Phospholipid Transfer Protein Sec14 Is Required for Trafficking from Endosomes and Regulates Distinct trans-Golgi Export Pathways*

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A protein known to regulate both lipid metabolism and vesicular transport is the phosphatidylcholine/phosphatidylinositol transfer protein Sec14 of *Saccharomyces cerevisiae*. Sec14 is thought to globally affect secretion from the *trans*-Golgi. The results from a synthetic genetic array screen for genes whose inactivation impaired growth of cells with a temperature-sensitive *SEC14* allele implied Sec14 regulates transport into and out of the Golgi. This prompted us to examine the role of Sec14 in various vesicular transport pathways. We determined that Sec14 function was required for the route followed by Bgl2, whereas trafficking of other secreted proteins, including Hsp150, Cts1, Scw4, Scw10, Exp1, Cis3, and Ygp1, still occurred, indicating Sec14 regulates specific *trans*-Golgi export pathways. Upon diminution of Sec14 function, the v-SNARE Snc1 accumulated in endosomes and the *trans*-Golgi. Its accumulation in endosomes is consistent with Sec14 being required for transport from endosomes to the *trans*-Golgi. Sec14 was also required for trafficking of Ste3 and the lipophilic dye FM4-64 from the plasma membrane to the vacuole at the level of the endosome. The combined genetic and cell biology data are consistent with regulation of endosome trafficking being a major role for Sec14. We further determined that lipid ligand occupancy differentially regulates Sec14 functions.

The composition of lipids within a membrane affects vesicle fission, transport, and fusion. Information on proteins that integrate lipid metabolism with vesicular transport is sparse. One protein that does so is Sec14 from *Saccharomyces cerevisiae*. Sec14 is an essential protein that extracts phosphatidylcholine (PC) and phosphatidylinositol (PI) from membranes in vitro and regulates PC and phosphoinositide metabolism in cells (1). Decreasing Sec14 function results in a reduction in Golgi PI 4-phosphate levels, an increase in the rate of PC syntheses, increased turnover of PC via the PC-phospholipase D Spo14, and decreased PC turnover by the PC-phospholipase B Nte1 (Fig. 1A) (2–5). The mechanisms by which Sec14 regulates PI 4-phosphate and PC metabolism are not known; however, this appears to be a major function in its regulation of cell biology as inactivation of numerous genes that mediate PC and phosphoinositide metabolism enhances or impairs growth of cells with defective Sec14 function (2, 4–6, 8–11).

Sec14 is known to be required for the transport of vesicles from the *trans*-Golgi to the plasma membrane and vacuole (equivalent to the lysosome in mammalian cells) as reduced Sec14 function results in a rapid decrease in Golgi-derived transport of invertase and acid phosphatase out of the cell and carboxypeptidase Y to the vacuole (2–6, 8–10, 12–15). Regulation of PC and phosphoinositide metabolism by Sec14 is hypothesized to provide the appropriate membrane environment for fission of vesicles from the *trans*-Golgi, although the precise step(s) where Sec14 regulates vesicular transport has yet to be determined.

The human genome contains at least 29 Sec14 domain-containing genes that code for more than 45 proteins (16). Mutations in several human Sec14 domain-containing proteins result in the onset of human diseases, including neurodegeneration, blindness, and cancer (17–22). Sec14 domains from higher eukaryotes are often embedded as part of a larger protein, many of which are guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs), indicating that regulation of small G protein activity is a unifying theme for several members of the Sec14 superfamily. Human Sec14 domains bind hydrophobic ligands, including phospholipids (16).

In this study, we used synthetic genetic array (SGA) analysis to determine more precisely how *S. cerevisiae* Sec14 regulates vesicular transport. Genetic interactions were observed between a *SEC14* allele with reduced function and GTPases that regulate vesicular transport in and out of the Golgi via endosomes. This is the first instance of Sec14 regulating import into the Golgi.

A main route for fusion of endosome-derived vesicles with the *trans*-Golgi is facilitated by the t-SNARE Tlg2 (23). Tlg2, along with Vps45 (a protein of the Sec1/Munc18 family), tethers endosome-derived vesicles to the *trans*-Golgi to facilitate membrane fusion (23) (Fig. 1B). The tethering complex that directly interacts with Tlg2 is the Golgi-associated retrograde protein (GARP) complex. The GARP complex is composed of four subunits Vps51–54 (23). The GARP complex directly
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The primary function of Sec14 is regulative function of vesicular transport through endosomes. We also demonstrate that ligand binding by Sec14 differentially regulates function.

MATERIALS AND METHODS

Synthetic Genetic Array Screen—SGA analysis was performed essentially as described (27, 28) with the below modifications. CMY503 (contains a temperature-sensitive SEC14 allele, sec14-ts) was mated with 4,800 S. cerevisiae single gene deletion strains at 25 °C; diploids were selected, and cells were sporulated for 5 days at 25 °C. To ensure that the haploid cells obtained were from mated diploids, cells were selected for histidine prototrophy followed by growth on medium containing G418 and nourseothricin. The resulting haploids were incubated at 25 or 35 °C. Three independent screens were performed. Mutants whose inactivation resulted in decreased growth when in combination with the sec14-ts allele in at least two of three screens were subjected to random spore analysis to determine whether the genetic interactions observed were true.

Yeast Strain Construction—The sec14-ts allele linked to the nourseothricin drug resistance cassette was constructed as follows (mutated sites in primer sequences are underlined). Plasmid-borne SEC14 was subjected to site-directed mutagenesis to convert Gln2-66 to Asp using primers 5′-CTTACCA-GTCAAAATTGGCAGTAAGTCTGAGTGTCA-3′ and 5′-GATTGCAATTCCAGACTTGACCGAATTTTTTQACTGGAAG-3′. This mutation confers temperature sensitivity to Sec14. To insert the natMX4 cassette next to the sec14-ts gene, an Sppl site was generated 300 bp downstream of the SEC14 allele in at least two of three independent screens. The resulting haploids were selected, and cells were sporulated to yield haploid cells obtained were from mated diploids, cells were selected for histidine prototrophy followed by growth on medium containing G418 and nourseothricin. The resulting haploids were incubated at 25 or 35 °C. Three independent screens were performed. Mutants whose inactivation resulted in decreased growth when in combination with the sec14-ts allele in at least two of three screens were subjected to random spore analysis to determine whether the genetic interactions observed were true.

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TACATGAGTATGCCCTGCCCCTA-3’ and 5’-TAGGGGCAGGGCATACTCATGTAGACGGC-3’, and this DNA fragment was subcloned into the 5’ph site downstream of the sec14α open reading frame. The sec14α::matMX4 disruption cassette was PCR-amplified from the resulting vector using primers 5’-CCCTTCTTGGATCCAGTCACTG-3’ and 5’-GGGACTTGATATCCTTAG-3’ to give a fragment containing both the sec14α mutation and the matMX4 cassette along with 5’ and 3’ SEC14-flanking DNA. This DNA fragment was transformed into the Y2454 yeast strain to replace the SEC14 gene with the sec14α allele linked to the natMX4 cassette to generate strain CMY503 (MATα mfa1Δ::MFA1pr-HIS3 can1Δ0 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 his3Δ1 sec14α::matMX4). The sec14α::natMX4 cassette was transformed into the indicated strains for the construction of isogenic sets of sec14α containing yeast (Table 1). Strain genotypes were confirmed by genomic PCR and/or genomic Southern blot using the digoxigenin High Prime DNA Labeling and Detection Kit II (Roche Applied Science).

Monitoring Bulk Protein Secretion and Mass Spectrometry Identification of Secreted Proteins—Cells were grown to mid-log phase at 25 °C, and an equal cell number was shifted to 37 °C for 10 min and incubated with [35S]methionine/cysteine for 10 min and then with 10 mCi unlabeled methionine/cysteine for 15 or 30 min. Proteins in the medium were precipitated with 10% trichloroacetic acid and separated by SDS-PAGE, and the gel was exposed to x-ray film. Similarly, equal numbers of log phase cells were shifted to 37 °C for 1 h in fresh YPD medium and proteins precipitated with 10% trichloroacetic acid, separated by SDS-PAGE, and the gel was stained with Gelcode (Thermo Scientific). Proteins contained in gel slices were reduced with dithiothreitol, carboxamidomethylated with iodoacetamide, and digested with trypsin. Peptides were extracted with 70% acetonitrile, 1% formic acid. The extraction solvent was removed under vacuum, and the tryptic peptides were resuspended in 5% methanol, 0.5% formic acid.

Liquid chromatography MS/MS was performed using an Ultimate pump and Famos auto-sampler (LC Packings, Amsterdam, Netherlands) interfaced to the nanoflow electrospray ionization source of a hybrid triple quadrupole linear ion trap mass spectrometer (QTrap, Applied Biosystems). Samples were injected onto a capillary column (0.10 × 150 mm Chromolith C18, monolithic, Merck) at a flow rate of 1.2 μl per min. Solvent A consisted of 98% water, 2% acetonitrile, 0.1% formic acid, and Solvent B consisted of 2% water, 98% acetonitrile, 0.1% formic acid, and the linear gradient was as follows: 5% solvent B to 35% solvent B over 35 min then 90% B for 6 min before re-equilibration at 5% solvent B. The sample was sprayed through distal coated fused silica emitter tips, 75-μm inner diameter with 15-μm inner diameter tip (New Objectives PicoTip). The capillary voltage was 2.10 kV with a declustering potential of 60 V, and the curtain gas was set to 15 (arbitrary units). Spectra were acquired using the information-dependent acquisition mode. The two most intense ions from the survey scan (375–1200 m/z) were selected for tandem MS, and the collision energy was set based on the mass of the precursor as determined by the m/z and charge. The raw MS/MS data were searched against NCBI yeast entries and against all SwissProt entries using the MASCOT algorithm (Matrix Science). Search parameters were peptide mass and fragment mass tolerance 0.8 and 0.5 Da, respectively, with one missed cleavage allowed. Oxidized methionines and carboxamidomethylated cysteines were chosen as variable modifications.

Invertase and Bgl2 Secretion Assays—The invertase secretion assay was performed as described (29) with modifications (13). Bgl2 secretion was determined as described (30). Mid-log phase cells were incubated at 25 °C or shifted to 37 °C for 15–60 min. Cells were then harvested by centrifugation at 1000 × g for 5 min. Cell pellets were resuspended in 10 mM NaN3, 10 mM KF solution, incubated on ice for 10 min, and then transferred to microcentrifuge tubes. Cells were centrifuged at 10,000 × g for 1 min, and pellets were resuspended in fresh pre-spheroplasting buffer (100 mM Tris-H2SO4, pH 9.4; 50 mM β-mercaptoethanol; 10 mM NaCl; 10 mM KF), incubated on ice for 15 min, centrifuged as before, washed with 0.5 ml of spheroplast buffer (50 mM K2HPO4-KOH, pH 7; 1.4 mM sorbitol; 10 mM NaCl), and pelleted. Cells were resuspended in spheroplast buffer containing 167 μg/ml Zymolyase 100T and incubated for 30 min at 25 °C. Spheroplasts were then pelleted at 5,000 × g for 10 min and resuspended in 2× SDS-PAGE sample buffer. Proteins were separated using a 10% SDS-polyacrylamide gel, and Bgl2 was detected by Western blot using a rabbit polyclonal antibody against Bgl2. Bgl2 antibodies were the kind gifts of Randy Schekman (University of California, Berkeley) and Wei Guo (University of Pennsylvania).

Ste3 Internalization—MATα cells were grown to mid-log phase at 25 °C and shifted to 37 °C for 2 h. Proteins were extracted, separated by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and probed with antibodies for Ste3 and Pkg1 (Molecular Probes). Ste3 antibodies were the kind gift of George Sprague (University of Oregon).

Microscopy—Live cells were observed using a Zeiss Axiovert 200 M microscope fitted with a plan-neofluor 100× oil immersion lens. Images were captured using a Zeiss Axio Cam HR using Axiovision 4.5 software. Cells were visualized using differential interference contrast (DIC), green fluorescent protein, or rhodamine filters as required. GFP-Snc1 subcloned into the plasmid pRS416 (pMJL1) was the kind gift of Hugh Pelham (MRC Laboratory of Molecular Biology, Cambridge, UK). The plasmid expressing the Vps27-GFP fusion was from Christopher Stefan (Cornell University, Ithaca, NY). The yeast strains expressing red fluorescent protein-tagged organelle marker proteins were from Erin O’Shea (University of California, San Francisco).

RESULTS

SGA Analysis of sec14α Cells Uncovers Novel Genetic Interactions—S. cerevisiae contains 6607 genes of which ~5000 are not essential. A haploid query strain containing a temperature-sensitive sec14α allele was mated to ~4800 viable single gene deletion haploid yeast strains of the opposite mating type using SGA technology (28). The resulting diploids were isolated and forced to undergo meiosis, and haploid cells were selected that contained the sec14α allele in combination with each single gene deletion. The resulting strains, obtained from three sepa-
TABLE 1
Yeast strains used in this study
UCSF is University of California, San Francisco.

| Strain              | Genotype                        | Source         |
|---------------------|---------------------------------|----------------|
| BY4741              | MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | EUROSCARF      |
| BY4742              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | EUROSCARF      |
| Y2454               | MATα mfa1Δ::MFA1pr-HIS3 can1Δ0 his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 | 49             |
| CMY503              | Y2454 sec14Δ::matMX4             | This study     |
| CMY505              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY506              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y01846              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY514              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y06583              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY515              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y02371              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY516              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y01709              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY517              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y05102              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY518              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY526              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY553              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY554              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y04662              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY327              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y05171              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY528              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y05091              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY530              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y05138              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY531              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y04707              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY532              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y05966              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY533              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y06772              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY547              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y05394              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY548              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y07277              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY549              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y04516              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY550              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y04796              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY551              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| Y04738              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY552              | YBY7471 gyp1Δ::kanMX4 sec14Δ::matMX4 | This study |
| CMY556              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| CMY577              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| CMY586              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| CMY594              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| CMY595              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| CMY596              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| CMY597              | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 (S288C) RFP-SN7 | UCSF |
| X2180-1A            | MATα mal mgal gal2 CLP1 SUIC2 | Randy Shekman |
| SF292-1A            | MATα sec14Δ-3 (sec14Δ-4) isogenic to X2180-1A | Randy Shekman |
| SF292-2A            | MATα sec14Δ-3 (sec14Δ-4) isogenic to X2180-1A | Randy Shekman |
| CBY926              | MATα ura3-52 his3Δ200 lys2Δ0 met15Δ0 ura3Δ0 (S288C) RFP-SN7 | This study |

rate screens, were incubated at permissive (25 °C) and semi-permissive (35 °C) temperatures for growth of cells containing the sec14Δ allele alone. We considered only strains with the sec14Δ gene in combination with each single gene deletion able to grow at 25 °C, but not 35 °C, to eliminate genes unable to grow because of defective mating, sporulation, or restoration of vegetative growth from spores.

Forty genes were isolated from the SGA screen whose inactivation agrivated growth of sec14Δ-containing cells (Fig. 2A and Table 2). Inactivation of SPO14 (PC phospholipase D) had been previously determined to diminish growth of sec14Δ cells (1, 5, 9), and it was isolated from our screen. Of the 40 genes isolated, the largest group contained genes known to regulate Golgi-dependent vesicular transport processes consistent with Sec14 acting as an essential regulator of this process (Fig. 2A). As genetic interactions can be representative of direct regulation of a protein/process, or may indicate processes whose functions are linked but by several degrees of separation, we focused on the genes isolated from the SGA screen with known roles in vesicular transport.

To independently confirm the results of the SGA screen, a set of isogenic strains was constructed whereby the wild type
genes identified by the SGA screen whose inactivation decreased growth of sec14ts membrane (35, 36). We monitored invertase secretion in from the invertase secretion from the cell. Invertase follows a pathway with successfully revealed previously unknown genetic interactions genes alone did not affect cell growth. The SGA analysis suc-
tive in Sec14 Function trans Sec14-mediated Vesicular Transport

FIGURE 2. Genes whose inactivation aggravates growth of sec14ts cells. A, pie chart of the functions of the 40 genes identified by the SGA screen whose inactivation decreased growth of sec14ts cells. A subset of these genes (SPO14, YPT31, TRS33, TLG2, GYP1, and VPS1) had been identified previously as genetic interactors in cells with decreased Sec14 function (2). B, isogenic mutant strains with the indicated genotypes were grown to mid-log phase in synthetic complete medium at 25 °C; identical numbers of cells were serial diluted, spotted onto agar plates, and grown for 3 days at the indicated temperatures. Invertase secretion is the ratio of external invertase enzyme activity to total (internal + external) invertase activity. Invertase data are the mean of three individual experiments performed in triplicate. Standard errors of the mean are indicated in the figure.

SEC14 gene was replaced with the sec14ts allele in strains with inactivated genes for SPO14 (PC phospholipase D) (1, 5, 9), YPT31 (Rab that regulates Golgi vesicle export and import) (31), TRS33 (a component of TRAPP complex that acts as a GEF for Ypt31 and its homologue Ypt32) (32, 33), GYP1 (GAP for the essential Rab Ypt1) (34), and TLG2 (syntaxin-like t-SNARE that mediates fusion of endosome-derived vesicles with the trans-Golgi) (34). Growth of cells containing the sec14ts allele alone was slightly slower than wild type at the semi-permissive temperature for this allele (35 °C) (Fig. 2B). This growth defect was exacerbated when a cell containing the sec14ts allele had in combination an inactivated gene for SPO14, YPT31, TRS33, GYP1, or TLG2. Inactivation of any of these genes alone did not affect cell growth. The SGA analysis success-

vated genes isolated from our SGA screen. In sec14ts cells invertase secretion was reduced to 66% at 35 °C (Fig. 2B) (compared with ~30% at 37 °C, which is the non-permissive temperature for the sec14ts allele; data not shown). In sec14ts cells that also contained inactivated SPO14, YPT31, TRS33, GYP1, or TLG2 invertase secretion was reduced to 22–48% of wild type at 35 °C (Fig. 2B). All strains secreted invertase similar to wild type at 25 °C and in addition all sin-
gle gene deletions secreted invertase at wild type levels at 35 and 37 °C (data not shown). Therefore, inacti-
vation of SPO14, YPT31, TRS33, GYP1, or TLG2 decreases Sec14-dependent vesicular transport of invertase from the trans-Golgi.

Two types of secretory vesicles have been isolated from cells based on differences in density (35, 36). The endosome-dependent route fractionates with higher density ves-
icles that contain invertase and acid phosphatase. These are the only two proteins that have been shown to be inefficiently secreted in sec14ts cells.

A second route to the plasma membrane is found in lower density vesicles containing Bgl2 and Pma1 and represent an endosome-inde-
dependent route from the trans-Golgi.

FIGURE 3. Secretion Pathways Are Differentially Affected in Cells Defec-
tive in Sec14 Function—Sec14 regulates fission of vesicles from the trans-Golgi. A hallmark of Sec14 deficiency is reduced invertase secretion from the cell. Invertase follows a pathway from the trans-Golgi via endosomes on its route to the plasma membrane (35, 36). We monitored invertase secretion in sec14ts cells and compared it with sec14ts cells containing inac-
to the plasma membrane. To assess if a deficiency in Sec14 function also affects secretion through the endosome-independent route, we monitored Bgl2 secretion. Bgl2 accumulated in sec14ts cells compared with wild type after incubation at the non-permissive temperature for the sec14ts allele. We observed a build up of Bgl2 in cells with defective Sec14 function as early as 15 min after temperature shift, and this was obvious within 1 h (Fig. 3A). The osbΔ osh4ts strain is known to accumulate Bgl2 and served as a positive control (30). Bgl2 observed outside the cell in all strains is standard for this assay as Bgl2 is present in the periplasmic space prior to onset of the secretory defect. The high and low density vesicles that contain invertase/acid phosphatase and Bgl2/Pma1, respectively, were isolated from cells with defects in fusion of Golgi-derived vesicles with the plasma membrane (36). These experiments did not rule out other routes to the plasma membrane from the trans-Golgi. To determine the extent and specificity of the protein secretion defect in sec14ts cells, wild type and sec14ts cells (from two different genetic backgrounds) were grown at 37 °C for 10 min, incubated with [35S]methionine/cysteine for 10 min to radiola-
bel cellular protein, and chased with 10 mM unlabeled methio-
nine/cysteine for various times, and radiolabeled proteins secreted into the medium were separated by SDS-PAGE. Wild
Sec14-mediated Vesicular Transport

| Gene     | Open reading frame | Description                                                                 | Cellular process                                      |
|----------|--------------------|-----------------------------------------------------------------------------|-------------------------------------------------------|
| SPO14    | YKR031C            | Phospholipase D, catalyzes hydrolysis of phosphatidylcholine to phosphatidic acid and choline | Vesicular transport/phospholipid metabolism           |
| YPT31    | YER031C            | GTPase of Rab family involved in the endocytic and exocytic pathways        | Vesicular transport                                   |
| TRS33    | YOR115C            | Protein of the TRAPP complex involved in endocytic and exocytic pathways; GEF activity toward Ypt31 | Vesicular transport                                   |
| GYP1     | YOR070C            | Golgi GTPase-activating protein for Rab family members                       | Vesicular transport                                   |
| TGL2     | YOL018C            | t-SNARE involved in endocytosis and maintenance of resident proteins in the trans-Golgi | Vesicular transport                                   |
| YEP1     | YER151C            | Ubiquitin-specific protease, interacts with Bre5                             | Vesicular transport                                   |
| BRE5     | YNR051C            | Required by Ubp3 to form an active de-ubiquitination complex which protects Sec23, a COP11 subunit, from degradation | Vesicular transport                                   |
| KEX2     | YNL238W            | Calcium-dependent serine protease involved in the activation of proproteins of the secretory pathway | Vesicular transport/Golgi function                    |
| VP51     | YRK001C            | Dynamin-like GTPase for vacuolar protein sorting and high density vesicle production for secretion of invertase | Vesicular transport                                   |
| TCB2     | YNL087W            | Synaptotagmin like protein; contains 3 C2 domains                            | Golgi localized                                       |
| INP51    | YIL002C            | PI-4,5-bisphosphate 5-phosphatase                                            | Phospholipid metabolism                               |
| YBR030W  | YBR030W            | Protein of unknown function; likely involved in phospholipid metabolism as it has Ino2/4-binding sites in promoter | Phospholipid metabolism                               |
| CLN2     | YPL256C            | G1/S-specific cyclin, interacts with Cdc28 kinase to control events at START | Cell cycle progression                                |
| CDH1     | YGL003C            | Cell cycle-regulated activator of the anaphase-promoting complex required for exit from mitosis | Cell cycle progression                                |
| WHI2     | YOR043W            | Protein involved in stress response and growth regulation; negative regulator of G1 cyclin expression | Cell cycle progression/stress response                |
| RIM9     | YMR063W            | Protein of unknown function; involved in proteolytic activation of Rim101 in response to pH | Stress response                                       |
| TLS1     | YLR425W            | GDP-GTP exchange factor that functions to modulate Rho1 activity as part of the cell integrity pathway | Stress response/cell wall organization               |
| SLG1     | YOR008C            | Plasma membrane protein required for maintenance of cell wall integrity and for the stress response | Cell wall organization                                |
| ECM33    | YBR078W            | Glycophosphatidylinositol anchor protein of unknown function; possible role in cell wall organization | Cell wall organization                                |
| NCS2     | YNL119W            | Role in urmulation                                                           | Cell polarity                                         |
| NCS6     | YGL211W            | Protein of unknown function; role in urmylation and invasive/pseudohyphal growth | Cell polarity                                         |
| PEA2     | YER149C            | Coiled-coil polarisome protein                                               | Cell polarity                                         |
| CRN1     | YLR429W            | Coronin, actin and microtubules associated protein                           | Actin patch assembly                                   |
| ICE2     | YIL090W            | Protein of unknown function; integral endoplasmic reticulum membrane protein | Endoplasmic reticulum organization                    |
| PER1     | YCR044C            | Vacular membrane protein; mutant is dependent on activation of the unfolded protein response | Protein Processing                                    |
| DSD1     | YGL196W            | d-Serine dehydratase                                                         | Amino acid metabolism                                 |
| HXT8     | YJL214W            | PM protein with strong similarity to hexose transporters; expression induced by low glucose | Hexose transport                                      |
| LSM1     | YIL124C            | Protein involved in mRNA degradation in cytoplasm                            | mRNA catabolism                                       |
| CBF1     | YJR060W            | Helix-loop-helix protein that binds to promoters in CDE1 and MET genes; required for chromosome stability | DNA/chromosome organization                         |
| RAD27    | YKL113C            | Single-stranded DNA endonuclease and 5'-3' exonuclease, 5'-flap endonuclease   | DNA/chromosome organization                         |
| EAF7     | YNL136W            | Subunit of the NuA4 histone acetyltransferase complex                       | DNA/chromosome organization                         |
| RAD34    | YDR314C            | Nucleotide excision repair                                                   | DNA repair                                            |
| YTS1     | YBR111C            | Nucleosidediphosphate sugar hydrolase                                        | Unknown                                               |
| CUE3     | YGL110C            | Protein of unknown function; has CUE domain that may facilitate intramolecular monoubiquitination | Unknown                                               |
| YFR045W  | YFR045W            | Protein with similarity to mitochondrial transporter family                    | Unknown                                               |
| YMR003W  | YMR003W            | Protein of unknown function                                                  | Unknown                                               |
| YGR064W  | YGR064W            | Protein of unknown function                                                  | Unknown                                               |
| YPL261C  | YPL261C            | Protein of unknown function                                                  | Unknown                                               |
| YIL007C  | YIL007C            | Protein of unknown function                                                  | Unknown                                               |
| YMR052C  | YMR052C            | Protein of unknown function                                                  | Unknown                                               |
| YMR052C-a| YMR052C-a          | Protein of unknown function                                                  | Unknown                                               |

The pattern of proteins secreted was similar but not completely identical (Fig. 3B). As a control we monitored the amount of radiolabeled protein secreted from sec18ts cells that possess a well characterized defect in secretion (Sec18 encodes the ATPase-N-ethylmaleimide-sensitive fusion protein that primarily regulates endoplasmic reticulum to Golgi transport). The sec18ts cells secreted significantly less protein.

To determine whether the radiolabeled bands could be due to protein breakdown, or whether they represented a series of proteins, proteins secreted into the medium were acid-precipitated and separated by SDS-PAGE, and gels were stained with Coomassie Blue and silver nitrate. Like the secretion of [35S]methionine/cysteine-labeled proteins, the banding pattern and amount were similar between wild type and sec14ts cells (data not shown). Identity was determined by mass spectrometry and included Hsp150, Cts1, Scw4, Scw10, Exg1, Cis3, and Ygp1, proteins known to be secreted by S. cerevisiae.

Decreasing Sec14 function does not appear to diminish all vesicular transport routes from the trans-Golgi, but it affects specific vesicular transport pathways emanating from this organelle.
GFP-Snc1 Trafficking Is Defective in Cells with Diminished Sec14 Function—Invertase transits through early endosomes on its way to the cell surface (35, 36). A possible explanation for the defect in delivery of invertase to the cell surface is that Sec14 is required for trafficking via endosomes. Consistent with this notion, genes isolated from the SGA screen (YPT31, TRS33, TLG2, and VPS1) regulate vesicular transport pathways through endosomes (Table 2). The endosome is a central organelle required for transit of a subset of proteins from the trans-Golgi to the plasma membrane, as well as trafficking of proteins from the plasma membrane back to the Golgi as well as the vacuole. To further assess if Sec14 regulated endosome requiring pathways we first monitored trafficking of the v-SNARE Snc1.

Snc1, the S. cerevisiae homologue of synaptobrevin, continually cycles between the plasma membrane and trans-Golgi. Snc1 is found in both low and high density Golgi-derived vesicles during transit to the plasma membrane (37), and thus the SNARE machinery is thought to be shared among vesicular transport pathways emanating from the trans-Golgi to the plasma membrane, as well as trafficking of proteins from the plasma membrane back to the Golgi as well as the vacuole. To further assess if Sec14 regulated endosome requiring pathways we first monitored trafficking of the v-SNARE Snc1.

A similar localization was observed in sec14Δ cells grown at the permissive temperature for function of this allele (25°C); however, when sec14Δ cells were shifted to the non-permissive temperature (37°C), GFP-Snc1 rapidly localized to large intracellular punctate spots. Localization of GFP-Snc1 with organelar marker proteins revealed that in the sec14Δ cells shifted to 37°C for 30 min GFP-Snc1 largely colocalized with the trans-Golgi in sec14Δ cells. Lat-A was added for 10 min to prevent GFP-Snc1 cycling back into the cell from the plasma membrane, and cells were grown for a further 15–30 min at 37°C. Live cells were visualized using DIC and fluorescence microscopy. The number of cells from random fields (four fields from two separate experiments totaling at least 100 cells) was visually assessed for GFP-Snc1 at the plasma membrane using a double-blinded protocol.
Golgi with a smaller fraction colocalizing with endosomes (Fig. 4B). GFP-Snc1 accumulation in endosomes indicates reduced endosome trafficking to the trans-Golgi.

Accumulation of GFP-Snc1 at the trans-Golgi could be due to either reduced fusion to or reduced transit from this organelle. To assess whether the GFP-Snc1 accumulating in the trans-Golgi upon reduction of function of Sec14 can exit this organelle, we treated cells with latrunculin-A (Lat-A). Lat-A blocks actin polymerization and blocks endocytosis from the plasma membrane to endosomes in *S. cerevisiae*. These conditions allowed us to monitor trans-Golgi to plasma membrane transport in the absence of recycling of GFP-Snc1 out of the plasma membrane. Cells were grown at 37 °C for 30 min to localize GFP-Snc1 to the trans-Golgi in sec14ts cells, and Lat-A was added to inhibit endocytosis, and cells were grown for a further 15–30 min at 37 °C to determine whether GFP-Snc1 could exit the trans-Golgi and traffic to the plasma membrane. As expected, wild type cells exhibit only plasma membrane GFP-Snc1 after treatment with Lat-A. A significant number of the Lat-A-treated sec14ts cells (58–67%) were able to transport GFP-Snc1 from the trans-Golgi to the plasma membrane (Fig. 4C). This is consistent with a Sec14-independent vesicular transport route from the trans-Golgi to the plasma membrane.

Endosome-dependent Routes from the Plasma Membrane Are Defective in Cells with Reduced Sec14 Function—Endosomes are also required for transport of vesicles from the plasma membrane to the vacuole. Ste3 is a mating factor receptor that, in the absence of mating factor, is transported from the plasma membrane via endosomes to the vacuole for degradation (39). Ste3 was observed in wild type cells at 25 °C with reduced levels at 37 °C (Fig. 5A). At increased temperature the rate of endocytosis is increased (40) resulting in the lower steady-state levels of Ste3 observed in wild type cells at 37 °C. In cells with defective Sec14, Ste3 was stabilized at 37 °C. Sec14 functions in the transport of Ste3 from the plasma membrane to the vacuole. We also monitored internalization of the lipophilic dye FM4-64 as a separate assay to determine whether there were defects in endosome to vacuole trafficking upon reduction of function of Sec14. FM4-64 incorporates into the plasma membrane and traffics to the vacuole via early and late endosomes (40). In wild type cells FM4-64 was transported to the vacuole in a matter of minutes at 37 °C, whereas in sec14ts cells shifted to 37 °C, FM4-64 accumulated in punctate spots in the cytoplasm and was unable to transit out of these punctate regions (Fig. 5B). These punctate structures colocalized with the endosomal marker Vps27-GFP (Fig. 5C). The inhibition of FM4-64 trafficking and stabilization of Ste3 upon inactivation of Sec14 is consistent with Sec14 regulating plasma membrane-derived endosome trafficking pathways.

Genetic Interactions of sec14ts with Genes That Regulate Endosome Trafficking—Our data are consistent with Sec14 regulating endosome function, and genes isolated from our SGA screen participate in this process. A main strength of the SGA strategy is comprehensiveness; however, SGA screens can miss genetic interactions depending on several factors, including the allele used in the screen, the nature of the screen, the fitness of each single gene deletion strain, and the effect of the gene(s) on mating, sporulation, and the transition from spore to growing cell (41). Therefore, we tested all nonessential genes involved in regulation of tethering and fusion of endosome-derived vesicles at the trans-Golgi for aggravating effects on growth of sec14ts cells (see Fig. 1B).

The wild type SEC14 gene was replaced with the sec14ts allele in an isogenic set of strains containing inactivated genes that regulate GARP-mediated fusion from endosome to the trans-Golgi, including VPS51, VPS52, VPS53, VPS54, VPS45, YPT6, RIC1, RGP1, ARL1, ARL3, or SYS1. Inactivation of any of these genes in sec14ts cells decreased growth at 35 and 33 °C compared with cells containing the sec14ts allele alone, except for cells containing an inactivated RGP1 gene that was inviable at any temperature (Fig. 6).

Inactivation of YPT31 was found by our SGA screen to aggravate growth of sec14ts cells. Ypt31/Ypt32 and the TRAPP II complex regulate both export from the trans-Golgi to the plasma membrane and endosome trafficking to the trans-Golgi (26, 31, 32). Inactivation of any of the nonessential subunits of the TRAPP complexes, including
thus inactivation of the cells. Ypt32 is found in cells at lower levels than Ypt31 (42), and TRS65 which is specific to the TRAPP II complex, aggravated

![FIGURE 6. Tethering complex defects aggravate Sec14 function. Isogenic mutant strains with the indicated genotypes were grown to mid-log phase in synthetic complete medium at 25 °C, and identical numbers of cells were serial diluted, spotted onto agar plates, and grown for 3 days at the indicated temperatures.](image)

TRS65 which is specific to the TRAPP II complex, aggravated growth of sec14ts cells (Fig. 6).

Inactivation of YPT32 did not aggravate growth of sec14ts cells. Ypt32 is found in cells at lower levels than Ypt31 (42), and thus inactivation of the YPT32 gene may have its phenotype masked by the Ypt31 still present in the cell. The converse would not be true as the low level of Ypt32 could not buffer the effect of inactivation of the YPT31 gene.

For all of the genes tested, growth of isogenic strains containing inactivating mutations for each gene in the presence of the wild type SEC14 gene was determined at 35, 33, and 25 °C. All grew at rates similar to wild type except strains with inactivated TRS85, YPT6, or RIC1 genes that displayed moderately reduced growth at 35 °C. However, the combination of the sec14ts allele along with inactivation of TRS85, YPT6, or RIC1 resulted in much more severe growth defects than inactivation of any one gene alone (data not shown). Combined, the cell biology and genetic evidence clearly shows that Sec14 regulates endosome trafficking from the plasma membrane to both the trans-Golgi and the vacuole.

Specific Lipid Ligands Regulate Discrete Vesicular Transport Processes—Sec14 extracts PC and PI from membranes, and this activity is essential for its function in cells (4, 10, 43). A mutant version of Sec14 (Sec14K266A,K239A known as Sec14-PC) that transfers PC with much higher affinity than PI in vitro was found to restore growth and secretion to cells lacking Sec14 function (7, 43). Yeast contain five other Sec14 homologues (Sfh1–5) capable of PI (but not PC) transfer, and overexpression of two of these, Sfh2 and Sfh4, restored growth and secretion to cells lacking Sec14 function.

To facilitate analysis of roles for PC versus PI binding by Sec14, we transformed plasmids expressing wild type Sec14, the Sec14-PC allele, or Sfh4 into the strains containing the sec14ts allele in combination with every gene deletion determined in this study to diminish Sec14-dependent growth. If there are separate functions for PC and PI binding by Sec14, then a subset may be rescued by only one or more of wild type Sec14, Sec14-PC and Sfh4.

Growth of sec14ts cells lacking VPS51-53, three of the four nonessential components of the endosome to Golgi tethering complex GARP, required Sec14 or Sfh4, whereas Sec14-PC was insufficient (Fig. 7). Growth of cells inactivated for the fourth member of the GARP complex, VPS54, was only restored by wild type Sec14. Inactivation of the GARP complex as a whole requires Sec14 that can exchange PC and PI in cis within the same Sec14 molecule.

Endosome to Golgi fusion by the GARP complex is facilitated by the Rab Ypt6, and sec14ts cells lacking YPT6 were similar to those lacking VPS54 in that growth was supported only by wild type Sec14 but not by Sec14-PC or Sfh4 (Fig. 7). Ypt6 is activated by the GEF Ric1, and growth of sec14ts cells with inactivated RIC1 was supported by Sfh4 but not by wild type Sec14 or Sec14-PC. A similar sec14ts rescue phenotype associated with loss of RIC1 was observed for loss of function of VPS45. Vps45 is required for the t-SNARE Tlg2 to tether the GARP complex to the Golgi. The sec14ts cells with inactivated Tlg2 could grow normally when expressing wild type Sec14 or Sfh4, with some growth observed if Sec14-PC was expressed.

The second complex capable of activating GARP for fusion of endosomes with the Golgi is mediated by Arl11, Arl3, and Sys1. Growth was restored upon inactivation of ARL3 or SYS1 in sec14ts cells by Sec14, Sec14-PC, and Sfh4 (Fig. 7). ARL1 was unique in that its inactivation in sec14ts cells was not restored by wild type Sec14, but it was restored by either Sec14-PC or Sfh4. The two pathways that mediate GARP complex fusion of endosomes with the Golgi have specific lipid ligand occupancy requirements by Sec14. Growth of sec14ts cells lacking YPT1 or TRS33 was restored by Sec14, Sec14-PC, or Sfh4, whereas growth of sec14ts cells with inactivated TRS85 required wild type Sec14 or Sec14-PC (although growth was restored to a lesser extent).

Growth of sec14ts cells lacking the GAP Gyp1 (has specificity for several small G proteins in vitro, and the essential Rab Ypt1 in vivo; with Ypt1 activity activated by the TRAPP complex) was restored by wild type Sec14, Sfh4, and to a lesser extent Sec14-PC. Processes influenced by Sec14 function are differentially affected depending on Sec14 lipid ligand occupancy.

**DISCUSSION**

Sec14 Regulates Specific trans-Golgi Export Pathways—Sec14 was known to be required for secretion through the endosome-
dependent route followed by invertase and acid phosphatase. In this study we demonstrate that Sec14 also regulates the endosome-independent route from the trans-Golgi to the plasma membrane used by Bgl2. Moreover, not all secretion was inhibited upon diminution of Sec14 function as many proteins were still secreted at a similar level to wild type, including Hsp150, Cts1, Scw4, Scw10, Exg1, Cis3, and Ygp1. This implies that there is at least one more route from the trans-Golgi to the plasma membrane, and it is independent of Sec14 (Fig. 8).

The v-SNARE Snc1 traffics from the trans-Golgi to the plasma membrane via both the endosome-dependent and -independent routes (35, 36). Decreasing Sec14 function resulted in ~50% of Snc1 still being able to traffic from the trans-Golgi to the plasma membrane, implying Snc1 may also transit to the plasma membrane by the route used by Hsp150, Cts1, Scw4, Scw10, Exg1, Cis3, and Ygp1. This is consistent with the idea that the SNARE machinery required for secretion is shared by the trans-Golgi to plasma membrane routes.

Regulation of Endosome Trafficking by Sec14—We also determined in this study that Sec14 was required for trafficking from the plasma membrane to the vacuole via endosomes. FM4-64 internalization was inhibited at the endosome stage of transport, and consistent with this Ste3 was stabilized in cells with diminished Sec14 function. Indeed, trafficking of FM4-64 was dramatically reduced within 5 min of reduction of Sec14 function. These findings imply a role for Sec14 is regulation of endosome trafficking from the plasma membrane to the vacuole.

We observed that inactivation of Sec14 resulted in the accumulation of a portion of GFP-Snc1 in endosomes implying a defect in endosome to trans-Golgi transport. We also present substantive genetic evidence pointing to an important role for Sec14 in this pathway. Cells with defective Sec14 function were further impaired for growth by inactivation of nonessential components of the GARP or TRAPP II complexes and their regulators, including Rabs, Arf-like proteins, and their GEFs. In total, this study has revealed that endosome trafficking is likely a major role for Sec14 (Fig. 8).

Sec14 Affects Phospholipid-mediated Regulation of Vesicular Trafficking—Sec14 binds PC and PI and affects the metabolism of both these ligands, although precisely how is not known (1–6, 8, 9, 11, 44, 45). It is believed that occupancy of Sec14 by PC regulates PC synthesis and turnover, whereas occupancy by PI regulates PI-4P levels (2, 4, 16, 45, 46). Two separate SGA screens using different temperature-sensitive alleles of the Golgi PI 4-kinase PIK1 determined that inactivation of many of the genes in the current study (as aggravating growth of sec14ts cells) exacerbated Pik1-dependent vesicular trafficking from the Golgi, including YPT31 (but not YPT32), TRS33, TRS65, ARL1, SYS1, VP51, VP54, YPT6, RIC1, and VP51 (47, 48). As inactivation of a similar set of genes was found to inhibit growth of pik1ts and sec14ts cells (this study), and the fact that Golgi PI-4P levels are reduced in cells with diminished Pik1 and Sec14 function (2, 11, 44), it is likely that inactivation of these genes inhibits growth when Golgi PI-4P levels are reduced. Indeed,
the majority of the genes we identified in the sec14<sup>ts</sup> SGA screen could be rescued by wild type Sec14 or Sfh4, suggesting that they are dependent on the function of PI-bound Sec14. Specifically, in the absence of Vps51–53, wild type Sec14 and Sfh4 restored life but Sec14-PC did not. This implies that normal phosphoinositide metabolism is required in the absence of these components of the GARP complex. Strains lacking Vps54, Ypt6, or Trs85 only grew when transformed with a plasmid expressing wild type Sec14 indicating that both PC and phosphoinositide metabolism have to be intact and/or Sec14 has to exchange PI and PC in cis within the same protein when these functions are absent. Remarkably, in the absence of some proteins (Arl1, Vps45, and Ric1) wild type Sec14 did not restore growth, with growth of cells lacking Arl1 restored by Sec14-PC or Sfh4, and growth of cells lacking Vps45 or Ric1 restored only by Sfh4. Although Sec14 and its analogues were expressed from their own promoters, they were plasmid-borne, with Sec14 and Sec14-PC on low copy plasmids (2–3 copies per cell) and Sfh4 on a high copy 2μ/H9262 plasmid (20 copies per cell). The inability of plasmid-borne Sec14 to restore growth to cells lacking Arl1, Vps45, or Ric1 could be due to inhibition of growth upon increased dose of a Sec14 that exchanges PI and PC in cis and regulates both PC and phosphoinositide metabolism, whereas either one of these functions alone is not growth-inhibitory. Regulation of components of these pathways by specific lipids or membrane properties needs to be investigated to allow for a more precise determination of how lipid metabolism interfaces with and regulates vesicular trafficking.

**FIGURE 8. Regulation of vesicular transport by Sec14.** A, Sec14 facilitates nonendosomal and endosomal transport pathways from the trans-Golgi to the plasma membrane (pink and blue dotted lines). At least one other route from the trans-Golgi to the plasma membrane is not facilitated by Sec14. Transport from the plasma membrane to both the trans-Golgi and vacuole via endosomes is also regulated by Sec14. B, genetic evidence suggest Sec14 regulates endosome trafficking to the trans-Golgi via all major routes into this organelle by regulating Golgi PI-4P levels. Many of the gene deletions that aggravate growth of sec14<sup>ts</sup> cells also aggravate growth of cells containing a temperature-sensitive allele of the Golgi PI 4-kinase, PIK1; Golgi PI-4P levels are decreased in sec14<sup>ts</sup> and pik1<sup>ts</sup> cells (2). This implies that regulation of Golgi PI-4P levels by Sec14 is necessary for regulation of import and export of vesicles from the trans-Golgi. The processes affected could include GARP complex (Vps51–54) binding to the t-SNARE Yig2 that, together with Vps45, aid in targeting and fusion of endosome-derived vesicles with the trans-Golgi. Fusion is promoted by interaction of the GARP complex with the Rab GTPase Ypt6 that is in turn activated by the heterodimeric GEF Ric1/Rgp1. GARP also binds the Arf-like GTPase Arl that is localized to the Golgi by a second Arf-like GTPase Arl3 that interacts with the Golgi resident protein Sys1. The function of the Rab GTPase Ypt31 appears to be to regulate trafficking both in and out of the Golgi. Ypt31 is activated by the 10-subunit TRAPP II complex of which Trs33, Trs65, and Trs85 are nonessential. Reduction of Sec14 function results in aberrant Golgi PI-4P levels directly confounding vesicle import and export. The defects in endosome trafficking from the plasma membrane to the vacuole could be due to improper endosome trafficking because of miscommunication between Rab cascades into and out of the trans-Golgi.
resulted in mislocalization of Rab11 implying a conserved role for PI-4P in regulation of Rab signaling as inactivation of YPT31 affected growth and vesicular transport in S. cerevisiae cells with reduced Ptk1 or Sec14 function (7).

Indeed, a similar coordination in Rab signaling appears to operate in S. cerevisiae. Inactivation of the S. cerevisiae Rab YPT6 was also aggravating for growth of sec14Δ cells. GTP-bound Ypt6 physically interacts with the GAP for Ypt31, Gyp2, while exhibiting no GAP activity toward Ypt6 (47). Regulation of timing of the Ypt31 and Ypt6 Rab cascades has been proposed to facilitate transport between endosomes and the trans-Golgi, similar to coordination of mammalian Rab and Rho pathways. There may be a conserved role for the Sec14 domain in regulation of Rab/Rho signaling cascades.

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