The Yeast SEC20 Gene Is Required for N- and O-Glycosylation in the Golgi

EVIDENCE THAT IMPAIRED GLYCOsylation DOES NOT CORRELATE WITH THE SECRETORY DEFECT

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The Golgi plays a fundamental role in posttranslational glycosylation, transport, and sorting of proteins. The mechanism of protein transport through the Golgi has been seen as controversial in recent years. During the characterization of N-glycosylation-defective mutants (ngd) previously isolated by this laboratory, it was found that ngd20 is allelic to sec20. SEC20 was reported to be required for transport from endoplasmic reticulum to Golgi, but its precise function remains to be determined. We show now that SEC20 is also required for N- and O-glycosylation in the Golgi but not in the ER, in a cargo-specific manner, and that the glycosylation defect does not correlate with the secretory defect. By pulse-chase labeling experiments in combination with mannose linkage-specific antibodies, invertase and carboxypeptidase were found to be efficiently secreted to their final compartment, even upon shift to the nonpermissive temperature, while glycosylation in the Golgi was severely impaired. Using microsomal membranes isolated from ngd20, we found that mannosyl transfer from GDP-Man to various mannose-oligosaccharides, indicative for Golgi mannosylation, was strongly diminished. Analysis of the carbohydrate component of chitinase, an exclusively O-mannosylated protein, or of the bulk mannoprotein indicates that O-mannosylation is also reduced. The results demonstrate that in addition to secretion SEC20 also affects glycosylation in the Golgi, presumably because it exerts a more general role in maintenance and function of the Golgi compartments.

The Golgi apparatus plays a fundamental role in posttranslational glycosylation, transport, and sorting of newly synthesized proteins within the secretory pathway. Morphological, biochemical, and genetic techniques have defined functionally distinct polarized subcompartments within this complex, each of which contains a set of resident proteins (1–3).

The fundamental mechanism of protein transport through the Golgi apparatus has been seen as controversial in recent years. Two models have been proposed. According to the stable compartment model, different Golgi proteins are anchored in distinct compartments, and secretory cargo is transported through the complex in anterograde vesicles. In view of the cisternal maturation concept, the cis-Golgi compartment is formed de novo by fusion of endoplasmic reticulum-derived vesicles, and the secretory cargo is carried forward by cisternal maturation, while retrograde vesicles recycle resident Golgi proteins. Each Golgi protein is concentrated in that part of the stack at which retrograde transport of this particular protein balances cisternal progression (1, 4–7).

Studies of the secretion pathway in Saccharomyces cerevisiae have been greatly facilitated by the isolation of a number of mutants with defects in secretion or glycosylation. The analysis of sec (secretion) mutants (8, 9) allowed dissection of the pathway into discrete steps, and mutants involved in protein glycosylation such as mnn (mannan) (10), och (outer chain) (11), ngd (N-glycosylation-defective) (12), and ldb (low dye binding) (13) were helpful in the functional description of the compartmental organization of the Golgi complex (14, 15). Following the initial core glycosylation in the ER (16, 17), the N-linked oligosaccharides of yeast are further elongated in a topologically ordered manner by Golgi-localized α,β,α,β, and α,β,α,β,mannosyltransferases (14, 15, 18–23). This so-called outer chain greatly varies in size depending on the protein. Whereas glycoproteins of the internal compartments have only a few mannose residues added, many of the proteins of the cell wall have attached a large, heterogeneous “mannan” structure up to 200 mannosides. To further our understanding of N-glycosylation in yeast, we screened for temperature-sensitive N-glycosylation-defective mutants (ngd) by using a tritium suicide selection procedure (12, 24). During the biochemical and genetic characterization of ngd20, it was found to be allelic to the secretory mutant sec20. The SEC20 gene was reported to encode a membrane glycoprotein required for endoplasmic reticulum to Golgi transport (25). Later it was found that Sec20p forms a complex with the cytosolic Tip20p (26, 27), and its primary function was assumed to be in retrograde transport from the Golgi to the ER rather than in the forward transport step (28). It was speculated that the complex acts in the ER to aid the uncoating, docking, or fusion of retrograde vesicles. However, its precise role remains to be determined. We show now that NGD20/SEC20 is also required for Golgi N- and O-glycosylation in a cargo-specific manner and that the glycosylation defect does not correlate with the secretion phenotype.

EXPERIMENTAL PROCEDURES

Yeast Strains, Genetic Methods, and Plasmids—The following yeast strains were used: X2180–1A (MATa SUC2 mal nes), SS330 (MATa ade2–101 ura3–52 his3–ΔD200 tyr1), DBY746 (his3–ΔD1 leu2–3 leu2–121)

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Isolation of the NGD20/SEC20 Locus—The mutant originates from a our glycosylation defective mutant collection isolated by [3H]mannose suicide selection (12). The original ngd20 was backcrossed twice into DBY746 to give ngd20–1. A genomic library, containing partially Sau3A-digested yeast chromosomal DNA ligated into vector YEp352 (32), was transformed into ngd20–1. Transformants were selected on YNB minimal medium supplemented with 0.5% casamino acids, 20 mM benzamidine) and broken with glass beads (0.45

Mannosyltransferase Assays—Mannosyltransferase activity was monitored in TNET buffer was incubated with the solubilized extract by rolling end over end for 2 h at 4 °C. The Sepharose beads were centrifuged for 1 min at 14,000 × g, washed four times with 1 ml of TNET buffer and once with 1 ml of 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, 1% Triton X-100, the appropriate antiserum was added, and the mixture was incubated at 4 °C overnight. For precipitation of the antigen-Ig G complexes, 5 mg of protein A-Sepharose equilibrated in TNET buffer was incubated with the solubilized extract and washing buffer (0.45 μm) for 10 min on a vibraphaker at 4 °C. After the lysate was collected, glass beads were washed with 1 ml of TNET buffer, and the extracts were centrifuged. The pellet was then centrifuged for 30 min at 40,000 × g, and the supernatant was incubated with 1% SDS for 5 min at 95 °C. SDS was then reduced to <0.2% by addition of TNET (50 mM Tris–HCl, pH 7.5, 3 mM EDTA, 150 mM NaCl, 1% Triton X-100), the appropriate antiserum was added, and the mixture was incubated at 4 °C overnight. For precipitation of the antigen-Ig G complexes, 5 mg of protein A-Sepharose equilibrated in TNET buffer was incubated with the solubilized extract by rolling end over end for 2 h at 4 °C. The Sepharose beads were centrifuged for 1 min at 14,000 × g, washed four times with 1 ml of TNET buffer and once with 1 ml of 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, then resuspended in 50 μl of SDS-PAGE sample buffer, and incubated at 95 °C for 5 min. Antigens to be reimmunoprecipitated with a secondary antibody were dissociated from the first antibody by boiling for 5 min in 1% SDS, 20 mM Tris–HCl, pH 7.5.

For endoglycosidase H (endoH) digestion immunoprecipitated samples were treated twice with 15 μl of 100 mM sodium citrate buffer, pH 5.5, containing 0.5% SDS and 0.5% β-mercaptoethanol and boiled for 5 min. Then 120 μl of 10 mM sodium citrate buffer containing 0.5% octylglucoside was added and boiled for another 3 min. The combined supernatants were incubated with 25 milliliters of endoH at 37 °C. After 10 min, 25 milliliters of water were added and boiled overnight. Control samples were similarly prepared without endoH.

Polyclonal antisera directed against unglycosylated cytoplasmic invertase and CPY were from laboratory stocks. Antiserum specific to α1,3- and α1,6-linked mannose were prepared by immunizing rabbits with heat-killed yeast cells (strains Y2180 and mnn1–3 mnn2–1, respectively). Antiserum specific to α1,3-linked mannose were purified by absorption of the antiserum to mnn1 cells; α1,6-specific antiserum was obtained by absorption to X2180 cells. 2. Antiserum against deglycosylated chitinase was a gift from W. Tanner, University Regensburg. Anti-HA tag antiserum was purchased from Babco (Berkeley, CA).

For subcellular fractionation labeled cells (10 A260 equivalents) were converted to spheroplasts by incubation at 25 °C for 30 min in 1 ml of 50 mM methionine, 10 mM cysteine, 4% yeast extract, 20% Bacto yeast extract, 2% Bacto peptone, 2% dextrose) or YNBD drop out medium (32), was transformed into DBY746 to give 2.14 mCi of [35S] label that was not incorporated was removed by washing cells once with the same buffer. The reaction was neutralized with HCl, and oligosaccharides were separated on a Bio-Gel P2 column (0.9 × 104 cm) equilibrated in 50 mM pyridine acetate, pH 5.2. Fractions of 33 drops were collected and analyzed for radioactivity.

Chitinase was analyzed by Western blotting to nitrocellulose membranes using the semi-dry blotting technique and visualized by the ECL method (Amerham Pharmacal Biotech). Cells were broken with glass beads as described above. A cell wall pellet was obtained from the lysate by centrifugation at 2,000 × g for 5 min. SDS sample buffer was added to 10 min for 95 °C, and centrifuged. From the supernatant the equivalent of 1–3 A260 was applied to a 6% polyacrylamide gel, transferred to nitrocellulose, and decorated with anti-chitinase antiserum. 

Mannosyltransferase Assays—Incubation mixtures contained 10 mM Tris–HCl buffer, pH 7.4, 10 mM MnCl2, GDP-[U-14C]mannose (0.1 μCi; specific activity, 303 mCi/mmol), 2 mM exogenous acceptor as indicated, and 0.2 mg of membrane protein in a final volume of 0.07 ml. The reaction mixtures were incubated for 20 min at 25 °C and terminated with 0.1 ml of NaOH overnight at 30 °C. The reaction was neutralized with HCl, and oligosaccharides were separated on a Bio-Gel P2 column. The neutral products were eluted with water, lyophilized, and analyzed by thin layer chromatography on silica gel 60 plates developed twice with the solvent acetone:butanol:water (70:15:15, v/v/v). Radioactivity was monitored and quantified with a TLC radioanalyzer (Berthold).

RESULTS

Growth Phenotype of ngd20 and Cloning of the Complementing Gene—Among the ngd mutant collection previously isolated (12), ngd20 showed a temperature-sensitive growth phenotype (Fig. 1) and a strong defect in glycosylation (see below). Compared with wild-type cells, ngd20 had a reduced growth at 30 °C and a complete block at 37 °C; addition of sorbitol improved growth at all temperatures. Yeast genomic clones, which complemented the temperature sensitivity of ngd20 at 36 °C, were isolated by transformation with a plasmid-based genomic library. From six colonies growing at the restrictive temperature one plasmid was identified, which also complemented the glycosylation defect. Sequence information from the 4-kilobase insert revealed that it contained SEC20 flanked by only incomplete sequences of ITR3 (myo-inositol transporter) and open reading frame YDR 499 (function unknown).

2 Lun Ballou, personal communication.
cysteine, and proteins were immunoprecipitated from cell extracts and analyzed by SDS-PAGE. As shown in Fig. 2, invertase in ngd20 at the permissive temperature of 25 °C is already severely underglycosylated compared with the wild type but still migrated as a diffuse band (lanes 1 and 4). By using antibodies specific for α1,3- and α1,6-linked mannose, 90 and 36%, respectively, of the label could be recovered in a second immunoprecipitation, similar to wild-type invertase (Fig. 2, compare lanes 2 and 3 with lanes 5 and 6). This indicates that in principle all Golgi compartments are functional at 25 °C, but the overall glycosylation activity is reduced and leads to shorter glycan chains. The slight increase in yield observed with the α1,6-mannose antiserum is probably due to a better access of the antigenic epitope on shorter chains. Labeling at 35 °C resulted in an invertase form that migrated as the core glycosylated ER form. As expected, it is poorly precipitated with the α1,3- and α1,6-mannose antiserum (Fig. 2, lanes 8 and 9). Similar precipitation values were also obtained for core invertase immunoprecipitated from och1Δ or sec18Δ cells at 36 °C (data not shown).

Examination of the glycosylation and maturation of CPY is depicted in Fig. 3A. Labeling of ngd20 cells for 30 min showed that in the mutant the ER form (p1-CPY) was the dominant species even at the permissive growth temperature (Fig. 3A, lanes 4–6). This indicates that processing to the mature, vacuolar form was retarded compared with wild-type yeast (Fig. 3A, lanes 1–3), in agreement with a sec phenotype. In addition, the mature form in ngd20 was smaller in size (Fig. 3A, lanes 7 and 8) and ran slightly faster than CPY immunoprecipitated from wild-type cells. Because the size of the p1 form was identical in both strains, we reasoned that ER glycosylation was normal, but subsequent Golgi glycosylation was defective. This could be substantiated further by pulse-chase experiments analyzing the glycosylation state with α1,6- and α1,3-mannose antibodies (Fig. 3B). Whereas 100% of m-CPY from wild-type cells could be precipitated with the α1,3-antiserum, in the mutant this amounted to only 33% at 25 °C and 16% at 35 °C, respectively (Fig. 3B, lanes 6 and 3).

**Invertase Is Efficiently Secreted in Its ER-glycosylated Form**—The results of Fig. 3, A and B indicate that processing of CPY to its mature form, and hence transport to the vacuole, occurred both at 25 °C (58%) and with reduced activity at 36 °C (30%). We therefore decided to extend the secretion analysis to invertase, which is secreted to the cell wall. Cells were pulse-labeled at 35 °C, chased at either 25 or 35 °C, converted to spheroplasts, and separated into I and E fractions, from which invertase was immunoprecipitated (Fig. 4). We found that at both the permissive and restrictive temperature invertase was secreted. Unexpectedly, at a 25 °C chase the glycosylation defect was not restored, and invertase had the size of the ER form. Even at 35 °C invertase was efficiently (32%) secreted. Reimmunoprecipitation of invertase with the α1,3-mannose linkage-recognizing antiserum (Fig. 4, lanes 5 and 8) indicated that only about 20% of the secreted fraction at 25 °C and 16% at 35 °C was modified with α1,3-mannose residues.

**Localization of Och1p in ngd20**—It has previously been shown that the cis-Golgi resident mannosyltransferase Och1p moves to later Golgi compartments as far as the trans Golgi network, after which it is recycled back to the cis-compartment using a retrograde transport system (30). We asked whether this early Golgi function is affected in ngd20 too. To examine this question a hemagglutinin (HA) epitope-tagged Och1p construct was used. The HA tag was found not to disturb the steady state distribution of the protein, and the protein was found to have a half-life of ~60 min (30). Cells were labeled for 15 min at 25 °C and chased at the same temperature, as indi-
As can be seen (Fig. 5A), Och1p-HA was degraded only slightly faster in ngd20 than in wild-type cells. To address the subcellular localization of Och1p-HA, a spheroplast lysate was examined by differential centrifugation. It has been reported that the majority of ER membranes are mainly recovered in a low speed 13,000 g pellet (P13), whereas the bulk of the Golgi membranes fractionates into a 100,000 g pellet (P100). The 100,000 g supernatant (S100) is composed of the cytoplasmic fraction and of osmotically sensitive soluble vacuolar content (38–40). When wild-type cells were labeled at 25 °C and chased at 25 °C, Och1p-HA (Fig. 5B) fractionated into both P13 and P100, in agreement with previous results (39). In the case of ngd20 about 12% of the pulse label was recovered in the S100 supernatant that was degraded during the chase (Fig. 5B, lanes 9 and 12). It has been suggested that Och1p degradation in wild-type cells occurs in the vacuole in a PEP4-dependent man-
shown in Fig. 6, proteolytic cleavage in the late Golgi compartment is functional secreted to the extracellular fraction. These data suggest that a PAGE. The data depicted in Fig. 6 demonstrate in the case of tated with invertase antibodies and digested with endoH to and chased at 25 °C. The fusion protein was immunoprecipi-

A

ngd20

wt

wt + pOCH1-HA

ngd20 + pOCH1-HA

pulse

chase:

30'

60'

90'

120'

wt + pOCH1-HA

ngd20 + pOCH1-HA

pulse

chase:

30'

60'

90'

120'

30'

60'

90'

120'

30'

60'

90'

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30'

60'

90'

120'

FIG. 5. Expression of HA epitope-tagged Och1p in ngd20 (A) and subcellular localization of HA epitope-tagged Och1p in ngd20 (B). A, wild-type (wt) and ngd20 cells transformed with pOCH1-HA were incubated for 30 min at 25 °C, labeled for 15 min with [35S]Pro-Mix, and chased for the times indicated. For immunoprecipitation of Och1p the anti-HA epitope antibody was used. Immunoprecipitates were analyzed by SDS-PAGE (10% gel) and visualized by autoradiography. The radioactivity incorporated was quantified by phosphorimaging analysis and is expressed as percent relative to Och1p synthesized in the pulse. B, cells transformed with pOCH1-HA were incubated for 30 min at 25 °C, labeled for 15 min with [35S]Pro-Mix, and chased for 60 min. Cells were converted to spheroplasts and fractionated into low speed pellet (P13), high speed pellet (P100), and high speed supernatant (S100) as described under “Experimental Procedures.” Och1p-HA was immunoprecipitated and analyzed by SDS-PAGE. Percent distribution of radioactivity in the three fractions was determined by phosphorimaging analysis.

er (30). Because in the experiment presented here an ngd20Δpep1Δprb1 strain was used, degradation occurred presumed independently of these proteases and/or at a different subcellular location. Altogether, the results indicate that local-

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ization and retrieval of Och1p from the trans Golgi network in the ngd20 mutant are not significantly altered.

Kex2p-dependent Cleavage in the Late Golgi Compartment Is Functional—The data so far indicate that ngd20 allows protein secretion despite a severe defect in Golgi glycosylation. We reasoned that this could be due to an anterograde transport bypassing the Golgi compartment or because Golgi glycosyl transferases are impaired due to improper sorting or as a result of an altered Golgi structure. We asked whether a function other than glycosylation, such as Kex2p-dependent proteolytic cleavage in the late Golgi, is defective. We took advantage of a gene fusion encoding the CPY vacuolar targeting signal (C) separated from enzymatically active invertase (I) by a Kex2p cleavage site (\(\alpha\)\(-\)\(\gamma\)\(-\)\(\gamma\)) present in mating pheromone \(\alpha\)-factor in either direction (C\(\alpha\)-1 or C\(\gamma\)-1) (31). It has previously been de-

scribed that 70% of invertase activity is secreted in a Kex2p-dep
tendent manner from cells expressing the triple fusion with the \(\alpha\)-fragment cloned in the proper orientation (C\(\alpha\)-1) but not when in the inverse orientation (C\(\gamma\)-1). ngd20 cells expressing either the C\(\alpha\) or C\(\gamma\) construct were pulse-labeled at 35 °C and chased at 25 °C. The fusion protein was immunoprecipi-
tated with invertase antibodies and digested with endoH to release the glycan chains for better analysis in subsequent PAGE. The data depicted in Fig. 6 demonstrate in the case of the C\(\alpha\)-1 fusion a Kex2p processing, indicated by a mobility shift from 63 to 59 kDa (Fig. 6, lanes 3–6), that amounts to 64%. As shown in Fig. 6, lanes 6 and 7, the cleaved invertase material is secreted to the extracellular fraction. These data suggest that proteolytic cleavage in the late Golgi compartment is functional in ngd20 despite the severe glycosylation defect.

CPY in ngd20 Is Transported to the Vacuole—To further examine the secretion pathway in ngd20, the subcellular dis-

tribution of CPY in wild-type and ngd20 cells was compared. In pulse-labeled wild-type yeast (Fig. 7, A–C), the three defined CPY forms indicative for the maturation process of this en-

zyme, i.e. p1-CPY (ER form), p2-CPY (Golgi form), and m-CPY (vacular form), showed the typical fractionation pattern, as reported (15, 31, 37). p1-CPY was most dominant in the P13 pellet, p2-CPY was preferentially present in the P100 pellet, and m-CPY was preferentially present in the soluble S100 fraction. As mentioned above, hardly any p2-CPY was detect-
able in ngd20 cells. Therefore, we considered the distribution of the p1 form during a chase (designated p1*-CPY; Fig. 7E) as indicative for CPY on transit to the vacuole. p1*-CPY was decreased in the P13 fraction, as expected, but did not follow the typical p2-CPY distribution and instead was highest in the S100 fraction. Because processing to the mature form occurs immediately upon arrival in the vacuole, we assume that S100 in ngd20 also contains, besides vacuolar material (see Fig. 7F), a light vesicle fraction as a result of a perturbed Golgi struc-
ture. However, mature CPY in ngd20 (Fig. 7F) has a similar distribution pattern as wild-type yeast, indicating that it has in fact reached the vacuole.

NGD20 Is Also Required for O-Glycosylation in the Golgi—Yeast glycoproteins contain in addition to N-glycan chains short unbranched mannose-oligosaccharides containing one to five mannosyl residues attached to serine or threonine. Whereas mannosyl transfer of the first mannose occurs in the ER, chain extension takes place in the Golgi apparatus (41). We asked, therefore, whether the glycosylation defect in ngd20 is only restricted to N-glycosylation or whether O-glycosylation is also perturbed. We analyzed the formation of O-linked chains by labeling cells with \([3H]mannose and releasing them by \(\beta\)-elimination or by investigation of mannosylation of chitinase, an exclusively O-mannosylated, extracellular protein (42). As shown in Table I, the overall mannose incorporation during a 2-h labeling period into serine/threonine chains was reduced at 36 °C to about 50% compared with that at 25 °C, and the mannose-oligosaccharide pattern was shifted to mannose in accord with a Golgi glycosylation defect. In addition, in a short term incubation of 15 min a decrease in formation of oligosaccharides was found, with a concomitant increase of single manno-

sede added in the ER (data not shown). Western analysis of chitinase from ngd20 (Fig. 8) revealed that it mi-

tered with a lower molecular mass, indicating again the O-

glycosylation defect. Transformation of the mutant with the
Complementing NGO20/SEC20 gene was able to restore the underglycosylation defect (Fig. 8, lane 4).

Golgi Mannosyltransferase Activities in ngd20 Are Decreased—Mannosyltransferases, involved in the biosynthesis of the carbohydrate outer chain of yeast mannoproteins, can be assayed in vitro using mannose and short mannose-oligosaccharides, respectively, as acceptors and GDP-mannose as glycosyl donor. The products formed are neutral oligosaccharides one mannose unit larger than the acceptors (10, 43, 44). Comparing the enzyme activity of membranes isolated from wild-type cells with that of ngd20 cells grown at the semipermissive temperature of 30 °C, we observed in the mutant a drastic reduction in mannosyl transfer to mannose (acceptor for α1,2 transferase), to α1,6-mannobiose and α1,2-mannobiose (acceptors for α1,6 transferase), and to α1,2-mannotriose (acceptor for α1,3 transferase) (Table II). These results clearly indicate that NGO20 is required for activity of mannosyltransferases in the Golgi.

DISCUSSION

In this paper we describe the biochemical and molecular genetic characterization of the temperature-sensitive glycosylation...
phenotype (8), whereas for The sec and from wild-type (wt) lanes 1 and 5, ngd20 lane 2, and ngd20 harboring the vector (lane 3) or the complementing plasmid (lane 4).

**TABLE II**

**Mannosyltransferase activities in membranes from wild-type and ngd20 cells**

In vitro mannosyltransferase activity was determined by measuring the transfer of radiolabeled mannose from GDP-mannose to the acceptors indicated as described under “Experimental Procedures.” Acceptor substrates α1,6-mannobiose, α1,2-mannobiose, and α1,2-mannotriose were obtained by partial acetolysis of bulk mannan fraction isolated from *mut2* and wild-type cells, respectively, and size-fractionated on Bio-Gel P2 according to Ballou (10). The data presented are average values of three experiments. The deviations from the average ranged between 5 and 20%.

| Acceptor         | Wild-type | ngd20 | Reaction product | cpm  |
|------------------|-----------|-------|------------------|------|
| Mannose          | 195       | 64    | (M₃)             |      |
| Methyl-mannose   | 2140      | 1074  | (M₃-CH₃)         |      |
| α1,6-Mannobiose  | 632       | 278   | (M₄)             |      |
| α1,2-Mannobiose  | 1078      | 517   | (M₄)             |      |
| α1,2-Mannotriose | 434       | 86    | (M₅)             |      |

It is shown in this paper that Sec20p also plays a role in Golgi glycosylation without affecting core glycosylation in the ER, and, most remarkably, the glycosylation defect is largely independent of the secretion phenotype. By pulse-chase cell labeling in combination with immunoprecipitation using α1,6- and α1,3-mannose linkage-specific antibodies and cell fractionation techniques, it was found that glycosylation of invertase and CPY in the Golgi is defective. In the case of invertase already at the permissive temperature, a strong reduced overall mannosyl transfer was observed, leading to shortened outer chains without affecting a specific manno linkage, however (Fig. 2). At the restrictive temperature complete inhibition of Golgi glycosylation took place. Analysis of invertase secretion showed that substantial traffic to the cell wall occurred at both temperatures during a 1-h chase period (45% secretion at 25 °C and 32% at 35 °C), without restoring the Golgi glycosylation defect; even at the maximal permissive temperature only 20% of the chains received an α1,3-linked mannose (Fig. 4). In the case of CPY impairment of glycosylation and secretion to the vacuole was more severe as compared with invertase and was affected already at the permissive temperature (Fig. 3). This may point to cargo-specific differences in ngd20/sec20. Evidence for cargo-selective transport in other yeast mutants already exists (40, 46). On the other hand, Kex2p proteolytic cleavage in the trans-Golgi network was found to be normal (Fig. 6), indicating that not all Golgi functions are impaired.

To explain the observed differences in the secretion and glycosylation phenotype, at least two scenarios are conceivable. (i) Forward vesicle traffic from the ER to either the plasma membrane or the vacuole follows a Golgi bypass; or (ii) the Golgi apparatus no longer retains functional integrity, thereby disturbing activity and/or proper localization of glycosyltransferases but still allowing secretion. Given the findings that Sec20p is mainly localized in the ER and cis-Golgi and that its primary role may be involved in retrieval from Golgi to ER (25–28), the ngd20 mutation could lead to a defect in the retrieval of transport factors required for normal packaging, budding, and/or docking of ER vesicles, thus impairing anterograde transport and circumventing the cis- to trans-Golgi function. Sorting of invertase and CPY to their target compartment, as well as Kex2p cleavage, both events occurring in the late secretory trans Golgi network compartment, remain undisturbed, however. One possibility to explain assumption (ii) is that ngd20 causes, in accord with the Golgi maturation model, a defect in retrieval of Golgi glycosyltransferases, thus disturbing outer chain formation. A sorting defect, e.g. of the OCH1-encoded α1,6-mannosyltransferase, assigned in the intermediate/cis-compartment would prevent initiation of outer chain, and thus further elongation and branching by α1,6-, α1,2-, and α1,3-mannosyltransferases would be indirect. Indeed it has been shown that Och1p involves retrograde transport from the trans-Golgi (30). Because we observed nearly normal Och1p localization in ngd20–1, this interpretation is rather unlikely to explain the strong glycosylation defect. On the other hand, by measuring several Golgi mannosyltransferase activities involved in outer chain formation, a severe decrease was detected in membranes from ngd20 cells. In addition, we found that O-glycosylation was also impaired. These observations suggest that Sec20p plays a more general role in

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the maintenance and/or function of the Golgi compartments either directly or indirectly by interacting with another, not yet identified protein different from Tip20. Our data do not allow us to distinguish between these possibilities. Nevertheless it is surprising that the ngd20/sec20 mutation causes such severe perturbations in the glycosylation machinery, with only minor effects on secretion through the Golgi complex. In a recent perturbations in the glycosylation machinery, with only minor effects on secretion through the Golgi complex. In a recent 

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