# Selective and Immediate Effects of Clathrin Heavy Chain Mutations on Golgi Membrane Protein Retention in *Saccharomyces cerevisiae*

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***Abstract.*** The role of clathrin in retention of Golgi membrane proteins has been investigated. Prior work showed that a precursor form of the peptide mating pheromone α-factor is secreted by *Saccharomyces cerevisiae* cells which lack the clathrin heavy chain gene (*CHC1*). This defect can be accounted for by the observation that the Golgi membrane protein Kex2p, which initiates maturation of α-factor precursor, is mislocalized to the cell surface of mutant cells. We have examined the localization of two additional Golgi membrane proteins, dipeptidyl aminopeptidase A (DPAP A) and guanosine diphosphatase (GDPase) in clathrin-deficient yeast strains. Our findings indicate that DPAP A is aberrantly transported to the cell surface but GDPase is not. In mutant cells carrying a temperature-sensitive allele of *CHC1* (*chc1-ts*), α-factor precursor appears in the culture medium within 15 min, and Kex2p and DPAP A reach the cell surface within 30 min, after imposing the nonpermissive temperature. In contrast to these immediate effects, a growth defect is apparent only after 2 h at the nonpermissive temperature. Also, sorting of the vacuolar membrane protein, alkaline phosphatase, is not affected in *chc1-ts* cells until 2 h after the temperature shift. A temperature-sensitive mutation which blocks a late stage of the secretory pathway, *sec1*, prevents the appearance of mislocalized Kex2p at the cell surface of *chc1-ts* cells. We propose that clathrin plays a direct role in the retention of specific proteins in the yeast Golgi apparatus, thereby preventing their transport to the cell surface.

PROTEINS that enter the secretory pathway are destined for the cell exterior, the plasma membrane, the major degradative organelle (lysosomes or vacuoles), and the secretory organelles themselves (Pfeffer and Rothman, 1987). In the case of several ER or Golgi proteins, domains have been identified which are required to maintain the proteins in residence. Without these sequences, the proteins usually continue along the secretory pathway to the cell surface. In a well-characterized example, retention of ER lumenal proteins appears to occur by means of a membrane-bound receptor which recognizes a carboxy-terminal four-amino acid retention signal on ER proteins (Pelham, 1990). The receptor has been proposed to dwell in an early Golgi compartment where it functions to return ER retention signal–bearing proteins back to the ER. The retention signals on several ER and Golgi membrane proteins have been recently identified (Machamer, 1991), but, in most cases, little else is known about other factors which influence membrane protein retention.

In the yeast *Saccharomyces cerevisiae*, the Golgi membrane protein encoded by the *KEX2* gene (Kex2p) requires its cytoplasmic domain for retention in the Golgi apparatus (Fuller et al., 1989). Insight into the mechanism of Kex2p retention has been provided by our studies of yeast mutants lacking the clathrin heavy chain gene (*CHC1*) (Payne and Schekman, 1989). In these mutants, Kex2p is mislocalized to the cell surface, a finding which suggests a novel role for clathrin as a participant in the retention process. The involvement of clathrin in receptor-mediated endocytosis in mammalian cells (Pearse and Robinson, 1990) provides a paradigm for clathrin's function in Kex2p retention. Lattices at the plasma membrane composed of clathrin heavy and light chains and associated proteins (APs, also known as adaptins) collect transmembrane receptors at sites undergoing vesiculation, leading to selective incorporation of the receptors into clathrin-coated endocytic vesicles. Collection of receptors at clathrin-coated plasma membrane sites depends on the receptors' cytoplasmic domains and is thought to occur by recognition of these domains by the clathrin APs. It has also been suggested that clathrin lattices on the *trans*-Golgi network (TGN) act in a similar fashion to recognize the cytoplasmic domains of mannose-6-phosphate receptors and direct packaging of the receptors carrying lysosomal precursors into vesicles targeted for prelysosomal compartments (Pearse and Robinson, 1990). By analogy, models for clathrin's role in retention posit interactions between the cytoplasmic tail of Kex2p and clathrin coats (Fuller et al.,

1. *Abbreviations used in this paper*: AP, associated protein; CHC1, clathrin heavy chain gene; CWP, cell wall protein; DPAP A, dipeptidyl aminopeptidase A; GDPase, guanosine diphosphatase; G6PD, glucose-6-phosphate dehydrogenase.

1989; Payne and Schekman, 1989). Such interactions could allow selective retention either by stable tethering of Kex2p in the Golgi apparatus or through a recycling event.

To further define the role of clathrin in Golgi membrane protein retention, we have used both clathrin-deficient (*chcl*Δ) mutants and strains carrying a temperature-sensitive allele (*chcl-ts*) of clathrin heavy chain to monitor the mislocalization of several Golgi membrane proteins. As an additional indicator of Golgi apparatus transport function, we have investigated the sorting and delivery of a vacuolar membrane protein in the *chcl* mutants. Our results are consistent with the model that clathrin plays a direct and selective role in the retention of certain Golgi membrane proteins.

## Materials and Methods

### Materials

Unless noted all reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

### Strains, Media, and Genetic Methods

*E. coli* strain used in this study was HB101 ($F^-$, *hsdS20*($r^-_B$, $m^-_B$), *recA13*, *ara-14*, *proA2*, *lacY1*, *galK2*, *rpsL20* ($Sm^r$), *xyl-5*, *mtl-1*, *supE44*, $\lambda^-$.) (Boyer and Roulland-Dussoix, 1969). Yeast strains used in this study are listed in Table I.

Strains carrying *chclΔ* and *dpp2Δ* were made by single step gene transplacement (Rothstein, 1983) using plasmid pchcl-Δ10 (Payne et al., 1987) and plasmid pGP6 (obtained from Tom Stevens, University of Oregon).

Yeast mating, sporulation, and tetrad analyses were conducted as described by Sherman et al. (1974). DNA transformations were performed by the lithium acetate procedure (Ito et al., 1982) or by spheroplast transformation (Hinnen et al., 1978).

YPD medium is 1% Bacto-Yeast extract, 2% Bactopeptone (Difco Laboratories, Inc., Detroit, MI) without amino acids (Difco Laboratories, Inc.), and 2% dextrose. SD is 0.67% yeast nitrogen base (Difco Laboratories, Inc.), 2% dextrose. Supplemented SD is SD with 20 μg/ml histidine, uracil, and tryptophan and 30 μg/ml leucine, adenine, and lysine. SD CAA medium is SD with 5 mg/ml vitamin assay casamino acid mix (Difco Laboratories, Inc.) with 15 μg/ml adenine, and 20 μg/ml methionine, histidine, uracil, and tryptophan. SD CAA-ura is SD CAA without uracil. SD CAA-trp is SD CAA without tryptophan. Cell densities in liquid culture were measured in a 1-cm plastic cuvette using a spectrophotometer (model DU-62; Beckman Instruments, Inc., Palo Alto, CA). One $A_{500}$ unit is equivalent to $2.3 \times 10^7$ cells per ml.

Polyclonal sera were raised against DPAP A fused to the first six amino acids of bacteriophage T4 lysozyme, expressed from pCJR24 and against alkaline phosphatase fused to T4 lysozyme as above, expressed from plasmid pGP100 (obtained from T. Stevens, University of Oregon). Fusion protein antigens were induced and prepared according to Roberts et al. (1989). Other antibodies were provided as follows: cell wall protein (Sanz et al., 1987); PGK antibody (T. Stevens); SEC63 (R. Schekman, University of California, Berkeley); Kex2p (R. Fuller, Stanford University).

### Isolation of CHC1 Temperature-sensitive Allele

YCpchc-521TRP is a derivative of YCpCHCTRP (Munn et al., 1991) which carries *chcl-521*, a temperature-sensitive allele of *CHC1* generated by hydroxylamine mutagenesis. YCpCHCTRP was mutagenized with hydroxylamine according to the procedure of Busby et al. (1982). Briefly, plasmid DNA was mutagenized in two aliquots of 10 μg each by incubation in 1 M hydroxylamine at 75°C for 2 h. The samples were then dialyzed overnight against TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) at 4°C. After dialysis, the DNA was precipitated with ethanol and resuspended in TE. This treatment resulted in roughly a 1,000-fold reduction in the number of bacterial transformants (selected for ampicillin resistance) when compared to an unmutagenized DNA sample carried through the procedure in parallel. Because clathrin heavy chain–deficient cells do not efficiently accept DNA by standard transformation protocols, a plasmid shuffle approach was adopted to score for *chcl-ts* alleles. Mutagenized DNA was introduced into strain GPY 112.1 (*chcl-Δ8* pCHCc102) using the lithium acetate procedure and transformants were selected for growth on SD-Trp medium at 24°C. $Trp^+$ transformants were replica-plated onto SD-Trp plates containing 1 mg/ml 5-fluoroorotic acid (5-FOA) (Sikorski and Boeke, 1991) at 24°C to select for cells having lost the pCHCc102 plasmid. The 5-FOA–resistant cells were then scored for growth at either 24 or 37°C on SD-Trp plates containing 30 μg/ml canavanine. The toxic arginine analogue canavanine enhances the growth rate differences between *chcl* cells and *CHC1* cells if the cells also harbor the *can1* allele. *CHC1* strains carrying *can1* are resistant to canavanine but *chcl can1* cells are sensitive and fail to grow (Payne et al., 1987). Of 1,249 $Trp^+$ transformants tested, 120 (9.6%) failed to grow at both 24 or 37°C on SD-Trp containing canavanine. One transformant grew at 24 but not at 37°C. Plasmid DNA from this strain was prepared and introduced into *E. coli* strain HB101. To confirm plasmid linkage of the *ts* phenotype, DNA isolated from a bacterial transformant was introduced into *chcl-Δ10* strain GPY146.2 at 24°C and four $Trp^+$ transformants were tested for growth on SD-Trp at 24 and 37°C. All four transformants displayed temperature-sensitive growth. The ts mutant allele was designated *chcl-521*. *chcl-521* was introduced into strain GPY55-10. This strain has wild type growth rates at 37°C indicating that *chcl-521* is recessive. *CHC1* was then disrupted to result in strain GPY176. GPY176 was mated to GPY154 and spores were analyzed for temperature-sensitive growth. From these, temperature-sensitive strain GPY268 was isolated.

The 3′ 1-kb region of *chcl-521* which is responsible for the *ts* phenotype was introduced into *CHC1* carried on YCp50 (selectable marker *URA3*) to generate pBP6 (Munn et al., 1991). pCHCc102 is CHC1 in YCp50 (Payne

*Table I. Yeast Strains Used in This Study*

| Strain | Genotype | Source |
|---|---|---|
| GPY55-10 | *MATalpha leu2-3,112 ura3-52 trp1-289 prb1 gal2* | Payne and Schekman (1989) |
| GPY112.1 | *MATa leu2-3,112 chc1-Δ8::LEU2 ura3-52 his4-519 trp1 can1 with pCHCc102* | This study |
| GPY146.2 | *MATalpha chc1-Δ10::LEU2 leu2-3,112 ura3-52 trp1 his4 can1 GAL2* | This study |
| GPY176 | *MATalpha leu2-3,112 ura3-52 trp1 prb1 gal2 chc1-Δ10::LEU2 YCpchc1-521TRP* | This study |
| SEY6210 | *MATalpha ura3-52 lys2-801 leu2-3,112 his3-Δ200 trp1-Δ901suc2-Δ9* | S. Emr |
| GPY154 | *MATa dpp2Δ::HIS3* transformant of SEY6210 | This study |
| GPY155 | *MATalpha dpp2Δ::HIS3* transformant of SEY6210 | This study |
| GPY242 | *MATalpha ura3-52 lys2-801 ade2-101 his3Δ200 dpp2Δ::HIS3 leu2-3,112 chcΔ8::LEU2* | This study |
| GPY268 | *MATalpha ura3-52 leu2-3,112 his3Δ200 dpp2Δ::HIS3 chc1-Δ10::LEU2 trp1* YCpchc1-521TRP | This study |
| GPY382 | pCHCc102 transformant of GPY268 cured of YCpchc1-521TRP | This study |
| GPY383 | pBP6 transformant of GPY268 cured of YCpchc1-521TRP | This study |
| GPY440 | *MATalpha trp1 ura3-52* pBP6 *leu2-3,112 chc1-Δ10::LEU2 sec1-1* | This study |
| GPY441 | *MATalpha trp1 ura3-52* pBP6 *leu2-3,112 chc1-Δ10::LEU2* | This study |
| GPY442 | *MATalpha trp1 ura3-52 leu2-3,112* pBP6 | This study |
| GPY443 | *MATalpha trp1 ura3-52 leu2-3,112* pBP6 *sec1-1* | This study |

et al., 1987). Further characterization of *chcl-521* will be described in detail elsewhere.

### Radiolabeling and Immunoprecipitations

For labeling with $Na^{125}I$ (Amersham, Arlington Heights, IL; New England Nuclear, Wilmington, DE), cells were grown to midlogarithmic phase in YPD at 30°C. Intact cells and cell lysates were prepared and labeled as described previously (Payne and Schekman, 1989) except that iodoacetamide was added to the lysates to a concentration of 10 mM and samples were incubated at 37°C for 10 min before labeling. Temperature-sensitive strains were grown in SD CAA-ura or SD CAA-trp at 24°C to midlogarithmic phase. Cells were shifted to 37°C for 0, 30, 60, or 120 min then harvested and labeled as above.

For metabolic labeling, cells were grown to midlogarithmic phase at 24°C in SD CAA-ura. Cultures were shifted to 30 or 37°C for 0, 15, 30, 60, or 120 min. $5 \times 10^6$ cells were harvested and washed twice in supplemented SD. Cells were resuspended in 250 μl of supplemented SD plus 0.2 mg/ml ovalbumin. Labeling was initiated by addition of 50 μCi of Trans$^{35}$S-Label (ICN, Irvine, CA), and incubated at 30 or 37°C for 10 min or at 24°C for the 0-min samples. $NaN_3$ (10 mM) was added and samples were placed on ice to terminate label incorporation. Labeled cells were sedimented and 200 μl of media was removed for immunoprecipitation of α-factor. 5 μl of 2% SDS and 0.2 mg/ml ovalbumin was added to the sample which was then heated to 100°C for 3 min. Samples were brought to 1 ml with PBS, 1% TX-100 (PT) 0.2 mg/ml ovalbumin.

All samples subjected to immunoprecipitation received 50 μl of 10% *S. aureus* suspension (IgSorb; The Enzyme Center, Malden, WA) and were incubated on ice for 15 min. The IgSorb was sedimented by centrifugation at 16,000 *g* for 15 min. To the supernatant was added antisera against α-factor, DPAP A, alkaline phosphatase, or Kex2p. For precipitation of DPAP A and Kex2p, 50 μl of a concentrated extract of cells (200 $A_{500}$ unit equivalents/ml) carrying deletions of *STE13* or *KEX2*, respectively, was also added. After an overnight incubation at 4°C, protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) 20% suspension was added to collect antibody. In the case of α-factor and alkaline phosphatase, beads were washed once with PBS, 1% TX-100, 0.1% SDS (PTS), once with 2 M urea, 2 M NaCl, 1% TX-100, 100 mM Tris-HCl, pH 7.4, again with PTS, and finally twice with 10 mM NaCl, 10 mM Tris, pH 6.8. Beads were resuspended in 25 μl Laemmli sample buffer (Laemmli, 1970) and heated at 100°C for 3 min. DPAP A and Kex2p immunoprecipitates collected by protein A-Sepharose were washed with PTS and twice with 10 mM NaCl, 10 mM Tris-HCl, pH 6.8. Beads were resuspended in 50 μl 2% SDS, 0.2% BME and heated at 100°C for 3 min. Immediately thereafter, 1 ml of PT containing 200 μg/ml ovalbumin was added. The beads were sedimented, and the supernatant was collected and subjected to a second round of immunoprecipitation. All samples were subjected to SDS-PAGE. In the case of α-factor, gels were treated with Amplify (Amersham) for 20 min prior to autoradiography. All other gels were incubated in 25% methanol, 10% acetic acid. Gels were exposed to x-ray film (Kodak, X-OMAT AR) at −70°C.

### Enzymatic Activity Assays

GDPase activity was determined according to the method of Abeijon et al. (1989). Cells were grown at 30°C in YPD to midlogarithmic phase. $1 \times 10^8$ cells were harvested and washed with water. Cell lysates were prepared by glass bead lysis (Payne and Schekman, 1989) in the absence of detergent. Membranes were sedimented at 100,000 *g* for 1 h. Intact cells or membranes from an equivalent number of cells were assayed in a reaction mix containing 20 mM imidazole, pH 7.4, 10 mM $CaCl_2$, 7 mM GDP. Samples were incubated at 37°C for 30 min. The reaction was stopped by addition of 5 μl 20% SDS. The volume of each sample was then brought to 1 ml with water. To assay for inorganic phosphate, 200 μl acid molybdate was added, then 50 μl Fiske-Subbarow reducer. Samples were mixed and the $A_{660}$ was measured after 15 min. To correct for interference from other NDPases, each sample was assayed in the presence of 7 mM ADP or CDP. This value was then subtracted from the value obtained with GDP to arrive at the activity due specifically to GDPase.

Activity of DPAP A was measured using a modification of the procedure described by Suarez-Rendueles et al. (1981). $5 \times 10^7$ cells were harvested and washed in 0.9% NaCl. Cells were resuspended in 50 mM potassium acetate, pH 5.0 (5 μl/$10^7$ cells). Cell lysates were prepared by harvesting and washing cells as above. Cell pellets were lysed by a sequential freeze/thaw treatment repeated three times. 50 mM potassium acetate, pH 5.0, was added to the lysed cells (5 μl/$10^7$ cells). Brij58 was then added to a final concentration of 1%. All samples were placed on ice for 30 min. The entire sample was brought to 200 μl with 50 mM potassium acetate and 250 μl 400 mM Hepes-Tris, pH 7.0, was added. 50 μl of Ala-Pro-*p*-nitroanilide (Bachem Bioscience, Philadelphia, PA) (3 mM in 25% methanol) was added. The assay mixture was incubated at 37°C for 30 min. The reaction was stopped by addition of 500 μl 5% $ZnSO_4$ and 100 μl 7.5% barium hydroxide. Samples were sedimented at 16,000 *g* for 10 min. The $A_{405}$ of the supernatants was measured to determine release of *p*-nitroaniline.

Glucose-6-phosphate dehydrogenase was measured in intact cells and cell lysates as described previously (Payne and Schekman, 1989).

All assays were linear over the time period and enzyme concentrations used.

## Results

### Mislocalization of Dipeptidyl Aminopeptidase A to the Cell Surface of Clathrin Heavy Chain–deficient Cells

Kex2p normally acts to initiate proteolytic maturation of the α-factor mating pheromone precursor in the Golgi apparatus (Fuller et al., 1988). Cleavage by Kex2p after basic amino acid pairs at four sites in the precursor releases four α-factor cassettes appended by short stretches of amino acids. Two additional proteases, dipeptidyl aminopeptidase A (DPAP A) and Kex1p carboxypeptidase, are necessary to convert the cassettes to the 13-amino acid, biologically active form of α-factor (Fuller et al., 1988). Like Kex2p, each of these proteases has three domains: a large lumenal domain which contains the protease activity; a single membrane spanning domain; and a cytoplasmic domain of ~100 amino acids (Fuller et al., 1988, 1989; Cooper et al., 1989). Kex2p and Kex1p are type I membrane proteins with the amino terminus located in the lumen; DPAP A, a type II membrane protein, has the opposite orientation. Since all three proteases appear to reside in the Golgi apparatus (personal communication, T. Stevens and H. Bussey; Redding et al., 1991) and have similar topologies, it seemed possible that clathrin would also be required for proper retention of DPAP A and Kex1p. We have focused on DPAP A and used solid phase radioiodination (Payne and Schekman, 1989) to determine whether the protease is mislocalized to the cell surface of clathrin heavy chain–deficient (*chcl*Δ) cells. In this procedure, *chcl*Δ and wild-type cells were iodinated either as intact cells or as cell lysates. After lysis of the labeled intact cells, DPAP A was precipitated from each sample with specific antisera, subjected to electrophoresis through SDS-PAGE and visualized by autoradiography. If DPAP A is present at the cell surface then it should be labeled in intact cells. The amount of DPAP A at the surface can be estimated by comparing the amount labeled in intact cells to that labeled in lysates where all the protease should be accessible to iodination. As shown in Fig. 1, DPAP A is clearly labeled in intact *chcl*Δ (Fig. 1, lane *3*) cells but not wild type cells (Fig. 1, lane *1*). Like Kex2p (Payne and Schekman, 1989), DPAP A sometimes undergoes a minor degree of degradation during the labeling period, resulting in a smaller labeled species.

As controls, glucose-6-phosphate dehydrogenase (G6PD) and a 33-kD cell wall protein (CWP) were also immunoprecipitated from each sample and analyzed by SDS-PAGE. G6PD, a cytoplasmic protein, serves as a control for cell lysis in intact cell samples. No labeling of G6PD in either mutant or wild-type intact cells was observed, but G6PD was readily detected when lysates were labeled (data not shown). This result eliminates the possibility that DPAP A is accessi-

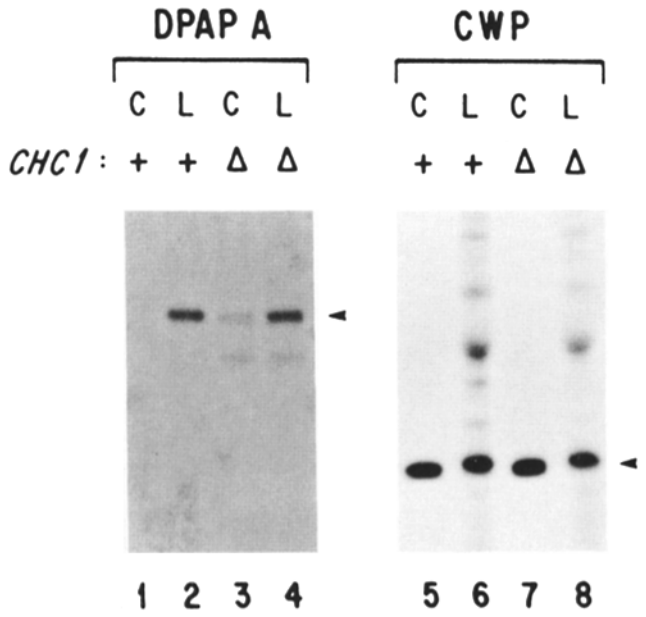


*Figure 1.* Mislocalization of DPAP A to the cell surface in clathrin-deficient cells. *CHC1* (GPY155) (lanes *1*, *2*, *5*, and *6*) and *chc1Δ* (GPY242) (lanes *3*, *4*, *7*, and *8*) intact cells (*C*) and cell lysates (*L*) were radiolabeled with $^{125}I$ as described in Materials and Methods. After labeling, intact cells were lysed and aliquots of all samples were treated with antisera against DPAP A (lanes *1–4*) or against the 33-kD cell wall protein (lanes *5–8*). Immunoprecipitated proteins were subjected to SDS-PAGE and autoradiography.

ble to iodination because *chc1Δ* cells lyse or are more permeable to the reactive iodide than wild-type cells. Labeling of the CWP occurred in all samples since it resides at the cell exterior (Fig. 1, lanes *5–8*) and the relative signal intensity in each sample was used to normalize labeling efficiency which can vary significantly (Fig. 1, lanes *5–8*). Using CWP to correct for labeling efficiency, densitometric analysis indicates that 30% of the DPAP A in *chc1Δ* cells is at the cell surface. Therefore, clathrin is necessary for retention of DPAP A as well as Kex2p although, compared to Kex2p, a smaller percentage of DPAP A reaches the surface (30% of DPAP A compared to 60–90% of Kex2p, Payne and Schekman, 1989; M. Seeger, unpublished observations).

### *Golgi-localized Guanosine Diphosphatase Is Retained in chc1Δ Cells*

Both proteins shown to be mislocalized to the cell surface of *chc1Δ* cells function in the maturation of α-factor and are probably located in the same late Golgi compartment. Two possibilities could account for the effect of *chc1* on retention of these proteins. First, in the absence of clathrin the integrity of the entire Golgi apparatus could be compromised resulting in haphazard expulsion of all Golgi membrane proteins. Alternatively, clathrin may play a selective role in the retention of a subset of Golgi membrane proteins.

Like DPAP A, guanosine diphosphatase (GDPase) is a Golgi-localized, type II membrane protein (Abeijon et al., 1989; C. Hirschberg, personal communication). Characterization of GDPase location in *chc1Δ* cells was undertaken to evaluate whether all Golgi membrane proteins are mislocalized to the cell surface. In the absence of antibody, we employed enzyme activity assays to assess cell surface localization. These assays rely on the same principle as iodinations, a membrane-impermeant probe is applied to intact and lysed cells. In this case the probe is guanosine diphosphate, a substrate for GDPase (Abeijon et al., 1989). If GDPase is at the surface of mutant cells then activity should be manifested in intact as well as lysed cell samples. Because of other, soluble nucleoside diphosphatases, total GDPase activity was measured in a membrane fraction obtained from lysed cells rather than in unfractionated extracts. Results of representative GDPase activity assays are shown in Table II. Only 2% of the total cell-associated GDPase activity was measureable in intact *chc1Δ* cells (Table II, row *1*) and no activity was present in intact wild-type cells. The slightly higher amount of GDPase detected in intact *chc1Δ* cells compared to wild-type cells (Table II, row *1*) can be accounted for by cell lysis, as measured by G6PD activity assays (Table II, row *6*). Although the absolute GDPase activity varied up to twofold between separate experiments, the percentage of GDPase activity at the surface was uniformly low. The lack of significant GDPase activity in intact mutant cells stands in contrast to measurements of DPAP A activity using the membrane-impermeant substrate, alanine-proline nitroanilide. In accordance with the results obtained by iodination, activity assays of DPAP A indicate that 25–30% of the protein is at the surface of *chc1Δ* cells (Table II, row *3*).

We have also determined by iodination whether an ER membrane protein, Sec63p (Deshaies et al., 1991; Sadler, 1989), can be detected at the surface of *chc1Δ* cells. No evidence for mislocalization was obtained (data not shown). Taken together, our results argue that the absence of clathrin heavy chain does not result in the indiscriminate export of secretory organelle membrane proteins and suggest that the

*Table II. GDPase Activity Is Not Found at the Surface of chc1Δ Cells*

| | *CHC1* | | | *chc1Δ* | | |
|---|---|---|---|---|---|---|
| | (U/$10^7$ cells) | | | (U/$10^7$ cells) | | |
| Enzyme | Cell surface activity | Total activity | % at Surface | Cell surface activity | Total activity | % at Surface |
| GDPase | 0 | 288 | 0 | 8 | 389 | 2.0 |
| G6PD | 0 | 149 | 0 | 3 | 195 | 1.6 |
| DPAP A | 1 | 25 | 4.0 | 10 | 30 | 33.3 |
| G6PD | 0 | 118 | 0 | 0 | 121 | 0 |

GDPase and DPAP A activity at the surface of *chc1Δ* cells. *CHC1* (GPY155) and *chc1Δ* (GPY242) cells were grown in YPD at 30°C, cells were washed, and activity at the cell surface (intact cells) or total activity (cell lysates for DPAP A or membrane fraction for GDPase) was measured. Activity is represented as Units/$10^7$ cells. G6PD activity was measured during each assay to control for cell lysis.

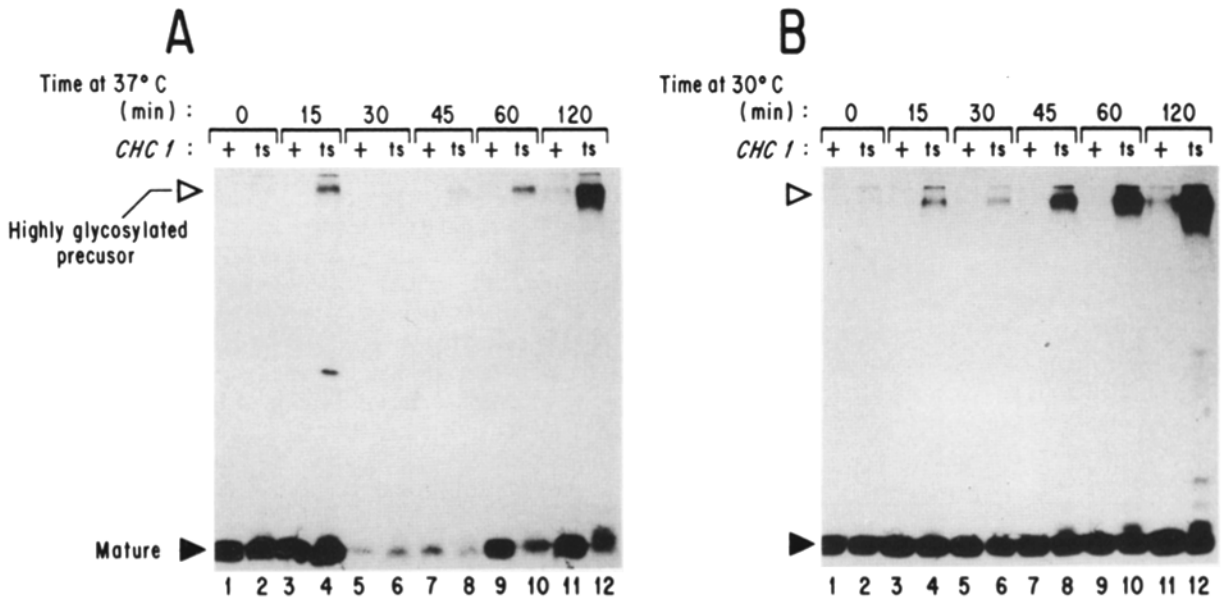


*Figure 2.* Secretion of unprocessed α-factor by *chcl-ts* cells. *CHCl* (GPY382) and *chcl-ts* (GPY383) cells were grown at 24°C and shifted to either 37°C (*A*) or 30°C (*B*) for 0, 15, 30, 45, 60, or 120 min. After the temperature shift, cells were metabolically labeled with Trans-$^{35}$S-label for 15 min, the media was collected, and the α-factor immunoprecipitated. Immunoprecipitated samples were analyzed as described in the legend to Fig. 1. Mature α-factor migrates at the bottom of the gel (*closed arrow*) while the highly glycosylated precursor form appears at the top of the gel (*open arrow*).

retention of only a subset of Golgi membrane proteins is influenced by clathrin.

### α-Factor Maturation in Cells Expressing a Temperature-sensitive Allele of CHC1

Cells which have sustained a deletion of *CHCl* grow slowly and exhibit accumulation of membranous structures when visualized by electron microscopy (Payne et al., 1987). Although the effect of *chcl*Δ on Golgi membrane protein retention is selective, it could result as a secondary consequence of slow growth or some other anomaly caused by the cells' continuous growth without clathrin heavy chain. Concern about indirect effects of a gene disruption can be addressed if cells carrying a conditionally defective (e.g., temperature-sensitive) allele of the gene are available. Commonly, the immediate appearance of a phenotype after such cells are transferred to nonpermissive conditions constitutes a reliable indicator of a primary and direct effect of a loss of gene product function. Based on this rationale, the phenotypes of cells carrying a temperature-sensitive allele of *CHCl* (*chcl-ts*; see Materials and Methods) were analyzed after shifting the cells to a nonpermissive temperature.

One of the most dramatic phenotypes which accompanies the mislocalization of Kex2p in *chcl*Δ cells is inefficient maturation of α-factor precursor (Payne and Schekman, 1989). The highly glycosylated form of the precursor which is secreted by *chcl*Δ cells can be conveniently and easily distinguished from mature α-factor by SDS-PAGE; the precursor migrates with an apparent molecular mass of ~125 kD whereas the mature form migrates as a 3.5-kD species. The secretion of precursor α-factor was monitored before and after shifting *chcl-ts* or wild-type cells from 24 to 37°C. Cells were labeled for 15 min with [$^{35}$S]methionine and cysteine and then secreted α-factor forms were collected from the medium by immuneprecipitation, separated by SDS-PAGE, and detected by autoradiography (Fig. 2 *a*). At 24°C both strains secreted only mature α-factor (Fig. 2 *a*, lanes *1* and *2*). However, when cells were shifted to 37°C and immediately labeled for 15 min, glycosylated precursor is apparent in the *chcl-ts* culture medium (Fig. 2 *a*, lane *4*) but not the wild-type culture medium (Fig. 2 *a*, lane *3*). Synthesis of α-factor is repressed by the heat shock which occurs when cells are shifted from 24 to 37°C (J. Finlay, unpublished observation) which precludes detecting maturation defects in samples harvested at 30 and 45 min after the temperature shift (Fig. 2 *a*, lanes *5–8*). When expression of α-factor begins to return at the 60-min time point, the maturation defect in the *chcl-ts* cells has worsened (compare the ratios of precursor to mature α-factor in Fig. 2 *a*, lanes *4* and *10*); and by the 2-h time point the defect is comparable to that observed in *chcl*Δ cells (Fig. 2 *a*, lane *12*).

In an attempt to circumvent the decrease in α-factor synthesis caused by shifting cells from 24 to 37°C, we conducted a parallel experiment in which cells were transferred to 30°C instead of 37°C (Fig. 2 *b*). The effect of the temperature shift on α-factor expression is significantly diminished using this protocol (Fig. 2 *b*, lanes *5–8*). Although the extent of the α-factor maturation defect is not as extreme when mutant cells are shifted to 30°C rather than 37°C (comparing ratios of mature to precursor forms), secretion of highly glycosylated precursor again occurs within the first 15 min after temperature shift (Fig. 2 *b*, lane *4*). Furthermore, this experiment more clearly reveals the progressive increase in precursor secretion by *chcl-ts* cells after imposition of the nonpermissive temperature (Fig. 2 *b*, *even-numbered lanes*). These results indicate that the α-factor maturation defect is an immediate consequence of reducing clathrin function.

As a means to evaluate the relationship between the α-factor maturation phenotype and cell growth, the effect of temperature shifts on growth rates of the *chcl-ts* and wild-type

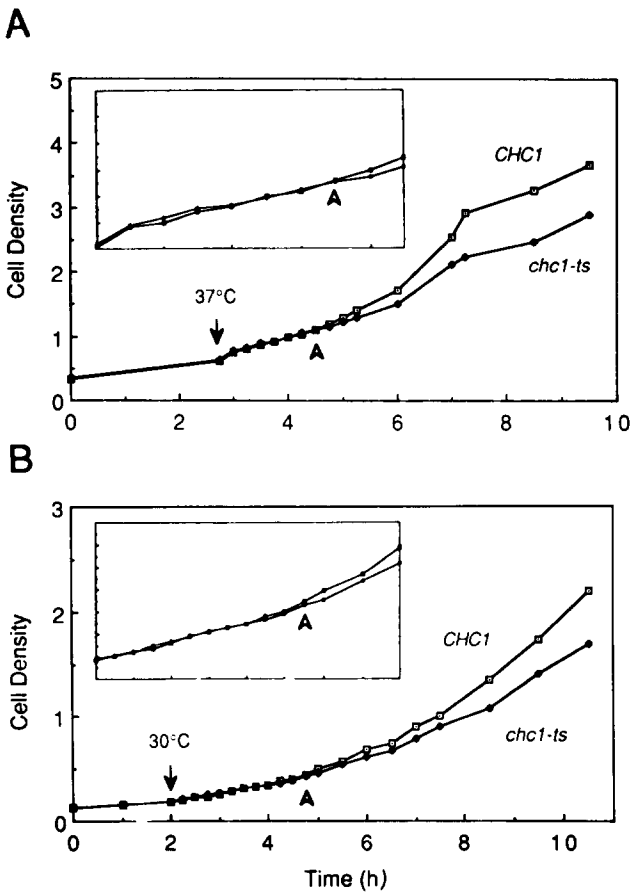


*Figure 3.* Growth of *CHC1* and *chcl-ts* at nonpermissive temperatures. *CHC1* (GPY382; *open squares*) and *chcl-ts* (GPY383; *closed squares*), grown in SD CAA-ura at 24°C, were shifted to 37°C (*A*) or 30°C (*B*). Cell densities were measured by optical density. Beginning at the time of the temperature shift (*arrow*) measurements were taken at 15-min intervals for a period of 2.5 h (*A*) or 3 h (*B*) and less frequently thereafter. The inset shows an expanded view of the growth immediately after the shift (2.75-6 h in *A* and 2–6.5 h in *B*). Open arrowheads beneath the growth curve mark the point at which *chcl-ts* cell growth begins to slow.

strains was examined (Fig. 3). The two strains grow equally well at 24°C and for at least 2 h after a shift to either 37 or 30°C (*arrows* mark the time of temperature shift). The growth rate of the *chcl-ts* cells begins to taper after 2 h at 37°C or after 2.75 h at 30°C (*open arrowheads*). The insets show an expanded view of the graphs over the period immediately after the temperature shift. Thus, at both 30 and 37°C, the α-factor maturation defect occurs immediately and reaches its maximum extent at a time when the retardation of cell growth just becomes apparent. This result argues that the α-factor maturation phenotype cannot be a consequence of slow growth.

### *Mislocalization of Golgi Membrane Proteins to the Cell Surface in chcl-ts Cells*

In *chcl*Δ cells, inefficient α-factor maturation is correlated with, and can be accounted for by, mislocalization of Kex2p to the cell surface (Payne and Schekman, 1989). The rapid appearance of an α-factor maturation defect in *chcl-ts* cells incubated at 37°C implies a similarly immediate effect on retention of Kex2p and DPAP A. Mislocalization of Kex2p and DPAP A to the cell surface was determined directly by solid phase iodination of intact *chcl-ts* cells at various times after a shift from 24 to 37°C (Fig. 4). At each time point, Kex2p, DPAP A, and CWP were immuneprecipitated from lysates of the labeled cells. Both Kex2p (Fig. 4, lanes *1–4*) and DPAP A (Fig. 4, lanes *5–8*) appeared at the cell surface within 30 min of the temperature shift and reached maximal levels (70% of total for Kex2p and 30% of total for DPAP A) at the plasma membrane between 1 and 2 h after incubation at 37°C was initiated. Analysis of CWP (Fig. 3, lanes *9–12*) indicates that the efficiency of surface iodination was commensurate in all samples. The temperature shift did not lead to cell surface mislocalization of Kex2p or DPAP A in wild-type cells (data not shown). These data show that mislocalization of Kex2p and DPAP A is also an immediate consequence of clathrin malfunction.

### *Sorting of a Vacuolar Membrane Protein in chcl-ts Cells*

Sorting of a vacuolar integral membrane protein, alkaline phosphatase (ALP, encoded by *PHO8*) (Kaneko et al., 1987), provides an additional diagnostic for Golgi apparatus function in *chcl* mutant cells. ALP is synthesized as a precursor which is translocated into the ER as a type II membrane protein, like DPAP A, and transported to the Golgi apparatus (Klionsky and Emr, 1989). ProALP is transferred from the Golgi apparatus to the vacuole where proteolytic maturation occurs. If *chcl* affects the ability of the Golgi apparatus to divert proALP from the secretory pathway then proALP might be expected to accumulate at the cell surface. Using the same iodination procedures used to detect cell surface Kex2p and DPAP A, mislocalization of proALP to the cell surface was evaluated in both *chcl*Δ cells and in *chcl-ts* cells incubated at 37°C. Immuneprecipitation of ALP from iodinated samples of *chcl*Δ cells (Fig. 5, lanes *1* and *2*) revealed a fraction of ALP, mostly the precursor form (Fig. 5, *open arrowhead*), which was accessible to the reactive iodide in intact cells (Fig. 5, lane *1*). The precursor form is not visible in the lysate sample, probably due to the high background. Using CWP to normalize labeling efficiencies, 20% of the total cell-associated ALP was labeled as the precursor form and 10% as the mature form in the intact cell sample. The mature ALP which is accessible to iodination (Fig. 5, lane *1, closed arrowhead*) could result from a low level of cell lysis or from limited proteolytic maturation of the precursor which reaches the cell surface.

To determine whether ALP missorting rapidly occurs upon a loss of clathrin function, we examined this phenomenon in the temperature-sensitive strain. ALP appearance at the cell surface was examined by iodination of *chcl-ts* and congenic *CHC1* cells grown at 24°C or shifted to 37°C for 30 min or 2 h (Fig. 5, lanes *3–14*). Not until 2 h after the temperature shift did a small amount (25%) of ALP precursor become accessible to iodination in intact mutant cells (Fig. 5, lane *13*). These data indicate that the missorting of ALP, like slow growth, is not an immediate effect of the loss of clathrin function.

### *A Late Secretory Pathway Block Prevents Appearance of Kex2p at the Surface of chcl-ts Cells*

We presume that, upon perturbing retention of Kex2p in the

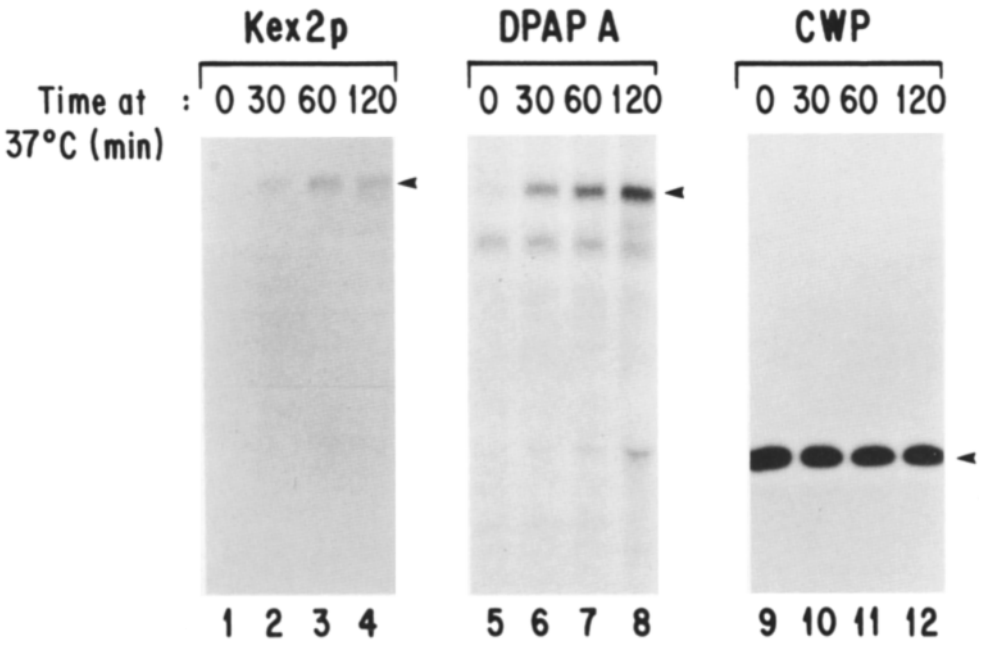


*Figure 4.* Kex2p and DPAP A are mislocalized to the cell surface rapidly after shifting to the nonpermissive temperature in *chcl-ts* cells. *chcl-ts* (GPY268) cells were grown at 24°C in SD CAA-trp to early log phase. Cells were shifted to 37°C for 30, 60 or 120 min. At each time point cells were harvested and intact cells radiolabeled with $^{125}I$ as described in Materials and Methods. After lysis, Kex2p (lanes *1–4*), DPAP A (lanes *5–8*), and CWP (lanes *9–12*) were immunoprecipitated and analyzed as described in the legend to Fig. 1. Arrow indicates the migration position of the relevant protein. The faster migrating band in the DPAP A panel is a nonspecifically precipitated protein.

Golgi, the protein moves to the surface via conventional secretory vesicles. A prediction of this hypothesis is that cell surface delivery of Kex2p should depend on gene products which are necessary for fusion of secretory vesicles to the plasma membrane (Novick et al., 1981). The inducible mislocalization of Kex2p in *chcl-ts* cells allowed us to test this prediction by introducing a second temperature-sensitive mutation, *secl*, which prevents fusion of secretory vesicles to the plasma membrane at 37°C (Novick and Schekman, 1979). A wild-type strain and strains carrying either *chcl-ts* or *secl* alone or in combination were shifted to 37°C for 1 h to eliminate clathrin heavy chain function and impose the *secl* block to secretory vesicle fusion. The cells were then radioiodinated either as intact cells or extracts and, after lysing the labeled intact cells, Kex2p was immuneprecipitated. As anticipated, Kex2p was mislocalized to the surface in *chcl-ts* cells (Fig. 6, lanes *3* and *4*) but not in wild-type (Fig. 6, lanes *1* and *2*) or *secl* cells (Fig. 6, lanes *5* and *6*). No Kex2p was iodinated in intact *secl chcl-ts* double mutant cells (Fig. 6, lane *7*). Thus, the *secl*-imposed block of secretory vesicle fusion to the plasma membrane prevents cell surface appearance of mislocalized Kex2p, suggesting that mislocalized Kex2p travels to the plasma membrane in secretory vesicles. It could be argued that the reason Kex2p is not mislocalized in the double mutant cells is that *secl* somehow suppresses the effect of *chcl-ts* on the retention of Kex2p so that Kex2p remains in the Golgi apparatus. However, in contrast to *secl CHCl* cells which accumulate mature α-factor (data not shown), the *secl chcl-ts* cells accumulate precursor α-factor indicating that *chcl-ts* still exerts an effect on Kex2p localization.

## Discussion

We have investigated clathrin's role in the retention of Golgi membrane proteins by analyzing selected properties of cells carrying mutations in the clathrin heavy chain gene. Prior work indicated that the Golgi membrane protein Kex2p is mislocalized to the cell surface in yeast cells devoid of clathrin heavy chain (Payne and Schekman, 1989). Here we

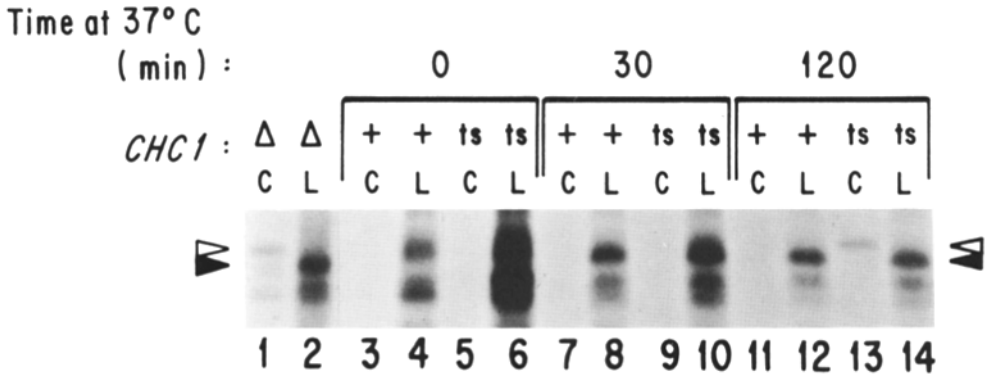


*Figure 5.* Sorting of alkaline phosphatase in *chcl* cells. (Lanes *1* and *2*) *chcl*Δ (GPY-242) cells were grown at 30°C in YPD to midlogarithmic phase. Intact cells (*C*) (lane *1*) and cell lysates (*L*) (lane *2*) were radiolabeled with $^{125}I$ and alkaline phosphatase was immunoprecipitated. Immunoprecipitations and analysis of immunoprecipitates were carried out as described in the Fig. 1 legend. The "pro" form of alkaline phosphatase is marked with an open arrow while the mature form is marked with the closed arrow. Other background bands are nonspecific. (Lanes *3–14*) *CHCl* (GPY382) and *chcl-ts* (GPY383) cells were grown at 24°C in selective media and shifted to 37°C for 0 (lanes *3–6*), 30 (lanes *7–10*), or 120 (lanes *11–14*) min then labeled with $^{125}I$ and processed as above.

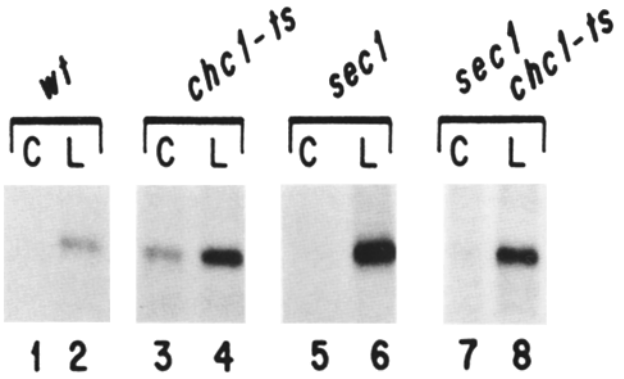


*Figure 6. secl* blocks the appearance of Kex2p at the cell surface in *chcl-ts* cells. Wild type (GPY442), *chcl-ts* (GPY441), *secl* (GPY-443), and *secl*, *chcl-ts* (GPY440) cells were grown at 24°C in selective media. Cells were shifted to 37°C for 1 h. At this time cells were harvested and labeled with $^{125}$I. Samples were processed as in the legend to Fig. 1 except antisera specific for Kex2p was used. C designates labeled intact cells. L designates labeled cell lysates.

have extended the analysis to include two additional Golgi membrane proteins: DPAP A, which functions after Kex2p in the proteolytic maturation of α-factor precursor (Novick and Schekman, 1979); and guanosine diphosphatase, which acts on GDP released from GDP-mannose during glycosylation reactions (Abeijon et al., 1989). An additional Golgi apparatus function, sorting of a vacuolar membrane precursor, has also been evaluated. We have found that DPAP A requires clathrin function to avoid reaching the cell surface whereas GDPase does not. Sorting of the vacuolar membrane protein, alkaline phosphatase, is partially defective in the absence of clathrin heavy chain. Mislocalization of Kex2p and DPAP A follows rapidly upon placing *chcl-ts* cells at the nonpermissive temperature, in contrast to growth retardation and the low level of ALP missorting which occur only after an extended period at the elevated temperature. Based on these phenotypic characterizations, we suggest that clathrin plays a role in the selective retention of certain Golgi membrane proteins, rather than acting to maintain the general integrity of the Golgi apparatus.

The choice of which proteins to analyze was influenced by the criteria of subcellular localization, protein topology, and ease of detection. Although not directly compared, immunofluorescent visualization of *STE13*-encoded DPAP A produces a staining pattern similar to that observed for Kex2p (T. Stevens, personal communication). Given that DPAP A acts with Kex2p in the maturation of α-factor precursor, it seems likely that both enzymes reside in the same Golgi compartment. However, it is also possible that DPAP A occupies a compartment distinct from that of Kex2p (Fuller et al., 1988). Although oppositely oriented, both Kex2p and DPAP A are integral membrane proteins which span the bilayer once and expose a cytoplasmic tail of ~100 amino acids. Iodination procedures and enzyme activity assays consistently demonstrate that 30% of DPAP A is mislocalized to the cell surface in *chcl* cells. Also, DPAP A and Kex2p reach the cell surface within 30 min after eliminating clathrin heavy chain function in *chcl-ts* cells. These findings clearly implicate clathrin in the Golgi retention of DPAP A as well as Kex2p. The explanation for the quantitative difference in mislocalization, 30% of DPAP A versus 60–90% of Kex2p, is not clear. The possibility that DPAP A mislocalization is an indirect effect of the *chcl* mutation is unlikely since both Kex2p and DPAP A respond quickly to the loss of clathrin function in *chcl-ts* cells. Different compartmental locations could explain the difference; this would mean that clathrin acts at more than one compartment, a possibility that cannot be discounted. Or, structural differences between DPAP A and Kex2p could allow Kex2p to leave the Golgi apparatus more efficiently than DPAP A.

GDPase is a type II integral membrane protein (C. Hirschberg, personal communication) which cofractionates with two other Golgi activities, GDP-mannose transport and α 1,2 mannosyl transferase (Abeijon, et al., 1989). Although antibodies were not available, enzyme activity assays indicate that GDPase is not mislocalized to the cell surface in *chcl* cells. This interpretation assumes that GDPase which reaches the cell surface retains activity. For both DPAP A and Kex2p, results from enzyme activity assays and cell surface iodinations are comparable. We therefore consider unlikely the possibility that inactive GDPase resides at the plasma membranes of *chcl* cells. Instead, we favor the idea that GDPase is unaffected by *chcl* mutations because the protein resides in a Golgi compartment distinct from that of Kex2p and DPAP A. Support for this model comes from the observation that GDPase activity can be separated from Kex2p activity by subcellular fractionation (Bowser and Novick, 1991). Alternatively, GDPase retention may be disrupted by *chcl* but unrestricted GDPase may not reach the cell surface. Although there is no reason to expect that GDPase in *chcl* cells would respond differently than Kex2p and DPAP A, this hypothesis cannot be dismissed at present.

The contrasting behavior of GDPase and the α-factor maturation enzymes in *chcl* cells argues that some specificity underlies the effect of clathrin mutations. We can only conclude at this point that proteins in different compartments are affected differently. However, it is tempting to speculate that the specificity of the *chcl* mutations is based on associations of the cytoplasmic tails of Kex2p and DPAP A with clathrin coats. This interaction could occur via associated protein complexes in a manner analogous to that proposed by Pearse (1988) to account for clustering of receptors in clathrin coated pits at the plasma membrane. The immediate effect of *chcl-ts* on Kex2p and DPAP A retention is consistent with this model. However, direct evidence for such interactions is needed to establish the mechanism of retention. In accord with the model, preliminary data shows that Kex2p cofractionates with coated vesicles enriched by Sephacryl S-1000 gel filtration chromatography (J. Finlay and G. Payne, unpublished observations). As more Golgi proteins are identified in yeast and their compartmental residence is established, we will be able to apply the same approaches used here to evaluate the effects of *chcl* mutations and further define the basis for clathrin's selective effects on Golgi membrane proteins.

### *The Fate of Mislocalized Golgi Membrane Proteins*

If interactions between clathrin coats and the cytoplasmic tails of Kex2p and DPAP A are responsible for proper Golgi retention then mutations in the tails might be expected to prevent retention. Recently, mutations have been introduced into the cytoplasmic domains of Kex2p, DPAP A, and Kex1p.

Indeed, many of the mutations affect the Golgi localization of the proteases, but, contrary to our observations with *chcl* cells, the mutant proteases appear to travel to the vacuole rather than the cell surface (C. Wilcox and R. Fuller; T. Stevens; and H. Bussey, personal communications). We can envision several ways to reconcile the different consequences of *chcl* and cytoplasmic tail mutations. At the outset, it should be noted that different assays have been used for assessing localization. We have used cell surface iodination while the other groups have used methods including immunofluorescence techniques, cell surface enzyme activity assays, and vacuole-dependent enzyme turnover measurements. Our iodination approach only distinguishes between cell surface and internal populations, the location of the internal pool cannot be evaluated. We have considered the possibility that much of the mislocalized Kex2p in *chcl* cells travels to the vacuole where it is degraded. This would lower the internal pool at the expense of cell surface Kex2p and skew upward our estimates of the amount mislocalized to the plasma membrane. This could also explain the different percentages of cell surface Kex2p and DPAP A since DPAP A is related to a vacuolar membrane dipeptidyl aminopeptidase (DPAP B) (Roberts et al., 1989) and might be more stable in the vacuole. To address this possibility, we examined Kex2p mislocalization in *chcl* cells also carrying the *pep4* and *prbl* mutations which drastically reduce the level of active vacuolar proteases (Jones, 1991). Such mutations stabilize Kex2p in mutants where the protein reaches the vacuole (K. Wilsbach and G. Payne, unpublished observations). The reduction in vacuolar protease activity does not, however, alter the level of cell surface Kex2p in *chcl* cells; we consistently measure 60–70% at the cell surface of these strains (data not shown). Yet another consideration is the possibility that the mutations in the Golgi membrane protein cytoplasmic domains alter the protein structure sufficiently to target (by unknown means) the abnormal protein to the vacuole for degradation. This possibility is reasonable for deletions that potentially change the protein structure in dramatic fashion but seems less credible for single amino acid changes which lead to vacuolar mislocalization (K. Redding and R. Fuller, personal communication).

Other explanations for the difference between cells expressing mutant Golgi membrane proteins and *chcl* cells could reflect more interesting aspects of intracellular traffic. It is evident from characterization of various transport pathways in *chcl* cells that clathrin acts at multiple points. For example, receptor-mediated endocytosis of $\alpha$-factor is reduced two to threefold in *chcl*$\Delta$ cells (Payne et al., 1988) and this effect occurs immediately in *chcl-ts* cells at the nonpermissive temperature (P. Tan and G. Payne, unpublished observations). Such pleiotropic effects of the mutation could alter an organelle(s) which is an intermediate between the Golgi apparatus and vacuoles. In this scenario, Kex2p retention would be disrupted in *chcl* cells and the protein would move to the intermediate organelle en route to the vacuole. Perturbation of the intermediate organelles in *chcl* cells could result in export of the Kex2p rather than delivery to the vacuole. On the other hand, mutant forms of Kex2p which leave the Golgi apparatus in otherwise wild-type cells would continue on to the vacuole. One finding which is inconsistent with this idea is that *secl* blocks cell surface appearance of Kex2p in *chcl-ts* cells. The simple interpretation of this result is that the unrestrained Kex2p in *chcl-ts* cells is packaged into secretory vesicles as it leaves the Golgi apparatus, since *secl* is known to block fusion of secretory vesicles to the plasma membrane (Novick et al., 1981). However, it could be proposed that the *SECI* gene product also functions in vesicular traffic from the putative intermediate compartment to the cell surface in which case it would still prevent delivery of Kex2p to the cell surface of the *chcl-ts secl* cells. A different explanation suggests that transport to the vacuole is a default pathway and some interaction must occur to keep Kex2p from moving to the vacuole. The important interaction may be binding of the cytoplasmic tail to other clathrin coat proteins (e.g., APs). Thus, in *chcl* cells, where Kex2p is not properly retained in the Golgi apparatus, interaction with these proteins might still act to exclude Kex2p from the vacuole pathway, in the absence of clathrin heavy chain, leading to transport to the plasma membrane. Mutations in the cytoplasmic tail of Kex2p would eliminate interaction with the clathrin APs and the mutant Kex2p would not be able to avoid delivery to the vacuole via the default pathway.

Analysis of the fate of mutant forms of Kex2p and DPAP A in *chcl* cells may aid in distinguishing among these numerous proposals. For example, if cell surface delivery is a consequence of an abnormal organelle between the Golgi apparatus and the vacuole in *chcl* cells, then mutant forms of Kex2p should be delivered to the cell surface rather than the vacuole. If, on the other hand, interaction of Kex2p with other clathrin coat proteins dictates cell surface delivery then the cytoplasmic tail mutants should travel to the vacuole in *chcl* cells. As other clathrin coat proteins are identified, their role in retention can also be investigated. Such analyses, based on the work described here, should allow definition of the potentially complex interactions which are necessary for the retention of membrane proteins in the Golgi apparatus.

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