Export of Major Cell Surface Proteins Is Blocked in Yeast Secretory Mutants

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ABSTRACT The transport of newly synthesized proteins to the yeast cell surface has been analyzed by a modification of the technique developed by Kaplan et al. (Kaplan, G., C. Unkeless, and Z. A. Cohn, 1979, Proc. Natl. Acad. Sci. USA, 76:3824-3828). Cells metabolically labeled with $^{35}$SO$_4^{2-}$ are treated with trinitrobenzenesulfonic acid (TNBS) at 0°C under conditions where cell-surface proteins are tagged with trinitrophenol (TNP) but cytoplasmic proteins are not. After fractionation of cells into cell wall, membrane and cytoplasmic samples, and solubilization with SDS, the tagged proteins are immunoprecipitated with anti-TNP antibody and fixed Staphylococcus aureus cells. Analysis of the precipitates by SDS gel electrophoresis and fluorography reveals four major protein species in the cell wall (S$_1$-S$_4$), seven species in the membrane fraction (M$_1$-M$_7$), and no tagged proteins in the cytoplasmic fraction.

Temperature-sensitive mutants defective in secretion of invertase and acid phosphatase (sec mutants; Novick, P., C. Field, and R. Schekman, 1980, Cell, 21:204-215) are also defective in transport of the 11 major cell surface proteins at the nonpermissive temperature (37°C). Export of accumulated proteins is restored in an energy-dependent fashion when secI cells are returned to a permissive temperature (24°C). In wild-type cells the transit time for different surface proteins varies from less than 8 min to about 30 min. The asynchrony is developed at an early stage in the secretory pathway.

All of the major cell wall proteins and many of the externally exposed plasma membrane proteins bind to concanavalin A. Inhibition of asparagine-linked glycosylation with tunicamycin does not prevent transport of several surface proteins.

Several independent lines of evidence suggest that eukaryotic cell surface proteins are transported via the secretory process. The most extensive evidence concerns the biosynthesis and export of viral membrane glycoproteins, among which the VSV G protein is best characterized. Biochemical, cell fractionation, immunoelectron microscopy, and genetic experiments all support a pathway in which VSV G protein is synthesized by endoplasmic-reticulum-associated ribosomes and transported to the plasma membrane via the Golgi-body and vesicles (1, 2, 3, 4). Less extensive but similar sorts of experiments support this mode of export for cellular plasma membrane proteins (5, 6).

Recent reports have suggested specialization of the Golgi apparatus to account for movement of distinct groups of exported proteins. Monensin, an ionophore that blocks transport of most proteins within the Golgi body (7), causes some glycoproteins to arrest before and some after, conversion of N-glycosidically linked oligosaccharides from the early high-mannose form to the mature complex form (8, 9). In one extreme case, influenza virus, which contains glycoproteins with complex oligosaccharides, buds normally from cells treated with monensin (10). Similarly, Gumbiner and Kelly (11) have proposed a branch point in the Golgi body to account for the production of ACTH granules in a pituitary cell line that secretes hormone in a regulated fashion, while exporting an endogenous viral glycoprotein constitutively.

We have developed an alternate approach to the study of secretion and cell surface assembly. The cellular components that execute the secretory process in yeast have been identified genetically by the isolation of temperature-sensitive lethal mutants that block secretion (12, 13). Secretory mutants (sec) accumulate glycoproteins inside one of three distinct organ...
elles: endoplasmic reticulum (ER), Golgi bodies, or secretory vesicles. Analysis of double sec mutants has shown that the blocks define a linear pathway along which glycoprotein oligosaccharides are assembled much as in mammalian cells (14, 15). The sec mutants are also defective in bud growth and in the export of at least four permease activities (SO₄²⁻, arginine, galactose, and proline-specific permease). These properties, together with the substantial accumulation of membrane within sec mutant cells, led us to propose an essential contribution of the secretory process to plasma membrane assembly (13).

Kaplan, Unkeless, and Cohn (16) devised a procedure for the analysis of plasma membrane synthesis and turnover in macrophages that we have adapted to evaluate yeast cell surface assembly. This procedure has now allowed a more specific appraisal of transport to the cell wall (secreted proteins) and to the external surface of the plasma membrane. The results presented here suggest that in yeast the sec gene products are responsible for export of the major cell surface proteins.

MATERIALS AND METHODS

Strains and Media: The Saccharomyces cerevisiae strain X2180-1A was from the Yeast Genetic Stock Center (Berkeley, CA). Mutant strains HMSF1 (sec1-1), SF294-2B (sec1-1), HMSF176 (sec16-1), and HMSF136 (sec4-4) were derived from X2180-1A (12, 13).

Wickerham’s minimal medium (17) was used with the following modification: the ammonium sulfate concentration was lowered to 50 mM unless otherwise indicated, ammonium chloride was used as the nitrogen source, and the potassium phosphate concentration was raised to 20 mM to increase the buffering capacity. The carbon source was 2% glucose unless otherwise indicated. Liquid cultures were grown at 25°C in flasks or tubes with agitation, and the experiments were initiated with exponentially growing cells at an A₆₀₀ of 3.0–4.5. The absorbance of cell suspensions was measured in a 1 cm quartz cuvette at 600 nm in a Zeiss spectrophotometer. 1 A₆₀₀ unit corresponds to 0.15 mg dry weight.

Reagents: Reagents were obtained as indicated: trinitrobenzenesulfonic acid, cycloheximide, tunicamycin, and concanavalin A coupled to Sepharose 4-B (8 mg protein/ml gel) from Sigma Chemical Co., St. Louis, MO; carrier-free H2 ¹³⁵SO₄ from Amersham, Arlington Heights, IL; Na ¹²⁵I and Enhance from New England Nuclear, Boston, MA; Iodo-gen from Pierce Chemical Co., Rockford, IL; fixed Staphylococcus aureus (IgG Sorb) from The Enzyme Center, Boston, MA. Anisterum prepared against TNP-keyhole limpet hemocyanin was generously provided by Dr. Ann Good. Anti-TNP IgG was purified by adsorption to and elution from DNP-bovine serum albumin coupled to Sepharose 4-B (18).

IODINATION: Cells (1–3 A₆₀₀ U) grown in low-sulfate minimal medium were sedimented in a clinical centrifuge, washed twice with 0.15 M NaCl, 20 mM sodium phosphate (pH 7.5), resuspended in 1 ml of the same buffer, and gently transferred to a tube in which 0.1 mg of iodogen had been dried down from a chloroform solution (1 mg/ml) (20). Na ¹²⁵I (100 μCi) was added and the iodination reaction was allowed to proceed for 15 min at 0°C. The cell suspension was removed and cells were washed three times with buffer. Cells were lysed by the techniques described in the legend to Fig. 1. Extracts diluted in sample buffer (2% SDS, 2% mercaptoethanol, 71 mM Tris-Cl [pH 6.8], 14% glycerol) were heated in a water bath at 94°C for 2 min. Aliquots (30 μl) were applied to 10% polyacrylamide slab gels (21) and electrophoresed at 30 mA for 2 h. Gels were dried and exposed on prefogged Kodak X-Omat R film.

RESULTS

Identification of Cell Surface Proteins

Yeast cells are surrounded by a rigid but porous wall consisting of glucan, β-1,3 and β-1,6 polymers of glucose, and mannann, a term given to a collection of secreted glycoproteins which contain large mannose-rich oligosaccharides. The plasma membrane forms the primary permeability layer of the cell. Secreted proteins, such as invertase and acid phosphatase, are trapped between the cell wall and the plasma membrane.

Cell surface proteins can be radioiodinated by catalytic iodination of intact yeast cells. Log-phase cells were labeled with ¹²⁵I and extracts were prepared by agitation of cells with glass beads. Iodinated proteins were solubilized by heating the extract in the presence of SDS, and analyzed by SDS-gel electrophoresis. Radioactive proteins with mobilities corresponding to 220, 155, 75, 51, 49, 33, 29, and 22 kdal were detected in SDS gels (Fig. 1).

Secreted and cell wall proteins are released and spheroplasts are formed when the β-1,3 glucan layer is degraded by a lytic glucanase in the presence of reducing agent and osmotic support (19). Iodinated cells were converted to spheroplasts with a purified glucanase or with lyticase (a lytic enzyme fraction that contains protease and glucanase activities) (19). Spheroplasts were sedimented, lysed by osmotic shock, and the resulting lysates were centrifuged to generate membrane and soluble cytoplasmic fractions. The membrane, membrane, and cytoplasmic fractions from each lytic enzyme digest were ex-
purified lytic glucanase or 500 U of lyticase, and incubated at 30°C for 1 h. Secreted and membrane fractions were prepared for gel electrophoresis as in the TNBS tagging procedure described in Materials and Methods. The third cell pellet was mixed with 0.3 g of glass beads (0.5 mm) and 50 μl of 2% SDS, 50 mM sodium phosphate (pH 7.5), 50 mM β-mercaptoethanol. Cells were broken by agitation on a vortex mixer for 1.5 min, followed by heating at 94°C for 2 min. All solubilized fractions were diluted with sample buffer to a final concentration of 150 U of enzyme in 1 ml, heated at 94°C for 2 min, and aliquots (30 μl) were applied to an SDS gel. An autoradiograph of the gel is shown.

To eliminate the possibility that TNP-tagged nonradioactive proteins aggregate with untagged radioactive proteins, we performed a control experiment in which cells were first treated with TNBS, washed, and then metabolically labeled with 35S. These cells contain proteins tagged with TNP and other proteins labeled with 35S but no proteins both tagged and radiolabeled. Aggregation was not a problem because neither the secreted nor the membrane fraction contained immunoprecipitated radioactive proteins (Fig. 2, pretagged). Addition of 50 mM β-mercaptoethanol during solubilization of the membrane fraction was essential for the success of this control. Nonspecific precipitation of radioactive proteins was reduced further by dilution of treated cells with a 10-fold excess of untreated cells before fractionation.

### Thermoreversible Export in Secretory Mutants

Wild-type and sec mutant cells were radiolabeled at a permissive temperature (25°C) and the pattern of exported proteins was examined by the TNBS-tagging procedure. Fig. 3 shows that the pattern of secreted (wall) and of plasma membrane surface proteins was similar in wild-type and mutant...
cells at 25°C. At the nonpermissive temperature (37°C), however, export of all major surface proteins was blocked in sec mutant cells (Fig. 4). Mutants sec1 (accumulates secretory vesicles), sec7 (accumulates Golgi bodies), and sec18 (accumulates endoplasmic reticulum) showed no detectable export of proteins S1 to S4, although a return to 25°C (in the presence of cycloheximide) allowed export (Fig. 4A). As was shown for invertase (15), reversible secretion of proteins S1 to S4 was blocked by an energy poison. Proteins M1 to M4; at the plasma membrane surface were exported at a much reduced level in mutants sec1 and sec7 at 37°C but were restored upon return to 25°C (Fig. 4B). The M6 species appeared not to be reduced as completely as the other major proteins in the sec1 and sec7 membrane fraction.

The pattern of export at 37°C was examined in representatives from all sec complementation groups and, although some were not blocked completely, most strains showed substantial reduction of all major surface proteins. Two exceptions were noted. sec11, a mutant which blocks secretion of acid phosphatase more completely than invertase (13), also showed anomalous behavior of the TNP-tagged secreted proteins: export of proteins S3 and S4 was blocked, while proteins S1 and S2 were secreted normally (data not shown). The membrane fraction from some of the mutants showed a series of TNP-tagged radioactive proteins that were not prominent in wild-type cells labeled at 37°C, while in other mutants, such as sec18, these bands were as prominent as the M3 to M7 species seen in wild-type cells (not shown). Similarly, a series of new bands appeared in the secreted fraction when sec18 cells were radiolabeled at 37°C (Fig. 4A). The abnormal proteins detected in the mutants may not be at the cell surface, or they may not be apparent unless the export of major proteins is blocked. Evidence suggesting an intracellular location for these proteins is reviewed in the Discussion.

Glycosylation and Export of Surface Proteins

The glycoprotein nature of isolated surface proteins was examined by binding to ConA-Sepharose. Wild-type cells were radiolabeled, tagged with TNBS, and TNP-modified proteins isolated with antibody. Immunoprecipitates were solubilized with SDS, diluted with buffer containing Triton X-100, and mixed with ConA-Sepharose. Proteins bound to ConA-Sepharose were sedimented, solubilized again with SDS, and analyzed by SDS-gel electrophoresis. Fig. 5 shows an SDS gel fluorograph in which ConA-Sepharose-bound samples were compared with equal fractions of the original immunoprecipitates. Proteins S1 to S3 and M2 and M4 were efficiently retained by ConA-Sepharose. Proteins M1, M3, and M5 showed partial binding, while M5 and M7 did not bind. Addition of more ConA-Sepharose did not enhance the recovery of any protein. In contrast, among the minor proteins recovered from the membrane fraction of sec7 cells (accumulates secretory vesicles) labeled at 37°C, none were bound by ConA-Sepharose.

The role of N-linked glycosylation in export of yeast surface proteins was examined with tunicamycin, a drug that blocks synthesis of the core oligosaccharide (22). Treatment of wild-type cells with tunicamycin during the period of radiolabeling resulted in altered electrophoretic profiles for the secreted and membrane fraction immunoprecipitates (Fig. 6). Tunicamycin treatment affected surface proteins in different ways. Protein S4 appeared to be secreted normally but with a reduced molecular weight; protein M6, which did not bind to ConA, appeared to be

**Figure 4** Mutant and wild-type cells labeled at 37°C. Cells were grown in low-sulfate minimal medium and labeled with 150 µCi of 35SO42- for 1 h at 37°C. Two additional cultures of sec7 cells were labeled and centrifuged; one cell pellet was resuspended in medium containing 0.1 mg/ml of cycloheximide, the other in medium with cycloheximide and 20 mM sodium azide but without glucose. These two cultures were incubated at 25°C for 2 h while the other samples were washed and stored at 0°C. All samples were then tagged with TNBS, fractionated, and solubilized immunoprecipitates (20-µl aliquots) were analyzed by SDS gel electrophoresis. (a) Cell wall fraction from: sec1 37°C; sec7 37°C; sec18 37°C; sec1 37°C shifted to 25°C with sodium azide, cycloheximide, without glucose; sec1 37°C shifted to 25°C with cycloheximide; X2180-1A 37°C. (b) Membrane fraction from: sec1 37°C, sec7 37°C, sec18 37°C, sec1 37°C shifted to 25°C with cycloheximide; X2180-1A 37°C.

**Figure 5** Binding of radiolabeled, TNP-tagged proteins to ConA-Sepharose. Solubilized immunoprecipitates, prepared from samples such as in Fig. 4, were diluted and treated with ConA-Sepharose. Bound material was released from the Sepharose and compared in adjacent lanes of an SDS gel with an equivalent volume of the original immunoprecipitate. A fluorograph of the gel shows (starting at the left): (a) immunoprecipitate from X2180-1A membrane fraction; (b) same material bound to ConA-Sepharose (66 µl); (c) same bound to ConA-Sepharose (33 µl); (d) immunoprecipitate from X2180-1A cell wall fraction; (e) same bound to ConA-Sepharose (66 µl); (f) immunoprecipitate from a sec6 membrane fraction; (g) same material bound to ConA-Sepharose (66 µl); (h) same bound to ConA-Sepharose (33 µl).
were analyzed by SDS gel electrophoresis; a fluorograph of the gel is shown.

exported normally with no change in mobility. It was not always clear whether a protein species was shifted or not exported; however, unlike the effect of the sec mutations, protein export was not generally blocked by inhibition of oligosaccharide synthesis.

Transit Time and Turnover of Surface Proteins

The transit time of newly synthesized protein to the cell surface was analyzed in a pulse-chase experiment. Wild-type cells were radiolabeled with $^{35}$SO$_4^{2-}$ for 2 min and transferred to nonradioactive medium for various chase periods. Cells were tagged with TNBS, fractionated, and analyzed as before. Fig. 7 shows that proteins S$_2$, M$_3$, M$_6$, and M$_7$ appeared at the cell surface between 3 and 8 min of chase. By 30 min, proteins S$_1$, S$_3$, S$_4$, and M$_1$ emerged. Proteins M$_2$ and M$_4$ appeared last and continued to increase in intensity between 58 and 88 min of chase.

The asynchronous appearance of surface proteins implied a differential delay in transport along the secretory pathway. sec Mutants blocked early (sec18, ER-blocked) and late (sec6, secretory vesicle-blocked) in the secretory pathway were used to evaluate the site of delay. Cells were labeled with $^{35}$SO$_4^{2-}$ for 4 min at 37°C and then chased at the same temperature for 45 min in nonradioactive medium. Aliquots were withdrawn at various times after return to 25°C, and secreted proteins were analyzed by TNBS tagging. If asynchrony developed before the secretion block, the chase at 37°C could allow proteins to be exported with similar kinetics during recovery at 25°C. Fig. 8 shows that, in sec6, proteins S$_2$ and S$_3$ were released coordinately at 25°C. Proteins M$_1$, M$_2$, and M$_3$, which appeared asynchronously in the experiment in Fig. 7, were exported coordinately in the sec6 experiment (not shown). The same experiment with sec18 showed a lag between proteins S$_2$ and S$_3$ just as was seen in the pulse-chase labeling of wild-type cells (data not shown). These results suggested that protein S$_1$ was delayed either in the ER (after the sec18 block) or in the Golgi body. Unfortunately, sec7, the Golgi-blocked mutant, was not thermoreversible under the conditions of this experiment (15).

Turnover of cell surface proteins was examined with $^{125}$I-labeled cells that were TNBS-treated before or after one generation of growth. TNP-tagged and iodinated cells were fractionated and aliquots of each sample were analyzed before and after precipitation with TNP-antibody. At time zero, 46% of the cell wall protein $^{125}$I and 73% of the plasma membrane surface $^{125}$I were immunoprecipitated. SDS gel analysis (Fig. 9) showed virtually identical patterns of total and TNP-tagged iodinated proteins. The principal loss of $^{125}$I label in the cell wall fraction was due to incomplete precipitation of protein S$_1$. After growth for 2 h (one doubling time), the yield of surface proteins was cooled to 25°C. At the indicated times, 0.25-ml aliquots were removed and chilled to 0°C. At the end of the experiment, all samples were processed for TNBS tagging. Aliquots of solubilized immunoprecipitates from the cell wall and membrane fractions were analyzed by SDS gel electrophoresis; a fluorograph of the gel is shown.

**FIGURE 6** Proteins exported in the presence of tunicamycin. X2180-1A cells (0.67 A$_{600}$ U) were suspended in two cultures of minimal medium; one contained tunicamycin (20 μg/ml). After 30 min at 25°C, 190 μCi of $^{35}$SO$_4^{2-}$ was added to each, and labeling continued for 45 min at the same temperature. Cells were tagged, fractionated, and solubilized material was immunoprecipitated. Aliquots of the solubilized immunoprecipitates were analyzed by SDS gel electrophoresis; a fluorograph of the gel is shown.

**FIGURE 7** Transit time of cell surface proteins. X2180-1A cells (4 A$_{600}$ U in 1 ml), grown in low-sulfate minimal medium at 25°C, were incubated at 37°C for 5 min and then labeled with 1.5 mCi of $^{35}$SO$_4^{2-}$ for 2 min. Ammonium sulfate (0.5 mM final concentration) was added and the cells were sedimented, resuspended in 1.5 ml of fresh medium, and incubation was continued at 37°C. At the indicated times, 0.25-ml samples were removed and chilled to 0°C. At the end of the experiment, all samples were processed for TNBS tagging. The chase was effective because the amount of total radioactive protein did not vary by >6% from the zero-time sample. Aliquots of solubilized immunoprecipitates from the cell wall and membrane fractions were analyzed by SDS gel electrophoresis; a fluorograph of the gel is shown.

**FIGURE 8** Synchronous export of secretory proteins accumulated in sec6. Cells (4 A$_{600}$ U in 1 ml), grown in low-sulfate minimal medium at 25°C, were incubated at 37°C for 2 min and then labeled with 0.4 mCi of $^{35}$SO$_4^{2-}$ for 4 min. Cells were sedimented, resuspended in 15 ml of minimal medium containing 0.5 mM ammonium sulfate, and incubation was continued for 45 min at 37°C. Cycloheximide (0.1 mg/ml) was added and the culture was cooled to 25°C. At the indicated times, 0.25-ml aliquots were removed and chilled to 0°C. At the end of the experiment, all samples were processed for TNBS tagging. Aliquots of solubilized immunoprecipitates from the cell wall fraction were analyzed by SDS gel electrophoresis; a fluorograph of the gel is shown.
proteins with apparent molecular weights of 220 (M₁), 155 (M₂), 49 (M₃), 33 (M₄), and 29 kdal (M₆). In addition, a 31 kdal reveals a 75 kdal protein that is detected only marginally by plasmic proteins. The cell wall fraction contains proteins with apparent molecular weights of >250 kdal (S₁; could be several subsets of exported proteins travel in different compartments. The components of the yeast cell surface have been defined by two techniques. The first, radioiodination of intact cells, identifies surface proteins with exposed tyrosine residues. The other, TNBS treatment and TNP-antibody precipitation, allows identification and isolation of radiolabeled surface proteins that have reactive amino groups. Both probes label secreted and plasma membrane surface proteins but not cytoplasmic proteins. The cell wall fraction contains proteins with apparent molecular weights of >250 kdal (S₁; could be several protein species), 140 (S₂), 51 (S₃), and 33 kdal (S₄). Iodination reveals a 75 kdal protein that is detected only marginally by the TNBS procedure with cells that are radiolabeled at 25°C. The membrane fraction contains proteins with apparent molecular weights of 220 (M₁), 155 (M₂), 49 (M₃), 33 (M₄), and 29 kdal (M₆). In addition, a 31 kdal protein (M₅) can often be resolved from protein M₆ (Fig. 2), although synthesis of both M₄ and M₅ is reduced at 25°C (compare Figs. 2 and 3). A protein with an apparent molecular weight of 19 kdal (M₇) is detected by the TNBS procedure but not by iodination. A good correlation between the two techniques is most apparent from a comparison of ¹²⁵I-labeled surface proteins from TNBS-tagged cells before and after TNP-antibody precipitation (Fig. 9). When the yield of ¹²⁵I in the TNP-antibody immunoprecipitate is taken into account, the cell wall protein accounts for 0.9%, and the plasma membrane surface protein accounts for 0.5%, of the total ³⁵S-O₂⁻⁻ incorpoated into cells during a 1-h incubation.

The TNBS procedure has allowed an evaluation of protein export in mutants that are temperature-sensitive for secretion and growth. Earlier work on the sec mutants suggested a pleiotropic defect in export of cell wall glycoproteins and a plasma membrane permease activity (13). The results presented here demonstrate that the conditional block is a general phenomenon. Furthermore, with the exceptions noted below, representative mutant alleles of 22/23 class A sec genes show the same pattern of blocked transport. In some of the mutants, export of the major surface proteins is reduced but not eliminated. This is best explained by mutations that are not completely restrictive at 37°C. On the other hand, sec11 blocks export of two secreted proteins, with no effect on two others. This mutant also shows unequal effects on secretion of acid phosphatase and invertase and, unlike the other sec mutants, sec11 does not accumulate organelles (13). The SEC11 gene may only be required for export of a subset of cell surface proteins.

The membrane fraction from sec mutant cells radiolabeled at 37°C contains TNP-tagged proteins that are less apparent in a comparable wild-type cell preparation, or in sec mutant cells labeled at 25°C (Figs. 3 and 4). Either these proteins are not really exposed at the cell surface and are recovered in the membrane fraction because of some artifact, or they are surface proteins that become apparent only when the export of major surface proteins is blocked. Several lines of indirect evidence support the first explanation. First, unlike many of the wild-type membrane surface proteins, none of the labeled proteins in a sec6 membrane fraction binds to concanavalin A (Fig. 5). Second, the labeling of these anomalous proteins varies irregularly among sec mutant strains; there is no pattern of appearance with respect to the stage in the secretory pathway that is blocked. Third, the sec mutants that show pronounced labeling of these species also reveal a series of new polypeptides in the cell wall fraction (sec18, Fig. 4A). We suspect that the anomalous proteins are of cytoplasmic origin. A small and variable percentage of sec mutant cells may become permeable during incubation at 37°C so that subsequent TNBS treatment allows tagging of intracellular proteins. Although the results with mutants sec1 and sec7 (Fig. 4) clearly indicate that transport of the major surface proteins is blocked at 37°C, the possibility that a minor class of membrane proteins is exported by a different mechanism deserves further analysis with more specific probes.

We have previously shown that the organelles and glycoproteins accumulated in single and double sec mutant strains define a unique, linear secretory pathway (14, 15). Although the TNBS-tagging experiments support this conclusion, the results are also consistent with parallel pathways in which subsets of exported proteins travel in different compartments. This possibility will be tested by analysis of secretory organelles purified from sec mutant cells.

Alternate, or parallel, transport pathways have been invoked to explain the widely varying rates at which newly synthesized proteins are exported. Strous and Lodish (9) reported transit
times of 23 min for albumin and VSV G protein and 40 min for transferrin in rat hepatoma cells. Monensin causes transport of transferrin to arrest before, and VSV G protein after, conversion of N-glycosidically linked oligosaccharides from the early high-mannose to the mature complex form. In spite of these differences, the two proteins accumulate in the same Golgi body vesicles (H. Lodish, personal communication). More dramatic, however, is the recent demonstration of two pathways for the secretion of ACTH (11). A pituitary cell line secretes mature ACTH by regulated exocytosis of granules; the transit time of mature ACTH in unstimulated cells is 3–4 h. The same cells display constitutive export of a viral membrane glycoprotein, and the precursor of ACTH, with a transit time of 40 min. Furthermore, the granules responsible for secretion of mature ACTH have been purified and contain neither ACTH precursor nor viral glycoprotein. Gumbiner and Kelly propose a branch point at the Golgi body to account for regulated and constitutive limbs of the secretory pathway.

There is no precedent for regulated secretion in yeast. Thin sections of budding yeast cells show very low levels of secretory organelles (12). Transport rates in yeast are generally greater than in mammalian cells: yeast invertase transits in 5 min or less (15), and a protease secreted by *Saccharomyces lipolytica* requires only 3 min for transport (D. Oglydziak, personal communication). The TNBS-tagging procedure has revealed major yeast surface proteins that are secreted rapidly, like invertase, and others that emerge more slowly (Fig. 7). The asynchrony appears to develop at a late step in the ER, or in the Golgi body. When sec mutant cells are pulse-labeled and chased at 37°C, synchronous secretion occurs upon return to 25°C if the mutant is blocked after, but not before, the Golgi body stage (Fig. 7). A similar conclusion was drawn by Strous and Lodish (9) who found that pulse-labeled glycoprotein precursors show transit time-dependent variation in the rate of oligosaccharide processing, reflecting asynchronous passage through the Golgi body.

Although many of the yeast surface proteins are glycosylated, as judged by their binding to ConA (Fig. 5), glycosylation appears not to be a general requirement for export (Fig. 6). A 30 kdal polypeptide, perhaps related to protein Sop, is synthesized and secreted when cells are treated with tunicamycin. Protein Mop, on the other hand, appears not to be exported without glycosylation. This heterogeneity in the effect of tunicamycin-treatment on protein transport has been noted in other systems (9, 23). Glycoproteins may vary in the degree to which oligosaccharides participate in protein folding, and this may be reflected in the rate of transport through the secretory pathway.

Turnover of yeast cell surface proteins is slow with respect to the generation time. All but one surface protein remains in place during one cell-doubling time (Fig. 9). Although endo-

cytosis has not been examined in yeast cells, the possibility remains that surface proteins are subject to internalization and recycling.

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REFERENCES

1. Rothman, J. E., and H. F. Lodish. 1977. Synchronized transmembrane insertion and glycosylation of a nascent membrane protein. Nature (Lond.) 269:775-780.
2. Katz, F. N., J. E. Rothman, D. M. Kuipe, and H. F. Lodish. 1977. Membrane assembly, synthesis and intracellular processing of the vesicular stomatitis viral glycoprotein. J. Cell Biol. 73:33-137.
3. Zilberstein, A., M. D. Studer, M. Porter, and H. F. Lodish. 1980. Mutants of vesicular stomatitis virus blocked at different stages in maturation of the viral glycoprotein. Cell 21:417-427.
4. Bergmann, J. E., K. T. Tokuyasu, and S. J. Singer. 1981. Passage of an integral membrane protein, the vesicular stomatitis virus glycoprotein, through the Golgi apparatus en route to the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. 78:1746-1750.
5. Farquhar, M. G., J. J. M. Bergeron, and G. E. Palade. 1974. Cytochemistry of Golgi fractions prepared from rat liver. J. Cell Biol. 60:25-28.
6. Tartakoff, A. D., H. H. P. Vassalli, and P. Vassalli. 1981. Intracellular transport of lymphoid surface glycoproteins: role of the Golgi complex. J. Mol. Biol. 150:525-535.
7. Tartakoff, A., and P. Vassalli. 1973. Plasma cell immunoglobin secretion. J. Exp. Med. 138:1333-1345.
8. Johnson, D., and M. Schlesinger. 1980. Vesicular stomatitis virus and Sindbis virus glycoprotein transport to the cell surface is inhibited by ionophores. Biochem. 103:807-824.
9. Strous, G., and H. F. Lodish. 1980. Intracellular transport of secretory and membrane proteins in hepatoma cells infected with vesicular stomatitis virus. Cell 22:709-717.
10. Alonso, F. V., and R. W. Compans. 1981. Differential effect of monensin on enveloped viruses that form at distinct plasma membrane domains. J. Cell Biol. 89:700-705.
11. Gumbiner, B., and R. Kelly. 1982. Two distinct intracellular pathways transport secreatory and membrane glycoproteins to the surface of pituitary tumor cells. Cell 28:51-59.
12. Novick, P., and R. Schekman. 1979. Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. 76:1858-1862.
13. Novick, P. C. Field, and R. Schekman. 1980. Identification of 23 complementation groups required for posttranslational events in the yeast secretory pathway. Cell 21:205-215.
14. Esmon, B. P., Novick, and R. Schekman. 1981. Compartmentalized assembly of oligosaccharide chains on exported glycoproteins in yeast. Cell 25:451-460.
15. Novick, P., S. Ferro, and R. Schekman. 1981. Order of events in the yeast secretory pathway. Cell 25:461-469.
16. Kaplan, G., J. C. Unkeless, and Z. A. Cohn. 1979. Insertion and turnover of macrophage plasma membrane proteins. Proc. Natl. Acad. Sci. U.S.A. 76:3824-3828.
17. Wickham, L. J. 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. 52:392-401.
18. Unkeless, J. C. 1977. The presence of two F, receptors on mouse macrophages: evidence from a variant cell line and differential tryptic sensitivity. J. Exp. Med. 150:941-947.
19. Scott, J., and R. Schekman. 1980. Lyticase: endoglucanase and protease activities that act together in yeast cell lysis. J. Bacteriol. 142:414-423.
20. Fraker, P. J., and J. C. Speck. 1979. Proteins and cell membrane iodinations with a sparingly soluble chloramide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril. Biochem. Biophys. Res. Commun. 90:349.
21. Lammli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
22. Tkacz, J., and J. O. Lampen. 1975. Tunicamycin inhibition of polyprenyl N-acyethylglucosaminyl pyrophosphate formation in calf liver microsomes. Biochem. Biophys. Res. Commun. 65:248-257.
23. Gebb, R., S. Schlesinger, and S. Kornfeld. 1979. The oligosaccharides of glycoprotein of vesicular stomatitis virus are temperature-sensitive and undergo intracellular aggregation at elevated temperatures. J. Biol. Chem. 254:3600-3607.