Localization of Components Involved in Protein Transport and Processing through the Yeast Golgi Apparatus

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Abstract. *Saccharomyces cerevisiae sec7* mutants exhibit pleiotropic deficiencies in the transit of proteins through the Golgi apparatus, and elaborate an array of Golgi apparatus–like cisternae at a restrictive growth temperature (37°C). The *SEC7* gene encodes an essential high-molecular weight protein (227 kD) that is phosphorylated in vivo. In cell lysates, Sec7 protein (Sec7p) is recovered in both sedimentable and soluble fractions. A punctate immunofluorescent pattern of Sec7p-associated structures seen in *SEC* cells coalesces in *sec14* mutant yeast that accumulate exaggerated Golgi cisternae at 37°C. Sec7p may function as a peripheral membrane protein that cycles between a soluble, cytosolic pool and a sedimentable, membrane-associated complex for its essential role in vesicular traffic through the Golgi apparatus.

The transmembrane Kex2 protease, which processes precursors of secreted peptides within the yeast secretory pathway, is also localized by indirect immunofluorescence to multiple structures in the yeast cell (Redding, K., and R. Fuller, manuscript submitted for publication). In double-immunofluorescence labeling experiments, significant colocalization of Sec7 and Kex2 proteins was found. Colocalization of the two antigens, one implicated in protein transport through the Golgi apparatus and the other in processing within a late Golgi compartment, supports the conclusion that we have visualized the yeast Golgi apparatus.

MEMBRANE traffic and protein compartmentalization in the secretory pathway of eukaryotic cells is maintained by vesicular intermediates. The complex processes of vesicular budding from one compartment with targeting and subsequent fusion to the next compartment involve a host of cytosolic and membrane protein components. The requirement for some cellular components is reiterated at multiple steps in the pathway, such as for the *N*-ethylmaleimide-sensitive factor (NSF)[1] in vesicle fusion with the target compartment (Wilson et al., 1989; Beckers et al., 1989; Diaz et al., 1989). Conversely, secretory pathway components involved in vesicle recognition and targeting to the various compartments are predicted to function at unique points in the pathway. Several approaches have been adopted to identify and to characterize proteins that facilitate these vesicular traffic processes. The biochemical reconstitution of different stages of intracellular protein transport has revealed several cellular components that participate in vesicle fusion in mammalian cells (Malhotra et al., 1988, 1989; Glick and Rothman, 1987; Block et al., 1988; Melancon et al., 1987; Weidman et al., 1989; Clary et al., 1990; Wattenberg et al., 1990).

Genetic selection in the yeast *Saccharomyces cerevisiae* was used to isolate a series of temperature-sensitive (*sec*) mutants affecting protein transport at various stages in the secretory pathway (Novick et al., 1980). Two mutations, *sec7* and *sec14* disrupt glycoprotein traffic from the Golgi apparatus and accumulate exaggerated Golgi cisternae at the non-permissive temperature (Novick et al., 1981). The *sec7* mutation abrogates protein traffic destined for both the lysosome-like vacuoles and the cell surface (Novick et al., 1981; Stevens et al., 1982; Franzusoff and Schekman, 1989). Examination of glycoprotein intermediates accumulated in *sec7* yeast at 37°C led us to propose that Sec7 protein (Sec7p) function was required at multiple steps in vesicular traffic through compartments of the Golgi apparatus (Franzusoff and Schekman, 1989). The sequence of the SEC7 gene predicts a high-molecular weight polypeptide (227 kD) with an unusual charged acidic domain near the amino terminus of the protein (Achstetter et al., 1988). In this report, we describe that Sec7p is found in both soluble and sedimentable protein pools from wild-type yeast lysates.

The Kex2 protease is a transmembrane glycoprotein that processes precursors of the α-factor and killer toxin polypeptides at pairs of basic residues during their transit of the secretory pathway (Fuller et al., 1988, 1989*a*, *b*). Accumulation of both pro-α-factor and mature α-factor species in *sec7* mutant cells at the non-permissive temperature suggested that transport to the processing, possibly late, compartment of the Golgi, depends on the *SEC7* gene product (Julius et

1. *Abbreviation used in this paper*: NSF, *N*-ethylmaleimide-sensitive factor.

al., 1984; Franzusoff and Schekman, 1989). Kex2 protease, like Sec7p, is shown by indirect immunofluorescence to identify multiple, discrete subcellular structures dispersed throughout the cell (Redding, K., and R. S. Fuller, manuscript submitted for publication). Evidence that both the sedimentable Sec7p species and the Kex2 protease are associated with the yeast Golgi apparatus is presented here. These data are consistent with a role for Sec7p in vesicular budding and traffic between compartments of the yeast Golgi apparatus.

Materials and Methods

Buffers and Growth Media

Isotonic lysis buffer consisted of 0.7 M sorbitol, 5 mM $MgSO_4$, 5 mM MES buffer, pH 6.5. Buffer H consisted of 0.7 M sorbitol, 5 mM $MgSO_4$, 1 mM EGTA, 1 mM DTT, 10 mM Hepes buffer pH 7.4, protease inhibitor mix, 0.05 mg/ml BSA. Buffer T was the same as Buffer H, except for the substitution of 10 mM Tris buffer pH 8.8. Buffer WT consisted of 50 mM Na-Hepes pH 7.5, 150 mM NaCl, 0.5 mg/ml BSA, 1% nonfat dry milk, 0.1% Tween 20, 5 mM sodium azide. Protease inhibitor mix consisted of 1 mM PMSF, 5 μg/ml each of leupeptin, pepstatin and chymostatin. YP media consisted of 1% yeast extract, 2% bacto-peptone (Difco Laboratories Inc., Detroit, MI). One OD_{600} unit of yeast represents 10^7 cells.

Immunoreagents

For experiments involving the Sec7p immunofluorescence alone, FITC-coupled goat anti-rabbit secondary antisera were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN) and used at 1:200 dilution. For the colocalization studies, the Fab fragment of affinity-purified goat anti-rabbit IgG (H + L) was generously donated by Jackson ImmunoResearch Laboratories (West Grove, PA). Affinity-purified mouse anti-rabbit IgG (H + L, minimal cross-reaction to goat and mouse serum proteins) and fluorescent antibodies [$F(ab')_2$] fragments of affinity-purified rabbit anti-goat IgG conjugated to Texas Red and affinity-purified rabbit anti-mouse IgG conjugated to fluorescein) were also from Jackson ImmunoResearch Laboratories. Affinity-purified anti-Kex2 antibody was approximately 0.8 mg/ml in specific IgG. An anti-peptide antiserum that recognizes yeast β-tubulin (anti-β-tubulin) was a gift from Lorraine Pillus (Division of Genetics, University of California, Berkeley) (Bond et al., 1986; Pillus and Solomon, 1986). Anti-Carboxypeptidase Y sera used in this study was described previously (Stevens et al., 1982).

Plasmids and Strains

Plasmids harboring the cloned *SEC7* gene have been previously described (Achstetter et al., 1988). *Escherichia coli* strains BMH 71-18 (Ruther and Muller-Hill, 1983), SE10 (Achstetter et al., 1988), and pop2136 (Stanley and Luzio, 1984) were used as bacterial hosts. The yeast strains 15-9B (Achstetter et al., 1988), GPY59 (Baker et al., 1988), SEY2102 (Emr et al., 1983), SF544 (*secl-1* yeast) and HMSF169 (*secl4* yeast) (Novick et al., 1980) were used in this study.

A 3,300-bp fragment containing the complete *KEX2* structural gene (Fuller et al., 1989*a*) was inserted behind the inducible *GAL1* promoter in vector pBM258 to create pBM-KX22. Plasmid pBM743 (gift of M. Johnston, Washington University, MO) served as a vector control, and differs from pBM258 by the inclusion of poly-linker sequences adjacent to the *GAL1* promoter. Plasmids pBM743 and pBM-KX22 are described in greater detail elsewhere.

Both copies of the *KEX2* gene were entirely deleted and replaced with selectable markers in the diploid yeast strain BFY125 (genotype: *MATa/MATα kex2Δ2::HIS3-A kex2Δ2::TRP1-S ade2/ade2 can1-100/can1-100 his3-11,-15/his3-11,-15, leu2-3,-112/leu2-3,-112 trp1-1/trp1-1 ura3-1/ura3-1*). Characterization of yeast strains lacking the Kex2 protease (*kex2Δ* strains) will be presented elsewhere. Plasmids were introduced by spheroplast transformation, as described (Burgers and Percival, 1987).

Anti-Sec7p Sera

Three hybrid-fusion proteins were prepared to immunize rabbits and to verify the specificity of the resultant sera. The Bgl I–Pst I fragment from the cloned *SEC7* gene (Achstetter et al., 1988) was inserted into the pUR290 vector (Ruther and Muller-Hill, 1983) to make an in-frame fusion of the bacterial *lacZ* and yeast *SEC7* genes. The resultant plasmid pTA50 was introduced into *Escherichia coli* strain BMH71-18 and the transformant was induced with isopropyl-thio β-galactoside to express a *lacZ-SEC7* fusion gene product (M_r ~270,000, Fig. 1 *A*). Extracts of the induced cells were prepared by adding 5× Laemmli sample buffer (Laemmli, 1970) plus 1 mM PMSF and 20 μg/ml pepstatin, and heating at 95°C 5 min. This hybrid protein was isolated from SDS gels and used for immunizing rabbits, as described by Nakano et al. (1988). Primary subcutaneous injections contained 50–100 μg of the protein in complete Freund's adjuvant. 6-wk after the initial immunization, boosts of similar amounts of hybrid protein in incomplete Fruend's adjuvant were given every 2 wk. Serum obtained after the fifth boost was used in experiments for this study without further purification, except as indicated.

The same fragment of the *SEC7* gene was also inserted into pATH10 (obtained from T. Koerner and A. Tzagoloff, Dept. of Biological Sciences, Columbia University) to make an in-frame fusion of the bacterial *trpE* and yeast *SEC7* genes. The resultant plasmid pTAF1-2 was introduced into *E. coli* strain SE10 and the transformant was induced by adding 10 μg/ml indole acrylic acid to exponentially growing cells for expression of the *trpE-SEC7* hybrid fusion protein (M_r ~190,000, Fig. 1 *A*). Extracts of the induced cells were prepared as described above. *E. coli* SE10 cells were also transformed with the pATH10 vector alone, and lysates were prepared in the same manner.

Another hybrid construct was created by inserting the 0.7-kb Eco RI–Eco RI fragment from the 5′ end of the *SEC7* gene into the pEX2 plasmid to generate a different *lacZ-SEC7* gene fusion. The resultant plasmid pAF9 was introduced into *E. coli* strain pop2136, and the hybrid-fusion protein was induced by shifting exponentially growing cells to 42°C for 2 h, as described by Stanley et al. (1984). The hybrid fusion protein was purified from SDS gels as above, and used for immunization of rabbits (M_r ~163,000, Fig. 1 *A*). This hybrid fusion protein showed anomalous (slower) electrophoretic mobility on SDS gels than predicted by the estimated molecular mass of the protein. This was presumably due to the high content of acidic residues in the Sec7 portion of the hybrid fusion polypeptide. Titer against yeast Sec7p epitopes was detected 2.5 mo after primary immunization (initial plus two booster injections).

Antiserum against the LacZ-antigen fragment 1 (anti-Sec7p[large] sera) was contaminated with antibodies that gave rise to speckled Western blots, but had no effect on the immunoprecipitation of Sec7p. This appeared to be a response to acrylamide and could be removed by preincubating the serum with pieces of Laemmli stacking gel in Western blocking buffer for at least 1 h at 23°C, or overnight at 4°C.

Competition experiments were performed to verify the specificity of anti-Sec7p[large] serum. Antiserum was preincubated overnight at 4°C in PBS and 0.5% Triton X-100, with either the *E. coli* extract induced for *trpE* alone (pATH10 plasmid) or extract induced for hybrid fusion *trpE-SEC7* gene product (pTAF1-2 plasmid). This mixture was further incubated with extracts of radiolabeled yeast for immunoprecipitation experiments or with formaldehyde-fixed cells for indirect immunofluorescence (see below).

The IgG component of both the pre-immune and anti-Sec7p[large] serum was purified for $[^{32}P]H_3PO_4$ labeling experiments to remove any source of phosphatases. The serum was loaded onto a protein A-Sepharose CL-4B column in phosphate buffered saline at 6 ml/h. IgG was eluted from the column with 0.1 M citrate pH 3 buffer (protein fractions monitored by A_{260}) and the eluate was immediately titrated to neutrality with 2M Tris base. The peak protein fractions were pooled, dialyzed against 20% glycerol, 5 mM sodium azide in PBS and stored at −80°C until use.

Fractionation and Western Immunoblotting Analysis

Yeast were grown overnight at 24°C in YP media containing 5% glucose. Cells (200 OD_{600} units) were harvested by centrifugation at 24°C, then converted to spheroplasts with 20 U lyticase per OD_{600} cells for 15 min at 30°C as described previously (Scott and Schekman, 1980). Spheroplasts were washed and resuspended in 0.1 ml ice-cold isotonic lysis buffer and set on ice. Acid-washed glass beads (0.3–0.5-mm diam) were added to the tube, and the suspension was agitated at half-maximal speed on a Vortex mixer (Scientific Industries, Bohemia, NY) 5 times for 10 s with 15 s intervals on ice. The lysate was diluted to 1 ml with lysis buffer, centrifuged at 750 *g* for 10 min at 4°C and the supernatant fraction (LSS) was transferred to a fresh tube on ice. Aliquots of the LSS (2 OD_{600} units) were diluted 10-fold into Buffer H or Buffer T plus urea, detergents or salts for treatment of the lysate (solutions are described above). The mixtures were incubated 30 min at 4°C, then centrifuged 12 or 24 min at 80 krpm at 4°C (forces

sufficient to bring down protein complexes >50 and 25S, respectively) in a TL100 ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA).

Proteins from the supernatant were precipitated in 9 vol of ice-cold methanol for 10 min at 4°C, then sedimented in a microcentrifuge for 5 min (12,000 *g*). We have found that Sec7p was not quantitatively precipitated by TCA. The precipitates were dried in a Speed-Vac (Savant Instruments, Hicksville, NY) for 5 min, then resuspended in 30 μl 3× Laemmli sample buffer and heated at 95°C for 5 min.

The ultracentrifugation pellet fractions were resuspended in 30 μl 3× Laemmli sample buffer and heated at 95°C for 5 min. The entire pellet and supernatant protein fractions were resolved on 7.5% SDS gels then transferred to nitrocellulose filters for Western immunoblotting analysis, as described previously (Burnette, 1981; Franzusoff and Schekman, 1989). [^{125}I]Protein A (prepared in this laboratory) was used for detection in the analysis. Autoradiograms were prepared by exposing the blots at −85°C to pre-flashed Kodak X-Omat AR films and an intensifying screen. Fluorograms were quantified by scanning densitometry with a Kratos model SD3000 spectrodensitometer coupled to a Kratos SD5300 density computer (Kratos Analytical Instruments, Ramsey, NJ) and an integrator (model no. 3380A; Hewlett-Packard Co., Palo Alto, CA).

Radiolabeling and Immunoprecipitation

The protocol for radiolabeling yeast strains with [^{35}S]H_2SO_4 and immunoprecipitation of labeled proteins is described elsewhere (Franzusoff and Schekman, 1989). 4 μl of anti-Sec7p sera (raised against either antigen) was sufficient to immunoprecipitate the protein from extracts of 1 OD_{600} yeast cells. Most of the strains used to study Sec7p were deficient in the *PEP4* gene product (or *PRA*), one of the vacuolar proteases, since Sec7p was degraded in lysates of wild type strains, even in the presence of protease inhibitors.

The protocol for radiolabeling yeast strains with [^{32}P]H_3PO_4 was similar to that with [^{35}S]SO_4 with the following modifications. Cells were grown overnight in sulfate- and phosphate-free synthetic minimal medium supplemented with 100 μM $(NH_4)_2SO_4$ and 100 μM KH_2PO_4 (Wickerham, 1946; Sherman et al., 1986). Yeast were labeled at 2 OD_{600} U/ml with 100 μCi [^{32}P]H_3SO_4 or 300 μCi [^{35}S]SO_4 per OD_{600} cells in the same medium supplemented with 5 μM $(NH_4)_2SO_4$, no phosphate for 1 h at 30°C. Extracts were prepared by glass-bead lysis as described above.

Indirect Immunofluorescence Microscopy

Sec7p-associated structures were visualized by indirect immunofluorescence as described previously (Jacobs et al., 1988). Yeast were fixed in minimal growth medium adjusted to 5% (vol/vol) formaldehyde for 2 h at 23°C. After washing the fixed cells with 1.2 M sorbitol, 0.1 M KH_2PO_4, 1% β-mercaptoethanol to remove the formaldehyde, the cells were gently spheroplasted with 10 units lyticase per OD_{600} cells for 10 min at 30°C. After washing in the same buffer without β-mercaptoethanol, the cells were prepared for indirect immunofluorescence. Both types of anti-Sec7p sera were used at 1:200 dilution in NP-40-containing blocking buffer and incubated for 1 h at 24°C with the fixed yeast. After incubation with the FITC-coupled secondary antibodies and DAPI solution (4′,6-diamidino-2-phenylindole, from Sigma Chemical Co., St. Louis, MO), mounting solution containing phenylamine diamine (Sigma Chemical Co.) was added to the wells to reduce fluorescence photobleaching during microscopy. Fluorescence photomicroscopy was performed using a Zeiss Photomicroscope III-RS with exciter barrier filter combinations suitable for DAPI and fluorescein detection onto either Kodak Professional Ektachrome P800/1600 film or T400-Max black-and-white film.

Double-Label Immunofluorescence

Kex2-overproducing yeast (BFY125[pBM-KX22]) and Kex2-deficient cells (BFY125[pBM743]) were grown at 30°C in synthetic complete growth medium (Sherman et al., 1986) lacking uracil, L-isoleucine, L-glutamic acid, L-aspartic acid, L-valine, and L-serine, and containing 2% galactose/0.1% glucose. Growth of strain BFY125[pBM-KX22] on this medium led to a 15-fold elevation of Kex2 protein relative to wild-type cells. Kex2 protein was overexpressed because the antibody amplification procedure needed to visualize wild-type levels of Kex2 protein was incompatible with double-labeling experiments. This extent of overproduction did not appear to alter the subcellular localization of Kex2 protein.

To reduce nonspecific immunofluorescence in the fixed yeast, antisera (except the anti-β-tubulin serum) were preadsorbed to formaldehyde-fixed spheroplasts or whole cells. Anti-Sec7p and preimmune sera were preadsorbed to fixed cells (BFY125). Anti-Kex2 sera was preadsorbed to fixed spheroplasts lacking Kex2 (BFY125[pBM743]), and the remaining antibodies to fixed spheroplasts overproducing Kex2 (BFY125[pBM-KX22]).

Because the available Sec7p-specific and Kex2-specific sera were raised in rabbits, a method was developed that permitted dual labeling without detectable cross-reaction. The two primary rabbit antibodies were saturated with secondary "linker" antibodies raised in different species. The key feature of this procedure was saturation of the first rabbit primary antibody with a monovalent goat anti-rabbit Fab before addition of the second rabbit primary antibody to the fixed yeast. Bivalent goat anti-rabbit antibodies caused artifactual cross-reaction of the two primary sera (data not shown). However, bivalent mouse anti-rabbit IgG was suitable as a linker for the second primary antibody, presumably because the tertiary fluorescent-coupled rabbit antibodies (anti-goat and anti-mouse $F(ab')_2$ fragments) were not bound significantly by the mouse anti-rabbit IgG. This protocol required serial incubations with the following five antibody-antiserum mixtures (15 μl in WT buffer): (*a*) 1:50 dilution of anti-Sec7p antiserum, preimmune serum, or a 1:200 dilution of anti-β-tubulin; (*b*) 10 μg/ml Fab fragment of goat anti-rabbit IgG (H + L); (*c*) 1:40 dilution of affinity-purified anti-Kex2 antibody; (*d*) 1.5 μg/ml mouse anti-rabbit IgG (H + L); and (*e*) 1.5 μg/ml Texas-Red conjugated rabbit anti-goat IgG (to detect Sec7 protein) and 1.5 μg/ml FITC-conjugated rabbit anti-mouse IgG (to detect Kex2 protein). Incubations were separated by five washes with 25 ml WT buffer. After the final washes, cells were stained with DAPI.

Fields of cells were observed using a Zeiss Axiophot Photomicroscope equipped for epifluorescence and photographed with Kodak Professional Ektachrome P800/1600 slide film, pushed to ASA 1600 in development. 450-490/FT510/LP520 filters were used for observing fluorescein fluorescence, BP546/FT580/LP590 filters for Texas red fluorescence, and G365/FT395/LP420 filters for DAPI fluorescence. Separate exposures were made of each field, using each fluorescence mode and also using Nomarski optics. To assess colocalization, photographic slides of fluorescein and Texas Red fluorescence were projected onto a whiteboard, and the positions of fluorescent spots marked. Landmarks in the field were used to align the photographs precisely. The relative positions of green (fluorescein, Kex2 protein) and red (Texas Red, Sec7p or β-tubulin) spots were noted, and the Nomarski and DAPI photographs were used to assign spots to individual cells. Analysis was limited to cells that stained well for Kex2 protein (61% of total). Cell-to-cell variation in the intensity of fluorescein staining was probably due to fluctuation of the plasmid-dependent expression of Kex2 protein rather than to variability in permeabilization of cells, because Sec7p staining was relatively consistent from cell to cell. Colocalization of a green spot and a red spot was noted when their positions were indistinguishable by visual inspection. Cells (197) from 9 fields were analyzed in this way (1,135 total spots scored).

Results

Identification of the SEC7 Gene Product, Sec7p

Analysis of the subcellular distribution of the Sec7 protein (Sec7p) from yeast was facilitated by the preparation of specific antisera. Three hybrid-fusion genes were constructed to generate two different types of polyclonal antisera and to verify the specificity of the sera (Fig. 1 *A*, see Materials and Methods). The product of the hybrid-fusion gene *lacZ*-Large Fragment 1 expressed in *E. coli* was used to generate anti-Sec7p[large] antibodies, which showed titer against both *E. coli* β-galactosidase and yeast Sec7p epitopes. For most of the experiments described in this study, the antisera were used without further purification, except as indicated.

The sequence of the *SEC7* gene predicts an unusual, highly acidic domain near the amino terminus of the encoded polypeptide (Achstetter et al., 1988). This 125-amino acid region is rich in serine (21%) and contains 47% acidic residues. This domain was fused in frame to the 3′ end of a *cro-lacZ* gene under control of the bacteriophage λP_R promoter in the fusion vector pEX2 (Stanley and Luzio, 1984). *E. coli* transformed with this gene fusion produced a novel 163-kD polypeptide species upon heat induction (*lacZ-Antigen Frag-*

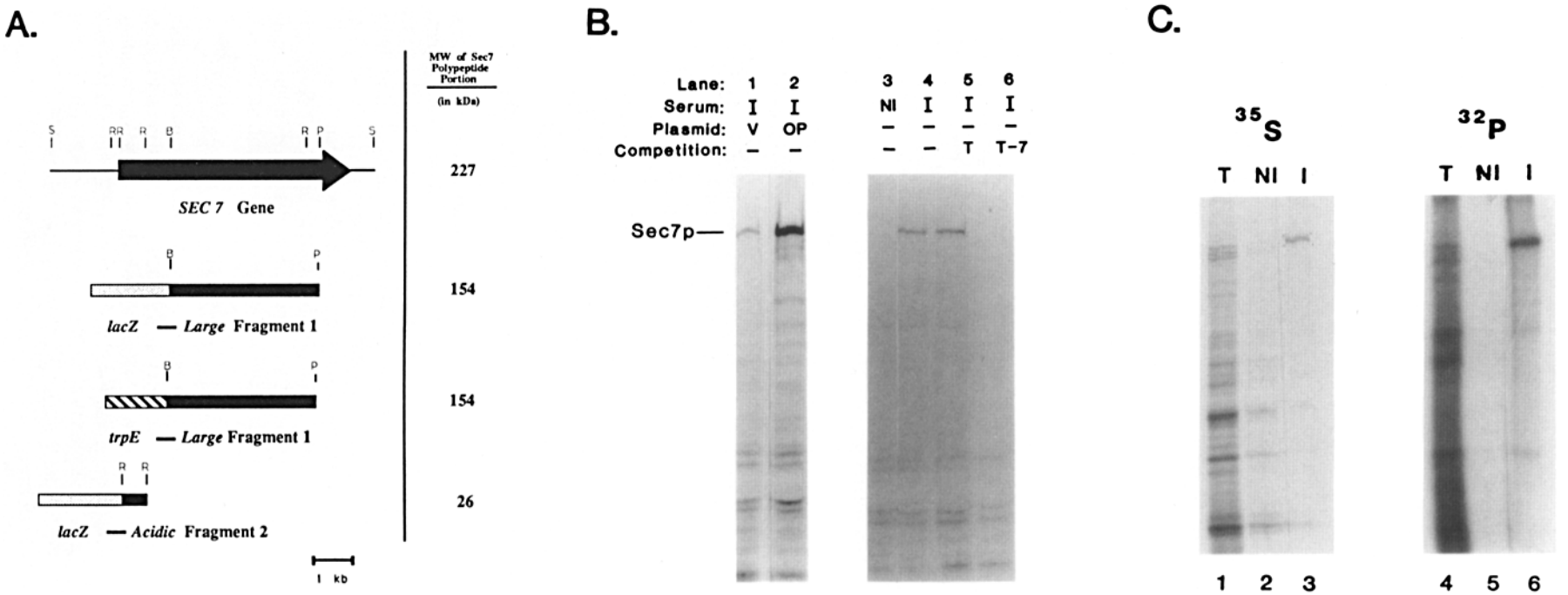

Figure 1. (*A*) Hybrid fusion gene constructs with the *SEC7* gene. A restriction map of the authentic *SEC7* gene and its coding region is shown at the top, with the expected size of the encoded polypeptide indicated on the right. Portions of the *SEC7* gene, highlighted in black, were fused in frame to either the *E. coli lacZ* gene or the *trpE* gene for induced expression in bacteria. (*B*) Specific immunoprecipitation of Sec7p. Yeast cells were labeled with $[^{35}S]SO_4^{2-}$ for 1 h at 30°C, vortexed with glass beads and 1% SDS then heated 5 min, 95°C. Anti-Sec7p[large] sera and protein A–Sepharose beads were incubated with the labeled whole cell extracts in 1 ml buffer overnight at 4°C. The precipitates were washed, boiled in Laemmli sample buffer, and resolved on 7.5% polyacrylamide SDS-gels. Lanes *1* and *2* show the immunoprecipitation of Sec7p from GPY59 or SEY2102 yeast harboring a 2μ–high-expression plasmid without or with the *SEC7* gene, respectively. Lanes *3–6* verify the specificity of the anti-Sec7p[large] sera. Pre-immune serum does not precipitate the 227-kD polypeptide species (lane *3*). Specific anti-Sec7p[large] sera were preincubated with no *E. coli* extract (lane *4*), *E. coli* extract containing TrpE protein (lane *5*), or *E. coli* extract containing the induced TrpE-Sec7p hybrid-fusion protein (lane *6*) in 0.1 ml buffer for 4 h at 24°C. Labeled yeast extracts and protein A–Sepharose beads were added to 1 ml final volume, and incubated at 4°C. Samples were processed as above. (*C*) Sec7p is a phosphoprotein. GPY59 yeast were labeled with either $[^{35}]SO_4^{2-}$ (lanes *1–3*) or $[^{32}P]PO_4^{3-}$ (lanes *4–6*). Sec7p was immunoprecipitated from whole cell extracts with anti-Sec7p[large] serum and protein A-Sepharose, and the precipitates were resolved on 7.5% SDS-gels, as described in the legend to Fig. 2 (*I*, lanes *3* and *6*). An aliquot of total labeled proteins is shown in lanes *1* and *4* (*T*). Lanes *2* and *5* (*NI*) show precipitates from the labeled cell lysates incubated with nonimmune rabbit antiserum and protein A–Sepharose.

Table 1. Colocalization of Sec7p and Kex2 Protein

Antigen	Number of spots that colocalize*	Number of spots that fail to colocalize‡	Colocalization
			%
Kex2p	577	134	81
Sec7p	577	424	58

* Number of Kex2p spots that colocalize with Sec7p spots or Sec7p spots that colocalize with Kex2p spots.
‡ Number of Kex2p spots that fail to colocalize with Sec7p spots or Sec7p spots that fail to colocalize with Kex2p spots.

ment 2, Fig. 1 *A*), which was used to immunize rabbits. The resultant polyclonal antiserum (hereafter referred to as anti-Sec7p[acidic] serum) also recognized *E. coli* β-galactosidase and yeast Sec7p epitopes.

Sec7p was immunoprecipitated from lysates of radiolabeled yeast cells. Wild-type yeast grown overnight in synthetic minimal sulfate medium was labeled with $[^{35}S]SO_4^{2-}$ at 30°C for 1 h. Extracts were mixed with anti-Sec7p[large] serum and protein A-Sepharose, and the precipitates were resolved on SDS-gels (Fig. 1 *B*, lane *1*). The apparent molecular mass (227 kD) of the immunoprecipitated polypeptide was in good agreement with the predicted molecular weight of the polypeptide deduced from DNA sequencing (Achstetter et al., 1988).

The specificity of the anti-Sec7p serum was tested by three criteria. First, the 227-kD species was not observed upon precipitation with preimmune sera (Fig. 1 *B*, lane *3*). Second, approximately eightfold higher levels of the high molecular weight polypeptide were immunoprecipitated from extracts of yeast harboring the *SEC7* gene on a multicopy plasmid (Fig. 1 *B*, compare lanes *1* and *2*). The third criterion for antibody specificity depended on competition for antigen recognition. A third hybrid fusion gene on the fusion vector pATH10 was constructed for these experiments. The promoter region and almost the entire coding sequence of the *E. coli trpE* gene was joined to the same large fragment of the *SEC7* gene described above. Lysates of *E. coli* expressing this hybrid-fusion protein were efficient in competing for recognition of the labeled yeast protein by anti-Sec7p[large] sera (Fig. 1 *B*, lane *6*). Extracts from bacteria induced for the production of the TrpE protein alone had no effect on the immunoprecipitation of Sec7p (Fig. 1 *B*, lane *5*). Immunoblotting of the total yeast extracts revealed Sec7p species when immune but not when preimmune sera was used (data not shown).

Biochemical fractionation of wild-type yeast extracts indicated that Sec7p exhibited the characteristics of both sedimentable and soluble proteins. Approximately 50% of Sec7p was recovered in high-speed pellet fractions of yeast lysates (see Materials and Methods). This figure varied from 40–60% in 12 separate experiments using three different strains of yeast.

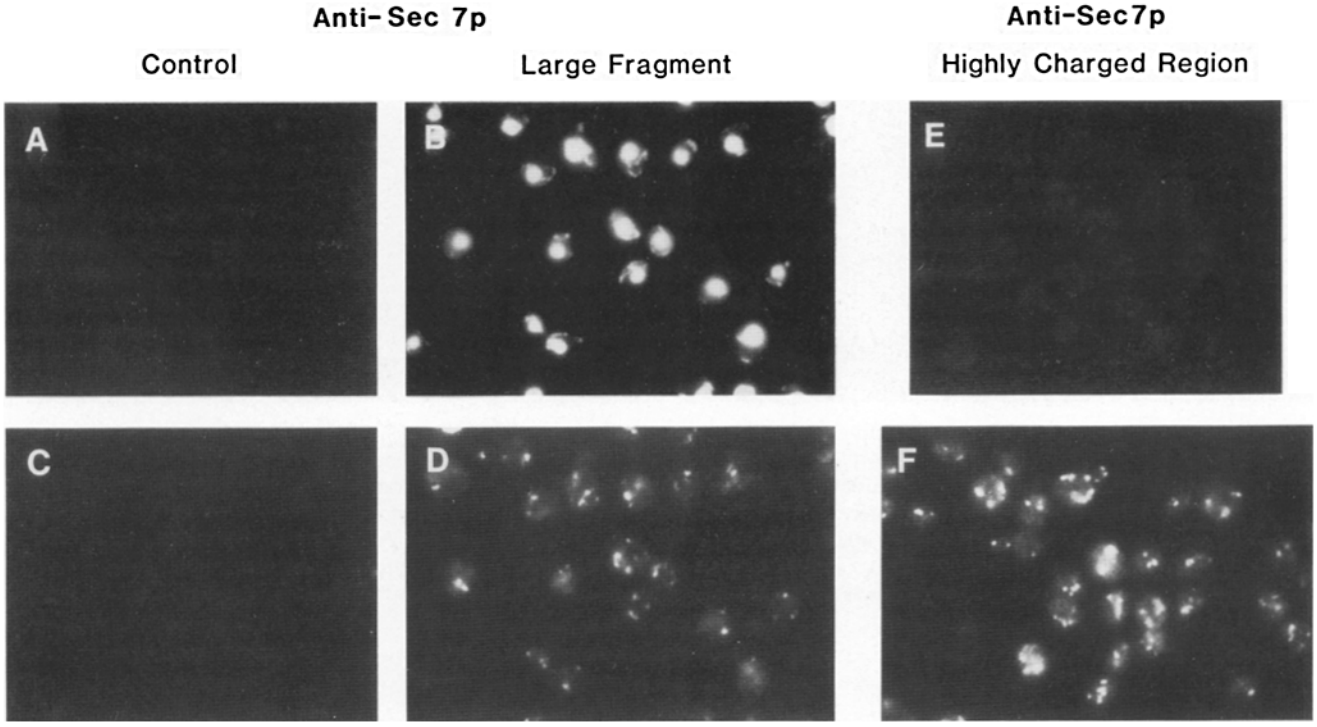

Figure 2. Subcellular distribution of Sec7p: indirect immunofluorescence. 15-9B yeast were fixed by formaldehyde addition to 5% final concentration in the growth medium. Fixed cells were briefly spheroplasted, then attached to polyethyleneimine-coated glass slides. Cells were incubated with primary antisera (1:400 dilution) for 2 h at 24°C. FITC-coupled goat anti-rabbit antisera were added to detect the primary antibody. Preimmune primary antisera were used in *A* and *E*. *B* shows DAPI staining of nuclear and mitochondrial DNA in the cells shown in *D*. Anti-Sec7p[large] serum was preincubated in 0.1 ml buffer overnight at 4°C with *E. coli* extract containing TrpE protein alone (*D*) or containing TrpE-Sec7p hybrid-fusion protein (*C*) before exposure with the fixed yeast. Anti-Sec7p[acidic] serum was also used to detect Sec7p in fixed cells (*F*). Exposure times for photomicrographs were 2.5 (*A–D*), and 2 min (*E* and *F*). Images are magnified ~1,000-fold.

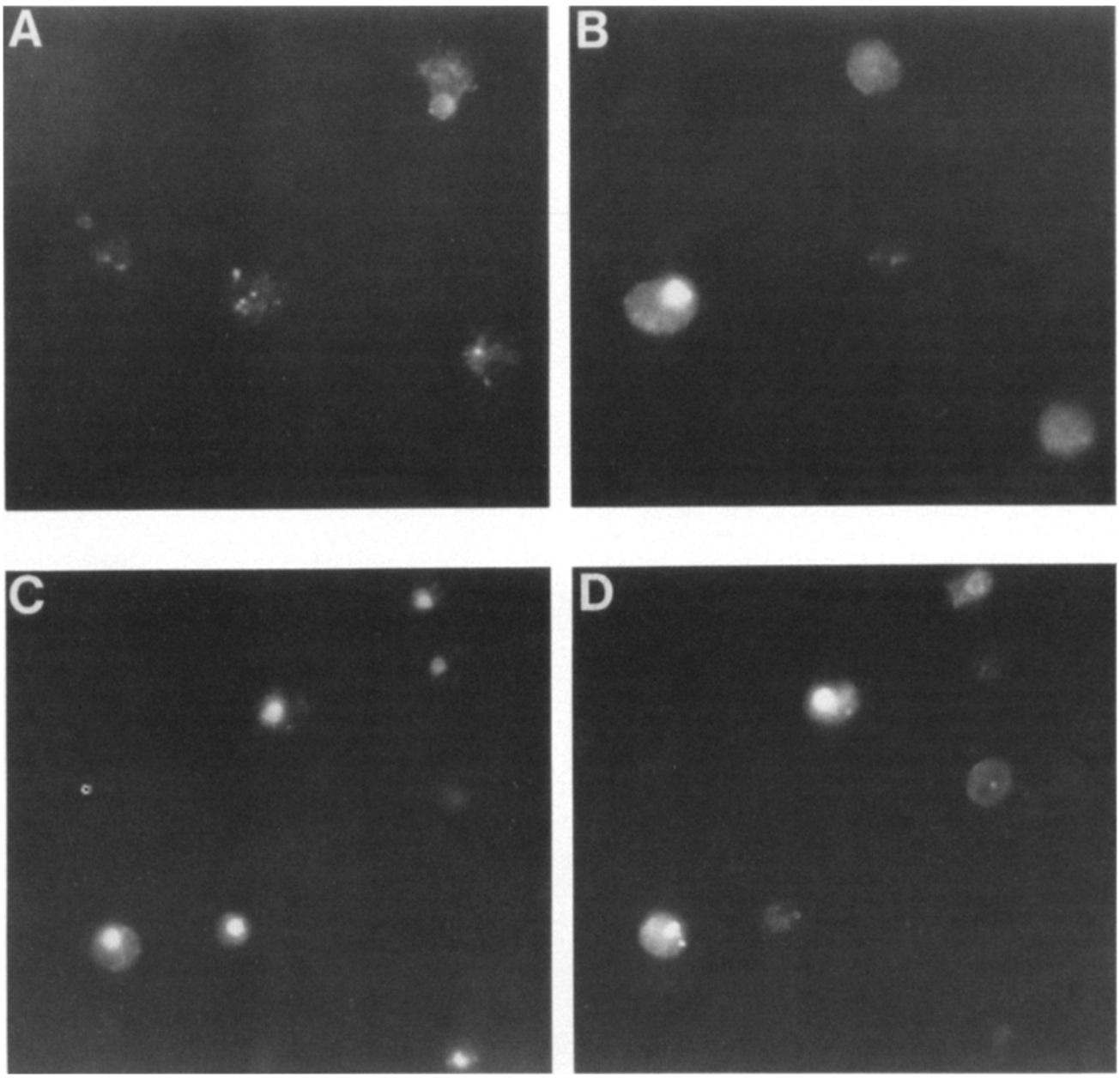

Figure 3. Indirect immunofluorescence of Sec7p-associated structures in *sec14* yeast. HMSF 169 (*sec14*) yeast were grown in synthetic defined media with 2% glucose overnight at 24°C, then harvested and resuspended in the same media with 0.1% glucose. Portions of the culture were shaken for 1 h at either the permissive (24°C) or restrictive (37°C) growth temperature before formaldehyde fixation. The cells were then prepared for indirect immunofluorescence with anti-Sec7p[large] serum as described in the legend to Fig. 2. *A* shows Sec7p-immunofluorescence in *sec14* yeast incubated at 24°C. The pattern of Sec7p-immunofluorescence is dramatically altered in *sec14* yeast shifted to the restrictive temperature (*B* and *D*). *C* shows the DAPI staining of the nuclear and mitochondrial DNA in the yeast cells from *D*. The exaggerated immunofluorescent structures seen in some of the cells of panels *B* and *D* were observed in all cells by adjusting the plane of focus.

The putative association of Sec7p with particulate subcellular structures was disrupted by incubation of the lysates with relatively low concentrations of urea (>2 M urea) causing the protein to be released into the soluble fraction. Under these conditions, carboxypeptidase Y, a soluble protease in the lumen of the vacuole was recovered in the pellet fractions. In contrast, treatment of the lysates with nonionic detergents or bile salts resulted in 40–60% Sec7p recovery in the particulate fractions, yet caused the solubilization of carboxypeptidase Y. Both Sec7p and carboxypeptidase Y were solubilized upon incubation of the lysates in 0.1 M carbonate buffer pH 11, a treatment known to release peripheral membrane proteins, but which also converts closed membrane compartments into open membrane sheets (Fujiki et al., 1982, data not shown). The sedimentation behavior of Sec7p in biochemical fractionation studies confirmed the polypep-

tide sequence predictions that Sec7p is not an integral membrane protein, nor that it appears to enter the lumen of secretory pathway compartments (Achstetter et al., 1988).

Posttranslational Modifications: Sec7p is a Phosphoprotein

Regulation of the distribution of Sec7p between soluble and sedimentable states could depend on post-translational modifications of the protein. The addition of long chain fatty acids has been postulated to promote the association of otherwise soluble proteins with membranes (e.g., Aderem et al., 1988). However, a canonical sequence for protein farnesylation, palmitoylation, or myristoylation was not evident in the predicted sequence of Sec7p. Furthermore, [^{3}H]palmitate labeling of wild-type yeast followed by immunoprecipitation of Sec7p from the lysates did not reveal a labeled polypeptide (data not shown).

An abundance of serine residues in the highly acidic domain of Sec7p suggested that the protein could be modified by phosphorylation. Yeast were grown in minimal phosphate-sulfate media, then incubated with [^{32}P]PO_4^{3-} or [^{35}S]SO_4^{2-} at 30°C for 1 h. Extracts were mixed with anti-Sec7p[large] or preimmune IgG and protein A-Sepharose beads and the immunoprecipitates were resolved on SDS-PAGE (Fig. 1 *C*). A single phosphorylated species was precipitated by the immune serum (Fig. 1 *C*, lane *6*), but not by the preimmune serum (Fig. 1 *C*, lane *5*). This species exhibited the same gel mobility as Sec7p labeled with [^{35}S]SO_4^{2-} (Fig. 1 *C*, compare lanes *3* and *6*). Neither the site(s) of phosphorylation on Sec7p, nor the phosphoamino acid species have been determined.

Fractionation of extracts prepared from ^{32}P- or ^{35}S-labeled cells failed to show enrichment of newly synthesized, or phosphorylated forms of Sec7p in either the particulate or soluble fractions (data not shown). The role of phosphorylation in the function of Sec7p remains to be determined.

Sec7p Is Associated with the Yeast Golgi Apparatus

Indirect immunofluorescence of formaldehyde-fixed yeast cells was also used to examine the subcellular localization of Sec7p. Both anti-Sec7p[large] and anti-Sec7p[acidic] sera were used to detect the protein in the wild-type cells (Fig. 2). Fluorescein isothiocyanate–coupled goat anti–rabbit serum was used to recognize the primary sera and provide the fluorescent signal. Sec7p was localized in a punctate pattern consisting of multiple large and small structures (approximately four to six per focal plane, Fig. 2, *D* and *F*). Nuclei and mitochondria, seen by DAPI staining of the DNA, showed distinct and nonoverlapping distributions (Fig. 2, compare *B* and *D*). Antibodies raised against the different portions of Sec7p showed similar punctate patterns, as did cells stained with rhodamine-coupled secondary antibodies (data not shown). The cytosolic form of Sec7p detected in the biochemical fractionation experiments may be represented by the diffuse cytoplasmic fluorescence in the images.

We verified the specificity of the immunofluorescence by two criteria. First, preimmune sera failed to produce any immunofluorescent signal (Fig. 2, *A* and *E*). Second, preincubation of the anti-Sec7p[large] serum with *E. coli* extracts containing the TrpE-Sec7p[large] hybrid fusion protein (Fig. 1 *A*) effectively competed for antibody recognition of Sec7p (Fig. 2 *C*). Preincubation of the immune serum with extracts of *E. coli* induced for the production of TrpE protein alone showed no effect on the pattern of immunofluorescence (Fig. 2 *D*).

As discussed above, two mutations (*sec7* and *sec14*) block protein traffic from the yeast Golgi apparatus (Novick et al., 1981; Stevens et al., 1982). Whereas morphologically identifiable stacks of Golgi cisternae are seldom observed in wild-type *S. cerevisiae* (Novick et al., 1981; Svoboda and Necas, 1987), *sec7* and *sec14* yeast accumulate exaggerated cisternae at the restrictive temperature. Correlation of the punctate pattern of Sec7p-associated structures in *SEC* cells with the nature of the yeast Golgi apparatus was assessed with immunofluorescence experiments in *sec14* mutant yeast. The punctate pattern of Sec7p immunofluorescence in wild-type yeast was also observed in *sec14* yeast grown at the permissive temperature (Fig. 3 *A*). When *sec14* cells were incubated at 37°C for 1 h before fixation, exaggerated Sec7p-associated structures were observed in each cell scanned at multiple focal planes (Fig. 3 *B*).

Colocalization of Sec7p and Kex2 Protease

Indirect immunofluorescence using antibody against the yeast Kex2 protein (Kex2p) also identified a punctate pattern of fluorescence (Redding, K., and R. S. Fuller, manuscript submitted for publication). Kex2p is a membrane-bound protease that cleaves the precursor of the mating pheromone α-factor at pairs of basic residues, a reaction thought to occur in a late Golgi compartment because unprocessed intermediates as well as mature peptides are observed in *sec7* yeast at the nonpermissive temperature (Julius et al., 1984; Franzusoff and Schekman, 1989). In order to evaluate the relationship between structures containing Sec7p and Kex2p, double-label immunofluorescence experiments were performed. Fig. 4 shows a typical field of Kex2p-overproducing cells [BFY125(pBM-KX22) strain] from Sec7p/Kex2p double-labeling experiments. The majority of Texas Red–labeled structures, corresponding to Sec7p (Fig. 4, *A* and *C*), and fluorescein-labeled structures, corresponding to Kex2p were coincident (Fig. 4, *B* and *D*). Additional sites labeled only by Texas Red or by fluorescein were observed in the photomicrographs (note *arrows* and *arrowheads* in Fig. 4, *C* and *D*). Photographic images of 197 cells were analyzed for colocalization of the fluorescent signals as described in Materials and Methods (Table I). Of the Sec7p signals, 58% were Kex2p positive, whereas 81% of the Kex2p signals corresponded to a Sec7p-labeled structure. At high magnification, individual Sec7p- and Kex2p-labeled signals often appeared to overlap only partially, suggesting differential organization of the two proteins within a larger extended structure.

Because both primary antibodies in the double-labeling methods were derived from rabbit antisera, two sets of control experiments were performed to ensure the fidelity of the procedure. First, the procedure was tested with anti-Kex2p serum and another rabbit antiserum that recognized morphologically distinct structures in a Kex2p-overproducing yeast strain. Anti-β-tubulin sera recognize both intranuclear and cytoplasmic microtubules emanating from opposite faces of the spindle pole body in the nuclear envelope (Kilmartin and Adams, 1984; Pringle et al., 1986; Pillus and Solomon,

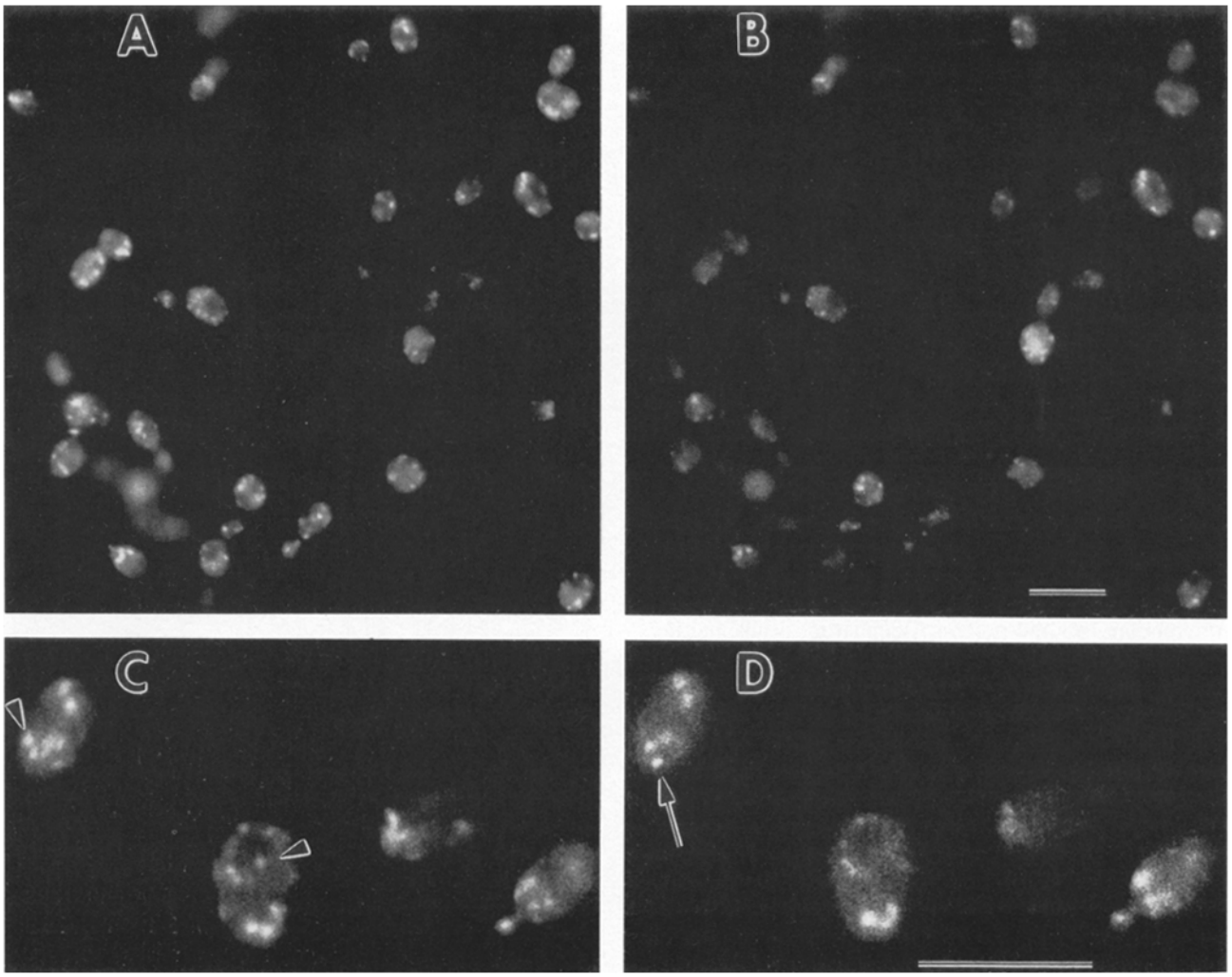

Figure 4. Double-immunofluorescence labeling of Sec7 and Kex2 proteins. Pairs of panels (*A* with *B* and *C* with *D*) represent individual fields of BFY125[pBM-KX22] cells subjected to the double-labeling protocol as described in Materials and Methods. Anti-Sec7p Ab was applied in the first incubation. (*A* and *C*) Texas Red fluorescence corresponding to Sec7 protein; (*B* and *D*) fluorescein fluorescence corresponding to Kex2 protein. Arrowheads correspond to Sec7-stained structures which do not label for Kex2 protein; the arrow corresponds to a Kex2-stained spot which does not label for Sec7 protein. Bar, 10 μm.

1986). With Texas Red–coupled antiserum marking the rabbit anti-β-tubulin serum, fluorescence was restricted to elongated structures typical of yeast microtubules (Fig. 5 *C*), whereas fluorescein fluorescence intended for the Kex2 protein highlighted the expected punctate pattern (Fig. 5 *F*). When the same protocol was applied to yeast mutants lacking Kex2 protease (BFY125(pBM743)), the fluorescein signal was absent, but Texas Red-labeled microtubules were still observed (data not shown).

A second set of controls was performed to assess possible cross-reactivity between the anti-Sec7p serum and anti-Kex2p antibody. In the Kex2p-deficient yeast strain, punctate Texas-Red fluorescence corresponding to Sec7p was observed (Fig. 5 *B*), but the fluorescein signal was absent (Fig. 5 *E*). Conversely, in a Kex2p-overproducing strain, when preimmune serum was used in place of the anti-Sec7p antiserum, no Texas Red–labeled structures were observed (Fig. 5 *A*), but the punctate Kex2p-fluorescein pattern was seen (Fig. 5 *D*). Omission of either primary antibody resulted in a fluorescent pattern corresponding to the other primary antibody (data not shown).

Discussion

We have shown by indirect immunofluorescence that the 227-kD Sec7p is associated with distinct multiple structures in the cell, probably corresponding to the yeast Golgi apparatus. The distribution of Sec7p by subcellular fractionation is divided between soluble and particulate states, and may be altered in vitro by conditions that interfere with protein–protein interactions, but not by detergent solubilization of membrane compartments. We conclude that Sec7p is a cytosolic protein that assembles into particulate complexes, presumably in proximity to the yeast Golgi compartments. Furthermore, Sec7p is phosphorylated in vivo. This posttranslational modification may play a role in the activity of Sec7p in intercompartmental protein transport.

Nature of the Yeast Golgi Apparatus

The *sec7* mutation causes accumulation of exaggerated Golgi apparatus–like cisternae and affects glycoprotein traffic through the yeast Golgi apparatus. Hence, the Sec7p-associated structures seen by indirect immunofluorescence

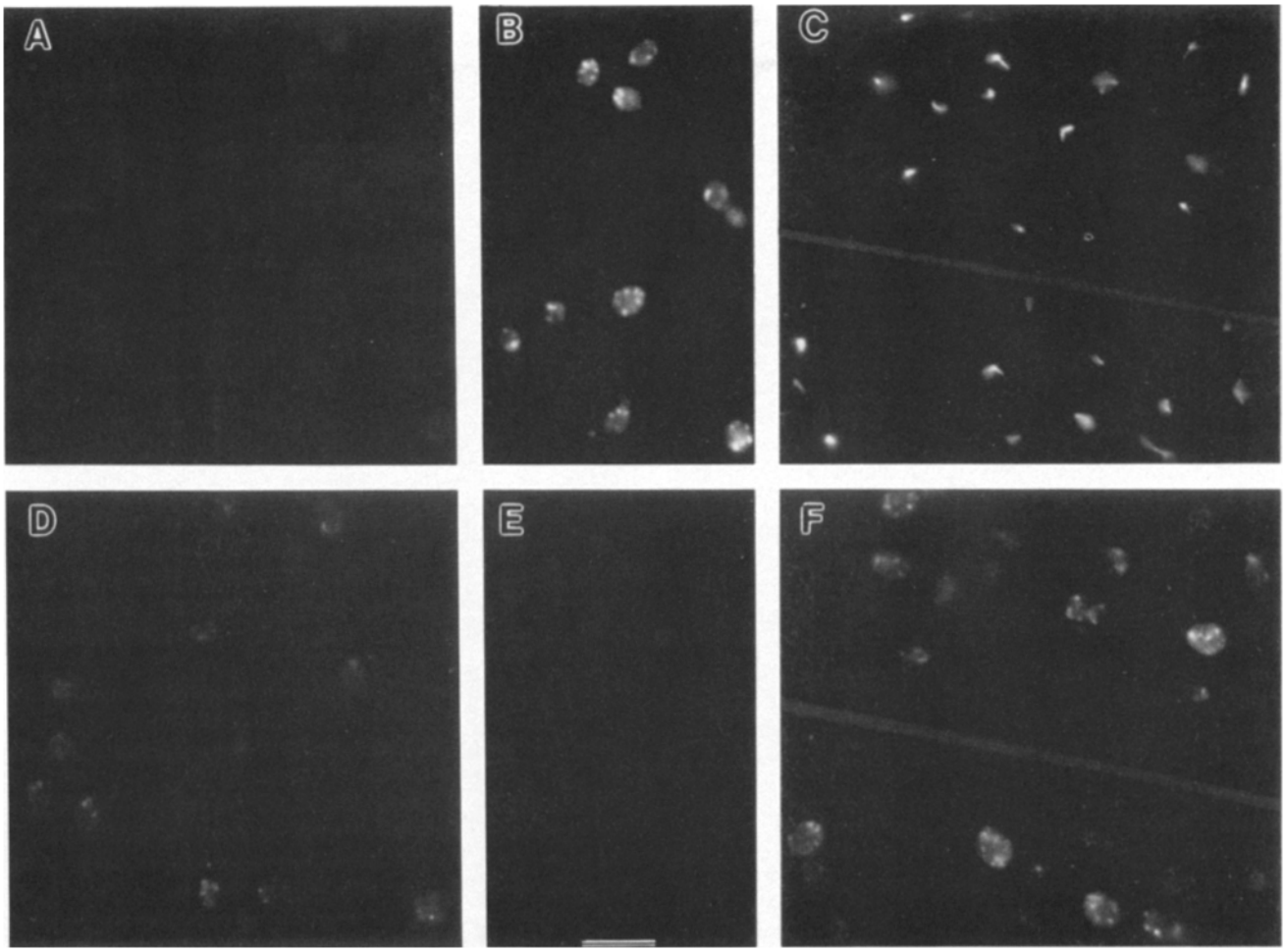

Figure 5. Controls for double-labeling experiments: (*A* and *D*) Control using Sec7 preimmune serum (*B* and *E*) control using a strain lacking Kex2 protein; (*C* and *F*) Control using anti-β-tubulin antiserum. Strains BFY125[pBM-KX22] (*A*, *C*, *D*, and *F*) and BFY125[pBM743] (*B* and *E*) were grown and processed as described in Materials and Methods. Pairs of panels (*A* and *D*, *B* and *E*, and *C* and *F*) represent epifluorescence of the same field of cells with different filters. *A–C* represent Texas Red fluorescence, corresponding to β-tubulin immunofluorescence in *C* and Sec7p-specific staining in *A* and *B*. *D*, *E*, and *F* represent fluorescein fluorescence, corresponding to staining for Kex2 protein. Cells adsorbed to multiwell slides were sequentially incubated with (*a*) anti-β-tubulin (*C* and *F*), anti-Sec7p antiserum (*B* and *E*), or preimmune serum (*A* and *D*); (*b*) Fab fragment of goat anti-rabbit IgG; (*c*) anti-Kex2p antibody; (*d*) mouse anti-rabbit IgG; and (*e*) a mixture of rabbit anti-goat-Texas Red and rabbit anti-mouse-fluorescein. Bar, 10 μm.

may represent either individual compartments of the Golgi apparatus, or small intact Golgi complexes. Since the detection of Sec7p is severely compromised in *sec7* mutant cells, we examined Sec7p distribution in *sec14* yeast that are also defective in glycoprotein traffic from the Golgi apparatus, and accumulate exaggerated membrane compartments and numerous vesicles (Novick et al., 1981). In *sec14* yeast at the permissive temperature for growth, the pattern of Sec7p immunofluorescence is identical to that in *SEC* yeast. However, at the restrictive growth temperature, the pattern of Sec7p-associated immunofluorescent structures changes, coalescing into large fluorescent bodies. It is unlikely that alteration of the Sec7p-associated fluorescent pattern in *sec14* yeast represents an artefact of the formaldehyde fixation procedures since wild-type immunofluorescent patterns were observed in the mutant cells at the permissive temperature. However, since the Sec14 protein may be involved in promoting proper membrane structure as a phosphatidyl inositol transfer protein (Bankaitis et al., 1990), the observed changes in *sec14* yeast may reflect aberrant membrane composition at the restrictive temperature. Furthermore, Sec7p-associated structures may represent only a subset of membrane compartments that appear by EM to be affected by the *sec14* mutation. These possibilities are nevertheless consistent with the hypothesis that Sec7p-containing bodies represent part of the yeast Golgi apparatus.

Sec7 and Kex2 proteins are largely localized to either identical or closely associated structures within the yeast cell. Given the evidence for functional interaction of each protein with the Golgi apparatus, it seems likely that the sites that contain both proteins represent some part of the Golgi apparatus.

What of the sites which appear to contain one and not the other protein? We do not believe that the incomplete colocalization observed is artefactual. Although cell-to-cell variability in Kex2p-specific staining occurred, such variability was not found for Sec7p staining. Only cells that stained well for Kex2p were included in the quantitative analysis of colocalization. Differences in the efficiency of staining within different regions of a single cell also seem un-

likely to account for the observed partial colocalization. For example, Kex2-positive and negative spots within cells were found generally to be stained to the same intensity with anti-Sec7 antibody.

Sites that contain Sec7p, but not Kex2p, might represent early (*cis*) Golgi compartments. At the non-permissive temperature, *sec7* mutants accumulate multiple forms of secretory proteins, suggesting arrest at several distinct stages of transport (Franzusoff and Schekman, 1989). The secretory protein invertase is accumulated intracellularly in *sec7* mutants as both a core-glycosylated ER-type form and as intermediates that have undergone extensive addition of mannosyl residues to Asn-linked oligosaccharides. The latter modifications are characteristic of molecules that have gained access to the Golgi apparatus in yeast cells (Esmon et al., 1981; Kukuruzinska et al., 1987). However, the outer chain carbohydrate chains on glycoproteins accumulated in *sec7* mutants lack terminal α-1,3-linked mannosyl residues (Franzusoff and Schekman, 1989). Ser/Thr-linked oligosaccharide in Kex2p is slowly modified during its lifetime in wild-type cells ($t_{1/2}$ ~90 min at 30°C), probably by addition of α-1,3-linked mannosyl residues, but in *sec7* yeast at the nonpermissive temperature, nascent Kex2p is not modified in this way (Wilcox, C. A., and R. S. Fuller, manuscript in preparation). This is consistent with the model that Sec7p function is required for transport of cargo molecules into each successive compartment of the Golgi apparatus, with Kex2p residing in a late (*trans*) compartment.

In mammalian cells, the Golgi apparatus "fragments" in mitosis, presumably to ensure that the daughter cells receive a complement of the Golgi compartments for proper cellular organization (Warren, 1985; Lucocq and Warren, 1987). The whole process of mitosis, changes in the nucleus and presumably other organelles during cell division requires up to 50% of the *S. cerevisiae* cell cycle (Wheals, 1987). Therefore, suspending fusion of the Golgi compartments in *S. cerevisiae* could maintain the probability that the yeast "bud" will acquire its complement of Golgi membrane. One prediction of this model is that individual Golgi compartments in yeast cells are not organized into stacks of successive compartments that are characteristic of the Golgi apparatus in mammalian cells and *Schizosaccharomyces pombe.* Indeed, stacks of multiple cisternae are rarely found in thin-section EM analysis of wild-type yeast cells (Novick et al., 1981; Svoboda and Necas, 1987). In contrast to the condensed perinuclear structure found in mammalian cells, the yeast Golgi apparatus appears to be a dispersed collection of structures. Sec7p-staining structures that lack Kex2p-staining may represent early Golgi compartments that are not physically associated with later compartments. Structures positively stained for Kex2 protein but not for Sec7 protein may represent the absence of Sec7 protein from a late Golgi compartment that contains Kex2 protein. Alternatively, these structures may be related to transport of Kex2 protein to its site of degradation by vacuolar proteases (Wilcox, C. A., and R. S. Fuller, manuscript in preparation).

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References

Achstetter, T., A. Franzusoff, C. Field, and R. Schekman. 1988. *SEC7* encodes an unusual high molecular weight protein required for membrane traffic from the yeast Golgi apparatus. *J. Biol. Chem.* 263:11711–11717.

Aderem, A. A., K. A. Albert, M. M. Krum, J. K. T. Wang, P. Greengard, and Z. A. Cohn. 1988. Stimulus dependent myristoylation of a major substrate for protein kinase C. *Nature (Lond.).* 332:362–365.

Baker, D., L. Hicke, M. Rexach, M. Schleyer, and R. Schekman. 1988. Reconstitution of SEC gene product-dependent intercompartmental protein transport. *Cell.* 54:335–344.

Bankaitis, V. A., J. R. Aitken, A. E. Cleves, and W. Dowhan. 1990. An essential role for a phospholipid transfer protein in yeast Golgi function. *Nature (Lond.).* 347:561–563.

Beckers, C. J., M. R. Block, B. S. Glick, J. E. Rothman, and W E. Balch. 1989. Vesicular transport between the endoplasmic reticulum and the Golgi stack requires the NEM-sensitive fusion protein. *Nature (Lond.).* 339: 397–398.

Block, M. R., B. S. Glick, C. A. Wilcox, F. T. Wieland, and J. E. Rothman. 1988. Purification of an N-ethylmaleimide-sensitive protein catalyzing vesicular transport. *Proc. Natl. Acad. Sci. USA.* 85:7852–7856.

Bond, J. F., J. L. Fridovich-Keil, L. Pillus, R. C. Mulligan, and F. Solomon. 1989. A chicken-yeast chimeric β-tubulin protein is incorporated into mouse microtubules *in vivo. Cell.* 44:461–468.

Burgers, P. M. J., and K. J. Percival. 1987. Transformation of yeast spheroplasts without cell fusion. *Anal. Biochem.* 163:391–397.

Burnette, W. N. 1981. Western blotting electrophoretic transfer of proteins from sodium.dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195–203.

Clary, D. O., I. C. Griff, and J. E. Rothman. 1990. SNAPs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. *Cell.* 61:709–721.

Diaz, R., L. S. Mayorga, P. J. Weidman, J. E. Rothman, and P. D. Stahl. 1989. Vesicle fusion following receptor-mediated endocytosis requires a protein active in Golgi transport. *Nature (Lond.).* 339:398–400.

Emr, S. D., R. Schekman, M. C. Flessel, and J. Thorner. 1983. *An MFα1-SUC2* (α-factor-invertase) gene fusion for study of protein localization and gene expression in yeast. *Proc. Natl. Acad. Sci. USA.* 80:7080–7084.

Esmon, B., P. Novick, and R. Schekman. 1981. Compartmentalized assembly of oligosacclarides on exported glycoproteins in yeast. *Cell.* 25:451–460.

Franzusoff, A., and R. Schekman. 1989. Functional compartments of the yeast Golgi apparatus are defined by the *sec7* mutation. *EMBO (Euro. Mol. Biol. Org.) J.* 8:2695–2702.

Fujiki, Y., A. L. Hubbard, S. Fowler, and P. B. Lazarow. 1982. Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. *J. Cell Biol.* 93:97–102.

Fuller, R., R. Sterne, and J. Thorner. 1988. Enzymes required for yeast prohormone processing. *Annu. Rev. Physiol.* 50:345–362.

Fuller, R. S., A. Brake, and J. Thorner. 1989*a*. Yeast prohormone processing enzyme (*KEX2* gene product) is a Ca^{2+}-dependent serine protease. *Proc. Natl. Acad. Sci. USA.* 86:1434–1438.

Fuller, R. S., A. J. Brake, and J. Thorner. 1989*b*. Intracellular targeting and structural conservation of a prohormone-processing endoprotease. *Science (Wash. DC).* 246:482–486.

Glick, B. S., and J. E. Rothman. 1987. Possible role for fatty acyl-coenzyme A in intracellular transport. *Nature (Lond.).* 326:309–312.

Jacobs, C. W., A. E. M. Adams, P. J. Szaniszlo, and J. R. Pringle. 1988. Function of microtubules in the *S. cerevisiae* cell cycle. *J. Cell Biol.* 107: 1409–1426.

Julius, D., R. Schekman, and J. Thorner. 1984. Glycosylation and processing of pre-pro-alpha-factor in the yeast secretory pathway. *Cell.* 36:309–318.

Kilmartin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces. J. Cell Biol.* 98:922–933.

Kukuruzinska, M. A., M. L. E. Bergh, and B. J. Jackson. 1987. Protein glycosylation in yeast. *Annu. Rev. Biochem.* 56:915–944.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lucocq, J. M., and G. Warren. 1987. Fragmentation and partitioning of the Golgi apparatus during mitosis in HeLa cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3239–3246.

Malhotra, V., L. Orci, B. S. Glick, H. Block, and J. E. Rothman. 1988. Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the Golgi stack. *Cell.* 54:221–227.

Malhotra, V., T. Serafini, L. Orci, J. C. Shepherd, and J. E. Rothman. 1989. Purification of a novel class of coated vesicles mediating biosynthetic protein transport through the Golgi stack. *Cell.* 58:329–336.

Melançon, P., B. S. Glick, V. Malhotra, P. J. Weidman, T. Serafini, M. L. Gleason, L. Orci, and J. E. Rothman. 1987. Involvement of GTP-binding "G" proteins in transport through the Golgi stack. *Cell.* 51:1053–1062.

Nakano, A., D. Brada, and R. Schekman. 1988. A membrane glycoprotein, Sec12p, required for protein transport from the endoplasmic reticulum to the Golgi apparatus in yeast. *J. Cell Biol.* 107:851–863.

Novick, P., C. Field, and R. Schekman. 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell.* 21:205–215.

Novick, P., S. Ferro, and R. Schekman. 1981. Order of events in the yeast secretory pathway. *Cell.* 25:461–469.

Pillus, L., and F. Solomon. 1986. Components of microtubular structures in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* 83:2468–2472.

Pringle, J. R., S. H. Lillie, A. E. M. Adams, C. W. Jacobs, B. K. Haarer, K. G. Coleman, J. S. Robinson, L. Bloom, and R. A. Preston. 1986. Cellular Morphogenesis in the Yeast Cell Cycle. *In* Yeast Cell Biology. J. Hicks, editor. Allan R. Liss, New York. 47–80.

Ruther, U., and B. Müller-Hill. 1983. Easy identification of cDNA clones. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:1791–1794.

Scott, J., and R. Schekman. 1980. Lyticase: endoglycanase and protease activities that act together in yeast cell lysis. *J. Bacteriol.* 142:414–423.

Sherman, F., G. R. Fink, and J. B. Hicks. 1986. Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Stanley, K. K., and J. P. Luzio. 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1429–1434.

Stevens, T., B. Esmon, and R. Schekman. 1982. Early stages in the yeast secretory pathway are required for transport of carboxypeptidase Y to the vacuole. *Cell.* 30:439–448.

Svoboda, A., and O. Necas. 1987. Ultrastructure of *Saccharomyces cerevisiae* cells accumulating Golgi organelles. *J. Basic Microbiol.* 27:603–612.

Warren, G. 1985. Membrane traffic and organelle division. *Trends Biol. Sci.* 10:439–443.

Wattenberg, B. W., R. R. Hiebsch, L. W. Le Cureux, and M. P. White. 1990. Identification of a 25 kD protein from yeast cytosol that operates in a prefusion step of vesicular transport between compartments of the Golgi. *J. Cell Biol.* 110:947–961.

Weidman, P. J., P. Melançon, M. R. Block, and J. E. Rothman. 1989. Binding of an N-ethylmaleimide-sensitive fusion protein to Golgi membranes requires both a soluble protein(s) and an integral membrane receptor. *J. Cell Biol.* 108:1589–1596.

Wickerham, L. J. 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. *J. Bacteriol.* 52:293–301.

Wheals, A. E. 1987. The biology of the cell cycle in yeasts. *In* The Yeasts. Vol. 1. A. H. Rose and J. S. Harrison, editor. Academic Press, New York. 283–390.

Wilson, C. W., C. A. Wilcox, G. C. Flynn, E. Chen, W.-J. Kuang, W. J. Henzel, M. R. Block, A. Ullrich, and J. E. Rothman. 1989. A fusion protein required for vesicle-mediated transport in both mammalian cells and yeast. *Nature (Lond.).* 339:335–359.