The Journal of Microbiology, September 2002, p.219-223 Vol. 40, No. 3
Copyright © 2002, The Microbiological Society of Korea

Characterization of Cell Wall Proteins from the *soo1-1/ret1-1* Mutant of *Saccharomyces cerevisiae*

Dong-Won Lee[1], Ki-Hyun Kim[1], Se-Chul Chun[2], and Hee-Moon Park[1,2]***

[1]*Department of Microbiology, Chungnam National University, Daejeon 305-764, Korea*
[2]*Institute of Biotechnology, Chungnam National University, Daejeon 305-764, Korea*

(Received May 30, 2002 / Accepted August 29, 2002)

In order to investigate the function of Soo1p/α-COP during post-translational modification and intracellular transport of cell wall proteins in *Saccharomyces cerevisiae*, cell wall proteins from the *soo1-1/ret1-1* mutant cells were analyzed. SDS-PAGE analysis of biotin labeled cell wall proteins suggested that the *soo1-1* mutation impairs post-translational modification of cell wall proteins, such as *N*- and/or *O*-glycosylation. Analysis of cell wall proteins with antibodies against β-1,3-glucan and β-1,6-glucan revealed alteration of the linkage between cell wall proteins and β-glucans in the *soo1-1* mutant cells. Compositional sugar analysis of the cell wall proteins also suggested that the *soo1-1* mutation impairs glycosylation of cell wall protein in the ER, which is crucial for the maintenance of cell wall integrity.

Key words: *soo1-1/ret1-1,* yeast cell wall protein, post-translational modification

The major wall components of *Saccharomyces cerevisiae* are mannoproteins, β-1,6-glucan, β-1,3-glucan, and chitin. The majority of the cell wall proteins of *S. cerevisiae* wild type cells are retained by covalent association with β-1,6-glucan which is itself coupled to β-1,3-glucan. β-1,6-glucan is bound to the cell wall protein through a glycosylphosphatidylinositol (GPI)-derived moiety containing five α-linked mannosyl residues. To the β-1,6 and β-1,3-glucan the chitin chains appear to be attached in a β-1,4 or β-1,2 linkage (Kollar *et al.*, 1997). Cell wall proteins are generally resistant to extraction with hot detergents such as SDS, however, 98 % of all SDS-resistance proteins in the cell wall are solubilized by β-1,3-glucanase digestion of cell wall (Kapteyn *et al.*, 1996).

The secretory pathway is heavily involved in the biosynthesis of cell surface components and in their transport to the cell surface. During their transport through the secretory pathway, cell wall proteins can be *N*-glycosylated and/or *O*-glycosylated; in addition, some of these glycoproteins can also receive a GPI-anchor in ER, and the resulting structures are modified or extended in later secretory compartments (Ballou, 1990; Orleans, 1997). Intracellular protein transport in eukaryotic cells is mediated by small transport vesicles that are defined by their coat proteins: COPII-coated vesicles allow exit from the ER; COPI vesicles carry proteins within the early secretory pathway (i.e. the ER and the Golgi apparatus) and clathrin-coated vesicles mediate transport from the *trans*-Golgi network and endocytic transport from the plasma membrane (Le Borgne and Hoflack, 1998; Rothman and Wieland, 1996; Schekman and Orci, 1996).

Yeast mutants harboring temperature-sensitive alleles of *SEC21* (γ-COP, one of seven different subunits of COPI) were recently shown to exhibit dramatic protein-specific anterograde transport defects at the nonpermissive temperature (Gaynor and Emr, 1997). Among the mutant alleles of *RET1* (α-COP, a subunit of COPI) characterized thus far in yeast, 4 alleles, *ret1-1*, *ret1-2*, *ret1-4*, and *ret1-5*, exhibit defects in KKXX protein retrieval and 2 alleles, *ret1-3* and *sec33-1*, exhibit defects in anterograde (forward) transport of CPY (Eugster *et al.*, 2000). A recent report demonstrated that transport of one of the GPI-anchored cell wall proteins, Gas1p, to the Golgi is blocked but the GPI-anchor attachment is still normal in *ret1-1* (Sutterlin *et al.*, 1997), yet no direct evidence for the effect of *ret1-1* mutation on post-translational modification, such as glycosylation, of cell wall proteins were demonstrated. Our recent reports show that the *soo1-1/ret1-1* mutation in Soo1p/α-COP, a subunit of COPI vesicle, is responsible for the thermo-sensitive osmo-fragile phenotype of *Saccharomyces cerevisiae*, and suggest that the α-COP (Soo1p) may play a crucial role in recognition and translocation of a protein(s) of the β-1,3-glucan synthase complex and, thus, may contribute to the maintenance of the osmotic integrity of the cells (Lee *et al.*, 1999; Lee *et al.*, 2001).

In this study, we analyzed cell wall proteins from the

* To whom correspondence should be addressed.
(Tel) 82-42-821-6417; (Fax) 82-42-822-7367
(E-mail) hmpark@cnu.ac.kr
**Present address: Department of Crop Science, Konkuk University, Seoul 143-701, Korea

wild type and mutant type strain and present evidence that the *sool-1* mutation causes defect(s) in post-translational modification and assembly of the yeast cell wall proteins, which may contribute to the maintenance of cell wall integrity.

Materials and methods

Strains and growth media

Saccharomyces cerevisiae strains, LP0353RS1 (*MATα ura3-52 lys2-801 SOO1 bgs2*) generated through the *URA* pop-out with 5-FOA of the strain LP0353IS1 (*MATα ura3-52 lys2-801 SOO1::URA3 sool-1 bgs2*) and LP0353 (*MATα ura3-52 lys2-801 sool-1 bgs2*) (Lee *et al.*, 1999) were used in this study. The yeast strains were grown in YEPD (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) or synthetic minimal medium, SD (0.67% Bacto-yeast nitrogen base without amino acids, 2% dextrose). D-sorbitol was added at the concentration of 1.2 M as an osmotic stabilizer, if necessary.

Fractionation of cell wall proteins labeled with NHS-LC-Biotin

Yeast cells were grown in 100 ml YEPD (≅O.D. 1.0) at 28°C, and then the phenotype was expressed at 37°C for 4 h. Cells were harvested and washed twice with 40 ml of 50 mM potassium-phosphate buffer (pH 8.0). Cell pellets were resuspended in 20 ml of the same buffer containing 10 mg NHS-LC-Biotin reagent (Sigma) and incubated for 90 min on ice, centrifuged, and washed twice with 40 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 50 mM $MgCl_2$ followed by an additional washing with 40 ml of 50 mM potassium-phosphate buffer (pH 8.0). Cell walls were obtained by breaking the cells mechanically using a glass bead beater in 50 mM potassium-phosphate buffer (pH 8.0), centrifuging cell debris at 5,000xg for 15 min, and washing them five times with the same buffer. SDS-soluble cell wall proteins were isolated by heating the cell wall twice with 1 to 2 ml of Laemmli sample buffer (Laemmli, 1970) at 95°C. After treatment with the Laemmli sample buffer, cell walls were washed five times with 0.1 M sodium-acetate buffer (pH 5.5). The remaining cell walls were resuspended in 0.1 M sodium-acetate buffer (pH 5.5) containing 60 units/g (wet weight of cell wall) of Quantazyme (Quantum), and incubated for 2 h at 37°C (Mrsa *et al.*, 1997).

Preparation of antisera against β-glucans

Pustulan (a β-1,6 glucose polymer with an average degree of polymerization of 120) was partly hydrolyzed in 0.1 M trifluoroacetic acid at 100°C for 60 min to obtain water-soluble β-1,6 glucan fragments. The hydrolysate was fractionated by GPC (gel permeation chromatography) on a TSK-GMPW column (TOSOH). The fractions with an average degree of polymerization of 15 hexose residues were pooled and lyophilized. Laminarin (a β-1,3-glucose polymer with an average degree of polymerization of 25) was oxidized in 0.25 M $NaIO_4$ at 20°C for 60 min. The reaction was stopped with ethylene glycol and the oxidized Laminarin was desalted and lyophilized (Meikle *et al.*, 1994). Both glucan preparations were conjugated to bovine serum albumin (BSA) by reductive amination (Roy *et al.*, 1984). After 1 h incubation at room temperature, the reaction was stopped and the neoglycoproteins were isolated on GPC on a TSK-GMPW column. Both glucan-BSA conjugates were used for raising antibodies in rabbits (Montijn *et al.*, 1994). Animals were injected with 0.5 ml antigen (25 μg protein) in PBS (phosphate buffered saline; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 $7H_2O$, 1.4 mM KH_2PO_4) containing complete/incomplete Freunds adjuvant at intervals of 2 weeks, and then boosted twice.

Western blot analysis of cell wall proteins

Protein samples were subjected to SDS-PAGE by Laemmlis method (1970) and electro-blotted onto an Immobilon-P membrane (Millipore) in a solution of 25 mM Tris, 192 mM glycine, 20% methanol, and 0.05% SDS (Towbin *et al.*, 1979). The obtained blots were blocked for 1 hr with 1% skim milk (Difco), 0.05% Tween-20 (Sigma) in PBS: Gels were stained with Coomassie brilliant blue R-250 (CBB; Bio-Rad). To visualize biotin-labeled proteins, the membrane was probed with streptavidin-horseradish peroxidase conjugate (Sigma) diluted 2,000-fold in PBS containing 1% skim milk, 0.05% Tween-20. The anti-β-1,3-glucan and anti-β-1,6 glucan antibodies probed in membranes were developed with anti-rabbit immunoglobulin G antibody conjugated with horseradish peroxidase (Sigma). After the reaction with corresponding antibodies, the membranes were washed three times with the PBS buffer and developed using an ECL kit (Amersham Pharmacia Biotech.).

Compositional sugar analysis of cell wall proteins

Cell wall proteins were dried in a microfuge tube. To release neutral sugars and amino sugars from the cell wall proteins, 400 μl of 2 M trifluoroacetic acid (Sigma) and 6 N HCl were added to the tubes, respectively. After incubating the tubes for 4 h at 100°C, the tubes were cooled to room temperature and samples were dried with a SpeedVac. The released sugars were dissolved in 100 μl water and used for qualitative and quantitative analysis by HPLC. For chromatographic analysis of sugars, BioLC DX-300 (Dionex) was used. After loading the sugar samples on CarboPac PA1 (4.6×250 mm, Dionex) with a guard column (4.6×50 mm), sugars were eluted with KOH gradient (16 mM to 100 mM) at 250 μl/min flow rate and detected by setting the pulsed electrochemical detector (PED2) in integrated amperometry mode.

Results and Discussion

Post-translational modification of cell wall proteins was altered by the sool-1 mutation

Since Soo1p/α-COP plays a critical role in intracellular protein trafficking, it is highly plausible to postulate that *soo1-1/ret1-1* mutation affects the processes for post-translational modification(s), such as glycosylation and GPI-anchor attachment, of proteins transported by COPI and, thus, leads to miss-incorporation of these proteins into the cell wall and alteration of cell wall integrity.

To address this issue, Laemmli-sample buffer soluble and β-1,3-glucanase-soluble cell wall proteins were labeled with biotin and analyzed by SDS-PAGE. With Laemmli-sample buffer soluble fractions from cell wall, hardly any detectable differences of band patterns between the *soo1-1* and the *SOO1* allele were observed (data not shown). With β-1,3 glucanase-soluble cell wall proteins, however, prominent differences in band patters were revealed (Fig. 1); both a band around 50 kDa and the bands lower than 120 kDa in the fraction from the *SOO1* allele (Lane 2) entirely disappeared from the *soo1-1* allele (Lane 1). Alteration in posttranslational modification of cell wall proteins, therefore, is apparent in the *soo1-1/ret1-1* mutation.

In mutant cell wall protein preparations, proteins smaller than 120 kDa could be extracted by Laemmli-sample buffer treatment prior to Quantazyme treatment of

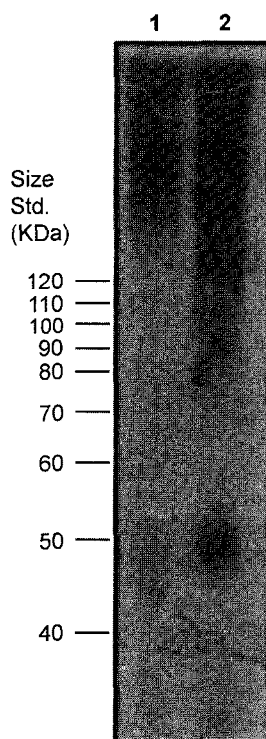

Fig. 1. SDS-PAGE analysis of biotin-labeled Quantazyme-soluble cell wall proteins. lane 1, LP0353; lane 2, LP0353RS1.

cell wall fractions. This result suggests that covalent links of a specific group of cell wall proteins to other cell wall components might be changed to a hydrogen bond link by defects in glycosylation and/or GPI-anchor attachment. Large molecular weight cell wall proteins detected in the mutant Quantazyme-soluble preparation (Fig. 1) indicates that these cell wall proteins seem to be still attached to other cell wall components via a covalent link. A previous report showed that GPI-anchor attachment to Gas1p is normal in the *ret1-1* mutation (Sutterlin *et al.*, 1997). Taking these together, it can be therefore postulated that during the intracellular transport, cell wall proteins are hypoglycosylated because of the defect in glycosylation steps caused by the *soo1-1* mutation; not all of the cell wall proteins are transported to the cell surface and/or to be modified post-translationally in a COPI-dependent manner. The latter could be partly supported by the recent report that cargo proteins such as carboxypeptidase Y (CPY) and pro-α-factor accumulated in the ER of *sec21-3* (γ-COP mutant of COPI) cells, but invertase and HSP150 were secreted with normal transport kinetics from the same cells (Gaynor and Emr, 1997).

Linkage between cell wall proteins and β-glucans is affected by soo1-1 mutation

The majority of the cell wall proteins of *S. cerevisiae* are retained by covalent association with β-1,6-glucan which is itself coupled to β-1,3-glucan (Kapteyn *et al.*, 1996). Cell wall mannoprotein is attached to β-1,6-glucan through a remnant of a GPI (glycosylphosphatidylinositol)-anchor containing five α-linked mannosyl residues. The β-1,6-glucan has some β-1,3–linked branches, and it is to these branches that the reducing terminus of chitin chains appears to be attached in a β-1,4 or β-1,2 linkage. Finally, the reducing end of β-1,6-glucan is connected to the non-reducing terminal glucose of β-1,3-glucan through a linkage that remains to be established. Most of SDS-resistant proteins in the cell wall could be solubilized by β-1,3 glucanase digestion of the cell wall (Kollar *et al.*, 1997).

To test the link between cell wall proteins and β-glucans, cell wall proteins extracted by Laemmli-sample buffer and Quantazyme treatment were analyzed by immunoblot with antibodie against Pustulan (β-1,6-glucan) and Laminarin (β-1,3-glucan), respectively. As shown in Fig. 2A, large amount of cell wall proteins was detected from the Laemmli-sample buffer soluble fractions of both strains. In these fractions, the covalent link of cell wall proteins to β-1,6 glucan (Lanes 1 and 2 of Fig. 2A) and β-1,3-glucan (Lanes 1 and 2 of Fig. 2B) is apparent. However, β-1,6 glucan attached cell wall proteins were hardly detected in Quantazyme soluble fractions in either strain (Fig. 2A Lane 3, 4). Notably, the molecular weight of proteins linked to β-1,6 glucan in Laemmli-sample buffer soluble fraction from the *soo1-1* allele was lower than that from the *SOO1* allele (Fig. 2A, Lane 1, 2). Also the amount of large molecular weight

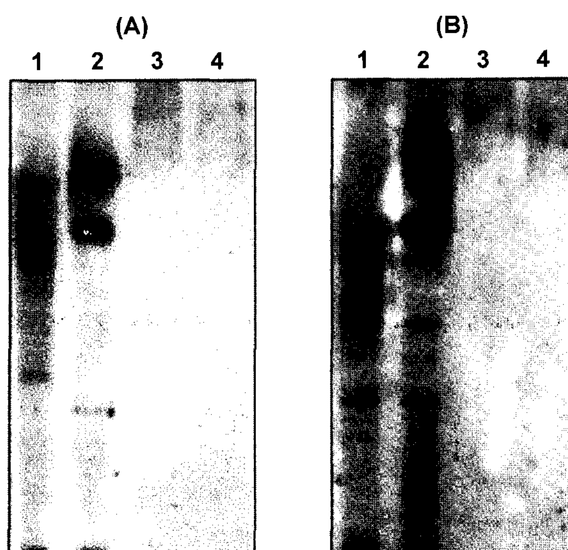

Fig. 2. Immunoblot analysis of cell wall proteins with anti-pustulan (panel A) and anti-laminarin (panel B) antiserum, respectively. lanes 1, 2, Laemmli-sample buffer soluble fractions; lanes 3, 4, Quantazyme-soluble fractions; lanes 1, 3, LP0353; lanes 2, 4, LP0353/RS1. All strains were grown in YEPD containing 1.2 M sorbitol at 37°C.

proteins associated with β-1,3-glucan was largely reduced (Fig. 2B, Lane 1, 2).

These results suggested that the downshift in the mobility of cell wall proteins linked to β-glucans from mutant cell walls might be mainly due to hypo-glycosylation of cell wall proteins, because the *ret1-1/soo1-1* mutation appears not to block the GPI-anchor attachment, which is crucial for β-glucan association to cell wall proteins. However, it can not be ruled out that the low level of covalent β-glucans (β-1,6 and/or β-1,3) linked to cell wall proteins occurs in the mutants, which shows a decrease in β-1,6-glucan and β-1,3-glucan (Song *et al.*, 1992; Lee *et al.*, 1999). In addition to the downshift of major protein bands, differences in several minor band patterns were also apparent (Fig. 2A, Lane 1). Although further experiments should be done to clarify the involvement of Soo1p in posttranslational modifications of cell wall proteins, such as glycosylation and the link to structural β-glucans, these results indicate that glycosylation of cell wall proteins and the linkage between cell wall proteins and β-glucans and/or other cell wall components may be impaired by the *soo1-1* mutation.

Sugar content of the cell wall proteins is altered by the soo1-1 mutation

In yeast, secretory proteins are heavily mannosylated in the ER by *N*-glycosylation and/or *O*-glycosylation. Biochemical analysis using an *in vitro* protein transport assay suggested that COPI-coated vesicles mediate *cis*-Golgi to medial Golgi protein transport (Ostermann *et al.*, 1993; Waters *et al.*, 1991), and is involved in the retrograde transport of ER resident proteins from the Golgi back to the ER (Cosson and Letourneur, 1994). The *ret1-1* mutation also impairs the retrograde transport (from the Golgi to the ER) of dilysine-tagged ER membrane proteins, such as Wbp1p, which is an essential subunit for the oligosaccharyltransferase (OST) complex performing *N*-glycosylation of the secretory proteins in the ER (Letourneur *et al.*, 1994; Schroder-Kohne *et al.*, 1998).

Table 1. Amount of D-mannose in the Laemmli-sample buffer extractable cell wall proteins

Strain	Temperature (°C)	D-mannose (nmol/μg protein)
LP0353RS1	28	52.68
(Wild Type)	37	55.73
LP0353	28	36.90
(Mutant Type)	37	9.40

Since results shown in Fig. 1 and 2 strongly suggest that impairment of glycosylation in cell wall proteins from the *soo1-1* mutant, the mannose content of the cell wall proteins extracted with the Laemmli-sample buffer was measured. In contrast to the wild type strains, which showed no significant change of mannose content in cell wall proteins even after upshifting of culture temperature, the mutant strain showed a dramatic reduction of mannose content upon shifting the culture to the nonpermissive temperature (Table 1). It is also noteworthy that phenotypes of OST mutant reported previously (Knauer and Lehle, 1999; Reiss *et al.*, 1997) shares many aspects in common with those of the *soo1-1* mutant. These results, therefore, suggest that the *soo1-1* mutation blocks retrograde transport of dilysine-tagged ER membrane protein, such as Wbp1p, and thus impairs glycosylation of cell wall proteins in the ER, which ultimately leads to weakening of cell wall integrity by altering linkage between cell wall proteins and other cell wall components.

In summary, although rigorous confirmation of this issue would demand further experimental evidence, the results shown here suggest that the *soo1-1* mutation impairs the glycosylation of cell wall proteins being transported through the endomembrane system, and thus, as mentioned previously (Lee *et al.*, 1999; 2001), that the Soo1p (α-COP) may play a crucial role in recognition and translocation of proteins for β-1,3-glucan synthesis and other proteins for the maintenance of cell wall integrity by modulating posttranslational modification of those proteins. In addition, our results suggest that use of the *soo1-1* mutant will facilitate the identification of cell wall proteins, which may affect the maintenance of cell wall integrity in yeast via differential cell wall proteomics.

Acknowledgment

This work was supported by Korea Research Foundation

Grant (KRF-2000-005-D00003).

References

Ballou, C.E. 1990. Isolation, characterization, and properties of *Saccharomyces cerevisiae mnn* mutants with nonconditional protein glycosylation defects. *Methods Enzymol.* 185, 440-470.

Cosson, P. and F. Letourneur. 1994. Coatomer interaction with dilysine endoplasmic reticulum retention motifs. *Science.* 263, 1629-1631.

Eugster, A., G. Frigerio, M. Dale, and R. Duden. 2000. COPI domains required for coatomer integrity, and novel interactions with ARF and ARF-GAP. *EMBO J.* 19, 3905-3917.

Gaynor, E.C. and S.D. Emr. 1997. COPI-independent anterograde transport: cargo-selective ER to Golgi protein transport in yeast COPI mutants. *J. Cell Biol.* 136, 789-802.

Kapteyn, J.C., R.C. Montijn, E. Vink, de la Cruz, J., A. Llobell, J.E. Douwes, H. Shimoi, P.N. Lipke, and F.M. Klis. 1996. Retention of *Saccharomyces cerevisiae* cell wall proteins through a phosphodiester-linked β-1,3-/β-1,6-glucan heteropolymer. *Glycobiol.* 6, 337-345.

Knauer, R. and L. Lehle. 1999. The oligosaccharyltransferase complex from *Saccharomyces cerevisiae*. Isolation of the *OST6* gene, its synthetic interaction with *OST3*, and analysis of the native complex. *J. Biol. Chem.* 274, 17249-17256.

Kollar, R., B.B. Reinhold, E. Petrakova, H.J. Yeh, G. Ashwell, J. Drgonova, J.C. Kapteyn, F.M. Klis, and E. Cabib. 1997. Architecture of the yeast cell wall. β(1,6)-glucan interconnects mannoprotein, β(1,3)-glucan, and chitin. *J. Biol. Chem.* 272, 17762-17775.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685.

Le Borgne, R. and B. Hoflack. 1998. Mechanisms of protein sorting and coat assembly: insights from the clathrin-coated vesicle pathway. *Curr. Opin. Cell Biol.* 10, 499-503.

Lee, D.W., G.W. Ahn. H.G. Kang, and H.M. Park. 1999. Identification of a gene, *SOO1*, which complements osmo-sensitivity and defect in *in vitro* β1,3-glucan synthase activity in *Saccharomyces cerevisiae. Biochim. Biophys. Acta* 1450, 145-154.

Lee, J.J., D.W. Lee, K.H. Kim, and H.M. Park. 2001. Isolation and analysis of the yeast mutant gene, *soo1-1*, which confers the defect in β-1,3-glucan biosynthesis. *Kor. J. Microbiol.* 37, 42-48 (written in Korean).

Letourneur, F., E.C. Gaynor, S. Hennecke, C. Demolliere, R. Duden, S.D. Emr, H. Riezman, and P. Cosson. 1994. Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. *Cell* 79, 1199-1207.

Meikle, P.J., N.J. Hoogenraad, I. Bonig, A.E. Clarke, and B.A. Stone, (1994) A (1→3,1→4)-β-glucan-specific monoclonal antibody and its use in the quantitation and immunocytochemical location of (1→3,1→4)-β-glucans. *Plant J.* 5, 1-9.

Montijn, R.C., J. van Rinsum, F.A. van Schagen, and F.M. Klis. 1994. Glucomannoproteins in the cell wall of *Saccharomyces cerevisiae* contain a novel type of carbohydrate side chain. *J. Biol. Chem.* 269, 19338-19342.

Mrsa, V., T. Seidl, M. Gentzsch, and W. Tanner. 1997. Specific labelling of cell wall proteins by biotinylation. Identification of four covalently linked *O*-mannosylated proteins of *Saccharomyces cerevisiae*. *Yeas.* 13, 1145-1154.

Orlean, P. 1997. Cell Cycle and Cell Biology, pp. 229-362. *In* J.R. Pringle, J.R. Broach, E.W. Jones (Eds.), The Molecular and Cellular Biology of the Yeast *Saccharomyces*, Vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ostermann, J., L. Orci, K. Tani, M. Amherdt, M. Ravazzola, Z. Elazar, and J.E. Rothman. 1993. Stepwise assembly of functionally active transport vesicles. *Cell* 75, 1015-1025.

Reiss, G., S. te Hessen, R. Gilmore, R. Zufferey, and M. Aebi. 1997. A specific screen for oligosaccharyltransferase mutations identifies the 9 kDa OST5 protein required for optimal activity *in vivo* and *in vitro*. *EMBO J.* 16, 1164-1172.

Rothman, J.E. and F.T. Wieland. 1996. Protein sorting by transport vesicles. *Science* 272, 227-234.

Roy, R., E. Katzenellenbogen, and H.J. Jennings. 1984. Improved procedures for the conjugation of oligosaccharides to protein by reductive amination. *Can. J. Biochem. Cell Biol.* 62, 270-275.

Schekman, R. and L. Orci. 1996. Coat proteins and vesicle budding. *Science* 271, 1526-1533.

Schroder-Kohne, S., F. Letourneur, and H. Riezman, 1998. α-COP can discriminate between distinct, functional di-lysine signals *in vitro* and regulates access into retrograde transport. *J. Cell Sci.* 111, 3459-3470.

Song, M.R., D.W. Lee, S.W. Park, K.S. Bae, and H.M. Park, 1992. Isolation and characterization of *Saccharomyces cerevisiae* mutants deficient in (1,3)-β-D-glucan synthase. *Kor. J. Appl. Microbiol. Biotechnol.* 20, 642-646.

Sutterlin, C., T.L. Doering, F. Schimmoller, S. Schroder, and H. Riezman. 1997. Specific requirements for the ER to Golgi transport of GPI-anchored proteins in yeast. *J. Cell Sci.* 110, 2703-2714.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76, 4350-4354.

Waters, M.G., T. Serafini, and J.E. Rothman. 1991. 'Coatomer': a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. *Nature* 349, 248-251.