**ALG9** mannosyltransferase is involved in two different steps of lipid-linked oligosaccharide biosynthesis

Christian G. Frank and Markus Aebi

Institute of Microbiology, Department of Biology, Swiss Federal Institute of Technology Zürich, ETH Hönggerberg, CH-8093 Zürich, Switzerland

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N-linked protein glycosylation follows a conserved pathway in eukaryotic cells. The assembly of the lipid-linked core oligosaccharide Glc3Man9GlcNAc2, the substrate for the oligosaccharyltransferase (OST), is catalyzed by different glycosyltransferases located at the membrane of the endoplasmic reticulum (ER). The substrate specificity of the different glycosyltransferases guarantees the ordered assembly of the branched oligosaccharide and ensures that only completely assembled oligosaccharide is transferred to protein. The glycosyltransferases involved in this pathway are highly specific, catalyzing the addition of one single hexose unit to the lipid-linked oligosaccharide (LLO). Here, we show that the dolichylphosphomannose-dependent **ALG9** mannosyltransferase is the exception from this rule and is required for the addition of two different α-1,2-linked mannose residues to the LLO. This report completes the list of lumen-oriented glycosyltransferases required for the assembly of the LLO.

Key words: endoplasmic reticulum/dolichol/Saccharomyces cerevisiae

Introduction

N-linked glycosylation in eukaryotic cells is an essential post-translational modification of proteins in the secretory pathway (Kornfeld and Kornfeld, 1985). A key feature is the transfer of the pre-assembled branched oligosaccharide Glc3Man9GlcNAc2. The assembly of Glc3Man9GlcNAc2 on the lipid-carrier dolichylphosphosphate (PP-Dol) at the membrane of the endoplasmic reticulum (ER) and the initial trimming reactions of the oligosaccharide after transfer to protein are highly conserved processes in eukaryotes (Herscovics and Orlean, 1993; Burda and Aebi, 1999). The N-linked oligosaccharide fulfills different functions in the maturation of the glycoprotein, such as glycoprotein quality control or lectin-mediated sorting in the secretory pathway (Helenius, 2001; Trombeta, 2003; Helenius and Aebi, 2004). Therefore, it is of central importance that only the complete Glc3Man9GlcNAc2 oligosaccharide is transferred to protein, not biosynthetic intermediates or incorrectly assembled structures. This is accomplished primarily by the substrate specificity of the oligosaccharyltransferase (OST) that transfers the oligosaccharide to protein and the individual glycosyltransferases involved in the substrate assembly (Murphy and Spiro, 1981; Burda et al., 1999; Cipollo and Trimble, 2000; Kaoaglu et al., 2001).

Lipid-linked oligosaccharide (LLO) biosynthesis starts on the cytoplasmic side of the ER membrane by transfer of GlcNAc-P to dolichylphosphate (P-Dol), followed by the addition of GlcNAc and five mannose residue-yielding Man5GlcNAc2-PP-Dol (Figure 1). Nucleotide-activated sugars serve as substrates for these reactions. After Rft1p-dependent translocation of the heptasaccharide moiety into the lumen of the ER (Helenius and Aebi, 2002), four more mannoses are added from the lipid-linked donor Man-P-Dol, and the 14mer oligosaccharide is completed by transfer of three glucoses from Glc-P-Dol. Finally, the multi-subunit complex OST recognizes the full-length substrate and transfers it to asparagine residues in the context Asn-X amino acid-Ser/Thr, where X amino acid can be any amino acid except proline (Gavel and Von Heijne, 1999; Knauser and Lehle, 1999; Yan and Lenmarz, 1999; Dempski and Imperiali, 2002).

Yeast *alg* (asparagine-linked glycosylation) (Huffaker and Robbins, 1982) mutants have been pivotal for elucidation of the pathway components, as all 10 known genes encoding glycosyltransferases involved in LLO biosynthesis were initially cloned by complementation of corresponding mutations (Burda et al., 1999). *alg* mutant strains are characterized by accumulation of the LLO substrate of the affected glycosyltransferases, suggesting the absence of redundant transferases. However, because of the branched structure of the oligosaccharide, LLO biosynthesis can proceed in some mutant strains, resulting in aberrant oligosaccharide structures that are then transferred to protein (Cipollo et al., 2001; Helenius and Aebi, 2002).

The high degree of conservation of this pathway in eukaryotes has enabled rapid elucidation of the molecular basis of several human diseases caused by deficiencies in the ER glycosylation pathway, known as congenital disorders of glycosylation (CDG) (Aebi and Hennet, 2001; Marquardt and Denecke, 2003). As mentioned above, the understanding of the LLO biosynthetic pathway is not complete. In particular, the mannosyltransferase responsible for the addition of the terminal α-1,2-linked mannose of the C-antenna (residue k in Figure 1B) has not been identified (Burda et al., 1999). On the basis of the current models (Figure 1), it is proposed that Man-P-Dol serves as a donor in this reaction. The known Man-P-Dol-dependent mannosyltransferases of the pathway, Alg3p, Alg9p, and Alg12p, are hydrophobic proteins with multiple predicted transmembrane

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1To whom correspondence should be addressed; e-mail: aebi@micro.biol.ethz.ch

2Present address: Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706-1544
domains. They belong to a group of related transferases which includes also enzymes involved in GPI-anchor formation (Oriol et al., 2002). Members of this family without assigned function would be prime candidates for the missing transferase. Yet in *Saccharomyces cerevisiae*, all family members have already a suggested function. Interestingly, no yeast mutant strain accumulating Man$_8$GlcNAc$_2$-PP-Dol (residues a–j in Figure 1) has been isolated so far, despite several genetic screens that have been conducted to identify mutants in this pathway (Huffaker and Robbins, 1982; Roos et al., 1994; Burda et al., 1996, 1999; Burda and Aebi, 1998).

The failure to isolate a Man$_8$GlcNAc$_2$-PP-Dol-accumulating mutant could be explained by a model in which a mannosyltransferase acting earlier in the pathway also catalyzes addition of the mannose in question. On the basis of the assumption that there is a high linkage specificity of the glycosyltransferases involved, the Man-P-Dol-dependent mannosyl transferases Alg3p and Alg12p can be ruled out as possible dual-function candidates, as these transferases add an $\alpha$-1,3- or $\alpha$-1,6-linked mannose, respectively. In contrast, the ALG9 mannosyltransferase has previously been shown to be required for the addition of the $\alpha$-1,2-linked seventh mannose which terminates the B-arm (residue i in Figure 1) (Burda et al., 1996; Cipollo and Trimble, 2000). Cells lacking Alg9p accumulate Man$_7$GlcNAc$_2$-PP-Dol, but an abnormal Man$_7$GlcNAc$_2$ LLO with the acceptor residue (j in Figure 1) for mannose k can be obtained by overexpression of *ALG12* in a $\Delta$alg9 strain (Burda et al., 1999). Interestingly, this Man$_7$GlcNAc$_2$ LLO lacks both terminal $\alpha$-1,2-linked mannose residues despite the absence of one single transferase, Alg9p. This implies that either the responsible mannosyltransferase has a strict requirement for presence of the terminal $\alpha$-1,2 mannose on the B-arm or that this transferase is Alg9p.

We have investigated the hypothesis that mannose k is added by Alg9p. Using an *in vitro* assay for elongation of LLO and by analysis of the effects of Alg9p overexpression, we provided evidence that Alg9p is involved in addition of both the seventh and the ninth mannose to the LLO of N-glycan biosynthesis. It is thus the first example of a transferase in this conserved pathway found to add two saccharides.

**Results**

First, we followed a biochemical approach to test whether Alg9p is required for addition of the ninth mannose to Man$_8$GlcNAc$_2$-PP-Dol. This employed an *in vitro* assay for elongation of Man$_7$GlcNAc$_2$-PP-Dol to Man$_9$GlcNAc$_2$-PP-Dol. We used [$^3$H]mannose-labeled Man$_7$GlcNAc$_2$-PP-Dol (residues a–i) isolated from a strain lacking Alg12p (Burda et al., 1999) as the acceptor LLO substrate. Crude membrane extracts isolated from wildtype $\Delta$alg9 or $\Delta$alg12 strains were mixed with LLO substrate in the presence of detergent. Addition of GDP mannose was required to allow formation of Man-P-Dol by dolichol-phosphate-mannose synthase *in vitro*, while UDP-Glc was left out to avoid formation of glucosylated LLOs. Alterations of the radioactive oligosaccharide were then analyzed by HPLC.

Wildtype membranes elongated the input substrate (Figure 2B) by one or two hexose units to species co-migrating with Man$_9$GlcNAc$_2$ and Man$_9$GlcNAc$_2$, respectively (Figure 2C). In contrast, membranes from $\Delta$alg9 strains elongated Man$_7$GlcNAc$_2$-PP-Dol only by one hexose unit (Figure 2D). No elongation of Man$_7$GlcNAc$_2$-PP-Dol was observed with $\Delta$alg12 membranes (Figure 2E). This demonstrates that the first elongation was Alg12p dependent and that the second elongation from Man$_7$GlcNAc$_2$-PP-Dol to Man$_9$GlcNAc$_2$-PP-Dol depended on Alg9p. Like *in vivo*,
also in the *in vitro* assay addition of the α-1,6-linked mannose on the C-arm (residue j) by Alg12p preceded addition of the terminal α-1,2-linked mannose.

To exclude that deletion of *ALG9* results in an inhibiting activity which would mask the α-1,2 mannosyltransferase activity, we mixed membranes from wildtype and ∆alg9 cells and looked for substrate elongation. A peak at position of Man9GlcNAc2 could still be observed (data not shown), which rules out the presence of an inhibiting activity.

To verify that the oligosaccharides generated in the assay had the presumed Manα9GlcNAc2 or Manα8GlcNAc2 structures, we collected individual oligosaccharide species, digested them with an α-1,2 specific exo-mannosidase and analyzed them by HPLC (Figure 3). The material from elongation assays with wildtype membranes showed the expected behavior (Figure 3B–D): Manα9GlcNAc2 shifted upon treatment to a Manα8GlcNAc2 isomer, while Manα9GlcNAc2 and Manα8GlcNAc2 both shifted to the same Manα9GlcNAc2 isomer, demonstrating that they only differed by a terminal α-1,2-linked mannose.

Digestion of the Manα9GlcNAc2 peak generated with ∆alg9 membranes produced Manα5GlcNAc2, demonstrating that only the Alg12p-dependent elongation occurred and not addition of an α-1,2-linked mannose. To confirm that the Manα9GlcNAc2 species produced in the assay by wildtype membranes was identical to the naturally occurring Manα9GlcNAc2, we compared it with a Manα9GlcNAc2 reference oligosaccharide isolated from a ∆alg5 strain. Cells lacking *ALG3* cannot produce Glc-P-Dol and therefore accumulate Manα9GlcNAc2-PP-Dol (te Heesen et al., 1994). The Manα5GlcNAc2 isomer of the digested assay products co-migrated with the Manα9GlcNAc2 isomer obtained after exo-mannosidase digestion of Manα9GlcNAc2 from ∆alg5.

To confirm our results by a genetic approach, we overexpressed *ALG9* in wildtype yeast and analyzed resulting changes in the LLO profile. Cells were transformed with a high-copy plasmid containing the *ALG9* gene under control of the endogenous promoter. We then analyzed the pulse-labeling LLO profile by HPLC (Figure 4) and compared it with LLO profiles of control cells bearing an empty plasmid. Three prominent glycan peaks are usually detected by the HPLC analysis if LLOs of our wildtype cells are labeled by a [3H]mannose pulse: [3H]Manα5GlcNAc2, [3H]Manα8GlcNAc2, and the full-length oligosaccharide [3H]Glc3Manα9GlcNAc2. Accumulation of Manα9GlcNAc2-PP-Dol can be explained by the potentially rate-limiting translocation step that precedes elongation, by the Alg3p mannosyltransferase. The full-length precursor likely accumulates because of lack of acceptor peptides or limiting OST activity. We envisioned that Manα9GlcNAc2-PP-Dol accumulates due to limited mannosyltransferase activity for elongation. In support of this hypothesis, we found that *ALG9* overexpression significantly reduces the relative abundance of the Manα9GlcNAc2 peak compared with the Manα5GlcNAc2 peak (Figure 4). Quantification of seven independent experiments revealed the Manα9GlcNAc2/Manα5GlcNAc2 ratio of 0.71 ± 0.15 in wildtype cells and 0.24 ± 0.06 in *ALG9*-overexpressing cells. Interestingly, we did not observe a concomitant increase in the level of the complete Glc3Manα9GlcNAc2 product upon *ALG9* overexpression. This phenotype has to be expected in a biosynthetic pathway where the flux through the pathway is controlled by the level of the product. Overexpression of biosynthetic enzymes in such a system results in an alteration of relative intermediate concentrations but not in an increase of the product level. Therefore, we concluded that Alg9p can add the ninth mannose *in vivo* as the bottleneck at the

![Fig. 2. In vitro assay for elongation of Man7GlcNAc2-PP-Dol. HPLC analysis of [3H]mannose-labeled lipid-linked oligosaccharide (LLO) substrate from ∆alg12 yeast cells after incubation with membranes isolated from different yeast strains. (A) Oligosaccharide standard mix from [3H]mannose-labeled yeast LLO. (B) LLO substrate incubated with heat-inactivated (5 min/95°C) wildtype membranes. (C) LLO substrate incubated with wildtype membranes. (D) LLO substrate incubated with ∆alg9 membranes. (E) LLO substrate incubated with ∆alg12 membranes. Peaks co-migrating with standard oligosaccharides and several selected standard oligosaccharides are indicated. M5–M9 refers to Manα5GlcNAc2–Manα9GlcNAc2.](image-url)
Discussion

We provided both biochemical and genetic evidence that Alg9p is a Dol-P-Man-dependent α-1,2 mannosyltransferase with dual function: the enzyme transfers the seventh mannose residue on the B-arm (i) (Burda et al., 1996) as well as the ninth mannose residue on the C-arm (k) to the LLO.

We have recently identified a mutation of the human ALG9 homologue as the cause of a novel type of CDG (CDG-Ii; Frank et al., 2004). Analysis of the LLO from the patient cells revealed that both ALG9 substrates Man₆GlcNAc₂ and Man₉GlcNAc₂ accumulated, together with detectable amounts of full-length Glc₃Man₉GlcNAc₂. In agreement with this observation, expression of human ALG9 cDNAs in yeast mutant strains indicated that the mutated ALG9 retained residual activity. This shows that the dual role of ALG9 is conserved also in mammals. ALG9 might also be bifunctional in amoebae, as Freeze and co-workers previously isolated a Dictyostelium glycosylation mutant accumulating Man₆GlcNAc₂, Man₈GlcNAc₂, and Glc₃ Man₉GlcNAc₂-P-Dol (HL244, Figure 4 in [Freeze et al., 1989]). This mutant was not studied further, but on the basis of our findings most likely the ALG9 locus is affected.

Trypanosomatids lack glucosylated LLOs and transfer unglycosylated oligosaccharides to protein (Parodi, 1993). Depending on the species, the mature LLO is Man₆GlcNAc₂-P-Dol, Man₇GlcNAc₂-P-Dol, or Man₉GlcNAc₂-P-Dol. Interestingly, no Trypanosoma species has been identified so far that transfers Man₈GlcNAc₂ to protein (Bosch et al., 1988). Our model predicts that cells of the Man₆GlcNAc₂-P-Dol synthesizing species do not have ALG9 activity, that those producing Man₇GlcNAc₂-P-Dol have ALG9 activity but no ALG12 activity, and that the Man₉GlcNAc₂-P-Dol-producing cells have both
activities. As the presence of \textit{ALG9} is required to form \textit{Man}_9\textit{GlcNAc}_2-\textit{PP-Dol} (as without \textit{ALG9} biosynthesis stops at \textit{Man}_6\textit{GlcNAc}_2-\textit{PP-Dol}), \textit{ALG9} will always elongate it further to \textit{Man}_9\textit{GlcNAc}_2-PP-Dol. This explains why no species with \textit{Man}_8\textit{GlcNAc}_2-PP-Dol as the mature LLO has been observed.

Removal of mannose i from protein-linked \textit{Man}_9\textit{GlcNAc}_2 by ER mannosidase I (which yields the \textit{Man}_8\textit{GlcNAc}_2 isomer B, different from the \textit{Man}_8\textit{GlcNAc}_2 isomer C formed during LLO biosynthesis [Camirand \textit{et al}., 1991; Weng and Spiro, 1996]) has an important function in glycoprotein folding quality control in the ER (Helenius \textit{et al}., 1996). Analysis of LLO glycosylation in various \textit{alg} mutants indicates that \textit{Man}_9\textit{GlcNAc}_2-PP-Dol is the optimal substrate for \textit{Alg6p} (Verostek \textit{et al}., 1993; Burda \textit{et al}., 1999; Cipollo and Trimble, 2000). Thus, capping with \(\alpha\)-1,2-linked mannoses by \textit{Alg9p} serves as a signal for \textit{Alg6p} for completion of the LLO.

Another key factor that ensures that only fully assembled \textit{Glc}_3\textit{Man}_9\textit{GlcNAc}_2 oligosaccharide is transferred to protein is the selectivity of the OST for glucosylated LLO (Murphy and Spiro, 1981; Burda and Aebi, 1998; Spiro, 2000; Karaoglu \textit{et al}., 2001) in combination with the specificity of the \textit{Alg6p} glucosyltransferase. \textit{Alg6p} is required for addition of the first glucose (l), the rate-limiting step in LLO glucosylation (Reiss \textit{et al}., 1996). Analysis of LLO glucosylation in various \textit{alg} mutants indicates that \textit{Man}_9\textit{GlcNAc}_2-PP-Dol is the optimal substrate for \textit{Alg6p} (Verostek \textit{et al}., 1993; Burda \textit{et al}., 1999; Cipollo and Trimble, 2000). Thus, capping with \(\alpha\)-1,2-linked mannoses by \textit{Alg9p} assembles the C-arm for optimal activity (Burda \textit{et al}., 1999).

The relaxed specificity of \textit{Alg9p} toward the mannose-acceptor structure rises an interesting question whether \textit{Alg9p} can add an \(\alpha\)-1,2-linked mannose (at position f) to the \textit{Man}_9\textit{GlcNAc}_2-PP-Dol observed in \textit{alg11} cells (Cipollo \textit{et al}., 2001). We have not carried out further studies on the substrate specificity so far. However, some conclusions on limitations of the substrate spectrum can be drawn from previously reported analyses of mutants affected in GPI-anchor biosynthesis. Besides \textit{Alg9p}, two other members of the Man-P-Dol-dependent \(\alpha\)-1,2 mannosyltransferase family, \textit{Smp3p} (Grimme \textit{et al}., 2001) and \textit{Gpi10p} (Canivenc-Gansel \textit{et al}., 1998; Sutterlin \textit{et al}., 1998), are known in yeast. Both are involved in GPI-anchor biosynthesis, act on a lumennally oriented substrate, and might have evolved from a common ancestor with \textit{Alg9p} (Oriol \textit{et al}., 2002). Overall sequence similarity to \textit{Alg9p} is low (\textit{Gpi10p} 20\% identical, 36\% similar over 616 amino acids; \textit{Smp3p} 19\% identical, 36\% similar over 555 amino acids), but they share a similar topology as judged from the hydrophobicity profiles (Kyte-Doolittle hydropathy algorithm). The \textit{gpi10}-1 mutant is affected in addition of the third mannose to GPI-anchor precursors but has a normal LLO profile (Canivenc-Gansel \textit{et al}., 1998). As demonstrated by the inability of \textit{SMP3-} or \textit{GPI10} deletion mutants (Sutterlin \textit{et al}., 1998), they are not redundant to each other. Assuming \textit{SMP3} and \textit{GPI10} do not have additional unknown functions indicates that the relaxed LLO substrate specificity of \textit{Alg9p} does not extend to GPI-anchor intermediates, as it could otherwise compensate for loss of \textit{SMP3} or \textit{GPI10}.

**Materials and methods**

**Yeast strains and media**

Standard yeast media and genetic techniques were used (Guthrie and Fink, 1991). Yeast strains used in this study are listed in Table I.
Table I. Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS328</td>
<td>MATα ade2-101 his3Δ200</td>
<td>Vijayraghavan et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>lys2-801 ura3-52</td>
<td></td>
</tr>
<tr>
<td>YG414</td>
<td>MATα ade2-101 ura3-52</td>
<td>Burda et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>his3Δ200 lys2-801 Δalg9::kanMX</td>
<td></td>
</tr>
<tr>
<td>YG840</td>
<td>MATα ade2-101 ura3-52</td>
<td>Burda et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>his3Δ200 lys2-801 Δalg12::kanMX</td>
<td></td>
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</tbody>
</table>

Plasmids

pALG9 (Jakob et al., 1998) is a derivative of YEp352 (Hill et al., 1986) and contains a 2.5 kbp BglII-KpnI fragment of yeast genomic DNA encompassing the ALG9 ORF plus 0.5 kbp upstream sequence in the BamHI-KpnI sites.

HPLC analysis of $[^{3}H]$-labeled oligosaccharides

HPLC analysis was carried out as described (Zufferey et al., 1995).

Isolation of $[^{3}H]$Man$_{7}$GlcNAc$_{2}$-PP-Dol substrate

Δalg12 yeast were grown in YPD medium at 30°C to late log phase and 80 OD546 cells pulse labeled for 12 min with 125 µCi $[^{3}H]$mannose (20 Ci/mmol; ICN Pharmaceuticals, Costa Mesa, CA) as described previously (Zufferey et al., 1995). Workup of LLO was stopped after the CHCl$_3$ : MeOH : H$_2$O (10:10:3) (v/v/v) extraction, and the glycolipids were stored at −20°C until use.

$[^{3}H]$mannose labeling of ALG9-overexpressing cells

Wildtype yeast (SS328) transformed with either pALG9 or YEp352 were grown in 50 mL YPD at 30°C to mid-log phase (OD546 1.0 ± 0.1) and pulse labeled for 12 min with 25 µCi $[^{3}H]$mannose as described previously (Zufferey et al., 1995). Half of the isolated hydrolyzed oligosaccharides were analyzed by HPLC (see Results).

For the assessment of the overexpression effect, individual peak areas were quantified with the FLO-One software (Packard Biosciences, Meridan, CT), and the ratios of the Man$_{9}$GlcNAc$_{2}$ and Man$_{5}$GlcNAc$_{2}$ peaks (M8/M5) were calculated for each trace.

Preparation of membranes

Yeast strains were grown to mid-log phase in YPD medium at 30°C.

200 OD546 cells were harvested and washed once in washing buffer (50 mM HEPES/NaOH pH 7.5, 3 mM MgCl$_2$). The cells were resuspended in cold lysis buffer (washing buffer supplemented with 1 mM DTT and protease inhibitors: 1 mM PMSF, 2 µg/mL Leupeptin, and 1 µg/mL E-64) and lysed by vortexing for 10 min with glass beads. The liquid was collected by puncturing the tubes with a hot needle and centrifugation at 1000 × g for 1 min. Intact cells, cell debris, and nuclei were removed by centrifugation for 5 min at 1000 × g. This supernatant was then centrifuged for 45 min at 50,000 × g to obtain the membrane pellet. The pellet was resuspended and homogenized in storage buffer (lysis buffer supplemented with 35% [v/v] glycerol). Membranes were shockfrozeed in liquid nitrogen and stored at −80°C until use. Protein concentration was determined using the bicinchoninic acid method.

In vitro assay for elongation of $[^{3}H]$Man$_{7}$GlcNAc$_{2}$-PP-Dol

Membranes (340 µg protein) were incubated with 1 mM GDP-Man and 1 mM CTP in 44 mM HEPES/NaOH pH 7.5, 3 mM MgCl$_2$, 1 mM DTT, 30% (v/v) glycerol in a volume of 20 µL.

After incubation for 10 min at 25°C, 100 µL of $[^{3}H]$Man$_{7}$GlcNAc$_{2}$-PP-Dol substrate (60,000–80,000 cpm) in 50 mM HEPES/NaOH pH 6.5, 60 mM NaCl, 5.8 mM MgCl$_2$, 4.8 mM CaCl$_2$, 4.8 mM MnCl$_2$, 1 mM DTT, 0.3% (w/v) NP40 was added. The reaction was stopped after incubation at 25°C for 20 min by addition of 800 µL CHCl$_3$ : MeOH (1:1) (v/v) and thorough vortexing. The supernatant after centrifugation for 5 min at 9,000 x g at 4°C was saved, and the pellet was re-extracted with 800 µL CHCl$_3$ : MeOH : H$_2$O (10:10:3) (v/v/v). Both supernatants were combined, dried under nitrogen, and subjected to mild acid hydrolysis and analyzed by HPLC as described previously (Zufferey et al., 1995).

Oligosaccharide digestion with exo-α-1,2 mannosidase

Single peaks from HPLC analysis of assay products or in vivo labeling were collected, dried, and resuspended in incubation buffer (100 mM HAc/NaOAc pH 5.0). The samples were split and incubated with or without 10 µL Trichoderma reesei exo-α-1,2 mannosidase (a gift from R. Contreras, Ghent) for 96 h at 37°C in a volume of 30 µL. After heat inactivation of the enzyme for 5 min at 95°C, the samples were spin-filtered and analyzed by HPLC as described above. Initial experiments were performed with an exo-α-1,2 mannosidase from Aspergillus saitoi (a gift from Dr. T. Butters, Oxford).

Protein sequence alignments

The protein sequences used have the accession numbers NP_014180 (Alg9p), NP_011373 (Gpi10p), NP_014792 (Alg11p), and NP_014350 (Alg12p). Sequences were aligned using the Clustal W algorithm with a BLOSUM scoring matrix.

Acknowledgments

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Abbreviations

ER, endoplasmic reticulum; GlcNAc, N-acetylglucosamine; LLO, lipid-linked oligosaccharide; OST, oligosaccharyltransferase; P-Dol, dolichylphosphate; PP-Dol, dolichylphosphorylcholine.
References


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