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Golgi Glycosylation

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Glycosylation is a very common modification of protein and lipid, and most glycosylation reactions occur in the Golgi. Although the transfer of initial sugar(s) to glycoproteins or glycolipids occurs in the ER or on the ER membrane, the subsequent addition of the many different sugars that make up a mature glycan is accomplished in the Golgi. Golgi membranes are studded with glycosyltransferases, glycosidases, and nucleotide sugar transporters arrayed in a generally ordered manner from the *cis*-Golgi to the *trans*-Golgi network (TGN), such that each activity is able to act on specific substrate(s) generated earlier in the pathway. The spectrum of glycosyltransferases and other activities that effect glycosylation may vary with cell type, and thus the final complement of glycans on glycoconjugates is variable. In addition, glycan synthesis is affected by Golgi pH, the integrity of Golgi peripheral membrane proteins, growth factor signaling, Golgi membrane dynamics, and cellular stress. Knowledge of Golgi glycosylation has fostered the development of assays to identify mechanisms of intracellular vesicular trafficking and facilitated glycosylation engineering of recombinant glycoproteins.

The Golgi is home to a multitude of glycosyltransferases (GTs), glycosidases, and nucleotide sugar transporters that function together to complete the synthesis of glycans from founding sugars covalently attached to protein or lipid in the endoplasmic reticulum (ER) (Fig. 1, sugars shaded in green). Thus, glycoproteins, glycosphingolipids (GSLs), proteoglycans, and glycophosphatidylinositol (GPI) anchors acquire their final sugar complement during passage through the Golgi. Most glycoproteins and proteoglycans are either secreted from the cell, or span the plasma membrane with their glycans becoming the molecular frontier of the cell (Fig. 1). GSLs and GPI-anchored proteins also reside in the plasma membrane, the latter being confined to the outer leaflet of the lipid bilayer. The forest of glycans at the cell surface is often called the glycocalyx and can be visualized by electron microscopy after staining for sugars.

Glycosylation is the most common post-translational modification of proteins. Mature glycans at any one glycosylation site may be as simple as a single sugar, or as complex as a polymer of more than 200 sugars, potentially modified with phosphate, sulfate, acetate, or phosphorylcholine. Most importantly, glycans are often branched. For example, a complex *N*-glycan (Fig. 1) may have up to six branches or antennae, and each antenna may contain many repeating disaccharide units. This article will describe the nature of resident Golgi GTs and other activities involved in Golgi glycosylation from entry into the *cis*-Golgi through passage to the *trans*-Golgi network (TGN). The

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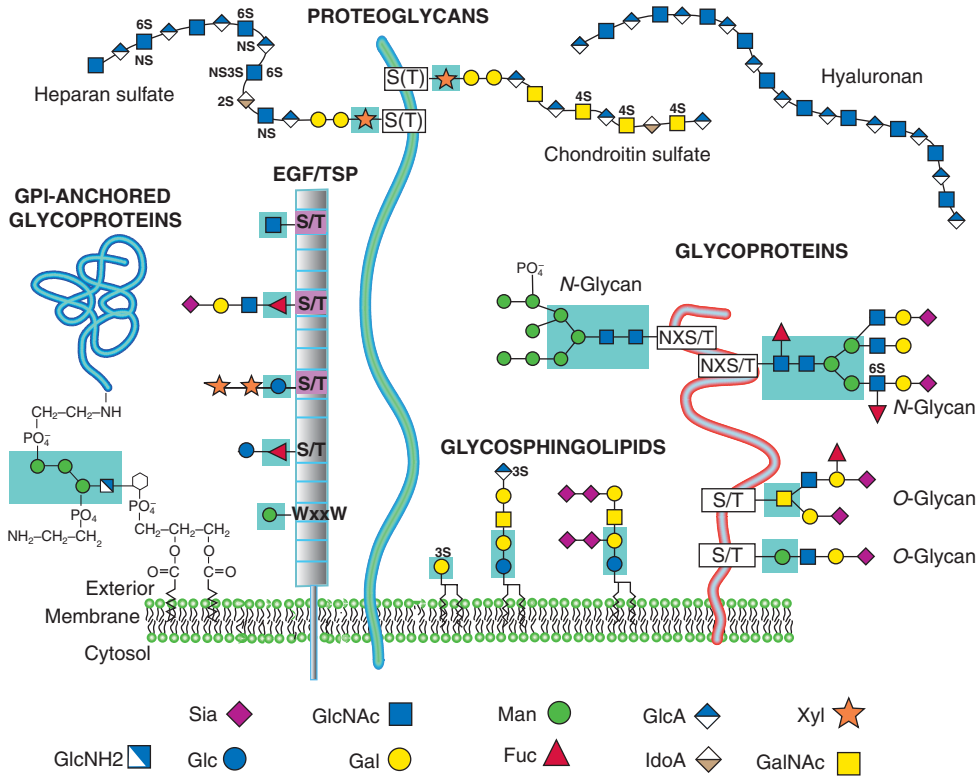

Figure 1. Glycans that mature in the Golgi. The diagram depicts simple *N*- and *O*-glycans attached to glycoproteins, proteoglycans, glycosphingolipids, and a GPI anchor in the plasma membrane. Rather rare *O*-glycans are found attached to EGF-like repeats (EGF; pink) or thrombospondin repeats (TSR; gray) with a particular consensus sequence. The WxxW motif in a TSR is C-mannosylated. Core regions boxed in teal are sugars added in the ER. The remaining sugars in each class of glycan are added during passage through the *cis*-, medial-, and *trans*-Golgi network (TGN) compartments of the Golgi. Abbreviations are: Man, mannose; Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; $GlcNH_2$, Glucosamine; GlcA, glucuronic acid; IdoA, iduronic acid; GalNAc, *N*-acetylgalactosamine; Xyl, xylose; Fuc, Fucose; Sia, sialic acid; 3S, 3-O-sulfated; 6S, 6-O-sulfated, PO_4^-, phosphate. (Modified from Figure 1.6 in *Essentials of glycobiology*, with permission from Varki and Sharon 2009.)

focus is on mammalian Golgi glycosylation but comparisons with yeast, *Caenorhabditis elegans*, and *Drosophila* are made where appropriate.

GOLGI GLYCOSYLTRANSFERASES

General Characteristics

In mammals, there are more than 250 GTs that reside in the Golgi where they catalyze the transfer of one sugar to another sugar on a glycan acceptor, generally covalently attached to protein or lipid. GTs are grouped into families of related catalytic or sequence activity in the Carbohydrate Active Enzymes database (CAZy) (Cantarel et al. 2009). There are, for example, 20 mammalian sialyltransferases (Takashima 2008). By contrast, *Drosophila* has a single sialyltransferase (Koles et al. 2004), and yeast have none. Most GTs transfer a single sugar to an acceptor but there are a few GTs that catalyze the transfer of two different sugars, typically to generate a polymer of repeating units, as in proteoglycans (Fig. 1). The Golgi GT is a type II transmembrane protein with a short amino-terminal cytoplasmic tail in the cytosol, a transmembrane domain, a stalk-like stem region, and a globular catalytic domain in the Golgi lumen

(Fig. 2). Some GTs contain two functional domains. For example, several polypeptide GalNAcTs have a lectin domain that binds to GalNAc and a catalytic domain that transfers GalNAc (Hassan et al. 2000; Fritz et al. 2006); a family of *N*-deacetylase/*N*-sulfotransferases are important in heparan sulfate biosynthesis (Aikawa et al. 2001); and the putative glycosyltransferase Large has two glycosyltransferase domains (Longman et al. 2003; Aguilan et al. 2009). GTs are often glycosylated by other GTs or, in some cases, by autocatalytic transferase activity. The glycans on some GTs are required for their correct folding during synthesis, and these GTs often lack catalytic activity when generated in bacteria. Interestingly, many GTs are cleaved in their stem region by proteases in the Golgi and secreted from the cell (Shifley and Cole 2008; Kitazume et al. 2009). This may be a mechanism to regulate their activity in the Golgi, or to allow them to act at the cell surface or in the extracellular environment. The latter would require nucleotide sugars to be available outside the cell. Intriguingly, nucleotide sugar

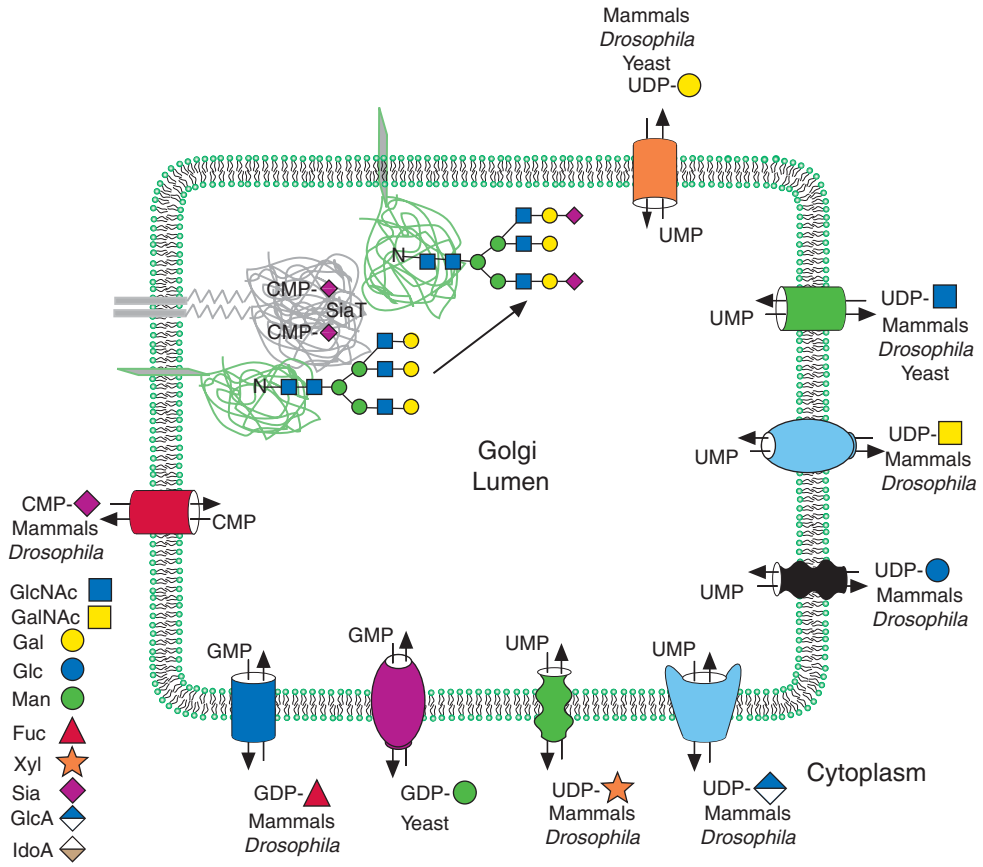

Figure 2. Glycosyltransferases and nucleotide sugar transporters of the Golgi. The diagram depicts a variety of nucleotide sugar transporters that transfer a nucleotide sugar from the cytoplasm into the Golgi lumen in exchange for a nucleotide monophosphate generated by hydrolysis of the nucleotide diphosphate released after transfer of the sugar to an acceptor. A typical type II transmembrane glycosyltransferase (a sialyltransferase; gray) with its short cytoplasmic tail, transmembrane domain, extended stem region, and globular catalytic domain is shown binding CMP-Sia from which it transfers Sia to Gal on a complex *N*-glycan on a glycoprotein (green). Abbreviations are defined in the legend to Figure 1. (Modified from Figure 4.4 in *Essentials of glycobiology*, with permission from Freeze and Elbein 2009.)

transporters of the Golgi have been found to facilitate nucleotide sugar release from the cell (Sesma et al. 2009).

General GT Reaction

The general reaction catalyzed by a Golgi GT is shown for a sialyltransferase (SiaT) in Figure 2. The sugar transferred by each GT comes from a high energy nucleotide sugar which is synthesized in the cytoplasm (or nucleus in the case of CMP-Sia), and must be imported into the Golgi lumen. This is achieved by nucleotide sugar transporters (Berninsone and Hirschberg 2000), families of multitransmembrane transporters (Cantarel et al. 2009) that reside throughout the compartments of the Golgi (Fig. 2). Importantly, mammals do not have a GDP-Man transporter but use Dolichol-P-Man as the donor for the transfer of core Man residues in the ER (Fig. 1, green shaded Man residues). Nucleotide sugar transport is driven by reverse transport of the monophosphate nucleotide generated by Golgi resident pyrophosphorylases that hydrolyse the released nucleotide diphosphate following sugar transfer (Berninsone and Hirschberg 2000). The addition of each sugar creates the substrate or acceptor of the next GT. Many glycosyltransferases require a metal ion to optimize catalysis. In the case of *N*-glycans linked via Asn to protein, the acceptor substrate is often a specifically branched glycan with a particular sugar composition (Stanley et al. 2009). Each sugar within a glycan may be substituted with another sugar at any carbon with a free hydroxyl group, and thus branched antennae are a feature of many glycans (Fig. 1). The specificity of each GT is defined not only by the sugar it transfers but also by the sugar transferred to and the glycosidic linkage formed. In fact, sequence comparisons group GTs based on the glycosidic linkage catalysed rather than the sugar transferred (Oriol et al. 1999).

Targeting GTs to the Golgi

Each Golgi GT transfers a sugar to a specific acceptor generated by preceding GTs, and must act at a particular stage in the glycosylation pathway. Thus, Golgi GTs must be appropriately localized in the *cis*-, medial-, *trans*-Golgi, or the TGN. This does not mean that a GT needs to be strictly confined to one Golgi compartment. In fact, Golgi GTs cycle from the Golgi to the ER, and at any time, a small proportion of those GTs tested have been found in the ER (Rhee et al. 2005). Nevertheless, an appropriate proportion of the enzyme population must be in the right place for a sufficient amount of time to act on glycoconjugates carrying the appropriate substrate. The factors that localize Golgi GTs to a particular compartment of the Golgi include sequence motifs located in the cytosolic domain, the transmembrane domain and/or the stem region, sometimes leading to oligomerization as shown for the sialyltransferase in Figure 2 (Colley et al. 1992; Fenteany and Colley 2005). Enzymes that act sequentially in a glycosylation pathway may also be found in a complex. For example, GlcNAcT-I, the GT that initiates the synthesis of complex *N*-glycans, interacts with α-mannosidase II, which removes two Man residues, and GlcNAcT-II which acts next in the pathway, by a mechanism termed kin recognition (Nilsson et al. 1993b, 1994, 1996; Opat et al. 2000). Golgi GTs generally have a shorter transmembrane domain than glycoproteins that span the plasma membrane, and this is proposed to promote retention of GTs in the Golgi (Munro 1995). In some cases, a Golgi GT associates with another protein, which is important for Golgi localization or transferase activity. For example, T-synthase must be synthesized together with the chaperone COSMC to acquire activity, and the complex constitutes the active enzyme in the Golgi (Ju and Cummings 2002; Aryal et al. 2010). The *Drosophila* protein pipe is similar to mammalian enzymes that modify glycosaminoglycans, and requires another protein termed "windbeutel" to localize to the Golgi (Sen et al. 2000). A glycoprotein inhibitor of complex and hybrid *N*-glycan synthesis (GnT1IP) forms a complex with GlcNAcT-I, α-mannosidase IIX and/or GlcNAcT-III, but inhibits the enzyme activity of only GlcNAcT-I (Huang and Stanley 2010). An extensive discussion of the mechanisms of Golgi resident

protein retention and compartmental localization is presented in Banfield 2011.

Sugars Added and Removed in the Golgi

The diagram in Figure 3 depicts which sugars are added by glycosyltransferases, may be removed by glycosidases, or modified by epimerases and sulfotransferases in the different Golgi compartments. The compartments are shown as overlapping to denote the anterograde and retrograde vesicular trafficking of Golgi GTs known to occur. Because of the dynamic and competitive nature of Golgi glycan synthesis, glycans on a mature glycoconjugate are a heterogeneous collection, even at a single glycosylation site. An immature glycan may be a substrate for many different GTs that compete, and a glycoconjugate may transit a Golgi compartment too quickly to be acted on by all GTs capable of using it as a substrate. Thus, a purified glycoprotein population will include many glycoforms and these may vary in functional activity. Nevertheless, in this sea of heterogeneity, there often exists a strategically placed glycan whose precise structure is the key to a biological activity of the molecule, as in the case of the P-selectin glycoprotein ligand (PSGL-1) (Somers et al. 2000). In PSGL-1, an amino-terminal core 2 *O*-glycan with a sialylated Lewis X glycan determinant and adjacent sulfated tyrosine(s) are required for the binding of PSGL-1 by P-selectin (Xia et al. 2003). The related L-selectin binds ligands with a sialylated Lewis X determinant that is sulfated (Hiraoka et al. 1999, 2004).

Sulfation of specific OH and NH_2 groups on sugars is a very important feature of proteoglycans (Selleck 2000; Fig. 1). Phosphorylation is required on high mannose *N*-glycans of lysosomal hydrolases for them to be recognized by mannose-6-phosphate receptors and routed to lysosomes (Kornfeld 1990; Fig. 1). Therefore, the glycans that emerge from the TGN may be quite simple or extremely complex with several branches that contain linear polymeric chains of sugars modified by sulfate, phosphate, or potentially other moieties. For example, in *C. elegans* some glycans contain phosphorylcholine (Cipollo et al. 2005). In additon, a glycoprotein often carries glycans of many different types. Thus, GPI-anchored proteins usually possess *N*- and/or *O*-glycans, glycoproteins may carry one or more glycosaminoglycan chains, proteoglycans may carry *N*- and *O*-glycans, and molecules with EGF-like (EGF) or thrombospondin (TSR) repeats may carry many different classes of glycan (Fig. 1). For example, Notch1 has 36 EGF repeats, some of which may carry *O*-fucose-, *O*-glucose- and *O*-GlcNAc glycans, and an *N*-glycan (Stanley and Okajima 2010).

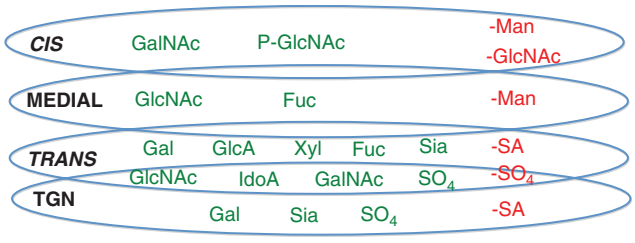

Figure 3. Compartmentalization of sugar addition and removal in the Golgi. Although Golgi membranes are dynamically recycling, compartments corresponding to *cis*-, medial-, and *trans*-Golgi and the TGN are identified by resident proteins that mostly localize to a particular compartment (Nilsson et al. 1993a; Rabouille et al. 1995). The diagram shows in which Golgi compartment particular sugars are added or generated (green) or may be removed (red). Compartments are shown overlapping to signify that distinctions are not precise. Sugars added in the ER or ERGIC compartments before the *cis*-Golgi are boxed in teal in Figure 1. Abbreviations are defined in the legend to Figure 1.

PROTEIN AND LIPID GLYCOSYLATION IN THE GOLGI

N-Glycosylation

Glycoproteins that are *N*-glycosylated arrive in the *cis*-Golgi carrying *N*-glycans added cotranslationally in the ER (Kelleher and Gilmore 2006) to a proportion of their Asn-X (not Pro)-Ser/Thr (rarely Cys) sites (Zielinska et al. 2010). The consensus motif for *N*-glycosylation is necessary but not sufficient for *N*-glycosylation to occur. The *N*-glycans on glycoproteins in the *cis*-Golgi are of the high mannose type and usually contain eight or nine mannose (Man) residues (Stanley et al. 2009). These may remain unchanged during passage through the Golgi and be present on cell surface or secreted glycoproteins (Fig. 1). However, *N*-glycans are often processed in the Golgi, initially in the *cis*-Golgi by a set of α-mannosidases that remove Man residues to generate the $Man_5GlcNAc_2Asn$ intermediate which is the substrate of the medial Golgi GT GlcNAcT-I. The transfer of GlcNAc to $Man_5GlcNAc_2Asn$ by GlcNAcT-I initiates the synthesis of hybrid and complex *N*-glycans. Hybrid *N*-glycans keep the five Man residues and extend the arm that received GlcNAc by adding Gal and sialic acid and/or other sugars. To become complex, *N*-glycans lose the terminal two of the five Man residues and acquire a second GlcNAc to form a biantennary, complex *N*-glycan (Fig. 1). This may be further branched up to six times and each branch may be elongated by the addition of different sugars including Gal, GlcNAc, GalNAc, Fuc, Sia, and disaccharide units (Stanley et al. 2009). Even a simple CHO cell has complex *N*-glycans that may carry 60 or more sugar residues (North et al. 2010).

Drosophila glycoproteins carry mainly high mannose *N*-glycans sensitive to cleavage by endoglycosidase H (Endo H), but may also carry complex *N*-glycans (Aoki et al. 2007; Koles et al. 2007). The complex *N*-glycans have few sugars. Some neural glycoproteins have *N*-glycans with terminal sialic acid, and other glycoproteins contain glucuronic acid in antennae and fucose in the core region of *N*-glycans (Aoki and Tiemeyer 2010; Rendic et al. 2010). *C. elegans* has high mannose and complex *N*-glycans with some specialized features such as the incorporation of phosphorylcholine on GlcNAc (Cipollo et al. 2005). Yeast glycoproteins enter the *cis*-Golgi with mainly $Man_8GlcNAc_2Asn$ at *N*-glycan sites, the same as *Drosophila* and mammals. However, these *N*-glycans quickly acquire additional Man residues and ultimately form yeast mannan with long chains of Man that may be substituted with phosphate (Munro 2001).

O-Glycosylation

The initiation of *O*-glycosylation also occurs in the ER for most *O*-glycans and consists of the addition of only a single sugar residue to Ser or Thr. The most abundant types of *O*-glycosylation are represented by the *O*-glycans termed mucins initiated by GalNAc-Ser/Thr and the glycosaminoglycan (GAG) chains on proteoglycans initiated by Xyl-Ser (Fig. 1). Mucin *O*-glycans are extended in the Golgi by the addition of Gal, GlcNAc, sialic acid and fucose to form linear or branched *O*-GalNAc glycans (Brockhausen et al. 2009)(Fig. 1). There are more than 15 polypeptide GalNAc transferases (ppGalNAcT) in mammals and several in *Drosophila* (Zhang and Ten Hagen 2010), but none in yeast. Much work has been devoted to determining consensus sites for *O*-GalNAc addition to Ser or Thr and criteria are beginning to appear (Caragea et al. 2007; Gerken et al. 2008).

GAG chains attached to Ser have a common core of four sugars (Fig. 1, green shaded) completed in the early Golgi and extended by disaccharide units to form long linear polymers to generate heparan sulfate and chondroitin sulfate (Fig. 1). Characteristic of GAGs is the modification of their sugars by sulfate at specific positions. These modifications occur in blocks that generate discrete regions of binding specificity and have been referred to in heparan sulfate and heparin as the "HS code" (Bulow et al. 2008). Sulfotransferases and sulfamidases of the Golgi may dynamically regulate such a code. *Drosophila* and *C. elegans* synthesize GAGs and proteoglycans, but yeast do not. The consensus site for addition of xylose and initiation of a

GAG chain is a-a-a-a-G-S-G-a-a/G-a ("a" representing Asp or Glu) (Roch et al. 2010).

The EGF-like repeats in the extracellular domain of Notch and other vertebrate proteins are modified by *O*-fucose and *O*-glucose glycans (Rampal et al. 2007; Stanley and Okajima 2010), as well as *O*-GlcNAc (Matsuura et al. 2008; Fig. 1). The first sugar in each case is added in the ER and requires a correctly folded EGF-like repeat that contains a specific consensus site (Panin et al. 2002; Takeuchi and Haltiwanger 2010; T. Okajima, personal communication). *O*-fucose and *O*-glucose glycans are known to be extended in the Golgi of mammalian cells (Fig. 1), and there is the potential for *O*-GlcNAc to acquire Gal, GlcNAc, sialic acid, and fucose. *Drosophila* also expresses these *O*-glycans on EGF-like repeats of Notch and other proteins. However, *O*-fucose glycans in *Drosophila* may have a glucuronic acid attached to the *O*-fucose (Aoki et al. 2008), and there is no evidence for the addition of Gal or sialic acid to the GlcNAc (Xu et al. 2007). *C. elegans* has protein *O*-fucosyltransferase 1 (Loriol et al. 2006), but no close homologue of Fringe, the transferase that adds GlcNAc to Fucose-*O*-EGF (Bruckner et al. 2000; Moloney et al. 2000). The *O*-glucose glycans on EGF repeats appear to have emerged with Notch signaling in the metazoa (Acar et al. 2008; Sethi et al. 2010).

Thrombospondins and other proteins with TSP repeats have consensus sites for two unusual glycans (Fig. 1). The addition of C-linked Man to tryptophan is thought to occur in the ER (Doucey et al. 1998) and no further modification in the Golgi has been observed to date (Wang et al. 2009). The *O*-fucose glycan of TSP repeats is also initiated in the ER, but it is extended in the Golgi with a single Glc (Kozma et al. 2006).

Yeast are not known to generate any of the *O*-glycans described above. However, yeast synthesize *O*-glycans initiated with *O*-mannose in the ER and extended with Man in the Golgi. Mammals and *Drosophila* also make *O*-mannose glycans, initiated from Dol-P-Man in the ER and extended in mammals in the Golgi (Fig. 1). *O*-mannose glycans are rare and concentrated in brain and other tissues that express α-dystroglycan. They are required on α-dystroglycan for it to functionally bind to the extracellular matrix, and disruptions of *O*-mannose glycan synthesis lead to muscular dystrophies (Moore and Hewitt 2009; Nakamura et al. 2010).

Glycosylation of Lipids

In mammals, the first sugar to be added to ceramide (Cer) is added in the ER. Glc-Cer may not be further modified during passage through the Golgi, may have one or two sugars added (Fig. 1), or may be extensively modified by a battery of GTs localized in different compartments of the Golgi (Schnaar et al. 2009). GSL may carry up to eight sugars attached to Glc-Cer. Another abundant glycolipid in certain tissues is sulfatide (Fig. 1) (SO_4-Gal-Cer).

GPI-Anchors

The synthesis and transfer of a GPI anchor occurs in the ER and the mature ER structure is shown in Figure 1 (teal shaded). A phosphatidylethanolamine is added in the Golgi in mammals (Fujita and Kinoshita 2010). In addition, in the Golgi the Man residues of the anchor may be substituted with additional Man residues or GlcNAc, which can then be extended with Gal and sialic acid (Ferguson et al. 2009). Novel Golgi GTs responsible for generating mature GPI anchors are still being uncovered (Izquierdo et al. 2009). The GPI-anchored protein is finally located in the outer leaflet of the plasma membrane (Fig. 1).

REGULATION AND USES OF GOLGI GLYCOSYLATION

Regulation of Golgi Glycosylation

Glycosylation in the Golgi is controlled by the spectrum of GTs, nucleotide sugar synthases, and transporters expressed by a cell, as well as factors that affect the lumenal Golgi environment, and the structure and organization of Golgi membranes. Changes of Golgi pH have long been known to alter glycosylation, and an

anion channel has now been identified that regulates Golgi pH (Maeda et al. 2008; Maeda and Kinoshita 2010). Cells lacking this ion channel transport glycoproteins slowly through the secretory pathway, and the glycosylation of both glycoproteins and glycolipids is truncated in these cells. Interestingly, passage through the Golgi is also slowed if the CMP-Sia or GDP-fucose transporters or both are knocked down using RNAi, or by CMP-Sia transporter mutation in Lec2 CHO cells (Xu et al. 2010). In cells with reduced nucleotide sugar transport, glycoproteins with truncated glycans accumulate in the Golgi rather than proceeding to the plasma membrane or to secretion. In addition, ER stress pathways are activated in cells in which nucleotide sugar transport is inhibited (Xu et al. 2010). In *Drosophila* cells in culture, separate knockdown of two different ppGalNAcTs causes a slowdown in secretion and an alteration in Golgi organization, as well as reduced transfer of GalNAc to mucin glycoproteins (Zhang and Ten Hagen 2010). Thus, Golgi resident proteins appear to contribute to the overall integrity and function of the Golgi.

Other factors in the Golgi lumen that are important for glycosylation are the nucleotide sugar pyrophosphorylases that hydrolyse released nucleotide diphosphates, as their number and activity will affect nucleotide sugar import (Berninsone and Hirschberg 2000; Fig. 2). Chaperones important for certain glycosyltransferases to leave the ER may also function in the Golgi. For example, COSMC is necessary for T-synthase to move along the secretory pathway and to be active in the Golgi (Wang et al. 2010). In another example, the putative glycosyltansferase Large must physically associate with α-dystroglycan to be functional in modifying this substrate in the Golgi (Kanagawa et al. 2004). Recently, inhibitors of glycosyltransferase activities have been discovered. One of these inhibits the activity of GlcNAcT-I, the transferase that initiates the synthesis of complex and hybrid *N*-glycans (Huang and Stanley 2010). It is a testis-specific glycoprotein that binds to GlcNAcT-I as well as some other medial Golgi enzymes, but appears to inactivate only GlcNAcT-I. It is termed GlcNAcT-I Inhibitory Protein (GnT1IP) and its expression is tightly regulated during spermatogenesis. Another example is a class of proteins of the transmembrane BAX inhibitor motif-containing (TMBIM) family that inhibit Gb3 synthase that generates the glycolipid Gb3, the receptor of Shiga toxin (Yamaji et al. 2010). Yet another mechanism of glycosylation regulation is to change the Golgi content of a glycosyltransferase by relocation. This has been recently described for several ppGalNAcTs that transfer GalNAc to Ser or Thr and initiate mucin glycan synthesis (Gill et al. 2010). Growth factor stimulation of Src tyrosine kinase activity, or injection of activated Src kinase, causes a large proportion of all ppGalNAcTs tested to shift from the Golgi to the ER, which results in an increase in total GalNAc addition to proteins. This is a COP1-mediated process and is dependent on the Arf1 GTPase. Medial Golgi enzymes tested, or the Golgi tethering protein giantin, were not redistributed by Src activation.

Mutations in proteins of the conserved oligomeric Golgi (COG) complex that provides a scaffold important for Golgi membrane structure and tethering of retrograde vesicles, also cause alterations in glycosylation (Smith and Lupashin 2008). The first member of the eight subunits of the COG complex was discovered in a CHO mutant ldlC that has defects in *N*- and *O*-glycan and glycolipid synthesis (Podos et al. 1994). Several COG subunits have now been shown to be mutated and to give rise to glycosylation defects in patients with congenital diseases of glycosylation (Zeevaert et al. 2008; Foulquier 2009; Lubbehusen et al. 2010). The mechanism by which COG defects alter multiple glycosylation pathways appears to be caused by partial relocation and degradation of Golgi glycosyltransferases and other glycosylation activities when COG is dysfunctional (see Freeze and Elbein 2009).

Golgi Glycosylation and the Cell Biologist

The fact that glycosyltransferase and processing glycosidase reactions are spaced along Golgi membrane compartments means that glycans are gradually built up before exit from the TGN,

and may be used as markers of passage through the Golgi. *N*-glycans in particular have been used extensively to monitor whether a glycoprotein traffics through the Golgi. The general rationale is shown in Figure 4. *N*-glycans of glycoproteins in the *cis*-Golgi are all high mannose and are susceptible to removal by cleavage using Endo H or peptide *N*-glycosidase (*N*-glycanase). Complex *N*-glycans with only three Man residues in the core, are resistant to removal by Endo H, but remain sensitive to *N*-glycanase. Because *N*-glycans usually contribute substantially to the molecular weight of a glycoprotein, simple comparisons by gel electrophoresis are used to detect glycosidase sensitivity. An important caveat however, is that only resistance to Endo H can be interpreted as evidence of passage through the Golgi. This is because a glycoprotein may fold in such a way that high mannose *N*-glycans never become complex, even though it passes through the Golgi. The same is true for hybrid *N*-glycans that contain five core Man residues and never become Endo H resistant.

Other glycosidases may also be used to detect exposure to a particular Golgi compartment. The presence of sialic acid residues on glycoproteins exposed to the *trans*-Golgi or TGN may be ascertained by sialidase treatment. Gal residues added in the *trans*-Golgi can be removed by treatment with a galactosidase and GlcNAc residues added in the medial Golgi

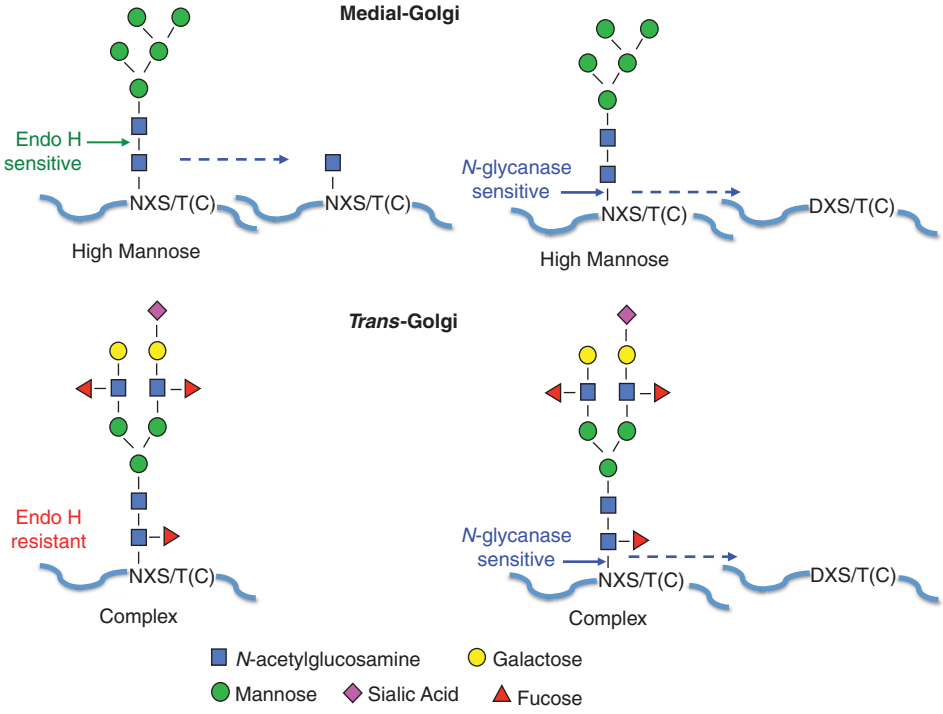

Figure 4. Golgi glycans as tags. Glycoproteins in the ER, ERGIC, and *cis*-Golgi have high mannose *N*-glycans that are all susceptible to release by Endo H. Processing α-mannosidases generate the $Man_5GlcNAc_2Asn$ *N*-glycan shown above in the medial-Golgi to which GlcNAcT-I transfers a GlcNAc on the left terminal Man residue. Hybrid *N*-glycans result from the extension of this GlcNAc but the $Man_5GlcNAc_2Asn$ core is not further processed and remains sensitive to Endo H. Complex *N*-glycans are formed by the removal of two Man residues following the action of GlcNAcT-I and the addition of a second GlcNAc by GlcNAcT-II. Further extension gives rise to the biantennary complex *N*-glycan shown in the diagram. Complex *N*-glycans are resistant to Endo H. Both high mannose and complex *N*-glycans are sensitive to removal by *N*-glycanase, whose action generates an Asp in place of Asn, a change that may be used to identify *N*-glycosylation sites by mass spectrometry.

can be removed using hexosaminidases. Monitoring sensitivity by gel electrophoresis is feasible provided at least two to three sugars are removed or sugars are specifically labeled.

Experimental approaches that have been very productive and still have much potential are the use of glycosylation mutants in intracellular trafficking assays. This approach was pioneered by James Rothman using the CHO mutant 15B (or Lec1) in mammalian cells (Fries and Rothman 1980). These mutants lack GlcNAcT-I activity in the medial Golgi, and thus by mixing Golgi membranes from mutant with membranes from wild type in vitro, glycoprotein transfer between them could be determined by the acquisition of the product of GlcNAcT-I activity only present in wild type Golgi membranes. This approach was widely adopted and several elegant trafficking assays were designed using CHO mutants with different glycosylation defects (Brandli 1991). Although these assays have now been superseded to a large extent by permeabilized cell and knockdown strategies, there are a battery of glycosylation mutants in CHO cells (Patnaik and Stanley 2006), BHK21 cells (Stojanovic et al. 1984), MDCK cells (Brandli et al. 1988), and HEK293T cells(Reeves et al. 2002; Crispin et al. 2009) that could be helpful. Mutant lines with a well-characterized defect in glycan biosynthesis have many applications such as determining functions for glycans in cell-cell and cell-pathogen interactions, and generating recombinant molecules with tailored glycans for functional studies and glycosylation engineering (Stanley 1992). For example, Lec1 CHO cells with inactive GlcNAcT-I (Chen and Stanley 2003), have an intact *N*-glycan pathway in the ER where *N*-glycans are important for folding and quality control (Maattanen et al. 2010), through the *cis*-Golgi and to the medial-Golgi, but glycoproteins made in Lec1 cells never acquire complex or hybrid *N*-glycans because their synthesis must be initiated by GlcNAcT-I. These high mannose *N*-glycans may be used to affinity purify a glycoprotein using lectins, to identify mannose binding proteins, to determine if complex *N*-glycans are necessary for function, and to generate correctly folded glycoproteins with only a single GlcNAc at most (if not all) *N*-glycan sites by Endo H treatment. The latter has been particularly helpful in generating crystals for structural studies (Bouyain et al. 2005).

CONCLUDING REMARKS

Golgi glycosylation is a complex and highly dynamic process that is essential for the production of fully functional glycoproteins, glycolipids, proteoglycans, and GPI-anchored proteins, and for the timely transport of membrane and secreted proteins. The enzymes, transporters, chaperones, and inhibitors that regulate glycosylation must function together in a crowded environment that is sensitive to perturbations in pH, Golgi peripheral membrane structure and the content of Golgi membrane proteins. Understanding each glycosylation step in all the different pathways, and the consequences of blocking glycosylation at any point, is important for the generation of tools and strategies in cell biology and Golgi research, and for understanding pathogenic mechanisms of Golgi glycosylation malfunction at the cellular and organismal level.

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