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# Trafficking and localization of Golgi-resident *N*-glycan processing enzymes in plants

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Asparagine (*N*)-linked glycosylation is a fundamental co- and post-translational modification of proteins, playing a crucial role in protein folding, stability and function, protein-protein interactions, biotic and abiotic stress response as well as glycan-dependent quality control processes in the endoplasmic reticulum (ER). Protein *N*-glycosylation is initiated in the ER and continued in the Golgi apparatus by *N*-glycan-processing glycosyltransferases and glycosidases, which are compartmentalized in a highly organized manner reflecting their function in the sequential modification of glycans. Therefore, the precise localization of these enzymes is crucial for the optimal functioning of the glycosylation process and the secretory pathway and hence must be tightly regulated to maintain protein function, cellular health, and overall organismal development. Here, we highlight recent developments that contribute to a better understanding of the localization mechanisms of this important class of Golgi residents and discuss future directions to move the field forward.

## KEYWORDS

plants, Golgi apparatus, glycosylation, *N*-glycan processing, *N*-glycan processing enzymes, protein transport, glycoengineering

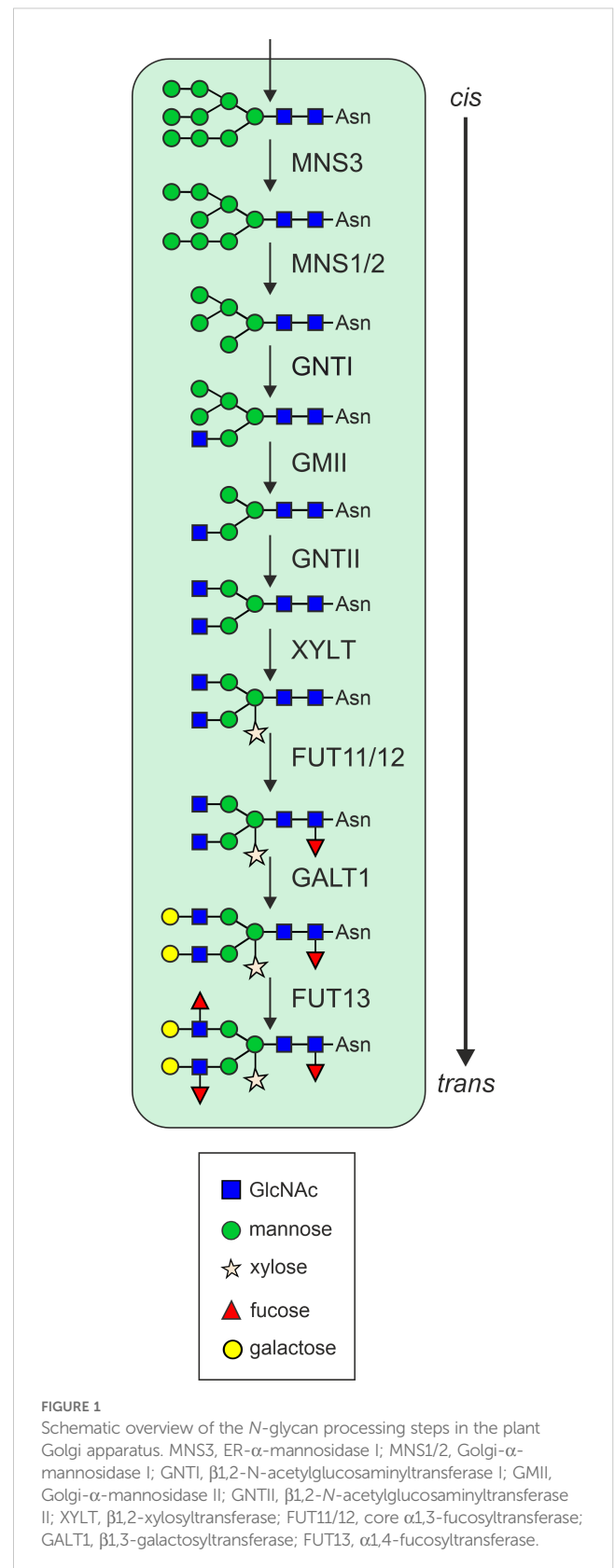
## Introduction

The *N*-glycosylation pathway begins in the ER, where the oligosaccharyltransferase complex catalyzes the *en bloc* transfer of a pre-assembled oligosaccharide precursor (Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>) from the lipid carrier dolichol pyrophosphate to asparagine (Asn or *N*) residues within the specific consensus sequence Asn-X-Ser/Thr (X can be any amino acid except proline) of nascent proteins (Helenius and Aebi, 2001; Moremen et al., 2012; Strasser, 2016). Subsequently, the two outmost glucose residues are cleaved by  $\alpha$ -glucosidase (GCS) I and GCSII, resulting in the generation of a monoglucosylated *N*-glycan. This glycan can interact with the lectins calnexin (CNX) and calreticulin (CRT), thereby promoting folding. The release from the CNX/CRT interaction depends on the trimming of the remaining glucose by GCSII. Proteins that fail to attain their final conformation are subjected to ER-associated degradation (ERAD), a process that involves the trimming of a specific mannose residue by the class I  $\alpha$ -mannosidases MNS4 and MNS5 (Hüttner et al., 2014). This processing step produces a glycan

degradation signal on the ERAD substrate, which is recognized by the lectin OS9 (Clerc et al., 2009; Quan et al., 2008; Strasser, 2018). Proteins that fold correctly, carry the  $\text{Man}_9\text{GlcNAc}_2$  oligosaccharide, which is subsequently subjected to further processing by the class I  $\alpha$ -mannosidases ER- $\alpha$ -mannosidase I (MNS3) and Golgi- $\alpha$ -mannosidase I (MNS1/2; Liebminger et al., 2009) in the Golgi apparatus (Figure 1). This collective action results in the removal of four  $\alpha$ 1,2-linked mannose residues from the *N*-glycan. The resulting  $\text{Man}_5\text{GlcNAc}_2$  *N*-glycan is the final product of early *N*-glycan processing steps and serves as the acceptor substrate for *N*-acetylglucosaminyltransferase I (GNTI), which adds a single *N*-acetylglucosamine (GlcNAc) residue, thereby initiating the formation of hybrid and complex *N*-glycans in the Golgi apparatus (Strasser et al., 1999). The product of this reaction is further processed by Golgi- $\alpha$ -mannosidase II (GMII), *N*-acetylglucosaminyltransferase II (GNTII),  $\beta$ 1,2-xylosyltransferase (XYLT) and core  $\alpha$ 1,3-fucosyltransferase (FUT11/12; Strasser et al., 2006). The final, mature *N*-glycans are of the complex type and are typically characterized by the presence of  $\beta$ 1,2-xylose and  $\alpha$ 1,3-fucose (GnGnXF), which is considered to be the predominant plant glycoform (Zeng et al., 2018; Strasser et al., 2021). Variations thereof may lack terminal GlcNAc on one of the two branches (GnMXF, MGnXF) and additionally lack xylose and/or fucose (GnGnF, GnGnX, GnMX, MGnX, GnMF, MGnF, GnGn, GnM, MGn). Complex *N*-glycans can be converted into paucimannosidic *N*-glycans, which lack the two terminal GlcNAc residues (MM, MMF, MMX or MMXF) in plants. This processing step occurs in post-Golgi compartments and is carried out by either the vacuolar  $\beta$ -*N*-acetylhexosaminidase 1 (HEXO1) or HEXO3, which mainly resides in the plasma membrane/apoplast (Strasser et al., 2007a; Liebminger et al., 2011; Alvisi et al., 2021). The most elaborate occurring glycoform in plants is the Lewis A structure, which is generated in the *trans*-Golgi by  $\beta$ 1,3-galactosyltransferase (GALT1) and  $\alpha$ 1,4-fucosyltransferase (FUT13) via the modification of GnGnXF structures (Strasser et al., 2007b). Lewis A *N*-glycans are ubiquitously found in plants (Fitchette et al., 1999; Wilson et al., 2001), but are only present on a few glycoproteins (Beihammer et al., 2021).

While the initial steps of *N*-glycosylation in the ER are highly conserved among eukaryotes, the processing steps in the Golgi show distinct differences between plants and mammals (Schoberer and Strasser, 2018). The presence of  $\beta$ 1,2-xylose and  $\alpha$ 1,3-fucose is a defining feature of plant *N*-glycosylation, as these sugars are absent in mammalian systems (Strasser, 2016). This structural difference may contribute to the antigenicity of some plant proteins and increase the risk of adverse reactions, such as allergic reactions or immunological responses, when plant-produced recombinant glycoproteins with  $\beta$ 1,2-xylose and  $\alpha$ 1,3-fucose are used in mammals in a therapeutic context (Jenkins et al., 1996; Bardor et al., 2003; Shaaltiel and Tekoah, 2016).

Our understanding of the role of *N*-glycans in plants is primarily derived from the study of mutants deficient in *N*-glycan biosynthesis and processing. These studies have demonstrated that defects in *N*-glycosylation can result in a range of adverse effects, including abnormal plant development and/or reduced stress



tolerance (Kang et al., 2008; Fanata et al., 2013; Pedersen et al., 2017; Kaulfürst-Soboll et al., 2021). Mutations in genes encoding enzymes involved in the biosynthesis and early processing of *N*-

glycans are embryo lethal (Strasser, 2014), whereas mutants of enzymes involved in *N*-glycan processing in the Golgi do not show any significant morphological phenotypes under normal growth conditions but may have conditional phenotypes (Strasser, 2022). This shows that the functions of *N*-glycans in the ER are essential, whereas Golgi-derived complex *N*-glycans are dispensable for plant survival but may have additional roles under non-physiological conditions.

## The plant Golgi apparatus and its role in *N*-glycan processing

The Golgi apparatus is the central biosynthetic organelle in the secretory pathway and the main site for the modification and sorting of proteins and lipids, and the biosynthesis of cell wall polysaccharides in plants (Foresti and Denecke, 2008). The plant Golgi apparatus has a unique morphology as it comprises numerous discrete stacks of flattened membrane compartments, classified into *cis*-, medial- and *trans*-Golgi cisternae. Plant Golgi stacks display an actin-myosin-driven mobility, distributing them throughout the

cytoplasm in higher plants (Faso et al., 2009; Hawes et al., 2010). Golgi cisternae compartmentalize specific populations of resident proteins that catalyze the step-wise processing of *N*-glycans (Hawes and Satiat-Jeunemaitre, 2005; Schoberer and Strasser, 2011) or the assembly of cell wall polysaccharides (Moore et al., 1991; Oikawa et al., 2013). Golgi-localized *N*-glycan processing enzymes are type II transmembrane proteins comprising an N-terminal cytoplasmic tail (C), a single transmembrane domain (T) and a luminal stem region (S), collectively referred to as the CTS domain, which is linked to the large catalytic domain in the Golgi lumen (Figure 2). The localization of these enzymes within the Golgi stack is highly organized and closely linked to their *in vivo* substrate specificities. Each enzyme produces the substrate for the next, thereby forming an assembly line that enables the sequential modification of glycoproteins (Figure 1). Consequently, an enzyme's specific role in the pathway is reflected by its enrichment in distinct regions of the Golgi stack, rather than by its precise confinement to a single cisterna (Saint-Jore-Dupas et al., 2006; Staehelin and Kang, 2008; Schoberer and Strasser, 2011). This results in the formation of a biochemical gradient across the stack from *cis* to *trans*, with enzymes that act early in the *N*-glycan processing pathway

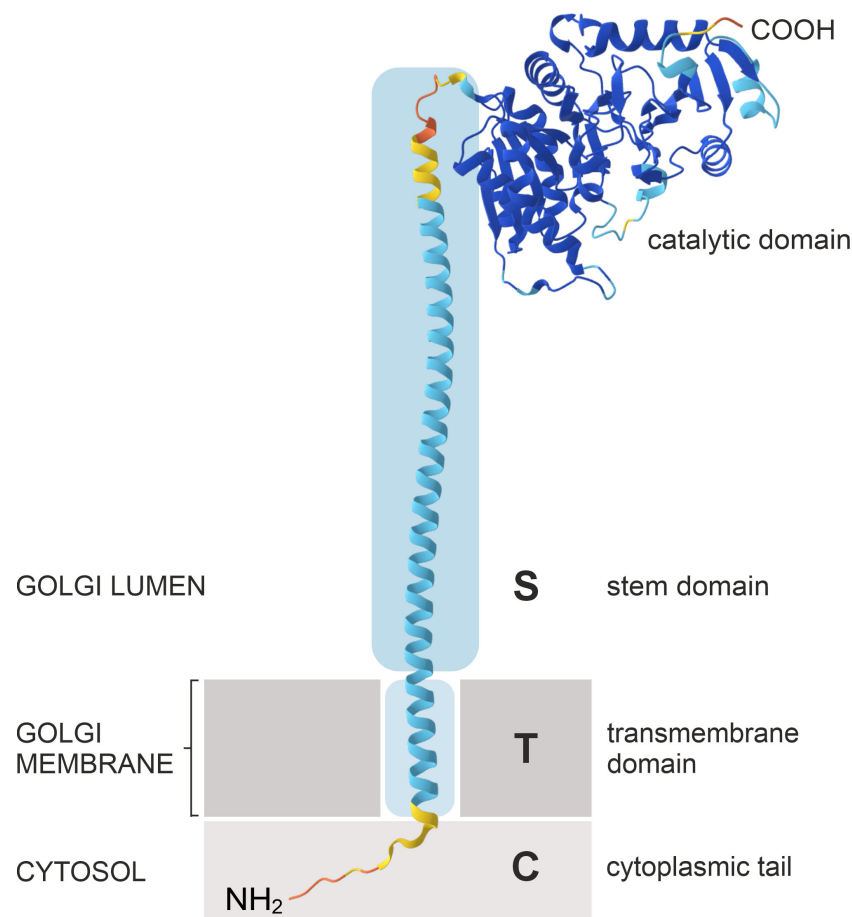


FIGURE 2

Schematic illustration of the predicted protein structure and domain organization of *N. tabacum* GNTI. NtGNTI is used as a representative model of plant Golgi *N*-glycan processing enzymes, which have a type II membrane topology, with the N-terminus (NH<sub>2</sub>) residing in the cytosol. This model is based on AlphaFold and DeepTMHMM (Hallgren et al., 2022) protein structure predictions.

residing in *cis*- and medial-Golgi cisternae, whereas later-acting enzymes concentrate in medial- and *trans*-Golgi cisternae or the *trans*-Golgi network (TGN). This non-uniform, subcompartment-specific distribution pattern of Golgi-localised glycosidases and glycosyltransferases remains constrained despite the constant flux of cargo molecules and the constant mobility of the Golgi apparatus (Boevink et al., 1998) and provides a valuable tool for studying various aspects of Golgi (protein) organization, trafficking and biogenesis (Hawes et al., 2010; Schoberer et al., 2010).

The initial evidence supporting the subcompartmentation of glycosidases and glycosyltransferases within the plant Golgi apparatus was based on the localization of the polysaccharide and glycoprotein products rather than the enzymes themselves (Moore et al., 1991; Lynch and Staehelin, 1992; Zhang and Staehelin, 1992). This was due to the absence of cloned plant Golgi-resident enzymes at that time. The cloning of the genes encoding plant glycosylation enzymes, coupled with the advent of fluorescent protein technology and confocal microscopy, has allowed the definitive determination of their subcellular localization within plant Golgi stacks (Essl et al., 1999; Dirnberger et al., 2002; Strasser et al., 2005; Saint-Jore-Dupas et al., 2006; Strasser et al., 2007b; Liebminger et al., 2009; Schoberer et al., 2009). The results of several studies have indicated that the fluorescent signals emitted by distinct co-expressed *N*-glycan processing enzymes do not entirely overlap within Golgi stacks, thereby substantiating the prevailing notion of a non-uniform distribution of glycosylation enzymes (Figure 3). This observation prompted extensive research to identify the precise signals and mechanisms by which this non-uniform Golgi distribution is established, maintained and regulated. It became evident early on that in plants the information required for the Golgi targeting and retention of plant *N*-glycan processing enzymes is present in their CTS region. The expression of the fluorescent protein-tagged CTS region of all plant *N*-glycan processing enzymes studied so far showed that the catalytic domain is not required for their targeting to the Golgi as their Golgi localization remained unchanged compared to the full-length enzymes (Essl et al., 1999; Dirnberger et al., 2002; Saint-Jore-Dupas et al., 2006; Schoberer et al., 2009, 2010; Schoberer and Strasser, 2011). This is in agreement with

mammalian and yeast studies that localised the necessary signals for Golgi targeting to the transmembrane domain (TMD) and neighboring polypeptide regions of the enzymes (Munro, 1991; Nilsson et al., 1991; Colley, 1997). Furthermore, a fluorescent protein fusion to the CTS region of the rat  $\alpha$ 2,6-sialyltransferase (ST) has become the most commonly used Golgi marker in plants, with the protein localised to the *trans*-half of Golgi stacks in leaves of *Nicotiana clelandii* as well as in callus tissue and root tips of *Arabidopsis* as demonstrated by electron microscopy (EM) (Boevink et al., 1998; Wee et al., 1998; Reichardt et al., 2007). This localization is consistent with the fact that the sialylation of glycoproteins by ST is a late *N*-glycan modification event in the Golgi of mammalian cells and it suggests that, despite the known differences between the plant and mammalian Golgi apparatus, the Golgi targeting signals and mechanisms are largely conserved between plants and mammals. This has been supported by studies showing that the plant and mammalian GNTs can complement each other (Gomez and Chrispeels, 1994; Bakker et al., 1999) and that Golgi-targeted mammalian glycosyltransferases such as ST are functional when transiently or stably expressed in plants (Castilho et al., 2010; Kallolimath et al., 2016).

## Models for protein traffic through the Golgi

Several models have been proposed to elucidate the distinct localization of glycosylation enzymes within *cis*-, medial-, and *trans*-Golgi cisternae. These models seek to elucidate how enzymes are sorted, retained or recycled within different Golgi cisternae, thereby ensuring the optimal functioning of glycosylation processes. Nevertheless, models that address the sorting and concentration of glycosylation enzymes fundamentally depend on the characteristics of protein transport through the Golgi apparatus (Glick and Nakano, 2009). This highly regulated process can entail anterograde (forward) movement from *cis*- to *trans*-Golgi cisternae and retrograde (backward) movement between cisternae or from the Golgi to the ER (Figure 4). Several mechanisms have been put

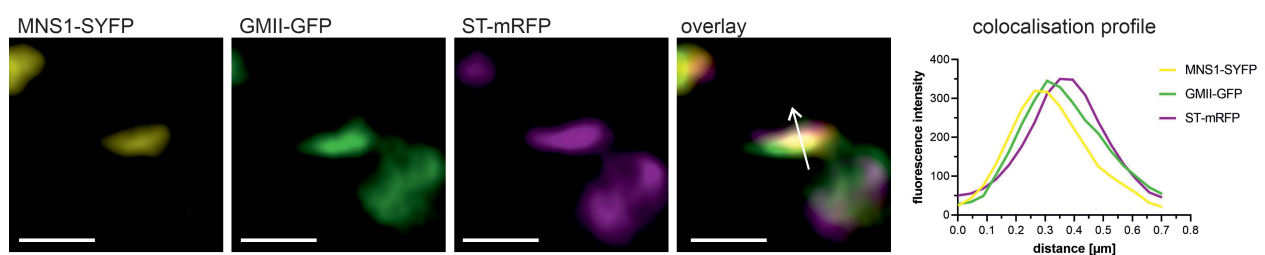


FIGURE 3

Co-localization of fluorescent protein-labelled *N*-glycan processing enzymes within the Golgi. Confocal microscopy images show a single, highly magnified Golgi stack triple-labelled with three different *N*-glycan processing enzymes with distinct intra-Golgi distributions. The fusion proteins MNS1-SYFP (CTS region fused to yellow fluorescent protein, in yellow), GMII-GFP (CTS region fused to green fluorescent protein, in green), and ST-mRFP (CTS region fused to red fluorescent protein, in magenta) were transiently co-expressed in *N. benthamiana* leaf epidermal cells and imaged using a Zeiss LSM980 Airyscan 2 confocal microscope. Images were further processed with the ZEN module Airyscan joint deconvolution. The co-localization profile shows the fluorescence intensities of the fusion proteins, plotted along the white arrow in the overlay image. Note the shifts in the fluorescence intensity peaks, indicating an enrichment of enzymes in distinct Golgi regions. Scale bar = 1  $\mu$ m.

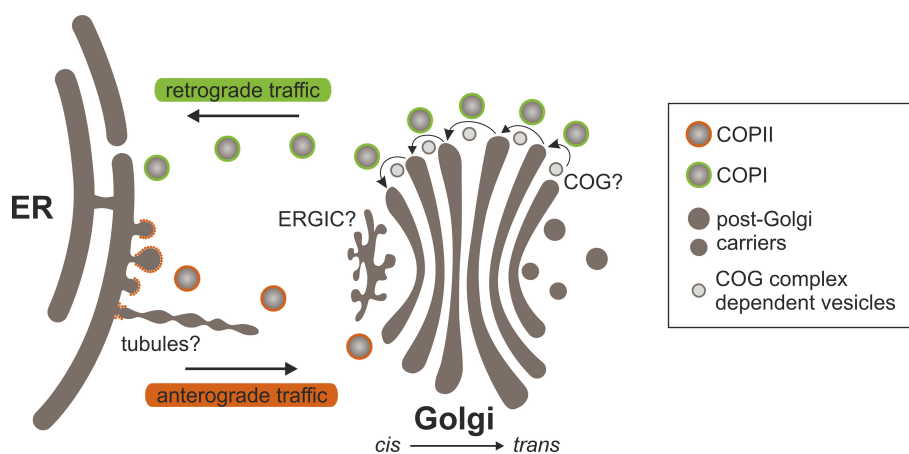


FIGURE 4

Protein transport between the ER and the Golgi. At ERES, cargo proteins are recruited and packaged into COPII transport carriers (vesicles and/or tubules) that bud from and exit the ER. COPII components can coat vesicles and/or decorate the neck of tubules. Carriers arrive at and fuse with *cis*-Golgi cisternae, releasing their contents. Cargo proteins move through the Golgi in a *cis*-to-*trans* direction in anterograde transport vesicles and/or via cisternal maturation. Anterograde transport probably involves elements of both models. Secretory proteins exit the Golgi at the *trans* face in post-Golgi transport carriers. Golgi residents are returned to earlier Golgi cisternae or the ER in COPI-coated vesicles that bud from Golgi cisternae. It is still unclear whether the COG complex plays a role in the retrograde intra-Golgi transport of Golgi-resident *N*-glycan processing enzymes. Another mystery is the functional role of the ERGIC in ER-Golgi transport. It may serve as an intermediate compartment where anterograde and retrograde transport pathways to and from the Golgi converge.

forth to explain the transport of glycosylation enzymes through the Golgi apparatus.

The vesicular transport model was influential in early mammalian cell biology and is based on the discovery “of machinery regulating vesicle traffic, a major transport system in our cells”, which led to the awarding of the 2013 Nobel Prize in Physiology or Medicine to James Rothman, Randy Schekman and Thomas Südhof (Zierath and Lendahl, 2013). It suggests that Golgi cisternae are stable compartments that house an unchanging set of Golgi-resident proteins (including glycosylation enzymes). Anterograde transport vesicles that form in the Golgi arrive at each cisterna with cargo proteins that are modified by resident enzymes within the cisterna. New vesicles with cargo proteins bud from the cisterna and then travel to the next stable cisterna in a *cis* to *trans* direction, where the next set of enzymes further modifies the cargo (Balch et al., 1984; Rothman and Wieland, 1996; Farquhar and Palade, 1998).

The cisternal maturation model suggests that each Golgi cisterna matures over time from the *cis* to the *trans* face of the Golgi, rather than being static, reflecting the ongoing process of protein modification and sorting. Proteins move as passengers within cisternae through the Golgi stack (Losev et al., 2006; Matsuura-Tokita et al., 2006; Emr et al., 2009). In this model, a new *cis*-Golgi cisterna matures into a medial- and then *trans*-Golgi cisterna before breaking apart as modified proteins are packaged for transport to their final destination, such as the plasma membrane or secretion outside the cell. Glycosylation enzymes are retained in specific cisternae by selective retrieval processes; enzymes that are needed in the earlier stages of glycosylation, are recycled from the later Golgi compartments back to earlier, younger cisternae by retrograde transport carriers, rather than being transported from one cisterna to another, to maintain the proper enzyme

composition (Glick et al., 1997; Pelham, 1998). Yeast provided the most direct evidence via real-time tracking of cisternal markers and cargo. Meanwhile, algal and mammalian studies highlighted the model’s applicability to diverse cargo types, including bulky proteins.

In the rapid partitioning model, the Golgi apparatus is considered a highly dynamic two-phase membrane system, in which secretory cargo proteins and resident glycosylation enzymes are continuously and rapidly partitioned into specific lipid domains of optimal composition, so-called “export domains”, which are enriched in cargo and “processing domains”, which are enriched in Golgi-resident enzymes (Patterson et al., 2008). Cargo moves forward by stochastically exiting from export domains at any cisternae. This model accounts for the high efficiency of protein sorting and processing in the Golgi while maintaining its compartmentalized structure; however, experimental evidence is currently limited to mammalian cells.

The cisternal progenitor model proposes that new cisternae are continuously formed at the *cis*-face as progenitors or “precursors” of mature cisternae (Pfeffer, 2010). These progenitor cisternae then gradually mature as they move through the Golgi stack from *cis* to *trans*, where they are eventually disassembled or recycled to form new cisternae. As the cisternae mature, they progressively acquire different enzymes that process cargo proteins in stages. RAB GTPases play a crucial regulatory role in this model by coordinating the formation, maturation, and function of Golgi cisternae as they move through the stack. RAB GTPases are a family of small GTP-binding proteins that act as molecular switches, switching between active (GTP-bound) and inactive (GDP-bound) states. Their primary role is to regulate vesicular trafficking including cargo sorting, vesicle budding, and fusion by recruiting specific effector proteins (Woollard and Moore, 2008;



Stenmark, 2009). In the cisternal progenitor model, RABs are thought to act in a cascade in which one RAB controls the recruitment of the next RAB, orchestrating the sequential maturation of cisternae. As a cisterna matures from *cis* to *trans*, another set of RABs takes over to regulate this progression. This cascade helps maintain the spatial organization of the Golgi and ensures efficient processing and sorting of proteins. This model takes into account both the maturation of cisternae and the dynamic recycling or exchange of membrane material, and it attempts to explain how the Golgi can maintain its structure and function while continuously processing large amounts of protein. This model has experimental support primarily in yeast (Rivera-Molina and Novick, 2009).

In plants, no experimental evidence has yet been presented to support the cisternal progenitor or rapid partitioning models. Instead, experimental evidence collectively favors the cisternal maturation model. For example, the interference with the retrograde transport machinery leads to the incorrect localization of Golgi residents and defects in Golgi stack morphology, which is consistent with impaired cisternal maturation, as the proper recycling of resident proteins and maintenance of cisternal identity are disrupted. Furthermore, large cargo molecules, such as cell wall polysaccharides, were retained in the cisternae and not recycled (Hawes and Satiat-Jeunemaitre, 2005; Glick and Nakano, 2009; Hawes et al., 2010; Robinson, 2020; Rui et al., 2022). These findings suggest that cisternal maturation may be a universal and evolutionarily conserved mechanism across all eukaryotes.

## Models for Golgi enzyme localization

Irrespective of the mechanisms by which Golgi proteins are transported through the Golgi, several models have been proposed to explain how the distinct sub-Golgi localization of glycosylation enzymes is established (Table 1).

## Golgi retention via enzyme oligomerization

In the kin recognition model, Golgi enzymes form multimeric enzyme complexes that are unable to enter anterograde transport carriers due to their size, leading to the retention within specific Golgi cisternae (Nilsson et al., 1993). This complex formation may also allow for coordinated enzyme activity, ensuring that glycosylation occurs in the correct sequence as glycoproteins move through the Golgi. There is ample experimental evidence that the signals influencing oligomerization reside in the TMD and/or stem domain; however, the requirements for enzyme oligomerization vary greatly (Tu and Banfield, 2010). For example, the stem region of human NAGTI is the primary determinant for the heteromerization with mannosidase II (MannII), which is critical for their localization to the medial-

Golgi in HeLa cells (Nilsson et al., 1994). The oligomerization of the sialyltransferase ST6Gal1, primarily via its TMD, is critical for its localization to the *trans*-Golgi (Chen and Colley, 2000). In plants, *N*-glycan processing enzymes such as MNS1, GNTI, GMII and XYLT have been shown to form *in planta* homo- and heterodimers in the *cis*- and medial-Golgi via their CTS region (Schoberer et al., 2013). Further biochemical approaches and confocal microscopy in *N. benthamiana* have demonstrated the role of the GNTI stem region in mediating homo- and heteromeric complex formation (Schoberer et al., 2014). A more recent study showed that a conserved nine-amino acid sequence motif in the stem was responsible for the GNTI-GNTI homodimerization, which led to a block in *N*-glycan processing increasing oligo-mannosidic *N*-glycans upon transient GNTI-CTS overexpression in *N. benthamiana* (Schoberer et al., 2023; Figure 5).

Of note, the formation of enzyme complexes is a common means of localizing enzymes involved in the biosynthesis of cell wall polysaccharides in the Golgi (Zabotina et al., 2021; Table 1). In contrast, homo- or heteromerization of *N*-glycan processing enzymes does not seem to be required for correct Golgi localization or catalytic activity.

## Golgi retention via transmembrane domains

The lipid bilayer model suggests that different membrane thicknesses within the Golgi stack naturally sort glycosylation enzymes into the appropriate locations based on their TMD length, preventing them from moving to the wrong compartment. The thickness of membranes increases progressively from the ER to the plasma membrane (PM). A mammalian study indicated that proteins localised in the ER and Golgi tend to have shorter TMDs than those in the PM, which suggests that shorter TMDs may result in retention in the Golgi apparatus, whereas longer TMDs facilitate progression to post-Golgi compartments and the PM (Welch and Munro, 2019). It has been demonstrated that the length of the TMD is a significant factor in the targeting of type I transmembrane proteins in plants (Brandizzi et al., 2002b). Immuno-EM studies demonstrated that an increase in TMD length from 16 to 23 residues resulted in the translocation of soybean  $\alpha$ -mannosidase I (GmManI) from the *cis*- to the *trans*-Golgi (Saint-Jore-Dupas et al., 2006). Furthermore, the TMD of ER-localised GCSI would direct the protein to the Golgi, but for ER localization it requires ER retention signals, such as four arginine residues within its 13-amino-acids cytoplasmic N-terminus or the 60-amino-acids luminal C-terminus (Saint-Jore-Dupas et al., 2006; Boulaflous et al., 2008). However, this model does not consider that the TMD length of Golgi-resident enzymes varies considerably (Figure 6A). The *trans*-Golgi enzymes ST and GALT1, for example, have a rather short membrane-spanning region with a length of 17 and 15 amino acids, respectively, which is in a similar range to the predicted TMD lengths of the *cis*/medial-located

TABLE 1 Sorting signals of various ER- and Golgi-resident membrane proteins.

Role in localisation	Protein	Organism	Topology	Interaction partner	Reference
<b>ER export via anterograde transport</b>					
Arginine/lysine-based cytoplasmic tail motifs					
R/K	prolyl 4-hydroxylase	plants	type II		Yuasa et al., 2005
KK	bZIP28	plants	type II	SAR1b	Srivastava et al., 2012
R/K	GNT1, GMII, XYLT	plants	type II		Schoberer et al., 2009
(RK)X(RK)	GalT2, GalNAcT, SialT2	mammals	type II	SAR1	Giraud and Maccioni, 2003; Quintero et al., 2010
Hydrophobic residues in the cytoplasmic tail					
FVXXXY	EMP12	plants	multi-TMD		Gao et al., 2012
FF	ERGIC-53	mammals	type I	SEC23/SEC24	Kappeler et al., 1997
YYXF	Emp47p, Emp46p	yeast	multi-TMD		Schröder et al., 1995; Sato and Nakano, 2002
YNNSN and LXXME	Sed5p	yeast	type II	SEC24	Mossessova et al., 2003
LXXLE plus dibasic R/K motif	BET12	plants	type II	SAR1	Chung et al., 2018
LXXLE	Bet1p	yeast	type II	SEC24	Mossessova et al., 2003
Diacidic cytoplasmic tail motifs					
(D/E)XE	GONST1	plants	multi-TMD		Hanton et al., 2005
	CASP	plants	type II		Hanton et al., 2005
	KAT1	plants	multi-TMD	SEC24A	Mikosch et al., 2006; Sieben et al., 2008
EXXD	SYP31	plants	type II		Chatre et al., 2009
<b>ER retention via retrograde transport</b>					
Dilysine-based cytoplasmic tail motifs					
KKXX	p24 family proteins	plants	type I	β'-COP, ARF1	Contreras et al., 2004; Langhans et al., 2008; Gao et al., 2014
KKXX	Cf-9	plants	type I		Benghezal et al., 2000
KXXXX	FAD3	plants	multi-TMD		McCartney et al., 2004
KK	GPAT8	plants	multi-TMD		Gidda et al., 2009
Arginine-based cytoplasmic tail motifs					
RR, RXR or RXXR (plus luminal domain)	GCSI	plants	type II		Boulaflous et al., 2008
RRXXXXR	M1-SAT-I (ST3GAL5)	mammals	type II		Uemura et al., 2009
Hydrophobic cytoplasmic tail motifs					
φ-X-X-K/R/D/E-φ	FAD2	plants	multi-TMD		McCartney et al., 2004
	GPAT9	plants	multi-TMD		Gidda et al., 2009
<b>Golgi retention</b>					
Cytoplasmic tail motifs					
LPYS	MNS3	plants	type II		Schoberer et al., 2019b
φ-(K/R)-X-L-X-(K/R)	GALNT3, GALNT8, C2GNT1	mammals	type II	β-, δ- and ζ-COP	Liu et al., 2018
	GALNT6	mammals	type II	β- and δ-COP	Liu et al., 2018

(Continued)

TABLE 1 Continued

Role in localisation	Protein	Organism	Topology	Interaction partner	Reference
<b>Golgi retention</b>					
WX(n1-6)(W/F)	GALNT4	mammals	type II	$\beta$ -, $\delta$ - and $\zeta$ -COP	Liu et al., 2018
Adaptor-binding via cytoplasmic tail					
(F/L)-(L/I/V)-X-X-(R/K)	<i>cis</i> - and medial Golgi mannosyltransferases, e.g.:				
	Kre2, Och1, Mnn9, Mnn2, Mnn5, Ktr6	yeast	type II	Vps74p	Tu et al., 2008, 2012
L-X-X-(R/K)	glycosphingolipid biosynthetic enzymes, O- and N-glycosylation enzymes, e.g.:				
	LCS, GD3S, GM3S, Gb3S	mammals	type II	GOLPH3	Rizzo et al., 2021
	POMGNT1, GALNT12	mammals	type II	GOLPH3	Pereira et al., 2014
	C1GALT1, GALNT2, GALNT7, GCNT1, MGAT1, MGAT2	mammals	type II	GOLPH3/GOLPH3L	Welch et al., 2021
	SiaT2 (ST6GAL1)	mammals	type II	GOLPH3/GOLPH3L	Eckert et al., 2014; Isaji et al., 2014; Welch et al., 2021
	C2GNTI (GCNT1)	mammals	type II	GOLPH3	Ali et al., 2012; Eckert et al., 2014
Via transmembrane domain					
glutamine (Q)	GNTI	plants	type II		Schoberer et al., 2014
cysteine (C) and histidine (H)	$\beta$ 1,4-GALT	mammals	type II		Aoki et al., 1992
length	ManI	plants	type II		Saint-Jore-Dupas et al., 2006
receptor interaction	ERManI	yeast	type II	Rer1p	Massaad et al., 1999
Via enzyme oligomerisation					
heteromerisation via stem	NAGTI	mammals	type II	MannII	Nilsson et al., 1994
homomerisation via stem	GNTI	plants	type II	GNTI	Schoberer et al., 2014, 2023
homomerisation via TMD	$\beta$ 1,4-GALT	mammals	type II	$\beta$ 1,4-GALT	Yamaguchi and Fukuda, 1995

ϕ are hydrophobic amino acid residues, X denotes any amino acid.

enzymes MNS1 and GNTI, but much shorter than the TMD of GMII in the medial-Golgi (Figure 6; Saint-Jore-Dupas et al., 2006; Strasser et al., 2006; Liebminger et al., 2009; Schoberer et al., 2009; Schoberer and Strasser, 2011). A study investigating the TMDs of integral membrane proteins in fungi and mammals revealed organelle-specific variations in the length and composition of these TMDs (Sharpe et al., 2010). However, the analysis showed no significant differences in mean residue hydrophobicity or amino acid volume between *cis*- and medial-Golgi proteins. Also, the dataset used to examine specific characteristics of transmembrane segments was relatively limited, as only a restricted number of proteins with confirmed sub-Golgi localization were available.

Nevertheless, it is evident that while the TMD is of vital importance for the anchoring and sorting of glycosylation enzymes in the Golgi, correct localization is largely dependent on the presence of additional signals. To illustrate, the cytoplasmic tail of XYLT is indispensable for its Golgi localization, as its TMD alone targeted the enzyme to the PM, PM-derived vesicles and the ER (Dirnberger et al., 2002). Similarly, the TMD of GNTI alone showed cytoplasmic localization and partial colocalization with the GFP-

tagged Golgi marker ER RETENTION DEFECTIVE 2 (ERD2). However, fusing the cytoplasmic tail of GNTI with the TMD restored its Golgi localization in *N. benthamiana* leaf cells (Schoberer et al., 2009). Furthermore, an N-terminal truncation of the cytoplasmic tail of GMII, comprising 10 amino acids most proximal to the TMD, was sufficient for its Golgi localization (Strasser et al., 2006). For the human GalNAc transferase family of mucin-type O-glycosylation enzymes, it was shown that a combination of the cytoplasmic tails and TMDs of GalNAc-T1, GalNAc-T2 and GalNAc-T7, respectively, and the combined TMD and luminal stem region of GalNAc-T7 and GalNAc-T10 resulted in Golgi localization (Becker et al., 2018).

Specific amino acids in the TMD have also been attributed a role in Golgi retention. For example, a conserved polar glutamine (Q) residue in the TMD of plant GNTI is crucial for maintaining its *cis*/medial-Golgi localization (Schoberer et al., 2019a; Figure 5). In contrast, the Golgi retention of human  $\beta$ 1,4-galactosyltransferase relies on a cysteine and histidine residue within its TMD, which confer homodimerization (Aoki et al., 1992; Yamaguchi and Fukuda, 1995).



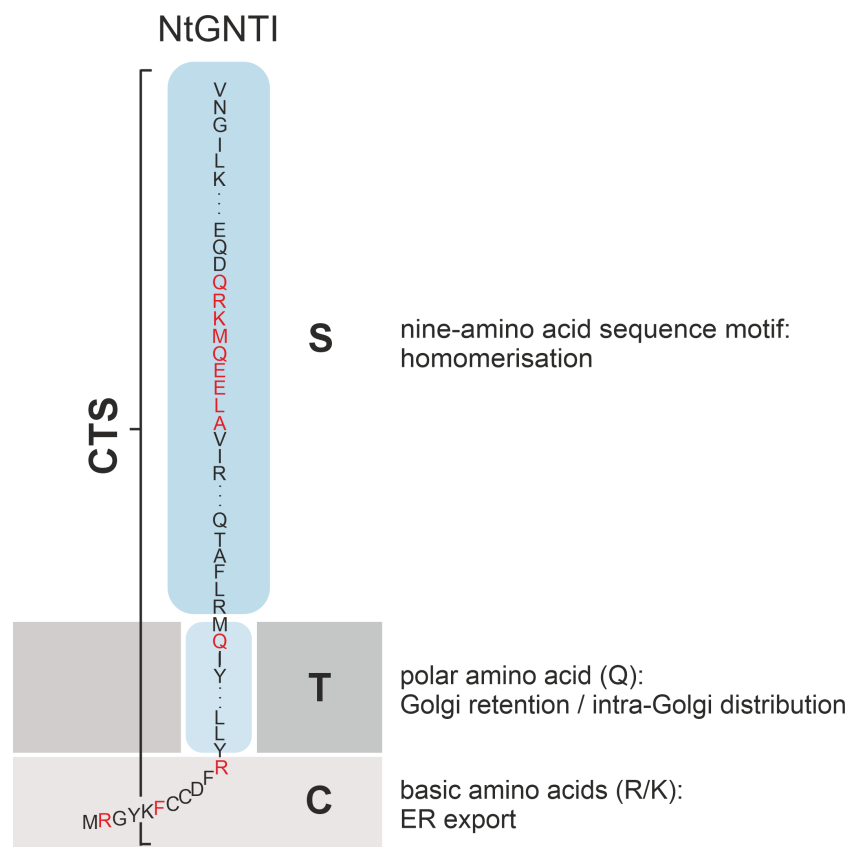


FIGURE 5

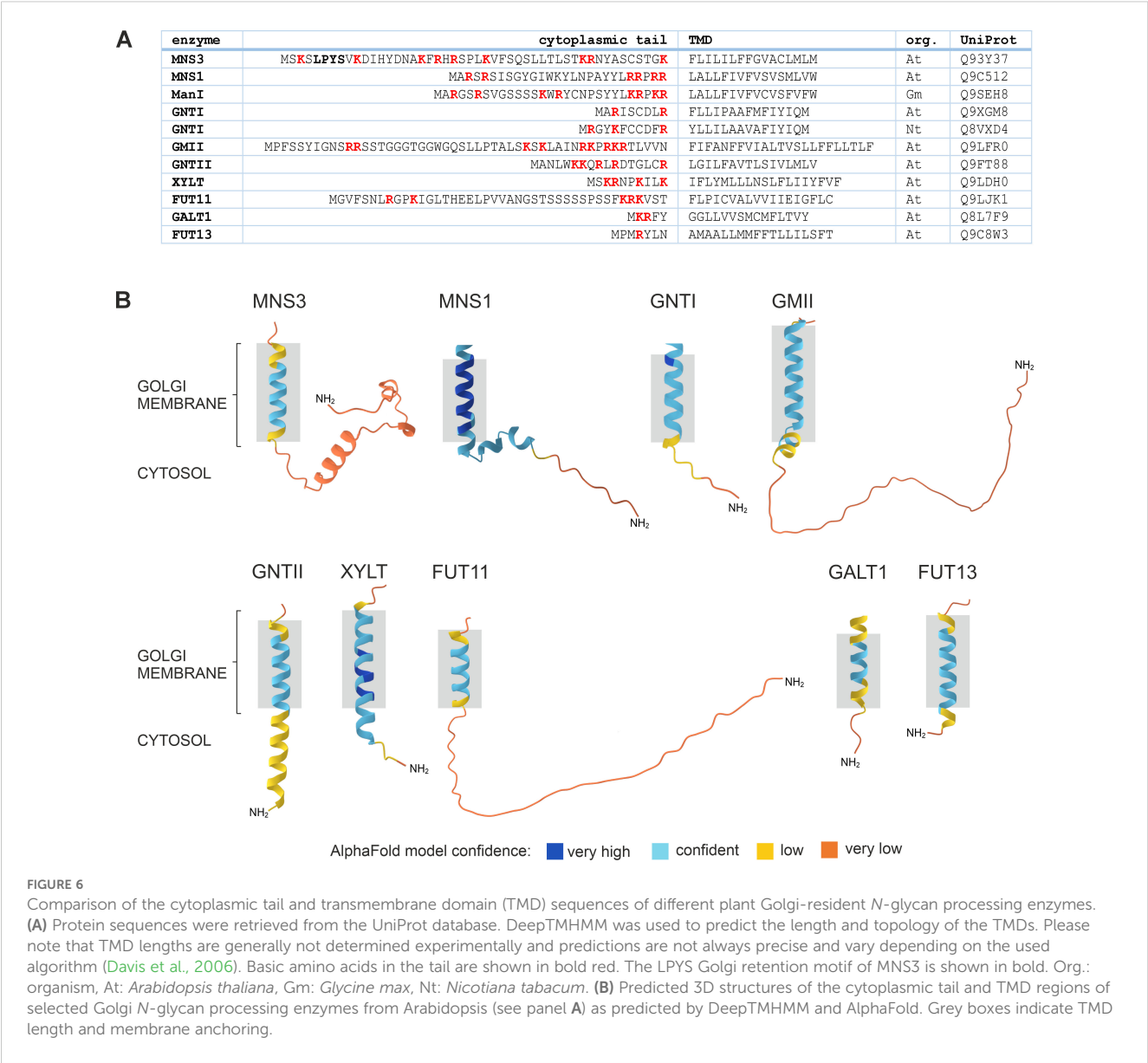
Schematic illustration showing the CTS region (cytoplasmic tail, transmembrane domain and stem domain) of NtGNTI. Amino acids and motifs in the different domains that are involved in the targeting and localization of NtGNTI to the Golgi are marked in red.

## ER export via anterograde transport

There is overwhelming evidence that the N-terminal cytoplasmic tail of *N*-glycosylation enzymes contains amino acid signal motifs that are recognized by the trafficking machinery (Table 1). The plant *N*-glycan processing enzymes GNTI, GMII and XYLT contain several arginine and/or lysine residues in their cytoplasmic tails, which are essential for ER export and Golgi localization (Figures 5, 6A). A comparison of the cytoplasmic tails of plant *N*-glycan processing enzymes reveals a clear enrichment of basic, positively charged arginine and lysine residues, which frequently occur in clusters. Interestingly, protein structure predictions suggest that the length of the tails varies considerably and that the majority lack a defined secondary structure. This is most likely to facilitate the interaction with the sorting and transport machinery via motifs therein (Figure 6B). Evidence for COPII-dependent transport was provided when the mutagenesis of the three basic amino acids in the cytoplasmic tail of GNTI impaired the recruitment of tobacco SAR1 to ER export sites (ERES) (Schoberer et al., 2009, Figure 5). SAR1 is a small GTPase that initiates the formation of coat protein complex II (COPII) carriers by recruiting coat proteins (SEC23/24, SEC13/31) to the ER membrane to form the COPII coat. This coat helps to select cargo proteins for transport to the Golgi apparatus. Upon hydrolysis of

GTP to GDP, SAR1 triggers the disassembly of the COPII coat, allowing the carrier to fuse with the Golgi. In mammals, the conserved dibasic amino acid motif (R/K)X(R/K) in the N-terminal cytoplasmic tail of mammalian glycosyltransferases involved in glycolipid synthesis has been shown to bind directly to SAR1 (Giraud and Maccioni, 2003). The same group demonstrated by cytoplasmic tail swap experiments that the information for the sub-Golgi concentration of the two glycolipid glycosyltransferases GalNAcT2 and SialT2 is present in their cytoplasmic tail (Uliana et al., 2006). Another study found that several basic amino acids located in the cytoplasmic tail of ST3Gal5, an enzyme involved in ganglioside synthesis, are important in both ER export and Golgi retention (Uemura et al., 2015). Replacement of two arginine residues (R2A/R3A) within the R/K-based motif localised the mutant not only in the Golgi but also in endosomes, resulting in the presence of immature *N*-glycans. This indicated that the R/K-based motif is essential for Golgi retention, and that retrograde transport is necessary for *N*-glycan maturation. The importance of cytoplasmic basic amino acids for ER export has also been demonstrated for a tobacco prolyl hydroxylase and the transcription factor bZIP28, both type II membrane proteins (Yuasa et al., 2005; Srivastava et al., 2012).

Despite the lack of a discernible function in the localization of *N*-glycosylation enzymes in the Golgi, cytoplasmic dihydrophobic



and diacidic signal motifs have also been shown to play a role in mediating Golgi localization. For example, hydrophobic residues in combination with a tyrosine residue (FVXXXY) play a role in the ER export of the Arabidopsis multi-TMD protein ENDOMEMBRANE PROTEIN 12 (EMP12; Gao et al., 2012). In yeast, the tyrosine-based motif YYXF found in the cargo receptors Emp47p and Emp46p, which shuttle between the ER and the cis-Golgi, is essential for their export from the ER and localization in the Golgi (Schröder et al., 1995; Sato and Nakano, 2002). Similarly, two phenylalanines facilitate the binding of the cargo receptor ERGIC-53, a membrane protein of the ER-Golgi intermediate compartment (ERGIC), to COPII, thereby enabling its ER exit and Golgi localization (Kappeler et al., 1997). In plants, diacidic sequence motifs (D/EXE) have been identified in the N-terminal cytoplasmic tails of type I multispansing membrane protein GONST1 (Golgi nucleotide sugar transporter 1) and the type II Golgi protein CASP (CCAAT-displacement protein alternatively

spliced product), both of which rely on diacidic motifs to exit the ER and reach the Golgi (Hanton et al., 2005). However, even after mutating this motif in both fusion proteins, they were still partially localised to the Golgi, suggesting that other factors contributed to their exit from the ER. When an ER-retained synthetic type I reporter protein with a 17-amino acid TMD was fused to a segment of the cytoplasmic region of CASP, it was localised to the Golgi. Mutation of the DXE motif in this reporter protein resulted in its complete retention in the ER, further supporting the role of the DXE motif in ER export. Similarly, ER export and Golgi targeting of the syntaxin of plants (SYP) 3 family protein SYP31, a type II soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) protein, relies on a diacidic EXXD motif (MELAD) in its N-terminal cytoplasmic tail (Chatre et al., 2009). The potassium channel protein KAT1 requires a diacidic signal for ER exit and subsequent localization to the PM in guard cells (Mikosch et al., 2006). The motif has been shown to interact

with SEC24A, a component of the COPII coat (Sieben et al., 2008). This is consistent with a mammalian study showing a preference of the SEC24A isoform for binding to DXE motifs (Chatterjee et al., 2021). SEC24 was also found to bind to an LXX(L/M)E motif in the cytoplasmic N-termini of the two yeast SNAREs Sed5p and Bet1p (Mosessova et al., 2003). The same LXXLE motif was identified in the N-terminus of BET12, the Arabidopsis homologue of Sed5p. This motif in combination with a dibasic motif near the TMD was shown to mediate ER export (Chung et al., 2018).

## ER retention via retrograde transport

Little is known about the presence and involvement of amino acid sequence motifs in the retrieval and retention of *N*-glycan processing enzymes within the Golgi stack. Mammalian Golgi glycosyltransferases and glycosidases were thought to be good examples of retained proteins (Munro, 1991; Nilsson et al., 1991; Machamer, 1993). However, many, if not all, Golgi proteins cycle continuously within the Golgi stack and/or between the Golgi and the ER (Lippincott-Schwartz et al., 2000; Ward and Brandizzi, 2004; Storrie, 2005; Schoberer et al., 2010). This could occur either by regular retrograde transport mediated by COPI-coated vesicles that form at the periphery of Golgi cisternae (Pimpl et al., 2000; Ritzenthaler et al., 2002), a COPI-independent mechanism involving tubules, direct transport to the ER, or a combination of these possibilities (Nichols and Pelham, 1998; Storrie, 2005; Schoberer et al., 2010). Evidence for the cycling of Golgi residents comes, for example, from fluorescence recovery after photobleaching (FRAP) experiments showing the exchange of GFP-labelled Golgi proteins between Golgi and ER pools (Zaal et al., 1999; Miles et al., 2001; Brandizzi et al., 2002a; Schoberer et al., 2010). The predominant means for retrograde transport are COPI vesicles, whose coat is composed of seven individual coat protein subunits,  $\alpha/\beta/\beta'/\delta/\epsilon/\gamma/\zeta$  (Pimpl et al., 2000; Ritzenthaler et al., 2002). In plants, two types of COPI vesicles have been identified based on their size. As shown by electron tomography analysis, COPIa vesicles exclusively bud from *cis*-Golgi cisternae and reside at the ER-Golgi interface in Arabidopsis (Donohoe et al., 2007). COPIb vesicles are present at the medial- and *trans*-Golgi cisternae and are most likely responsible for retrograde transport within the Golgi stack.

The best-characterized COPI binding signals include dilysine motifs (KKXX and KKKXX) as described for the p24 protein family and the K/HDEL retrieval signal, which are found on many ER-resident proteins to direct them back to the ER (Contreras et al., 2004; Langhans et al., 2008; Gao et al., 2014). The Arabidopsis glycerol-3-phosphate acyltransferase 8 (GPAT8) and fatty acid desaturase 3 (FAD3) contain C-terminal KK and KKKXX motifs, respectively, that differ slightly from the canonical dilysine motif (McCartney et al., 2004; Gidda et al., 2009). Arginine-based motifs have also been shown to play a crucial role in mediating ER localization. For example, the N-terminal cytoplasmic tail of Arabidopsis GCSI contains four arginine residues that are constitutive for three di-arginine motifs (RR, RXR or RXXR),

each of which can confer ER localization most likely through retrieval from the Golgi as mutating all four arginines shifted GCSI localization from the ER to the Golgi (Boulaflous et al., 2008). Fusing the arginine-rich tail of GCSI to the first 35 N-terminal amino acids of XYLT (encompassing the cytoplasmic tail and TMD) redirected the enzyme from the Golgi to the ER. In mammals, the arginine-rich motif RRXXXXR has been demonstrated to retain the GM3 synthase isoform M1-SAT-I, a glycosyltransferase involved in ganglioside synthesis, within the ER. Mutating two of the three arginine residues redirected the enzyme to the Golgi, where its two homologs M2-SAT-I and M3-SAT-I are located (Uemura et al., 2009). ER retention can also be conferred by the hydrophobic pentapeptide motif  $\phi$ -X-X-K/R/D/E- $\phi$  (where  $\phi$  denotes a hydrophobic amino acid), which is found in the C-termini of Arabidopsis GPAT9 and FAD2 (McCartney et al., 2004; Gidda et al., 2009).

## Golgi retention via retrograde transport

A recent study showed that certain mammalian *cis*-Golgi glycosyltransferases are capable of directly binding to COPI subunits via the specific amino acid motif  $\phi$ -(K/R)-X-L-X-(K/R) in their N-terminal cytoplasmic tails. This interaction is critical for maintaining their correct steady-state location in the *cis*-Golgi (Liu et al., 2018). The same study also showed that  $\delta$ -COP can bind to the N-terminal tail of GALNT4 via a WX(n1-6)(W/F) motif, which is an evolutionarily conserved  $\delta$ -COP  $\mu$ -homology domain (MHD)-interacting motif (Suckling et al., 2015). Notably, most medial and *trans*-Golgi enzymes did not bind to COPI subunits. This suggests that this mechanism may be unique to *cis*-Golgi proteins that require recycling from late Golgi compartments to maintain their steady-state distribution.

Furthermore, glycosyltransferases may interact with COPI via an adaptor-mediated mechanism. In yeast, the peripheral membrane protein Vps74p has been shown to facilitate the sorting and recycling of multiple Golgi-localised glycosyltransferases by binding to a semi-conserved FLS-like motif (F/L-L/I/V-X-X-R/K) present in their cytoplasmic tail and the COPI coat (Tu et al., 2008, 2012). Vps74p thus functions as an adapter linking these enzymes to the COPI machinery, which facilitates the transport of glycosylation enzymes back to earlier Golgi cisternae, thereby enabling their dynamic localization in the Golgi (Schmitz et al., 2008; Tu et al., 2008). For example, the mutation of one or two residues within this motif resulted in the mislocalization of the mannosyltransferase Kre2p to the vacuole (Tu et al., 2008). Golgi phosphoprotein 3 (GOLPH3), the mammalian homologue of Vps74p, has been demonstrated to perform a comparable function (Ali et al., 2012; Eckert et al., 2014; Pereira et al., 2014). Although mammalian glycosyltransferases lack the yeast consensus motif, the two isoforms GOLPH3 and GOLPH3L have been demonstrated to partially compensate for the Vps74p mutant phenotypes (Tu et al., 2008). The amino acid sequence LxxR has been identified in the cytoplasmic tails of potential GOLPH3 binding partners that are involved in glycosphingolipid biosynthesis

(Rizzo et al., 2021). For example, GOLPH3 was shown to bind to the N-terminal cytoplasmic tail of the lactosylceramide synthase (LCS) that contains an LPRR motif, which led to its retention at the *trans*-Golgi, where it is sorted into retrograde transport vesicles (Rizzo et al., 2021). Knockdown of GOLPH3 led to its mislocalization to the TGN and then lysosome for its degradation. It has been put forth that TMD- and GOLPH3-dependent sorting occurs primarily in the late Golgi, whereas direct interactions with COPI are observed predominantly in the early Golgi (Welch and Munro, 2019). The human mannosidase ERManI has two di-basic arginine motifs (RRXX) in its N-terminal cytoplasmic tail that are critical for binding to  $\gamma$ -COP, the gamma subunit of COPI (Pan et al., 2013), but has also been proposed to interact with GOLPH3 (Welch et al., 2021). Despite the important function of Vps74p/GOLPH3 in the retrieval and retention of Golgi-resident glycosylation enzymes in yeast and mammals, respectively, no homologues of Vps74p/GOLPH3 have been identified in plants. The only study suggesting adaptor-mediated retrieval of Golgi-resident *N*-glycan processing enzymes in plants is that of the *cis*/medial- enzyme GNTI (Schoberer et al., 2019a). When a conserved polar glutamine (Q) residue in the TMD of AtGNTI or NtGNTI was replaced, the fluorescent fusion protein was mislocalized to the vacuole and *N*-glycan processing was impaired *in vivo*, indicating a TMD-based sorting mechanism. The glutamine residue may facilitate an interaction with an unknown Golgi-resident adaptor protein/complex or may be crucial for a specific protein-lipid interaction to partition GNTI into a specific lipid/membrane domain, which is likely to promote COPI-mediated retrograde transport and thereby maintain the steady-state localization of GNTI in the *cis*/medial Golgi of plants. The newly identified mechanism, which is active in both Arabidopsis and Nicotiana, differs from previous models such as the bilayer thickness model, kin recognition and the more recent cytoplasmic tail-dependent sorting (Tu and Banfield, 2010).

Further support for the involvement of the COPI machinery in the steady-state localization of Golgi-resident glycosylation enzymes comes from the study of the Arabidopsis *cis*-Golgi resident ER- $\alpha$ -mannosidase I (MNS3; Schoberer et al., 2019b). MNS3-GFP was partially translocated to the vacuole when coexpressed with RNAi constructs that silence endogenous  $\delta$ -COP and  $\epsilon$ -COP, the delta and epsilon subunits of COPI, in *N. benthamiana* leaves (Schoberer et al., 2019b). These results suggested the involvement of COPI-mediated recycling from *trans*- to *cis*-Golgi cisternae as proposed by the cisternal maturation model, while the trafficking pathway from the Golgi to the vacuole may highlight the default degradation pathway for glycosyltransferases that are no longer needed in a plant cell. A similar shift to the vacuole was shown for GNTI-mRFP upon knockdown of endogenous  $\delta$ -COP and  $\epsilon$ -COP (Schoberer et al., 2019a). Although the cytoplasmic tails of MNS3 and GNTI contain numerous basic amino acids that are essential residues of the binding motifs in the cytoplasmic tails of Golgi glycosyltransferases and glycosidases, there is no recognizable canonical COPI binding motif present that could account for the observed mislocalization (Figure 6A). This is in contrast to a study

of the mammalian MNS3 ortholog, human ERManI, which contains two di-basic arginine motifs (RRXX) required for binding to  $\gamma$ -COP (Pan et al., 2013). Interestingly, the ER localization of the yeast MNS3 ortholog ER  $\alpha$ 1,2-mannosidase I relies on the interaction of its TMD with retention in endoplasmic reticulum sorting receptor 1 (RER1), a Golgi-localised retrieval receptor that cycles the protein from the Golgi back to the ER (Massaad et al., 1999). Arabidopsis possesses three RER1 family members that can complement the mislocalization of cargo proteins in the yeast mutant  $\Delta$ *rer1*. A role for RER1 in intra-Golgi transport or the presence of RER1-interacting proteins has yet to be described in plants.

## Golgi retention via hydrophobic residues

A Golgi retention motif containing an important leucine residue (LPYS) was identified in the cytoplasmic tail of Arabidopsis MNS3 (Schoberer et al., 2019b). Despite its high level of conservation among plant ER- $\alpha$ -mannosidases, this motif does not appear to be related to any of the Golgi localization signals that have been described thus far in yeast, mammals, and plants. When MNS3-GFP was treated with brefeldin A (BFA), a secretory inhibitor, or tagged with the ER-retrieval signal HDEL, which both commonly result in the relocation of Golgi enzymes to the ER, the fluorescent fusion protein remained on dispersed punctate structures resembling Golgi remnants. However, the deletion of the LPYS motif or the exchange of the leucine residue for alanine in the full-length cytoplasmic tail of MNS3 resulted in the relocation of the protein to the ER. This Golgi-to-ER shift may indicate a malfunction in the Golgi retention mechanism, potentially due to the disruption of the binding interaction between the cytoplasmic motif and hitherto unidentified determinants within the Golgi apparatus. Only recently, a conserved di-leucine motif (LXL) near the cytoplasmic C-terminus of the Arabidopsis K/HDEL receptor ERD2 was identified as a crucial determinant of its Golgi residency and biological function, mediating ER retention of soluble ligands (Silva-Alvim et al., 2018). Replacement of the leucine residues resulted in a significant relocation of AtERD2 to the ER. Subsequently, it was demonstrated that the LXL motif functions as a Golgi retention signal, preventing its recycling to the ER, and is required to inhibit COPI-mediated receptor recycling (Alvim et al., 2023).

## Golgi retention via COG-mediated retrograde transport

An important role in the intra-Golgi retrograde trafficking of Golgi-resident *N*-glycan processing enzymes in mammalian and yeast cells has been attributed to the COG complex (Conserved Oligomeric Golgi complex). This multi-protein tethering complex



consists of eight subunits, named COG1 to COG8, that are organized into two subcomplexes, lobe A and lobe B. The COG complex is essential for tethering retrograde vesicles within the Golgi, helping to ensure that enzymes and other proteins remain in the correct Golgi compartments and thereby maintaining Golgi homeostasis (Smith and Lupashin, 2008; Blackburn et al., 2019). Live-cell super-resolution microscopy has recently shown that lobe A is preferentially Golgi-bound, whereas lobe B is mainly found on vesicles (Willett et al., 2016). This localization facilitates association between vesicles and target membranes, allowing SNARE complex formation and vesicle fusion. Disruptions in the COG complex can lead to severe diseases known as congenital disorders of glycosylation (COG-CDG), which result from the mislocalisation of *N*-glycosylation enzymes, affecting glycosylation patterns and leading to improperly glycosylated proteins. Although Arabidopsis homologs of all eight COG subunits have been identified in plants (Latijnhouwers et al., 2005), only COG3, COG6, COG7 and COG8 have been functionally studied in Arabidopsis. Studies of *cog3*, *cog6*, *cog7* and *cog8* mutants suggest that the Arabidopsis COG complex plays an important role in maintaining the structural and functional integrity of the Golgi apparatus during pollen tube tip growth (Ishikawa et al., 2008; Tan et al., 2016; Rui et al., 2020). This seems reasonable since pollen tube growth relies on the targeted secretion of vesicles containing cell wall components, such as pectins and hemicelluloses, which are synthesized in the Golgi. Mislocalisation of Golgi-resident enzymes, which are involved in the synthesis of these cell wall polysaccharides, and a defective Golgi stack morphology result in their incorrect deposition, leading to weakened cell walls and impaired pollen tube elongation (Tan et al., 2016). Interestingly, mutations in other proteins involved in vesicle tethering, such as the Golgi-resident Qa-SNAREs SYP31 and SYP32 or the RAB GTPases RABD2b and RABD2c, also result in defects in pollen development and pollen tube growth (Peng et al., 2011; Rui et al., 2021). Rui and colleagues recently showed that SYP31 and SYP32 interact with COG6 and are responsible for its Golgi localization in Arabidopsis (Rui et al., 2021).

In summary, the CTS region is essential for the correct localization of *N*-glycosylation enzymes in yeast, mammals and plants (Tu and Banfield, 2010; Schoberer and Strasser, 2011). However, the determinants required for proper Golgi targeting and retention are highly variable, as reflected by the lack of sequence similarity between different glycosylation enzymes (Figure 6).

## Significance of *N*-glycan processing enzyme localization in plant glycoengineering

Glycoengineering is the process of manipulating the glycosylation pathways of organisms to create tailored glycoproteins with precise glycan modifications. This is particularly important for the production of glycoproteins used in a therapeutic context, where specific glycan profiles can influence their efficacy, stability, and immunogenicity. For example,

protective glycan residues, such as sialic acid, can prolong the half-life of glycoproteins, which is critical for creating stable, long-lasting therapeutic proteins, making them more effective in clinical applications. Plants are an attractive platform for glycoengineering and are increasingly being used as biofactories for the production of therapeutic proteins (Tekoah et al., 2015; Hager et al., 2022; Whaley and Zeitlin, 2022; Zahmanova et al., 2023), because they are scalable, cost-effective, and can be grown in large quantities. However, control of the glycosylation process is essential for the use of plants as commercial platforms for the production of non-plant glycoproteins due to differences in plant-specific glycan structures and their potential incompatibility with human applications. Plants add  $\beta$ 1,2-xylose and core  $\alpha$ 1,3-fucose to complex *N*-glycans, which are absent in humans and potentially immunogenic, limiting the therapeutic use of plant-produced glycoproteins. For example, antibodies used in monoclonal antibody therapy require fucose-depleted glycans to enhance antibody-dependent cell-mediated cytotoxicity, a mechanism that helps the immune system kill target cells. Therefore, glycoengineering efforts have focused on inhibiting the activity of  $\beta$ 1,2-xylosyltransferase and core  $\alpha$ 1,3-fucosyltransferase through knock-down/knock-out strategies, resulting in the generation of plant species that synthesize complex *N*-glycans composed of the GnGn oligosaccharide devoid of plant-specific glycan residues (Castilho and Steinkellner, 2012).

By removing plant-specific glycosylation enzymes, introducing human or mammalian glycosylation enzymes that plants naturally lack, into the appropriate Golgi cisternae and manipulating the localization of resident enzymes within the Golgi stack, scientists can control the sequence of glycan processing, thereby 'humanizing' the glycosylation pathways in plants to produce therapeutic proteins that meet regulatory standards for use in humans without the need for costly downstream processing to alter the glycan structures. Knowledge of the critical determinants of Golgi enzyme localization is essential as the mislocalisation of enzymes could result in incomplete or incorrect glycosylation, leading to undesired protein properties. To illustrate, human  $\beta$ 1,4-galactosyltransferase ( $\beta$ 1,4-GALT), which is absent in plants, is targeted to a distinct Golgi compartment in plants, where it inhibits biantennary complex *N*-glycan formation, resulting in augmented *N*-glycan heterogeneity (Bakker et al., 2001; Strasser et al., 2009). The previous chapters established that the localization of *N*-glycan processing enzymes in the plant Golgi is controlled by the CTS region and that the catalytic domain is responsible for glycan processing activity. Swapping the CTS regions and catalytic domains between enzymes has shown considerable potential for gaining more precise control over glycosylation pathways and for adding additional functions to plant pathways. This is exemplified by the successful generation of branched and galactosylated structures (Castilho et al., 2011; Nagels et al., 2011; Castilho and Steinkellner, 2012). Furthermore, the expression of a chimeric protein comprising the CTS region of ST and the catalytic domain of human  $\beta$ 1,4-GALT led to the efficient production of di-galactosylated *N*-glycan structures in *N. benthamiana* (Strasser et al., 2009). In contrast, the CTS region of XYLT fused to the



catalytic domain of  $\beta$ 1,4-GALT resulted in predominantly mono-galactosylated and hybrid *N*-glycan structures (Bakker et al., 2006). Since galactosylation of *N*-glycans prevents further processing by GMII and GNTII, this incomplete processing of *N*-glycans suggests that the ST-CTS region, in contrast to the XYLT-CTS region, directs targeting to a later Golgi compartment. In addition, the transient expression of the GNTI-CTS region led to a block in *N*-glycan processing on a co-expressed glycoprotein in *N. benthamiana* (Schoberer et al., 2023). The identified sequence motif in the GNTI stem region acts as a dominant-negative sequence motif that can be used in transient glycoengineering approaches for the recombinant production of oligo-mannosidic *N*-glycans.

In addition to mimicking human glycosylation, glycoengineering can create novel glycan structures that do not occur naturally in plants or humans (Kallolimath et al., 2016; König-Beihammer et al., 2021), allowing the synthesis of novel glycoforms with specific biological properties that can be exploited for therapeutic or industrial applications.

## Challenges and future directions

Studying the trafficking of Golgi-resident *N*-glycan processing enzymes in plants is a complex task due to the unique challenges posed by plant cellular architecture and the dynamic nature of the Golgi apparatus. The plant Golgi apparatus is highly dynamic and spatially complex compared to its mammalian and yeast counterparts. It consists of numerous mobile Golgi stacks that move along the actin cytoskeleton, often interacting with the rapidly remodeling ER. Golgi-localised *N*-glycan processing enzymes are also not static, constantly cycling between the ER and the Golgi and within the Golgi. This makes it difficult to capture, track and quantify protein dynamics and determine the specific Golgi compartments where *N*-glycan processing takes place. While mammalian and yeast systems have well-established tools for studying and visualizing protein trafficking, plant-specific tools are still being developed. For example, high-resolution imaging techniques are essential for studying the fine details of enzyme trafficking. However, many fluorescence-based imaging techniques have limitations when applied to plant cells due to the presence of a cell wall that limits the penetration of fluorescent probes, autofluorescence in chlorophyll-containing leaves, a large vacuole that reduces the density of target structures, the thickness of plant tissues, and the rapid movement of organelles. Numerous super-resolution imaging techniques, such as stochastic optical reconstruction microscopy (STORM), photoactivated localization microscopy (PALM) and stimulated emission depletion microscopy (STED) have increased spatial resolution, but are limited at the level of temporal resolution as they necessitate prolonged acquisition times and are therefore incompatible with the imaging of mobile phenomena, such as the rapid remodeling of the ER and movement of Golgi stacks in plants.

A tool that was missing in plants until very recently was the RUSH (Retention Using Selective Hooks) system, which has been used in mammalian cells to study and quantify protein trafficking and

dynamics in living cells by allowing researchers to control the retention, release and movement of cargo proteins from a donor compartment within cells in real-time (Boncompain and Perez, 2012). Using the RUSH system in combination with real-time high-resolution imaging, it has been shown that ERES expand into a tubular network connected to the ER, containing secretory cargo but no COPII components. COPII components do not accompany departing cargo containers but instead remain on ERES by decorating the neck of tubules, which form an interwoven network connected to the ER (Westrate et al., 2020; Shomron et al., 2021; Weigel et al., 2021). This suggests that vesicles are not the only means of anterograde transport and is consistent with a recent study performed in *N. tabacum* leaves, which found that ER tubules are often associated with punctate structures where ERES and Golgi markers are co-localised (McGinness et al., 2022). The immobile nature of the mammalian ER and Golgi apparatus is a major advantage when monitoring fluorescent proteins in real time at high resolution. Recently, a version of the RUSH system has been established in plants (Fougère et al., 2025). Utilizing the RUSH system and high-resolution microscopy, the authors have identified a highly dynamic, Golgi-independent *cis*-Golgi tubulo-vesicular network in Arabidopsis, which was proposed to constitute an early station of the ERGIC in plants (Figure 4). This study significantly updates previous models of the plant secretory pathway. The existence of a plant ERGIC has been widely debated (Robinson et al., 2015), and this study raises the question of whether the proposed plant ERGIC acts as a new sorting hub for both anterograde and retrograde traffic, as is the case in mammalian cells (Appenzeller-Herzog and Hauri, 2006). This finding certainly introduces additional layers of regulation and complexity to the localization mechanisms of Golgi-resident *N*-glycan processing enzymes by introducing extra steps of sorting, retention, and retrieval steps that could affect the distribution or trafficking dynamics of enzymes. The ERGIC could facilitate more precise spatial and temporal control over the entry of enzymes into the Golgi, potentially impacting the overall accuracy of glycan processing.

The presence of multiple isoforms for glycosylation enzymes and a variety of components of the trafficking machinery, such as RABs, SNAREs, COPI and COPII proteins, adds another layer of complexity in plants. This redundancy makes it difficult to study the function and trafficking of a single enzyme, as knocking out or disrupting one protein of interest may be compensated for by another. This may also explain why knockouts of single proteins in plants are neither lethal nor result in specific phenotypes. The significance of multiple copies for proteins involved in trafficking is unknown but may be related to specificity for a particular type of cargo.

Future research may focus on gaining a mechanistic understanding of the sub-Golgi targeting and protein retrieval and retention mechanisms by studying how specific motifs and domains in *N*-glycan processing enzymes are recognized by the plant Golgi trafficking machinery. To date, little is known about how *N*-glycan processing enzymes are recycled and positioned in the cisternae where they function. The precise role of the COG complex within the plant Golgi is not well understood. Notably, a

*cog7* mutant with an amino acid substitution in the conserved domain of the protein displays a highly accelerated dark-induced senescence phenotype (compared to wildtype) that is accompanied by enhanced protein *N*-glycosylation, thereby linking COG function to both *N*-glycosylation and plant stress responses (Choi et al., 2023). Also, unlike in mammals and yeast, plants lack certain sorting factors (e.g. Vps74p/GOLPH3), and the motifs responsible for COPI-mediated retrieval are not conserved. These findings suggest the existence of unique or as-yet-undiscovered plant-specific mechanisms for Golgi enzyme localization and retention.

Similarly, the impact of variations in the lipid environment within Golgi membranes on the localization and function of glycosylation enzymes in plants remains relatively unexplored. Lipids are active participants in the sorting of membrane proteins in the Golgi, rather than passive components. They create specialized membrane environments, regulate vesicle formation, and interact with proteins to ensure the accurate delivery of cargo throughout the cell. Inhibition of glucosylceramide biosynthesis was linked to decreased protein secretion and perturbations of Golgi structure, although the localization of the *trans*-Golgi marker ST remained unaffected (Melser et al., 2010). Only recently, it was reported that the maturation of ERGICs into Golgi cisternae depended on C24-ceramide sphingolipids (Fougere et al., 2025).

Even small changes in the localization of *N*-glycan processing enzymes can lead to abnormal glycosylation, which can have significant effects on protein folding, stability, and function. These changes may not be readily observable, especially in the early stages of mislocalisation, making it difficult to link trafficking defects to physiological effects. Molecular or proteomic studies using mutants with glycosylation and/or trafficking defects will help to elucidate the regulatory mechanisms that control enzyme localization.

## Concluding remarks

The trafficking and precise localization of Golgi-resident *N*-glycan processing enzymes and the precise control of these processes in plants are vital for maintaining proper glycoprotein biosynthesis and function. Understanding these mechanisms is crucial for manipulating glycosylation processes in plants, which can be important for producing glycoproteins for pharmaceutical purposes. Studying the trafficking of Golgi-resident *N*-glycan processing enzymes in plants is challenging due to the dynamic and complex organization of the plant Golgi, the difficulty of distinguishing between distinct compartments within the Golgi stack at the nanoscale and the redundancy of the key components of the protein trafficking machinery. Advanced imaging techniques that combine high-resolution and real-time imaging, tools like the RUSH system, and genetic/proteomic screens of mutants with aberrant glycosylation will provide a deeper understanding of plant-specific trafficking pathways and help overcome these challenges to unravel the precise mechanisms governing the

localization and function of glycosylation enzymes in plants, which in turn will expand our understanding of plant cell biology.

## Author contributions

KD: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. JS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

- Ali, M. F., ChaChadi, V. B., Petrosyan, A., and Cheng, P. W. (2012). Golgi phosphoprotein 3 determines cell binding properties under dynamic flow by controlling Golgi localization of core 2 N-acetylglucosaminyltransferase 1. *J. Biol. Chem.* 287, 39564–39577. doi: 10.1074/jbc.M112.346528
- Alvim, J. C., Bolt, R. M., An, J., Kamisugi, Y., Cuming, A., Silva-Alvim, F. A. L., et al. (2023). The K/HDEL receptor does not recycle but instead acts as a Golgi-gatekeeper. *Nat. Commun.* 14, 1612. doi: 10.1038/s41467-023-37056-0
- Alvisi, N., van Noort, K., Dwiani, S., Geschiere, N., Sukarta, O., Varossieau, K., et al. (2021).  $\beta$ -Hexosaminidases along the secretory pathway of *Nicotiana benthamiana* have distinct specificities toward engineered helminth N-glycans on recombinant proteins. *Front. Plant Sci.* 12, 638454. doi: 10.3389/fpls.2021.638454
- Aoki, D., Lee, N., Yamaguchi, N., Dubois, C., and Fukuda, M. N. (1992). Golgi retention of a trans-Golgi membrane protein, galactosyltransferase, requires cysteine and histidine residues within the membrane-anchoring domain. *Proc. Natl. Acad. Sci. U.S.A.* 89, 4319–4323. doi: 10.1073/pnas.89.10.4319
- Appenzeller-Herzog, C., and Hauri, H. P. (2006). The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function. *J. Cell Sci.* 119, 2173–2183. doi: 10.1242/jcs.03019
- Bakker, H., Bardor, M., Molthoff, J. W., Gomord, V., Elbers, I., Stevens, L. H., et al. (2001). Galactose-extended glycans of antibodies produced by transgenic plants. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2899–2904. doi: 10.1073/pnas.031419998
- Bakker, H., Lommen, A., Jordi, W., Stiekema, W., and Bosch, D. (1999). An *Arabidopsis thaliana* cDNA complements the N-acetylglucosaminyltransferase I deficiency of CHO Lec1 cells. *Biochem. Biophys. Res. Commun.* 261, 829–832. doi: 10.1006/bbrc.1999.1117
- Bakker, H., Rouwendal, G. J., Karnoup, A. S., Florack, D. E., Stoopen, G. M., Helsper, J. P., et al. (2006). An antibody produced in tobacco expressing a hybrid beta-1,4-galactosyltransferase is essentially devoid of plant carbohydrate epitopes. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7577–7582. doi: 10.1073/pnas.0600879103
- Balch, W. E., Dunphy, W. G., Braell, W. A., and Rothman, J. E. (1984). Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine. *Cell* 39, 405–416. doi: 10.1016/0092-8674(84)90019-9
- Bardor, M., Faveeuw, C., Fitchette, A. C., Gilbert, D., Galas, L., Trottein, F., et al. (2003). Immunoreactivity in mammals of two typical plant glyco-epitopes, core alpha(1,3)-fucose and core xylose. *Glycobiology* 13, 427–434. doi: 10.1093/glycob/cwg024
- Becker, J. L., Tran, D. T., and Tabak, L. A. (2018). Members of the GalNAc-T family of enzymes utilize distinct Golgi localization mechanisms. *Glycobiology* 28, 841–848. doi: 10.1093/glycob/cwy071
- Beihammer, G., Maresch, D., Altmann, F., Van Damme, E. J. M., and Strasser, R. (2021). Lewis A glycans are present on proteins involved in cell wall biosynthesis and appear evolutionarily conserved among natural. *Front. Plant Sci.* 12, 630891. doi: 10.3389/fpls.2021.630891
- Benghezal, M., Wasteneys, G. O., and Jones, D. A. (2000). The C-terminal dilysine motif confers endoplasmic reticulum localization to type I membrane proteins in plants. *Plant Cell* 12, 1179–1201. doi: 10.1105/tpc.12.7.1179
- Blackburn, J. B., D'Souza, Z., and Lupashin, V. V. (2019). Maintaining order: COG complex controls Golgi trafficking, processing, and sorting. *FEBS Lett.* 593, 2466–2487. doi: 10.1002/1873-3468.13570
- Boevink, P., Oparka, K., Santa Cruz, S., Martin, B., Betteridge, A., and Hawes, C. (1998). Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. *Plant J.* 15, 441–447. doi: 10.1046/j.1365-3113.1998.00208.x
- Boncompain, G., and Perez, F. (2012). Synchronizing protein transport in the secretory pathway. *Curr. Protoc. Cell Biol.* doi: 10.1002/0471143030.cb1519s57
- Boulaflous, A., Faso, C., and Brandizzi, F. (2008). Deciphering the golgi apparatus: from imaging to genes. *Traffic* 9, 1613–1617. doi: 10.1111/j.1600-0854.2008.00769.x
- Brandizzi, F., Frangne, N., Marc-Martin, S., Hawes, C., Neuhaus, J., and Paris, N. (2002b). The destination for single-pass membrane proteins is influenced markedly by the length of the hydrophobic domain. *Plant Cell* 14, 1077–1092. doi: 10.1105/tpc.000620
- Brandizzi, F., Snapp, E., Roberts, A., Lippincott-Schwartz, J., and Hawes, C. (2002a). Membrane protein transport between the endoplasmic reticulum and the Golgi in tobacco leaves is energy dependent but cytoskeleton independent: evidence from selective photobleaching. *Plant Cell* 14, 1293–1309. doi: 10.1105/tpc.001586
- Castilho, A., Gattinger, P., Grass, J., Jez, J., Pabst, M., Altmann, F., et al. (2011). N-glycosylation engineering of plants for the biosynthesis of glycoproteins with bisected and branched complex N-glycans. *Glycobiology* 21, 813–823. doi: 10.1093/glycob/cwr009
- Castilho, A., and Steinkellner, H. (2012). ). Glyco-engineering in plants to produce human-like N-glycan structures. *Biotechnol. J.* 7, 1088–1098. doi: 10.1002/biot.201200032
- Castilho, A., Strasser, R., Stadlmann, J., Grass, J., Jez, J., Gattinger, P., et al. (2010). In planta protein sialylation through overexpression of the respective mammalian pathway. *J. Biol. Chem.* 285, 15923–15930. doi: 10.1074/jbc.M109.088401
- Chatre, L., Watelet-Boyer, V., Melsers, S., Maneta-Peyret, L., Brandizzi, F., and Moreau, P. (2009). A novel di-acidic motif facilitates ER export of the syntaxin SYP31. *J. Exp. Bot.* 60, 3157–3165. doi: 10.1093/jxb/erp155
- Chatterjee, S., Choi, A. J., and Frankel, G. (2021). A systematic review of Sec24 cargo interactome. *Traffic* 22, 412–424. doi: 10.1111/tra.12817
- Chen, C., and Colley, K. J. (2000). Minimal structural and glycosylation requirements for ST6Gal I activity and trafficking. *Glycobiology* 10, 531–583. doi: 10.1093/glycob/10.5.531
- Choi, H. S., Bjornson, M., Liang, J., Wang, J., Ke, H., Hur, M., et al. (2023). COG-imposed Golgi functional integrity determines the onset of dark-induced senescence. *Nat. Plants* 9, 1890–1901. doi: 10.1038/s41477-023-01545-3
- Chung, K. P., Zeng, Y., Li, Y., Ji, C., Xia, Y., and Jiang, L. (2018). Signal motif-dependent ER export of the Qc-SNARE BET12 interacts with MEMB12 and affects PR1 trafficking in. *J. Cell Sci.* 131, jcs202838. doi: 10.1242/jcs.202838
- Clerc, S., Hirsch, C., Oggier, D., Deprez, P., Jakob, C., Sommer, T., et al. (2009). Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. *J. Cell Biol.* 184, 159–172. doi: 10.1083/jcb.200809198
- Colley, K. (1997). Golgi localization of glycosyltransferases: more questions than answers. *Glycobiology* 7, 1–13. doi: 10.1093/glycob/7.1.1-b
- Contreras, I., Yang, Y., Robinson, D. G., and 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. doi: 10.1093/pcp/pch200
- Davis, M. J., Zhang, F., Yuan, Z., and Teasdale, R. D. (2006). MemO: a consensus approach to the annotation of a protein's membrane organization. *In Silico Biol.* 6, 387–399. doi: 10.3233/ISB-00252
- Dirnberger, D., Bencúr, P., Mach, L., and 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. doi: 10.1023/A:1016061815748
- Donohoe, B. S., Kang, B. H., and Staehelin, L. A. (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. doi: 10.1073/pnas.0609818104
- Eckert, E. S., Reckmann, I., Hellwig, A., Röhling, S., El-Battari, A., Wieland, F. T., et al. (2014). Golgi phosphoprotein 3 triggers signal-mediated incorporation of glycosyltransferases into coatomer-coated (COPI) vesicles. *J. Biol. Chem.* 289, 31319–31329. doi: 10.1074/jbc.M114.608182
- Emr, S., Glick, B., Linstedt, A., Lippincott-Schwartz, J., Luini, A., Malhotra, V., et al. (2009). Journeys through the Golgi-taking stock in a new era. *J. Cell Biol.* 187, 449–453. doi: 10.1083/jcb.200909011
- Essl, D., Dirnberger, D., Gomord, V., Strasser, R., Faye, L., Glössl, J., et al. (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. doi: 10.1016/S0014-5793(99)00712-7
- Fanata, W. I., Lee, K. H., Son, B. H., Yoo, J. Y., Harmoko, R., Ko, K. S., et al. (2013). N-glycan maturation is crucial for cytokinin-mediated development and cellulose synthesis in *Oryza sativa*. *Plant J.* 73, 966–979. doi: 10.1111/tjp.12087
- Farquhar, M., and Palade, G. (1998). The Golgi apparatus: 100 years of progress and controversy. *Trends Cell Biol.* 8, 2–10. doi: 10.1016/S0962-8924(97)01187-2
- Faso, C., Boulaflous, A., and Brandizzi, F. (2009). The plant Golgi apparatus: Last 10 years of answered and open questions. *FEBS Lett.* 583, 375–3757. doi: 10.1016/j.febslet.2009.09.046
- Fitchette, A., Cabanes-Macheteau, M., Marvin, L., Martin, B., Satiat-Jeunemaitre, B., Gomord, V., et al. (1999). Biosynthesis and immunolocalization of Lewis A-containing N-glycans in the plant cell. *Plant Physiol.* 121, 333–344. doi: 10.1104/pp.121.2.333
- Foresti, O., and Denecke, J. (2008). Intermediate organelles of the plant secretory pathway: identity and function. *Traffic* 9, 1599–1612. doi: 10.1111/j.1600-0854.2008.00791.x
- Fougère, L., Grison, M., Laquel, P., Montrazi, M., Cordelières, F., Fernández-Monreal, M., et al. (2025). ER-to-Golgi trafficking through a dynamic intermediate cis-Golgi tubular network in *Arabidopsis*. *Nat. Cell Biol.* 27, 424–437. doi: 10.1038/s41556-025-01624-x
- Gao, C., Cai, Y., Wang, Y., Kang, B. H., Aniento, F., Robinson, D. G., et al. (2014). Retention mechanisms for ER and Golgi membrane proteins. *Trends Plant Sci.* 19, 508–515. doi: 10.1016/j.tplants.2014.04.004
- Gao, C., Yu, C. K., Qu, S., San, M. W., Li, K. Y., Lo, S. W., et al. (2012). The Golgi-localized *Arabidopsis* endomembrane protein12 contains both endoplasmic reticulum export and Golgi retention signals at its C terminus. *Plant Cell* 24, 2086–2104. doi: 10.1105/tpc.112.096057
- Gidda, S. K., Shockey, J. M., Rothstein, S. J., Dyer, J. M., and Mullen, R. T. (2009). *Arabidopsis thaliana* GPAT8 and GPAT9 are localized to the ER and possess distinct ER retrieval signals: functional divergence of the dilysine ER retrieval motif in plant cells. *Plant Physiol. Biochem.* 47, 867–879. doi: 10.1016/j.plaphy.2009.05.008



- Giraud, C. G., and Maccioni, H. J. (2003). Endoplasmic reticulum export of glycosyltransferases depends on interaction of a cytoplasmic dibasic motif with Sar1. *Mol. Biol. Cell* 14, 3753–3766. doi: 10.1091/mbc.e03-02-0101
- Glick, B., Elston, T., and Oster, G. (1997). A cisternal maturation mechanism can explain the asymmetry of the Golgi stack. *FEBS Lett.* 414, 177–181. doi: 10.1016/S0014-5793(97)00984-8
- Glick, B. S., and Nakano, A. (2009). Membrane traffic within the Golgi apparatus. *Annu. Rev. Cell Dev. Biol.* 25, 113–132. doi: 10.1146/annurev.cellbio.24.110707.175421
- Gomez, L., and Chrispeels, M. J. (1994). Complementation of an Arabidopsis thaliana mutant that lacks complex asparagine-linked glycans with the human cDNA encoding N-acetylglucosaminyltransferase I. *Proc. Natl. Acad. Sci. U.S.A.* 91, 1829–1833. doi: 10.1073/pnas.91.5.1829
- Hager, K. J., Pérez Marc, G., Gobeil, P., Diaz, R. S., Heizer, G., Llapur, C., et al. (2022). Efficacy and safety of a recombinant plant-based adjuvanted covid-19 vaccine. *N Engl. J. Med.* 386, 2084–2096. doi: 10.1056/NEJMoa2201300
- Hallgren, J., Renna, L., Tsigos, K. D., Pedersen, M. D., Almagro Armenteros, J. J., Marcattili, P., et al. (2022). DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. *bioRxiv preprint*. doi: 10.1101/2022.04.08.487609
- Hanton, S., Renna, L., Bortolotti, L., Chatre, L., Stefano, G., and Brandizzi, F. (2005). Diacidic motifs influence the export of transmembrane proteins from the endoplasmic reticulum in plant cells. *Plant Cell* 17, 3081–3093. doi: 10.1105/tpc.105.034900
- Hawes, C., and Satiat-Jeunemaitre, B. (2005). The plant Golgi apparatus—going with the flow. *Biochim. Biophys. Acta* 1744, 466–480. doi: 10.1016/j.bbamer.2005.03.009
- Hawes, C., Schoberer, J., Hummel, E., and Osterrieder, A. (2010). Biogenesis of the plant Golgi apparatus. *Biochem. Soc. Trans.* 38, 761–767. doi: 10.1042/BST0380761
- Helenius, A., and Aebi, M. (2001). Intracellular functions of N-linked glycans. *Science* 291, 2364–2369. doi: 10.1126/science.291.5512.2364
- Hüttner, S., Veit, C., Vavra, U., Schoberer, J., Liebming, E., Maresch, D., et al. (2014). Arabidopsis Class I  $\alpha$ -Mannosidases MNS4 and MNS5 Are Involved in Endoplasmic Reticulum-Associated Degradation of Misfolded Glycoproteins. *Plant Cell* 26, 1712–1728. doi: 10.1105/tpc.114.123216
- Ishikawa, T., Machida, C., Yoshioka, Y., Ueda, T., Nakano, A., and Machida, Y. (2008). EMBRYO YELLOW gene, encoding a subunit of the conserved oligomeric Golgi complex, is required for appropriate cell expansion and meristem organization in Arabidopsis thaliana. *Genes Cells* 13, 521–535. doi: 10.1111/j.1365-2443.2008.01186.x
- Isaji, T., Im, S., Gu, W., Wang, Y., Hang, Q., Lu, J., et al. (2014). An oncogenic protein Golgi phosphoprotein 3 up-regulates cell migration via sialylation. *J. Biol. Chem.* 289, 20694–20705. doi: 10.1074/jbc.M113.542668
- Jenkins, N., Parekh, R. B., and James, D. C. (1996). Getting the glycosylation right: implications for the biotechnology industry. *Nat. Biotechnol.* 14, 975–981. doi: 10.1038/nbt0896-975
- Kallolimath, S., Castilho, A., Strasser, R., Grünwald-Gruber, C., Altmann, F., Strubl, S., et al. (2016). Engineering of complex protein sialylation in plants. *Proc. Natl. Acad. Sci. U.S.A.* 113, 9498–9503. doi: 10.1073/pnas.1604371113
- Kang, J. S., Frank, J., Kang, C. H., Kajiu, H., Vikram, M., Ueda, A., et al. (2008). Salt tolerance of Arabidopsis thaliana requires maturation of N-glycosylated proteins in the Golgi apparatus. *Proc. Natl. Acad. Sci. U.S.A.* 105, 5933–5938. doi: 10.1073/pnas.0800237105
- Kappeler, F., Klopfenstein, D. R., Foguet, M., Paccaud, J. P., and Hauri, H. P. (1997). The recycling of ERGIC-53 in the early secretory pathway. ERGIC-53 carries a cytosolic endoplasmic reticulum-exit determinant interacting with COPII. *J. Biol. Chem.* 272, 31801–31808. doi: 10.1074/jbc.272.50.31801
- Kaufmann-Soboll, H., Mertens-Beer, M., Brehler, R., Albert, M., and von Schaeuwen, A. (2021). Complex. *Front. Plant Sci.* 12, 635962. doi: 10.3389/fpls.2021.635962
- König-Belhammer, J., Vavra, U., Shin, Y. J., Veit, C., Grünwald-Gruber, C., Gillisch, Y., et al. (2021). In planta production of the receptor-binding domain from SARS-CoV-2 with human blood group A glycan structures. *Front. Chem.* 9, 816544. doi: 10.3389/fchem.2021.816544
- Langhans, M., Marcote, M. J., Pimpl, P., Virgili-López, G., Robinson, D. G., and Ariento, F. (2008). In vivo trafficking and localization of p24 proteins in plant cells. *Traffic* 9, 770–785. doi: 10.1111/j.1600-0854.2008.00719.x
- Latijnhouwers, M., Hawes, C., and Carvalho, C. (2005). Holding it all together? Candidate proteins for the plant Golgi matrix. *Curr. Opin. Plant Biol.* 8, 632–639. doi: 10.1016/j.pbi.2005.09.014
- Liebming, E., Hüttner, S., Vavra, U., Fischl, R., Schoberer, J., Grass, J., et al. (2009). Class I alpha-mannosidases are required for N-glycan processing and root development in Arabidopsis thaliana. *Plant Cell* 21, 3850–3867. doi: 10.1105/tpc.109.072363
- Liebming, E., Veit, C., Pabst, M., Batoux, M., Zipfel, C., Altmann, F., et al. (2011). Beta-N-acetylhexosaminidases HEXO1 and HEXO3 are responsible for the formation of paucimannosidic N-glycans in Arabidopsis thaliana. *J. Biol. Chem.* 286, 10793–10802. doi: 10.1074/jbc.M110.178020
- Lippincott-Schwartz, J., Roberts, T., and Hirschberg, K. (2000). Secretory protein trafficking and organelle dynamics in living cells. *Annu. Rev. Cell Dev. Biol.* 16, 557–589. doi: 10.1146/annurev.cellbio.16.1.557
- Liu, L., Doray, B., and Kornfeld, S. (2018). Recycling of Golgi glycosyltransferases requires direct binding to coatamer. *Proc. Natl. Acad. Sci. U.S.A.* 115, 8984–8989. doi: 10.1073/pnas.1810291115
- Losev, E., Reinke, C. A., Jellen, J., Strongin, D. E., Bevis, B. J., and Glick, B. S. (2006). Golgi maturation visualized in living yeast. *Nature* 441, 1002–1006. doi: 10.1038/nature04717
- Lynch, M. A., and Staehelin, L. A. (1992). Domain-specific and cell type-specific localization of two types of cell wall matrix polysaccharides in the clover root tip. *J. Cell Biol.* 118, 467–479. doi: 10.1083/jcb.118.2.467
- Machamer, C. E. (1993). Targeting and retention of Golgi membrane proteins. *Curr. Opin. Cell Biol.* 5, 606–612. doi: 10.1016/0955-0674(93)90129-E
- Massaad, M. J., Franzusoff, A., and Herscovics, A. (1999). The processing alpha1,2-mannosidase of Saccharomyces cerevisiae depends on Rer1p for its localization in the endoplasmic reticulum. *Eur. J. Cell Biol.* 78, 435–440. doi: 10.1016/S0017-9335(99)80070-3
- Matsuura-Tokita, K., Takeuchi, M., Ichihara, A., Mikuriya, K., and Nakano, A. (2006). Live imaging of yeast Golgi cisternal maturation. *Nature* 441, 1007–1010. doi: 10.1038/nature04737
- McCartney, A. W., Dyer, J. M., Dhanoa, P. K., Kim, P. K., Andrews, D. W., McNew, J. A., et al. (2004). Membrane-bound fatty acid desaturases are inserted co-translationally into the ER and contain different ER retrieval motifs at their carboxy termini. *Plant J.* 37, 156–173. doi: 10.1111/j.1365-313X.2004.01949.x
- McGinness, A. J., Schoberer, J., Pain, C., Brandizzi, F., and Krichbaum, V. (2022). On the nature of the plant ER exit sites. *Front. Plant Sci.* 13, 1010569. doi: 10.3389/fpls.2022.1010569
- Melser, S., Batailler, B., Peypelut, M., Poujol, C., Bellec, Y., Watelet-Boyer, V., et al. (2010). Glucosylceramide biosynthesis is involved in Golgi morphology and protein secretion in plant cells. *Traffic* 11, 479–490. doi: 10.1111/j.1600-0854.2009.01030.x
- Mikosch, M., Hurst, A., Hertel, B., and Homann, U. (2006). Diacidic motif is required for efficient transport of the K<sup>+</sup> channel KAT1 to the plasma membrane. *Plant Physiol.* 142, 923–930. doi: 10.1104/pp.106.087064
- Miles, S., McManus, H., Forsten, K., and Storr, B. (2001). Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block. *J. Cell Biol.* 155, 543–555. doi: 10.1083/jcb.200103104
- Moore, P. J., Swords, K. M., Lynch, M. A., and Staehelin, L. A. (1991). Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the Golgi apparatus of plants. *J. Cell Biol.* 112, 589–602. doi: 10.1083/jcb.112.4.589
- Moremen, K. W., Tiemeyer, M., and Nairn, A. V. (2012). Vertebrate protein glycosylation: diversity, synthesis and function. *Nat. Rev. Mol. Cell Biol.* 13, 448–462. doi: 10.1038/nrm3383
- Mossessova, E., Bickford, L. C., and Goldberg, J. (2003). SNARE selectivity of the COPII coat. *Cell* 114, 483–495. doi: 10.1016/S0092-8674(03)00608-1
- Munro, S. (1991). Sequences within and adjacent to the transmembrane segment of alpha-2,6-sialyltransferase specify Golgi retention. *EMBO J.* 10, 3577–3588. doi: 10.1002/j.1460-2075.1991.tb04924.x
- Nagels, B., Van Damme, E. J., Pabst, M., Callewaert, N., and Weterings, K. (2011). Production of complex multiantennary N-glycans in Nicotiana benthamiana plants. *Plant Physiol.* 155, 1103–1112. doi: 10.1104/pp.110.168773
- Nichols, B., and Pelham, H. (1998). SNAREs and membrane fusion in the Golgi apparatus. *Biochim. Biophys. Acta* 1404, 9–31. doi: 10.1016/S0167-4889(98)00044-5
- Nilsson, T., Hoe, M., Slusarewicz, P., Rabouille, C., Watson, R., Hunte, F., et al. (1994). Kin recognition between medial Golgi enzymes in HeLa cells. *EMBO J.* 13, 562–574. doi: 10.1002/j.1460-2075.1994.tb06294.x
- Nilsson, T., Lucocq, J. M., Mackay, D., and Warren, G. (1991). The membrane spanning domain of beta-1,4-galactosyltransferase specifies trans Golgi localization. *EMBO J.* 10, 3567–3575. doi: 10.1002/j.1460-2075.1991.tb04923.x
- Nilsson, T., Slusarewicz, P., Hoe, M. H., and Warren, G. (1993). Kin recognition. A model for the retention of Golgi enzymes. *FEBS Lett.* 330, 1–4. doi: 10.1016/0014-5793(93)80906-B
- Oikawa, A., Lund, C. H., Sakuragi, Y., and Scheller, H. V. (2013). Golgi-localized enzyme complexes for plant cell wall biosynthesis. *Trends Plant Sci.* 18, 49–58. doi: 10.1016/j.tplants.2012.07.002
- Pan, S., Cheng, X., and Sifers, R. N. (2013). Golgi-situated endoplasmic reticulum  $\alpha$ -1, 2-mannosidase contributes to the retrieval of ERAD substrates through a direct interaction with  $\gamma$ -COP. *Mol. Biol. Cell* 24, 1111–1121. doi: 10.1091/mbc.e12-12-0886
- Patterson, G. H., Hirschberg, K., Polishchuk, R. S., Gerlich, D., Phair, R. D., and Lippincott-Schwartz, J. (2008). Transport through the Golgi apparatus by rapid partitioning within a two-phase membrane system. *Cell* 133, 1055–1067. doi: 10.1016/j.cell.2008.04.044
- Pedersen, C. T., Loke, I., Lorentzen, A., Wolf, S., Kamble, M., Kristensen, S. K., et al. (2017). N-glycan maturation mutants in Lotus japonicus for basic and applied glycoprotein research. *Plant J.* 91, 394–407. doi: 10.1111/tpj.13570
- Pelham, H. R. (1998). Getting through the Golgi complex. *Trends Cell Biol.* 8, 45–49. doi: 10.1016/S0962-8924(97)01185-9
- Peng, J., Ilarslan, H., Wurtele, E. S., and Bassham, D. C. (2011). AtRabD2b and AtRabD2c have overlapping functions in pollen development and pollen tube growth. *BMC Plant Biol.* 11, 25. doi: 10.1186/1471-2229-11-25
- Pereira, N. A., Pu, H. X., Goh, H., and Song, Z. (2014). Golgi phosphoprotein 3 mediates the Golgi localization and function of protein O-linked mannose  $\beta$ -1,2-N-

- acetylglucosaminyltransferase 1. *J. Biol. Chem.* 289, 14762–14770. doi: 10.1074/jbc.M114.548305
- Pfeffer, S. R. (2010). How the Golgi works: a cisternal progenitor model. *Proc. Natl. Acad. Sci. U.S.A.* 107, 19614–19618. doi: 10.1073/pnas.1011016107
- Pimpl, P., Movafeghi, A., Coughlan, S., Denecke, J., Hillmer, S., and Robinson, D. (2000). *In situ* localization and *in vitro* induction of plant COPI-coated vesicles. *Plant Cell* 12, 2219–2236. doi: 10.1105/tpc.12.11.2219
- Quan, E., Kamiya, Y., Kamiya, D., Denic, V., Weibezahn, J., Kato, K., et al. (2008). Defining the glycan destruction signal for endoplasmic reticulum-associated degradation. *Mol. Cell* 32, 870–877. doi: 10.1016/j.molcel.2008.11.017
- Quintero, C. A., Giraudo, C. G., Villarreal, M., Montich, G., and Maccioni, H. J. F. (2010). Identification of a site in SAR1 involved in the interaction with the cytoplasmic tail of glycolipid glycosyltransferases. *J. Biol. Chem.* 285, 30340–30346. doi: 10.1074/jbc.M110.128868
- Reichardt, I., Stierhof, Y., Mayer, U., Richter, S., Schwarz, H., Schumacher, K., et al. (2007). Plant cytokinesis requires *de novo* secretory trafficking but not endocytosis. *Curr. Biol.* 17, 2047–2053. doi: 10.1016/j.cub.2007.10.040
- Ritzenthaler, C., Nebenführ, A., Movafeghi, A., Stussi-Garaud, C., Behnia, L., Pimpl, P., et al. (2002). Reevaluation of the effects of brefeldin A on plant cells using tobacco Bright Yellow 2 cells expressing Golgi-targeted green fluorescent protein and COPI antisera. *Plant Cell* 14, 237–261. doi: 10.1105/tpc.010237
- Rivera-Molina, F. E., and Novick, P. J. (2009). A Rab GAP cascade defines the boundary between two Rab GTPases on the secretory pathway. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14408–14413. doi: 10.1073/pnas.0906536106
- Rizzo, R., Russo, D., Kurokawa, K., Sahu, P., Lombardi, B., Supino, D., et al. (2021). Golgi maturation-dependent glycoenzyme recycling controls glycosphingolipid biosynthesis and cell growth via GOLPH3. *EMBO J.* 40, e107238. doi: 10.15252/emboj.2020107238
- Robinson, D. G. (2020). Plant golgi ultrastructure. *J. Microsc.* 280, 111–121. doi: 10.1111/jmi.12899
- Robinson, D. G., Brandizzi, F., Hawes, C., and Nakano, A. (2015). Vesicles versus Tubes: Is Endoplasmic Reticulum-Golgi transport in plants fundamentally different from other eukaryotes? *Plant Physiol.* 168, 393–406. doi: 10.1104/pp.15.00124
- Rothman, J., and Wieland, F. (1996). Protein sorting by transport vesicles. *Science* 272, 227–234. doi: 10.1126/science.272.5259.227
- Rui, Q., Tan, X., Liu, F., and Bao, Y. (2022). An update on the key factors required for plant Golgi structure maintenance. *Front. Plant Sci.* 13, 933283. doi: 10.3389/fpls.2022.933283
- Rui, Q., Tan, X., Liu, F., Li, Y., Liu, X., Li, B., et al. (2021). Syntaxin of plants 31 (SYP31) and SYP32 is essential for Golgi morphology maintenance and pollen development. *Plant Physiol.* 186, 330–343. doi: 10.1093/plphys/kiab049
- Rui, Q., Wang, J., Li, Y., Tan, X., and Bao, Y. (2020). Arabidopsis COG6 is essential for pollen tube growth and Golgi structure maintenance. *Biochem. Biophys. Res. Commun.* 528, 447–452. doi: 10.1016/j.bbrc.2020.05.189
- Saint-Jore-Dupas, C., Nebenführ, A., Boulaflos, A., Follet-Gueye, M., Plasson, C., Hawes, C., et al. (2006). Plant N-glycan processing enzymes employ different targeting mechanisms for their spatial arrangement along the secretory pathway. *Plant Cell* 18, 3182–3200. doi: 10.1105/tpc.105.036400
- Sato, K., and Nakano, A. (2002). Emp47p and its close homolog Emp46p have a tyrosine-containing endoplasmic reticulum exit signal and function in glycoprotein secretion in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 13, 2518–2532. doi: 10.1091/mbc.e02-01-0027
- Schmitz, K. R., Liu, J., Li, S., Setty, T. G., Wood, C. S., Burd, C. G., et al. (2008). Golgi localization of glycosyltransferases requires a Vps74p oligomer. *Dev. Cell* 14, 523–534. doi: 10.1016/j.devcel.2008.02.016
- Schoberer, J., Izadi, S., Kierein, C., Vavra, U., König-Belhammer, J., Ruocco, V., et al. (2023). The tobacco GNT1 stem region harbors a strong motif for homomeric protein complex formation. *Front. Plant Sci.* 14, 1320051. doi: 10.3389/fpls.2023.1320051
- Schoberer, J., König, J., Veit, C., Vavra, U., Liebminger, E., Botchway, S. W., et al. (2019b). A signal motif retains Arabidopsis ER- $\alpha$ -mannosidase I in the cis-Golgi and prevents enhanced glycoprotein ERAD. *Nat. Commun.* 10, 3701. doi: 10.1038/s41467-019-11686-9
- Schoberer, J., Liebminger, E., Botchway, S. W., Strasser, R., and Hawes, C. (2013). Time-resolved fluorescence imaging reveals differential interactions of N-glycan processing enzymes across the golgi stack in planta. *Plant Physiol.* 161, 1737–1754. doi: 10.1104/pp.112.210757
- Schoberer, J., Liebminger, E., Vavra, U., Veit, C., Castilho, A., Dicker, M., et al. (2014). The transmembrane domain of N-acetylglucosaminyltransferase I is the key determinant for its Golgi subcompartmentation. *Plant J.* 80, 809–822. doi: 10.1111/tj.12671
- Schoberer, J., Liebminger, E., Vavra, U., Veit, C., Grünwald-Gruber, C., Altmann, F., et al. (2019a). Golgi localization of GNTI requires a polar amino acid residue within its transmembrane domain. *Plant Physiol.* 180, 859–873. doi: 10.1104/pp.19.00310
- Schoberer, J., Runions, J., Steinkellner, H., Strasser, R., Hawes, C., and Osterrieder, A. (2010). Sequential depletion and acquisition of proteins during Golgi stack disassembly and reformation. *Traffic* 11, 1429–1444. doi: 10.1111/j.1600-0854.2010.01106.x
- Schoberer, J., and Strasser, R. (2011). Sub-compartmental organization of Golgi-resident N-glycan processing enzymes in plants. *Mol. Plant* 4, 220–228. doi: 10.1093/mp/ssq082
- Schoberer, J., and Strasser, R. (2018). Plant glyco-biotechnology. *Semin. Cell Dev. Biol.* 80, 133–141. doi: 10.1016/j.semdb.2017.07.005
- Schoberer, J., Vavra, U., Stadlmann, J., Hawes, C., Mach, L., Steinkellner, H., et al. (2009). Arginine/lysine residues in the cytoplasmic tail promote ER export of plant glycosylation enzymes. *Traffic* 10, 101–115. doi: 10.1111/j.1600-0854.2008.00841.x
- Schröder, S., Schimmöller, F., Singer-Krüger, B., and Riezman, H. (1995). The Golgi-localization of yeast Emp47p depends on its di-lysine motif but is not affected by the ret1–1 mutation in alpha-COP. *J. Cell Biol.* 131, 895–912. doi: 10.1083/jcb.131.4.895
- Shaaltiel, Y., and Tekoah, Y. (2016). Plant-specific N-glycans do not have proven adverse effects in humans. *Nat. Biotechnol.* 34, 706–708. doi: 10.1038/nbt.3556
- Sharpe, H. J., Stevens, T. J., and Munro, S. (2010). A comprehensive comparison of transmembrane domains reveals organelle-specific properties. *Cell* 142, 158–169. doi: 10.1016/j.cell.2010.05.037
- Shomron, O., Nevo-Yassaf, I., Aviad, T., Yaffe, Y., Zahavi, E. E., Dukhovny, A., et al. (2021). COPII collar defines the boundary between ER and ER exit site and does not coat cargo containers. *J. Cell Biol.* 220, e201907224. doi: 10.1083/jcb.201907224
- Sieben, C., Mikosch, M., Brandizzi, F., and Homann, U. (2008). Interaction of the K (+)-channel KAT1 with the coat protein complex II coat component Sec24 depends on a di-acidic endoplasmic reticulum export motif. *Plant J.* 56, 997–1006. doi: 10.1111/j.1365-3113X.2008.03658.x
- Silva-Alvim, F. A. L., An, J., Alvim, J. C., Foresti, O., Grippa, A., Pelgrom, A. J. E., et al. (2018). Predominant Golgi-residency of the plant K/HDEL receptor is essential for its function in mediating ER retention. *Plant Cell* 30, 2174–2196. doi: 10.1105/tpc.18.00426
- Smith, R. D., and Lupashin, V. V. (2008). Role of the conserved oligomeric Golgi (COG) complex in protein glycosylation. *Carbohydr. Res.* 343, 2024–2031. doi: 10.1016/j.carres.2008.01.034
- Srivastava, R., Chen, Y., Deng, Y., Brandizzi, F., and Howell, S. H. (2012). Elements proximal to and within the transmembrane domain mediate the organelle-to-organelle movement of bZIP28 under ER stress conditions. *Plant J.* 70, 1033–1042. doi: 10.1111/j.1365-3113X.2012.04943.x
- Staehelein, L., and Kang, B. (2008). Nanoscale architecture of endoplasmic reticulum export sites and of Golgi membranes as determined by electron tomography. *Plant Physiol.* 147, 1454–1468. doi: 10.1104/pp.108.120618
- Stenmark, H. (2009). Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell Biol.* 10, 513–525. doi: 10.1038/nrm2728
- Storrie, B. (2005). Maintenance of Golgi apparatus structure in the face of continuous protein recycling to the endoplasmic reticulum: making ends meet. *Int. Rev. Cytol.* 244, 69–94. doi: 10.1016/S0074-7696(05)44002-4
- Strasser, R. (2014). Biological significance of complex N-glycans in plants and their impact on plant physiology. *Front. Plant Sci.* 5, 363. doi: 10.3389/fpls.2014.00363
- Strasser, R. (2016). Plant protein glycosylation. *Glycobiology* 26, 926–939. doi: 10.1093/glycob/cww023
- Strasser, R. (2018). Protein quality control in the endoplasmic reticulum of plants. *Annu. Rev. Plant Biol.* 69, 147–172. doi: 10.1146/annurev-arplant-042817-040331
- Strasser, R. (2022). Recent developments in deciphering the biological role of plant complex. *Front. Plant Sci.* 13, 897549. doi: 10.3389/fpls.2022.897549
- Strasser, R., Bondili, J. S., Schoberer, J., Svoboda, B., Liebminger, E., Glössl, J., et al. (2007a). Enzymatic properties and subcellular localization of Arabidopsis beta-N-acetylhexosaminidases. *Plant Physiol.* 145, 5–16. doi: 10.1104/pp.107.101162
- Strasser, R., Bondili, J. S., Vavra, U., Schoberer, J., Svoboda, B., Glössl, J., et al. (2007b). 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. doi: 10.1105/tpc.107.052985
- Strasser, R., Castilho, A., Stadlmann, J., Kunert, R., Quendler, H., Gättinger, P., et al. (2009). Improved virus neutralization by plant-produced anti-HIV antibodies with a homogeneous beta1,4-galactosylated N-glycan profile. *J. Biol. Chem.* 284, 20479–20485. doi: 10.1074/jbc.M109.014126
- Strasser, R., Mucha, J., Schwihla, H., Altmann, F., Glössl, J., and Steinkellner, H. (1999). Molecular cloning and characterization of cDNA coding for beta1,2N-acetylglucosaminyltransferase I (GlcNAc-TI) from Nicotiana tabacum. *Glycobiology* 9, 779–785. doi: 10.1093/glycob/9.8.779
- Strasser, R., Schoberer, J., Jin, C., Glössl, J., Mach, L., and 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. doi: 10.1111/j.1365-3113X.2005.02648.x
- Strasser, R., Seifert, G., Doblin, M. S., Johnson, K. L., Ruprecht, C., Pfrengle, F., et al. (2021). Cracking the “Sugar code”: A snapshot of. *Front. Plant Sci.* 12, 640919. doi: 10.3389/fpls.2021.640919
- Strasser, R., Stadlmann, J., Svoboda, B., Altmann, F., Glössl, J., and Mach, L. (2005). Molecular basis of N-acetylglucosaminyltransferase I deficiency in Arabidopsis thaliana plants lacking complex N-glycans. *Biochem. J.* 387, 385–391. doi: 10.1042/BJ20041686



- Suckling, R. J., Poon, P. P., Travis, S. M., Majoul, I. V., Hughson, F. M., Evans, P. R., et al. (2015). Structural basis for the binding of tryptophan-based motifs by  $\delta$ -COP. *Proc. Natl. Acad. Sci. U.S.A.* 112, 14242–14247. doi: 10.1073/pnas.1506186112
- Tan, X., Cao, K., Liu, F., Li, Y., Li, P., Gao, C., et al. (2016). Arabidopsis COG complex subunits COG3 and COG8 modulate golgi morphology, vesicle trafficking homeostasis and are essential for pollen tube growth. *PLoS Genet.* 12, e1006140. doi: 10.1371/journal.pgen.1006140
- Tekoah, Y., Shulman, A., Kizhner, T., Ruderfer, I., Fux, L., Nataf, Y., et al. (2015). Large-scale production of pharmaceutical proteins in plant cell culture-the Protalix experience. *Plant Biotechnol. J.* 13, 1199–1208. doi: 10.1111/pbi.12428
- Tu, L., and Banfield, D. K. (2010). Localization of golgi-resident glycosyltransferases. *Cell Mol. Life Sci.* 67, 29–41. doi: 10.1007/s00018-009-0126-z
- Tu, L., Chen, L., and Banfield, D. K. (2012). A conserved N-terminal arginine-motif in GOLPH3-family proteins mediates binding to coatamer. *Traffic* 13, 1496–1507. doi: 10.1111/j.1600-0854.2012.01403.x
- Tu, L., Tai, W. C., Chen, L., and Banfield, D. K. (2008). Signal-mediated dynamic retention of glycosyltransferases in the Golgi. *Science* 321, 404–407. doi: 10.1126/science.1159411
- Uemura, S., Shishido, F., Kashimura, M., and Inokuchi, J. (2015). The regulation of ER export and Golgi retention of ST3Gal5 (GM3/GM4 synthase) and B4GalNAcT1 (GM2/GD2/GA2 synthase) by arginine/lysine-based motif adjacent to the transmembrane domain. *Glycobiology* 25, 1410–1422. doi: 10.1093/glycob/cwv071
- Uemura, S., Yoshida, S., Shishido, F., and Inokuchi, J. (2009). The cytoplasmic tail of GM3 synthase defines its subcellular localization, stability, and *in vivo* activity. *Mol. Biol. Cell* 20, 3088–3100. doi: 10.1091/mbc.e08-12-1219
- Uliana, A. S., Giraudo, C. G., and Maccioni, H. J. (2006). Cytoplasmic tails of SialT2 and GalNAcT impose their respective proximal and distal Golgi localization. *Traffic* 7, 604–612. doi: 10.1111/j.1600-0854.2006.00413.x
- Ward, T., and Brandizzi, F. (2004). Dynamics of proteins in Golgi membranes: comparisons between mammalian and plant cells highlighted by photobleaching techniques. *Cell Mol. Life Sci.* 61, 172–185. doi: 10.1007/s00018-003-3355-6
- Wee, E., Sherrier, D., Prime, T., and Dupree, P. (1998). Targeting of active sialyltransferase to the plant Golgi apparatus. *Plant Cell* 10, 1759–1768. doi: 10.1105/tpc.10.10.1759
- Weigel, A. V., Chang, C. L., Shtengel, G., Xu, C. S., Hoffman, D. P., Freeman, M., et al. (2021). ER-to-Golgi protein delivery through an interwoven, tubular network extending from ER. *Cell* 184, 2412–2429.e2416. doi: 10.1016/j.cell.2021.03.035
- Welch, L. G., and Munro, S. (2019). A tale of short tails, through thick and thin: investigating the sorting mechanisms of Golgi enzymes. *FEBS Lett.* 593, 2452–2465. doi: 10.1002/1873-3468.13553
- Welch, L. G., Peak-Chew, S. Y., Begum, F., Stevens, T. J., and Munro, S. (2021). GOLPH3 and GOLPH3L are broad-spectrum COPI adaptors for sorting into intra-Golgi transport vesicles. *J. Cell Biol.* 220, e202106115. doi: 10.1083/jcb.202106115
- Westrate, L. M., Hoyer, M. J., Nash, M. J., and Voeltz, G. K. (2020). Vesicular and uncoated Rab1-dependent cargo carriers facilitate ER to Golgi transport. *J. Cell Sci.* 133, jcs239814. doi: 10.1242/jcs.239814
- Whaley, K. J., and Zeitlin, L. (2022). Emerging antibody-based products for infectious diseases: Planning for metric ton manufacturing. *Hum. Vaccin Immunother.* 18, 1930847. doi: 10.1080/21645515.2021.1930847
- Willet, R., Blackburn, J. B., Climer, L., Pokrovskaya, I., Kudlyk, T., Wang, W., et al. (2016). COG lobe B sub-complex engages v-SNARE GS15 and functions via regulated interaction with lobe A sub-complex. *Sci. Rep.* 6, 29139. doi: 10.1038/srep29139
- Wilson, I. B., Zeleny, R., Kolarich, D., Staudacher, E., Stroop, C. J., Kamerling, J. P., et al. (2001). Analysis of Asn-linked glycans from vegetable foodstuffs: widespread occurrence of Lewis a, core alpha1,3-linked fucose and xylose substitutions. *Glycobiology* 11, 261–274. doi: 10.1093/glycob/11.4.261
- Woollard, A. A., and Moore, I. (2008). The functions of Rab GTPases in plant membrane traffic. *Curr. Opin. Plant Biol.* 11, 610–619. doi: 10.1016/j.pbi.2008.09.010
- Yamaguchi, N., and Fukuda, M. N. (1995). Golgi retention mechanism of  $\beta$ -1,4-galactosyltransferase. Membrane-spanning domain-dependent homodimerization and association with  $\alpha$ - and  $\beta$ -tubulins. *J. Biol. Chem.* 270, 12170.12176. doi: 10.1074/jbc.270.20.12170
- Yuasa, K., Toyooka, K., Fukuda, H., and Matsuoka, K. (2005). Membrane-anchored prolyl hydroxylase with an export signal from the endoplasmic reticulum. *Plant J.* 41, 81–94. doi: 10.1111/j.1365-3113X.2004.02279.x
- Zaal, K., Smith, C., Polishchuk, R., Altan, N., Cole, N., Ellenberg, J., et al. (1999). Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* 99, 589–601. doi: 10.1016/S0092-8674(00)81548-2
- Zabotina, O., Zhang, N., and Weerts, R. (2021). Polysaccharide biosynthesis: Glycosyltransferases and their complexes. *Front. Plant Sci.* 12, 625307. doi: 10.3389/fpls.2021.625307
- Zahmanova, G., Aljabali, A. A. A., Takova, K., Minkov, G., Tambuwala, M. M., Minkov, I., et al. (2023). Green biologics: harnessing the power of plants to produce pharmaceuticals. *Int. J. Mol. Sci.* 24. doi: 10.3390/ijms242417575
- Zeng, W., Ford, K. L., Bacic, A., and Heazlewood, J. L. (2018). N-linked glycan microheterogeneity in glycoproteins of arabidopsis. *Mol. Cell Proteomics* 17, 413–421. doi: 10.1074/mcp.RA117.000165
- Zhang, G. F., and Staehelin, L. A. (1992). Functional compartmentation of the Golgi apparatus of plant cells: immunocytochemical analysis of high-pressure frozen- and freeze-substituted sycamore maple suspension culture cells. *Plant Physiol.* 99, 1070–1083. doi: 10.1104/pp.99.3.1070
- Zierath, J. R., and Lendahl, U. (2013). *Machinery regulating vesicle traffic, a major transport system in our cells* (The 2013 Nobel Prize in Physiology or Medicine - Advanced Information).