Factors controlling the glycosylation potential of the Golgi apparatus

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Most of the biosynthetic reactions that generate the oligosaccharide structures of eukaryotic cells occur in compartments of the Golgi apparatus. This article provides a brief outline of the major glycosylation pathways of the Golgi, and discusses current understanding of the many factors that can control the glycosylation potential of this organelle. Old and new approaches towards elucidating the organization of glycosylation machinery in the Golgi are also considered.

Most cell surfaces and extracellular macromolecules carry sugar chains (oligosaccharides or glycans) that were initially attached or modified in the Golgi apparatus. There are now numerous examples of specific biological functions attributed to these glycans, including cell–cell and cell–matrix interactions, intra- and inter-cellular trafficking, and signalling[1]. A colleague recently commented on how little attention is paid by cell biologists to the details of this glycosylation machinery, despite the fact (as she put it) that 'glycosylation is, after all, what the Golgi apparatus does'. While this may be an overstatement (the Golgi mediates other major functions such as lipid biosynthesis, and transport and trafficking of proteins and lipids), much of the complexity and specialization of the Golgi is, in fact, directed towards achieving the correct glycosylation of proteins and lipids. This review provides a brief outline of these pathways and then considers the many factors that can determine the final glycosylation product found on a given protein or a lipid that has passed through the Golgi. It also considers approaches aimed at elucidating these factors, including a recently developed 'freeze-frame' technique. Although the focus is mostly on glycosylation reactions occurring in higher animal cells, the principles described should be generally applicable to Golgi function in most eukaryotic cells.

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The major classes of glycans

Of the many dozens of possible monosaccharide units, only a few are actually utilized to make vertebrate glycoconjugates[2–10]. Unlike nucleic acids and proteins, sugar chains can also have multiple branches. A full exploration of the details of glycosylation and oligosaccharide function requires a working knowledge of the structure and stereochemistry of each of the monosaccharides and their linkages and modifications. For the biologist beginning to explore the field of glycobiology, it is often sufficient just to remember the abbreviations for each monosaccharide, to know that they are structurally and functionally different from one another and to appreciate that α- or β-linkages confer a completely different presentation. The classification of glycan chains that pass through the Golgi is based on the linkage region to the protein or lipid, rather than on the monosaccharide units that comprise the rest of the chain (see Fig. 1)[2–10]. This makes sense since the protein or lipid often determines the initiation of glycosylation and can also influence the further extension and modification of the chains. An individual protein can carry more than one class of glycan. There are minimal requirements to initiate the synthesis of each class. In some instances, initiation occurs by *en bloc* transfer of a preformed oligosaccharide chain, whereas, in others, chains are initiated by the addition of a single monosaccharide. In all pathways, the chains subsequently grow by simple addition of monosaccharide units, catalysed by specific glycosyltransferases that utilize specific sugar-nucleotide donors. In a few cases, the chains can also be trimmed by specific glycosidases. Competing pathways for branching of some chains can result in very different final structural outcomes. Note that, in contrast to the distinctive inner 'core' regions of the different types of glycans, many outer chain residues and sequences are shared (see Table 1 for some common examples of outer sequences found on three classes of glycans). These regions are added to the different classes by overlapping and, in some cases, identical transferases. The sugar chains are also subject to further modification by substituents such as phosphate, sulfate, acetate or methyl groups. While these modifications add further complexity to an already complex system, they might also impart specific functions to the sugar chains[1]. For more details, the interested reader is referred to some of the many reviews and book chapters written on these subjects[2–10].

The types of glycans presented here are the most common ones encountered in the Golgi apparatus – there are many other less common forms of protein–carbohydrate linkages (e.g. *O*-linked mannose or *N*-linked glucose). While these are less well studied, they should not be forgotten, particularly because they are often restricted to a particular cell type or class of proteins, and hence more likely to mediate unique biological function(s)[1,11]. The completely separate universe of nuclear and cytoplasmic glycosylation[12,13] is also not considered in this review since the Golgi plays little or no part in the biosynthesis of these glycans.

Topological issues

Biosynthesis of glycans in the Golgi requires a variety of nucleotide and lipid-linked donors, many glycosyltransferases (enzymes that transfer specific sugar residues) and some glycosidases (enzymes that remove specific residues). While some glycan classes are made initially on the cytoplasmic face of endoplasmic reticulum (ER) or Golgi membranes and 'flipped' across to the lumenal side, most monosaccharides are added to

0962-8924/98/$19.00
PII: S0962-8924(97)01198-7

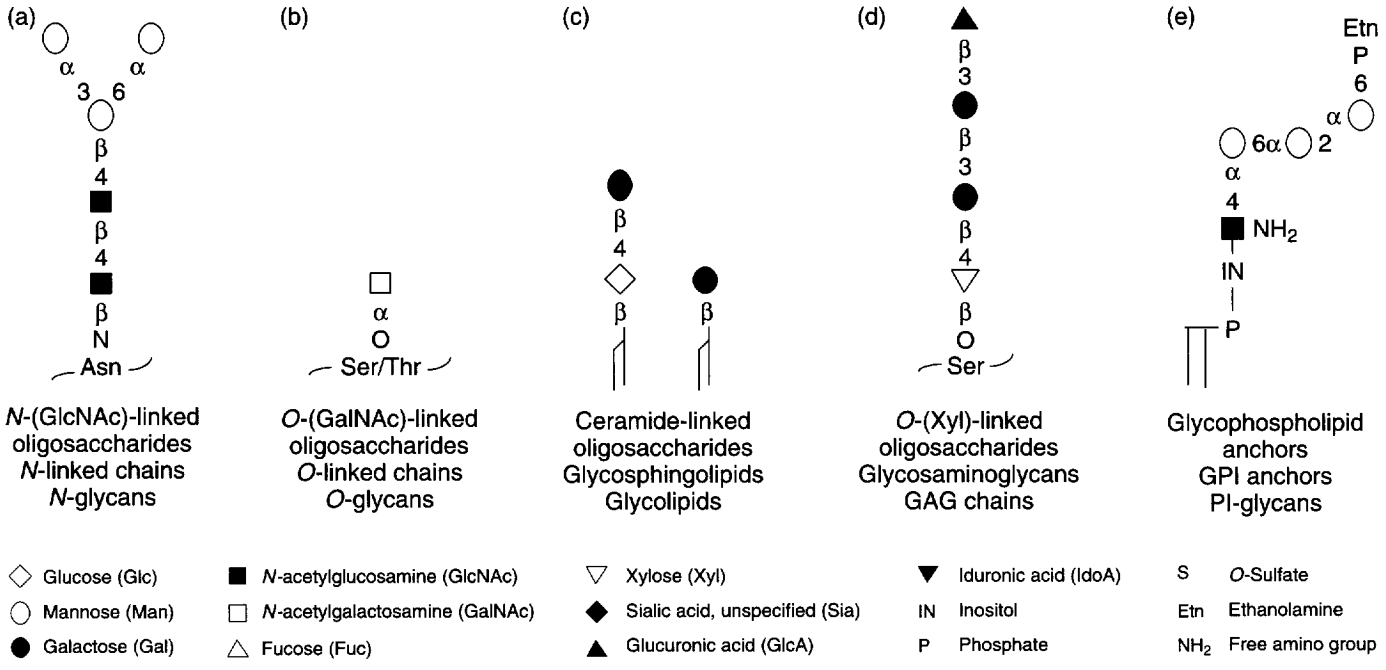

FIGURE 1

The major classes of vertebrate glycoconjugates passing through the Golgi apparatus. The invariant core structures of the major classes and the commonly used terms for each class are shown. (a) *N*-glycans originate from a lipid-linked oligosaccharide donor that is transferred to acceptor proteins in the endoplasmic reticulum (ER). While early steps in biosynthesis are shared among almost all eukaryotes, continued processing of these chains leads to many diverse structures in different evolutionary lineages, including high-mannose type, complex type and hybrid structures. In higher animals, the addition of GlcNAc-P-residues to high-mannose chains of lysosomal enzymes eventually generates the mannose 6-phosphate (M6P) recognition marker that targets the newly synthesized enzymes to the lysosome. Alternatively, removal of mannose residues and the addition of branching β-GlcNAc residues results in the formation of multiple 'antennae'. Thereafter (see Fig. 2), each GlcNAc residue can potentially have added to it a β-Gal residue, with subsequent decoration or termination by a variety of termini that can then be further modified – for example, by sulfation or *O*-acetylation. (b) *O*-glycans are initiated in the ER or the Golgi on Ser or Thr residues by the transfer of α-linked GalNAc residues. These chains are often found in clusters on the polypeptide backbone, generating 'mucin-type' molecules. The early steps in these pathways generate a series of distinctive options called 'core' units. The final steps of extension and termination of *O*-glycans are very similar to those of *N*-glycans (see Fig. 2). (c) Glycosphingolipids are so called because of the sphingosine unit that is part of their ceramide lipid tail. The initial addition of glucose or galactose to ceramide in the ER or Golgi occurs on the cytoplasmic leaflet (in some lower animals, the initiating monosaccharide can be different). The resulting monoglucosylceramide is then flipped into the lumen of the Golgi for further stepwise addition of monosaccharides. As with *O*-glycans, there are many different classes of glycolipid cores, each defined by addition of specific monosaccharide units in particular sequences. While the early-acting transferases are specific for the glycosphingolipids, many of the later ones (including the terminating enzymes) are probably shared with *N*- and *O*-glycans (see Fig. 2). (d) Most glycosaminoglycan chains are initiated in the Golgi by the transfer of β-linked xylose to serine residues on a variety of 'core protein' polypeptides that have arrived from the ER. A distinct linkage region, shown in the figure, is then formed and extended with alternating disaccharide units of either GlcA–GlcNAc (generating heparan sulfate and heparin) or GlcA–GalNAc (generating chondroitin sulfate and dermatan sulfate). The finished product, a core protein with one or more glycosaminoglycan chains, is called a proteoglycan. Some core proteins can carry either chondroitin/dermatan sulfate or heparin/heparan sulfate glycans, depending upon the cell type. Thereafter, the chains are polymerized by the sequential addition of monosaccharides, and can be modified simultaneously to varying extents by sulfation and epimerization. (e) Glycophospholipid anchors are preformed in the ER and then used to replace the C-terminal membrane-spanning regions of certain proteins – i.e. providing an alternative means to anchor proteins to cell surfaces. In some cell types, an additional fatty acyl chain can sometimes be attached to the core inositol group. The common core structure can also be elongated or modified in several ways in the Golgi apparatus.

the growing chain on the inside of the compartments. Therefore, the catalytic sites of most of the glycosyltransferases and remodelling glycosidases face the lumen of various functional compartments in the Golgi. The low-molecular-weight sugar-nucleotide donors for the glycosyltransferases are made in the cytosol. Thus, for glycosylation to occur, these donors must be specifically transported into the lumen of the Golgi[14].

Traditional approaches for exploring the organization of Golgi glycosylation mechanisms

A few of the numerous glycan-processing steps taking place in the Golgi have been used by cell biologists as convenient signposts for the passage of glycoproteins through the Golgi. However, the speed with which these reactions actually occur makes it impossible to trap most of the true biosynthetic intermediates. Hence, understanding of each glycosylation step has required analysis of mutants[15,16], purification, cloning and characterization of Golgi enzymes[5,7–10] or the use of specific glycosylation-pathway inhibitors[17]. While such studies are valuable, they still provide an incomplete picture of the complexity of oligosaccharide processing as it actually occurs in the Golgi. A variety of techniques have therefore been developed to study the organization of glycosylation in the Golgi, each with its own advantages and limitations.

The localization of carbohydrates, hydrolases and transferases *in situ* provided some of the earliest evidence for the compartmental organization of the Golgi. These approaches include the detection of

TABLE 1 – SOME COMMON OUTER CHAIN SEQUENCES FOUND ON DIFFERENT TYPES OF VERTEBRATE GLYCANS[a]

Structure	Attached to[b] N	O	G	Common name(s)
●β(3)4■	+	+	+	Lactosamine
◆α6●β4■	+	+	+	6'Sialyl-lactosamine
◆α3●β(3)4■	+	+	+	3'Sialyl-lactosamine
◆α8◆α3●β4■	+	+	+	Oligosialic acid
$(◆α8)_n$◆α3●β4■	+	+	+	Polysialic acid
●α3●β4■	+	+	+	Alpha-Gal epitope
△α2●β(3)4■	+	+	+	H structure
$△α_3$ ◆α3●β4■	+	+	+	Sialyl Lewis X
◆α(3)6●β4■ 6S	+	+		6'sulfated Sialyl-lactosamine
3S●β4■	+			
3S▲β3●β4■	+		+	HNK-1 antigen
$(●β4■β3)_n$●β4■	+	+	+	Polylactosamine (i antigen)
$△α_3$ $△α_3$ ◆α3●β4■β3●β4■	+	+	+	Sialyl dimeric Lewis X
◆α(3)6(●β4■β3)$_n$ 6S 6S	+	+		Keratan sulfate
□β4■	+			LacdiNAc
4S□β4■	+			Pituitary hormone sulfation
◆α6□β4■	+			
□(●)$α_3$ $△α_4$ △α2●β3■	+	+	+	Blood group A(B)
$△α_4$ ◆α3●β3■	+	+	+	Sialyl Lewis A
$◆α_6$ ◆α3●β3■	+		+	
●β3□		+	+	PNA receptor
◆α3●β3□		+	+	
◆α8◆α3●β3□			+	

[a]See Fig. 1 for the symbol key. GlcNAc or GalNAc residues added to *N*-glycans, *O*-glycans or glycosphingolipids during their biosynthesis can be further elongated and/or terminated in a variety of ways, only a few of which are shown here. The common names for some of these chains are indicated. Parentheses indicate alternative types of linkages or residues. Thus, some glycosyltransferases might add very similar structures to different classes of glycan acceptors. [b]N, *N*-glycans; O, *O*-glycans; G, glycosphingolipids.

enzymes by *in situ* substrate development, autoradiography following monosaccharide labelling, detection of terminal sugars by lectin binding, and direct immunolocalization of Golgi proteins[18,19]. However, even with well-defined probes, these approaches can only reveal the major site of concentration of a given enzyme or product. For reasons outlined below, the compartment of greatest enzyme concentration is not necessarily the site of its maximum action *in vivo*. The results obtained also do not rule out the presence of smaller amounts of the same molecules in adjacent compartments, where conditions might actually be more optimal for their action. For example, the distribution of the ST6Gal I sialyltransferase in rat liver Golgi is broader than that of the fully competent α2–6 sialylation machinery[20].

Another approach involves fractionation of Golgi compartments on continuous or discontinuous density gradients, monitoring the distribution profiles of various markers and enzymes[21,22]. When complete separation of two enzymes is detected, this can be taken to represent complete separation of the relevant compartments within the cell. However, in comparing Golgi enzymes, there is often overlap between the profiles. This could be either because two different compartments happen to overlap in density or because there is genuine overlap in distribution of the enzymes in the compartments. Thus, the lack of coincidence of peak fractions cannot be taken as proof of complete compartmental separation of the activities in the intact cell.

Intercompartmental transport assays were carried out originally between fractions isolated from mutant cells (whose oligosaccharides are incompletely processed) and those from wild-type cells. The processing of the mutant oligosaccharide is taken as evidence for entry into a wild-type compartment with the correct enzyme complement[23]. While this approach has been of enormous value in uncovering the detailed biochemistry of vesicular trafficking[24], interpretation is somewhat limited with regard to compartmental organization. The only result that can be scored is a positive one, which is presumed to arise from transport to a wild-type compartment containing the correct enzyme. There is also an alternative interpretation of such assays – what is being scored might instead be the retrograde transport of the glycosylation machinery to the compartment containing the target glycoprotein[25,26]. This controversial subject is dealt with elsewhere in this issue[27].

Perturbation of Golgi apparatus organization by drugs such as monensin and brefeldin A (BFA) has also provided very useful information on glycosylation[28–30]. However, such drugs substantially disorganize the Golgi itself, making interpretation somewhat limited. Moreover, studies of glycosylation proteins after BFA treatment have reached somewhat variable conclusions regarding the organization of the Golgi.

Multiple factors regulate each step of Golgi oligosaccharide processing.

None of the above methods for the study of Golgi organization simultaneously takes into account the many factors that can affect glycosylation reactions in the Golgi. In a Golgi compartment competent to add a specific monosaccharide or modification to a glycan, a variety of factors can affect the final biosynthetic outcome (see also Fig. 2).

- Cytosolic monosaccharide precursors are activated into sugar-nucleotide donors (Table 2), which are used by glycosyltranferases[7–9] for extension of sugar chains. Other cytosolic nucleotide donors are used for modifications such as sulfation, methylation and acetylation (see Table 2). While much is known about the pathways of nucleotide donor synthesis in the cytosol and the feedback regulatory loops

that control this process, actual steady-state concentrations in the cytosol and Golgi are unknown for most of these molecules.

- The nucleotide donors are transported from the cytosol into the lumen of the Golgi by specific nucleotide transporters (generally antiporters) that mediate electroneutral exchange with the corresponding mononucleotides[14]. This mechanism simultaneously provides the nucleotide donor for a lumenally oriented transferase and removes breakdown products that could inhibit the enzyme (Fig. 2). An additional benefit is the increased concentration of the nucleotide within the lumen. Thus, for CMP-Sia, the transporter has an apparent K_m of ~2 μM and is capable of concentrating the nucleotide at least 50-fold within the lumen[14,31]. This should achieve an effective lumenal concentration of >100 μM. Since the K_ms of many of the sialyltransferases for CMP-Sia are in the 50–200 μM range[32], this concentrating effect could be crucial in driving sialylation reactions, even if cytosolic concentrations of the donor are quite low. An exception to this general mechanism may be sialic acid *O*-acetylation, in which the acetyl group from the acetyl coenzyme A (AcCoA) donor seems to undergo a transmembrane transfer reaction[33]. Several of these transporter proteins have been cloned very recently and have proven to be highly hydrophobic proteins with multiple predicted membrane-spanning regions[14,34–39]. As antibodies to these transporters become available, it will be interesting to know their precise subcellular localization and distribution in Golgi compartments.
- As shown in Figure 2, the donor nucleotides, once transported, are also susceptible to breakdown in the Golgi by specific nucleotide-degrading enzymes. The UDP and GDP sugar dinucleotides are hydrolysed by a pyrophosphatase activity[40], and the mononucleotide CMP-sialic acid by a distinct and specific hydrolase[41]. The former is probably the same as the 'thiamine pyrophosphatase' activity that has traditionally been used to mark the *trans* side of the Golgi. While a Golgi GDPase has been cloned from yeast[42], these enzymes have not been well characterized in vertebrate systems. Their potential role in regulating glycosylation has also not been much investigated. It should be noted that, while breakdown of a sugar nucleotide would have a negative effect on glycosylation, the breakdown of UDP and GDP dinucleotide byproducts of glycosylation reactions is actually needed to drive the antiporter function by mononucleotides[42]. Indeed, the yeast GDPase mutant is defective in glycosylation[43].
- It is estimated that more than two hundred distinct glycosyltransferases and glycosidases are involved in the biosynthesis of the different types of known glycans[7–10]. While many of these enzymes carry out similar reactions, there is surprisingly limited sequence homology among different classes. However, they all share a common structural motif, being type 2 membrane-bound proteins with short cytosolic N-terminal sequences, followed by hydrophobic membrane-anchor domains and a 'stem' and catalytic region within the Golgi lumen. Even the Golgi glycosidases and the yeast GDPase share this structure and topological orientation. A number of these enzymes have been solubilized, stabilized, purified and characterized, and many have been cloned[7–10]. Given the sequential and potentially competing nature of their action, the precise localization of these enzymes within the Golgi is obviously of great significance – the factors that determine this localization are discussed elsewhere in this issue[44]. It is worth emphasizing that there are substantial variations between cell types with regard to localization, and that overall levels of expression can affect the extent of functional distribution. Also, the presence of a given enzyme in a given compartment does not mean that it actually functions in that location because the appropriate sugar nucleotide and acceptor might not be present[20,45].
- Transport of nucleotides into the Golgi lumen does not require divalent cations, but some of the Golgi transferases need Mn^{2+} and/or Mg^{2+} for optimal action. Indeed, addition of micromolar concentrations of Mn^{2+} to isolated intact Golgi cisternae can increase incorporation of label from some radioactive sugar nucleotides into endogenous acceptors[46–49]. While it is assumed that the Mn^{2+} gains access to the lumen of the Golgi to exert its effects, there is no direct evidence as yet for a Mn^{2+} transporter. Overall, the role of divalent cations in regulating glycosylation reactions in the Golgi is not well understood. Note that, in contrast to the reactions involving GDP and UDP-sugar nucleotides, the late steps of sialic acid addition (utilizing CMP-Sia) and the *O*-acetylation of these residues (utilizing AcCoA) do not require divalent cations.
- Most Golgi enzymes recognize one or more residues of the underlying sugar chain and are hence variably specific for their oligosaccharide acceptors. For example, the α2–6 sialyltransferase ST6Gal I recognizes *N*-acetyl-lactosamine (Galβ1–4GlcNAc), a disaccharide unit found on the outer antennae of complex-type *N*-glycans, some *O*-glycans and glycosphingolipids[10]. By contrast, the β1–4 galactosyltransferase GalT galactosylates any terminal

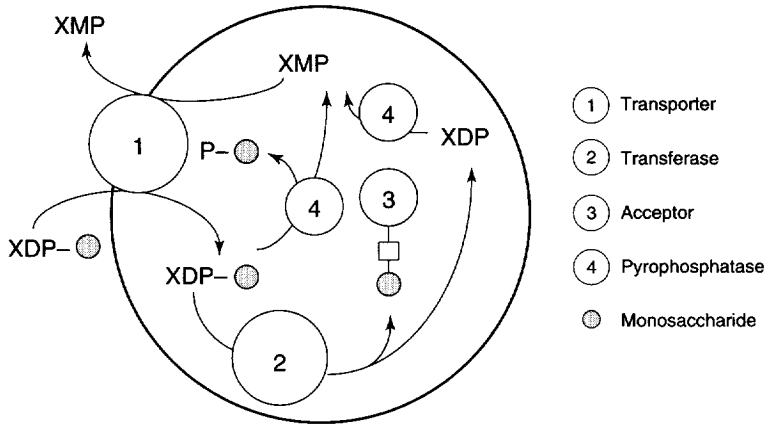

FIGURE 2

Some factors involved in Golgi glycan processing. Many factors can affect the glycosylation potential of the Golgi apparatus. Some of the major factors that are potentially involved in a single step of Golgi glycan processing are shown here. See text for details, as well as for consideration of some of the other factors.

TABLE 2 – NUCLEOTIDE DONORS FOR GOLGI GLYCOSYLATION AND MODIFICATION REACTIONS

Nucleotide donor (abbreviation)	Donor for:
Guanosine diphosphate-mannose (GDP-Man)	Addition of Man residues[a,b]
Guanosine diphosphate-fucose (GDP-Fuc)	Addition of Fuc residues[a]
Uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc)	Addition of GlcNAc residues[a,b]
Uridine diphosphate-glucose (UDP-Glc)	Addition of Glc residues
Uridine diphosphate-*N*-acetylgalactosamine (UDP-GalNAc)	Addition of GalNAc residues[b]
Cytidine monophosphate-sialic acid (CMP-Sia)	Addition of Sia residues[c]
Acetyl coenzyme A (AcCoA)	*O*- and *N*-acetylation
Phosphoadenosine phosphosulfate (PAPS)	*N*- and *O*-sulfation
S-adenosylmethionine (AdoMet)	*O*-methylation
Unknown	Pyruvylation
Unknown	*O*-lactoylation

[a]Man, Glc and GlcNAc residues can also be added via dolichol-linked intermediates.
[b]UDP-GlcNAc and GDP-Man (and possibly UDP-GalNAc) can also donate monosaccharide-1-P units. With GlcNAc-1-P units added to Man residues, removal of the outer GlcNAc results in formation of Man 6-P units.
[c]Only some sialic acids are known to be transferred from CMP-sialic acid donors. Others are created by further modification of a basic sialic acid type that is already transferred to a sugar chain.

GlcNAc residue, provided that sufficient time and UDP-Gal donor are available[5]. However, in all cases (including GalT), significant preferences are noted for the different antennae (branches) of *N*-glycans[5,8]. Other control points in the elongation and branching of *N*-glycans include the effect of β-Gal addition or 'bisecting' GlcNAc addition (both block further action of other GlcNAc-transferases)[5], and the phosphorylation of Man residues (which stops further Man removal by Golgi α-mannosidases)[4].

- In a few instances, the underlying polypeptide chain of glycoprotein is known to specifically dictate the action of a particular Golgi transferase[50]. For example, GlcNAc-phosphotransferase recognizes subtle motifs in the secondary structure of lysosomal enzymes and selectively phosphorylates the *N*-glycans on these proteins[4,51], ProXaaArg/Lys sequences in pituitary glycoprotein hormones dictate the addition of specific GalNAc residues that subsequently become substrates for 4-*O*-sulfation[52], and features of the core protein dictate whether a proteoglycan receives a heparan sulfate or chondroitin sulfate chain[53]. However, for most proteins, there is no clear evidence for recognition by transferases. Despite this, specific glycan structures are found at specific glycosylation sites of most proteins from normal tissues. To some degree, this could be dictated by accessibility of sites to the processing and extending enzymes[54]. A recent study of direct *in vitro* competition between three terminal transferases indicates that there must be additional features by which glycoproteins can regulate their own glycosylation[55].
- Given competing substrates, and limiting transferases and/or donor concentrations, the rapid transit of glycoconjugates through the Golgi could limit the time available for some of the reactions to occur. This is perhaps the most difficult factor to evaluate. In this regard, studies utilizing temperature shifts to alter transport rates can be somewhat difficult to interpret, since the enzyme reaction rates can also be affected by temperature changes.
- The internal pH of the secretory pathway is known to fall from the ER to the *trans*-Golgi network (TGN), probably determined by the action of one or more ATP-dependent proton pumps[56]. Changes in lumenal pH could thus affect the relative activity of the different enzymes, which do have somewhat different pH optima. Overall changes in extracellular and intra-lumenal pH also seem to perturb the organization and function of the Golgi[57].

In an intact cell, many or all of these factors probably interact to determine the final structures of the glycans synthesized on a particular glycoconjugate that is passing through the Golgi apparatus. Thus, while studies of isolated purified glycosyltransferases have been valuable, they provide a very incomplete picture of the functional organization of glycosylation in the Golgi. Indeed, even measuring the activity level of an enzyme in a cell cannot predict the extent to which it contributes to the final output of the Golgi. Moreover, it is not possible to do detailed pulse–chase analysis of the Golgi glycan processing because of the rapidity with which the events occur in the intact cell. It is therefore necessary to devise different ways to explore the complexities of Golgi glycosylation reactions.

'Freeze-frame' analysis of Golgi glycosylation reactions

At any one time, a given Golgi compartment should have the complete machinery to carry out a specific glycosylation reaction, provided that the nucleotide donor is made available (Fig. 2). Indeed, earlier studies noted that some glycosylation reactions could be studied by adding labelled sugar nucleotide donors to microsomal preparations *in vitro* even in the absence of detergents[58–61]. More recently, we and others have shown that, when adding radioactive nucleotides to isolated intact (nontrafficking) Golgi compartments, the resulting labelled glycans on endogenous glycoprotein acceptors provide a 'freeze-frame' picture of the biosynthetic capabilities of individual Golgi compartments[20,45,47–49,62–65]. Essentially, the steps outlined in Figure 2 are being reconstituted *in vitro*. Since labelling is dependent upon specific nucleotide transport and increased intralumenal concentration, the compartments are defined functionally as those to which a specific nucleotide has access and that already contain the appropriate transferases and endogenous acceptors. Thus, there is no need for complete physical separation of the compartments to be studied. The results can be used to evaluate the pathways for biosynthesis of glycans and to understand the compartmental organization of the Golgi. Rare or novel biosynthetic intermediates might also be encountered during such an analysis. Reconstitution of transport between functional compartments might be studied

using this approach[20]. Finally, this provides a way to compare two cell types for the fidelity of organization of the Golgi – for example, between normal and transformed cells.

Protease and detergent treatments show that the labelled glycans arising from such *in vitro* labellings are derived primarily from reactions occurring on the inside of intact compartments and not from those taking place between components of broken compartments[47–49]. Also, neither biological nor mechanical fusion is a major factor during the labelling reactions[47]. In this regard, the complete segregation seen for some (but not all) biosynthetic steps is reassuring[20,47–49,62]. A limitation is that the availability of endogenous acceptors cannot be controlled. However, as long as the tissue or cell type being studied is chilled rapidly prior to homogenization, it is reasonable to assume that there will always be some itinerant molecules that are caught in an incompletely glycosylated state. It is also reasonable to study the whole mixture of endogenous glycoprotein acceptors rather than a single protein. Indeed, this has several merits, including an increase in the amount of label available for study, and the ability to obtain a broad view of all the possible events that can occur during the biosynthesis of glycans in an intact Golgi compartment. A variation of this approach is the addition of an exogenous acceptor that is capable of penetrating into the intact compartments, becoming trapped there by virtue of addition of acidic sugars such as sialic acid[20,45,63,66]. Overall, the 'freeze-frame' technique appears to be a useful adjunct to pre-existing methods for understanding the organization and function of the Golgi. For example, this approach has shown that there is considerable overlap between compartments previously thought to be discrete and that the rules derived from one glycosylation pathway cannot be extrapolated to another (e.g. sialylation of glycolipids can take place very early in the Golgi pathway). Furthermore, some novel biosynthetic intermediates have been uncovered by this approach.

Conclusion and perspectives

Much progress has been made in understanding the organization and structure of the Golgi apparatus. In the final analysis, no single method can provide a complete picture of the many complex events and factors that affect glycosylation reactions in the Golgi. Indeed, given the non-template-driven nature of these Golgi reactions and the many variables that can affect them, *in vitro* studies must always be complemented by studies in intact cells and animals and should perhaps be modelled eventually from a mathematical point of view[67]. It is humbling to realize that, at the present time, there is no reliable way to predict the precise type of glycosylation that will be bestowed upon on a given glycoconjugate by the Golgi apparatus of a given cell type. Indeed, numerous factors, even so much as slight changes in extracellular fluid composition, can alter dramatically the glycosylation potential of the Golgi[57,68,69]. All of this is a source of great frustration for biotechnologists intent on producing recombinant glycoproteins with defined glycosylation. Conversely, it indicates the remarkable versatility of the Golgi apparatus and represents a fertile opportunity for innovative research by cell biologists interested in Golgi function.

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Acknowledgements

Some of the concepts in this review are modified from earlier work done with Hud Freeze. I thank him as well as Jeff Esko, Jamey Marth, Bill Balch, Marilyn Farquhar and George Palade for helpful comments and suggestions.

Golgi division and membrane traffic

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The Golgi complex has a distinctive morphology in mammalian cells, comprising a ribbon of closely apposed, stacked cisternae located adjacent to the nucleus and often the centrioles. Observations since the turn of the century have revealed dramatic changes in Golgi structure as cells undergo mitosis, and more recent microscopic analyses have confirmed that the Golgi ribbon is converted to clusters of vesicles and tubules dispersed throughout the mitotic cell. We have long been interested in this fragmentation since it offers a unique opportunity to study organelle division at the molecular level. Here, we describe the way in which our understanding has developed through another dramatic change to membrane function in mitosis, namely the inhibition of membrane traffic.

All membrane trafficking steps that have been studied so far are inhibited during mitosis[1]. This was observed initially for endocytosis when dividing erythroblasts did not endocytose bound ferritin[2]. Mitotic inhibition was later confirmed for other ligands and cell types, and extended to other membrane trafficking steps[1]. In this review, we focus on the secretory pathway and in particular on transport to and through the Golgi complex.

The absence of newly synthesized vesicular stomatitis virus glycoprotein (VSV-G) from the plasma membrane of infected mitotic cells was the first indication that exocytosis was inhibited in mitosis[3]. Further studies showed that VSV-G failed to reach the Golgi complex and that transport of endogenous proteins from the endoplasmic reticulum (ER) to the Golgi complex was similarly inhibited[4–6]. Transport of lipids and proteins through the Golgi complex has also been shown to be inhibited in mitosis both *in vitro* and *in vivo*[7–9]. In all cases, as cells exit mitosis, transport rapidly resumes.

Mitotic fragmentation of the Golgi complex was first observed in the early 1900s when light microscopy and the original silver-impregnation technique of Camillo Golgi were used to follow dictyokinesis (Golgi division)[10]. One particularly good example is the study by Ludford in 1924, in which the Golgi was described as forming 'rodlets' that were scattered throughout the dividing cell and that, at a late stage in cell division, reassociated to form two separate Golgi complexes, one in each daughter cell (Fig. 1, top panel)[11]. Early electron microscopy (EM) studies showed that the number of Golgi stacks decreased significantly during mitosis, leaving behind what appeared to be clustered tubular and vesicular elements[12]. These were shown by immunoelectron microscopy to be derived from the Golgi complex[13], and serial section analysis showed that there were approximately 150 of these clusters per dividing cell (Fig. 2)[14]. Immunolabelling also suggested the additional presence of variable numbers of free vesicles, ranging from tens to thousands[15]. There was an

0962-8924/98/$19.00
PII: S0962-8924(97)01189-6