Human SEC13Rp Functions in Yeast and Is Located on Transport Vesicles Budding from the Endoplasmic Reticulum

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Abstract. In the yeast *Saccharomyces cerevisiae*, Sec13p is required for intracellular protein transport from the ER to the Golgi apparatus, and has also been identified as a component of the COPII vesicle coat structure. Recently, a human cDNA encoding a protein 53% identical to yeast Sec13p has been isolated. In this report, we apply the genetic assays of complementation and synthetic lethality to demonstrate the conservation of function between this human protein, designated SEC13Rp, and yeast Sec13p. We show that two reciprocal human/yeast fusion constructs, encoding the NH_2-terminal half of one protein and the COOH-terminal half of the other, can each complement the secretion defect of a *sec13-1* mutant at 36°C. The chimera encoding the NH_2-terminal half of the yeast protein and the COOH-terminal half of the human protein is also able to complement a *SEC13* deletion. Overexpression of either the entire human SEC13Rp protein or the chimera encoding the NH_2-terminal half of the human protein and the COOH-terminal half of the yeast protein inhibits the growth of a *sec13-1* mutant at 24°C; this growth inhibition is not seen in a wild-type strain nor in other *sec* mutants, suggesting that the NH_2-terminal half of SEC13Rp may compete with Sec13-1p for a common target. We show by immunoelectronmicroscopy of mammalian cells that SEC13Rp (like the putative mammalian homologues of the COPII subunits Sar1p and Sec23p) resides in the region of the transitional ER. We also show that the distribution of SEC13Rp is not affected by brefeldin A treatment. This report presents the first demonstration of a putative mammalian COPII component functioning in yeast, and highlights a potentially useful approach for the study of conserved mammalian proteins in a genetically tractable system.

PROTEINS secreted by eukaryotic cells are vectorially transported from the ER through the Golgi apparatus to the cell surface in a series of steps mediated by membrane-bounded vesicles (Palade, 1975). The ability to study vesicle budding and fusion events in both yeast and mammalian systems has greatly facilitated the detailed molecular study of intracellular protein transport (Pryer et al., 1992; Rothman and Orci, 1992; Kaiser, 1993).

Vesicular transport between adjacent Golgi stacks has been extensively investigated in mammalian systems, and a clear model has now emerged (Ostermann et al., 1993). In this scheme, transport is initiated by the attachment of the small molecular weight GTP-binding protein, ADP-ribosylation factor (ARF),[1] to the donor compartment (Donaldson et al., 1992; Helms and Rothman, 1992; Helms et al., 1993). Bound ARF-GTP stimulates the recruitment of coatomer, a seven-subunit protein complex, from the cytosol, and budding occurs when coatomer binds (Donaldson et al., 1992; Orci et al., 1993*b*,*c*; Palmer et al., 1993; Hara-Kuge et al., 1994). Finally, the hydrolysis of ARF-GTP, presumably at the target membrane, results in coat disassembly, and permits the vesicle to fuse (Tanigawa et al., 1993; Elazar et al., 1994).

Both ARF and the β-COP subunit of coatomer have also been implicated in ER to Golgi transport. Transport of the vesicular stomatitis virus glycoprotein (VSV-G) from the ER is inhibited by the overexpression of a dominant negative ARF mutant (T31N) in cultured cells (Dascher and Balch, 1994). VSV-G transport from the ER is also inhibited by β-COP–specific antibodies in both microinjected (Pepperkok et al., 1993) and digitonin-permeabilized (Peter et al., 1993) cells. These data suggest a possible role for coatomer in ER to Golgi transport.

Vesicular transport from the ER to the Golgi has been extensively studied in the yeast *Saccharomyces cerevisiae*, and more than 20 genes have been implicated in this process (Kaiser, 1993). One of these genes, *SEC21*, is homologous

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1. *Abbreviations used in this paper*: ARF, ADP-ribosylation factor; CPY, carboxypeptidase Y; VSV-G, vesicular stomatitus virus glycoprotein.

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to the γ-subunit of coatomer (Hosobuchi et al., 1992). However, purification of *S. cerevisiae* transport-competent vesicles synthesized in vitro has revealed a coat complex containing five proteins previously implicated in vesicle formation – Sec13p, Sec23p, Sec24p, Sec31p, and Sar1p – but not Sec21p (Barlowe et al., 1994). Sec13p and Sec23p were initially identified by temperature-sensitive mutations which block vesicle formation at the non-permissive temperature (Novick et al., 1980; Kaiser and Schekman, 1990). Sec24p and Sec31p were identified by the physical association with Sec23p and Sec13p, respectively (Hicke et al., 1992; Salama et al., 1993). Sar1p was initially isolated as a suppressor of a mutation in the vesicle formation gene *SEC12* (Nakano and Muramatsu, 1989). Since none of these proteins show any apparent homology to subunits of the mammalian coatomer, the yeast coat complex has been designated COP II (Barlowe et al., 1994).

Mammalian genes showing significant sequence similarity to three COPII proteins have now been identified. Using degenerate PCR, Kuge et al. (1994) isolated two different mammalian clones encoding proteins with predicted amino acid sequences 61% identical to the sequence of the yeast Sar1p protein. The export of VSV-G protein from the ER is inhibited by overexpression of a dominant negative Sar1 mutant (T39N), as well as by the incubation of semi-intact cells with Sar1-specific antibody. Immuno-EM analysis reveals that Sar1 is highly enriched on vesicular carriers in the transitional region of the ER, consistent with a role in ER to Golgi transport. A similar distribution was also seen in mammalian cells for Sec23p, using cross-reacting antibodies raised against the yeast Sec23p protein (Orci et al., 1991). A mouse Sec23p homologue, encoding a product 40% identical to Sec23p, was recently discovered (Wadhwa et al., 1993); the relationship between this protein and the protein that cross-reacts with the anti-yeast-Sec23p antibody has not yet been established. Finally, Swaroop et al. (1994) have identified a human gene, *SEC13R*, that encodes a protein with 53% identity and 70% similarity to the amino acid sequence of the yeast COPII protein Sec13p.

The extensive genetic study of secretion provides us with the tools to explore the relationship between the function of a yeast COPII component and the function of a potential mammalian homologue. Perhaps the most direct way to study this relationship is to examine the behavior of the human protein in yeast – particularly yeast bearing a mutation in the corresponding endogenous gene. Not only is this approach useful in helping us understand and compare the process of vesicular transport in yeast and mammalian cells, but evidence of interchangeability is also required for the rigorous demonstration of homology (Tugendreich et al., 1994).

To investigate the functional relationship between the human protein SEC13Rp and yeast Sec13p, we examined the effect of human *SEC13R* expression in mutant and wild-type yeast. Through the application of two different genetic criteria – complementation (Benzer, 1962) and synthetic lethality (Dobzhansky, 1946; Sturtevant, 1956; Huffaker, 1987) – we have been able to show that *SEC13R* exhibits *SEC13* function, and therefore may be designated a mammalian homologue of *SEC13*. Although *SEC13R* itself does not complement the temperature-sensitive yeast mutant *sec13-1*, two reciprocal human/yeast chimeras, encoding the NH_2-terminal of one protein and the COOH-terminal half of the other, are each able to rescue the *sec13-1* secretion defect at 36°C; one of the chimeras can also complement a *SEC13* deletion. Furthermore, overexpression of the entire mammalian gene exhibited a negative effect in *sec13-1* mutants but not in either wild-type yeast or in other *sec* mutants; this result, an example of the genetic phenomenon of synthetic lethality, strongly suggests that in yeast, Sec13p and SEC13Rp participate in the same pathway. Together, the genetic data provide compelling evidence that human SEC13Rp can function in yeast; SEC13Rp thus represents the first putative mammalian homologue of a yeast COPII component to fulfill this criteria.

Immuno-EM analysis of pancreatic cells using antibodies raised against SEC13Rp demonstrates that SEC13Rp is concentrated in the transitional ER, in a distribution indistinguishable from that previously observed for Sec23 in the same cell type (Orci et al., 1991, 1993*a*). The distribution of SEC13Rp is not affected by brefeldin A treatment, in contrast to the result seen for coatomer (Orci et al., 1993*a*).

As a whole, our data not only support the existence of a mammalian COPII structure, but also emphasize the utility of heterologous gene expression as tool to study conserved proteins (Whiteway et al., 1993; Thukral et al., 1993).

Materials and Methods

Strains, Materials, and General Methods

Yeast strains used in this study are CKY8 (*MATα leu2-3,112 ura3-52*), RHY305 (*MATα leu2-3,112 ura3-52 sec13-1 Gal⁺*), DSY174 (*MATa leu2-3,112 ura3-52 Gal⁺* [pRS315, pGAL-SEC13R]), DSY223 (*MATα sec12-4 leu 2-3,112 ura3-52 Gal⁺*[pRS315, pGAL-SEC13R]), DSY216 (*MATα sec13-1 leu2-3,112 ura3-52 Gal⁺*[pRS315, pGAL-SEC13R]), DSY228 (*MATa sec16-2 leu2-3,112 ura3-52 Gal⁺*[pRS315, pGAL-SEC13R]), DSY232 (*MATa sec17-1 leu2-3,112 ura3-52 Gal⁺*[pRS315, pGAL-SEC13R]), DSY236 (*MATa sec18-1 leu2-3,112 ura3-52 Gal⁺*[pRS315, pGAL-SEC13R]).

Yeast culture, genetic manipulations, and molecular techniques were as described (Sambrook et al., 1989; Rose et al., 1990). Mammalian cell extracts were prepared by standard methods (Harlow and Lane, 1988). Materials were obtained from Sigma Chemical Company (St. Louis, MO) unless stated otherwise.

Gel electrophoresis was performed according to the Laemmli SDS-PAGE method using 10% polyacrylamide (Laemmli, 1970). For Western blotting affinity-purified anti-SEC13Rp antibody was used at 1:10,000 dilution. Secondary antibody was goat anti-rabbit IgG, conjugated to HRP (Amersham Corp., Arlington Heights, IL), at a 1:10,000 dilution. Filter-bound antibodies were then detected by peroxidase-catalyzed chemiluminescence (ECL kit; Amersham Corp.).

The monkey fibroblast cell line COS was grown in complete medium consisting of Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin. The Chinese hamster ovary cell line CHO was grown in complete medium consisting of Ham's F12 medium with 5% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin. Both cells lines were maintained at 37°C in a 5% CO_2 cell incubator.

Generation of Chimeric Constructs

The cloning of the *SEC13R* gene has been previously described (Swaroop et al., 1994). A ClaI site was introduced at nucleotide 449 in the *SEC13R* cDNA, corresponding to the ClaI site present in *SEC13* at nucleotide 488 (Pryer et al., 1993), using the oligonucleotide-directed mutagenesis method of Kunkel et al. (1987). The (antisense) primer used for this mutagenesis was: 5'-GGCAATGGTGTGAGCATCGATGATCTTCTTTACTTC-3'; altered nucleotides are underlined. In addition to introducing a restriction site, this procedure also altered two amino acids, changing Asn146→Ile and Asn147→Asp (see Fig. 1). Both mutagenized and wild-type forms were cloned into the vector pCD43 directly downstream of the *GAL10* promoter. Since both forms behaved identically in all assays described, only the strain

containing the mutagenized form (designated pGAL-SEC13R) is shown. pCD43 is a modified pRS316 (*ARS CEN URA3*) vector (Sikorski and Hieter, 1989) in which divergent *GAL1* and *GAL10* promoters have been introduced between the BamHI and EcoRI sites in the polylinker. The NH_2-terminal Sec13p/COOH-terminal SEC13Rp chimera was constructed by linking a BamHI/ClaI *SEC13* 5' fragment with a ClaI-HindIII *SEC13R* 3' fragment. The reciprocal chimera was constructed by linking a 5' EcoRI-ClaI *SEC13R* fragment with a ClaI-KpnI *SEC13* fragment. The *SEC13* plasmid was generated by first introducing a BamHI site immediately 5' to the initial ATG by PCR, using pCK1313 as a template (Pryer et al., 1993) and 5'-GCGGATCCAACCATGGTCGTCATAGCTAATGC-3' as the (sense) primer. The resulting BamHI/SacI fragment was cloned into pCD43, and was able to rescue *sec13-1* mutants at the restrictive temperature.

Chimera Complementation/Inhibition Assays

All constructs were transformed into RHY305. The vector pRS315 (Sikorski and Hieter, 1989) was co-transformed in all experiments, rendering all strains effectively prototrophic. Transformants were then assayed at 36°C (complementation) or at 24°C (inhibition) on synthetic minimal media (Difco Laboratories, Inc., Detroit, MI) supplemented with either 2% glucose or 2% galactose.

Radiolabeling and Immunoprecipitations

Cells were pre-grown at 24°C in selective SC medium containing 2% raffinose, and were induced by the addition of 2% galactose 3 h prior to the start of labeling. 1 h before labeling, cells were shifted to 36°C. 8×10^7 exponentially growing cells (4 OD_{600} units) were radiolabeled in supplemented SD medium by incubating with 30 μCi[^{35}S]methionine per OD_{600} unit (express protein labeling mix [NEN, Boston, MA], sp. act. 1,200 Ci/mmol). Samples were chased by the addition of 1/100 vol of a solution containing 0.1 M ammonium sulfate, 0.3% cysteine, 0.4% methionine. Labeled samples of 1 OD_{600} unit of cells were collected into chilled tubes containing 1 vol of 40 mM sodium azide. Protein extracts were prepared in 30 μl ESB by vigorous agitation with glass beads. Extracts were diluted with 1 ml IP buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.2% SDS), absorbed for 20 min with 50 μl 10% *Staphylococcus aureus* cells (Sigma Chemical Co.), and cleared by centrifugation at 12,000 *g* for 5 min. 0.5 μl anti-CPY antibody was added, and extracts were rotated for 1 h at room temperature. Immune complexes were collected by adding 25 μl 50% protein A/Sepharose (Pharmacia, Piscataway, NJ) per sample, and incubating an additional hour at room temperature. Protein A/Sepharose pellets were washed twice with IP buffer, and twice with detergent-free IP buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl). Protein was released into 30 μl ESB by heating to 100°C for 2 min. 12 μl of the supernatant were separated by SDS-PAGE and visualized by fluorography (Harlow and Lane, 1989).

Generation of SEC13-deletion Strain

RHY297 (*MATα sec13-Δ1 ura3-52 leu2-3,112 ade2-101 ade3-24* [pKR4 (*ARS CEN LEU2 ADE3 SEC13*)]) carries a *sec13* allele (*sec13-Δ1*) in which the entire *SEC13* coding sequence is deleted. RHY297 was constructed by R. Hammer as follows. A 50-nucleotide oligomer (SEC13-N1: 5'-CAT TTT AAA TTC TTG ATA CTC TTC ACG GAT CCG TAT GCT GAT ATA AAA TTA TCT GTT ATC-3') consisting of 28 and 26 nucleotides complementary to the 5' and 3' untranslated region of *SEC13*, respectively, and six nucleotides creating a BamHI site (underlined) was used to mutagenize pKR1 (*CEN SEC13 URA3*) (Roberg, K., unpublished results) using the protocol of Kunkel et al. (1987). pRH102 is a mutagenesis product that lacks the entire *SEC13* coding region as judged by restriction mapping, but retains *SEC13* flanking DNA. A 5-kb *hisG-URA3-hisG* marker cassette (modified from Alani et al., 1987; Elledge, S., unpublished results) was inserted into the BamHI site of pRH102 to make pRH104. The 6-kb EcoRI-SacI fragment of pRH104 was transformed into KRY5p4 (*MATα sec13-1 ura3-52 leu2-3,112 ade2-101 ade3-24* [pKR4 (*ARS CEN LEU2 ADE3 SEC13*)]) (Roberg, K., unpublished results). Ura$^+$ transformants that are unable to sector at 24°C were selected. RHY297 is a non-sectoring, Ura$^-$ derivative, selected by plating transformants on medium containing 5-fluoro-orotic acid (Boeke et al., 1984).

The chimera-suppressed *SEC13*-deletion strain was obtained by transformation of RHY297 using the construct encoding the NH_2-terminal Sec13p/COOH-terminal SEC13Rp chimera, described above, and then isolating white (pKR4$^-$) colonies.

Generation of Antibodies to the SEC13Rp Protein

SEC13Rp antiserum was elicited against a hybrid protein composed of the entire *SEC13R* coding region fused to Staphyloccocal protein A, using the pRIT33 vector (Nilsson and Abrahmsen, 1990). Hybrid protein was prepared from *Escherichia coli* extracts and antibody to this protein produced in rabbits, as described previously (Griff et al., 1992). Antiserum was affinity-purified using a β-galactosidase-SEC13Rp hybrid protein constructed by fusing the entire coding sequence of *SEC13R* to the *lacZ* gene in the pEX2 vector (Stanley and Luzio, 1984). The hybrid protein was isolated and used for affinity purification of the antibody as described (Pryer et al., 1993). The affinity-purified anti-sera was concentrated using a Centricon-30 microconcentrator (Amicon Corp.).

Immunofluorescence

COS cells or CHO cells were grown in complete medium on 12 mm glass coverslips for 2 d prior to recovery. Cells were fixed in 2% paraformaldehyde, and permeabilized in 0.1% Triton X-100, 0.02% SDS. Affinity-purified anti-SEC13Rp was used at a dilution of 1:50 in PBS/10% FBS in the presence of 0.2% saponin; secondary antibody (FITC-conjugated goat anti-rabbit; Boehringer-Mannheim Biochemicals, Indianapolis, IN) was added at a concentration of 1:250, using the same buffer composition. Coverslips were viewed by epifluorescence with a Zeiss Axioskop.

Immunoelectronmicroscopy

Rat pancreatic acinar tissue and isolated islets of Langerhans fixed in 1%, phosphate-buffered glutaraldehyde were processed for cryoultramicrotomy according to Tokuyasu (1986). SEC13Rp was localized by the protein A-gold method (Roth et al., 1978). Antibody dilution = 1.50; gold particle size = 10 nm. After immunolabeling, the cryosections were stained with uranyl acetate. Brefeldin A (BFA) treatment of insulin cells was performed as previously described (Orci et al., 1993*a*).

Results

Complementation of sec13-1 Defect by Human/Yeast Chimeras

The high degree of sequence similarity between Sec13p and SEC13Rp (Fig. 1), distributed along the length of the two proteins, encouraged us to ask whether *SEC13R* could func-

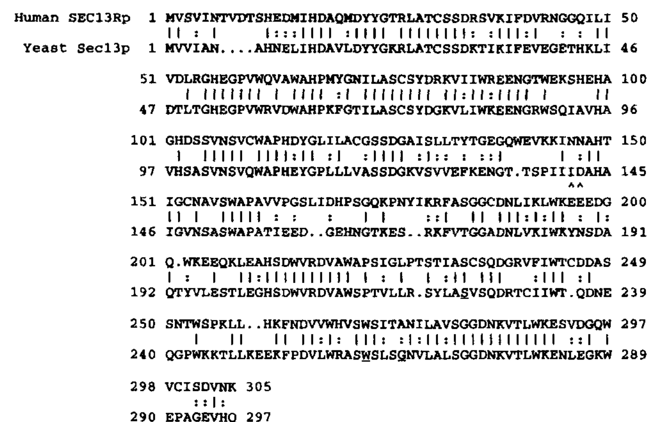
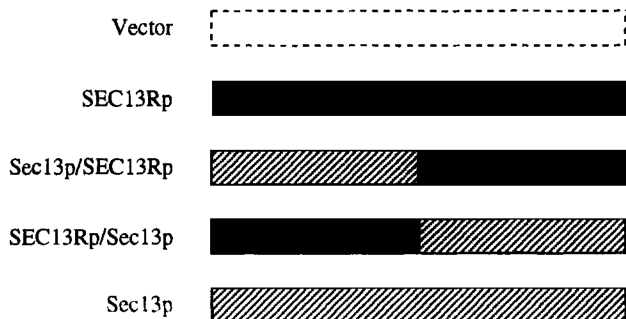


Figure 1. Sequence comparison of yeast Sec13p and human SEC13Rp. Identities are indicated by solid lines, similarities are indicated by dotted lines. The two amino acids altered during site-directed mutagenesis, corresponding to the junction site for chimera construction (see Materials and Methods), are indicated by carats. The Sec13p amino acids altered in the three known *SEC13* temperature-sensitive alleles (*sec13-1, sec13- 4, sec13-5*) are underlined (Pryer et al., 1993).



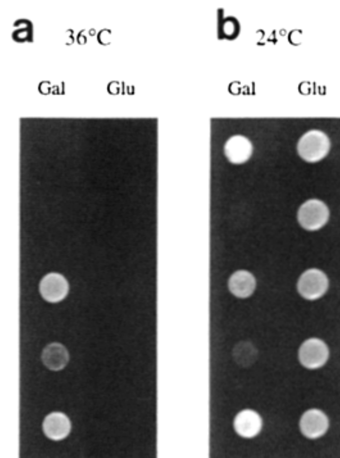


Figure 2. Effect of human/yeast chimeras in a *sec13-1* mutant strain. (*a*) Chimeras between human *SEC13R* and yeast *SEC13* can complement a *sec13-1* temperature-sensitive allele at the non-permissive temperature (*36°C*). *SEC13R*, *SEC13*, or chimeric constructs were fused to a galactose-inducible promoter in a centromeric vector, transformed into a *sec13-1* mutant, and assayed for growth in the presence of galactose (induced) or glucose (uninduced) at non-permissive conditions (*36°C*). (*b*) SEC13Rp overexpression inhibits the growth of a *sec13-1* strain. Assay performed at 24°C, but otherwise conditions identical to *a*.

tionally substitute for a defective *SEC13* gene. The *sec13-1* allele represents a single point mutation in *SEC13*, and cannot support growth at temperatures above 30°C (Pryer et al., 1993). The overexpression of *SEC13R* cDNA from a galactose-inducible promoter in a *sec13-1* strain did not restore viability at 36°C (Fig. 2 *a*). However, significant growth was observed at 36°C upon the galactose-induced overexpression of a chimeric construct encoding the NH_2-terminal half of yeast Sec13p and the COOH-terminal half of human SEC13Rp. This chimera was constructed by first creating a ClaI restriction site at nucleotide 449 of the human cDNA, corresponding to a naturally occurring ClaI site in nucleotide 488 of the yeast gene; each ClaI site occurs roughly in the middle of the protein coding sequence. The NH_2-terminal-encoding half of yeast *SEC13* was then fused to the COOH-terminal-encoding half of human *SEC13R*, using the ClaI site as a junction (Fig. 1). Induction of a construct encoding only the NH_2-terminal half of yeast Sec13p was unable to complement a *sec13-1* defect (data not shown; Pryer et al., 1993), suggesting that the COOH-terminal region of Sec13p is functionally required, and is not dispensable (see Discussion). The galactose-induced overexpression of the reciprocal chimeric construct, encoding the NH_2-terminal half of human SEC13Rp and the COOH-terminal half of yeast Sec13p, also complemented the *sec13-1* defect, though somewhat less efficiently (Fig. 2 *a*).

To demonstrate that both chimeras correct the *sec13* secretion defect, the transport of the marker protein carboxypeptidase Y (CPY) was monitored by pulse–chase analysis. CPY is targeted to the vacuole via the ER and Golgi. The core-glycosylated pl form of the enzyme can be resolved from the form that has received Golgi-specific modification (p2) and the mature form (*M*) that has been proteolytically cleaved in the vacuole (Stevens et al., 1982). Cells were grown in exponential phase at 24°C in medium containing raffinose, induced for 2 h with galactose, then shifted to 36°C for 1 h. Cells were next pulse labeled for 10 min, and then chased with excess methionine and cysteine. Lysates were prepared, and immunoprecipitated with anti-CPY antibodies. In *sec13-1* mutants, at 36°C, CPY is unable to exit the ER, and remains almost exclusively in the pl form (Fig. 3). However, upon the galactose-induced overexpression of either the NH_2-terminal yeast Sec13p/COOH-terminal human SEC13Rp chimera or the NH_2-terminal human SEC13Rp/COOH-terminal yeast Sec13p chimera, CPY is able to exit the ER, progress through the Golgi apparatus, and arrive in the vacuole (Fig. 3). The observation that either half of SEC13Rp can supply sufficient Sec13p activity to permit a yeast/human chimeric protein to complement the temperature-sensitive secretion defect of a *sec13-1* mutant suggests that SEC13Rp and Sec13p are functionally similar.

The ability of SEC13Rp to exhibit Sec13p function was illustrated further by the viability of a yeast strain expressing an NH_2-terminal yeast Sec13p/COOH-terminal human SEC13Rp chimeric protein, but containing no endogenous Sec13p (Fig. 4). To determine whether a yeast/human chi-

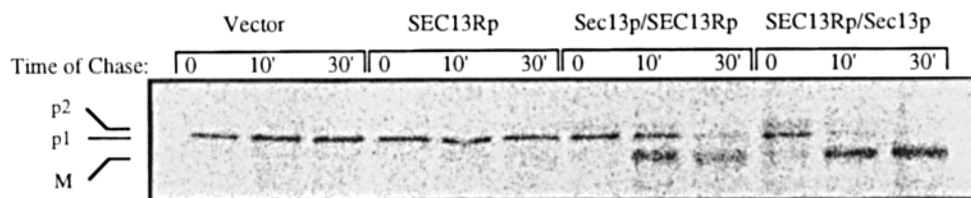


Figure 3. Complementation of the *sec13-1* secretion defect by both Sec13p/SEC13Rp and SEC13Rp/Sec13p chimeras. *sec13-1* cells carrying the indicated plasmid were induced for 2 h in galactose at 24°C, shifted to 36°C for one hour, pulse-labeled with Tran^{35}S-label for 10 min, and then chased by the addition of excess cysteine and methionine. Glass-bead extracts corresponding to the indicated time points were prepared; immunoprecipitation was performed using anti–CPY antibodies, and the precipitates subjected to SDS-PAGE and fluorography. Positions of the pl, p2, and mature forms of CPY are indicated.

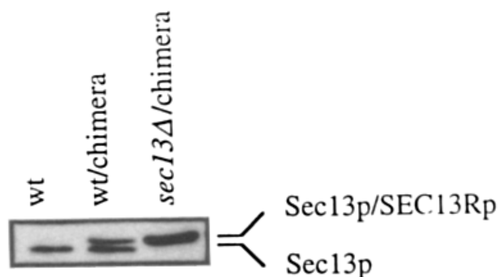


Figure 4. Complementation of a *SEC13* deletion by the Sec13p/SEC13Rp chimera. 50 μg of protein extract from the indicated yeast strains was analyzed by Western blotting using affinity-purified anti-Sec13p antibody, as described previously (Pryer et al., 1993). Lane *1*, extracts from wild-type yeast; lane *2*, from wild-type yeast expressing the Sec13p/SEC13Rp chimera; lane *3*, from a *SEC13*-deletion strain expressing the Sec13p/SEC13Rp chimera.

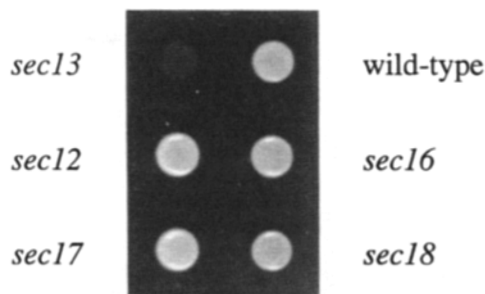


Figure 5. Specificity of SEC13Rp-induced growth inhibition. Indicated yeast strains containing a galactose-inducible SEC13Rp construct were incubated at 24°C in the presence of galactose. Strains used were: DSY174 (wild-type), DSY223 (*sec12*), DSY216 (*sec13*), DSY228 (*sec16*), DSY232 (*sec17*), and DSY236 (*sec18*).

mera could complement a *SEC13* deletion, the NH_2-terminal Sec13p/COOH-terminal SEC13Rp construct was transformed into the indicator strain RHY297. RHY297 is an *ade2- ade3-* strain in which the entire *SEC13* coding sequence has been deleted; the strain carries a plasmid bearing *SEC13*, *LEU2*, and *ADE3*. The strain is normally dependent upon the plasmid-encoded *SEC13*, and is also colored, reflecting the accumulation of a red intermediate resulting from *ade2*-blocked adenine biosynthesis (Jones and Fink, 1981; Koshland et al., 1985). However, upon transformation with a plasmid capable of complementing the *SEC13* deletion, the *SEC13*, *ADE3*, *LEU2* plasmid is no longer required, and can be lost during colony growth. Plasmid loss is detected by the appearance of white sectors; in the absence of the *ADE3* gene product, the adenine biosynthetic pathway is blocked at an earlier stage, prior to the *ade2* block, and the red-colored intermediate is not produced.

RHY297 was transformed with the construct encoding the NH_2-terminal Sec13p/COOH-terminal SEC13Rp chimeric protein, grown on plates containing galactose but not leucine (to maintain selection for the *LEU2* marker), and analyzed by Western blot using affinity-purified anti-Sec13p antibodies as a probe (Pryer et al., 1993). Yeast Sec13p itself has a predicted molecular weight of approximately 33 kD. The yeast/human chimera, however, has a slightly heavier predicted molecular weight, since the human COOH-terminal region is 20 amino acids longer than the corresponding region of the yeast protein. Extracts from wild-type cells revealed a single band at 33 kD, representing endogenous Sec13p (Fig. 3). Extracts from RHY297 cells transformed with the yeast/human chimeric construct revealed two bands: a 33-kD band representing Sec13p, and a slower-migrating band representing the chimeric construct.

When the RHY297 strain containing the chimeric construct was then grown on plates containing galactose plus rich medium (thus no longer maintaining selection for the *LEU* marker on the *SEC13* plasmid), white sectors appeared, indicating that in the presence of the chimeric construct, the Sec13p-encoding plasmid was no longer necessary. When colonies from the white sectors were isolated and analyzed by Western blot, a single band appeared at the location expected for the chimeric protein (Fig. 4). These data demonstrate that the NH_2-terminal Sec13p/COOH-terminal SEC-13Rp chimeric protein can functionally substitute for an absent Sec13p protein. Furthermore, since the chimeric product can functionally replace the endogenous protein, the *SEC13R*-encoded COOH-terminal region must exhibit activity similar to that found in the COOH-terminal region of Sec13p. Complementation of a *SEC13* deletion was not noticeably detected in a yeast strain transformed with the reciprocal chimera, a result consistent with the weaker activity exhibited by this construct in the suppression of a *sec13-1* growth defect (Fig. 2 *a*).

Human SEC13R Specifically Inhibits Growth of a sec13-1 Mutant

In the course of the complementation experiments, we discovered that the galactose-induced overexpression of SEC13Rp inhibited the growth of a *sec13-1* mutant strain at permissive temperatures. Although this strain normally grows well at 24°C, cell growth was dramatically inhibited by the induction of SEC13Rp (Fig. 2 *b*). Galactose-induced overexpression of SEC13Rp produced no evident phenotype in a wild-type strain or in strains carrying temperature-sensitive alleles of *SEC12*, *SEC16*, *SEC17*, or *SEC18*, suggesting that the observed effect was specific for the *sec13-1* strain (Fig. 5). Overexpression of the chimera encoding the NH_2-terminal half of SEC13Rp and the COOH-terminal half of Sec13p also inhibited growth of the *sec13-1* strain at 24°C (Fig. 2 *b*), implying that the NH_2-terminal half of SEC13Rp was responsible for this growth inhibition. Consistent with this interpretation, the NH_2-terminal Sec13p/COOH-terminal SEC13Rp chimera produced no obvious phenotype at 24°C. The inhibitory effect of SEC13Rp expression on the growth of a *sec13-1* strain at 24°C suggests that both SEC13Rp and Sec13-1p may interact with the same protein or substrate, and thus may participate in the same step or pathway. For example, SEC13Rp may titrate out a Sec13p target by binding to it unproductively.

Subcellular Localization of SEC13Rp

To explore further the function of SEC13Rp in mammalian cells, we raised rabbit serum against a hybrid protein of *Staphylococcus* protein A fused to SEC13Rp. Antibodies specific for SEC13Rp were affinity-purified using a hybrid protein composed of *E. coli* β-galactosidase fused to SEC13Rp. Purified antibodies recognized a single prominent band with

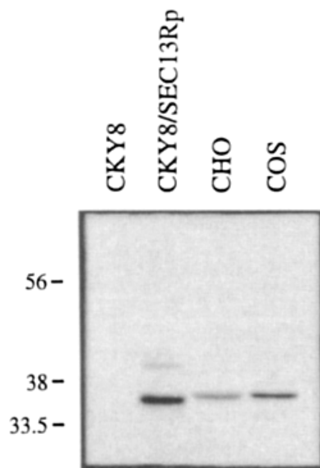


Figure 6. Specificity of antisera to the Sec13Rp protein. 50 μg of the indicated cell extracts were subjected to Western analysis and probed using the affinity-purified anti-SEC13Rp antibody. Lane *1*, wild-type yeast; lane *2*, wild-type yeast+pGAL-SEC13Rp; lane *3*, CHO cells; lane *4*; COS cells. The anti-SEC13Rp antibody recognizes a single predominant species of the predicted molecular weight, 36 kD.

the expected molecular weight of approximately 36 kD on immunoblots of lysates from wild-type yeast overexpressing SEC13Rp or from CHO or COS cells (Fig. 6). Immunoblots of wild-type yeast lysates did not show any pronounced bands; this shows that the affinity-purified anti-SEC13Rp antibodies do not cross-react with the yeast Sec13p protein.

The affinity-purified antibody was used to determine the subcellular localization of SEC13Rp. By indirect immunofluorescence, SEC13Rp exhibited a "diamond ring" appearance, encircling the nucleus in both CHO and COS cells (Fig. 7, *a* and *b*). To determine more precisely the subcellular distribution of SEC13Rp, we used immunoelectronmicroscopy, and focused on two cell types with well-developed secretory compartments, the insulin and the acinar cells of the pancreas (Fig. 8, *a* and *b*). In both of these cell types, SEC13Rp was found concentrated in the transitional area of the ER (Table I), in a distribution indistinguishable from that previously observed for mammalian Sec23p in the same cells (Orci et al., 1991). Furthermore, as previously observed for mammalian Sec23p (Orci et al., 1993*a*), the distribution of SEC13Rp was not altered by brefeldin A treatment, and remained excluded from coatomer-rich areas of "BFA bodies" (Fig. 9). These data are consistent with the view that SEC-13Rp and mammalian Sec23p are not constituents of the coatomer, but rather are both components of a different structure (most likely, mammalian COPII) involved in ER to Golgi transport.

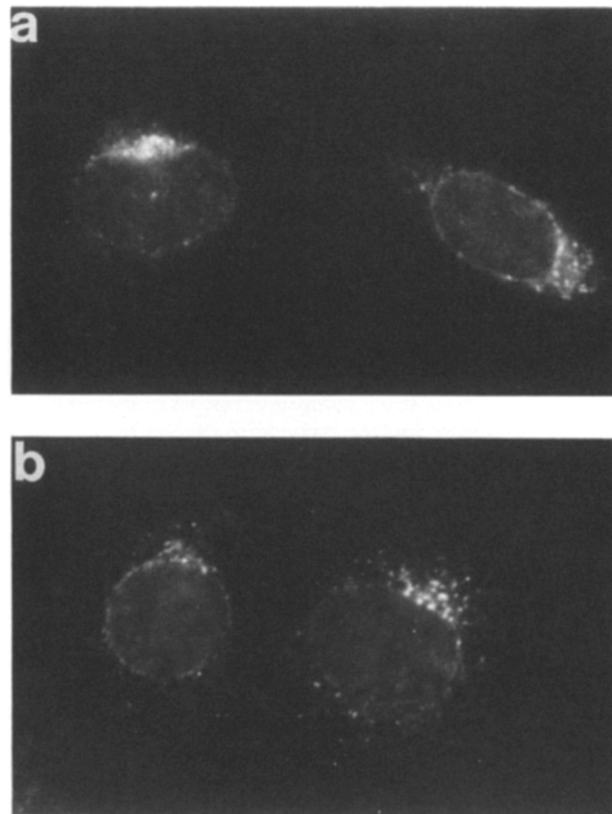


Figure 7. Localization of SEC13Rp by immunofluorescence. SEC13Rp appears in a perinuclear distribution in (*a*) CHO and (*b*) COS cells. Immunofluorescence performed using affinity-purified anti-SEC13Rp antibodies. Magnification: ×700.

Discussion

This report provides strong evidence that the function of Sec13p has been highly conserved through evolution. In yeast, Sec13p is required for vesicle budding from the ER, and has been identified as a constituent of the vesicle-coating protein complex designated COPII (Pryer et al., 1993; Barlowe et al., 1994). We have shown that two reciprocal human/yeast chimeric Sec13 constructs can each complement a *sec13-1* mutant, and can rescue both the growth defect and the secretion defect. We have also shown that one of these chimeric constructs can complement a deletion of *SEC13*. In addition, we have demonstrated that the human SEC13Rp protein itself inhibits the growth of a *sec13-1* mutant, but not of either wild-type yeast or mutants in *SEC12*, *SEC16*, *SEC17*, or *SEC18*. We also show that SEC13Rp is located in the transitional ER, in a distribution identical to that previously observed for mammalian Sec23. Finally, we show that the cellular distribution of SEC13Rp is insensitive to BFA treatment, consistent with the behavior previously observed for mammalian Sec23, but not for coatomer components.

Complementation of the growth defect of a *sec13-1* mutant at 36°C represents a stringent assay that requires no presumptions about the specific function of Sec13p. The ability of both yeast/human chimeric proteins to complement a *sec13-1* mutant implies that in both cases, the human region of the chimeric protein is fulfilling the function or functions normally performed by the corresponding region of the yeast protein. The ability of both chimeras to restore CPY transport in a *sec13-1* strain specifically demonstrates complementation of the ER to Golgi secretion defect characteristic of *sec13-1* mutants.

If the two reciprocal chimeras complement both the growth defect and the secretion defect of a *sec13-1* mutant, then why does the entire *SEC13R* cDNA not complement either defect? We believe that there are at least two contributing factors. First, we would suggest that although both halves of human SEC13Rp can function in yeast, they do not function at quite the same level of wild-type Sec13p; this would explain why the full-length SEC13Rp protein, representing the sum of two sub-optimal halves, cannot rescue the *sec13-1* secretion defect. Second, we would propose that in a *sec13-1* strain, the NH_2-terminal region of SEC13Rp is toxic, resulting in the growth defect observed at 24°C in strains

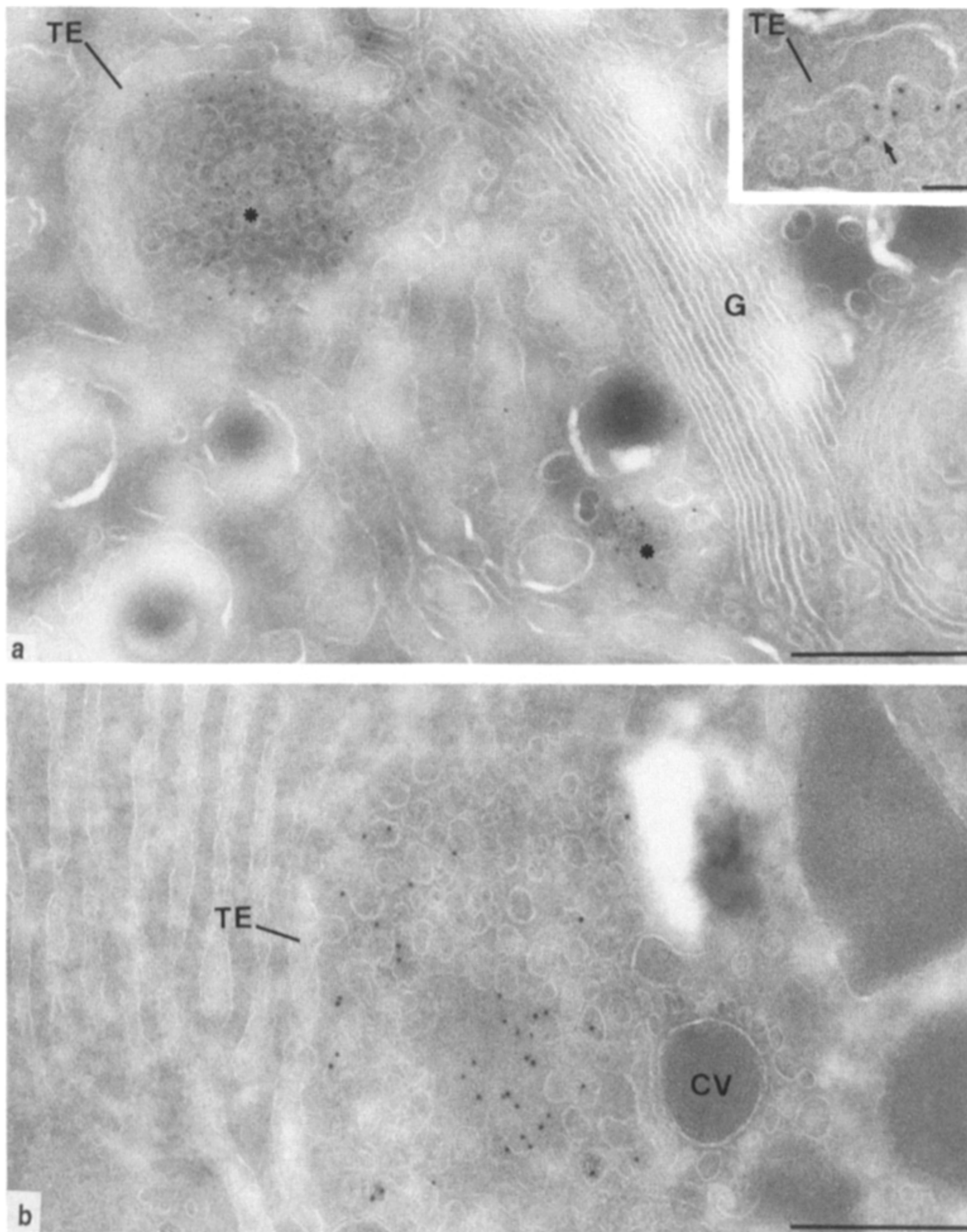


Figure 8. By electron microscope immunolabeling of (*a*) insulin or (*b*) acinar rat pancreatic cells, SEC13Rp is restricted to the transitional area of the ER. Transitional elements (TE) of the ER with associated transfer vesicles (*asterisks*); the arrow in the inset indicates a labeled bud on a transitional cisterna; *G*, Golgi complex; *CV*, condensing vacuole in the Golgi region. Note that the dense cytosolic matrix in the transitional area of *b* is also labeled in addition to transfer vesicles. See Table I for the quantitation of the immunogold labeling. Magnifications: (*a*) ×53,000 (*inset*, ×72,000); (*b*) ×54,000. Bars: (*a* and *b*) 0.5 μm; (*inset*) 0.1 μm.

Table I. SEC13Rp Immunogold Labeling of Transitional Area, ER, and Golgi of Pancreatic Acinar and Insulin Cells

	Number of gold particles per μm^2 ± SEM	
	Acinar cell	Insulin cell
ER (n = 10)	0.34	0.18
Transitional area* (n = 10)	61 ± 13 (54 ± 6 vesicles per μm^2; 28% ± 4% of vesicles labeled)	110 ± 23 (105 ± 15 vesicles per μm^2; 48% ± 7% of vesicles labeled)
Golgi (n = 10)	1 ± 0.7	2 ± 1

n, number of pictures evaluated. Quantitation was performed as described previously (Orci et al., 1991).
* Including the budding front of the transitional ER, vesicles, and intervening cytosol.

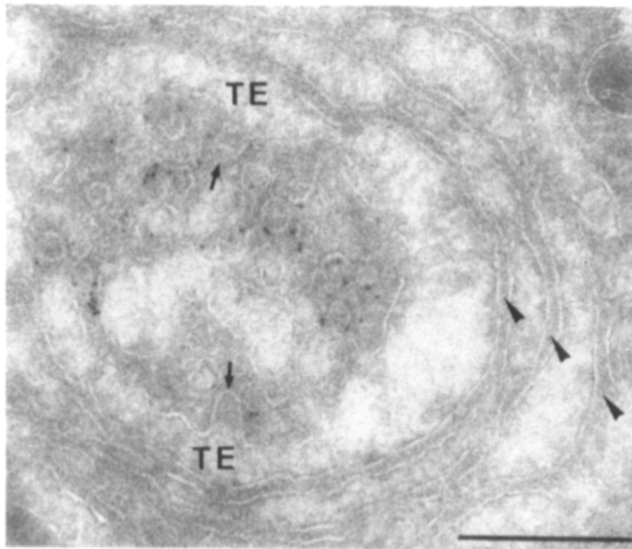


Figure 9. SEC13Rp labeling of a BFA body. Immunogold particles are present on the buds (arrows) and vesicles associated with the transitional endoplasmic reticulum cisterna (TE), but are absent from the dense bands of cytosol (arrowheads) enriched in coatomer (Orci et al., 1993a), situated between transitional and non-transitional ER cisternae. BFA-treated insulin cell. Magnification: ×47,600. Bar, 0.5 μm.

expressing either the full-length SEC13Rp protein or the NH_2-terminal SEC13Rp/COOH-terminal Sec13p protein. Although the precise reason for this toxicity is not known, the observation that it is only seen in a *sec13* mutant, and not in either a wild-type strain or in other *sec* mutants, suggests a synthetic lethal interaction between *SEC13R* and *sec13-1*, and implies that sec13-1p and SEC13Rp participate in the same pathway, and perhaps compete for a common target. The ability of the NH_2-terminal SEC13Rp/COOH-terminal Sec13p protein to rescue a *sec13-1* secretion defect at 36°C suggests that the toxicity associated with the NH_2-terminal region of SEC13Rp develops over a period of time longer than that encompassed by the pulse–chase assay.

Localization of SEC13Rp to the transitional ER and associated transport vesicles represents an important observation. These data not only situate SEC13Rp in precisely the area expected for a mammalian protein involved in ER to Golgi transport, but also emphasize that SEC13Rp is concentrated at this level. This is strong evidence that SEC13Rp is specifically involved in ER to Golgi transport. If SEC13Rp is in fact a component of a mammalian COPII complex, then these data would suggest that COPII is involved solely in transport between the ER and the Golgi apparatus, and, unlike coatomer, is not involved in intra-Golgi transport.

The observation that SEC13Rp does not redistribute upon BFA treatment is encouraging because it places SEC13Rp and mammalian Sec23p in a different category from all the known coatomer components. Furthermore, since BFA is known to inhibit the binding of ARF to donor membranes, it is tempting to speculate that ARF is not involved in the recruitment of SEC13Rp and mammalian Sec23p; perhaps this function is fulfilled by Sar1.

Functional complementation of a yeast mutant by a human/yeast chimera has been reported for a number of different genes. Mutants in yeast genes encoding the transcription factor SWI2p (Khavari et al., 1993), the nucleotide exchange factor CDC25p (Wei et al., 1992), the RNA-binding protein SNP1p (Smith and Barrell, 1991), and the ABC-transporter STE6p (Teem et al., 1993) have all been complemented by chimeric constructs consisting of fused regions of the wild-type yeast gene and its putative mammalian homologue. Not only does such complementation demonstrate the conservation of function between yeast and human gene products, but it can also allow for the detailed study of the mammalian protein, as is illustrated by the work by Teem et al., (1993) on the cystic fibrosis transmembrane conductance regulator.

Together, the data presented in this report argue that the function of Sec13p has been conserved from yeast to humans, and also support the existence of a mammalian COPII complex. More generally, our results emphasize that chimera studies represent a useful, easily-adaptable approach for applying the tools of yeast genetics to the study of conserved mammalian proteins.

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