Gene Dosage-dependent Secretion of Yeast Vacuolar Carboxypeptidase Y

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Abstract. The structural gene for yeast vacuolar carboxypeptidase Y (PRC1) has been cloned by complementation of the prcl-I mutation. As much as an eightfold elevation in the level of carboxypeptidase Y (CPY) results when a multiple-copy plasmid containing the PRC1 gene is introduced into yeast. Unlike the situation with a single copy of PRC1 in which newly synthesized CPY is efficiently localized to the vacuole, plasmid-directed overproduction results in secretion of >50% of the protein as the precursor form. Secretion is blocked in a mutant that is defective at a late stage in the transport of periplasmic proteins. Unlike normal cell surface glycoproteins, secreted CPY precursor acquires no additional oligosaccharide modifications beyond those that accompany normal transport to the vacuole. In the periplasm, the CPY precursor is proteolytically activated to an enzymatically active form by an enzyme that is unrelated to the vacuolar processing enzyme. These findings suggest that proper sorting and transport of CPY is saturable. This may reflect limiting amounts of a CPY-sorting receptor, or of CPY-modifying machinery that is essential for recognition by such a receptor.

The secretory pathway in yeast, as in more complex eukaryotic cells, is required for the transport of hydrolytic enzymes to the lysosome-like vacuole (22, 23). For example, yeast sec mutants blocked in transport from the endoplasmic reticulum or from the Golgi body fail to transport carboxypeptidase Y (CPY)† to the vacuole (29). Precursor forms of this protein accumulate at a restrictive temperature in this class of sec mutant cells and transport is restored when cells are returned to a permissive temperature. CPY transport to the vacuole proceeds normally in sec mutant cells blocked after the Golgi body step. These findings indicate that sorting of vacuolar and secretory proteins occurs at or before the Golgi body.

Unlike the targeting of lysosomal proteins in animal cells, transport of CPY to the vacuole in yeast does not require a carbohydrate determinant (26). In mammalian fibroblasts, a mannos-6-phosphate determinant directs transport of most lysosomal glycoproteins from the Golgi body to the lysosome (7, 10, 16). Nevertheless, protein structure must ultimately be responsible for targeting proteins to the lysosome. Lang et al. (18) have shown that lysosomal glycoproteins are much better substrates for the transfer of the N-acetyl-glucosamine-phosphate lysosomal targeting signal than similarly modified secretory glycoproteins. In order to develop a genetic means of identifying the signal(s) on CPY involved in transport of the polypeptide to the vacuole, we have cloned the CPY structural gene (PRC1).

In this report we show that when the PRC1 gene is introduced into yeast on a multiple-copy 2-micron plasmid (2α), the resulting overproduction of CPY leads to secretion of a large fraction of the newly synthesized enzyme. These results suggest that CPY transport may be mediated by a saturable component.

Materials and Methods

Strains, Growth Conditions, and Materials

A prcl-I strain (34) was obtained from G. Fink (Whitehead Institute, Boston, MA), and strain SEY5016a, (sec-1, leu2-3, leu2-112, ara3-52, gal2) was provided by R. Fink (Caltech, Pasadena, CA). SF838-5Aa (leu2-3, leu2-112, ura3-52, ade6), SF838-1Da (araABOIC-leu) 7679A(lac)X74 galU galK rpsL) was obtained from G. Fink (Whitehead Institute, Boston, MA). Strain SF838-1Da was grown on 3% Bacto-yeast extract peptone dextrose medium contained 1% Bacto-yeast extract (Difco Laboratories, Inc., Detroit, MI). Yeast strains were con-
corresponds to 0.15 mg dry weight. Radiolabeling experiments were initiated with exponentially growing cells at an OD_{600} of 0.5-2.

N-CBz-L-Phenylalanyl-l-leucine, L-amino acid oxidase, horseradish peroxidase, and o-dianisidine were from Sigma Chemical Co. (St. Louis, MO); 35S-H_{2}SO_{4} was from ICN K&K Laboratories Inc. (Plainview, NY); IgG Sorb was from the Enzyme Center (Boston, MA); restriction endonucleases, acetylated BSA, and DNA ligation were from Bethesda Research Laboratories, Gaithersburg, MD; endoglycosidase F (endo F) was from Boehringer Mannheim Diagnostics Inc. (Houston, TX). Fraction II Lyticase was used in spheroplast preparation (27). Affinity-purified CPY antibody was described previously (29).

**Plasmid Vectors and Recombinant DNA Methodology**

Restriction endonuclease digestions and ligations with T4 DNA ligase were carried out as recommended by the suppliers. Plasmid purification, agarose gel electrophoresis, and DNA-mediated transformations of bacteria were performed by standard methods (1, 19). A yeast genomic clone bank constructed in the LEU2 vector YEpl3 (21) was used to complement the pre-1 mutation in cloning PRC1. The YEpl3 plasmid containing the genomic PRC1 insert is pTYS1. Plasmid pTYS3 was constructed by subcloning the 3.2-kb pBR322-Sal I fragment from pTYS1 into the Pvu II and Sal I sites of the multiple-copy 2μ circle plasmid YEp24 (2μ-PRC1, 2) and pTYS1000 (single-copy centromere-containing plasmid (CEN)-PRC1) was constructed by inserting the 3.7-kb EcoRI-Sal I fragment into the EcoRI and Sal I sites in the single-copy plasmid YCp50 (17, 31) which contains the centromere region of chromosome 4 (CEN4). Subcloned plasmids were introduced into yeast cells by the lithium acetate transformation method (13).

A chromosomal PRC1 deletion was constructed by transposing (25) the genomic copy with the cloned gene into which the LEU2 gene was inserted in place of a majority of the PRC1 coding region. pTYS1000 was treated with Bal31 nuclease followed by S1 nuclease. A 2.2-kb Sal I-Xho I fragment containing the LEU2 gene on YEpl3 was removed and treated with S1 nuclease. The two cut plasmid samples were mixed and treated with T4 DNA ligase. A plasmid conferring Amp resistant strain was selected in a suitable marked E. coli strain; restriction analysis showed that the LEU2 fragment had been inserted in the PRC1 gene in pTYS1000. A HindIII-Sal I fragment containing the LEU2 gene with plasmid vector was cut from this plasmid and used to transform a PRC1 pep4-3 leu2-3 yeast strain. Stable Leu+ transformants were purified and screened for immunoprecipitable CPY. One was selected that produced no detectable immunoreactive CPY (∆prc1) and this strain was used to generate yeast crude extract lacking CPY. To prepare a ∆prc1 cell extract ∆prc1 cells were centrifuged, resuspended to 10^6 cells/ml in 25 mM potassium phosphate, pH 7.5, 0.2 M NaCl, followed by cell breakage in a homogenizer (Manton-Gaulin, Everett, MA). SDS was added to 1% final concentration, the extract was heated in a boiling water bath for 5 min, followed by a 15-min centrifugation in a microfuge to yield a supernatant that was used as a ∆prc1 crude extract.

**Results**

**Cloning of PRC1**

Hemmings et al. (12) demonstrated that the gene identified by Wolf and Fink (34) was the structural gene for CPY (PRC1) by isolating strains bearing nonsense mutations at the PRC1 locus, at least one of which produces a truncated immunoreactive fragment of the enzyme. In order to clone the PRC1 gene, a pre-1 leu-2-3 yeu-2-112 yeast strain was transformed with a YEpl3 (LEU2) yeast genomic bank (21) and Leu+ transformants were selected. About 20,000 Leu+ transformants were tested for CPY activity using the CPY plate stain (13); 16 positive colonies were detected. Quantitative assays of extracts of these transformants showed only one that contained CPY activity. Plasmid DNA was isolated from this transformant and introduced into E. coli. One class of plasmid was obtained (pTSY1), and a restriction map of the genomic insert in this plasmid is shown in Fig. 1. pTSY1 propagated in E. coli restored the wild-type Prc+ phenotype when introduced into the pre-1 yeast strain. Enzyme activity measurements revealed an approximately fourfold increase in CPY activity.
Secretion of CPY is Dependent on PRC1

The smallest subcloned region of the PRC1 insert that complemented the prcl-1 mutation was the 2.6-kb Pvu II-Cla I fragment. This DNA sequence actually includes the PRC1 gene since it maps genetically to the PRC1 locus: a strain deleted for most of the putative PRC1 open reading frame and carrying the LEU2 marker (see Materials and Methods) was crossed to a prcl-1 strain, diploids were selected, sporulated, and asci dissected. In 20 asci the Prc phenotype segregated 0+4-, demonstrating that the integrated cloned gene is tightly linked to the PRC1 (CPY structural gene) locus. In addition, the DNA sequence of the cloned PRC1 gene confirms that it codes for the vacuolar protease CPY (Valls, L. A., and T. H. Stevens, manuscript in preparation).

**Secretion of CPY is Dependent on PRC1 Gene Copy Number**

To determine if any overproduced CPY escapes the normal sorting reaction in the Golgi body, cells transformed with pTSY3 or YEp24 were fractionated into I, P, and M fractions. In yeast, the cell wall retains most of the secreted proteins in the periplasmic space. These proteins can be released in a soluble form by converting cells to spheroplasts with a lytic enzyme in an osmotically supportive medium. Yeast cells were labeled with 35S-H2SO4 in the presence of 0.5 mg/ml BSA for 15 min, followed by a 45-min chase period and fractionated into I and extracellular (E) fractions. Fractions corresponding to the spheroplast pellet (I), spheroplast supernatant (P), and medium (M) were analyzed by SDS polyacrylamide gel electrophoresis and fluorography. Cells not carrying a PRC1 plasmid have only a little cellular CPY was observed whether the nitrogen source in the growth medium was ammonium chloride or proline (G. L. Vails et al., manuscript in preparation). Thus, between two- and sixfold enhanced synthesis of CPY appears to overload the CPY-sorting apparatus. Experiments in which the PRC1 gene was placed under the transcriptional control of the highly active PHO5 or ADH1 promoters did not result in levels of CPY synthesis significantly higher than those obtained with the PRC1 promoter.

### Table I. Overproduction of CPY Results In Secretion of the Protein

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Estimated PRC1 gene copy number</th>
<th>Relative CPY synthesis</th>
<th>CPY secreted %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2μ</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>CEN-PRC1</td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>2μ-PRC1</td>
<td>&gt;5</td>
<td>6–8</td>
<td>50–55</td>
</tr>
</tbody>
</table>

Cultures of ISY1-7Ba carrying these plasmids (2μ-YEp24, CEN-PRC1/pTSY1000, 2μ-PRC1/pTSY3) were labeled with 35S-H2SO4 for 15 min, chased in medium-containing excess Na2SO4 for 45 min, centrifuged, and fractionated into I, P, and M fractions as before. Samples were analyzed for CPY by immunoprecipitation and electrophoresis. Relative CPY synthesis was calculated by quantifying the intensity of the CPY bands (22) expressed for each strain normalized for total 35S-labeled protein. The percent CPY secreted was determined by quantitation (15) of the CPY bands for the I, P, and M fractions from Fig. 2, where the percent secreted is 100 (%).
Secreted CPY Transits the Full Secretory Pathway

We have shown previously (29) that proCPY transits the early portion of the secretory pathway. CPY fails to be transported to the vacuole in endoplasmic reticulum-blocked and Golgi-blocked sec mutant cells incubated at the restrictive temperature (37°C); sec mutations that result in the accumulation of secretory vesicles (vesicle-blocked mutations) do not block transport of proCPY to the vacuole. If overproduced CPY transits the remainder of the secretory pathway (Golgi → secretory vesicles → periplasm), then a vesicle-blocked mutant (sec1-1) should not secrete proCPY at 37°C. However, if the presence of extracellular CPY is due either to cell lysis or to mechanisms independent of secretion, then the sec1 mutation should not suppress the appearance of extracellular CPY.

Yeast strain SEY5016a (sec1-1) carrying plasmid pTSY3 was labeled with 35S-H2SO4 at 25°C and 37°C. Cells were converted to spheroplasts and the three fractions examined as before. At 25°C, as expected, overproduced CPY was secreted (Fig. 3). At 37°C, however, secretion of proCPY was blocked and a portion of CPY (~50%) accumulated intracellularly as proCPY, whereas sec1+ cells carrying plasmid pTSY3 were found to secrete a high percentage of CPY (40–50%) at 37°C (not shown). Presumably the intracellular proCPY seen in sec1 cells at 37°C resides within secretory vesicles that accumulate in such cells (23). Thus, these results indicate that extracellular CPY must reach the periplasmic space via the secretory pathway.

Stability and Activation of Secreted proCPY

Gene dosage-dependent appearance of external CPY could be accounted for by rapid degradation of low levels of the protein secreted by cells with only one or two copies of the PRC1 gene. This possibility was examined in a pulse-chase experiment designed to test the stability of secreted CPY. Cells were labeled for 15 min in the presence of 1 mg/ml BSA and chased for either 45 or 165 min (Fig. 4). Even in cells containing one copy of the PRC1 gene, a small percentage of proCPY (6%) escaped proper sorting, and was stable in both the periplasm and medium fractions over a 165-min chase period (Fig. 4). The stability of CPY in the medium fraction was greatly increased when BSA was present during the labeling and chase period (not shown). Very short chase periods did not reveal any additional forms of secreted CPY. Hence, the stability of the secreted proenzyme argues strongly against saturation of extracellular CPY degradation as an explanation for the gene-dosage response.

Processing of p2 CPY to mature CPY in the vacuole requires the PEP4 gene. In the experiment shown in Fig. 4 periplasmic material with a mobility similar to mature CPY was detected after a 45-min chase and its relative abundance appeared to increase slightly during the 165-min chase. If this mature-size form is produced by a PEP4-dependent reaction, then periplasmic conversion should not occur in pep4 cells. To test this possibility, pep4 cells carrying the multiple-copy PRC1 plasmid were 35S-H2SO4 labeled for 15 min without BSA and chased in the presence of excess Na2SO4 for either 45 or 165 min. After a 45-min chase, very little 61 kD CPY was observed in the I or E fractions (Fig. 5). In contrast, after a 165-min chase period, the P fraction contained immunoreactive protein with a mobility similar to mature CPY (Fig. 5). While BSA stabilized proCPY in the medium during labeling and chase (~35% CPY in the medium in Fig. 4 vs. <5% CPY in the medium in Fig. 5), it slowed the appearance of the mature-like CPY in the periplasmic space (not shown), possibly by inhibition of some cell-surface enzyme. Extracellular conversion of proCPY thus occurs by a reaction that is independent of the PEP4 gene, and can be inhibited by BSA in the labeling medium.

The experiment shown in Fig. 5 also demonstrated that secreted CPY (P and M fractions) had the same electrophoretic mobility as vacuolar p2 CPY, which accumulates in the

![Figure 3](https://example.com/fig3.png)  
*Figure 3. Secretory vesicle-accumulating mutant (sec1-1) blocks secretion of overproduced CPY. Yeast strain SEY5016a (sec1-1, PEP4) carrying plasmid pTSY3 was labeled with 35S-H2SO4 in the presence of BSA as described in Fig. 2 at 25°C and 37°C. Cells were collected, fractionated, and analyzed as before.

![Figure 4](https://example.com/fig4.png)  
*Figure 4. Low level of CPY secreted by wild-type yeast cells is stable in the extracellular fractions. Yeast strain ISY1-7Ba (PEP4) was labeled with BSA present as described in Fig. 2 except that one-half of the culture was chased for 165 min. Cells were collected, fractionated, and analyzed for CPY as before, except that P and M samples were adjusted to have 20-fold more material loaded on the gel than the I samples to enhance detection of secreted CPY.

![Figure 5](https://example.com/fig5.png)  
*Figure 5. Secreted p2 CPY is cleaved in the periplasm. Yeast strain SF838-1Da (pep4-2) carrying plasmid pTSY3 (2μ-PRC1) was labeled and chased as described in Fig. 4 except that BSA was not added during the labeling or chase periods. Cells were collected, fractionated, and analyzed for CPY as before.*
vacuole (I fraction) of pep4 cells as a result of deficient proteolytic processing of the proenzyme (12, 29). To confirm that the polypeptide components of the secreted and vacuolar CPY precursors are similar, CPY from the I and E fractions of pep4 cells carrying plasmid pTSY3 was treated with Endo F to remove carbohydrate (4, 29) and electrophoresed on polyacrylamide gels. Fig. 6 shows that the deglycosylated extracellular and intracellular proCPY forms comigrate, indicating that the carbohydrate on secreted proCPY is indistinguishable (by electrophoretic mobility) from that on vacuolar proCPY. This result shows that no additional detectable post-translational modifications accompany proCPY transport through the late stages of the secretory pathway.

To determine if secreted periplasmic CPY was converted to an enzymatically active form whole cells were assayed using conditions in which substrate penetrated into the periplasm, but not into the cell. N-CBZ-L-phenylalanyl-L-leucine, a specific substrate for CPY (35), was used to measure periplasmic activity. For comparison, cells were permeabilized with Triton X-100 to allow detection of all active CPY. Cells with a single copy of PRC1 (Table II, strains without the PRC1 plasmid) show background levels of periplasmic CPY activity. In contrast, PEP4 or pep4 cells carrying the multiple-copy PRC1 plasmid (2uPRC1) show appreciable periplasmic CPY activity (Table II). Confirming the results in Fig. 5, which show converted CPY only in the P fraction, most of the enzymatically active CPY in the pTSY3-transformed pep4 strain is periplasmic (Table II).

Active secreted CPY was seen in transformed cells of both mating types and in bar1 cells carrying pTSY3 (not shown).

Figure 6. The molecular weights of deglycosylated secreted and vacuolar CPY precursors are similar. Yeast strain SF838-1Dα (pep4-3) carrying plasmid pTSY3 (2uPRC1) was labeled with 35S-H2SO4 in the presence of BSA as described in Fig. 2. Cells were collected, fractionated, and analyzed for CPY as before except that the P and M fractions were combined into the E fraction. The I and E fractions were treated with endo F as described, and subjected to electrophoresis and autoradiography as before. Protein standards are given in kilodaltons.

Table II. Enzymatically Active CPY in the Periplasm

<table>
<thead>
<tr>
<th>Strain</th>
<th>PEP4</th>
<th>Plasmid</th>
<th>CPY activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intact cells</td>
</tr>
<tr>
<td>SF838</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1Dα</td>
<td>-</td>
<td>2u</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>-1Dα</td>
<td>-</td>
<td>2uPRC1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>-5Aα</td>
<td>+</td>
<td>2u</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>-5Aα</td>
<td>+</td>
<td>2uPRC1</td>
<td>15 ± 3</td>
</tr>
</tbody>
</table>

Cells carrying the indicated plasmids were grown to mid-log phase, centrifuged, washed (±1% Triton X-100), and resuspended in either substrate or substrate plus 0.2% Triton X-100. CPY activity is given in milliunits per milligram dry weight. The 2u plasmid is YEp24 and the 2uPRC1 plasmid is pTSY3. The results are the average (and range) of five repeat assays.

Thus, the BARI gene product, a secreted protease that may proteolytically degrade α-factor (3, 20), appears not to be responsible for activation of the CPY precursor in the periplasm.

Discussion

We have shown that a high percentage of CPY is secreted when the dosage of its structural gene (PRC1) is increased. Although CPY is ordinarily localized almost exclusively to the vacuole (∼95%), the percentage of proenzyme that is secreted increases dramatically with elevated expression of the gene. It is possible that at some overproduction level any additional CPY synthesis results in secretion of the excess newly-synthesized protein; at six- to eightfold higher-than-normal levels of CPY production, >50% of the protein is secreted. These results are consistent with titration of some component essential for transport of CPY to the vacuole. A similar situation may explain the recent report of a mammalian cell line that overproduces and secretes a lysosomal protein in maligantly transformed but not in normal cells (6).

One interpretation of this data is that a limiting component, such as a proCPY receptor in the Golgi body, is not sufficiently abundant to handle much more than a twofold increase in CPY levels. When the receptor is saturated the unbound proenzyme is secreted. An equally plausible explanation is that some other component required for sorting, such as an enzyme that modifies CPY in a way that is essential for sorting, is limited, and unmodified CPY is secreted. Since glycosylation is not required for sorting of CPY, oligosaccharyl modification is unlikely to be the limiting reaction for proper transport (26, 29).

An alternative explanation for the gene dosage effect is that CPY overproduction results in the saturation of an extracellular protease that rapidly degrades high levels of CPY secreted by cells with new copies of PRC1. However, with a short labeling period designed to detect newly synthesized molecules, radiolabeled CPY is found in the E fractions even in normal cells, and this low level (6%) of secreted CPY is stable during a subsequent 165-min chase period. Hence even low levels of CPY are stable in the E fractions. These results argue strongly against limited extracellular turnover of CPY as an explanation for observing secretion at high PRC1 gene dosage.

We have also considered the possibility that overproduction leads to aggregation of proCPY within the lumenal space of the endoplasmic reticulum or Golgi. Aggregated protein may not expose the targeting signals to the sorting component(s). However, secreted proCPY is accessible to the Golgi mannosyl transferases that act to convert p1 to p2 CPY during normal transport to the vacuole. In addition, a second yeast vacuolar enzyme, proteinase A, also exhibits overproduction-induced aggregation at high PRC1 gene dosage.

We have also considered the possibility that overproduction leads to aggregation of proCPY within the lumenal space of the endoplasmic reticulum or Golgi. Aggregated protein may not expose the targeting signals to the sorting component(s). However, secreted proCPY is accessible to the Golgi mannnosyl transferases that act to convert p1 to p2 CPY during normal transport to the vacuole. In addition, a second yeast vacuolar enzyme, proteinase A, also exhibits overproduction-induced aggregation (24), and thus proteinase A would also have to aggregate to the same extent at the same level of overproduction as CPY. Nevertheless, though we consider saturation of a limited quantity of transport sites a more likely explanation of the data, a sorting-deficient aggregate cannot be ruled out. Detection of a saturable binding component by in vitro reconstitution will allow these possibilities to be distinguished.

If a single receptor is involved in the transport of a family of vacuolar proteins, saturation by overproduction of one member should result in secretion of the related members. This remains a possibility, although in strains carrying PRC1
on a multicopy plasmid we have not detected secretion of enzymatically active alkaline phosphatase, or immunoreactive proteinase A polypeptide, two other vacuolar proteins (Rothman, J. H., and T. H. Stevens, unpublished observations). A more general approach may be needed to monitor secretion of a wider range of vacuolar proteins.

In the normal transit of CPY to the vacuole a 67-kD glycoprotein precursor form (produced in the endoplasmic reticulum) is glycosylated further in the Golgi body to yield a 70-kD species (p2 CPY). When CPY is secreted, the same 70-kD form is detected in the periplasm (with the same complement of carbohydrate) where it is slowly converted to an enzymatically active form. Invertase, a normally secreted enzyme, undergoes considerably more extensive glycosylation in the Golgi body where a 79–83-kD form is converted to heterogeneous forms of 100–150 kD (5). While the sec1 experiment suggests that CPY likely transits the entire secretory pathway, the extent of glycosyl modification appears to be an inherent property of CPY itself and not simply a result of spatial separation from the glycosyl transferases that act on secreted enzymes. However, this conclusion assumes that the sec1 block defines a unique secretory pathway and not multiple parallel pathways, a point yet to be resolved.

This situation differs from that observed in the mislocalization of lysosomal enzymes in fibroblasts from I-cell disease (mucolipidosis II) patients. Unlike secretory glycoproteins, lysosomal enzyme oligosaccharides are phosphorylated in the Golgi body. This modification allows targeting to the lysosome by interaction with a mannose-6-phosphate receptor, and prevents conversion of lysosomal oligosaccharide chains from the high-mannose to complex form (28). I-cell fibroblasts lack the enzyme that phosphorylates lysosomal enzyme oligosaccharides (11). In the absence of this modification, lysosomal enzymes undergo the normal high mannose to complex oligosaccharide conversion and are secreted (9, 28).

Conversion of the secreted proCPY to active CPY is mediated by an unknown activity; this process occurs independently of the PEP4 gene product which is required for activation of many hydrolytic enzymes within the vacuole. Since trypsin can convert proCPY to a mature-like CPY species (8) that is enzymatically active (36), periplasmic maturation may be mediated by a secretory or plasma membrane surface endoprotease of similar specificity. The activation of the secreted precursor allows a genetic selection for mutants that mislocalize CPY. Mutations in the sorting apparatus (e.g., CPY receptor) may be obtained by selecting for cells capable of growth on a peptide which must be cleaved by periplasmic CPY to provide a necessary nutritional supplement (30). This approach should aid in the identification of molecules that participate in the sorting reaction.

In conclusion, by modulating the level of CPY synthesis through PRC1 gene dosage experiments, we have demonstrated that proper transport of this glycoprotein to the vacuole can be overloaded. Our results suggest that CPY transport to the vacuole may be mediated by a saturable component such as a receptor. Unlike the mannose-6-phosphate receptor of mammalian cells, the yeast receptor would most likely recognize protein determinants since transport of CPY does not require glycosylation. Although excess CPY transits the entire secretory pathway, it does not undergo the glycosyl modifications characteristic of typical secreted yeast glycoproteins.

This result indicates that glycosyl modifications in yeast may be dictated by structural properties inherent in the protein undergoing the modifications, and not merely by exposure to the transferases.

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