Clathrin-dependent Localization of α1,3 Mannosyltransferase to the Golgi Complex of *Saccharomyces cerevisiae*

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**Abstract.** Posttranslational modification of yeast glycoproteins with α1,3-linked mannose is initiated within a Golgi compartment analogous to the medial Golgi cisternae of higher eukaryotes. We have characterized the synthesis, posttranslational modification, and localization of the yeast α1,3 mannosyltransferase (Mnnlp) using antibodies prepared against a segment of this protein expressed in bacteria. Mnnlp is initially synthesized as a 98.5-kD, type II integral membrane glycoprotein that is modified with both N- and O-linked oligosaccharides. It is subject to a slow, incremental increase in molecular mass that is dependent upon protein transport to the Golgi complex. Self-modification of Mnnlp with α1,3 mannose epitopes, primarily on O-linked oligosaccharides, is at least partly responsible for the incremental increase in molecular mass.

Mnnlp is a resident protein of the Golgi complex and colocalizes with guanosine diphosphatase to at least two physically distinct Golgi compartments by sucrose gradient fractionation, one of which may be a late Golgi compartment that also contains the Kex2 endopeptidase. Surprisingly, we found that a significant fraction of Mnnlp is mislocalized to the plasma membrane in a clathrin heavy chain temperature sensitive mutant while guanosine diphosphatase remains intracellular. A mutant Mnnlp that lacks the NH2-terminal cytoplasmic tail is properly localized to the Golgi complex, indicating that clathrin does not mediate Mnnlp Golgi retention by a direct interaction with the Mnnlp cytoplasmic tail. These results indicate that clathrin plays a broader role in the localization of Golgi proteins than anticipated.

The initial events of protein glycosylation in yeast and mammalian cells are essentially the same, but *Saccharomyces cerevisiae* lacks the glycosyltransferases for complex sugars and so only produces glycoproteins with extended chains of mannose. Maturation of oligosaccharides on yeast glycoproteins requires the sequential action of α1,6, α1,2, and α1,3 mannosyltransferases within the Golgi complex of the secretory pathway. These enzymes catalyze the linkage-specific transfer of mannose from a GDP-mannose donor to N- and/or O-linked oligosaccharides (reviewed in reference 22). The isolation and characterization of mutants that display abnormal mannan structures (*mnn* mutants) has facilitated the elucidation of these biosynthetic pathways (3). The *mnnl* mutant was isolated by an immunological enrichment procedure in which mutagenized cells were precipitated with an antiserum specific to α1,3 linked mannosyl epitopes. Nonreactive cells remained in suspension and were enriched for mutants that failed to display the α1,3 linked mannosyl epitope on their cell walls (46). The *mnnl* mutant lacks α1,3 mannosyltransferase activity, suggesting that the *MNN1* gene encodes this enzyme (35). The *MNN1* gene was recently cloned and its sequence predicts a type II integral membrane protein of 88.6 kD (66). While yeast has only one α1,3 mannosyltransferase, there appear to be multiple α1,6 and α1,2 mannosyltransferases (22). The genes that encode an initiating α1,6 mannosyltransferase (*OCH1*) (36), and an α1,2 mannosyltransferase (*MNT2/KRE2*) (19, 23) have been cloned and sequenced. Surprisingly, these proteins do not exhibit sequence homology to each other or to Mnnlp, although all three proteins are predicted to be type II integral membrane proteins with short cytoplasmic tails. This structure is also a feature of several mammalian glycosyltransferases (43).

In previous work, we proposed that the yeast Golgi complex is divided into at least three functionally distinct compartments (16). Within these compartments are catalyzed, from cis to trans, the α1,6 mannosylation, α1,3 mannosylation, and Kex2p-mediated proteolytic processing of glycoproteins. This conclusion was based on the requirement for the Sec18/NSF intercompartmental protein transport factor for each successive modification in vivo (16). These experiments indicated that the α1,3 mannosyltransferase activity is...
first encountered in a medial-Golgi compartment. Based on these observations, we reasoned that Mnnlp should be a resident marker protein for the yeast medial- and perhaps trans-Golgi compartments.

One approach towards understanding the biogenesis of the Golgi complex is to ask how the resident proteins are localized to this organelle. In the case of the late Golgi enzymes Kex1p, Kex2p, and dipeptidylaminopeptidase A (DPAP A) that are required for the final proteolytic maturation of the α-factor mating pheromone precursor, it was found that all of these proteins have Golgi localization signals within their cytoplasmic tails (10, 39, 63). The Kex2p and DPAP A Golgi localization signals are short peptide segments containing essential tyrosine or phenylalanine residues that are very similar to the signals within mammalian receptor proteins that mediate clustering into clathrin coated pits (reviewed in 40, 64). A role for clathrin in the Golgi localization of Kex2p and DPAP A was demonstrated using yeast strains harboring a temperature-sensitive allele of the clathrin heavy chain gene (chcl) (44, 53). Loss of clathrin function in these strains results in the mislocalization of Kex2p and DPAP A to the plasma membrane. Guanosine diphosphatase (GDPase), another Golgi enzyme that appears to mark earlier compartments of the Golgi complex, is not mislocalized to the plasma membrane in the clathrin mutants (53). It is thought that binding of the cytoplasmic tails of Kex2p and DPAP A to clathrin and associated protein (AP-1) coat complexes either mediates direct retention in the late Golgi by a tethering mechanism, or the recycling of these proteins from either the endosome or the yeast equivalent of a conditional secretory granule. It does not appear that these proteins are recycled to the Golgi complex by endocytosis from the plasma membrane (reviewed in 64). Surprisingly, deletion of the Golgi localization signals of Kex1p, Kex2p and DPAP A result in the mislocalization of these proteins to the vacuole rather than the plasma membrane. In addition, overexpression of these proteins also leads to their partial mislocalization to the vacuole (10, 48, 63). These observations have led to the proposal that in yeast, the default (signal-independent) destination of integral membrane proteins is the vacuole, rather than the plasma membrane as is the case in mammalian cells (48).

Analysis of Golgi localization signals within two mammalian glycosyltransferases (34, 37) and the coronavirus E1 protein (58) has resulted in the unexpected finding that transmembrane domains in these proteins can target a reporter enzyme to the Golgi complex. The localization of these proteins is not easily saturable by overexpression, suggesting that specific interaction with a receptor may not be involved in the retention mechanism. Evidence exists to support two hypotheses for the mechanism of transmembrane domain mediated Golgi retention. One model suggests that the length of a transmembrane domain will specify Golgi localization by selectively associating with membranes of the appropriate thickness. The lipid and sterol composition of the Golgi membrane would determine the membrane thickness and would consequently specify the membrane proteins that are retained within each compartment (7). The second model suggests that Golgi resident membrane proteins form aggregates within the appropriate compartment resulting in a protein matrix that is too large to enter budding transport vesicles (kin recognition hypothesis, references 38, 45). Neither model would require trans-acting protein receptors such as the End2 protein which recycles HDEL containing ER proteins from the Golgi back to the ER (32, 54). Moreover, it would seem unlikely that clathrin would mediate the Golgi retention of these mammalian glycosyltransferases via a tethering or recycling mechanism, because the primary localization signal is within the transmembrane domain rather than the cytoplasmic tails of these proteins.

In this work, we provide the first detailed analysis of the synthesis, posttranslational modification, and sorting of a yeast mannosyltransferase. Our data indicate that Mnnlp is a type II integral membrane, resident protein of the yeast Golgi complex. The Golgi marker enzyme GDPase colocalizes with Mnnlp in sucrose density gradients, but Kex2p colocalizes with only a denser membrane fraction of Mnnlp and GDPase. We also present evidence that clathrin is required for efficient Golgi localization of the Mnnlp by a mechanism independent of the Mnnlp cytoplasmic tail.

Materials and Methods

Strains and Media

The yeast strains used were XCY42-30D (MATα ura3 leu2-3,112 trpl lys2 ade2-101 adeX suc2-A9), XYCY42-30D Δmnn1::LEU2 (MATTα ura3 leu2-3,112 trpl lys2 ade2-101 adeX suc2-A9 Δmnn1::LEU2 (this study)), SEY-6201 (MATTα ura3-52 leu2-3,112 his3-D200 trpl-D908 lys2-801 suc2-A9 [50]), TVY1 (SEY6210 Δpep4-1::LEU2 [57]) SEY5188 (MATTα sec7-1 leu2-3,112 ura3-52 suc2-A9 [16]), SF274 3A (MATTα sec7-4), SF294-2B (MATTα sec7-1), HMSF-1 (MATTα sec7-1) [Randy Schekman, University of California, Berkeley, CA], GY382 (MATTα ura3-52 leu2-3,112 his3-D200 trpl-dpp2::HIS3 chcl-chcl::LEU2 YEPCHIC02) GY385 (MATTα ura3-52 leu2-3,112 his3-D200 trpl-dpp2::HIS3 chcl-chcl::LEU2 YEPCHIC02), FYG1 (XCY42-30D/XCY42-30D p2V236), TGY32 (XCY42-30D/XCY42-30D), TGY33 (XCY42-30D) Δmnn1::LEU2/XCY42-30D Δmnn1::LEU2). Isogenic MATTα diploid strains were prepared by transforming the corresponding haploid strain with the HO gene carried on pHo-cl2 (51) to induce mating type switching and mating within transformed colonies. After restreaking transformants twice on selective plates, individual colonies were picked randomly and tested for the secretion of α or mating factors on lawns of supesensitive yeast. Diploid colonies that secreted neither mating factor were cured of pHo-cl2 to give TGY11-TGY35.

Standard rich (YPD) and synthetic minimal (SD) media for yeast was used (55). The SD medium was supplemented with 0.2% yeast extract (SDYE) and other supplements as needed for growing cells in liquid culture before labeling experiments. Standard rich medium for Escherichia coli (33) was used.

Reagents

Zymolyase-100T (Kirin Brewery Co.) was obtained from Seikagaku Kogyo Co. (Tokyo, Japan), endoglucosidase H (endo H) was from New England Nuclear Corp. (Boston, MA), DNA modifying enzymes were from New England Biolabs (Beverly, MA), proteinase K was from Boehringer Mannheim Biochemicals (Indianapolis, IN), Protein A-Sepharose and CNBr activated Sepharose 4B was from Pharmacia (Piscataway, NJ), Trans 5'S-label was from ICN radiochemicals (Irvin, CA), the ECL detection kit was from Amersham (Arlington Heights, IL) and 0.2-0.3 mm glass beads were from Glen Mills Inc. (Maywood, NJ). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Antiser to α-3 mannose linkages, alkaline phosphatase, GDPase, and Ochlp were from R. Schekman (UC Berkeley, Berkeley, CA), Gregome Payne (UCLA, Los Angeles, CA), Carlos Hirschberg (University of Massachusetts, Worcester, MA) and Yoshifumi Jigami (National Institute of Bioscience and Human
Plasmid Construction

To prepare pMNN1-XCT, a 1.4-kb BamHI-EcoRI fragment containing the 5' end of the MNN1 gene was subcloned from pVZ236 (Yelpl3 harboring a complementing 5.2-kb BamHI fragment of the MNN1 gene, V. L. MacKay, unpublished observation) to Mtl114B to produce pTI04. Site-directed oligonucleotide mutagenesis was performed on pTI04 as previously described (31) to delete the sequences encoding amino acids 2-18 of MNN1 and replace them with sequences derived from the cytoplasmic tail of dipetidyl peptidase IV (24) (gol10, see Fig. 9) to generate pTI04-XCT. The BamHI-EcoRI fragment from pTI04-XCT was used to replace that of pRGS24-MNN1 in TGY222A to generate pMNN1-XCT (gol, URA3) and pTG224 (gen, URA3) respectively. To prepare pRGS24-MNN1, pRS246 (56) was digested with EcoRI; the ends were filled in using T4 DNA polymerase and the plasmid was re-circularized to generate pRS426R1. A 3.7-kb BamHI-BglII complementing fragment of the MNN1 gene was subcloned from pVZ236 into the BamHI site of pRS426R1 to generate pRS426-MNN1. TGY222A was constructed similarly to pRS426-MNN1 except pHYC18 (20) was the starting plasmid. pATH2-MNN1 was prepared by subcloning a 1.19-kb HindIII fragment of MNN1 (predicted to encode amino acids 118-514) from pVZ236 into the HindIII site of pATH2 (30) such that the reading frame was maintained between trpE and the MNN1 fragment.

Immunological Techniques

To prepare a bacterially expressed Mnnlp antigen, E. coli harboring pATHT2-MNN1 were induced to synthesize the trpE-Mnnlp fusion protein as described by Kjeld et al. (27) and the fusion protein was purified as described by Kleid et al. (27) and the fusion protein was emulsified with Freund's complete adjuvant and injected intramuscularly into a male New Zealand white rabbit. The rabbit was boosted subcutaneously every three weeks with 0.05 mg of fusion protein in Freund's incomplete adjuvant. Bleeds were collected one week after each boost. Affinity purified anti-Mnnlp antibodies were prepared by chromatography on a trpE-Mnnlp column. Approximately 0.8 mg of trpE-Mnnlp was coupled to CNBr activated Sepharose 4B following the manufacturer's protocol. 3 ml of anti-Mnnlp antisera were diluted: 1:1 with buffer A as described by Redding et al. (47) and circulated through the fusion protein column for 3 h. The column was washed as previously described (47), and eluted with 0.1 M glycine-HCl, pH 2.75. The eluate fractions were neutralized by the addition of 0.1 vol of Tris-HCl, pH 9.4, and tested against dot blots of the fusion protein to identify antibody containing fractions. These fractions were pooled and stored in aliquots at −75°C.

Indirect immunofluorescence analysis was done as described by Redding et al. (47) with the following modifications. Fixed spheroplasts were permeabilized by incubation in SHA buffer (1 M sorbitol, 0.1 M Na Hepes pH 7.5, 5 mM Na Azide) plus 1% SDS for 10 min at room temperature. Fixed, permeabilized cells were incubated for 3-4 h at room temperature with a 1:50 dilution of affinity purified anti-Mnnlp antibodies and bound primary antibody was detected using FITC conjugated goat anti-rabbit secondary antibodies (1:1,000 dilution). Cells were observed using a Zeiss Axio phot microscope with a 100x objective and photographed with Tmax 400 film.

For labeling experiments, yeast cells were grown to mid-logarithmic phase in SD-YE, then washed twice in water and resuspended in SD media with appropriate supplements at 5 OD600/ml. To initiate labeling, Tran35S label was added to a final concentration of 150 uCi/ml and labeling was quenched by adding a 50x chase solution (50 mM methionine, 10 mM cysteine and 5% yeast extract) to a 1x concentration. The chase was subsequently terminated by adding TCA to a final concentration of 10%. Processing of TCA pellets for immunoprecipitation and size fractionation of Mnnlp in 7% SDS-polyacrylamide gels were done as previously described (17). For endo H treatment of immunoprecipitates, washed protein A-Sepharose immune pellets were dried, then resuspended in 64 μl of 1% β-mercaptoethanol, 0.2% SDS and heated at 95°C for 4 min. 16 μl of 250 mM Na citrate buffer, pH 5.5, was added to each tube, then 0.5 μl of endo H was added. The samples were incubated for 3.5 h at 37°C. The reactions were stopped by the addition of 4x Laemmli sample buffer and heating to 95°C for 5 min. For the experiment shown in Fig. 4, a Molecular Dynamics PhosphorImager was used to quantitate the amount of 35S present within defined areas of the polyacrylamide gel.
Figure 1. Synthesis and posttranslational modification of Mnnlp.
(A) Strains XCY42-30D Δmnl (Δ), XCY42-30D (WT) and XCY42-30D pZV236 (2µ) were labeled for 10 min at 30°C as described in Materials and Methods and immunoprecipitations were performed using anti-Mnnlp (a-Mnnlp) or preimmune serum with anti-alkaline phosphatase (a-ALP) as indicated above each lane. pZV236 is a multicopy, 2µ plasmid harboring the MNN1 gene. Lane 3 was loaded with 1/10 the amount of sample as that in lanes 1, 2 and 4. (B) Strain XCY42-30D (wild type) was labeled for 10 min at 30°C, then chased for 0, 1.5, or 3 h (lanes 1–3) and processed for coimmunoprecipitation with antisera to Mnnlp and alkaline phosphatase (ALP). (C) Strain XCY42-30D (wild type) was labeled for 10 min at 30°C and chased for the times indicated. Labeled Mnnlp was recovered from each sample by immunoprecipitation and was eluted from the primary antibody by boiling in 1% SDS. The samples were split in half and reimmunoprecipitated with α-Mnnlp and antisera specific to α,3 linked mannose epitopes. Bands on the autoradiograms were quantitated by densitometry and the amount of Mnnlp recovered in the anti-α,3 linked mannose immunoprecipitate was divided by the amount of Mnnlp recovered in the anti-Mnnlp immunoprecipitate and expressed as the % of Mnnlp with α,3 mannose epitopes.

Two lines of evidence argue that Mnnlp is the Saccharomyces cerevisiae α,3 mannosyltransferase. First, the level of α,3 mannosyltransferase activity in yeast membrane preparations corresponds to dosage of the MNN1 gene. A strain bearing a Δmnl null allele lacks any measurable α,3 mannosyltransferase activity and a strain that overexpresses Mnnlp exhibits ~10-fold higher level of α,3 mannosyltransferase activity as compared to wild-type extracts. Second, native immunoprecipitations using the anti-Mnlp antiserum described in this report, precipitate α,1 mannosyltransferase activity from detergent solubilized membrane preparations (reference 18, preliminary report, Verostek, M. F., T. R. Graham, and R. B. Trimble, manuscript in preparation).

The late Golgi enzymes Kex1p and Kex2p are subject to a slow posttranslational modification that results in a gradual increase in apparent molecular mass of these proteins (9, 62). As shown in Fig. 1 B, Mnnlp also exhibited a slow, incremental increase in molecular mass during a pulse/chase analysis. The 98.5-kD Mnnlp had increased in relative molecular mass to 102.5 kDa at 1.5 h and 106 kDa at 3 h of chase (Fig. 1 B, lanes 1–3). This is in contrast to ALP for which the 76-kDa precursor undergoes a rapid PEP4-dependent proteolytic processing step to produce the 72-kDa mature form (29), but does not increase in molecular mass thereafter (Fig. 1 B, lanes 1–3). In other experiments in which more time points were taken, we found that the gradual increase in mass of the Mnnlp was linear over a 3-h chase with a slope of ~2.5 kDa per hour at 30°C. To test for the presence of α,3 linked mannose on Mnnlp, we subjected this protein to a second immunoprecipitation using an antiserum specific to α,3 linked mannose epitopes. Wild-type cells were labeled for 10 min and chased for the times indicated in Fig. 1 C. Mnnlp was recovered from the cells by immunoprecipitation and then was eluted from the primary antibody by boiling in 1% SDS. The samples were split in half and immunoprecipitated a second time with either anti-Mnnlp antiserum, or the linkage-specific antiserum. The amount of Mnnlp recovered in each pair of immunoprecipitates were compared over time. We found that the percentage of Mnnlp that could be precipitated with the linkage-specific antiserum also increased gradually with increasing time after synthesis (Fig. 1 C). The correlation of the slow rate of α,3 mannose epi-
topo acquisition with the slow rate of change in molecular mass suggests that self-modification of Mnnlp (auto-mannosylation) is at least partly responsible for the observed increase in molecular mass.

To determine if the auto-mannosylation of Mnnlp was occurring on O- or N-linked oligosaccharides, we treated Mnnlp with endo H which specifically cleaves N-linked oligosaccharides. A sec18 strain was labeled and chased for 0 and 3 h at the permissive temperature, then Mnnlp was recovered from cell lysates by immunoprecipitation and half of each sample was treated with endo H (Fig. 2, lanes 1–4). At 24°C, Mnnlp increased in apparent molecular mass from 98.5 kD (0 h, lane 1) to 104 kD (3 h, lane 2). If this post-translational increase in molecular mass of Mnnlp was due to elaboration of N-linked oligosaccharides, then both forms of the enzyme should be converted to the same molecular mass after endo H treatment. This was not the case. Endo H treatment converted the 98.5-kD form to 92.5 kD (lanes 1 and 3) and the 104-kD form to 98 kD (lanes 2 and 4). The endo H treated Mnnlp from the 0 and 3 h chase times still differed by 5.5 kD (compare lane 3 with lane 4), as did the untreated 0 and 3 h forms. The molecular mass of both forms decreased by ~6 kD, which suggests that three of the four predicted N-linked glycosylation sites are used in vivo. After removal of N-linked oligosaccharides by endo H treatment, Mnnlp from the 3 h chase point was still precipitable with the α1,3 linkage specific antiserum (although not as efficiently as the untreated protein) indicating the presence of O-linked oligosaccharides on Mnnlp (data not shown).

O-linked glycosylation is thought to be initiated in the ER by the addition of a single mannose residue to Ser and Thr residues which are then extended in the Golgi complex with α1,2 and α1,3 linked mannose (22). To determine if the post-translational increase in molecular mass of Mnnlp required protein transport to the Golgi complex, Mnnlp was also immunoprecipitated from sec18 cells labeled at a restrictive temperature (34°C) where ER to Golgi transport is blocked. Under these conditions, Mnnlp failed to increase in molecular mass during the chase period, demonstrating that protein transport out of the ER is required for this posttranslational modification (Fig. 2, lanes 5–8). These data indicate that the slow posttranslational increase in molecular mass of Mnnlp is primarily due to auto-mannosylation of O-linked oligosaccharides in a post-ER compartment, presumably the Golgi complex. The slow increase in molecular mass of Kex2p has also been shown to result from modification of O-linked oligosaccharides on Kex2p, and the extent of this modification on Kex2p was decreased in a mnnl mutant (62).

**Membrane Association of Mnnlp**

The sequence of the MNN1 gene predicts a 762-amino acid protein with a single NH2-terminal hydrophobic domain encompassing amino acids 19–37 (66). This signal sequence is predicted to be uncleaved by signal peptidase based on the empirical rules of von Heijne (61), suggesting a dual role for the transmembrane domain as a signal sequence and membrane anchor which would result in the protein adopting a type II integral membrane topology. We addressed the membrane association and topology of Mnnlp by extraction of intracellular membranes with alkaline carbonate buffer and Triton X-100, and by a protease protection assay. Wild-type cells were labeled for 10 min, then converted to spheroplasts and lysed by osmotic shock. The lysate was subjected to centrifugation at 100,000 g to pellet membranes and one fifth of the pellet (P) and supernatant (S) fractions were immediately TCA precipitated (Fig. 3, lanes 2 and 3). The remaining membrane pellet was divided into four equal portions which were then treated with 0.1 M Na carbonate, pH 11.0 (lanes 4 and 5), 1% Triton X-100 (lanes 6 and 7), proteinase K (lane 8), or proteinase K plus 1% Triton X-100 (lane 9). The carbonate and Triton X-100 treated samples were centrifuged again at 100,000 g to produce pellet and supernatant fractions and the proteinase K treated samples were stopped by the addition of PMSF and TCA as described in Materials and Methods. Mnnlp, carboxypeptidase Y and glucose-6-phosphate dehydrogenase were recovered from each sample by sequential immunoprecipitation and subjected to SDSPAGE. Nearly all the Mnnlp fractionated with a crude membrane high speed pellet (Fig. 3, lanes 2 and 3), while 95% of a cytoplasmic enzyme, glucose-6-phosphate dehydrogenase, remained in the supernatant (data not shown). Mnnlp sedimented in the membrane pellet after treatment of the membranes with high pH carbonate buffer (lanes 4 and 5), but was extracted from the membrane into the supernatant fraction with the detergent Triton X-100 (lanes 6 and 7). Precursors of carboxypeptidase Y that were present in the ER and Golgi were completely extracted from the membranes by the carbonate buffer, indicating that the carbonate had effectively removed soluble components from the lumen of subcellular compartments (lanes 4 and 5) as expected (14). These data indicate that Mnnlp is membrane associated and suggests that the NH2-terminal signal sequence was not cleaved. In addition, a large fragment of Mnnlp was protected from degradation by proteinase K added to the cytoplasmic face of the membranes (Fig. 3, lane 8). A small decrease in molecular mass of Mnnlp was observed in the proteinase K treated sample, indicating a portion of Mnnlp crossed the membrane and was accessible to the cytoplasm. This molecular mass shift (~1 kD) is consistent with the removal of the exposed NH2-terminal cytoplasmic tail. In the presence of proteinase K and Triton X-100, Mnnlp was completely degraded (lane 9) showing that an intact lipid bilayer was required for protection from the protease, and that this protein is not inherently protease resistant. These results, together with the hydrophathy analysis of the MNN1 gene predicts a 762-amino acid protein with a single NH2-terminal hydrophobic domain encompassing amino acids 19–37 (66).
In order to assess the subcellular compartment in which the functional modification of Mnnlp is catalyzed, we analyzed the extent to which Mnnlp was subcellularly processed after 30 min before labeling to inactivate the Mnnlp. The complete block in Mnnlp modification exhibited in wild-type cells, and to an accumulation of Kex2p in the vacuole by the sec2 mutant, strongly suggest that the slow modification of Mnnlp was catalyzed within the Golgi complex. Taken together, these data suggest that Mnnlp is a resident of the yeast Golgi where the carboxymannosylation with αl,3 mannose is catalyzed over a prolonged period of time.

**Turnover of Overexpressed Mnnlp**

Kex2p has a half-life of ~80 min and is turned over in the vacuole by PEP4-dependent proteases. Overexpression of Kex2p leads to a two- to threefold increased rate of degradation in wild-type cells, and to an accumulation of Kex2p in the vacuole of pep4 mutant cells (63) which are pleiotropically deficient in the activities of the major vacuolar proteases (67). Kex1p and DPAP A also accumulate in the vacuole upon overexpression in pep4 cells (10, 48). We tested if overexpression would lead to mislocalization of Mnnlp and degradation in the vacuole. Wild-type cells with or without MNN1 overexpression would lead to mislocalization of Mnnlp and degradation in the vacuole. Wild-type cells with or without MNN1 on a multicopy plasmid were labeled and chased for 0, 1.5, or 3 h and then Mnnlp and ALP were coimmunoprecipitated. The level of overexpression from the multicopy plasmid ranged from 35–50-fold relative to Mnnlp expressed from a single copy gene in these experiments (see Fig. 1A). While Mnnlp expressed from the genomic MNN1 gene was very stable (Fig. 1B and Fig. 5, MNN1), overexpression led to a significant increase in the rate of degradation of this protein such that after three hours of chase ~80% of the labeled Mnnlp had been degraded (Fig. 5, 2μ MNN1). Even with the increased rate of degradation, there was still an 8–10-fold higher level of Mnnlp in the overexpressing strain relative to wild-type at the three hour time point. To assess the role of vacuolar proteases in degradation of overexpressed Mnnlp, the pulse chase analysis was also done in an isogenic pep4 strain. The degradation rate of overexpressed Mnnlp was nearly identical in the Pep4+ and Pep4− strains (Fig. 5, 2μ MNN1 vs. 2μ MNN1 pep4). Although the PEP4-dependent vacuolar proteases were not involved in the

**Compartmental Site of Mnnlp Modification**

In order to assess the subcellular compartment in which the functional modification of Mnnlp is catalyzed, we analyzed the extent to which Mnnlp was subject to this modification in sec mutants that block protein transport at various stages of the secretory pathway (41). The sec mutants indicated in Fig. 4 were preincubated at 37°C for 30 min before labeling to inactivate the sec gene products. The sec12 mutant exhibits a block in ER to Golgi protein transport and would be expected to accumulate newly synthesized Mnnlp in the lumen of the ER (26). As expected, this mutant exhibited a complete block in the modification of Mnnlp (Fig. 4, sec12). The sec7 mutant exhibits a partial block in ER to Golgi protein transport as well as protein transport steps between Golgi cisternae (13). This mutant exhibited a nearly complete block in the modification of Mnnlp. The sec14 mutant harbors a defective phosphatidylinositol/phosphatidylcholine exchange factor which results in an altered phospholipid composition of the Golgi at 37°C and an inability to form transport vesicles from the late Golgi complex (4). Although not as defective as the sec12 or sec7 mutants, the sec14 mutant also exhibited a partial block in modification of Mnnlp. The sec1 mutant, which exhibits a block in secretory vesicle fusion with the plasma membrane, did not exhibit a defect in the modification of Mnnlp. The complete block in Mnnlp modification exhibited by the sec12 and sec18 mutants (Figs. 2 and 4) demonstrate a requirement for transport of Mnnlp to the Golgi complex for this modification to occur. The partial inhibition of this

Figure 3. Membrane association of Mnnlp. Strain XCY42-30D was labeled for 15 min, then the cells were converted to spheroplasts and lysed by osmotic shock. A portion of the lysate was TCA precipitated (lane 1) and the remainder was subjected to centrifugation at 100,000 g to generate a pellet (P) and supernatant (S) fraction. One fifth of the pellet and supernatant fractions were TCA precipitated and processed for immunoprecipitation (lanes 2 and 3), then equal portions of the remainder of the pellet were extracted with Na carbonate, pH 11.0 (lanes 4 and 5), Triton X-100 (lanes 6 and 7), or treated with protease K (lane 8) or protease K and Triton X-100 (lane 9) as described in Materials and Methods. The Mnnlp immunoprecipitates are shown, the other immunoprecipitates were quantitated and expressed as the % total recovered in each pair of fractions (pellet and supernatant).

Figure 4. Slow modification of Mnnlp in sec mutants. Strains harboring the sec mutation indicated above each pair of lanes were preincubated for 30 min at 37°C, then labeled for 10 min and chased for 0 or 1.5 h at 37°C. Mnnlp was recovered from each sample by immunoprecipitation and size fractionated by SDS-PAGE. The strains used are listed in Materials and Methods, and the stage of the secretory pathway where each mutant exhibits a block in protein transport is shown.
Figure 5. Turnover of overexpressed Mnnlp in isogenic Pep4+ and Pep4− cells. Strains SEY6210 (MNN1), SEY6210 pZV236 (2μ MNN1) and TYY1 pZV236 (2μ MNN1 pep4) were labeled, chased, and subjected to immunoprecipitation as in Fig. 1. Bands on the autoradiograms were quantitated by densitometry as described in Materials and Methods and the recovery of Mnnlp was normalized to the recovery of ALP in the coimmunoprecipitates. ALP is subject to PEP4-dependent proteolytic processing and so the mobility of ALP in the gel also served as an internal control to confirm the genotype of the strains being used. The amount of Mnnlp in the immunoprecipitate at each time point was divided by the amount present at the 0 h chase point for each strain and expressed as the % initial Mnnlp.

Figure 6. Subcellular localization of Mnnlp by indirect immunofluorescence gives a punctate staining pattern typical of the yeast Golgi complex. Immunofluorescent staining was done as described in Materials and Methods using affinity purified antibodies to Mrm1p (α-Mnnlp). The top panels (2μ MNN1) are diploid cells (TGY31) harboring MNN1 on a multicopy plasmid. The bottom panels (Δmnnl) are diploid Δmnnl cells (TGY33). The indented structures seen in the Nomarski images are vacuoles and the arrows indicate the position of the nucleus as determined by costaining of the samples with DAPI.
the p100 fraction as compared to the lysate (data not shown). Most of the ER, nuclei, vacuole membrane and plasma membrane are sedimented at 13,000 g (21, 25). The Golgi membranes collected on the sucrose cushion following the 100,000 g centrifugation step were loaded on top of a sucrose step gradient and centrifuged to equilibrium. We found three peaks of Mnnlp that migrated with sucrose densities of 1.11, 1.14, and 1.18 g/ml, respectively. GDPase exhibited the same distribution in these gradients, but membranes containing the Kex2 endopeptidase cofractionated with only the denser peaks of Mnnlp and GDPase (Fig. 7). These data suggest that the Mnnlp and GDPase are localized to at least two physically distinct compartments and that these enzymes may reside within the same, or at least very similar Golgi compartments. This is not surprising as α,3 mannosyltransferase activity is linked to GDPase function; GDPase is required to hydrolyze the GDP product of mannosyltransferase reactions to GMP which is thought to be exchanged for GDP-mannose by a specific cotransporter in the Golgi membrane. Strains harboring a null allele of the GDPase gene exhibit a defect in glycosylation (1) as well as GDP-mannose transport into Golgi vesicles (5). The two peaks in the more dense part of the gradient were not significantly different in the distribution of the three enzymes that we tested, so it is not clear if these peaks represent two distinct compartments or fragmented portions of the same compartment. We have also examined the distribution of Ochlp (an α,6 mannosyltransferase, reference 36) in these gradients and found that this protein was also present in the same membrane fractions as Mnnlp, but was somewhat more enriched in the lighter fraction (data not shown). These results suggest that the lighter membrane fraction that contained Mnnlp, GDPase and Ochlp, but lacked Kex2p, corresponds to the early Golgi compartments (cis and medial). From these experiments it appears that the late Golgi compartment that contains Kex2p may also contain a significant fraction of Mnnlp and GDPase.

Clathrin-dependent Localization of Mnnlp

The absence of clathrin function in a strain bearing a disruption of the clathrin heavy chain gene results in the mislocalization of Kex2 and DPAP A to the plasma membrane (44, 53). GDPase remains intracellular in the mutant cells suggesting that this protein is retained in the Golgi by a mechanism distinct from that employed by Kex2p and DPAP A (53). To test if Mnnlp is mislocalized to the plasma membrane when clathrin function is lost, a temperature-sensitive chcl

mutant (chcl-ts) and an isogenic wild-type strain (CHCI) were shifted to 37°C for 0, 30, or 60 min, then subjected to cell surface iodination (Fig. 8 C) as previously described (52). Half of the cells treated at each temperature were lysed

Figure 7. Subcellular localization of Mnnlp by sucrose gradient fractionation. An enriched Golgi membrane fraction (p100) was prepared from strain XCY42-30 by differential centrifugation, and was subjected to fractionation in a sucrose gradient as described in Materials and Methods. The relative amount of Mnnlp in each fraction was determined by Western blot and densitometry, and the concentration of Kex2, GDPase and protein in each fraction was determined by specific assays as described in Materials and Methods. The density of sucrose in the peak fractions 6, 12 and 14 were 1.11, 1.14, and 1.18 g/ml, respectively. The specific activity of GDPase in fractions 6 and 12 was enriched 25- and 50-fold, respectively, as compared to the initial cell lysate.
(Fig. 8L) before iodination to provide an estimate of the total amount of Mnnlp present in these cells. After iodination, Mnnlp was recovered from the cells by immunoprecipitation and analyzed by SDS-PAGE (Fig. 8). In addition, a portion of each sample was also subject to immunoprecipitation with antiserum to GDPase to control for the integrity of the intact cells. We could not detect any Mnnlp on the cell surface of chcl-ts cells grown at the permissive temperature (Fig. 8, chc-ts 0°) or of wild-type cells incubated at 37°C for 1 h (Fig. 8, CHe 60). Yet, we found that Mnnlp appeared on the cell surface by 30 min after temperature shift and ~40% of the Mnnlp was mislocalized to the plasma membrane of the clathrin mutant after 60 min at 37°C. As previously reported (53), GDPase did not mislocalize to the cell surface in these experiments under any of the conditions tested (Fig. 8, GDPase). In other experiments, as much as 80% of Mnnlp was mislocalized to the cell surface of the chcl-ts strain after 2 h at the nonpermissive temperature. The kinetics and extent of mislocalization of Mnnlp in the clathrin mutant is very similar to that previously described for Kex2p (44). In addition, Mnnlp was also mislocalized to the plasma membrane of a Δchcl strain to a similar extent as seen in the chcl-ts strain (data not shown).

Both Kex2p and DPAP A have large cytoplasmic tails with Golgi localization signals roughly defined as a (Y/F)X(Y/F)-XX(I/L) motif that is similar to clathrin coated pit localization signals found within the cytoplasmic tails of mammalian plasma membrane receptor proteins (reviewed in reference 40). Mutations in the cytoplasmic tails of Kex2p and DPAP A result in the mislocalization of these proteins to the vacuole (40). The Mnnlp cytoplasmic tail does not have an amino acid sequence that fits this motif well, although there is a FIL sequence that could possibly serve as this type of Golgi localization signal and mediate the clathrin-dependent Golgi localization of Mnnlp (Fig. 9 A). If this is the case, then removal of the Mnnlp cytoplasmic tail should result in the mislocalization of this protein to either the cell surface or vacuole. In order to maintain the type II topology of a Mnnlp cytoplasmic tail mutant, we exchanged the sequences that code for the Mnnlp 18-amino acid cytoplasmic tail for the six-amino acid cytoplasmic tail of dipeptidyl peptidase IV (gpl10, reference 24), a rat plasma membrane protein with the same topology as the Mnnlp, to produce pMNN1-XCT (Fig. 9 A). Other than the required initiator methionine, a threonine residue is the only common amino acid between these two cytoplasmic tails, and there are no tyrosines or phenylalanines in the dipeptidyl peptidase IV cytoplasmic tail.

As shown in Fig. 9 B by immunofluorescence, the Golgi retention of Mnnlp is not mediated through its cytoplasmic tail. Cells overexpressing this protein bearing the heterologous cytoplasmic tail exhibited a typical Golgi staining pattern (Fig. 9 B). We also found that the MNN1-XCT allele complemented a Δmnnl null allele when expressed from a single copy plasmid (data not shown). Moreover, the Mnnl-XCT protein exhibited wild-type stability and slow posttranslational modification in pulse-chase experiments. We could find no differences in the activity, localization, stability or modification of the Mnnlp cytoplasmic tail mutant. These data argue that the Golgi localization of Mnnlp is not mediated by a direct interaction of clathrin/AP-1 complexes with the Mnnlp cytoplasmic tail.

Discussion

The Golgi complex plays a central role in the transport, modification and sorting of proteins in the secretory pathway. Towards a better understanding of the organization of this multicompartment organelle and the mechanisms used to localize the intrinsic proteins of the Golgi complex, we have initiated an analysis of the yeast αl,3 mannosyltransferase. This work represents the first detailed characterization of the biosynthesis, modification and localization of a yeast mannosyltransferase. The predicted amino acid sequence of Mnnlp suggested that the protein would be a type II integral membrane protein (66). Consistent with this prediction, Mnnlp is resistant to extraction from membranes with alkaline carbonate buffer, but is readily extracted from the membrane with the detergent Triton X-100 indicating that it is tightly associated with the membrane. Proteinase K treatment of an intact membrane fraction indicates that the bulk of the Mnnlp is in the lumen of the Golgi, but also that this protein traverses the membrane such that a small cytoplasmic tail is susceptible to exogenously added protease (Fig. 4). These data indicate that the Mnnlp is an integral membrane protein, and combined with the hydrophathy analysis that shows a single NH2-terminal hydrophobic domain, suggests that this protein adopts a type II integral membrane topology.

Several lines of evidence demonstrate that Mnnlp is localized to the Golgi complex. (a) It has been shown previously that the modification catalyzed by the Mnnlp (αl,3 mannos addition to N- and O-linked oligosaccharides) is restricted
to the Golgi complex (12, 16). (b) Analysis of the slow auto-
mannosylation of Mnnlp in sec mutants indicates that it is
catalyzed within the Golgi complex, suggesting that the
primary site of residence of the Mnnlp is the Golgi complex
(Fig. 3). (c) In situ detection of Mnnlp by immunofluores-
cence reveals a punctate staining pattern typical of the yeast
Golgi complex (Fig. 6). (d) Mnnlp colocalizes with other
Golgi enzymes through subcellular fractionation of mem-
branes by differential centrifugation and sucrose gradient
fractionation (Fig. 7). Subcellular fractionation of Golgi
membranes in sucrose gradients suggests that Mnnlp is
localized to at least two discrete compartments of the
Golgi complex. The distribution of marker proteins in these
sucrose gradients suggests that the lighter fraction represents
the early Golgi compartments (cis and medial) and the
denser peaks the late, or trans Golgi.

A remarkable aspect of the Mnnlp biosynthesis is the slow,
incremental increase in molecular mass of this glycoprotein.
Newly synthesized Mnnlp has a molecular mass of 98.5 kD,
but the protein slowly increases in apparent molecular mass
to 106 kD over a 3-h period of time. We have shown that the
slow increase in molecular mass of Mnnlp is at least partly
due to a slow acquisition of α1,3 mannose residues (auto-
mannosylation), primarily on endo H resistant O-linked
oligosaccharides. Mnnlp could not auto-mannosylate when
accumulated in the ER of a sec12 or sec18 mutant at the non-
permissive temperature, indicating that transport to the
Golgi complex is required for this modification. In addition,
the kinetics of this modification is unaffected when protein
transport from the Golgi to the cell surface is blocked in a
sec1 mutant. These data suggest that transport to the cell
surface and recycling by endocytosis is not part of the normal
itinerary of this protein.

The slow acquisition of α1,3 mannose epitopes on Mnnlp
is surprising, as other yeast glycoproteins such as carboxy-
peptidase Y, proteinase A, invertase, and α-factor, can be
quantitatively immunoprecipitated with the anti-α1,3 man-
nose linkage-specific antiserum within 5–15 min after syn-
thesis (references 16, 28 and unpublished observations). For
this group of soluble proteins, the α1,3 mannose epitope is
added to N-linked oligosaccharides. It is not clear why the
N-linked oligosaccharides of Mnnlp fail to be rapidly mod-
ified with α1,3 mannose residues, although it is possible that
Mnnlp exists in a protein complex that hinders access to its
own N-linked oligosaccharides. We presume that O-linked
α1,3 mannose residues may be weakly recognized by the
linkage-specific antiserum, such that the accumulation of
many of these epitopes are required for efficient immunopre-
cipitation with the linkage specific antiserum.

We can suggest three models to explain the slow kinetics
of Mnnlp posttranslational modification. (a) The α1,3 man-
nosylation of O-linked oligosaccharides is inherently a slow
reaction; therefore, the slow increase in mass of Mnnlp
would be a measure of the mannosyltransferase's enzyme ki-
etics towards O-linked substrates. (b) Aggregation of Golgi
proteins into complexes could sterically hinder the accessi-
bility of Mnnlp to its own oligosaccharides. (c) The Mnnlp
is continuously trafficking through a distinct Golgi compart-
ment that has the appropriate concentration of substrates
(Mnnlp and GDP-mannose) and effectors of enzyme activity
(ions, pH, inhibitors or activators) to allow this modification
to occur. Consistent with this latter model, the Mnnlp ap-
ppears to be localized to at least two distinct Golgi compart-
ments, and may require recycling from the Kex2 compart-
ment into the earlier compartment for auto-mannosylation.
An α1,3 mannose residue is normally added onto an α1,2
linked mannose to extend the O-linked oligosaccharide
chain. It is also possible that the addition of α1,2 mannose
is limiting, and retrograde trafficking of Mnnlp into earlier
Golgi compartments is required to form the appropriate sub-
strate for auto-mannosylation.

We found that Mnnlp was very stable when expressed
from a single copy gene, but showed a dramatic increase in
the rate of degradation when overexpressed from a multicopy
plasmid (Fig. 5). The rate of degradation of overexpressed
Mnnlp was nearly the same in isogenic Pep4+ and Pep4−
strains. The pep4 strain is pleiotropically deficient in the
activities of the major vacuolar proteases; therefore, it is
unlikely that the degradation of overexpressed Mnnlp was
the result of mislocalization to the vacuole and degradation in
this organelle. Moreover, immunofluorescence data indi-
ated that the Mnnlp is not localized to the vacuole in wild-
type or pep4 cells overexpressing this protein. At this time,
we do not know where the degradation of overexpressed
Mnnlp is occurring within the cell, but analysis of this event
in sec mutants should define more precisely the organelle
where the degradation is catalyzed.

Western blot analysis of Mnnlp suggested that the steady-
state level of overexpressed Mnnlp in these strains is 10–
15-fold higher than wild-type strains (data not shown).
Therefore, the mechanism used to retain Mnnlp in the Golgi
complex is apparently not saturated by a 10–15-fold increase
in the amount of this protein. These results are in contrast
to the findings that a significant fraction of Kex1p, Kex2p and
DPAP A all mislocalize to the vacuole when overexpressed,
where they are subsequently degraded in a PEP4-dependent
manner (10, 49, 63). This result is more similar to mali-
nalian glycosyltransferases, which upon overexpression are
predominantly Golgi localized and do not appear to mis-
localize to the plasma membrane or lysosome, although ER
accumulation was sometimes observed (38). Overexpression
of Mnnlp does not significantly affect the turnover of Kex2p,
nor does overexpression of Kex2 affect the turnover of Mnnlp
(Chen and Graham, unpublished data). These data suggest
that overexpression of Mnnlp does not lead to general turn-
over of Golgi membrane, and that Kex2p and Mnnlp are not
competing for a limiting component of a common retention
apparatus.

We have found that 40–80% of Mnnlp was mislocalized
to the cell surface in a temperature sensitive chcl-ts strain incu-
bated at the nonpermissive temperature for 1–2 h (Fig. 8).
Clathrin is also required for the efficient retention of Kex2p
and DPAP A in the yeast Golgi complex (44, 53). It has been
suggested that clathrin mediates the retention of Kex2p and
DPAP A by a direct association of clathrin coats with the cy-
toplasmic tails of these Golgi proteins (64). The cytoplasmic
tail of Mnnlp is very short (18 amino acids) relative to Kex2p
(115 amino acids) or DPAP A (118 amino acids). Although
there is a single aromatic amino acid in the cytoplasmic tail
of Mnnlp (F7), it does not appear to be in the appropriate
sequence context to fit the Golgi localization motif described
for Kex2 and DPAP A (40). The cytoplasmic tail of Mnnlp
is more similar to that of GDPase (1), which is predicted to
have a nine–amino acid NH2-terminal cytoplasmic tail with
two aromatic residues (F5 and Y8). Moreover, Mnnlp cofractionated with GDPase in sucrose gradients which suggests that these two proteins are localized to the same Golgi compartments. However, GDPase is not mislocalized to the plasma membrane in the clathrin mutant (cells (Fig. 8, reference 53), and defects in outer chain mannosylation has not been observed in clathrin mutants (44)). Therefore, we were surprised to find that a significant fraction of Mnnlp was mislocalized to the plasma membrane of clathrin disrupted cells, and that the extent of Mnnlp mislocalization in this mutant was very similar to what had previously been shown for Kex2p (44).

The finding that clathrin is required for Golgi localization of Mnnlp suggested that the Mnnlp cytoplasmic tail may contain a localization signal that mediates this retention, analogous to the signals within the Kex2p and DPAP A cytoplasmic tails. To test this, we replaced the Mnnlp cytoplasmic tail with that of dipetidyl peptidase IV, a mammalian plasma membrane protein with a six-amino acid tail containing no tyrosine or phenylalanine residues (24). The mutant Mnnl-xct protein was expressed in a Δmnnl strain and was found to be localized normally to the Golgi complex (Fig. 9 B). If clathrin mediates the retention of the Mnnlp in the Golgi by a direct interaction with the cytoplasmic tail, then deletion of the tail should result in the mislocalization of the Mnnlp to either the plasma membrane, as occurs in the clathrin mutant, or to the vacuole as occurs with cytoplasmic tail mutants of Kex2p and DPAP A. These results suggest that the clathrin requirement for Mnnlp Golgi localization is not mediated by a direct interaction of clathrin coats with the cytoplasmic tail of this protein. Moreover, this data argues for a different clathrin-dependent mechanism for the Golgi localization of the Mnnlp than that employed by Kex2p and DPAP A.

There are three models we can suggest to explain how clathrin is required for Mnnlp Golgi localization. (a) The entire Kex2p compartment may be lost to the plasma membrane upon disruption of clathrin function. Therefore, any protein contained in the Kex2p compartment would be mislocalized to the plasma membrane in the clathrin mutant. (b) Localization of the Mnnlp to the Golgi complex is mediated by a protein(s) that directly requires clathrin for Golgi localization by an interaction of the cytoplasmic tail(s) of this hypothetical protein with clathrin coat complexes. (c) Proteins required to maintain the appropriate balance of lipid and lumenal constituents of the late Golgi are mislocalized in the clathrin mutant causing significant changes in the physical characteristics of this compartment and possibly earlier compartments as well. These changes would then indirectly disrupt the mechanisms used to localize the Mnnlp. The first model seems least likely because our data suggests that a fraction of GDPase was also contained within the Kex2p compartment, but was not mislocalized to the plasma membrane in the clathrin mutant. In addition, protein transport through the secretory pathway is relatively unaffected in the clathrin mutants which would be surprising if an entire Golgi compartment was lost. Others have reported a greater extent of separation of GDPase and Kex2 in sucrose gradients (6, 8), but we have found a peak of GDPase activity that cofractionated with Kex2p in each of 15 sucrose gradient fractionation experiments. Models 2 and 3 are difficult to discriminate at present; however, we have recently found that a fusion protein containing only the transmembrane domain of the Mnnlp can function to localize a reporter enzyme to the yeast Golgi complex (manuscript in preparation). Therefore, the mechanism for Golgi localization of Mnnlp might be similar to that used by several mammalian glycosyltransferases (see introduction). Current models used to explain the transmembrane domain-mediated retention of Golgi enzymes do not implicate specific interactions with a receptor, or tethering protein, but do require specific local environments of the membrane or compartment. Clathrin may be required for these proposed retention mechanisms by localizing enzymes required to maintain the appropriate lipid or lumenal environment of the late Golgi (model 3). It is also possible that Mnnlp forms transmembrane domain mediated aggregates with late Golgi proteins (the kin recognition hypothesis) that are stabilized by a direct interaction of clathrin with the cytoplasmic tails of a subset of these Golgi proteins. Loss of clathrin might destabilize these compartment-specific aggregates resulting in the mislocalization of the late Golgi proteins, including Mnnlp, to the plasma membrane (model 2). Additional studies currently in progress are aimed at distinguishing between these possible models for the mechanism of Mnnlp retention within the appropriate Golgi compartment(s).

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