutants described above were tagged with GFP by integration. The exponentially growing cells of these strains at 30°C were examined for actin structures and Sla1p-GFP localization. In wild-type cells, most of the Sla1p signals were localized to cortical actin patches that were well polarized in small- and medium-size-budded cells (Fig. 6A, Vps1WT). In all four vps1Δ mutants, however, actin and Sla1p signals were both disturbed. The cell-cortex-associated Sla1p was evidently diminished and most of the signals were not concentrated at the site of polarized growth (Fig. 6A). While the actin structures and the distribution patterns became abnormal, as described earlier, the co-localization between actin and Sla1p was no longer obvious in these mutants (Fig. 6A). Based on these findings, it is concluded that the normal cellular location of Sla1p depends on the proper function of Vps1p.

**Overexpression of the C-terminal region of Vps1p leads to detrimental effects**

The N-terminal region of Vps1p is the GTPase domain and the C-terminal region is the GED domain based on its sequence homology with the mammalian dynamin (Fig. 7A). Both domains are required for the function of Vps1p in vacuolar protein sorting (Vater et al., 1992). Using the two-hybrid assay system, the regions within Vps1p that are involved in the interaction with Sla1p were mapped. As shown in Table 5, Vps1p interacted with Sla1p through a C-terminal region comprising the GED domain (amino acids 357-704 and amino acids 566-704). The N-terminal GTPase domain of Vps1p (amino acids 1-355) also showed a positive, albeit weaker, interaction (Table 5).

To analyze the importance of the C-terminal region of Vps1p, we first examined whether the ectopic expression of the C-
terminal region could cause any actin defects. Various regions of Vps1p (Fig. 7A) were tagged with CFP and placed under the inducible promoter pGAL1. All constructs gave rise to similar expression levels in vivo when induced by galactose (data not shown). As shown in Fig. 7B, expression of a region containing the central and the C-terminal parts of Vps1p (amino acids 276-704) resulted in temperature sensitivity at 37°C. Deletion of the most-C-terminal GED domain from this construct mitigated this growth inhibitory effect (Fig. 7B, amino acids 276-616). Thus, the GED domain was at least part of the cause for the cell death upon over-expression at 37°C. Conversely, the GED domain alone was not sufficient (Fig. 7B, amino acids 614-704), and it had to be combined with some 200 amino acids of the upstream sequence to result in a cell growth defect (Fig. 7B, amino acids 357-704). In comparison, over-expression of either the GTPase domain or the full-length protein did not cause a temperature sensitive phenotype (Fig. 7B).

The cellular localization of various regions of Vps1p was also examined in parallel with that of Sla1p. Consistent with the finding that Vps1p and Sla1p could be immunoprecipitated as a complex, the full-length Vps1p (amino acids 1-704) was found to be partially co-localized with Sla1p after 4 hours of induction by galactose at 30°C (Fig. 7C, row 6). Under the same condition, the two GED-domain-containing constructs that caused temperature sensitivity (amino acids 276-704 and amino acids 357-704) both gave rise to more irregular or aggregated Vps1p signals that remained largely overlapped with the abnormally accumulated Sla1p (Fig. 7C, rows 1 and 4). Cells over-expressing the central region without the GED domain (amino acids 276-616) only showed one or two dots of the Vps1p signal in the cytosol, which did not alter the normal localization of Sla1p (Fig. 7C, row 2). Furthermore, over-expression of the N-terminal-most GTPase domain (amino acids 1-355) or the very C-terminal GED domain (amino acids 614-704), both had no cell growth inhibitory effect, yielded no discrete Vps1p signals at all (Fig. 7C, rows 3 and 5). These patterns of domain localization indicated that the GED domain along with some upstream sequence in the central region of Vps1p is indispensable for the protein’s co-localization with Sla1p. This is consistent with results from the two-hybrid interaction demonstrating this region as being involved in the interaction with Sla1p.

As the proper localization of Sla1p is important for actin cytoskeleton organization, it was anticipated that the over-expression of the two GED domain containing constructs (amino acids 276-704 and amino acids 357-704) that caused cell lethality at 37°C and mislocalization of Sla1p will also compromise the function of the actin cytoskeleton. This was ascertained by testing the LAT-A sensitivity of these cells. As shown in Fig. 7D, over-expression of these two constructs indeed led to a hypersensitivity to LAT-A (Fig. 7D, 4 and 7).

Correlation between the defects in actin organization and vacuolar protein sorting in vps1 mutants
We have shown that Vps1p is required for actin cytoskeleton organization in yeast. As Vps1p is well known for its functions in the protein transport process at the trans-Golgi network (TGN), it is important to find out whether the roles of Vps1p in vesicle transport and actin cytoskeleton are connected. In an initial attempt to address this question, we compared the ability of the various vps1 mutants (used in above) to perform vacuolar protein transport. Vacuolar protein transport in yeast can be assayed by monitoring the traverse of a vacuolar protein, carboxypeptidase Y (CPY), which is first synthesized and glycosylated in the endoplasmic reticulum (ER). Upon translocation to TGN, CPY acquires further glycosylation before travelling from Golgi to vacuole through the endosomal systems (Stevens et al., 1982; Howard et al., 2002; Gourlay et al., 2003). The vps1 mutant is known to be defective in clathrin-mediated vesicle formation at TGN. As a result, CPY in vps1 cells is mis-sorted and secreted into extracellular fractions that can be detected by anti-CPY antibody in a simple colony blot assay (Vater et al., 1992). Using this assay, we obtained several interesting findings. First, we found that the GTPase activity of Vps1p was required for the normal sorting of CPY. As shown in Fig. 8, none of the GTPase mutants of vps1 expressed under its native promoter was able to correct the CPY mis-sorting defect in the vps1Δ cells (Fig. 8A). Second, over-expression of the vps1 GTPase mutants also promoted CPY secretion in wild-type cells (Fig. 8B), once again demonstrating the dominant-negative effects of these mutants. Third, over-expression of some truncated forms of Vps1p also led to mis-sorting of CPY in wild-type cells (Fig. 8C). Significantly, the two Vps1p truncation constructs that caused temperature sensitivity and the hypersensitivity to LAT-A upon over-expression, amino acids 276-704 and amino acids 357-704, were also the only two constructs that promoted CPY secretion in wild-type cells in this experiment (Fig. 8C, 4 and 6). It is worth pointing out that the results of the above experiment cannot be attributed to cell lyses as these mutants maintained their viability under the assay conditions (data not shown). These results demonstrated that the function of Vps1p in vesicle protein sorting might be associated with its role in actin cytoskeleton organization, and possibly involves the interaction with Sla1p.

Discussion
Modulation of actin cytoskeleton is now appreciated to be a critical aspect of dynamin’s function in endocytosis. In yeast, the issue of whether a dynamin-like factor acts to link actin cytoskeleton to vesicle formation has been left unresolved. The recent evidence for clathrin to be required for actin cytoskeleton organization in yeast prompted us to carry out this study to investigate the possibility that Vps1p, a yeast dynamin-like protein that functions cooperatively with clathrin in vesicle formation at TGN, might also have a role in actin cytoskeleton organization. The analysis of various vps1 mutants confirmed our speculation.

Vps1p is required for normal actin organization in yeast
So far, studies of Vps1p have been mainly focused on its role in protein trafficking at TGN. Here we demonstrate for the first time that Vps1p also affects the organization of cortical actin patches. Even though the defects of actin cytoskeleton organization in the vps1 mutants were more severe at the non-permissive temperature, the majority of the mutant cells already exhibited a phenotype of actin patch depolarization at a lower temperature of 30°C. It has been shown previously that the actin depolarization is a specific phenotype and cannot be
Fig. 7. The effects of over-expression of the vps1 C-terminal region. (A) The structures of Vps1p and its deletion constructs used in the experiments below. Numbers on the top indicate the amino acid positions. (B) Over-expressions of the C-terminal region of Vps1p caused cell death at 37°C. Wild-type cells containing various deletion constructs of VPS1 (pGALI-VPS1-C1–C4, pGALI-VPS1-N1), as well as the full length VPS1 (pGALI-VPS1) were grown on glucose containing medium and replicated to galactose plates and incubated at 37°C for one day. (C) The localization of Sla1p was affected by the over-expression of the C-terminal regions of Vps1p. The endogenous copy of SLA1 in wild-type cells was tagged with YFP tag at its C-terminus and the resulting integrant was transformed with various deletion constructs of VPS1 indicated in A. The resulting transformants were cultured in raffinose-containing media to the mid-log phase before galactose was added and incubated for another 3 hours at 30°C. The localization of the various domains of Vps1p along with that of Sla1p was examined under Leica microscopy. Note the partial co-localization of Sla1p with Vps1p and its domains in 1, 4 and 6 (bar, 5 μm). (D) The strains as in (B) were analyzed for their sensitivities to LAT-A. The cells were plated on the galactose-containing medium at 30°C and the LAT-A with the indicated concentrations was applied (left panel). The results were summarized graphically in the right panel.
simply attributed to poor cell growth (Karpova et al., 1998). Some populations of the vps1 cells also accumulated abnormal actin aggregates in the cytosol at 30°C, and this phenotype became a dominant one among the cells incubated at 37°C, with over 70% of the total cells displaying aggregations of actin structures. These aberrant actin structures were somewhat similar to the actin abnormalities previously observed in other actin defective mutants, such as sla1 and pan1. In comparison, disruption of the other two yeast dynamin encoding genes, DNM1 and MGM1, did not result in any obvious actin defects under all the conditions they were examined, suggesting that the actin-related function is unique to Vps1p among the three yeast dynamin-like proteins. The importance of Vps1p for the actin cytoskeleton was also illustrated by the fact that the vps1Δ mutants were hypersensitive to the actin cytoskeleton toxin LA T-A. The sensitivity to LA T-A has been regarded as an indicator of the actin cytoskeleton integrity, and has been applied to studies of the actin cytoskeleton in a variety of mutants (Ayscough et al., 1997; Belmont and Drubin, 1998; Singer et al., 2000). Therefore, there is little doubt that Vps1p is required for normal actin cytoskeleton organization in yeast.

The similar actin defects, such as aggregated actin structures and hypersensitivity to LA T-A, were manifested by cells of the putative GTPase mutants of vps1, as well as the cells over-expressing these mutants. The three GTPase mutations used in our studies were created according to mutations characterized previously, which abolished the GTPase activity in mammalian dynamins, although whether they indeed resulted in a loss of the GTPase activity of Vps1p has not been determined. Nevertheless, it could be at least tentatively concluded that the GTPase activity of Vps1p is important for its role in actin organization.

The vps1Δ cells were also found to be defective in bud site selection and chitin deposition at all temperatures tested, a phenotype that is often associated with the perturbation of actin cytoskeleton (Novick and Botstein, 1985; Yang et al., 1997). However, as mutations in genes involved in vesicular transport, such as vesicle formation at TGN, transport between ER and Golgi, or later steps of secretion, also affected budding patterns (Ni and Snyder, 2001), this phenotype of the vps1 cells is probably due to a combination of defects in actin cytoskeleton and vesicular transport.

Vps1p is required for the efficient internalization of some membrane proteins

It has been well known that the integrity of actin

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<tr>
<th>Prey</th>
<th>Vector (pGADT7)</th>
<th>Vps1p (aa 1-704)</th>
<th>Vps1p (aa 1-355)</th>
<th>Bait Vps1p (aa 357-704)</th>
<th>Vps1p (aa 276-616)</th>
<th>Vps1p (aa 566-704)</th>
<th>Vps1p (aa 614-704)</th>
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<tr>
<td>Vector (pGADT7)</td>
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<td>Sla1p (aa 1-1244)</td>
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<td>64±0.5</td>
<td>12±0.2</td>
<td>46±0.8</td>
<td>&lt;0.1</td>
<td>37±1.3</td>
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*In units of β-galactosidase activity. Abbreviation: aa, amino acids.
cytoskeleton is important for membrane protein endocytosis. In order to gather additional evidence for the role of Vps1p in actin cytoskeleton, we also analyzed the half-life of Ste3p-GFP and the internalization of Fur4p in the vps1 mutant. At 30°C, the turnover of Ste3p in vps1Δ cells was somewhat slower than in the wild-type. This phenomenon, however, can be explained by the known vacuolar sorting defect of the mutant (Raymond et al., 1992), rather than attributed to an endocytosis defect, because the internalization of Fur4p was found to proceed with virtually the same kinetics as that of wild-type cells at this temperature. Conversely, it is likely that the significant retardation of the uracil uptake and the marked increase of the half-life of the Ste3p at 37°C were principally due to the retardation of actin cytoskeleton dynamics through the interaction with Sla1p. This phenomenon, however, can be explained by the known vacuolar sorting defect of the mutant (Raymond et al., 1997), rather than attributed to an endocytosis defect, because the internalization of Fur4p was found to proceed with virtually the same kinetics as that of wild-type cells at this temperature.

Vps1p may function in actin cytoskeleton through interaction with Sla1p

Among the three yeast dynamin knockout mutants, vps1Δ was not only the only mutant that showed actin cytoskeleton defects, but also the only mutant that conferred a synthetic lethality with sla1Δ. Synthetic lethality is a common phenomenon among mutants with actin cytoskeleton defects and has been observed in many cases such as abp1 sla1 and abp1 sla2 (Holtzman et al., 1993), pan1 end3 (Tang et al., 1997), pan1 sla1 (Tang and Cai, 1996), to name a few. This phenotype is thought to reflect, at least in part, a functional relationship of the cytoskeleton machinery components. The relationship between Vps1p and Sla1p was further revealed by the experiments that established the physical interaction of the two proteins.

The interaction between Vps1p and Sla1p was further revealed by the experiments that established the physical interaction of the two proteins.

Is the actin-related function of Vps1p required for protein transport?

Although Vps1p has been known to be required for a number of cellular processes, its most well-known function is in the protein trafficking at the Golgi complex. It is postulated that Vps1p promotes vesicle formation at the Golgi in an analogous way to dynamin functioning in endocytosis at the plasma membrane (Nothwehr et al., 1995; Conibear and Stevens, 1995). Similar to endocytosis, protein transport from Golgi might also involve actin cytoskeleton. In mammalian cells, it is known that the actin cytoskeleton network is required for the morphology and cellular positioning of the Golgi apparatus (Valderrama et al., 1998), and disruption of the actin cytoskeleton network causes a concomitant collapse of the Golgi complex (Valderrama et al., 2000). The actin regulators Cdc42 and Arp2/3 are also known to be important for Golgi trafficking processes (Kroschewski et al., 1999; Musch et al., 2001; Stannes, 2002). In yeast, the Golgi complex has been linked to actin cytoskeleton by the identification of proteins that are required both for the protein trafficking at the TGN and for the integrity of actin cytoskeleton. These include Chc1p, Vps54p, Pik1p and Grd20p. Like the vps1 mutant, mutants of chc1, grd20 and vps54 all exhibited depolarization and aggregation of cortical actin patches at 37°C and, in the case of grd20, a significant delay in the turnover of the membrane receptor Ste3p (Spelbrink and Nothwehr, 1999; Walch-Solimena and Novick, 1999; Henry et al., 2002; Fiedler et al., 2002). The identification of Vps1p as a new factor from TGN to be required for normal actin organization further supports the connection between protein transport and actin cytoskeleton. The mechanism by which the actin assembly is integrated into the process of protein transport at TGN remains unknown. Thus far, we have not been able to separate the actin-related function of Vps1p from the function required for vacuolar transport by mutations. The same region of Vps1p was responsible, upon over-expression, for inciting actin
structural abnormalities and for promoting CPY secretion, suggesting that both processes required an unimpared Vps1p-Sla1p interaction. However, it remains possible that the actin cytoskeleton and protein transport processes involve two different populations of Vps1p molecules, and Sla1p might only interact with one of them.

Whether or not Sla1p participates in the protein sorting or cargo transport at the Golgi apparatus has not been formally addressed in this report. The limited evidence available so far suggests that it does. A recent report demonstrates that Sla1p interacts with a cis-Golgi membrane protein Kre6p and is required for the correct localization of this protein (Li et al., 2002). We also found that Sla1p was required for the correct localization of another Golgi resident protein Kex2p (data not shown). Nevertheless, this is an important question and certainly worth of further studies.

We are grateful to Hsin-yao Tang and Guisheng Zeng for various plasmid constructs. We also thank Alan Munn for helping with the Fur4p internalization assay. Wang Jun and Neo Suat Peng are thanked for general technical assistance. This work was supported by the Agency for Science, Technology and Research of Singapore. M.C. holds an adjunct faculty appointment from the Department of Biochemistry, Faculty of Medicine, National University of Singapore.

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Li, H., Page, N. and Bussey, H. (2002). Actin patch assembly proteins Las17p and cytoskeleton and protein transport processes involve two different populations of Vps1p molecules, and Sla1p might only interact with one of them. Whether or not Sla1p participates in the protein sorting or cargo transport at the Golgi apparatus has not been formally addressed in this report. The limited evidence available so far suggests that it does. A recent report demonstrates that Sla1p interacts with a cis-Golgi membrane protein Kre6p and is required for the correct localization of this protein (Li et al., 2002). We also found that Sla1p was required for the correct localization of another Golgi resident protein Kex2p (data not shown). Nevertheless, this is an important question and certainly worth of further studies.

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References


Drosophila melanogaster


and Slalp restrict cell wall growth to daughter cells and interact with cis-Golgi protein Kcd1p. Yeast 19, 1097-1112.


