Running title:

Arrangement of glycosylation enzymes

Correspondence:

R. Strasser

Department of Applied Genetics and Cell Biology, University of Natural Resources and

Applied Life Sciences, BOKU-Vienna, Muthgasse 18, 1190 Vienna, Austria.

Tel: 0043 1 36006 6700

Fax: 0043 1 36006 6392

E-mail: richard.strasser@boku.ac.at

Localization of plant N-glycan processing enzymes along the secretory pathway

Richard Strasser

Abstract

N-glycosylation is an abundant covalent protein modification in all eukaryotic cells. The biosynthesis and processing of protein N-linked glycans results from a series of highly co-ordinated step-by-step enzymatic conversions occurring mainly in the endoplasmic reticulum (ER) and Golgi apparatus. N-glycan processing enzymes are thought to act on cargo glycoproteins in a highly ordered fashion in an assembly line. Thus, the subcellular localization of these enzymes together with their *in vivo* substrate specificity determines the carbohydrate structures of glycoproteins transported through the secretory pathway. While the substrate specificities of many plant N-glycan processing enzymes are fairly well characterized the molecular mechanisms underlying enzyme localization to the ER and Golgi have remained largely elusive so far. This review discusses current data on ER and Golgi localization of plant N-glycan processing enzymes.

Key words: ER exit, glycosidase, glycosyltransferase, Golgi targeting, N-glycosylation

Introduction to the N-glycan processing pathway

N-glycosylation of proteins is initiated in the ER by transfer of the $Glc_3Man_9GlcNAc_2$ oligosaccharide from a lipid-linked precursor to the asparagine residue of a nascent polypeptide chain (Kornfeld and Kornfeld, 1985; Lerouge et al., 1998). Processing in the ER starts by removal of the terminal α1,2-linked glucose residue by α-glucosidase I (GCSI, Figure 1) (Boisson et al., 2001; Gillmor et al., 2002). The subsequent two α1,3-linked glucose residues are cleaved off by α-glucosidase II (GCSII) (Taylor, 2000; Burn, 2002). The next steps of the pathway are the removal of α1,2-linked mannose residues. These reactions are catalyzed by class I mannosidases (Mast and Moremen, 2006), which includes ER- and Golgi-located enzymes in yeast and mammals. Although candidates for these enzymes have been proposed in plants (Nebenführ et al., 1999; Preuss et al., 2004), their role in N-glycan processing remains to be shown.

In the Golgi the formation of complex and hybrid N-glycans is initiated by β1,2-N-acetylglucosaminyltransferase I (GnTI), which transfers a single GlcNAc residue to the β1,3-linked mannose of the $Man_5GlcNAc_2$ acceptor substrate to create the $GlcNAcMan_5GlcNAc_2$ N-glycan structure (Strasser et al., 1999a). In the main route of N-glycan processing two mannose residues are subsequently cleaved off from the latter substrate by Golgi-α-mannosidase II (GMII, Figure 1) (Strasser et al., 2006). The resulting N-glycan structure ($GlcNAcMan_3GlcNAc_2$) is the substrate for β1,2-N-acetylglucosaminyltransferase II (GnTII), in order to produce diantennary complex N-glycans (Strasser et al., 1999b). Further modification of the N-glycans arising from GnTI, GMII and GnTII action can occur by β1,2-xylosyltransferase (XylT), which is capable of transferring xylose residues to various N-glycan acceptor substrates *in vitro* (Strasser et al., 2000; Bencúr et al., 2005) and *in vivo* (Strasser et al., 2004; Strasser et al., 2006) and by core α1,3-fucosyltransferase (FUT11/FUT12) (Leiter et al., 1999; Wilson et al., 2001). The final step in the N-glycan processing pathway is the generation of a trisaccharide [Fucα1-4(Galβ1-3)GlcNAc-R] known as the Lewis a (Le^a) epitope. It has

been proposed that two enzymes are required for the synthesis of the Le^a epitope on complex plant N-glycans (Lerouge et al., 1998). First, β1,3-galactosyltransferase (GALT1) transfers galactose to the terminal GlcNAc residue in β1,3-linkage and second an α1,4-fucosyltransferase (FUT13) transfers fucose in α1,4-linkage to the same GlcNAc residue to complete the synthesis of the Le^a structure (Strasser et al., 2007a). Analysis of N-glycans from *galt1* and *fut13* knockout lines as well as *in vitro* substrate specificity of FUT13 show that α1,4-fucosylation is the final elongation reaction of plant complex N-glycans. Finally the presence of large amounts of truncated paucimannosidic N-glycans (MMXF structures) on plant glycoproteins indicates that terminal GlcNAc residues on N-glycans are removed by β-N-acetylglucosaminidases, which presumably takes place in a post Golgi compartment like the vacuole, the plasma membrane, cell wall or apoplast (Vitale and Chrispeels, 1984; Strasser et al., 2007b).

Compartmentalization of N-glycan processing enzymes

N-glycan processing enzymes are not uniformly distributed within the early secretory pathway to allow the sequential modification of passing glycoproteins (Nilsson et al., 1993a; Rabouille et al., 1995; Roth, 1996). To generate and maintain this non-uniform distribution, the glycosylation enzymes must be trafficking to specific ER or Golgi subdomains and their steady-state localization is maintained through a combination of retention and retrieval. In plants evidence for compartmentalization of N-glycan processing enzymes was first obtained indirectly by electron microscopy using antibodies against distinct N-glycan structures (Lainé et al., 1991; Fitchette-Lainé et al., 1994; Fitchette et al., 1999). These data indicated that the attachment of β1,2-xylose by XylT takes place mainly in the medial-Golgi, while core α1,3-fucosylation and formation of the Le^a epitope are *trans*-Golgi reactions. Additional electron microscopy data were obtained by detection of tagged enzymes. The full-length soybean GMI fused to GFP displayed ER and predominantly *cis*-Golgi labelling when stably expressed in BY-2 cells (Nebenführ et al., 1999). A similar subcellular localization was detected for a truncated

form of soybean GMI fused to GFP (Saint-Jore-Dupas et al., 2006) and a tagged form of XylT was mainly associated with the medial-Golgi (Pagny et al., 2003). Recently the Staehelin group provided some evidence that endogenous *A. thaliana* GMI localizes predominantly to medial-Golgi cisternae in root meristems, without any significant labelling of ER or *cis*-Golgi cisternae (Staehelin and Kang, 2008). These data, which were generated using an antibody against native *A. thaliana* GMI isoforms, are clearly in contrast to previous findings obtained with GFP tagged GMI (Nebenführ et al., 1999; Saint-Jore-Dupas et al., 2006) and suggest that the *cis*-Golgi is not a compartment where trimming of N-glycans to $Man_5GlcNAc_2$ takes place. The latter finding is consistent with the limited processing of N-glycans found on HDEL/KDEL-containing proteins. These proteins are transported back from the *cis*-Golgi to the ER by the KDEL-receptor ERD2 and they normally contain as major glycoforms $Man_7GlcNAc_2$ and $Man_8GlcNAc_2$ N-glycans (e.g. Sriraman et al., 2004), indicating that the *cis*-Golgi is devoid of GMI activity.

Mechanisms of localization of N-glycan processing enzymes

Although it is generally agreed that N-glycan processing enzymes are arranged in a sequential manner it is still not well understood how they are compartmentalized within the ER and Golgi (Colley, 1997; Opat et al., 2001a; de Graffenried and Bertozzi, 2004; Saint-Jore-Dupas, 2006). With the exception of GCSII, all of the known plant glycosidases and glycosyltransferases are predicted to be type II membrane proteins consisting of a short N-terminal cytoplasmic tail, a single pass transmembrane domain and a stem region (together the CTS region) orienting the catalytic domain into the ER and Golgi lumen (Figure 2). The importance of the CTS region for localization of plant glycosylation enzyme has been demonstrated (Essl et al., 1999; Dirnberger et al., 2002; Saint-Jore-Dupas et al., 2006; Strasser et al., 2006; Strasser et al., 2007a; Schoberer et al., 2008) and is similar to mammalian enzymes (Colley, 1997; Grabenhorst and Conradt, 1999; Opat, et al., 2001a). Importantly, the CTS region from the *trans*-Golgi-resident mammalian enzyme α2,6-sialyltransferase (ST-GFP) is also sufficient to provide *trans*-Golgi

targeting in plant cells as also observed for a tagged form of the full-length enzyme stably expressed in *A. thaliana* (Boevink et al., 1998; Wee et al., 1998), indicating that basic mechanisms for localization are conserved between mammals and plants.

The subcellular localization of GCSII has yet to be established, but it has been postulated that *A. thaliana* GCSII consists of a catalytic α-subunit (At5g63840), which is devoid of a transmembrane domain and other ER retention signals and a proposed β-subunit (At5g56360) (Soussillane et al., 2008). The β-subunit contains a conserved HDEL motif for retrieval from the Golgi to the ER and could be responsible for ER localization of the α-subunit.

Three models have been proposed to describe the underlying mechanism for arrangement of glycosylation enzymes in the ER or Golgi. In the "bilayer thickness" model, the length of the transmembrane domain determines the retention of single-pass membrane proteins in different compartments of the secretory pathway (Bretscher and Munro, 1993). In this sorting model, proteins with shorter transmembrane domains (e.g. ER-resident proteins) are excluded from transport vesicles by partitioning effects (Ronchi et al., 2008). The length of the transmembrane domain was sufficient to dictate the export of a type I membrane protein from the ER in tobacco (Brandizzi et al., 2002). A transmembrane length of 17 amino acid residues resulted in ER retention, while an increase to 20 amino acids led to accumulation in the Golgi and the protein with a 23 amino acid long transmembrane domain was transported to the plasma membrane. An elongation of the transmembrane domain length from 16 to 23 residues resulted also in forward transport of GMI to the medial/*trans*-Golgi (Saint-Jore-Dupas et al., 2006), indicating a similar role of the transmembrane domain length for Golgi-resident type II membrane proteins. However, a comparison of the predicted transmembrane domain length of individual plant N-glycan processing enzymes is not in favour of such a model because the predicted transmembrane domain length is not consistent between the different enzymes and does not increase from ER-resident to *trans*-Golgi-resident proteins (see Table I for comparison of

transmembrane domain length). The transmembrane domain of late Golgi enzymes like FUT11 or GALT1 is only 18 amino acids and the widely used *trans*-Golgi marker ST-GFP has also a rather short domain of 17 amino acids, suggesting that the transmembrane domain length alone does not play a significant role for localization of these enzymes.

In the second model for Golgi localization of mammalian enzymes, the "kin recognition" model, it has been proposed that Golgi-resident proteins form large homo/hetero-oligomers (Nilsson et al., 1993b). ER-retention of the medial-Golgi enzyme GMII resulted also in ER-localization of the *cis*- to medial-Golgi enzyme GnTI, due to specific interactions occurring mainly in the stem regions (Nilsson and Warren, 1994; Nilsson et al., 1996). Evidence for the occurrence of the kin recognition model and the role of hetero- or homo-oligomerization of glycosylation enzymes has so far not been provided in plants. It has been shown that the CT-domains of GnTI, XylT and GMII are sufficient for Golgi targeting without any contribution from the lumenal stem region (Dirnberger et al., 2002; Saint-Jore-Dupas et al., 2006; Strasser et al., 2006; Schoberer et al., 2008). However, these findings do not exclude the possibility of oligomerization as an additional mechanism for retention in the Golgi or for sub-Golgi localization. Early Golgi-resident mammalian enzymes like GnTI and GnTII form high molecular weight complexes, while late Golgi enzymes have been found as monomers or dimers (Opat et al., 2000). These differences in oligomer-formation could significantly contribute to the differential localization of these N-glycan processing enzymes.

In a third and more recent model it has been demonstrated that the cytoplasmic tail of glycosyltransferases carries information for ER exit and for sub-Golgi localization (Giraudo and Maccioni, 2003; Osman et al., 1996; Milland et al., 2001). Basic amino acids in the N-terminal cytoplasmic tail are required for direct interaction with the small guanosine triphosphatase (GTPase) Sar1p, suggesting that they provide a binding motif for COPII-dependent ER-to-Golgi transport (Giraudo and Maccioni, 2003). Recently we have shown that a single basic amino acid in the cytoplasmic tail of three Golgi-resident N-glycan processing enzymes (GnTI, GMII, XylT) is sufficient to promote rapid Golgi targeting (Schoberer et al., 2008). Importantly as

demonstrated for GnTI this intact ER export motif is essential for proper *in vivo* function of GnTI as determined by complementation of the *A. thaliana cgl1* mutant, which lacks GnTI activity. All Golgi-resident N-glycan processing enzymes contain at least one single basic amino acid residue in their N-terminal cytoplasmic tail (Table I), suggesting that a common selective mechanism, which seems to involve a COPII-dependent transport, is functional for Golgi targeting of these enzymes (Schoberer et al., 2008).

The first 13 amino acids in the cytoplasmic tail of *A. thaliana* GCSI have also been shown to be essential for ER retention or efficient ER retrieval, since a deletion of these residues resulted in targeting of a tagged GCSI protein to the Golgi (Saint-Jore-Dupas et al., 2006). Interestingly, these N-terminal amino acids contain also a basic amino acid motif (Table I), which could be involved in COPI-mediated retrograde transport from the Golgi back to the ER, like it was also shown for plant type I membrane proteins (Contreras et al., 2004). Together these data indicate that basic amino acids in the cytoplasmic tail can serve as a functional ER exit signal for Golgi-resident enzymes (Schoberer et al., 2008) and as an ER retention/retrieval signal for ER-resident glycosylation enzymes.

Apart from their putative function as described above basic amino acid residues could also play an important role for the steady-state localization of enzymes in different Golgi cisternae. Cytoplasmic tails of mammalian glycosyltransferases have been found to influence their sub-Golgi distribution (Osman et al., 1996; Milland et al., 2001; Uliana et al., 2006). Exchange of the cytoplasmic tails resulted in respective changes of the sub-Golgi localization of reporter proteins. Golgi-resident glycosylation enzymes (like GMII) have been detected in COPI vesicles in mammalian cells (Martinez-Menárguez et al., 2001) and retrograde transport of human GnTI from *trans*- to medial-Golgi cisternae has been suggested to play an important role for its steady-state localization (Opat et al., 2001b). However, a conserved COPI-binding motif or a mechanism for packaging of these enzymes into COPI vesicles has not been identified. The existence of COPI vesicles for retrograde transport from *trans*- to *cis*/medial-Golgi cisternae has also been proposed for plants (Donohoe et al., 2007), but evidence for the presence of N-glycan

processing enzymes in these vesicles has not been provided so far. For yeast glycosyltransferases it has been shown recently that Golgi localization is dependent on a protein called Vps74p, since loss of Vps74p resulted in defects in N-glycan processing in the Golgi (Schmitz et al., 2008). Furthermore, it was demonstrated that Vps74p binds to the cytoplasmic tails of Golgi-resident glycosyltransferases as well as to COPI subunits (Tu et al., 2008). Although there are human homologues of Vps74p no obvious plant homologue is present in the *A. thaliana* genome and the proposed Vps74p-binding motif is not conserved in the cytoplasmic tails of plant N-glycan processing enzymes, suggesting that other proteins or another mechanism are responsible for the sub-Golgi localization of plant N-glycan processing enzymes.

The proposed models for ER and Golgi localization of N-glycan processing enzymes are not mutually exclusive and more than one domain (e.g. transmembrane domain length for retention and cytoplasmic tail sequences for retrieval of N-glycan processing enzymes) may in fact contribute for efficient steady-state localization of these enzymes.

Conclusions and outlook

Although the plant N-glycan processing pathway is now fairly well characterised at a biochemical level we are just beginning to understand the mechanisms for localization of the involved enzymes and important signals e.g. for sub-Golgi localization are yet to be elucidated. Despite the unique structural characteristics of the plant ER and Golgi (Robinson et al., 2007; Hawes et al., 2008; Staehelin and Kang, 2008) localization mechanisms of N-glycan processing enzymes seem to be conserved in mammals and plants. Future work must address the question whether a COPI-mediated mechanism of dynamic localization of glycosyltransferases and glycosidases similar to the one proposed for yeast exists, which would be consistent with the widely postulated cisternal maturation model. In this respect, a recently described high-throughput screen for aberrant localization of the well-known *trans*-Golgi marker ST-GFP in *A.*

thaliana could result in the identification of key players for sub-Golgi localization of glycosylation enzymes (Boulaflous et al., 2008). In addition, the signals for ER export and the role of complex formation in localisation of these enzymes have to be explored in more detail to provide a deeper understanding of the early secretory pathway and the underlying mechanisms for the maintenance of its functionality.

Acknowledgements

Work in my group is supported by grants from the Austrian Science Fund (P19092 and P19494). I thank Herta Steinkellner for comments and discussions and Jennifer Schoberer (all from BOKU) for preparation of Figure 2.

References

Bencúr P, Steinkellner H, Svoboda B, Mucha J, Strasser R, Kolarich D, Hann S, Köllensperger G, Glössl J, Altmann F, Mach L. (2005). *Arabidopsis thaliana* beta1,2-xylosyltransferase: an unusual glycosyltransferase with the potential to act at multiple stages of the plant N-glycosylation pathway. Biochem J 388: 515-525.

Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C. (1998). Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. Plant J 15: 441-447.

Boisson M, Gomord V, Audran C, Berger N, Dubreucq B, Granier F, Lerouge P, Faye L, Caboche M, Lepiniec L. (2001). Arabidopsis glucosidase I mutants reveal a critical role of N-glycan trimming in seed development. EMBO J 20: 1010-1019.

Boulaflous A, Faso C, Brandizzi F. (2008). Deciphering the Golgi Apparatus: From Imaging to Genes. Traffic 9: 1613-1617.

Brandizzi F, Frangne N, Marc-Martin S, Hawes C, Neuhaus J, Paris N. (2002). The destination for single-pass membrane proteins is influenced markedly by the length of the hydrophobic domain. Plant Cell 14: 1077-1092.

Bretscher M, Munro S. (1993). Cholesterol and the Golgi apparatus. Science 261: 1280-1281.

Burn J, Hurley U, Birch R, Arioli T, Cork A, Williamson R. (2002). The cellulose-deficient Arabidopsis mutant rsw3 is defective in a gene encoding a putative glucosidase II, an enzyme processing N-glycans during ER quality control. Plant J 32: 949-960.

Colley K. (1997). Golgi localization of glycosyltransferases: more questions than answers. Glycobiology 7: 1-13.

Contreras I, Yang Y, Robinson D, Aniento F. (2004). Sorting signals in the cytosolic tail of plant p24 proteins involved in the interaction with the COPII coat. Plant Cell Physiol 45: 1779-1786.

de Graffenried C, Bertozzi C. (2004). The roles of enzyme localization and complex formation in glycan assembly within the Golgi apparatus. Curr Opin Cell Biol 16: 356-363.

Dirnberger D, Bencúr P, Mach L, Steinkellner H. (2002). The Golgi localization of *Arabidopsis thaliana* beta1,2-xylosyltransferase in plant cells is dependent on its cytoplasmic and transmembrane sequences. Plant Mol Biol 50: 273-281.

Donohoe B, Kang B, Staehelin L. (2007). Identification and characterization of COPIa- and COPIb-type vesicle classes associated with plant and algal Golgi. Proc Natl Acad Sci U S A 104: 163-168.

Essl D, Dirnberger D, Gomord V, Strasser R, Faye L, Glössl J, Steinkellner H. (1999). The N-terminal 77 amino acids from tobacco N-acetylglucosaminyltransferase I are sufficient to retain a reporter protein in the Golgi apparatus of *Nicotiana benthamiana* cells. FEBS Lett 453: 169-173.

Fitchette A, Cabanes-Macheteau M, Marvin L, Martin B, Satiat-Jeunemaitre B, Gomord V, Crooks K, Lerouge P, Faye L, Hawes C. (1999). Biosynthesis and immunolocalization of Lewis a-containing N-glycans in the plant cell. Plant Physiol 121: 333-344.

Fitchette-Lainé A-C, Gomord V, Chekkafi A, Faye L. (1994). Distribution of xylosylation and fucosylation in the plant Golgi apparatus. Plant J 5: 673-682.

Gillmor C, Poindexter P, Lorieau J, Palcic M, Somerville C. (2002). Alpha-glucosidase I is required for cellulose biosynthesis and morphogenesis in Arabidopsis. J Cell Biol 156: 1003-1013.

Giraudo C, Maccioni H. (2003). Endoplasmic reticulum export of glycosyltransferases depends on interaction of a cytoplasmic dibasic motif with Sar1. Mol Biol Cell 14: 3753-3766.

Grabenhorst E, Conradt H. (1999). The cytoplasmic, transmembrane, and stem regions of glycosyltransferases specify their in vivo functional sublocalization and stability in the Golgi. J Biol Chem 274: 36107-36116.

Hawes C, Osterrieder A, Hummel E, Sparkes I. (2008). The plant ER-Golgi interface. Traffic 9: 1571-1580.

Kornfeld R, Kornfeld S. (1985). Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 54: 631-664.

Lainé A, Gomord V, Faye L. (1991). Xylose-specific antibodies as markers of subcompartmentation of terminal glycosylation in the Golgi apparatus of sycamore cells. FEBS Lett 295: 179-184.

Leiter H, Mucha J, Staudacher E, Grimm R, Glössl J, Altmann F. (1999). Purification, cDNA cloning, and expression of GDP-L-Fuc:Asn-linked GlcNAc alpha1,3-fucosyltransferase from mung beans. J Biol Chem 274: 21830-21839.

Lerouge P, Cabanes-Macheteau M, Rayon C, Fischette-Lainé A, Gomord V, Faye L. (1998). N-glycoprotein biosynthesis in plants: recent developments and future trends. Plant Mol Biol 38: 31-48.

Martinez-Menárguez J, Prekeris R, Oorschot V, Scheller R, Slot J, Geuze H, Klumperman J. (2001). Peri-Golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. J Cell Biol 155: 1213-1224.

Mast S, Moremen K. (2006). Family 47 alpha-mannosidases in N-glycan processing. Methods Enzymol 415: 31-46.

Milland J, Taylor S, Dodson H, McKenzie I, Sandrin M. (2001). The cytoplasmic tail of alpha 1,2-fucosyltransferase contains a sequence for golgi localization. J Biol Chem 276: 12012-12018.

Nebenführ A, Gallagher L, Dunahay T, Frohlick J, Mazurkiewicz A, Meehl J, Staehelin L. (1999). Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. Plant Physiol 121: 1127-1142.

Nilsson T, Pypaert M, Hoe M, Slusarewicz P, Berger E, Warren G. (1993a). Overlapping distribution of two glycosyltransferases in the Golgi apparatus of HeLa cells. J Cell Biol 120: 5-13.

Nilsson T, Rabouille C, Hui N, Watson R, Warren G. (1996). The role of the membrane-spanning domain and stalk region of N-acetylglucosaminyltransferase I in retention, kin recognition and structural maintenance of the Golgi apparatus in HeLa cells. J Cell Sci 109: 1975-1989.

Nilsson T, Slusarewicz P, Hoe M, Warren G. (1993b). Kin recognition. A model for the retention of Golgi enzymes. FEBS Lett 330: 1-4.

Nilsson T, Warren G. (1994). Retention and retrieval in the endoplasmic reticulum and the Golgi apparatus. Curr Opin Cell Biol 6: 517-521.

Opat A, Houghton F, Gleeson P. (2000). Medial Golgi but not late Golgi glycosyltransferases exist as high molecular weight complexes. Role of luminal domain in complex formation and localization. J Biol Chem 275: 11836-11845.

Opat A, Houghton F, Gleeson P. (2001b). Steady-state localization of a medial-Golgi glycosyltransferase involves transit through the trans-Golgi network. Biochem J 358: 33-40.

Opat A, van Vliet C, Gleeson P. (2001a). Trafficking and localization of resident Golgi glycosylation enzymes. Biochimie 83: 763-773.

Osman N, McKenzie I, Mouhtouris E, Sandrin M. (1996). Switching amino-terminal cytoplasmic domains of alpha(1,2)fucosyltransferase and alpha(1,3)galactosyltransferase alters the expression of H substance and Galalpha(1,3)Gal. J Biol Chem 271: 33105-33109.

Pagny S, Bouissonnie F, Sarkar M, Follet-Gueye M, Driouich A, Schachter H, Faye L, Gomord V. (2003). Structural requirements for Arabidopsis beta1,2-xylosyltransferase activity and targeting to the Golgi. Plant J 33: 189-203.

Preuss M, Serna J, Falbel T, Bednarek S, Nielsen E. (2004). The Arabidopsis Rab GTPase RabA4b localizes to the tips of growing root hair cells. Plant Cell 16: 1589-1603.

Rabouille C, Hui N, Hunte F, Kieckbusch R, Berger E, Warren G, Nilsson T. (1995). Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. J Cell Sci 108: 1617-1627.

Robinson DG, Herranz M-C, Bubeck J, Pepperkok R, Ritzenthaler C. (2007). Membrane Dynamics in the Eearly Secretory pathway. Critical Reviews in Plant Sciences 26: 199-225.

Ronchi P, Colombo S, Francolini M, Borgese N. (2008). Transmembrane domain-dependent partitioning of membrane proteins within the endoplasmic reticulum. J Cell Biol 181: 105-118.

Roth J. (1996). Protein glycosylation in the endoplasmic reticulum and the Golgi apparatus and cell type-specificity of cell surface glycoconjugate expression: analysis by the protein A-gold and lectin-gold techniques. Histochem Cell Biol 106: 79-92.

Saint-Jore-Dupas C, Nebenführ A, Boulaflous A, Follet-Gueye M, Plasson C, Hawes C, Driouich A, Faye L, Gomord V. (2006). Plant N-glycan processing enzymes employ different targeting mechanisms for their spatial arrangement along the secretory pathway. Plant Cell 18: 3182-3200.

Schmitz K, Liu J, Li S, Setty T, Wood C, Burd C, Ferguson K. (2008). Golgi localization of glycosyltransferases requires a Vps74p oligomer. Dev Cell 14: 523-534.

Schoberer J, Vavra U, Stadlmann J, Hawes C, Mach L, Steinkellner H, Strasser R. (2008). Arginine/lysine residues in the cytoplasmic tail promote ER export of plant glycosylation enzymes. Traffic Nov 1. [Epub ahead of print].

Soussillane P, D'Alessio C, Paccalet T, Fitchette A, Parodi A, Williamson R, Plasson C, Faye L, Gomord V. (2008). N-glycan trimming by glucosidase II is essential for Arabidopsis development. Glycoconj J Oct 30. [Epub ahead of print].

Sriraman R, Bardor M, Sack M, Vaquero C, Faye L, Fischer R, Finnern R, Lerouge P. (2004). Recombinant anti-hCG antibodies retained in the endoplasmic reticulum of transformed plants lack core-xylose and core-alpha(1,3)-fucose residues. Plant Biotechnol J 2: 279-287.

Staehelin L, Kang B. (2008). Nanoscale architecture of endoplasmic reticulum export sites and of Golgi membranes as determined by electron tomography. Plant Physiol 147: 1454-1468.

Strasser R, Altmann F, Mach L, Glössl J, Steinkellner H. (2004). Generation of *Arabidopsis thaliana* plants with complex N-glycans lacking beta1,2-linked xylose and core alpha1,3-linked fucose. FEBS Lett 561: 132-136.

Strasser R, Bondili J, Schoberer J, Svoboda B, Liebminger E, Glössl J, Altmann F, Steinkellner H, Mach L. (2007b). Enzymatic properties and subcellular localization of Arabidopsis beta-N-acetylhexosaminidases. Plant Physiol 145: 5-16.

Strasser R, Bondili J, Vavra U, Schoberer J, Svoboda B, Glössl J, Léonard R, Stadlmann J, Altmann F, Steinkellner H, Mach L. (2007a). A unique beta1,3-galactosyltransferase is indispensable for the biosynthesis of N-glycans containing Lewis a structures in *Arabidopsis thaliana*. Plant Cell 19: 2278-2292.

Strasser R, Mucha J, Mach L, Altmann F, Wilson I, Glössl J, Steinkellner H. (2000). Molecular cloning and functional expression of beta1, 2-xylosyltransferase cDNA from *Arabidopsis thaliana*. FEBS Lett 472: 105-108.

Strasser R, Mucha J, Schwihla H, Altmann F, Glössl J, Steinkellner H. (1999a). Molecular cloning and characterization of cDNA coding for beta1,2N-acetylglucosaminyltransferase I (GlcNAc-TI) from *Nicotiana tabacum*. Glycobiology 9: 779-785.

Strasser R, Schoberer J, Jin C, Glössl J, Mach L, Steinkellner H. (2006). Molecular cloning and characterization of *Arabidopsis thaliana* Golgi alpha-mannosidase II, a key enzyme in the formation of complex N-glycans in plants. Plant J 45: 789-803.

Strasser R, Steinkellner H, Borén M, Altmann F, Mach L, Glössl J, Mucha J. (1999b). Molecular cloning of cDNA encoding N-acetylglucosaminyltransferase II from *Arabidopsis thaliana*. Glycoconj J 16: 787-791.

Taylor M, Ross H, McRae D, Stewart D, Roberts I, Duncan G, Wright F, Millam S, Davies H. (2000). A potato alpha-glucosidase gene encodes a glycoprotein-processing alpha-glucosidase II-like activity. Demonstration of enzyme activity and effects of down-regulation in transgenic plants. Plant J 24: 305-316.

Tu L, Tai W, Chen L, Banfield D. (2008). Signal-mediated dynamic retention of glycosyltransferases in the Golgi. Science 321: 404-407.

Uliana A, Giraudo C, Maccioni H. (2006). Cytoplasmic tails of SialT2 and GalNAcT impose their respective proximal and distal Golgi localization. Traffic 7: 604-612.

Vitale A, Chrispeels M. (1984). Transient N-acetylglucosamine in the biosynthesis of phytohemagglutinin: attachment in the Golgi apparatus and removal in protein bodies. J Cell Biol 99: 133-140.

Wee E, Sherrier D, Prime T, Dupree P. (1998). Targeting of active sialyltransferase to the plant Golgi apparatus. Plant Cell 10: 1759-1768.

Weinstein J, Lee E, McEntee K, Lai P, Paulson J. (1987). Primary structure of beta-galactoside alpha 2,6-sialyltransferase. Conversion of membrane-bound enzyme to soluble forms by cleavage of the NH2-terminal signal anchor. J Biol Chem 262: 17735-17743.

Wilson I, Rendić D, Freilinger A, Dumić J, Altmann F, Mucha J, Müller S, Hauser M. (2001). Cloning and expression of cDNAs encoding alpha1,3-fucosyltransferase homologues from *Arabidopsis thaliana*. Biochim Biophys Acta 1527: 88-96.

Table I. Comparison of cytoplasmic tail sequences (C-tail) and predicted transmembrane domain length (T-length) of plant N-glycan processing enzymes. Enzymes are arranged according to their predicted location in the pathway (see Figure 1). Basic amino acids motifs putatively involved in ER-exit are highlighted in bold in the C-tail sequences. For abbreviations of enzyme names see Figure 1.

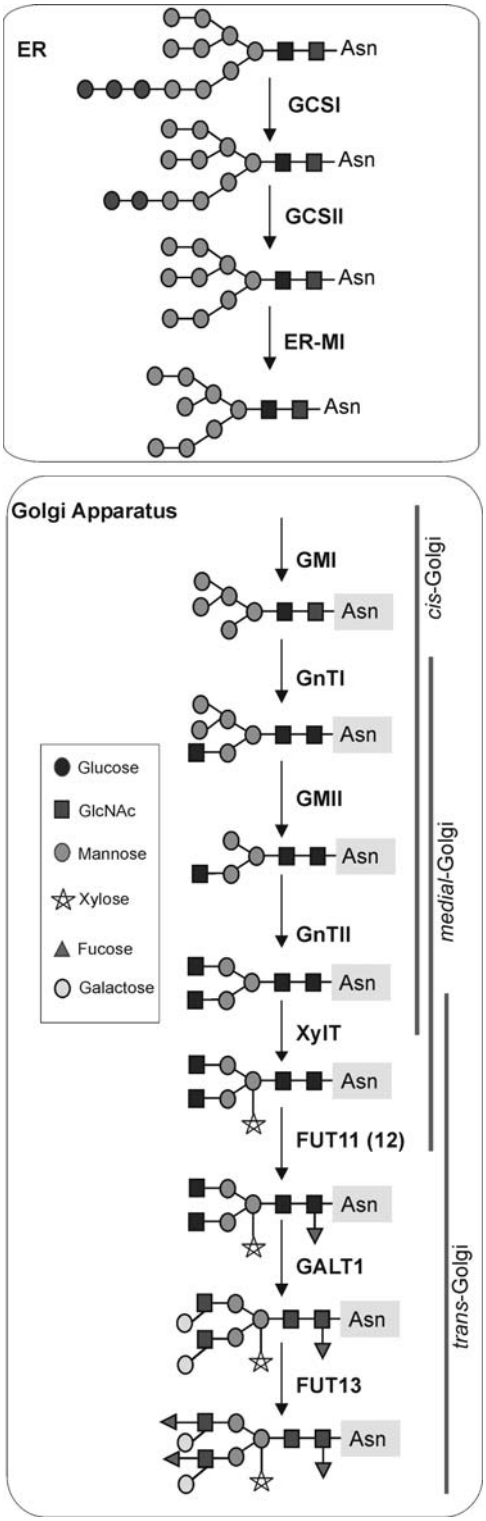
Enzyme	C-tail sequence	T-length[1]	Gene ID or Acc. Nr.	Reference
GCSI	MTGAS**RR**SA**R**G**RIK**SSSLSPGSDEGSAYPPSI**RR**G**K**G**K**ELVSIGAF**K**TNL**K**	18	At1g67490	(Saint-Jore-Dupas et al., 2006)
GMI	MA**R**GS**R**SVGSSSS**KWR**YCNPSYYL**KR**P**KR**	16	AAF16414.1	(Nebenführ et al., 1999)
GnTI	M**R**GY**K**FCCDF**R**	18	CAB53347	(Saint-Jore-Dupas et al., 2006; Schoberer et al., 2008)
GMII	MPFSSYIGNS**RR**SSTGGGTGGWGQSLLPTALSKS**K**LAIN**RK**P**RKR**TLVVN	22	At5g14950	(Strasser et al., 2006)
GnTII	MANLW**KK**Q**R**L**R**D	23	At2g05320	(Strasser et al., 1999a)
XylT	MS**KR**NP**K**IL**K**	23	At5g55500	(Strasser et al., 2000)
FUT11	MGVFSNL**R**GP**K**IGLTHEELPVVANGSTSSSSSPSSF**KRK**	18	At3g19280	(Wilson et al., 2001)
GALT1	M**KR**FY	18	At1g26810	(Strasser et al., 2007a)
FUT13	MPM**R**	23[2]	At1g71990	
ST	MIHTNL**KKK**	17	AAA41196	(Weinstein et al., 1987)

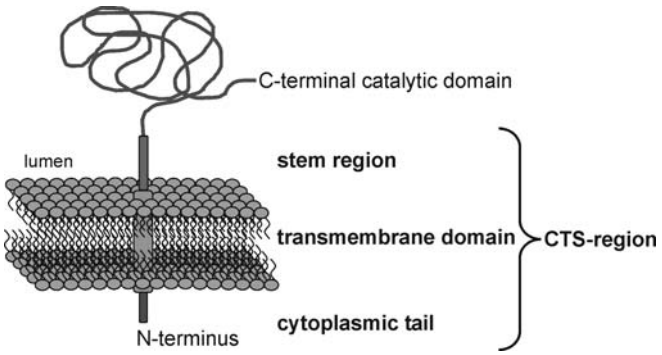
[1]as described in the references or [2]shown as consensus prediction at http://aramemnon.botanik.uni-koeln.de/

Figure legends

Figure 1. Processing steps of N-glycans in the ER and Golgi apparatus are shown. GCSI: α-glucosidase I; GCSII: α-glucosidase II; ER-MI: ER-α-mannosidase I; GMI: Golgi-α-mannosidase I; GnTI: β1,2-N-acetylglucosaminyltransferase I; GMII: Golgi-α-mannosidase II; GnTII: β1,2-N-acetyl-glucosaminyltransferase II; XylT: β1,2-xylosyltransferase; FUT11(12): core α1,3-fucosyltransferase; GALT1: β1,3-galactosyltransferase; FUT13: α1,4-fucosyltransferase. Based on data from the analyses of substrate specificity of recombinant enzymes (GMII, GnTII and XylT) and N-glycan profiles in knockout plants an alternative processing route is also possible in the medial-Golgi (Bencúr et al., 2005; Strasser et al., 2006).

Figure 2. The modular domain architecture of plant glycosyltransferases and glycosidases involved in N-glycan processing. These enzymes are typically type II membrane proteins with an N-terminal cytoplasmic tail, a single pass transmembrane domain, a luminal stem and C-terminal catalytic domain. The cytoplasmic tail, transmembrane domain and stem region (abbreviated CTS region) are usually sufficient for ER or Golgi localization in mammalian and plant cells.


ER
Asn
GCSI
Asn
GCSII
Asn
ER-MI
Asn
Golgi Apparatus
GMI
Asn
GnTI
Asn
GMII
Asn
GnTII
Asn
XylT
Asn
FUT11 (12)
Asn
GALT1
Asn
FUT13
Asn
Glucose
GlcNAc
Mannose
Xylose
Fucose
Galactose
cis-Golgi
medial-Golgi
trans-Golgi


C-terminal catalytic domain
lumen
stem region
transmembrane domain
CTS-region
cytoplasmic tail
N-terminus