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Glycosylation quality control by the Golgi structure

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Abstract

Glycosylation is a ubiquitous modification that occurs on proteins and lipids in all living cells. Consistent with their high complexity, glycans play crucial biological roles in protein quality control and recognition events. Asparagine-linked protein N-glycosylation, the most complex glycosylation, initiates in the endoplasmic reticulum (ER) and matures in the Golgi apparatus. This process not only requires an accurate distribution of processing machineries, such as glycosyltransferases, glycosidases and nucleotide sugar transporters, but also needs an efficient and well-organized factory that is responsible for the fidelity and quality control of sugar chain processing. In addition, accurate glycosylation must occur in coordination with protein trafficking and sorting. These activities are carried out by the Golgi apparatus, a membrane organelle in the center of the secretory pathway. To accomplish these tasks, the Golgi has developed into a unique stacked structure of closely aligned flattened cisternae in which Golgi enzymes reside; in mammalian cells, dozens of Golgi stacks are often laterally linked into a ribbon-like structure. Here, we review our current knowledge of how the Golgi structure is formed and why its formation is required for accurate glycosylation, with the focus of how the Golgi stacking factors GRASP55 and GRASP65 generate the Golgi structure and how the conserved oligomeric Golgi (COG) complex maintains Golgi enzymes in different Golgi subcompartments by retrograde protein trafficking.

Graphical Abstract

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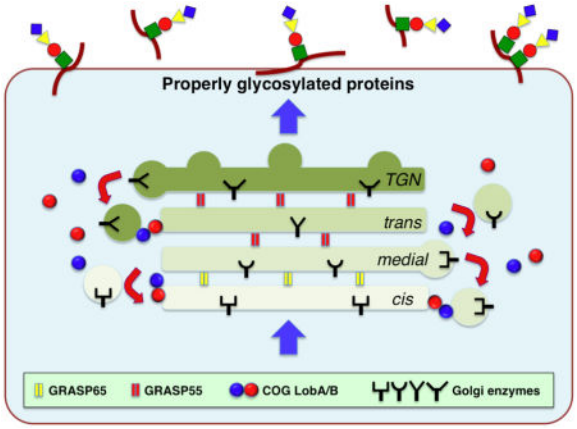

Keywords

Protein glycosylation; Golgi stacks; GRASPs; COG complex; CDG

Introduction

Eukaryotic cells benefit from segregation of cellular functions into a variety of membrane compartments including the endoplasmic reticulum (ER), the Golgi apparatus, the endosomal/lysosomal system, mitochondria, and peroxisomes. This compartmentalization increases the efficiency of cellular functions, but challenges rapid and appropriate communications between organelles or subcompartments within an organelle. The exocytic pathway is responsible for synthesis, modifications, and transport of all secretory and membrane proteins along the ER-Golgi-plasma membrane trafficking axis. The Golgi apparatus is the central hub of the secretory pathway; it receives the output from the ER, including proteins and lipids, modifies and delivers them to their final destinations in the endomembrane system or outside of the cell by regulated or constitutive secretion [1]. During transport through the Golgi, proteins and lipids are subjected to extensive modifications, such as glycosylation, sulfation, phosphorylation, and proteolysis [2]. Proteins derived from over one third of human genes travel through this secretory pathway [3], thus proper functioning of the Golgi is required for a variety of cellular activities. To fulfill these functions, the Golgi has developed a multilayer stacked structure of 5–8 closely arranged flat cisternae found in almost all eukaryotic cells, including those of animals, plants, and fungi [4]. In mammals, each cell contains about 100 Golgi stacks that often line up and laterally link to form a ribbon localized in the pericentriolar region [5–7]. This review discusses the structural-functional relationship of the Golgi in an effort to explain how the Golgi structure takes part in the most prominent protein modification, glycosylation.

Glycosylation is the most common post-translational modification of proteins [8–11]. There are two main forms of protein glycosylation depending on where in the cell proteins are glycosylated. In the cytosol and nucleus, proteins could be modified with one sugar, β-N-acetylglucosamine (GlcNAc), attached to a serine or threonine residue. This so-called O-GlcNAcylation impacts protein-protein interactions, protein stability and activity, and

regulates protein transcription, metabolism, apoptosis, organelle biogenesis, and transport [12, 13]. In the lumen of the ER and Golgi, secretory and transmembrane proteins can be modified with oligosaccharides (glycans) attaching to the side chains of a specific amino acid. Depending on where the sugar chains are attached to a protein, lumenal glycosylation can be further divided into four groups: 1) N-glycosylation, attached to the amide group of asparagine (Asn); 2) O-glycosylation, linked to the hydroxyl group of Serine (Ser), Threonine (Thr), hydroxylysine [14] or Tyrosine (Tyr) [15]; 3) C-mannosylation, a mannose is attached to the C2 atom of tryptophan (Trp) through an C-C bond [16]; and 4) Glypiation, in which a glycan acts as a linker to bridge a protein to a glycosylphophatidylinositol (GPI) anchor in the membrane [17].

N-glycosylation is the best-characterized form of protein glycosylation. Approximately half of human proteins are glycoproteins and most of them contain N-glycan structures [18]. N-glycans are initially synthesized as a lipid-linked oligosaccharide (LLO) precursor, and then the 14-sugar chain $GlcNAc_2Man_9Gluc_3$ of the LLO is transferred *en bloc* by the oligosaccharyltransferase (OST) to the amide group of Asn of a nascent protein cotranslationally on the lumenal face of the ER [19–21]. Before the proteins are delivered to the Golgi, three glucose and one mannose residues are removed in the ER. The resulting high mannose type sugar chains, similar to those prevalent in lower eukaryotes, rarely reach the cell surface of more differentiated vertebrate cell types as they are extensively modified in the Golgi during transport to the plasma membrane [22, 23]. High mannose N-glycans derived from the ER are further trimmed in the *cis*-Golgi. Addition of GlcNAc on mannose allows for generating sugar branches in the medial Golgi. Decoration of galactose (Gal), sialic acid (NeuAc) and/or fucose (Fuc) in late Golgi (or *trans*-Golgi) creates complex N-glycans [11]. A single protein may bear multiple sugar chains attached to different amino acid residues, and sometimes not all sugar chains are processed equally in the Golgi, resulting in a hybrid N-glycan in which some branches keep the high mannose characteristics, whereas others are decorated with complex products [11]. Therefore the diverse glycan structures are created by the elaborate trimming and processing of the glycan chains in the Golgi.

In contrast to the single origination of N-glycosylation, lumenal O-glycosylation is more diverse but the exact mechanism is less well established. There are two main forms of O-glycans in higher eukaryotic cells: shorter mucin type glycans and longer glycosaminoglycan (GAG) chains on proteoglycans, both of which are synthesized in the Golgi. Mucin synthesis starts with the attachment of N-acetylgalactosamine (GalNAc) to the side chain of Ser/Thr and then are extended by the addition of Gal, GlcNAc, sialic acid, and fucose to form linear or branched glycans [24]. GAG chains are attached to Ser through a common core of four sugars [xylose-Gal-Gal-glucuronic acid (GlcA)] in the early Golgi and then extended with repetitive disaccharide units, GlcNAc-GlcA or GlcNAc-iduronic acid, to form long linear polymers. The hallmark of GAG chains is the frequent modification of their sugars with sulfate in the *trans*-Golgi [8]. Rather rare O-Glycans are found attached to EGF-like repeats or thrombospondin repeats (TSR). These two kinds of peptide repeats could be modified by O-fucose and O-glucose on Ser/Thr and extended in the Golgi [25–27]. Another less frequent but important O-glycosylation is O-mannosylation. It is initiated with O-mannose addition in the ER and extended with modifications in the Golgi. The best-known

O-mannose glycan is attached to α-dystroglycan that is required for its functional binding to the extracellular matrix [28, 29]. C-mannosylation is unusual since the sugar is added to a carbon, and it is thought to occur in the ER [30]. Another special form of glycosylation is the formation of GPI anchor that is also named glypiation, initiated in the ER and matured in the Golgi [31, 32].

It is not surprising that diverse protein glycosylation plays critical roles in multiple cellular activities, including protein folding, stability and sorting, protein-protein interactions, signal transduction, cell-cell communications, and immunity [8–11]. Glycosylation defects have been implicated in a large number of human diseases. Congenital disorders of glycosylation (CDG) are rare genetic diseases in which both N-glycan and O-glycan biosynthesis may be defective [33]. Glycosylation defects have also been linked to the pathogenesis of diabetes [34], cancer [35], and cystic fibrosis [36, 37]. For this reason, the fidelity of glycosylation is highly essential. However, as opposed to protein and DNA, there is no template for the synthesis of glycan polymers and it has been estimated that about 700 proteins are needed to generate the diverse glycan structures, including glycosyltransferases (addition of sugars), glycosidases (removal of sugars) and nucleotide sugar transporters (supply of sugar substrates) [10]. Therefore, protein glycosylation has to be a highly ordered and sequential process. As the main sugar chain factory, the Golgi apparatus is responsible for utilizing every element to secure this highly efficient enzymatic event.

GRASPs and the mechanism of Golgi structure formation

The Golgi is the home to a series of glycosyltransferases, glycosidases, and nucleotide sugar transporters that function corporately to complete the synthesis of various glycans. These enzymes must act on the glycoconjugates in the right place at the right time. To fulfill this complex task, the Golgi provides specialized membrane-bound compartments for the Golgi enzymes to reside and function. The flattening of the cisternae reduces the volume in the lumen, which may increase the concentration of the enzymes to substrates, and help maintain a defined microenvironment optimized for the enzymes to work (Fig. 1A). In mammalian cells, dozens of Golgi stacks often laterally link with each other to form a ribbon-like structure, which is thought to increase the efficiency of Golgi function in protein trafficking and glycosylation [38]. The Golgi structure is not static; rather, it is highly dynamic, undergoes rapidly disassembly and reassembly during mitosis and under stress and physiological conditions [39]. In the last a few decades, the unique stacked morphology and the dynamic properties of the Golgi have prompted numerous studies focusing on the mechanism of Golgi structure formation and function. Morphological and biochemical research revealed proteinaceous connections that cross-link adjacent cisternae [40–43]; removal of these proteins by mild proteolysis resulted in Golgi unstacking [44]. Subsequently, efforts have been made to identify key components in the “Golgi matrix”, resulting in the discovery of GRASPs (GRASP55 and GRASP65) and golgins, which work together to maintain Golgi structure and function [45].

GRASP65 (Golgi ReAssembly Stacking Protein of 65 kD) was first discovered as a Golgi stacking protein that is accessible to the alkylating reagent N-ethylmaleimide (NEM) only when the Golgi stack is disassembled [46]. Subsequently, GRASP55 was identified as the

homologue of GRASP65 by database searching [47]. GRASPs are evolutionally conserved and their orthologous and homologues have been identified in different species, including flies [48], yeast [49], and parasites [50–52], but not in plants [53]. Both GRASP65 and GRASP55 contain an N-terminal GRASP domain, which is highly conserved between the two and across species, and a C-terminal Serine/Proline-Rich (SPR) domain, which is more divergent. Both GRASPs are peripheral proteins on the cytoplasmic surface of the Golgi, targeted to the Golgi membranes via a myristic acid attached to the N-terminal glycine residue [46]. Cryo-EM revealed that GRASP65 is present in *cis*-Golgi, while GRASP55 is more concentrated in the *medial/trans*-cisternae [47]. Both GRASPs are required for the formation of the polarized stacked structure (Fig. 1A) [54].

The first evidence for GRASP65 to function as a stacking factor came from a cell-free assay that mimics Golgi disassembly and reassembly during the cell cycle. Inhibition of GRASP65 using recombinant proteins or antibodies in the reassembly reaction blocked the formation of Golgi stacks but not the generation of single cisternae [46]. Further biochemical studies revealed that GRASP65 forms homodimers through the GRASP domain; dimers from adjacent membranes oligomerize in *trans* and *trans*-oligomers function as a “glue” to hold the cisternae together into stacks [55, 56].

Oligomerization of the GRASP proteins is regulated by phosphorylation in the cell cycle, which provides an explanation for Golgi disassembly and reassembly during cell division [57]. *In vitro,* treatment of Golgi stacks with mitotic kinases that phosphorylate GRASPs led to cisternal unstacking. *In vivo,* microinjection of purified mitotic kinases into interphase cells resulted in Golgi fragmentation. GRASP65 is a major target of mitotic kinases on the Golgi [55, 58], it is phosphorylated by mitotic kinases Cdk1 (Cyclin-dependent kinase 1) and Plk1 (Polo-like kinase 1) at multiple phosphorylation sites in the SPR domain, which inhibits GRASP oligomerization and results in mitotic Golgi disassembly [56]. At the end of mitosis, GRASP65 dephosphorylation by PP2A [59] allows the reformation of GRASP *trans*-oligomers and restacking of newly formed cisternae [60]. GRASP55 is regulated in a similar way [54], though phosphorylated by the MAP (mitogen-activated protein) kinase ERK2 instead [61–63].

The role of GRASPs in Golgi stack formation has also been assessed in cells. Microinjection of affinity purified GRASP65 antibodies into mitotic cells inhibited Golgi stack formation in the daughter cells [55, 64]. Depletion of either GRASP by RNA interference (RNAi) reduced the number of cisternae per stack [65], which was rescued by expressing exogenous GRASP proteins [60]; while simultaneous depletion of both GRASPs leads to the disassembly of the entire stack [54].

Recent structural studies of the GRASP domain also suggested that GRASPs are ideal candidates for Golgi stacking. First, in addition to N-myristoylation, GRASP65 and GRASP55 also interacts with GM130 [66] and Golgin-45 [67], respectively. This dual anchoring of GRASPs onto the Golgi membranes restricts the orientation of the protein to favor *trans* pairing over *cis* [68], thus ensuring membrane tethering by forming *trans*-oligomers [55]. Second, the size of the GRASP proteins fits the tight gap between the cisternae. The crystal structures confirmed that the GRASP domain of GRASP55 is globular,

with a 6.5 nm diameter, and that this domain forms oligomers [69–71]. The size of GRASP65 *trans*-oligomers fits well the 11 nm inter-cisternal gap [44]. Together, these findings show that GRASP55/65 are necessary and sufficient for Golgi stack assembly.

In other reports, RNAi-mediated depletion of GRASP65 or GRASP55 also resulted in Golgi ribbon unlinking, suggesting that GRASPs may link Golgi stacks into a ribbon [38, 72]. It is possible that GRASPs function in both Golgi stacking and ribbon linking by forming *trans*-oligomers; however, since the gaps between the stacks are relatively large and heterogeneous (10s to 100s nm), it was speculated that other proteins might help GRASPs in ribbon linking. Indeed, a recent study has provided evidence that the actin elongation factor, Mena, interacts with GRASP65 to promote local actin polymerization and GRASP65 oligomerization, both of which facilitate Golgi ribbon linking [73]. Taken together, these studies provide strong evidence that GRASPs mediate Golgi structure formation through *trans*-oligomerization.

Golgi structure formation and protein glycosylation

It is generally believed that organelle structure formation is required for proper functioning. However, whether the formation of Golgi stacks and ribbon is important for different Golgi functions has remained largely a mystery in the field for many decades. Golgi cisternae do not normally form stacks in the budding yeast (*Saccharomyces cerevisiae*), suggesting that stacking is not absolutely required for cell survival. However, Golgi stacking is a pronounced feature in all metazoans and many unicellular eukaryotes, implying that it must have important functional consequences. The best way to address these questions is to disrupt the Golgi stacks and assess the subsequent effects on the major functions of the Golgi, including protein trafficking, glycosylation, and sorting.

Disruption of the Golgi structure by disrupting GRASPs resulted in accelerated protein trafficking. It has been long thought, without any experimental evidence, that Golgi stack formation increases the efficiency of protein trafficking [74], as the close spatial arrangement of cisternae in stacks minimizes the distance that molecules must travel, and local tethering proteins facilitate vesicle fusion with Golgi membranes [75]. However, studies from the Rothman group and ours have demonstrated that Golgi destruction by the depletion of both GRASPs enhanced trafficking of CD8 [76], the vesicular stomatitis virus G glycoprotein (VSV-G), the cell adhesion protein integrin, and the lysosomal enzyme cathepsin D [77]. In addition, inhibition of stacking by microinjection of GRASP65 antibodies also resulted in accelerated CD8 trafficking [64]. These observations are in contradictory to the original hypothesis that Golgi stack formation facilitates protein trafficking [74]. A plausible explanation for these results is that stacking (and thus the narrow gap between the cisternae) restricts vesicle budding and fusion to the rims of the cisternae and so it may delay trafficking (Fig. 1A); in contrast, unstacking increases the accessibility of coat proteins to Golgi membranes for vesicle budding and fusion, thereby increasing the rate of protein transport (Fig. 1B) [39, 40]. In support of this hypothesis, unstacking increased the rate of COPI vesicle formation from Golgi membranes in an *in vitro* budding assay [64], and disruption of the Golgi structure in cells by GRASP depletion increased the association of coat proteins with Golgi membranes [77]. This finding is

relevant to human disorders where the Golgi structure is fragmented. In Alzheimer's disease, Golgi fragmentation resulted from GRASP65 phosphorylation by activated Cdk5 accelerated the trafficking of the amyloid precursor protein (APP) and thus increases amyloid beta (Aβ) production, which could be reversed by expressing non-phosphorylatable GRASP proteins [78–80].

Golgi destruction by GRASP depletion impaired accurate protein glycosylation, although the expression level and localization of Golgi enzymes did not significantly change [77]. Multiple-stage mass spectrometry (MS^n) analysis demonstrated that depletion of both GRASP55 and GRASP65 resulted in a substantial decrease in N-linked glycoprotein glycans compared to that from control cells (Fig. 2A). Both high-mannose and complex glycans were reduced, but the decrease in complex glycans was significantly greater than that of high-mannose glycans (Fig. 2B), consistent with the notion that GRASP depletion affects glycan processing in the Golgi. Thus, GRASP depletion exerted two significant effects on glycosylation: 1) decreased global N-linked glycoprotein glycosylation, and 2) decreased N-linked glycan complexity. It is not immediately obvious why total N-linked glycan amount should be reduced in GRASP knockdown cells. However, decreased LLO abundance but increased free oligosaccharide (FOS) suggests that GRASP-depletion increases LLO hydrolysis [77]. Therefore, we speculate that a feedback pathway monitors Golgi processing and adjusts the flux of protein trafficking and processing through early secretory compartments such as the ER. In addition, lectin staining for cell surface glycans also supports that GRASPs depletion alters protein glycosylation [77].

It is apparent that Golgi stack formation is required for accurate protein glycosylation. One reasonable explanation is that stacking controls the sequence and speed of protein transport through the Golgi, allowing the cargo to remain in each subcompartment for a sufficient time period to ensure proper glycosylation in the stack (Fig. 1A); unstacking increases the membrane surface for vesicle formation and protein transport, but causes glycosylation defects (Fig. 1B). Thus, Golgi cisternal stacking mediated by GRASP55/65 negatively regulates exocytic transport to ensure more complete and accurate protein glycosylation. In this regard, Golgi stack formation may function as a quality control mechanism for accurate protein glycosylation (Fig. 1A). Unlike the ER that contains a high concentration of folding chaperones that retain improperly modified cargos [81], the Golgi lacks a rigorous system to control the fidelity of its biosynthetic processes. Additionally, the concentration of cargo proteins inside the Golgi is considerably higher than that in the ER. Therefore, a controlled slow cargo flow through the Golgi could be critical for cells in which accurate glycosylation is important. In yeast and some other fungi, N-glycosylation in the Golgi mainly involves only the addition of mannoses [82]. In multi-cellular organisms, N-glycosylation and O-glycosylation of membrane and secretory proteins are more complex and critical. Accurate glycosylation is essential for their cellular functions, including cell adhesion and migration, cell-cell communication, and immunity [83]. This may explain why stacking is not required in yeast, but is essential for many cellular activities in higher order organisms.

An alternative explanation for the glycosylation defects caused by GRASP depletion is Golgi ribbon unlinking. Although the Golgi is a dynamic structure, each subcompartment, corresponding to *cis*-, *medial*-, and *trans*-Golgi and the *trans*- Golgi network (TGN),

processes specific properties and resident components, especially glycosylation enzymes (Fig. 1A) [84, 85]. Therefore, the Golgi needs to develop an effective molecular mechanism to maintain its characteristic subcompartments. Consistent with the results that GRASP65 and GRASP55 are differentially localized to *cis*- and *medial*/*trans*-cisternae, acute inactivation of GRASP65 or GRASP55 led to a loss of *cis*- or *trans*-Golgi integrity, respectively [86]. Thus it was proposed that Golgi ribbon formation mediated by the GRASP proteins allows Golgi enzymes in the *cis*, *medial,* and *trans* compartments to synchronize between stacks. When one GRASP protein was substituted by the other, the Golgi ribbon was intact, but the membranes were de-compartmentalized and glycosylation became defective. Thus the two GRASPs specifically link analogous cisternae to ensure Golgi compartmentalization, enzyme localization, and proper glycan processing [86]. Additionally, knockout of GRASP65 in mice also resulted in glycosylation defects at the cell surface [87]. In summary, GRASP proteins function as the glue that holds Golgi cisterna and stacks together to form the Golgi structure, which controls the cargo flux through the Golgi stack, and thus ensures accurate protein glycosylation (Fig. 1A).

GRASP depletion also caused missorting of cathepsin D precursor to the extracellular space [77]. So far it is unclear whether missorting is caused by defects in protein glycosylation (and thus defects in mannose-6 phosphorylation, a signal for lysosomal sorting), or by dysfunction of the sorting machinery in the TGN. In polarized cells such as neurons and epithelial cells, N- and O-linked glycosylation serve as apical sorting signals [88]. These results indicate that stacking may ensure that sorting occurs only when cargo molecules are properly glycosylated and have reached the TGN, but not in earlier subcompartments.

Golgi trafficking machinery and protein glycosylation

As the Golgi is a dynamic structure, Golgi enzymes must stay in the right place where they perform their functions while substrate molecules constantly flow through the membrane stacks (Fig. 1A). In addition to the flattened cisternae, each Golgi stack is surrounded by multiple transport vesicles, which are thought to carry Golgi enzymes to the proper subcompartments for function. In the last decade, the Conserved Oligomeric Golgi (COG) complex, a membrane tether essential for intra-Golgi trafficking [89–92], has been highlighted for its role in maintaining the Golgi enzymes to their right locations within the Golgi stack, which is critically important for accurate protein glycosylation. Defects of the COG complex have been linked to CDG type II, a growing family of diseases involving misregulation of the processing of N- and O-linked glycans.

In the secretory pathway, vesicles are tethered to the target membranes to ensure that the vesicles do not diffuse away. Tethering factors also contribute to the specificity and efficiency of membrane fusion. Together with small Rab GTPases, they dock the vesicle to its target compartment, promote v-SNARE/t-SNARE alignment, and thus facilitate membrane fusion. Within the Golgi stack, there are mainly two types of membrane tethers. One is the long coiled-coil domain containing golgins that utilize their extended coiled domains for long-range vesicle capturing, which is mostly essential for anterograde trafficking [93–95]; the other is a group of proteins known as multi-subunit tethering

complexes (MTCs) including the COGs, most likely important for retrograde trafficking and recycling of Golgi enzymes.

The best-characterized MTC in the Golgi is the COG complex, a hetero-oligomer consisting of eight subunits, COG1-COG8, which display as two lobes, COG1–COG4 in lobe A, and COG5–COG8 in lobe B; these two lobes are interconnected by a COG1–COG8 interaction [96–102]. COGs are peripheral membrane proteins that exist in cells either in soluble or membrane-bound forms. Membrane-bound COGs are present in different arrangements [103], including the complete COG1-8 complex, lobe A and lobe B sub-complexes. The COG complex interacts with all classes of molecular machinery maintaining intra-Golgi trafficking, including SNAREs, SNARE-interacting proteins, Rabs, coiled-coil tethers, vesicular coats, and molecular motors, and is proposed to play three possible roles: direct function in vesicle tethering [98, 104, 105], assembly of vesicle docking stations [92, 106], and stabilization of SNARE complexes [106–109]. The characteristics and functions of the COG complex have been well summarized by Lupashin and colleagues in [110].

Most importantly, the COG complex plays essential roles in targeting and recycling of Golgi glycosylation enzymes (Fig. 1A). Most of the CDGs have been linked to defective enzymes or transporters. However, around one-third of the remaining patients have mutations in the subunits of the COG complex [90]. So far mutations in seven out of the eight COG subunits (except COG3) have been identified in clinical phenotypes and glycosylation abnormalities [33]. Several COG subunits are either severely truncated or rapidly degraded in some CDG type II diseases [33]. Loss of one COG subunit also destabilizes the other subunits and reduces their expression; and a series of studies on COG-deficient CDG patients showed that the glycosylation deficiencies are widespread, extending from very early demannosylation defects to late sialylation defects [111].

Studies on COG3 and COG7 in HeLa cells indicated that depletion of these components caused extensive Golgi fragmentation and led to an accumulation of COG-dependent vesicles carrying Golgi enzymes MAN2A1 and GALNT2 (Fig. 1B) [104, 112], indicating that in COG deficient cells a significant fraction of Golgi glycosylation enzymes are separated from the target proteins that travel through the Golgi stack. COG mutations impair retrograde but not anterograde trafficking of Golgi enzymes [113–115]. Therefore, COG-mediated retrograde trafficking is crucial for maintaining the accurate localization of glycosylation enzymes in the Golgi stack. These results further suggest that Golgi structure formation and function in trafficking and glycosylation are tightly coupled.

Conclusion and perspectives

The Golgi is an essential subcellular organelle with a complex stacked structure and delicate functions in protein trafficking, sorting, and glycosylation; the structural-functional relationship of this membrane organelle is particularly intriguing. Recent studies on the GRASP proteins provided a possibility to understand the molecular mechanism of Golgi structure formation, and also a tool to disrupt the Golgi structure and thereby to dissect the biological significance of Golgi structure formation in relation to Golgi functions such as glycosylation. Although GRASP depletion resulted in glycosylation defects, no evidence has

been provided for an interaction between GRASPs and Golgi enzymes. Thus it is unlikely that GRASPs play direct roles in protein glycosylation. In contrast to GRASPs, the COG complex plays a direct role in targeting and recycling Golgi enzymes into the different subcompartments within the Golgi stack. It is possible that GRASPs set up the infrastructure of the Golgi stacks for the glycosylation enzymes to reside and work, COGs locate the enzymes to specific subcompartments, and other Golgi structural proteins, such as golgins, control the transport of substrate molecules through the Golgi stack (Fig. 1A), although the precise mechanism that coordinates these machineries to ensure accurate glycosylation needs to be elucidated.

Many questions regarding Golgi structure and function still remain. For example, GRASP depletion leads to dramatic and broad glycosylation defects, but so far no GRASP mutants have been identified in CDG patients. One possibility is that GRASP functions and Golgi structure formation may be critically important for cellular functions; and thus deletion of GRASPs may be lethal. Another more likely possibility is that GRASPs may be posttranslationally modified rather than mutated and degraded in diseases, which so far has not been studied. For instance, GRASPs phosphorylation occurs not only in mitosis as a way to regulate Golgi disassembly and reassembly during the cell cycle, but also in abnormal pathological conditions [78] and upon stimulation with growth factors for cell migration [116]. Therefore future studies should determine whether GRASPs are phosphorylated or down regulated in human diseases where Golgi fragmentation and protein glycosylation defects have been observed. In addition, whether Golgi structural defects affect other type of glycosylation in addition to the N-type remains unexploited. Thus further investigation of GRASPs and COGs in relation to Golgi structure formation and function may provide meaningful insights into disease therapy.

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Abbreviations

Aβ	amyloid beta
APP	amyloid precursor protein
CDG	Congenital disorders of glycosylation
Cdk1	Cyclin-dependent kinase 1
COG	Conserved Oligomeric Golgi
ER	endoplasmic reticulum
FOS	free oligosaccharide
Fuc	fucose

GAG	glycosaminoglycan
Gal	galactose
GalNAc	N-acetylgalactosamine
GlcA	glucuronic acid
GlcNAc	β-N-acetylglucosamine
Gluc	glucose
GPI	glycosylphophatidylinositol
LLO	lipid-linked oligosaccharide
Man	mannose
MAP	mitogen-activated protein
MTCs	multi-subunit tethering complexes
NEM	N-ethylmaleimide
Plk1	Polo-Like Kinase 1
OST	oligosaccharyltransferase
RNAi	RNA interference
SPR	Serine/Proline-Rich
TGN	*trans*- Golgi network
TSR	thrombospondin repeats
VSV-G	vesicular stomatitis virus G glycoprotein

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Highlights

- The Golgi is a central organelle for protein trafficking and glycosylation.
- GRASPs play essential roles in Golgi structure formation.
- Golgi stack formation decelerates protein trafficking.
- Golgi stack formation ensures accurate protein glycosylation.
- COGs target glycosylation enzymes to the precise locations in the Golgi stack.

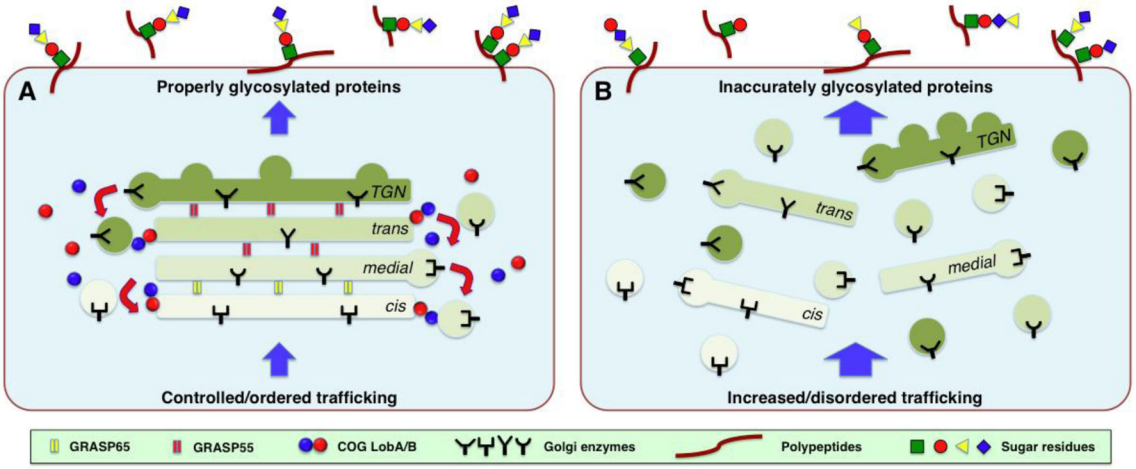

Figure 1. The Golgi structure ensures accurate glycosylation

A. A schematic model showing the significance of Golgi structure for proper protein glycosylation. GRASP-mediated Golgi cisternal stacking plays three roles: 1) it sets up a station for the Golgi enzymes to reside and work; 2) it ensures the cargo molecule travel through the Golgi stack step-by-step to be sequentially processed by different Golgi glycosylation enzymes; and 3) it restricts vesicle budding and fusion to the rims of the stack, which slows down trafficking to allow sufficient time for the enzymes to modify the glycoproteins and enforce accurate glycosylation. COG proteins tethers vesicles that contain Golgi glycosylation enzymes to Golgi cisternal membranes for fusion, and thus maintain Golgi enzymes in the right subcompartments of the Golgi stack. The flattening of the Golgi cisternae reduces the lumenal volume within the cisternae, which increases the concentration of Golgi enzymes to cargo proteins, and helps maintain a microenvironment optimized for the Golgi enzymes. B. Disruption of the Golgi structure results in glycosylation defects. Golgi disruption by GRASP depletion increases the accessible areas for vesicles budding and fusion and accelerates cargo transport through the Golgi membranes, while COG deficiency results in Golgi enzyme mistargeting and accumulation in vesicles, both of which lead to inaccurate protein glycosylation.

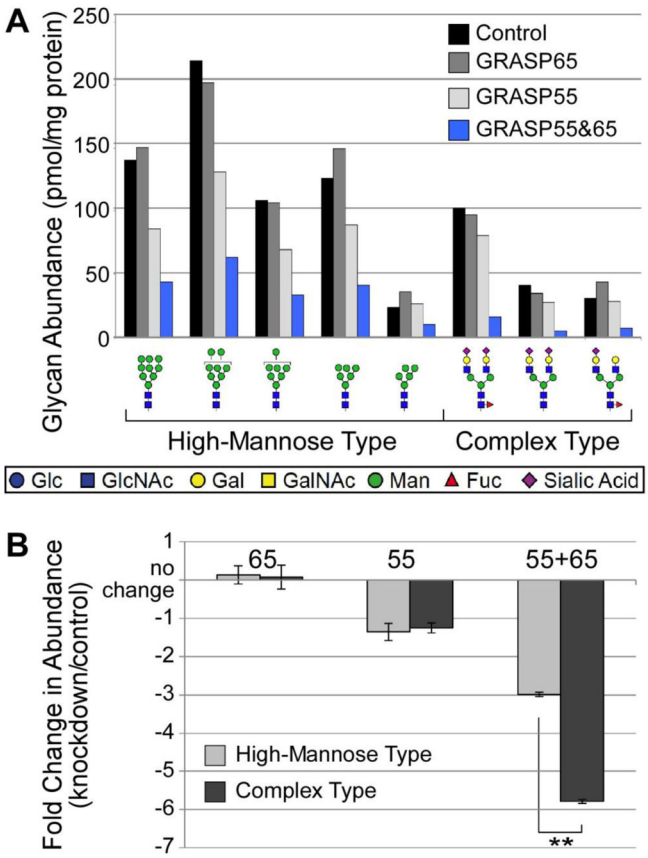

Figure 2. Glycomic analysis of total protein glycosylation in GRASP-depleted cells

HeLa cells treated with RNAi to the indicated GRASP proteins were processed for glycomic analysis by mass spectrometry. **A.** The major high-mannose and complex glycans were quantified relative to an external standard and normalized to protein content. **B.** The fold-change for each glycan indicated in panel A was calculated (knockdown/control). Average fold-changes ± SEM are plotted; negative values indicate decreased abundance relative to control. **, $p < 0.001$ (adapted and modified from [77]).