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# Glycosylation disorders of membrane trafficking

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**Abstract** During evolution from prokaryotic to eukaryotic cells, compartmentalization of cellular functions has been achieved with a high degree of complexity. Notably, all secreted and transmembrane proteins travel through endoplasmic reticulum (ER) and Golgi apparatus, where they are synthesized, folded and subjected to covalent modifications, most particularly glycosylation. N-glycosylation begins in the ER with synthesis and transfer of glycan onto nascent protein and proceeds in Golgi apparatus where maturation occurs. This process not only requires the precise localization of glycosyltransferases, glycosidases and substrates but also an efficient, finely regulated and bidirectional vesicular trafficking among membrane-enclosed organelles. Basically, it is no surprise that alterations in membrane transport or related pathways can lead to glycosylation abnormalities. During the last few years, this has particularly been highlighted in genetic diseases called CDG (Congenital Disorders of Glycosylation). Alterations in mechanisms of vesicle formation due to COPII coat component SEC23B deficiency, or in vesicles tethering, caused by defects of the COG complex, but also impaired Golgi pH homeostasis due to ATP6V0A2 defects have been discovered in CDG patients. This mini review will summarize these fascinating discoveries.

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## Introduction

The basic principle underlying the evolution from the prokaryotic to the eukaryotic cell is the segregation of cell functions into a complex network of membrane-enclosed organelles. This compartmentalization has considerably increased efficiency, but also generated the need for rapid communication among organelles to ensure the correct coordination of cellular functions. This communication is mediated by the exchange of cytosolic signals and by the direct transfer of components via vesicular and non vesicular transport. Central is the endoplasmic reticulum (ER)-Golgi plasma membrane pathway that takes part in the synthesis and modifications of all secreted and transmembrane proteins. The Golgi apparatus is often considered as a processing and sorting hub in the transport and targeting of transmembrane and soluble cargo proteins, to their final destinations [1]. Associated with their transport through and out of the Golgi, proteins and lipids are subjected to extensive covalent modifications such as glycosylation. Within the Golgi, the action of glycosyltransferases and glycosidases generates a broad spectrum of glycan structures that will depend on their abundance, affinity and subcellular localization [2]. All these factors will favor or prevent the synthesis of specific glycan structures as cargo proteins move through the Golgi. Extensive bidirectional traffic between organelles involves vesicular intermediates that play crucial roles in the targeting of ER and Golgi glycosylation enzymes, endocytosis processes and membrane biogenesis. This protein traffic to and through the Golgi is finely regulated and relies on a large ensemble of proteins and protein complexes [3].

## The ER and Golgi N-glycosylation process

Among the many different post-translational modifications that can occur onto proteins, one of the most prominent is protein glycosylation. Glycosylation stands for an enzymatic reaction that allows the chemical linkage synthesis of monosaccharides or polysaccharides (glycans) onto proteins or lipids. Highly conserved during evolution, this process is involved in a multitude of cellular functions such as immune response, protein folding, degradation and intracellular targeting [4]. In mammals, the different glycosylation types can be divided into two major forms: cytosolic glycosylation and compartmentalized glycosylation occurring in specific cellular organelles. In 1984, Torres and Hart identified the first type of glycosylation occurring outside the secretory pathway, the *O*-β-*N*-acetylglucosaminylation that links a GlcNAc residue to serine and/or threonine residues [5]. All other types of glycosylation occur in two different organelles of the cell: the ER and/or the Golgi compartment. Among these types, three major forms can be distinguished: 1)- N-glycosylation or the addition of glycans to the amide group of asparagine residues, 2)- O-glycosylation in which the glycan is linked to the hydroxyl group of serine or threonine residues and 3)- C-glycosylation or the binding of a glycan to the C2 atom of tryptophan via an unusual C-C bond. In this review, we will mainly focus on N-glycosylation process.

N-glycosylation, consisting in the linkage of glycans to asparagine residues of the protein is the most widespread form of glycosylation responsible for the modification of secreted and transmembrane proteins. During evolution, N-glycosylation evolved from a process implicated in pathogenesis and host invasion in archae and bacteria to a complex and multifunctional protein modification in eukaryotic cells [6]. In bacteria and archae, N-glycans are added onto proteins at the cell surface while this process occurs in specialized organelles in eukaryotes. In eukaryotes, N-glycans are added to nascent polypeptide chain in the ER lumen by means of an oligosaccharide precursor consisting of 3 Glc, 9 Man and 2 GlcNAc residues (Fig. 1). This precursor is preassembled onto a lipid carrier, the dolichol, through a metabolic cycle named the dolichol cycle. Paradoxically, after protein transfer, the oligosaccharide is trimmed and processed in the ER and Golgi compartments to build it up again with different monosaccharides! Why? Part of the answer resides in the fact that glycan structures produced by trimming and/or addition are not biosynthetic intermediates but have essential biological functions.

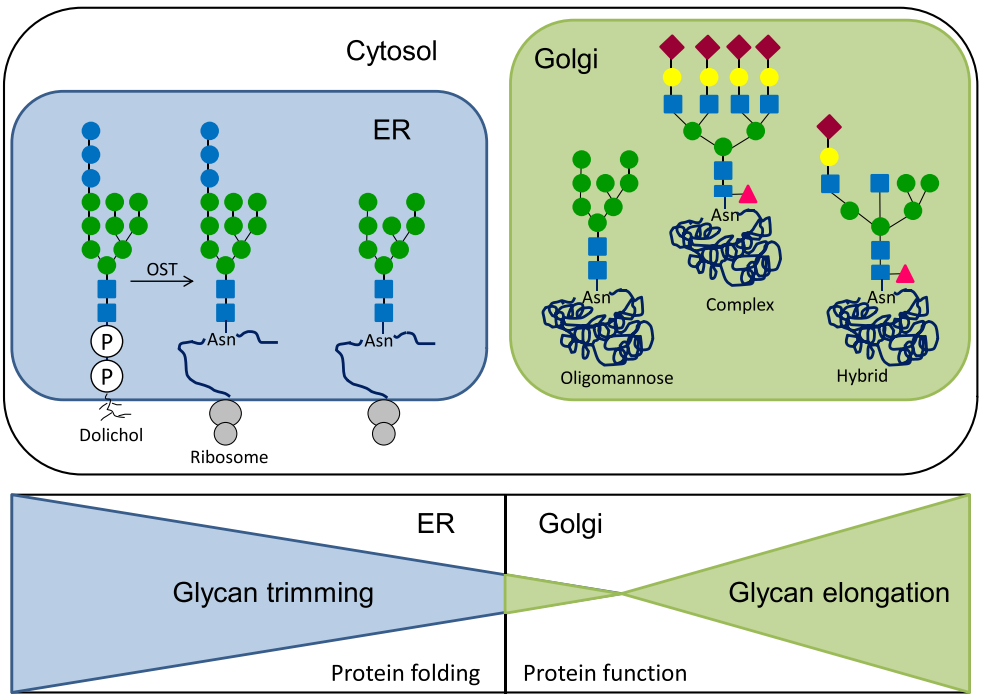


**Fig. 1** Schematic representation of N-glycan maturation inside ER and Golgi apparatus. In eukaryotes, N-glycans are added to nascent proteins in the ER by means of an oligosaccharide precursor, which is subsequently trimmed in the ER and Golgi compartment to build it up again with different monosaccharides. The glycans produced are not biosynthetic intermediates but have specific functions in protein folding and determining glycoproteins' fate in the ER as well as in modulating protein activity and protein function in the Golgi compartment. Several sugars will be added with a high degree of specificity in order to give a precise function, for example a complex N-glycan here with addition of four GlcNAc (*blue square*), four Gal (*yellow circle*), four sialic acids (*fushia diamond*) and one fucose (*pink triangle*). *Blue and green circles* depict Glc and Man, respectively

In the ER, partial trimming of the oligosaccharide precursor by ER glucosidases and mannosidases [7] plays a role in glycoprotein folding and quality control. Since glycan trimming differs according to the folding state of the protein, glycans serve as tags to control the fate of the newly synthesized glycoproteins [7]. Depending on the folding state, glycoproteins are targeted to the Golgi, retained in the ER or translocated to the cytosol for degradation (Fig. 1).

Once correctly folded, the glycoprotein is sent to the Golgi apparatus and becomes the substrate for a number of Golgi enzymes such as glycosidases and glycosyltransferases. Further processing involving demannosylation as well as *N*-acetylglucosaminylation, galactosylation, fucosylation and sialylation will occur [8] (Fig. 1).

The glycans will then acquire more complex and diversified structures then generating a new set of functions to fine tune protein functions thereby generating species- and cell type specific characteristics. The final structures are divided in three groups depending on their composition: high mannose, hybrid or complex type N-glycans (Fig. 1). In contrast to the ER, the Golgi compartment does not seem to develop a rigorous system to control the fidelity of its biosynthetic processes. The correct oligosaccharide structure is not only the result of a dynamic process depending on the activities of glycosyltransferases and remodeling enzymes but also depends on external factors such as the correct Golgi localization of Golgi enzymes and/or transporters [8].

## Protein transport to and through the Golgi

A fundamental basis in understanding the steady state localization of Golgi enzymes is the mechanism for distribution of

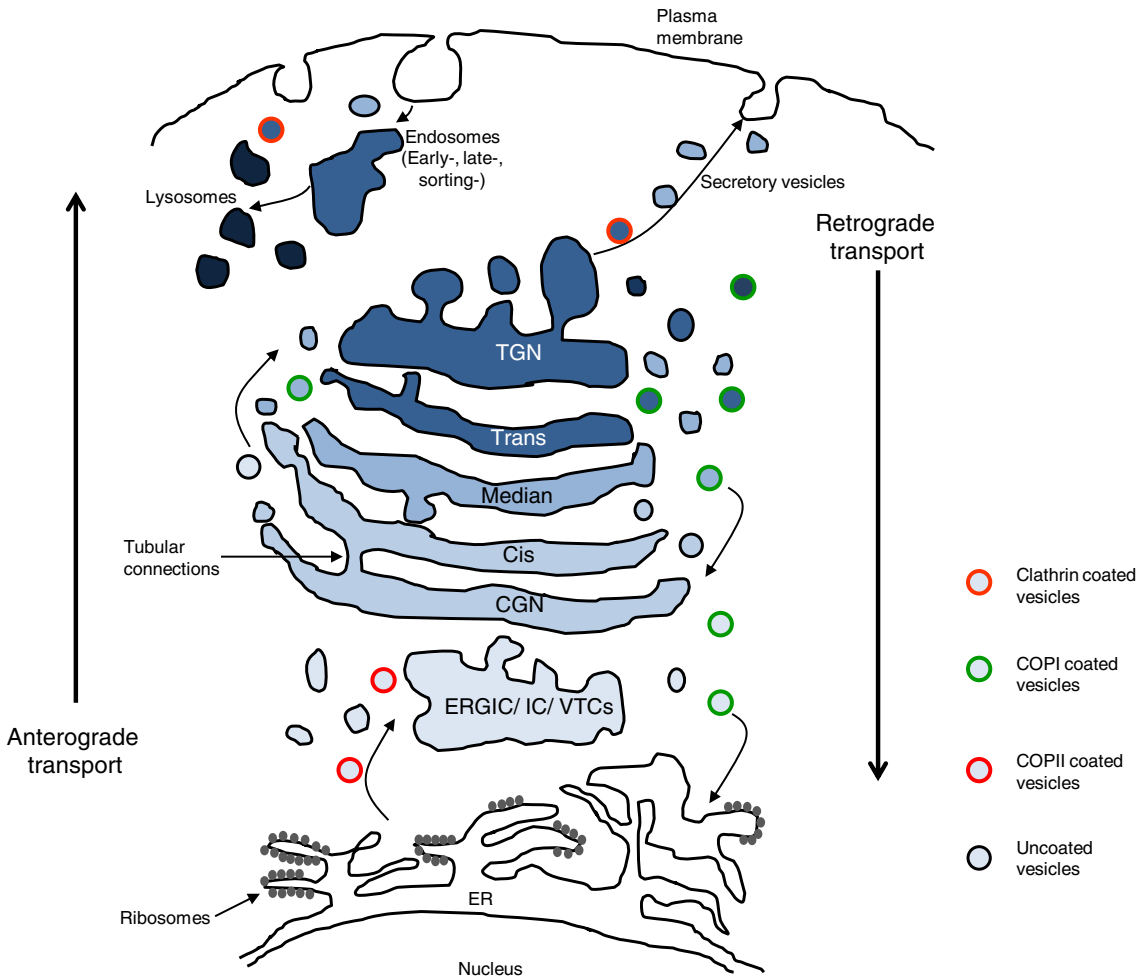


**Fig. 2** The secretory pathway. Cargo protein travels from endoplasmic reticulum (ER) to the plasma membrane or endosomes/lysosomes through ERGIC, cis, medial and Trans-Golgi. As it is illustrated on the left side of the figure, anterograde traffic from ER to ERGIC is assumed by COPII coated vesicles, while retrograde traffic inside Golgi apparatus and from Golgi to ER, as well as anterograde traffic between ERGIC and Golgi are assumed by COPI coated vesicles. Endocytosis and secretory pathway vesicles are clathrin coated vesicles

cargo proteins. Once synthesized in the ER lumen, glycosylation enzymes are transported to specific Golgi stacks and maintained through a combination of retention and retrieval. This suitable compartmentalization within the Golgi is achieved by a finely regulated balance between anterograde and retrograde trafficking of resident Golgi proteins (Fig. 2). Basically, if the balance is disturbed by a defect in vesicular trafficking, the glycosylation process is affected, leading to glycosylation abnormalities.

In the mid to late 1960s, several studies highlighted that eukaryotic secreted proteins are first segregated in the lumen of the ER before to travel within membranous structures to reach the cell surface. These studies placed the ER and the Golgi compartment at the beginning point of an undiscovered pathway, deciphered few years later by Palade (1975) and nowadays known as the secretory pathway [9]. A schematic overview of the transport route from ER through the different Golgi cisternae and other organelles is shown in Fig. 2. The constant communication between these compartments is achieved by sets of membrane enclosed transport vesicles or convoluted compact structures of tubules and vesicles, termed vesicular-tubular clusters (VTCs) [10, 11]. Evidences also exist for the existence of direct connections between these two compartments [12].

Three models of protein transport between Golgi cisternae are currently described (Fig. 3). The vesicular transport model, in which Golgi stacks are considered as a series of distinct sub-organelles, is certainly the "simplest" one. In this model, secretory cargoes travel through golgian static structures following anterograde vesicular trafficking (from the ER to the plasma membrane), while inadvertently mis-targeted enzymes are relocated using retrograde trafficking [13] (Fig. 3a). In the cisternal maturation model, Golgi stacks are conversely considered as dynamic structures formed from vesicles coming from the ER and maturing along secretory pathway to the *trans* Golgi network (TGN) [14]. According to this view, secretory cargoes move forward together with maturing cisternae, then involving a retrograde vesicular trafficking in order to recycle resident Golgi proteins from *trans* to *cis* sub-compartment (Fig. 3b). A third model has recently been proposed based on protein partitioning following a two lipid phases gradient (Fig. 3c). In this model, secretory cargoes and enzymes are rapidly shared through the Golgi apparatus according to their affinity for a specific ratio between glycerophospholipids and sphingolipids in the membrane composition [15]. Secretory cargoes and enzymes further travel through the Golgi following their suitable gradient in opposite directions (Fig. 3c). Although the Golgi traffic mechanism remains controversial, the cisternal maturation model is the most accepted [16]. Indeed, this model fits with the observed morphological observations of cisternae formation at the *cis*-face and their disintegration at the *trans*-face [17], as well as with the transport of large cargo molecules [18, 19].

Whatever the model, the predominant transport intermediates through and from the Golgi apparatus are vesicular carriers, which bud from a donor compartment and fuse with an acceptor compartment. This way of communication is easy to understand but raises some thorny issues particularly about the molecular mechanisms involved in such processes.

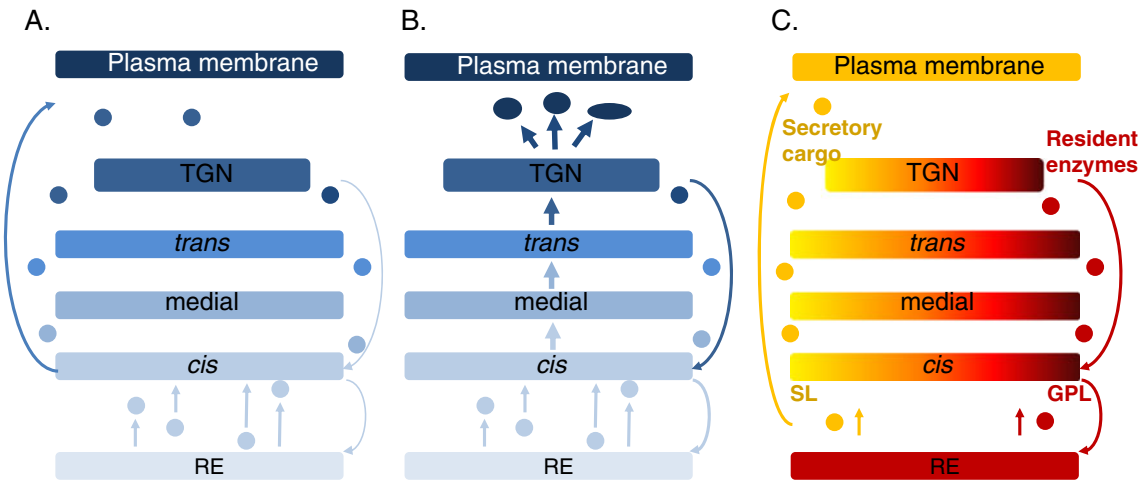


**Fig. 3** Schematic representation of Golgi transport models. Three models have been proposed to explain membrane trafficking through the Golgi apparatus. **A** The vesicular transport model, in which cargoes and enzymes travel through static Golgi cisternae using vesicular trafficking. **B** The cisternal maturation model, where Golgi stacks progressively mature from *cis* to *trans* Golgi apparatus, while Golgi enzymes/proteins are relocated backward using retrograde vesicular trafficking. **C** Two lipid phase repartition model, in which enzymes and cargoes are segregated between different ratios of sphingolipids/glycerophospholipids (SL/GPL). Resident enzymes follow GPL gradient along retrograde pathway, while cargo proteins travel through cisternae via SL gradient on the opposite direction. This scheme has been modified from Glick and Nakano [55] and from Patterson *et al.* [15]

What controls the specificity of vesicle targeting and docking? How is it regulated? This concept indeed implies a draconian regulation where the rate of delivery to, and removal from individual organelles is tightly controlled to prevent either an uncontrolled disappearance or a swelling of a particular organelle/cisternae. Once formed, uncoated vesicles need to be selective in recognizing the correct target compartment. This process relies on an extremely large number of proteins and protein complexes [20]. Golgi glycosylation enzymes move between the ER and Golgi in transport vesicles that are coated with cytosolic proteins forming the COPI and COPII coats [21]. COPII coated vesicles mediate the anterograde transport from the rough endoplasmic reticulum to the intermediate compartment/*cis* Golgi [10, 22]. The COPI coated vesicles mediate retrograde transport from the Golgi apparatus and pre-Golgi compartments to the ER, but also retrograde transport within the Golgi cisternae (Fig. 2). Controversial studies, however, highlight that COPI vesicles could also be involved in anterograde trafficking from ERGIC to the Golgi apparatus by transporting Golgi enzymes [23, 24]. Although the existence of two types of COPI vesicles that travel in two opposite directions is not excluded [13], no solid evidences have been obtained for anterograde COPI-dependant trafficking from *cis* to *trans* Golgi cisternae. This hence would be in favor of the cisternal maturation model where the retrograde trafficking would be achieved by COPI vesicles [25].

COPI coat assembly shows many similarities with that of COPII, both involving small GTPases and different cytosolic coat components. Coats serve to select cargo proteins and are required to deform a membrane into a shape that can bud off and become a vesicle. After budding, the vesicles are rapidly uncoated due to the hydrolysis of GTP (Fig. 4). Once formed, uncoated transport vesicles need to be selective in recognizing the correct target compartment. This selectivity is ensured by two types of proteins: the soluble NSF attachment protein receptor (SNARE) family and the Rab GTPases family [26, 27]. The basic mechanism for vesicle tethering and fusion includes activation of Rab proteins, which recruit long-range tethering proteins such as golgins and multi-subunit tethering complexes (MTC) [3]. This recruitment allows the formation of a SNARE complex by interaction of a single v-SNARE and three t-SNARE proteins that provide binding specificity and catalyzing fusion of vesicles with their target membrane (Fig. 4).

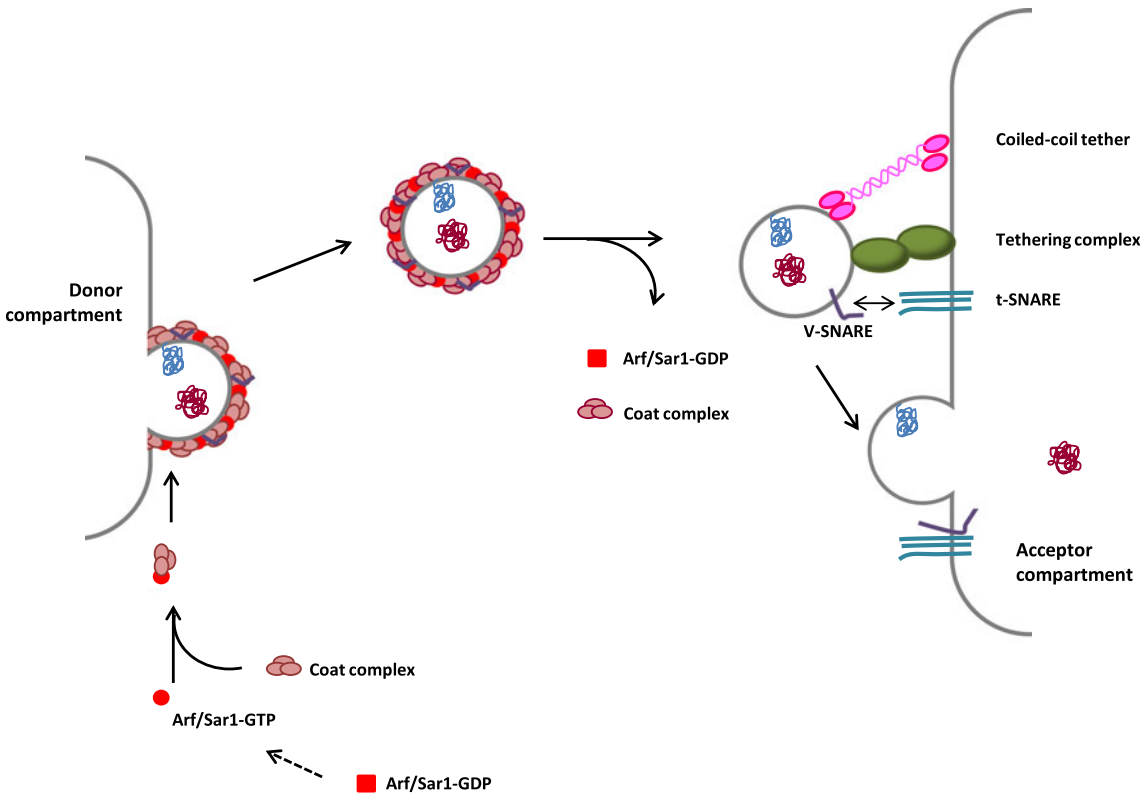


**Fig. 4** Vesicle budding and fusion. The coat assembly is initiated at the donor compartment by the recruitment of membrane coat components, which bind to a small membrane activated GTPase (*red circle*). All coat components polymerize to induce membrane deformation, and subsequent budding of the vesicle off the donor compartment. Close to the acceptor compartment, destabilization of the coat leads to uncoating of the vesicle. The "naked" vesicle moves to the acceptor compartment and tethers to the acceptor compartment membrane by interacting with Rab-activated tethering factors. The v- and t-SNARE interact together to form a four-helix bundle, bringing the vesicle in close proximity to the target membrane. This trans-SNARE complex finally promotes the fusion of the vesicle and acceptor membranes

## Glycosylation diseases characterized by defects in intracellular protein transport

### Defects in the machinery for the docking and fusion of transport vesicles

Diseases of glycosylation and in particular Congenital Disorders of Glycosylation (CDG) have revealed the importance of tethering factors in regulating Golgi-glycosylation. The COG complex is a multisubunit tethering complex of emerging interest assumed to act as a tether for Golgi recycling for vesicles containing glycosyltransferases. This mechanism is in favor of the cisternal maturation model in which retrograde COPI vesicles are tethered by the COG complex to the targeted cisternae [28, 29] (Fig. 5). The COG complex is a peripheral hetero-octameric Golgi localized complex composed of eight subunits arranged in two lobes: lobe A (Cog1-4) and lobe B (Cog5-8) [30]. The identification of the first CDG case relative to COG subunits deficiencies by the group of Hudson Freeze in 2004 [31] increased the awareness about this complex and its importance, making the obvious link between inherited deficiencies in intracellular protein trafficking and an altered glycosylation in human diseases. Since the description of the first Cog7 deficient patient, patients with deficiencies in Cog1, Cog4, Cog5, Cog6 and Cog8 have additionally been described broadening the spectrum of cellular and clinical identified phenotypes [31–42]. All patients described so far present defects in glycoconjugates synthesis (N- and O-glycosylproteins), in the stability and/or localization of resident Golgi proteins, in the ultrastructure of the Golgi apparatus, and in retrograde intra-Golgi trafficking. All the studies highlight the central role of the COG complex in establishing or maintaining the normal structure and functions of the Golgi apparatus [43–45]. Nevertheless, although substantial evidence suggested that the COG complex could act by directly influencing retrograde intra Golgi vesicular trafficking, the precise mechanism by which the COG tethering factor influences the proper localization/stability of certain Golgi glycosylation enzymes has not been determined yet. Very recently, medial Golgi enzymes MGAT1 and MAN2A1 have been reported to be extensively mislocalized in lobe A and lobe B depleted cells [46]. In

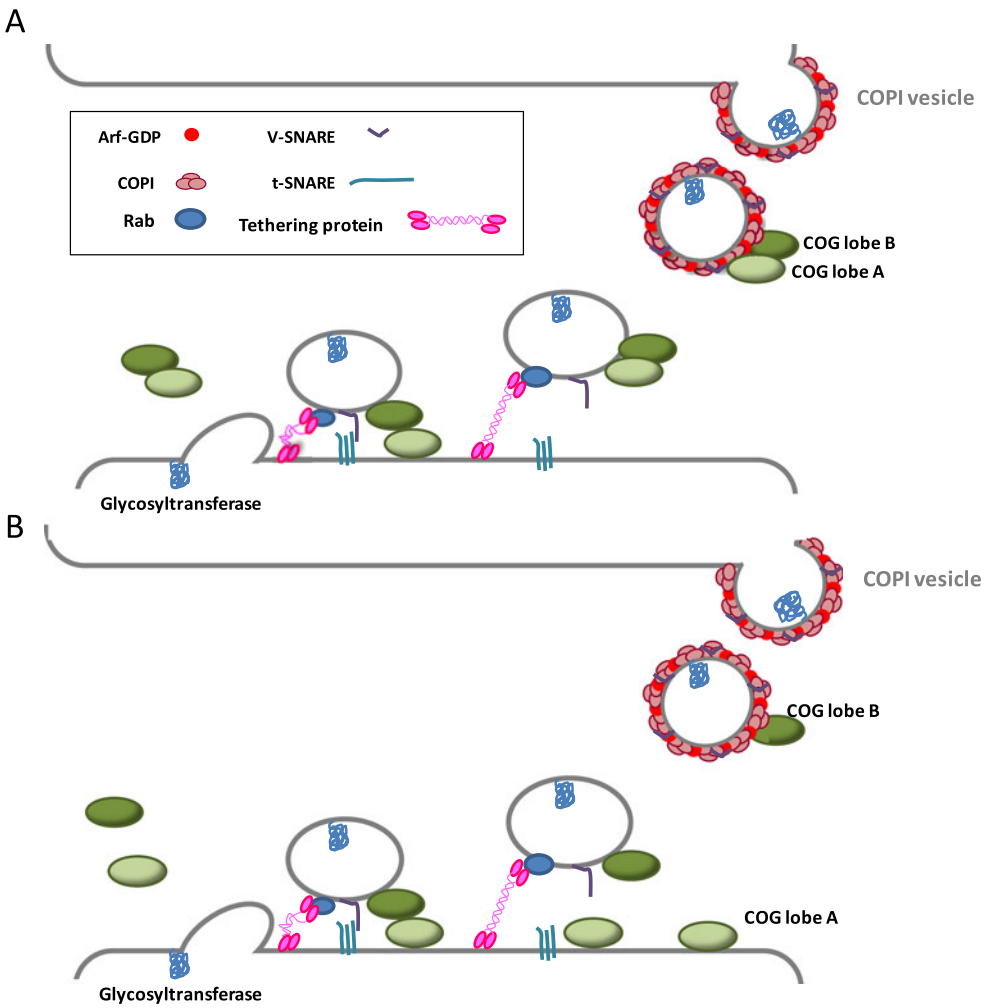


**Fig. 5** Schematic model of the action of the COG complex in intra-Golgi retrograde trafficking. A long range tether associated to a Rab-GTP protein mediates the initial tethering of a COPI derived vesicle. The COG complex (*light and dark green circles*) likely facilitates the docking and/or the fusion of COPI transport vesicles to their correct Golgi cisterna, presumably by interacting with SNARE proteins. Two different models of COG action can be illustrated: (**A**) lobe A and lobe B of the COG complex form a hetero-octameric complex linked to the COPI vesicles or (**B**) lobe A and lobe B act separately. In this model, lobe B would tether the COPI vesicles while lobe A would be linked to the acceptor compartment

addition, we highlighted that the steady state stability of both ST6GAL1 and B4GALT1 (two glycosylation enzymes involved in final steps of N-glycan processing) was drastically reduced in absence of lobe B. These results tend to prove that lobe A and lobe B could play distinct roles in controlling the stability and/or localization of some Golgi glycosylation enzymes [47]. However, a role of the whole COG complex in Golgi enzymes targeting, localization and stability can't be excluded [46] (Fig. 5).

### Defects affecting the protein sorting apparatus

Another group of defects are those affecting protein sorting apparatus. SEC23B is a member of Sec23/24 family, a component of the COPII coat machinery allowing the budding of COPII coat vesicles and then the transport of cargo proteins from the ER to the Golgi (Fig. 2). Mutations affecting SEC23B have been shown to cause congenital dyserythropoietic anemia type II, a disease affecting the differentiation of cells of the erythroid lineage [48, 49]. While SEC23B and SEC23A have redundant functions, in erythrocytes the SEC23B defect is not sufficiently compensate by SEC23A, then accounting for the observed restricted phenotype [48]. Interestingly, many abnormalities affecting glycosylation and/or levels of erythrocyte glycoconjugates were observed related to SEC23B mutations. The MALDI-TOF analysis of band 3 derived N-glycans revealed for example the presence of high mannose and hybrid glycans compatible with a general impairment of *cis*, medial and *trans* Golgi processing [50].

### Defects affecting Golgi pH homeostasis and/or membrane fusion

Deficiencies in ATP6V0A2 have been found to cause cutis laxa type II and wrinkly skin syndrome [51, 52]. This gene encodes a subunit of the vacuolar ATPase that is a heteromultimeric enzyme allowing the acidification of membrane enclosed organelles such as vacuoles, lysosomes, endosomes, coated vesicles and Golgi compartments [53]. This protein is composed of two domains, the V1 domain hydrolyzing ATP and the V0 domain that translocates protons from the cytosol to the lumen. This genetic defect is associated with strong Golgi glycosylation abnormalities leading to both N- and O-glycosylation deficiencies. To date, the exact mechanism by which mutations in ATP6V0A2 subunit affects Golgi glycosylation remains unclear. Two mechanisms can be proposed. The first one implies the disturbance of the Golgi pH. A lack of V0-ATPase activity would indeed certainly enhance the pH of the Golgi compartment thus affecting the activity of certain Golgi glycosyltransferases. The other mechanism related to intracellular transport would be a defect in membrane fusion. A recent study by Andreas Mayer's group highlighted that the V0 transmembrane domain of the V-ATPase could be involved in membrane fusion [54]. This could also explain the glycosylation deficiency resulting from a lack of fusion of vesicles containing Golgi glycosyltransferases.

## Conclusion and future perspectives

The past couple of years have seen a transition in the field of human glycosylation disorders with the identification of deficient proteins involved in trafficking. In this growing field, deficiencies are for instance limited to deficiencies in Golgi trafficking or abnormalities in COPII formation. Intriguingly, the identification of such deficiencies is growing but can rather be considered as moderate taking into account the huge number of known genetic disorders of membrane trafficking where glycosylation seems to be normal. This has to urge us in the future to understand why only specific defects lead to Golgi glycosylation abnormalities.

Another topic of emerging interest is the impact of other proteins and lipid components on vesicular Golgi trafficking and Golgi glycosylation. In literature, numerous proteins, non-associated with human genetic diseases yet, have been shown to affect Golgi glycosylation. These proteins such as GM130, GPHR, Sac1 can be considered as extremely good candidates for unsolved human glycosylation diseases. In the future we expect to see the discovery of many other trafficking defects affecting Golgi glycosylation. This will give us new insights into the molecular mechanisms for localization and retention of Golgi glycosylation enzymes and thus learn more about how Golgi glycosylation is regulated.

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