SED5 Encodes a 39-kD Integral Membrane Protein Required for Vesicular Transport Between the ER and the Golgi Complex

Kevin G. Hardwick and Hugh R. B. Pelham

MRC Laboratory of Molecular Biology, Cambridge, CB2 2QH, United Kingdom

Abstract. The *ERD2* gene, which encodes the yeast HDEL (His-Asp-Glu-Leu) receptor, is essential for growth (Semenza, J. C., K. G. Hardwick, N. Dean, and H. R. B. Pelham. 1990. *Cell.* 61:1349–1357; Lewis, M. J., D. J. Sweet, and H. R. B. Pelham. 1990. *Cell.* 61:1359–1363). *SED5*, when present in multiple copies, enables cells to grow in the absence of Erd2p. Sequence analysis of *SED5* reveals no significant homology with *ERD2* or other known genes. We have raised antibodies to Sed5p which specifically recognize a 39-kD integral membrane protein. A stretch of hydrophobic residues at the COOH terminus is predicted to hold Sed5p on the cytoplasmic face of intracellular membranes.

Cells that are depleted of Sed5p are unable to transport carboxypeptidase Y to the Golgi complex, and stop growing after a dramatic accumulation of ER membranes and vesicles. We conclude that the *SED5* gene is essential for growth and that Sed5p is required for ER to Golgi transport. When Sed5p is overexpressed the efficiency of ER to Golgi transport is reduced, vesicles accumulate, and cellular morphology is perturbed. Immunofluorescence studies reveal that the bulk of Sed5p is not found on ER membranes but on punctate structures throughout the cytoplasm, the number of which increases upon *SED5* overexpression. We suggest that Sed5p has an essential role in vesicular transport between ER and Golgi compartments and that it may itself cycle between these organelles.

THE secretory pathway of eukaryotic cells consists of a number of distinct compartments and the transfer of proteins from one to another is thought to be mediated by transport vesicles (Palade, 1975). Genetic and biochemical analyses have identified many of the molecular components involved in the budding, transfer, targeting, and fusion of forward transport vesicles (Rothman and Orci, 1992). These steps are likely to involve complex molecular machines: numerous genetic interactions have been documented and a number of multicomponent protein complexes have been identified.

We are interested in the early stages of the secretory pathway, particularly the transfer and sorting of proteins between the ER and the Golgi complex. Genetic studies have already identified around 20 genes whose products are required for transport from the ER to the Golgi (Novick et al., 1980; Newman and Forro-Novick, 1987; Segev et al., 1988; Nakano et al., 1989; Nakano and Muramatsu, 1989; Stearns et al., 1990; Nakajima et al., 1991; Ossig et al., 1991; Shim et al., 1991), some of which have been assigned to specific steps (Kaiser and Schekman, 1990; Rexach and Schekman, 1991). For example, *SEC12* and *SAR1* have been suggested to interact directly in the formation of ER-derived transport vesicles (d'Enfert et al., 1991) and *SEC17* and *SEC18* appear to form part of a complex involved in the attachment or fusion of such vesicles with Golgi acceptor membranes (Wilson et al., 1992).

Upon reaching the Golgi complex certain proteins are diverted from the "bulk flow" secretory pathway and are recycled back to the ER (Pelham, 1988, 1989; Dean and Pelham, 1990). Such sorting allows soluble ER proteins that enter transport vesicles to be retrieved from later compartments. A COOH-terminal sorting signal, usually KDEL (Lys-Asp-Glu-Leu) in mammalian cells or HDEL (His-Asp-Glu-Leu) in *S. cerevisiae*, has been shown to be necessary and sufficient for such retrieval (Munro and Pelham, 1987; Pelham et al., 1988). To date only two membrane proteins, Sec20p and Sed4p, with such a signal have been described (Sweet and Pelham, 1992; Hardwick et al., 1992). However, it seems likely that other membrane components, such as those of the ER–Golgi transport vesicles, will also be recycled.

The *ERD2* gene encodes the HDEL receptor and is normally essential for growth (Semenza et al., 1990). We have carried out a screen for multicopy suppressor genes (*SED* genes, suppressors of the *erd2*-deletion) that can allow cells to grow in the absence of Erd2p. We have found that *SED1*, *SED2* (*SEC12*), *SED3* (*DPM1*), and *SED4* all encode membrane proteins, some of which have essential roles within the secretory pathway (Hardwick et al., 1992). In this report we present the sequence of *SED5* and a functional analysis of its

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product. We show that *SED5* encodes an essential 39-kD integral membrane protein that is required for ER–Golgi transport. We describe defects apparent in cells expressing abnormal levels of Sed5p, and suggest possible roles for this protein within the secretory pathway.

Materials and Methods

Plasmids

Derivatives of the original *SED5* library isolate (p*SED5*) were made by subcloning fragments into the multicopy expression vector ZUC13. Fragments were also subcloned into Bluescript (Promega Biotec, Madison, WI) for construction of deletion series and sequencing. Deletion series were made with Exonuclease III and Exonuclease VII (Yanisch-Perron et al., 1985). The *SED5* gene disruption construct (pS5KO) was made by replacing the internal MscI–NcoI fragment with XhoI–BamHI fragment containing the *LEU2* marker, which removes nucleotides 236–833 of the *SED5* gene. EcoRI and BamHI sites were introduced at the 5′ and 3′ ends of *SED5*, respectively, via polymerase chain reaction (PCR)[1] mediated, oligonucleotide-directed mutagenesis (Higuchi, 1989). PCR products were cloned into a derivative of the integration vector YIP56X (Pelham et al., 1988) under the control of the *GAL1* promoter to produce p*GAL1-SED5* and into a derivative of the multicopy expression plasmid pJS209 (Semenza et al., 1990), under the control of the triose phosphate isomerase (*TPI*) promoter to produce p*TPI-SED5*. Myc-tagged *MNT1* (kindly provided by Rowan Chapman, LMB) was cloned as a XhoI–XbaI fragment into a YIP56X derivative under the control of the *TPI* promoter.

Yeast Strains

Saccharomyces cerevisiae strain JCB102 (*MAT***a** *ade2 ade3 his3-Δ200 leu2-3, -112 ura3-52 TRP1 erd2Δ; pLE26A [Kluyveromyces lactis ERD2* expressed from the *TPI* promoter, *CEN6, URA3, ADE3*]) (kindly provided by John Boothroyd) was used for the isolation of the *SED5* gene, from a multicopy library lacking the *ERD2* gene (Hardwick et al., 1992). Overexpression of *SED5* was carried out in SEY6210 (*MATα; his3-Δ200; leu2-3, -112; ura3-52; trp1-Δ901; lys2-801; suc2-Δ9*) (kindly provided by S. Emr, University of California, San Diego, CA). For the depletion of Sed5p, a strain was constructed by first disrupting one copy of *SED5* using pS5KO (cut with XbaI) in a SEY6210/CH1305 diploid (CH1305 was kindly provided by Connie Holm, Harvard University, Cambridge, MA). Leu$^+$ transformants were then transformed with p*GAL1-SED5* linearized with XhoI to integrate the construct at the *URA3* locus. Transformants were then sporulated and ura$^+$, leu$^+$ haploid progeny were isolated, after tetrad dissection, on galactose-containing plates.

Yeast cultures were grown in rich medium containing 1% Bacto yeast extract, 2% Bacto peptone (Difco Laboratories Inc., Detroit, MI), and 2% glucose, or in minimal medium containing 0.7% yeast nitrogen base without amino acids (Difco Laboratories Inc.), 2% glucose, and supplements as described by Sherman et al. (1974). 4% galactose and 0.1% sucrose were used as carbon source for expression from p*GAL1-SED5*. Yeast transformations were carried out as described by Ito et al. (1983). Tetrads were dissected using a Singer MSM System (Singer Instrument Co. Ltd., Somerset, UK).

Antibody Production

Nucleotides 1–958 of *SED5* were amplified by PCR, thereby omitting the coding region for the hydrophobic COOH terminus of Sed5p, and inserted as a BamHI–EcoRI fragment into pGEX-2T (kindly provided by Ross Camidge, LMB), producing an inframe fusion with glutathione-S-transferase (GST). For production of GST-Sed5 fusion protein *E. coli* containing this construct were grown overnight; diluted 1:10 in fresh medium and grown for 1 h at 37°C; isopropyl β-D-thiogalactopyranoside was added to 0.1 mM and cells incubated for a further 4 h. Cells were spun down at 5,000 *g* for 10 min and resuspended in ice-cold PBS. Triton X-100 was added to 1% and the cells lysed by sonication. Insoluble material was removed by centrifugation, glutathione-agarose beads added to the supernatant, and mixed at room temperature for 5 min. The beads were collected by centrifugation and washed three times with ice cold PBS before eluting the fusion protein with 5 mM reduced glutathione in 50 mM Tris (pH 8.0). The elution was repeated three times and supernatants pooled before dialyzing against PBS. The yield of GST-Sed5 fusion protein was ~3 mg/liter. Rabbits were injected with an emulsion of Freund's complete adjuvant and fusion protein and boosted at 6-wk intervals in incomplete adjuvant (~0.5 mg of fusion protein was used per injection). Antiserum was affinity purified by first removing nonspecific antibodies by passing the serum twice over a 2-ml Affi-Gel column to which GST had been coupled, and then binding specific antibodies to a similar column to which GST-Sed5 fusion protein had been coupled, following manufacturer's instructions (Bio-Rad Laboratories, Richmond, CA). The column was washed with 0.5 M NaCl in 10 mM Tris, pH 7.5, and specific antibodies eluted with 100 mM glycine, pH 2.5.

Membrane Extractions and Western Blotting

Membranes were made by glass bead lysis as described by Ossig et al. (1991). Lysis buffer was 200 mM Tris, pH 8.0, 6 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 mM benzamidine, and 3 μg/ml pepstatin A. Extractions were performed on ice for 30 min with either: 1 ml of lysis buffer, lysis buffer containing 1% Triton X-100, 100 mM NaCl in water, or 100 mM sodium carbonate in water, pH 11.5. Membranes were pelleted at 100,000 *g* in a TL100.2 in an ultracentrifuge (TL100; Beckman Instruments, Inc., Palo Alto, CA) at 4°C. Supernatants were removed, proteins precipitated in 10% TCA for 30 min, pelleted, washed with ice-cold acetone, and resuspended in SDS sample buffer (80 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, bromphenol blue, 1 mM PMSF, 1 mM benzamidine, and 3 μg/ml pepstatin A), as were the membrane pellets. Whole cell extracts were made from log phase cells: cells were spun down, washed in water, resuspended in SDS sample buffer, glass beads (Ballotini no. 8) added, samples vortexed at 4°C for 3 min, and then boiled for 2 min. After SDS-PAGE proteins were transferred to nitrocellulose. Antibody incubations were carried out in 3% milk in PBS at room temperature. Anti-CPY serum was diluted to 1/5,000 (kindly provided by Neta Dean), affinity-purified anti-Sed5p serum to 1/200, peroxidase-conjugated goat anti-rabbit Ig to 1/4,000 (Sigma Chemical Co, St. Louis, MO). Blots were incubated for 1 min with ECL detection reagents (Amersham International, Amersham, UK) before autoradiography.

Immunofluorescence

10 ml of log phase cells were spun down and fixed with 3.7% formaldehyde in 100 mM potassium phosphate buffer, pH 6.5, for 60–90 min. Cells were washed four times with phosphate buffer and once in spheroplasting buffer (1.2 M sorbitol, 100 mM phosphate-citrate, pH 5.9) before resuspending in 5 vols of spheroplasting buffer containing 1/10 glusulase and 1/100 1% zymolyase 20T and digesting for 90 min at 30°C. Cells were then washed with spheroplasting buffer and pipetted on to polylysine-coated microscope slides. Slides were plunged into methanol (−20°C) for 6 min and acetone (−20°C) for one min and air dried before the addition of diluted antiserum. Anti-BiP serum (J. Vogel, Princeton University, NJ) was diluted to 1/30,000 in 3% milk in PBS; 9E10 to 1/500 (S. Munro, LMB) and affinity-purified anti-Sed5 serum to 1/20, incubations were carried out overnight at 4°C. Slides were washed and incubated in appropriate secondary antibodies (FITC or Texas red–conjugated sheep anti-mouse Ig or FITC-conjugated donkey anti-rabbit Ig [Amersham International] diluted to 1/50) for 1 h at room temperature, washed with PBS, and mounted as described by Kilmartin and Adams (1984). Images were obtained using an MRC-600 confocal laser-scanning microscope (Bio-Rad Laboratories).

Electron Microscopy

Yeast cells were fixed with 1.5% potassium permanganate, sectioned, and poststained as described by Sweet and Pelham (1992).

Results

We have previously reported the isolation and analysis of four *SED* genes that enable *S. cerevisiae* to grow in the absence of Erd2p (Hardwick et al., 1992). Further screening for suppressors of the *erd2Δ*, using the sectoring assay described previously, yielded a new multicopy suppressor gene that we have named *SED5*.

1. *Abbreviations used in this paper*: CPY, carboxypeptidase Y; PCR, polymerase chain reaction; *TPI*, triose phosphate isomerase.

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            M  N  I  K  D  R  T  S  E  F  Q  Q  S  V  L  S
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 Y  K  K  R  N  K  N  F  R  E  Q  Q  R  E  R  L  Q  E  K  E
TACAAGAAAAGAAACAAAAACTTTAGAGAACAGCAGAGGGAACGTCTACAAGAGAAAGAA
                                    50
 S  E  N  F  A  N  N  T  T  G  N  G  K  S  V  S  E  F  Q  K
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 K  A  S  G  I  A  H  E  I  S  S  T  A  Q  L  L  S  K  L  A
AAGGCTTCTGGCATAGCTCACGAAATTTCATCCACCGCACAATTATTATCGAAATTAGCA

 V  L  A  K  R  K  P  M  F  N  D  N  P  V  E  I  A  E  L  S
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 F  L  I  K  R  K  I  Y  A  I  E  Q  S  L  V  Q  L  S  Q  L
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                                    150
 Q  H  S  K  N  V  V  N  L  L  N  T  Q  M  K  N  I  S  G  S
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         200
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TCAGAAAAGAATAACAACTCGTCCAACCAAGGAGAACTGTCTTTCCCTCAAAACGACTCT
                                    250
 Q  L  M  L  M  E  E  G  Q  L  S  N  N  V  Y  L  Q  E  R  N
CAACTAATGTTAATGGAAGAGGGACAGTTATCCAATAACGTCTACTTACAAGAAAGAAAT

 R  A  V  E  T  I  E  S  T  I  Q  E  V  G  N  L  F  Q  Q  L
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         300
 I  D  L  N  I  S  G  A  Q  R  E  L  L  K  Y  F  D  R  I  K
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 S  N  R  W  L  A  A  K  V  F  F  I  I  F  V  F  F  V  I  W
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         340
 V  L  V  N  *
GTTTTAGTCAATTAAAAGCAAAGTAAAAGAAAAAAAGATACATATTTGATTATTTATTTT
TTTTCCTTCTCTCATCTTTTCTGTACAATAATATAGCAATAACAGATAAAAATAATGAAT
GCACATTCCCTTC
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Figure 1. Nucleotide sequence and predicted product of the *SED5* gene. The underlined amino acids represent the putative membrane anchor. These sequence data are available from EMBL under accession number X66980.

SED5 Is Predicted to Encode a Novel Membrane Protein

The *SED5* library plasmid was restriction mapped and regions of the insert were subcloned into the multicopy yeast expression vector ZUC13. The suppressing activity was found to be contained within a 3-kb XbaI fragment. When the NcoI site in this fragment was filled in it no longer suppressed the *erd2Δ*, indicating that this restriction site was within or near to the *SED5* gene. Overlapping restriction fragments were cloned into bluescript and deletion series made and sequenced. This analysis revealed a 1,020-bp reading frame (Fig. 1) containing the NcoI site at position 833. This open reading frame was amplified by PCR and cloned into a multicopy expression vector downstream of the *TPI* promoter. This construct, p*TPI-SED5*, was able to suppress

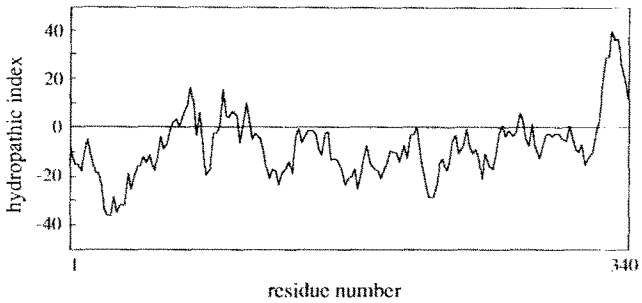

Figure 2. Hydropathy plot of the predicted *SED5* product. Hydrophibicity was calculated as in Kyte and Doolittle (1982) using a window of 11 amino acids.

the *erd2Δ*, confirming that the reading frame that had been amplified was the *SED5* gene.

Sequence analysis predicts that the *SED5* gene encodes a 39-kD protein, with 10 potential N-linked glycosylation sites. The hydropathy profile of Sed5p (Fig. 2) reveals a single hydrophobic stretch, of sixteen residues, at the COOH terminus of the protein that might be capable of attaching Sed5p to membranes. As there is no signal peptide and the bulk of Sed5p is hydrophilic, with over one third of the protein consisting of serine, threonine, glutamine, and asparagine residues, we suggest that the bulk of Sed5p is cytoplasmic.

Sed5p Is an Integral Membrane Protein

To allow the above prediction about the membrane attachment of Sed5p to be tested, we raised polyclonal antibodies to a Sed5–GST fusion protein. The bulk of the *SED5* reading frame, from nucleotides 1–958, was amplified by PCR and cloned into the pGEX-2T vector to produce a GST fusion gene. This construct was used to make a fusion protein in *E. coli* that was purified and injected into rabbits. The serum produced was affinity-purified and used to probe a Western blot of two yeast extracts, one of which was made from a strain containing p*TPI-SED5* (Fig. 3). A 39-kD band was specifically recognized by this serum and was being overex-

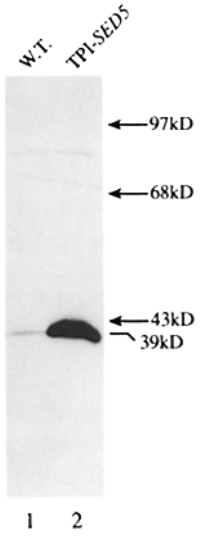

Figure 3. Immunological detection of Sed5p. Total yeast extracts were immunoblotted with affinity-purified anti-Sed5p serum. The wild-type strain SEY6210 (*W.T.*) was analyzed (lane *1*), along with the same strain containing p*TPI-SED5* (lane *2*).

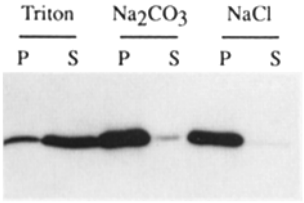

Figure 4. Sed5p is membrane associated. Cells containing p*TPI-SED5* were lysed by vortexing with glass beads and extracted with 1% Triton X-100, 0.1 M Na_2CO_3, pH 11.5, or 1 M NaCl. After centrifugation, Sed5p was detected in pellet (*P*) and supernatant (*S*) fractions by immunoblotting with affinity-purified anti-Sed5p serum.

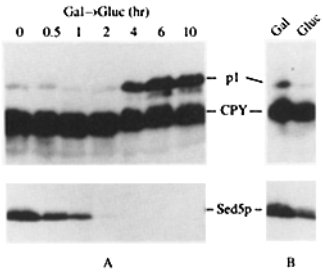

Figure 5. Depletion of Sed5p leads to the accumulation of the ER form of CPY. (*A*) A *sed5*-disrupted strain containing p*GAL1-SED5* was grown to log phase in galactose medium, then washed and switched to medium containing 2% glucose. At the time points indicated cell extracts were made and analyzed by immunoblotting with anti-CPY (*upper panel*) and anti-Sed5p (*lower panel*) antibodies. (*B*) A wild-type strain containing p*GAL1-SED5* was analyzed in a similar manner, after growth in galactose and glucose media. Positions of the pl and mature forms of CPY, and Sed5p are indicated.

pressed in the strain containing p*TPI-SED5*. One other protein, of significantly higher molecular weight, was recognized by the affinity-purified antiserum, suggesting that it may share an epitope with Sed5p. Although Sed5p contains ten potential N-linked glycosylation sites none of these appear to be used (endoglycosidase H digestion did not result in a shift in Sed5p gel mobility [data not shown]), supporting the prediction that the bulk of the protein is cytoplasmic. In addition, fusion of pro-alpha factor to the COOH terminus of Sed5p produced a protein of ~60 kD that was glycosylated, as determined by endoglycosidase H digestion (not shown), suggesting that the COOH terminus of Sed5p is membrane-associated and indeed is capable of traversing a membrane.

To test the prediction that Sed5p might be tightly associated with membranes we made glass bead extracts of whole yeast cells and treated them with a range of buffers before spinning at 100,000 *g* to pellet membrane fractions. The results (Fig. 4) indicate that neither a 1 M NaCl buffer nor a 0.1 M Na_2CO_3 buffer, pH 11.5, which would be expected to release peripheral membrane proteins, extracted Sed5p from membranes whereas 1% Triton X-100 did so efficiently. The cross-reacting protein was extracted from membranes with 1 M NaCl (not shown). Such behavior strongly supports the prediction that Sed5p is an integral membrane protein.

SED5 Is Essential for Transport between the ER and Golgi Compartments

To analyze the function of *SED5* we first constructed a disruption plasmid in which coding sequence for 200 residues of Sed5p was deleted (see Materials and Methods). This plasmid was used to transform a diploid yeast strain and a number of the resulting leu^+ colonies were sporulated. Tetrad analysis indicated that the *SED5* gene is essential for growth, as the leu^+ haploid progeny containing a disrupted *sed5* gene were unable to germinate and grow.

To examine the terminal phenotype of cells depleted of Sed5p we made an inducible *GAL1-SED5* construct and used it to transform a diploid strain containing one disrupted copy of *sed5*. A number of the ura^+ transformants were then sporulated and leu^+/ura^+ haploid progeny were selected on plates containing galactose. These cells have their *sed5* gene disrupted with the *LEU2* marker and are kept alive by the inducible *GAL1-SED5* construct integrated at the *URA3* locus. Such cells were grown to log phase in galactose medium, washed, and then resuspended in medium containing 2% glucose which would repress expression from the *GAL1* promoter. At 2-h intervals cell samples were taken and analyzed by Western blotting and thin-section EM. Growth slowed after 4 h and stopped 2 h later. Immunoblotting of whole cell extracts (Fig. 5 *A*) revealed a gradual depletion of Sed5p which was complete after 4 h of growth in glucose-containing medium. In parallel with this depletion of Sed5p, the ER-modified form (pl) of the vacuolar protease carboxypeptidase Y (CPY) was seen to accumulate. This suggests that Sed5p is required for the transport of proteins from the ER to the Golgi complex. In agreement with this, analysis of cells by thin-section EM revealed the gradual accumulation of ER membranes and scattered vesicles within cells depleted of SED5p (Fig. 6). After 6 h of growth in glucose when Sed5p was no longer detectable by immunoblotting, large clusters of vesicles were observed in many cells.

The accumulation of ER membranes suggests that Sed5p is important in vesicle budding from the ER, but the accumulation of clusters of vesicles, most noticeable at later time points, suggests a role in vesicle targeting or fusion. One way to account for both phenotypes would be if Sed5p is a component of transport vesicles and has multiple roles in budding, targeting, and/or fusion reactions. Alternatively, the primary defect in cells depleted of Sed5p may be in vesicle fusion, and the accumulation of ER membranes could be an indirect effect. For example, the accumulation of transport vesicles could lead to the depletion from the ER of another recycling protein that is required for vesicle formation or budding. Analysis of the time course of Sed5p depletion favors the former model as the accumulation of ER membranes was apparent at early time points and the clusters of vesicles were only seen after 6 h of Sed5p depletion.

Dominant Effects of the Overexpression of SED5

Multiple defects were observed when Sed5p was overproduced. The strain analyzed in Fig. 5 *B* contains both a wild-type *SED5* gene and the integrated *GAL1-SED5* construct. In the first lane, where the cells had been grown in galactose medium, Sed5p was being overexpressed and significantly higher levels of the pl CPY intermediate were present, when compared with the second lane where cells were grown in glucose and only the wild-type gene was being expressed. We have observed a similar reduction in the efficiency of ER to Golgi transport when *SED5* was expressed, either from its own promoter (p*SED5*) or the *TPI* promoter (p*TPI-SED5*), on a multicopy vector.

To determine whether Sed5p has any dominant effects on intracellular membrane organization we have analyzed sev-

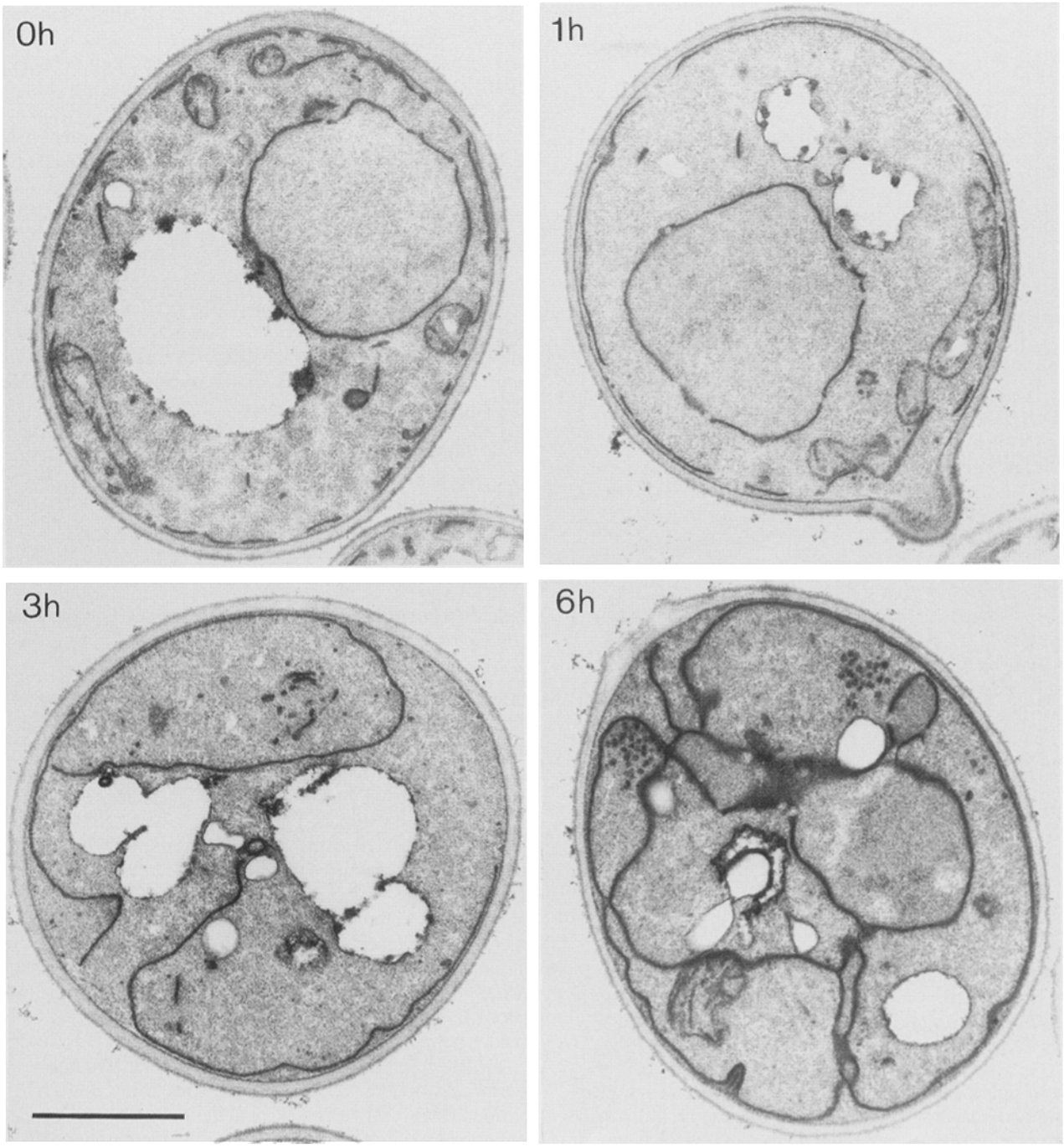

Figure 6. Sed5p-depleted cells accumulate ER membranes and putative transport vesicles. A *sed5*-disrupted strain containing p*GALI-SED5* was grown to log phase in galactose medium, then washed and switched to medium containing 2% glucose. At the time points indicated cells were fixed with potassium permanganate. Bar, 1 μm.

eral strains overexpressing Sed5p by thin-section EM. Cells containing p*TPI-SED5* contained an increase in the number of vesicular structures (Fig. 7 *A*). These may be transport vesicles of one or more stages of the secretory pathway or result from the fragmentation of compartments. No accumulation of ER membranes was apparent.

We have previously described membrane defects in cells depleted of the HDEL receptor (Semenza et al., 1990). We found that upon depletion of Erd2p, ER to Golgi transport continues, but that Golgi-modified precursors and membranes accumulate. The Golgi complex increases in size, presumably due to the accumulation of ER proteins and lipids that are normally recycled. This accumulation appears to impair the transport of proteins through the Golgi complex and eventually halts growth. The Golgi membranes that accumulated were not stacked as in *sec7* cells (Novick et al., 1981) and no accumulation of transport vesicles was observed. We have found that overexpression of Sed5p enables cells to grow in the absence of Erd2p. EM analysis of the *erd2Δ*.p*SED5* strain (Fig. 7 *B*) revealed a dramatic accumu-

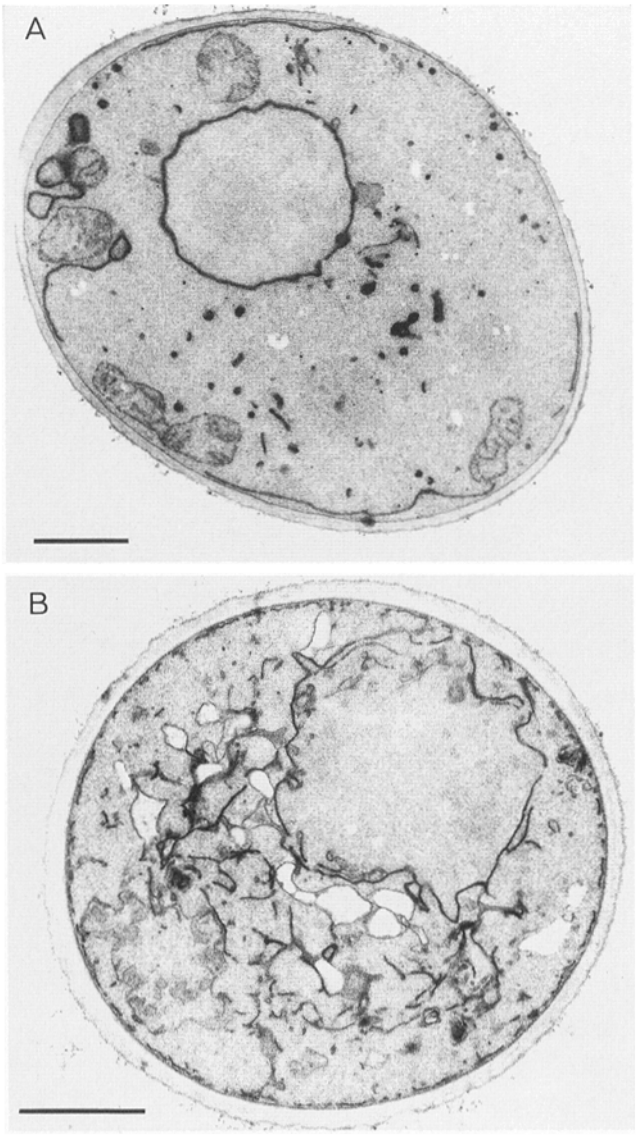

Figure 7. Thin-section electron micrographs of cells overexpressing *SED5*. (*A*) SEY6210, a wild-type strain, containing p*TPI-SED5*. (*B*) An *erd2*-disrupted strain containing p*SED5*. Bars, 1 μm.

lation of intracellular membranes and vesicles. Such membranes and vesicles were not observed in most other *SED*-suppressed-*erd2Δ* strains (Hardwick et al., 1992), nor in cells depleted of Erd2p, and we believe that they mainly reflect the ability of Sed5p overexpression to lead to membrane fragmentation and vesicle production. Indeed, this could be the mechanism by which Sed5p allows cells to grow without the HDEL receptor. The production of vesicles from the Golgi complex would reduce its size and could deplete it of ER components interfering with its function.

A number of other, perhaps indirect, defects were apparent in cells containing very high levels of Sed5p. Transformants containing p*TPI-SED5* grew at slower rates than control strains (the doubling time was increased ~threefold), and analysis of cells in the light microscope revealed a significant increase in cell size (compare Fig. 8, *A* and *B*). We also observed an increase in the level of secretion of the luminal ER protein BiP (greater than a twofold increase during 2 h of log phase growth). Such an *erd* phenotype has previously been observed in *sec17, sec18, sec20,* and *sec22* alleles grown at their permissive temperature (Semenza et al., 1990). These strains form the vesicle-accumulating class of ER–Golgi *sec* mutants. It has been suggested that the accumulation of transport vesicles leads to a depletion of Erd2p within the Golgi complex and thereby a reduction in the efficiency of HDEL sorting. The accumulation of vesicles seen upon overexpression of Sed5p supports this hypothesis, although we can not rule out the possibility that excess Sed5p leads to other targeting defects within the cell and the fusion of transport vesicles with inappropriate compartments. The slow growth and increase in cell size may reflect a reduction in the efficiency of the targeting of late secretory vesicles to the bud. Preliminary experiments suggest that the actin cytoskeleton, which is known to be important for the targeting of late secretory vesicles, becomes disrupted upon Sed5p overexpression. However, other early *sec* mutants show similar phenotypes (Sweet, D., personal communication) suggesting that this may be an indirect effect caused by other changes within the secretory pathway, rather than reflecting a direct interaction between Sed5p and late secretory vesicles or the cytoskeleton.

SED5 Is Localized to Punctate Structures Throughout the Cytoplasm

In light of the multiple defects described above we examined the intracellular distribution of Sed5p. We carried out a number of immunofluorescent studies with the affinity-purified anti-Sed5p antibodies. In wild-type cells we observed a small number of brightly staining punctate structures, together with a fainter background of punctate staining throughout the cytoplasm (Fig. 8 *A*). Upon Sed5p overexpression the cells became larger, the staining brighter, and the number of structures that were labeled increased (Fig. 8 *B*). When the *sed5Δ* strain containing p*GAL1-SED5* was analyzed by immunofluorescence after growth in galactose medium, punctate structures were observed. When this strain was analyzed after growth in glucose medium, when *SED5* was not expressed, there was no punctate staining (not shown). We conclude that the punctate staining observed in wild-type cells and in cells containing p*TPI-SED5* is Sed5p.

To determine the nature of these punctate structures we performed double labeling experiments using anti-HDEL antibodies to localize the ER, and the mAb 9E10 to localize epitope-tagged Mntlp and thereby Golgi membranes. *MNT1* encodes an α-1,2-mannosyltransferase (Hausler and Robbins, 1992) which, although Mntlp has not previously been localized by immunofluorescence, is thought from biochemical and genetic studies to function in a Golgi compartment (Franzusoff and Schekman, 1989; Hausler and Robbins, 1992). Fig. 8 *C* shows the typical ER pattern, of nuclear envelope plus a ribbon-like structure at the periphery of the cell, seen using anti-BiP serum. The Sed5p-containing structures were found throughout the cytoplasm and therefore cannot wholly consist of ER-derived membranes. In fact we were unable to detect any significant staining of ER structures with the anti-Sed5p antibody (double labeling with anti-HDEL mAb, 2E7, not shown). The staining observed for Mntlp, using the 9E10 mAb, did resemble that of Sed5p: numerous punctate structures were found throughout the

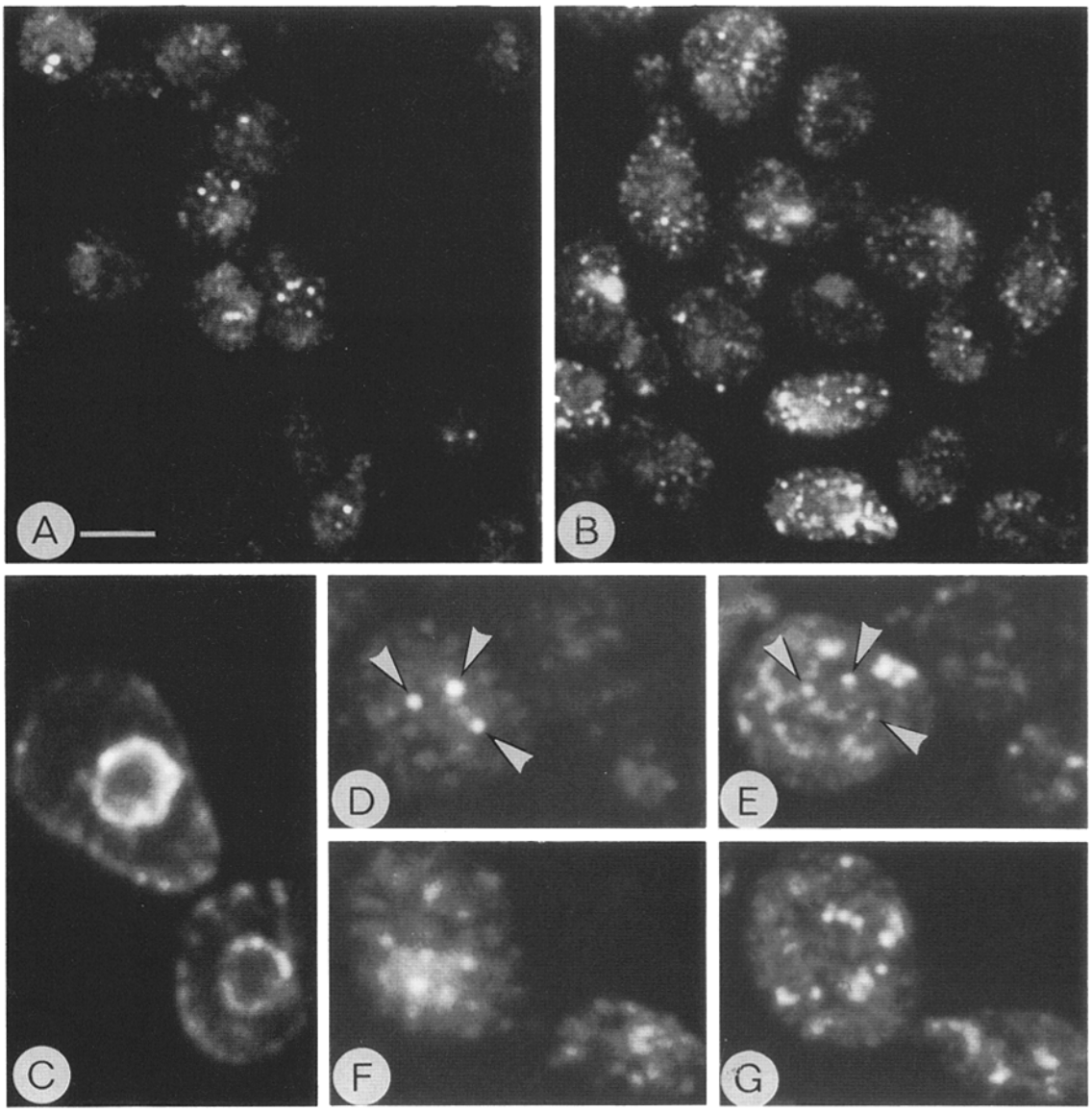

Figure 8. Sed5p is localized to punctate structures throughout the cytoplasm. (*A*) Immunofluorescent analysis of SEY6210 (wild-type strain) with anti-Sed5p serum; (*B*) SEY6210 containing p*TPI-SED5*, with anti-Sed5p serum; (*C*) SEY6210 with anti-BiP serum; (*D* and *F*) SEY-6210 containing p*TPI-MNTI* with anti-Sed5p serum; and (*E* and *G*) the same cells with 9E10 to detect myc-tagged Mntlp. *C–G* are at twice the magnification of *A* and *B*. Brightness levels have been normalized, and therefore should not be compared. Bar, 5 μm.

cytoplasm (Fig. 8, *E* and *G*). However, double labeling revealed that only a few of these, and in some cells none, colocalized with Sed5p-containing structures (Fig. 8, *D–G*). We conclude that the bulk of Sed5p is not found in the ER, and that at least a fraction of it is localized within the Golgi complex but that most of it is not in the same compartment as Mntlp. These studies would be unable to visualize a low level of Sed5p dispersed throughout the ER; a localization one might predict if it were a cycling constituent of transport vesicles.

When the strain containing p*TPI-SED5* was studied by thin-section EM an accumulation of vesicles was observed (Fig. 7 *A*), and immunoblotting revealed a reduction in the efficiency of ER to Golgi transport. Our immunofluorescence studies revealed an increase in the numbers of punctate structures labeled with anti-Sed5p antibodies in this strain (Fig. 8 *B*) when compared to SEY6210 (Fig. 8 *A*). We therefore suggest that many of these structures, found throughout the cytoplasm, could be ER–Golgi transport vesicles. The overexpression of Sed5p may titrate out other proteins required for vesicle consumption and thereby lead to the accumulation of transport vesicles and a reduction in the efficiency of ER to Golgi transport. Without further Golgi markers we cannot rule out the possibility that Sed5p is also found on multiple Golgi compartments, due to the dispersed nature of this organelle in *S. cerevisiae* (Franzusoff et al., 1991).

Discussion

We have shown that *SED5* encodes a novel integral membrane protein essential for ER to Golgi transport. Immunofluorescence studies revealed that the bulk of Sed5p is not found on ER membranes but on punctate structures

throughout the cytoplasm. Depletion of Sed5p leads to the accumulation of the ER form of CPY along with ER membranes and clusters of putative transport vesicles. Overexpression of Sed5p leads to a number of defects including a reduction in the efficiency of ER to Golgi transport.

What Is the Function of Sed5p?

At early time points of Sed5p-depletion ER membranes and scattered vesicles accumulated, indicting that there were multiple defects in vesicle budding and targeting/fusion. At later time points of Sed5p-depletion clusters of vesicles were observed, highlighting the defects in vesicle consumption. It is possible that Sed5p is an essential constituent of transport vesicles and that it is involved in both vesicle budding and targeting/fusion reactions. Depletion of Sed5p would lead to defects in vesicle budding and thereby an accumulation of ER membrane. In addition, any vesicles that did bud off from the ER but that lacked Sed5p would be defective in targeting/attachment/fusion reactions and would therefore accumulate. The bulk of Sed5p is not found in the ER, as judged by immunofluorescence. Therefore, to have a role in vesicle budding from the ER, Sed5p must cycle through this compartment, presumably via transport vesicles. These could be the faint punctate structures containing Sed5p visualized by immunofluorescence. Alternatively, Sed5p may be a constituent of Golgi membranes with a role in vesicle docking or fusion. This could account for the vesicle accumulation observed upon depletion of Sed5p. The accumulation of ER membranes would then be an indirect effect, possibly because of the sequestration of another protein, that is required for vesicle budding, within the accumulated transport vesicles. Our EM analysis of the time course of Sed5p depletion favors the former model as the accumulation of ER membranes was apparent at early time points whereas the clusters of vesicles were only seen after 6 h of Sed5p depletion.

When Sed5p was overexpressed the efficiency of ER to Golgi transport was reduced and an increase in the numbers of putative transport vesicles was observed by EM. This correlated well with the increase in the numbers of Sed5p-containing structures observed by immunofluorescence, supporting the model that Sed5p is a constituent of transport vesicles. Overexpression of Sed5p may lead to the production of vesicles that, although they do contain Sed5p, do not contain other proteins required for their efficient transfer to the Golgi complex. Alternatively, if Sed5p is a constituent of Golgi membranes, its overexpression may affect the structure and function of this "acceptor" compartment and thereby reduce the efficiency of vesicle consumption.

It is also possible that some of the vesicles seen to accumulate upon Sed5p depletion are retrograde transport vesicles. It is difficult to determine genetically whether Sed5p has a role in Golgi to ER retrograde transport because of its significant role in the forward pathway. However, using the anti-Sed5p antibodies we should now be able to determine whether Sed5p is enriched within transport vesicle fractions or Golgi membranes, and define more precisely its role in vesicular transport.

The *ERD2* gene encodes the yeast HDEL receptor and is essential for growth (Semenza et al., 1990). When Erd2p is depleted in cells their Golgi complex becomes severely disrupted, both functionally and morphologically. We believe that the essential function of Erd2p is to promote retrograde transport and thus maintain a balance in the traffic of membrane constituents to and from the Golgi complex. There are two ways that overexpression of Sed5p could enable cells to balance Golgi membrane traffic in the absence of Erd2p. One would be to reduce the flow of membrane into the Golgi complex, by reducing the efficiency of ER to Golgi transport. The other way would be to stimulate transport from the Golgi complex, either back to the ER or to the cell surface. Here we have shown that *SED5* overexpression leads to a reduction in the efficiency of ER to Golgi transport, and also to the production of transport vesicles some of which may be Golgi derived. We are at present uncertain which property of Sed5p it is that allows cells to grow without the HDEL receptor.

Although Sed5p is not closely related to any known protein, it shares some features with other proteins that are thought to be involved in vesicular transport. Adjacent to the COOH-terminal membrane anchor of Sed5p is a stretch of predicted amphipathic helix (residues 252–315) similar to those found in proteins with a coiled-coil motif, carrying a net charge of −6. A similar acidic coiled-coil motif, adjacent to a COOH-terminal membrane anchor, is found in the yeast *PEP12* product (K. Becherer and E. Jones, GenBank sequence YSCPEP12P), and in the rat protein syntaxin (Bennett et al., 1992). Both of these proteins show ~33% sequence identity to Sed5p in this region; they are not obviously homologous in other regions, although they are quite similar in size and each has other stretches of predicted amphipathic helix. Pep12p is required for transport of proteins to the vacuole; it is found predominantly on the vacuolar membrane, and possibly also in the Golgi complex (op. cit. by Preston et al., 1991). Syntaxin has been implicated in the targeting of synaptic vesicles to the plasma membrane, and is thought to be at least partly located on the plasma membrane (Bennett et al., 1992). These analogies in structure and function suggest that Sed5p may be a member of a family of proteins that perform similar roles in vesicular transport, perhaps in the targeting step. The overall structure of Sed5p is also reminiscent of that of Bos1p, Sly2p, and Sly12p, yeast membrane proteins involved in ER–Golgi transport, which are themselves similar to the synaptobrevins found on synaptic vesicles (Shim et al., 1991; Ossig et al., 1991).

To date few integral membrane proteins of the secretory pathway have been described. However, it is clear that a number of such proteins are likely to exist with important functions in vesicular transport. For example, an integral membrane protein is thought to act as a receptor for the SNAP proteins complexed with NSF protein on the surface of uncoated intra-Golgi transport vesicles (Wilson et al., 1992). In addition, it seems likely that peripheral membrane proteins of coated membrane structures, such as the ARF and COP proteins (Rothman and Orci, 1992), will interact with integral membrane proteins. Further biochemical analysis should indicate whether Sed5p performs one of these, or some other essential role in vesicular transport. Analysis of further *SED* genes is in progress and may lead to the identification of further novel membrane components of the secretory pathway.

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