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REVIEW

Localization of Golgi-resident glycosyltransferases

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Abstract For many glycosyltransferases, the information that instructs Golgi localization is located within a relatively short sequence of amino acids in the N-termini of these proteins comprising: the cytoplasmic tail, the transmembrane spanning region, and the stem region (CTS). Also, one enzyme may be more reliant on a particular region in the CTS for its localization than another. The predominance of these integral membrane proteins in the Golgi has seen these enzymes become central players in the development of membrane trafficking models of transport within this organelle. It is now understood that the means by which the characteristic distributions of glycosyltransferases arise within the subcompartments of the Golgi is inextricably linked to the mechanisms that cells employ to direct the flow of proteins and lipids within this organelle.

Keywords COG · Coatomer · Protein sorting · Glycosyltransferase · Golgi

Introduction

Proteins enter the secretory pathway following their translocation into the lumen of the endoplasmic reticulum (ER). Folded, core-glycosylated proteins exit the ER in transport vesicles transiting the Golgi apparatus via the *cis*, *medial*, and *trans* cisternae (Fig. 1). In addition to being a protein-sorting hub for the cell, the organization of the Golgi apparatus non-uniformly compartmentalizes the enzymes involved in the covalent attachment of glycans to proteins and lipids—a process collectively referred to as glycosylation. In eukaryotic cells, glycosylation results in the generation of glycoproteins, glycolipids, and proteoglycans [1].

Glycosylation is ubiquitous among eukaryotes and is catalyzed by a group of enzymes called glycosyltransferases. However, although some aspects of glycosylation pathways are evolutionarily conserved, there is also considerable variation amongst organisms. Understanding how these enzymes are directed to, and retained in, the sub-compartments of the Golgi in which they function remains an important question in cell biology. Glycosyltransferases are the predominant integral membrane protein constituents of Golgi membranes and, as such, a detailed understanding of the mechanisms involved in establishing and maintaining the steady-state distribution of these enzymes within the Golgi promises to offer tremendous insight into the mechanisms that govern protein and lipid trafficking through this organelle.

The majority of glycosyltransferases are type II integral membrane proteins comprised of a cytoplasmic tail (CT), a transmembrane (TM) spanning region, a stem region, and an enzymatic domain (Fig. 2). The relative position an enzyme occupies in the Golgi (i.e., whether its steady-state localization is predominantly in the *cis*, *medial*, or *trans* cisternae) often reflects the sequential order in which these enzymes act in glycan synthesis, and appears to be an important contributing factor to the structure of the glycans that a cell produces.

Glycosylation plays a plethora of roles in the cell biology of single-celled and multi-cellular eukaryotic organisms. These include protein folding, cell polarization, cell–cell communication, cell wall biosynthesis, development, and cell differentiation/specialization. In addition, defects in

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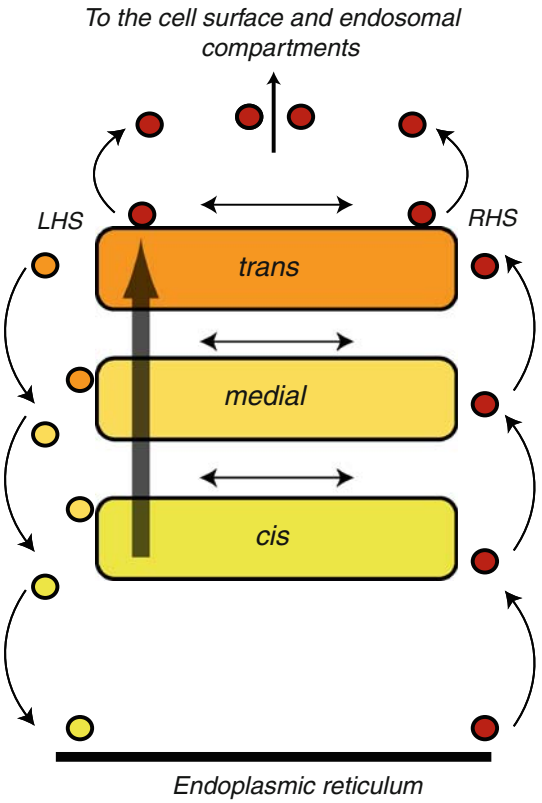

Fig. 1 Mechanisms of protein and membrane trafficking in the Golgi (left-hand side, *LHS*). Retrograde transport vesicles carrying glycosyltransferases and transport machinery in COPI-coated vesicles. *Vertical arrows* indicate the direction of movement of proteins within the Golgi whereas *horizontal arrows* denote the lateral segregation of proteins and lipids within individual cisterna. The *thicker arrow* denotes the movement of proteins transiting the Golgi via cisternal maturation. The distinct protein and lipid compositions of individual cisterna may be established by cisternal maturation and/or rapid partitioning [28]. Similarly, proteins transiting the Golgi apparatus may do so in forward moving COPI-generated transport vesicles (right-hand side, *RHS*), by rapid partitioning and/or via cisternal maturation [28]. Vesicles on the LHS carry enzymes retrieved from the *trans*, *medial* and *cis* cisternae whereas vesicles on the RHS (*red* in color) are transporting proteins transiting the Golgi

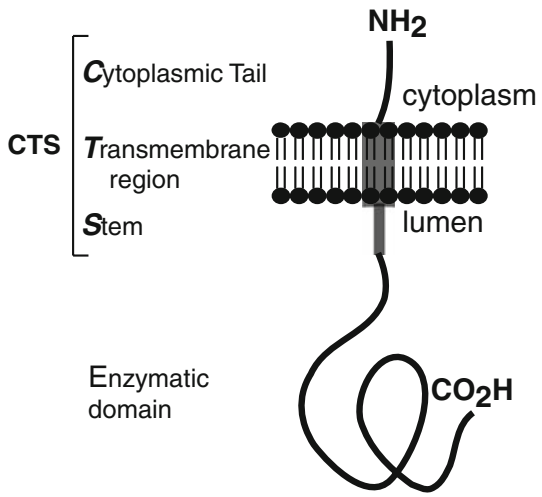

Fig. 2 The modular organization of glycosyltransferases. The region corresponding to the cytoplasmic domain, the transmembrane spanning region, and luminal stem region are herein collectively referred to as the *CTS*

glycosylation cause severe congenital disorders in humans. Thus, understanding the requirements for the non-uniform distribution of these enzymes in the Golgi also has important implications for the development of therapeutics; in particular where recombinant glycoproteins with human N- and O-linked modifications are desirable [2–6]. Here, we focus on what is currently understood about how glycosyltransferases are localized to the Golgi.

An overview of the action of glycosyltransferases

Glycosyltransferases catalyze the transfer of a specific sugar, from a sugar-nucleotide donor to a particular hydroxyl position of a monosaccharide in a growing glycan chain via an α- or *β*-linkage [7]. In single-celled eukaryotic organisms such as the budding yeast *Saccharomyces cerevisiae*, only two nucleotide donors, UDP-*N*-acetylglucosamine (GlcNAc) and GDP-mannose, are used [7]. UDP-GlcNAc and GDP-mannose are required for core glycosylation of proteins in the ER whereas only GDP-mannose is required for N- and O-linked mannosylation reactions that occur in the Golgi [8–10]. In contrast, in mammalian cells, nine monosaccharide nucleotide donors are known to be employed, reflecting the overall increase in the complexity of glycan synthesis reactions in higher eukaryotes [1, 10]. The type of glycans produced by cells is influenced by a number of factors including: the variety of enzymes expressed by the cell and their substrate affinity, the distribution of enzymes within the Golgi, and the diversity of sugar nucleotide donors and the respective affinity of enzymes for these donors.

Proteins can be modified by sugars that are attached either to the amino group side chain of asparagine residues (N-linked glycosylation) in the context of N-X-S/T (where X is any amino acid except proline) or, most commonly, to the hydroxyl group side chains of serine (S) or threonine (T) residues (O-linked glycosylation) [7]. The initiation of both N- and O-glycosylation of proteins occurs in the ER, and this process is extended as glycoproteins transit the Golgi apparatus. Although some protein glycosylation processes are fairly well conserved throughout eukaryotic evolution (in particular N-linked glycosylation and O-linked mannosylation), a substantial degree of diversity in the repertoire of carbohydrate modifications of proteins and lipids exists with respect to the glycan components, the chemical mode of the carbohydrate linkages (α- or *β*-),

branching of the glycans, and the overall length of the glycan chains.

Glycolipids are lipids with monosaccharides covalently linked to ceramide. After ceramide is either glucosylated by a ceramide-specific glucosyltransferase or galactosylated by a specific galactosyltransferase, further sugar side chain elongation of the lipid bound oligosaccharides is carried out in the ER and Golgi by glycolipid glycosyltransferases, many of which also act on proteins [11]. Like their protein glycosyltransferase counterparts, glycolipid glycosyltransferases are also type II integral membrane proteins comprised of a CTS and enzymatic domain (Fig. 2) [12]. Unless stated otherwise, glycolipid glycosyltransferases will hereafter be referred to as glycosyltransferases.

Overview of ER–Golgi transport of glycosyltransferases

Glycosyltransferases reach the Golgi apparatus via vesicle-mediated transport from the ER. The packaging of these enzymes into ER-derived (COPII-coated) transport vesicles requires amino acids with positively charged side chains (R and K) located on the cytoplasmic side of the membrane proximal region. These residues have also been shown to mediate the interaction between glycosyltransferases and the COPII coat [13, 14]. In addition, the cytoplasmic tails of some glycosyltransferases may associate with additional proteins that function in the transport of enzymes from the ER to the Golgi. For example, in *Drosophila*, an N-acetylgalactosaminyltransferase (β4GalNacTB) requires the presence of a multi-spanning membrane protein (GABPI) to form the active enzyme, as well as to direct its steady-state distribution to the Golgi [15]. The features of this region of β4GalNacTB required for this interaction have not been determined.

ER-derived transport vesicles tether and subsequently fuse with the *cis* cisternae of the Golgi. Once at the Golgi, glycosyltransferases migrate to the cisternae on which they function. The distribution of enzymes throughout the Golgi is not homogeneous, and often reflects the sequential order in which particular glycosyltransferases act in glycan synthesis. Mislocalization of glycosyltransferases within the Golgi can have profound effects on glycan synthesis and in turn on the normal physiology of cells. The cell biological, developmental, and physiological significance of the action of glycosyltransferases is highlighted by the consequences of deletion or knock-down of members of this protein family, which results in developmental anomalies as well as in congenital defects in glycosylation (CDG) in humans [1, 16–23].

Mechanisms of compartmentalization of glycosyltransferases in the Golgi

Precisely how glycosyltransferases establish their non-uniform distributions within the Golgi is a question that has puzzled cell biologists for several decades [24]. In many cases, understanding the requirements for the localization of glycosyltransferases has been complicated by the method of assessment used: such as the use of chimeric enzymes, truncated proteins, different cell types, or non-physiological reporters [12, 25]. Localization studies have been further confounded by the observation that different cell types from the same organism can show distinct localization patterns for the same enzyme [12, 25].

In addition, the models proposed for the mechanism(s) employed by cells to localize proteins to the Golgi have understandably been influenced by the prevailing view of the mode of transport through this organelle. Several models have been proposed whereby membrane proteins, such as glycosyltransferases, are retained in the Golgi apparatus, and several reviews covering the localization and retention of Golgi membrane proteins are available [12, 25–27]. Figure 1 summarizes the current views of transport within the Golgi.

Golgi localization of glycosyltransferases mediated by enzyme oligomerization/aggregation

Glycosyltransferases can exist as monomers, homo-dimers, hetero-dimers, or hetero-oligomers, and the observation that some glycosyltransferases can form oligomers led to the proposal that these enzyme aggregates might be too large to enter transport vesicles and thus they remain in their respective cisternae. Until relatively recently, the turn of this century, it was believed that Golgi-resident enzymes were confined to their resident cisternae as only anterograde (forward moving) proteins were thought to be incorporated into transport vesicles (Fig. 1, RHS; [28]). A key requirement of this aggregation/kin-recognition model of glycosyltransferase Golgi retention was that formation of oligomers had to occur in the (cisternal) vicinity in which the steady-state localization of the enzymes was observed. For example, aggregation of *trans*-resident glycosyltransferases in the *cis* Golgi would presumably preclude their arrival at the *trans*-most cisternae. However, this is only a mechanistic issue where transport within the Golgi is exclusively mediated by forward vesicle-mediated transport, which is unlikely to be the case (Fig. 1; [25, 28, 29]).

Glycosyltransferase associations appear to contribute to both the localization of these enzymes in the Golgi as well as to their enzymatic activity. In addition, for some

complexes, it is known that members are co-dependent for both localization and biochemical activity [30, 31]. For some glycosyltransferases, homo-dimerization has been shown to result from disulphide bond formation within the transmembrane spanning region [32–34]. On the other hand, for other enzymes, this may come about as a result of interactions between amino acids in the TM region bearing uncharged polar side-chains [35, 36]. In any case, the formation of enzyme hetero-oligomers presumably ensures that enzymes will function sequentially, as the product formed by one member of the complex serves as a substrate for another member. For example, the sequential action of Mannosidase II (ManII) and N-acetylglucosaminyl transferase I (GlcNAcT1) is crucial to the synthesis of N-linked glycans in mammalian cells [31]. Man II and GlcNacT1 bind to one another, and the localization of one enzyme can influence the localization of the other [37, 38].

The formation of complexes comprised of more than one glycosyltransferase plays an important role in the sequential modification of proteins and lipids. For example, in *S. cerevisiae* glycosyltransferases involved in N- and O-linked glycan synthesis form hetero-oligomers [39, 40]. Whereas, studies in mammalian cells have highlighted the necessity for Golgi enzyme oligomerization in glycolipid [41], ganglioside [42], and glycosylaminoglycan [30] biosynthesis. For some glycosyltransferase hetero-oligomers, the presence of each partner is required for their function, as well as for their correct localization [30]. Regions of glycosyltransferases influencing oligomerization are known to reside in the stem region and/or the transmembrane (TM) region, and the requirements among enzymes and complexes are by no means ubiquitous [12, 25, 31, 43]. In addition, the physiochemical environment (e.g., the pH) of particular cisternae may also influence the ability of glycosyltransferases to form oligomers [44] as well as protein localization [45]. In one case, oligomerization between two glycosyltransferases was shown to occur through the stem region, although disrupting oligomerization did not result in mislocalization of either protein [38]. This example illustrates that the formation of glycosyltransferase oligomers is not necessarily a prerequisite for proper localization, and therefore aggregation cannot account for the localization of all enzymes.

The role of the transmembrane domain in glycosyltransferease localization

Several studies have revealed either an exclusive or combinatorial requirement for the TMs of glycosyltransferases in the Golgi targeting of these enzymes [12, 25, 34, 46–52]. As discussed above, the ability of some glycosyltransferases to form aggregates is a contributing factor for Golgi localization. Features of the membrane spanning region that are known to contribute to the oligomerization of enzymes include the formation of disulphide bonds [34] as well as associations between uncharged polar side chains of amino acids [35, 36]. Amino acids with uncharged polar side chains are common in the membrane spanning regions of glycosyltransferases [53] (Table 1). In yeast, at least two mannosyltransferases are palmitoylated on cysteine residues adjacent to the cytoplasmic side of the transmembrane spanning region, although the functional significance of this modification has not been explored [54]. However, by analogy to SNAREs proteins [55], palmitoylation may mask features of the TMs of glycosyltransferases that might otherwise result in their mislocalization and degradation, or alternatively act to sequester these enzymes into particular cisternae. Thus, the presence of amino acids with uncharged polar side chains may contribute to Golgi localization by: promoting enzyme aggregation/oligomerization, restricting the movement of enzymes within or out of the Golgi, enriching enzymes within a particular lipid environment of the organelle, facilitating export from the ER [56], or by preventing enzyme mislocalization and degradation (Fig. 3) [29].

To date, two lipid-based sorting mechanisms have been proposed to account for the localization of glycosyltransferases within the Golgi [29, 53, 57, 58] (Fig. 3). One mechanism is based on the observations that: (1) Golgi localized proteins tend to have shorter membrane spanning regions than those located in the plasma membrane; (2) the membrane spanning regions of Golgi localized proteins tend to be enriched in amino acids with aromatic side chains (Table 1); and (3) that the composition of lipids in membranes of the secretory pathway are not homogeneous [59] and consequently membrane bilayers of the Golgi are thinner than their plasma membrane counter-parts (Fig. 3). This proposal has received experimental support from studies that revealed that altering the composition, but not the length of the TM, had no effect on the Golgi-localization of a mammalian glycosyltransferase (alpha-2,6 sialyltransferase I), whereas increasing the length of this enzyme's TM region resulted in mislocalization of the protein to the plasma membrane [50]. However, it is unlikely that the length of the membrane spanning region can entirely account for the localization of these enzymes, as other glycosyltransferases show no obligate dependence on this region for their steady-state distributions [25, 51, 60], and there is significant variation in the length of the TM regions among Golgi-resident glycosyltransferases (Table 1).

The other and more recently proposed mechanism suggests a more prominent role for lipid composition in the Golgi-localization of glycosyltransferases (Fig. 3) [28, 29]. This mechanism relies on the segregation of integral membrane proteins into membrane domains on the basis of

Table 1 The distribution of amino acids with aromatic [amino acids with aromatic side chains (F, Y, and W) are underlined] and uncharged polar side chains [amino acids with uncharged polar side chains (S, T, C, P, N and Q) are in bold and italics] in the TM spanning regions of yeast glycosyltransferases

Enzyme	Amino acid sequence of the transmembrane spanning region	Localization	Enzymatic activity	References
Kre2	-F***T***VIAGAVIVLLL***T***L***NSNS***- (19)	*Medial*-Golgi	α1,2-Mannosyltransferase	[60, 93, 94]
Ktr1	-LGLLLVAVF***T***VYVFF- (15)	Golgi	α1,2-Mannosyltransferase	[93, 95, 96]
Ktr2	-LLFV***S***LLF***C***LIA***QTC***WLALVPY***Q***- (23)	Golgi	N-linked protein glycosylation	[95]
Ktr3	-***SS***FIGLIIVL***S***FLFFM***S***G***S***- (19)	Early-Golgi	Putative α1,2- mannosyltransferase	[93, 97]
Ktr4	-***P***VL***S***VIILI***S***IAV***T***VVLYFL***T***A***N***- (23)	Golgi	Putative mannosyltransferase	[97, 98]
Ktr5	-***CNLT***A***TC***ILIAFVI***T***MYVVLV***S***- (22)	Golgi	Putative mannosyltransferase	[99, 100]
Ktr6	-FLLI***S***FVFVLALMV***T***I***N***- (16)	Golgi	Putative mannosylphosphate transferase	[75, 101, 102]
Ktr7	-YGFLFLG***C***IFAILY***C***MG***T***W***P***FFA- (23)	Golgi	Putative mannosyltransferase	[99, 100]
Yur1	-GG***S***LYIVGIFL***P***IW***T***FMIYIFG- (22)	Golgi	N-linked protein glycosylation	[95]
Mnt2	-LFILVVLLGIVLVVYY***SQLNS***L- (22)	Golgi	α1,3-Mannosyltransferase	[103]
Mnt3	-LV***T***LLL***S***LFF***S***YLIF***S***A***S***- (18)	Golgi	α1,3-Mannosyltransferase	[103]
Mnt4	-LA***P***LIF***TS***LL***S***LIVLF- (16)	ND	Putative α1,3- mannosyltransferase	[103]
Mnn1	-***SCTIP***ILVGALIIILVLF***Q***LV***T***- (22)	*Medial*-Golgi	α1,3-Mannosyltransferase	[104, 105]
Mnn2	-L***T***FIVLIL***C***GLFVI***TN***- (16)	*Cis*-Golgi	α1,2-Mannosyltransferase	[106]
Mnn4	-ILL***P***LILL***Q***IIII***T***FIW***SNSPQ***- (22)	ND	Putative positive regulator of Ktr6p	[102, 107]
Mnn5	-IL***Q***VIV***S***AVVLILFF***CS***V- (18)	*Cis*-Golgi	α1,2-Mannosyltransferase	[106]
Mnn9	-***NP***WV***N***IFL***P***VLAIFLIYIIFF***Q***- (22)	*Cis*-Golgi	α1,2- and contributes to α1,6- mannosyltransferase	[39, 73]
Anp1	-LVL***S***FF***S***I***S***LF***Q***LV***T***F***Q***GIF- (20)	*Cis*-Golgi	Contributes to α1,6- mannosyltransferase	[39]
Hoc1	-LMIFAIIALI***S***LAFGV- (18)	*Cis*-Golgi	Contributes to α1,6- mannosyltransferase	[39]
Gnt1	-FIVFILFGVL***T***VFVV***S***- (18)	*Medial*-Golgi	N-acetylglucosaminyltransferase	[108]
Och1	-***T***IVV***T***VLLIY***S***LL***T***F- (17)	*Cis*-Golgi	α1,6-Mannosyltransferase	[109, 110]

The length of the membrane spanning sequence is given in parentheses

ND Not determined

the properties of individual membrane compartments. The authors propose that differences in the ratios of lipids, in particular, glycerophospholipids (GPLs) and sphingolipids, could account for observed protein distributions; SL/GPL ratios are lowest in the *cis* and highest in the *trans* Golgi [28, 29]. Segregation of sphingolipids and sterols in the *trans* Golgi has also been observed in yeast cells [61]. Thus, integral membrane proteins with different SL/GPL preferences would be enriched in different Golgi cisternae. Presumably, the features of membrane proteins that could account for such SL/GPL preferences reside in their TM spanning regions and may include the preponderance of amino acids with uncharged polar side chains and/or aromatic side chains (Table 1). As this model was developed based on experiments carried out in mammalian cells, it will be important to access the universality of these observations in cells from other organisms.

The role of the cytoplasmic tail in glycosyltransferease localization

The importance of the cytoplasmic tail (CT) in the localization of Golgi-resident glycosyltransferases and glycosidases (which are often topologically similar to glycosyltransferases) has been highlighted by several studies [62–68]. However, precisely how the CT might affect the localization of these enzymes remains elusive.

Glycosyltransferases are known to cycle both within the Golgi and between the Golgi and the ER [29, 69, 70] by both COPI-dependent [71] and COPI-independent [72] mechanisms. The localization of many integral membrane proteins to the Golgi apparatus at steady state appears to rely on a dynamic process wherein proteins cycle within the Golgi cisternae or between the Golgi cisternae and the ER [28]. Such dynamic localization has been observed for glycosyltransferases and, for some enzymes at least, this process is likely to be mediated by the transport of enzymes within COPI-coated vesicles. For example, in yeast cells, Och1p (which encodes an α 1,6 mannosyltransferase) is predominantly located in the *cis* Golgi at steady state but can reach the *trans* compartment from where it is retrieved in a COPI-dependent manner [69]. Similarly, members of the yeast Man-Pol-I and Man-Pol-II complexes (comprised of Mnn9p and Van1p; and Mnn9p, Hoc1p, Anp1, Mnn10p, and Mnn11p; respectively [39, 40, 73]) cycle between the Golgi and ER in a COPI- and SNARE-dependent manner

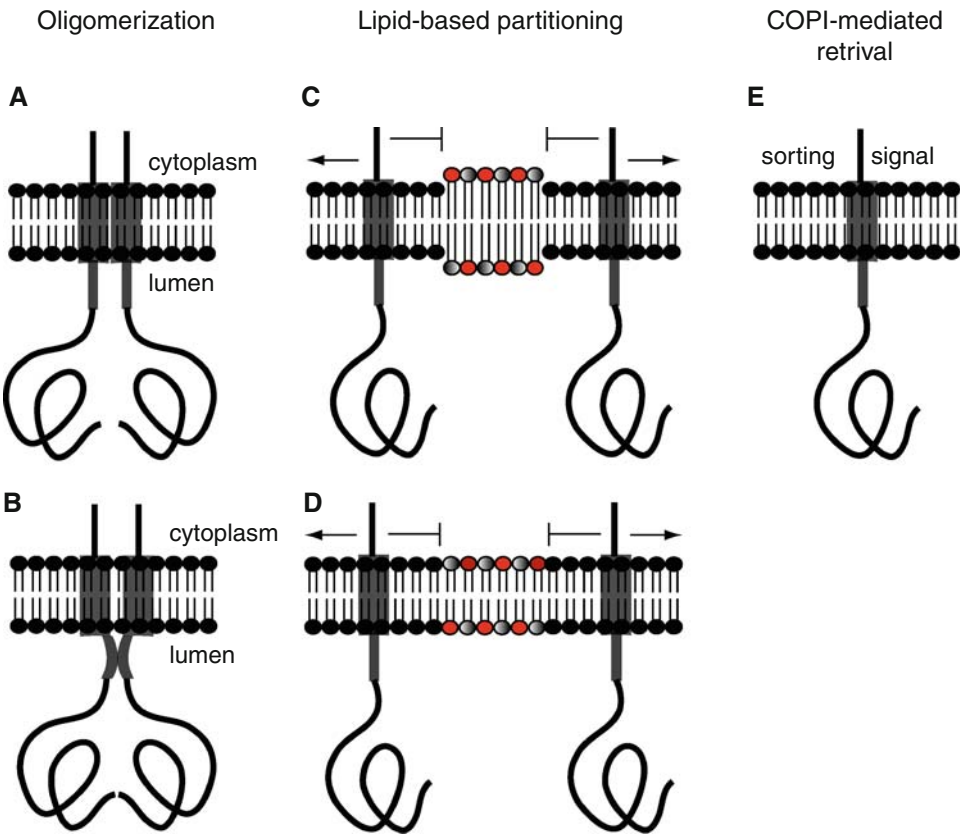

Fig. 3 Mechanisms and features mediating retention of glycosyltransferases in the Golgi. **a** Enzyme oligomerization/aggregation mediated by the TM region through disulphide bond formation or associations between uncharged polar side chains of amino acids. **b** Enzyme oligomerization/aggregation mediated by associations within the stem region. Enzyme oligomerization/aggregation may also result from a combination of the mechanisms depicted in (**a**) and (**b**); see text for details. **c** Lipid-based partitioning of glycosyltransferases on the basis of differences in bilayer thickness and width of the TM region. The *colored regions* within the membrane denote differences in the lipid composition of the bilayer. **d** Lipid-based partitioning of glycosyltransferases based on the affinity of the TM region for membranes of a particular SL/GPL ratio [28, 29]. **e** Motif-based sorting of glycosyltransferases. RXR-based motifs and/or Vps74p-binding motifs in the CT of enzymes mediates the direct and indirect association, respectively of glycosyltransferases with the COPI vesicle coat complex. These modes of localization are not necessarily mutually exclusive and the localization of a particular enzyme within the Golgi may be the result of a combination of one or more modes

[71]. However, the CTs of glycosyltransferases lack canonical COPI-binding motifs so how can these proteins be selected for packaging into COPI coated vesicles?

Several recent studies have identified motifs in the CTs of glycosyltransferases that appear to be important for maintaining their steady state distributions [66, 74, 75], and proteins that bind to the CTs [75–77].

In yeast cells, the mannosyltransferase Och1p cycles between the *trans* and *cis* Golgi [69, 71], whereas the mannosyltransferase Mnn9p cycles between the Golgi and the ER [71]. Unlike Mnn9p, Och1p is not retrieved from the Golgi to the ER [71]. Okamoto and colleagues [66] showed that the differences in the sorting repertoires of Mnn9p and Och1p reside in the CTs of these proteins. When the CT of Och1p was replaced with that of Mnn9p, the chimeric protein could be retrieved from the Golgi to the ER [66]. Amino acid substitution analysis revealed that arginine (R) residues within the CT of Mnn9p, conforming to a canonical R-based COPI-binding motif [66, 78], were required for retrieval. Similarly, a recent study by Uemura et al. [74] identified an R-based motif in the CT of GM3 synthase that was required for the robust re-cycling of this enzyme from the Golgi to the ER.

Using the CT of β-1,3-galactosyltransferase (GalT2) as bait in a yeast two-hybrid screen, calsenilin and calsenilin-like protein (CALP), two members of the recoverin-neuronal calcium sensor family of calcium-binding proteins, were identified and subsequently shown to influence the Golgi-localization of β-1,3- GalT2 by binding to its CT [76]. While expression of GalT2 in CHO-K1 cells gave a typical Golgi localization pattern, co-expression of CALP/calsenilin with GalT2 in CHO-K1 cells resulted in the appearance of GalT2 in the ER as well as in the Golgi [76]. Calsenilin and CALP also affected the distribution of β-1,4-N-acetylgalactosaminyltransferase (GalNAcT) and sialytransferase (SialT2); however, as GalNAcT has been

shown to bind to GalT2, and SialT2 can bind to GalNAcT, the influence of CALP and calsenilin on GalNAcT and SialT2 may be mediated by GalT2 ([76] and references therein). Precisely how CALP/calsenlin affects the localization of glycosyltransferases awaits further study.

Studies examining the function of *VPS74* established that deletion of the gene resulted in defects in both N- and O-linked mannosylation in yeast [75, 77]. Closer examination of the fate of mannosyltransferases in cells lacking *VPS74* lead to the identification of a peptide motif ([F/L][L/I/V]XX[R/K]) in the cytoplasmic tails of numerous yeast glycosyltransferases (Table 2). The presence of this peptide sequence in the CT was shown to be required for binding to Vps74p [75]. Vps74p bound both the CT of glycosyltransferases as well as to the COPI coat, providing a mechanism by which these enzymes can be incorporated into retrograde transport vesicles [75]. Even though the Vps74p-binding motif is not present in the CTs of mammalian glycosyltransferases, the human orthologs of Vps74p (i.e., Gpp34/GmX33α and Gpp34R/Gmx33β [79, 80]) could still functionally substitute for Vps74p when they where expressed in yeast cells [75]. Although Vps74-like proteins are present in the majority of eukaryotes, which suggests a conserved mechanism, homologs have yet to be identified in higher plants and protozoa, an observation that supports the operation of multiple mechanisms in the retention of glycosyltransferases in the Golgi. Taken together, these studies suggest a sorting repertoire for glycosyltransferases in which both RXR-based motifs and Vps74p-binding motifs influence the steady-state distribution of glycosyltransferases in cells via COPI-coated vesicle retrograde transport (Fig. 4). Depending on the context, the same set of basic amino acids can function to promote glycosyltransferase export from the ER and retrieval of these enzymes from the Golgi [13, 14, 66, 74, 78].

Nevertheless, not all glycosyltransferases contain Vps74p-binding motifs or RXR-based motifs, so how then are these proteins incorporated into COPI vesicles? As many glycosyltransferases form hetero-oligomers [39, 40], perhaps it is possible that such complexes can be retrieved en masse by taking advantage of members that contain RXR-based motifs and/or Vps74p-binding motifs. For example, Mnn9p has both a Vps74p-binding motif [75] and an RXR-based motif [66], and is a component of both the Man-Pol-I (together with Van1p) and Man-Pol-II (together with Hoc1p, Anp1p, Mnn10p, and Mnn11p) complexes. Thus, in addition to its enzymatic role, Mnn9p may also be required to maintain the steady-state distributions of the Man-Pol-I and Man-Pol-II complexes in the yeast Golgi.

Table 2 Vps74p-binding motifs in the cytoplasmic tails of yeast Golgi resident glycosyltransferases

Enzyme[a]	Amino acid sequence of the cytoplasmic tail[b]
Kre2	MAL***FLSKR***LLR- - - - - - - - - - - - - - - - - - - -
Ktr1	MAKIMIPASKQPVYKK- - - - - - - - - - - - - - - - -
Ktr2	MQICKVFLTQVKK- - - - - - - - - - - - - - - - - -
Ktr3	MSVHHKKKLMPKSA***LLIRK***YQKGIR- - - - - - -
Ktr4	MR***FLSKR***ILK- - - - - - - - - - - - - - - - - - - -
Ktr5	M***LLIRR***TINAFLGCIH- - - - - - - - - - - - - - - -
Ktr6	MHV***LLSKK***IAR- - - - - - - - - - - - - - - - - - -
Ktr7	MAIRLNPKVRR***FLLDK***CRQKR- - - - - - - - -
Yur1	MAK- -
Mnt2	MRRKNR- -
Mnt3	MLKSLKSRR***LILKR***- - - - - - - - - - - - - - - - -
Mnt4	M***VLRIR***RIKK- - - - - - - - - - - - - - - - - - - -
Mnn1	MLALRRF***ILNQR***SLR- - - - - - - - - - - - - - - -
Mnn2	M***LLTKR***FSKLFK- - - - - - - - - - - - - - - - - -
Mnn4	MLQRISSKLHRRFLSG***LLRVK***HYPLRR- - - - -
Mnn5	M***LIRLK***KRK- -
Mnn9	MSLS***LVSYR***LRK- - - - - - - - - - - - - - - - - - -
Anp1	MKYNNRKLSFNPTTVSIAGTLLTVFFLTR- - - - -
Hoc1	MAKTTKRASSFRR- - - - - - - - - - - - - - - - - - - -
Gnt1	MR***LISKR***RIR- -
Och1	MSRKLSH***LIATR***KSK- - - - - - - - - - - - - - - - -

[a] Glycosyltransferases bearing presumptive Vps74p-binding motifs [(F/L)(L/I/V)-X-X- (R/K)] motifs are highlighted in bold

[b] Their corresponding amino acid residues are underlined and in italics

The role of vesicle tethering complexes in glycosyltransferase localization

The non-uniform distribution of glycosyltransferases in the Golgi is maintained through a combination of retention and recycling mechanisms. It is generally accepted that proteins are recycled in COPI-coated vesicles, and this has been shown to be the case for both yeast and mammalian glycosyltransferases [25, 69–71]. Thus, glycosyltransferases maintain their steady-state localization to the Golgi dynamically, through iterative rounds of retrieval and forward transport.

The COG complex is evolutionarily conserved and localizes peripherally to the cytoplasmic side of Golgi membranes. Support for a role of the COG complex in the intra-Golgi retrograde transportation of glycosyltransferases comes from multiple findings. Defects in components of the hetero-octameric COG complex in yeast and mammalian cells result in mislocalization and instability of glycosyltransferases [81–84]. Mammalian cell lines deficient in COG subunits show pleiotrophic defects in both N- and O-linked protein glycosylation, as well as deficiencies in biosynthesis of glycolipids, suggesting that

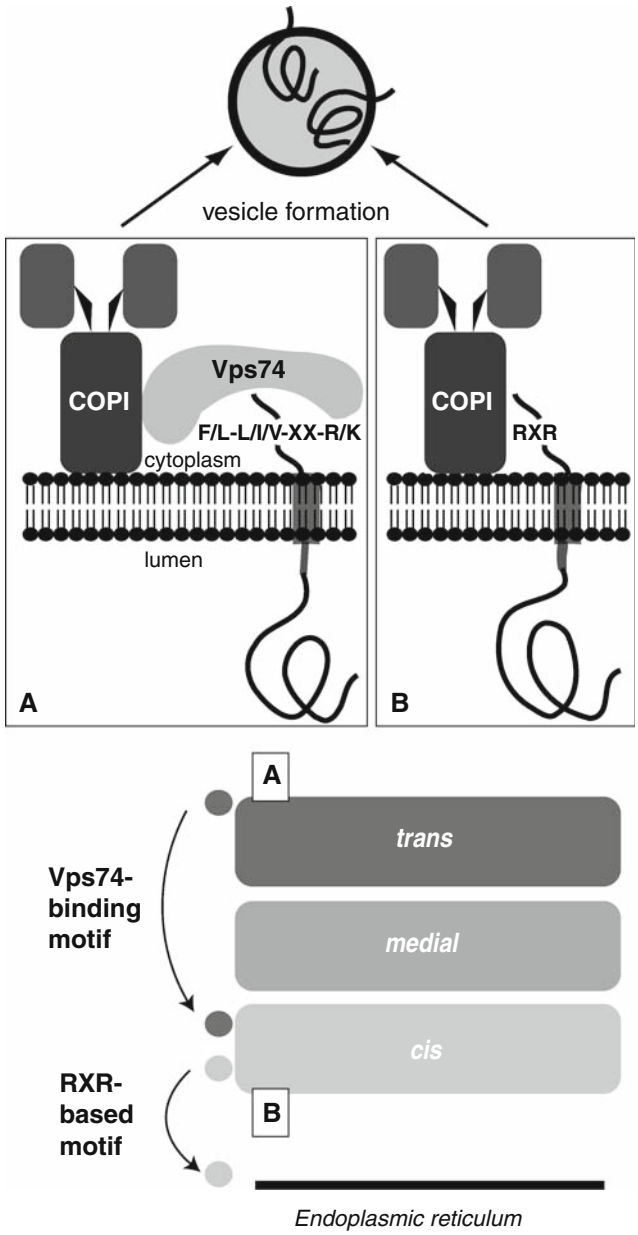

Fig. 4 Motif-based selection of Golgi-resident glycosyltransferases for COPI-mediated retrograde transport. Glycosyltransferases bearing Vps74p-binding motifs and or RXR-based motifs are sequestered into COPI-coated vesicles. Current evidence suggests that RXR-based motifs mediate retrieval from the *cis* cisterna to the ER. The presence of a Vps74p-binding motif in Och1p together with the observation that Och1p does not cycle through the ER is consistent with the Vps74p-binding motif mediating intra-Golgi retrograde transport of glycosyltransferases. Although not indicated, it is possible that enzymes may also be retrieved from the *medial* to *cis* cisterna and from the *trans* to *medial* cisterna

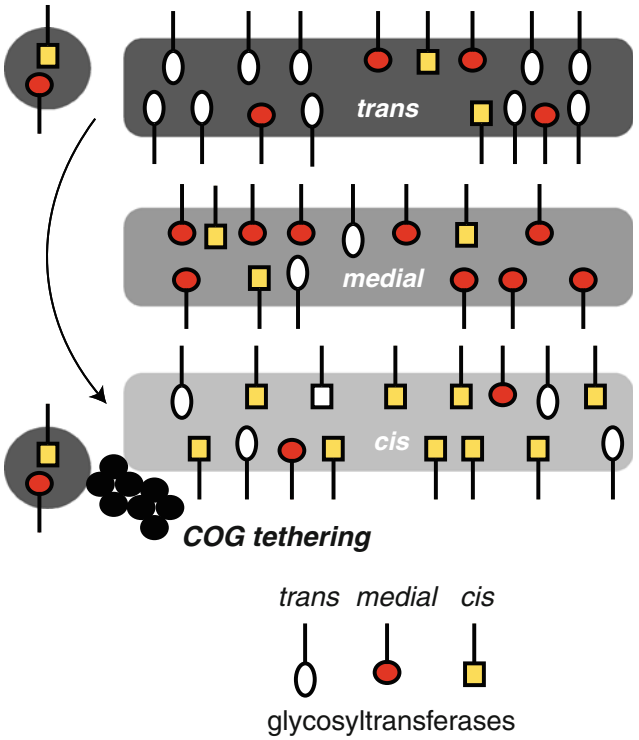

Fig. 5 COG-mediated retrograde transport of Golgi-resident glycosyltransferases. *Medial*- and *cis*-resident glycosyltransferases are predominantly retrieved from the *trans* cisterna. COPI-generated vesicles dock with the *cis* cisterna through association with COG, a presumptive vesicle-tethering complex. Although not indicated, it is possible that enzymes may also be retrieved from the *medial* to *cis* cisterna and from the *trans* to *medial* cisterna

COG is important for the integrity of glycan synthesis in general [83, 84]. COG is also required for the retrograde transport of Och1p within the yeast Golgi [81]. Temperature-sensitive alleles of COG component genes accumulate vesicles at the restrictive temperature and prolonged RNAi-mediated knockdown of COG constituents in mammalian cells results in the accumulation of vesicles containing retrograde transport machinery (SNAREs) as well as glycosyltransferases [83, 84]. It is well established that COPI-mediated retrograde transport requires the action of SNAREs as well as GTPases of the Rab/Ypt family, and COG has been implicated genetically and physiologically with COPI, SNAREs, and Rabs [83, 84]. Together, these findings are consistent with COG acting as a tether for vesicles containing recycling glycosyltransferases (Fig. 5). The importance of vesicle-mediated cycling of glycosyltransferases is highlighted by the severity of the phenotypes of cells carrying mutations in components of the COG complex. Congenital disorder of glycosylation (CDG) type II (CDG-II) is linked to defects in the expression and/or function of COG components [23, 85–89]. CDG-II has severe consequences, resulting in death shortly after birth, and is associated with a wide spectrum of developmental anomalies reflecting the importance of glycan synthesis in eukaryotic cell biology [22, 23, 83].

The impact of glycosyltransferase Golgi localization requirements on synthetic glycobiology

Concerns over the use of bovine serums for the propagation of mammalian cell lines in culture has lead to the development of alternate systems for the expression of human protein therapeutics. For a variety of reasons, the use of eukaryotic microorganisms such as yeasts is an attractive alternative. However, after the addition and processing of the core glycan ($Man_8GlcNAc_2$) in the ER, N-linked glycan biosynthetic pathways in yeast and human cells diverge. In yeast cells, the core glycan is elaborated by the addition of mannose glycans, whereas in human cells this structure is modified to yield complex N-glycans. Many therapeutic proteins require proper N-glycosylation to facilitate their folding, as well as for their pharmacokinetic efficacy and stability [2]. Consequently, human proteins produced in yeast cells as potential therapeutic agents are unsuitable for use in humans [3]. Additionally, the expression of human integral membrane proteins in yeast cells is often problematical.

In addition to their intrinsic substrate specificities, the relative position a glycosyltransferase occupies in the Golgi has a significant impact on the types of glycan structures produced by cells. Details regarding the localization requirements of Golgi-resident glycosyltransferases has provided the information necessary to generate libraries of yeast-human glycosyltransferase chimeras that have allowed yeast cells to be redesigned for the production of human-specific glycans [3–6]. The humanization of yeast glycosylation pathways has been dependent on the removal of certain yeast mannosyltransferases and the introduction of libraries of yeast-human chimeras in which the CTS region (Fig. 2) of yeast glycosyltransferases have been fused to the catalytic domains of human enzymes [2–6]. Such approaches have resulted in the generation of yeast strains capable of recapitulating the biosynthesis of human glycans [3, 4, 6]. Additionally, the recent discovery of glycosylation in some prokaryotic species has also opened up the prospect of generating humanized glycoproteins in bacterial cells [5].

A holistic view of Golgi-resident glycosyltransferase localization: the combinatorial action of localization attributes within the CTS

Examination of the mechanisms and signals that dictate the characteristic non-uniform distribution of glycosyltransferases across cisternae of the Golgi have revealed that, for many glycosyltransferases, their steady-state localizations are often mediated by a number of features within the CTS region of the protein (Fig. 2). While the relative importance of the CT, TM, and stem regions in the localization of glycosyltransferases may vary from one enzyme to another, it is apparent that the features that direct Golgi localization reside exclusively within the CTS region. Indeed, this conclusion is exemplified by the success of strategies to humanize yeast strains through the expression of yeast CTS–human catalytic domain hybrids [2, 4, 6].

The fact that a single feature within the CTS cannot satisfy the localization requirements of all of an organism's glycosyltransferases is highlighted by studies in plants [65] and in yeast cells. In yeast, Mnn1p appears to rely on its TM region and luminal sequences for its Golgi retention [51], whereas Kre2p requires its CT as well as its TM region and luminal sequences for localization to the Golgi [60, 75]. Similar observations have also been reported for the localization of mammalian glycosyltransferases [12, 25, 90–92].

Features within the CTS may or may not function in a mutually exclusive fashion, and for some enzymes one feature may play a more prevailing role than another. In the case of Kre2p, amino acid substitutions within the Vps74p-binding motif are sufficient to re-direct the enzyme to the vacuole for degradation [75], but the TM region appears to be crucial as well ([60]; Tu and Banfield, unpublished observations). It is also conceivable that some features may contribute in different ways to different enzymes, or in different ways on the same enzyme in different cell lines or tissues.

How can the apparently disparate contributions of localization determinants be reconciled in cell biological terms? The apparent redundancy might reflect the molecular evolution of glycan synthesis pathways and/or serve as a failsafe to ensure that this very important class of enzymes is not dependent on a single localization strategy. We suggest that it is perhaps more instructive to consider features in the CTS of glycosyltransferases as functioning in a combinatorial manner, and that each plays a particular and significant role. For example, an enzyme bearing a Vps74p-binding motif in its CT may be reliant on its stem region for oligomerization and on the amino acid composition of its TM region to ensure its successful sequestration into a lipid environment that subsequently guarantees its incorporation into (rather than exclusion from) COPI-coated vesicles. In principle, a deficiency in any one of these three aspects could result in the mislocalization of the glycosyltransferase. From such a standpoint, a variety of combinatorial sorting scenarios, dictated by features of the CTS, can be envisioned (Fig. 3), which ultimately reflect the mechanisms that cells employ to control transport within the Golgi apparatus itself (Fig. 1).

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