Glycomics and Lipidopics

Extended Abstract

Sugary Logistics Gone Wrong: Membrane Trafficking and Congenital Disorders of Glycosylation

Peter T. A Linders

Abstract:

Glycosylation is an important post-translational modification for both intracellular and secreted proteins. For glycosylation to occur, cargo must be transported after synthesis through the different compartments of the Golgi apparatus where distinct monosaccharides are sequentially bound and trimmed, resulting in increasingly complex branched glycan structures. Of utmost importance for this process is the intraorganellar environment of the Golgi. Each Golgi compartment has a distinct pH, which is maintained by the vacuolar H^+-ATPase (V-ATPase). Moreover, tethering factors such as Golgins and the conserved oligomeric Golgi (COG) complex, in concert with coatomer (COPI) and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated membrane fusion, efficiently deliver glycosylation enzymes to the right Golgi compartment. Together, these factors maintain intra-Golgi trafficking of proteins involved in glycosylation and thereby enable proper glycosylation. However, pathogenic mutations in these factors can cause defective glycosylation and lead to diseases with a wide variety of symptoms such as liver dysfunction and skin and bone disorders. Collectively, this group of disorders is known as congenital disorders of glycosylation (CDG). Recent technological advances have enabled the robust identification of novel CDGs related to membrane trafficking components. In this review, we highlight differences and similarities between membrane trafficking-related CDGs. The conjugation of oligosaccharide structures to proteins, glycosylation, is a ubiquitous and fundamental post-translational modification found in all domains of life. Glycosylation is not only important for the structure and function of proteins, but also for their transit and selective targeting through the secretory pathway. In mammals, approximately 700 proteins are necessary for generating the full diversity of over 7000 glycan structures. The addition of glycan structures in vertebrates is a sequential process and involves both the addition of monosaccharides via glycosyltransferases and the trimming of glycans by glycosidases. Only ten different monosaccharides are required to build the full glycan spectrum: fucose (Fuc), galactose (Gal), glucose (Glc), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), glucuronic acid (GlcA), mannose (Man), sialic acid (SA, also known as neuraminic acid), xylose (Xyl), and recently identified ribitol. In vertebrates, N-glycan synthesis is initiated in the ER as a 14 monosaccharide precursor on the carrier lipid dolichol. During translation, this glycan is transferred by oligosaccharyltransferase (OST)from dolichol to the nascent polypeptide at acceptor peptide sequons, generally consisting of an Asn-X-(Ser/Thr) motif. Distal glucose moieties of these immature, high glucose and mannose containing, glycan structures are subsequently trimmed before Golgi entry; an important step in the control of misfolded glycoproteins in the ER. Glycoproteins then exit the ER via, for instance, cargo receptor ERGIC-53 and are transported to the Golgi apparatus for further processing. In the Golgi, glycoproteins are trimmed, extended, and branched until they reach their final glycan form. The mammalian Golgi apparatus is a single large perinuclear organelle, organized into discrete compartments or cisternae. The Golgi can be subdivided into *cis*-Golgi, closest to the ER, medial-Golgi, *trans*-Golgi, and the *trans*-Golgi network (TGN), furthest away from the nucleus. Furthermore, mammals have a pre-Golgi compartment known as the ER-Golgi intermediate compartment (ERGIC, previously known as the vesicular-tubular cluster (VTC)). Newly synthesized glycoproteins emanating from the ER enter the Golgi apparatus at the *cis*-Golgi, sequentially pass through medial- and *trans*-Golgi, and finally, exit the Golgi at the TGN. The compartmentalization of the Golgi allows for distinct environments containing subsets of glycosylation enzymes, enabling sequential modifications for the formation of completely mature glycoproteins. The organization of Golgi-resident enzymes and the Golgi apparatus itself differs between cell types, contributing to glycoprotein diversity. Two examples are the distribution of α-mannosidases I and II, which primarily localize to the *trans*-Golgi in intestinal goblet cells, but are distributed over all Golgi cisternae in hepatocytes, the functional consequences of which are currently unknown . Efficient glycosylation fully relies on the correct localization of glycosylation enzymes, as well as on the delivery of other glycosylation machinery, such as nucleotide sugar transporters, and cargo proteins to be glycosylated to the correct Golgi compartment. An important factor involved in the correct trafficking of glycosylation enzymes is the maintenance of pH within the Golgi apparatus. In eukaryotic cells, the principal proton pump for the regulation of

Peter T. A Linders

Tumor Immunology Lab, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands

intraorganellar pH is the vacuolar H^+-ATPase (V-ATPase). The membrane V_0 domain anchors this complex in the membrane, and the V_1 domain is cytosolic. The V_0 domain contains six different subunits (a, d, e, c, c', and c''). This domain functions as a proton translocator across the membrane, which not only results in a pH gradient, but also in a change in membrane potential, which is neutralized by counter ions such as K^+ and Cl^-. The cytosolic V_1 domain contains eight subunits (A-H), and its main function is ATP hydrolysis to provide the energy needed for the pH gradient. In mammals, the specificity of V-ATPase localization is encoded in the V_0a subunit, as four unique isoforms exist (V_0a1-4). This is in contrast to *Saccharomyces cerevisiae*, which has only two unique isoforms (Vph1p and Stv1p) . The diversity in V_0a subunits is likely important for specific cell type-dependent functions and differential regulation of the pH in different organelles. Isoform V_0a1 is targeted to secretory vesicles and V_0a2 to the Golgi and endosomes, and V_0a3, highly expressed in macrophages and osteoclasts, is enriched in late endosomes and lysosomes, while V_0a4 is mainly expressed in the kidney, inner ear, and ocular ciliary body. The V-ATPase ensures a constant pH in the various Golgi compartments, which ranges from 6.7 for the *cis*-Golgi to 6.0 for the *trans*-Golgi . Given the pH optima of glycosylation enzymes, this pH gradient could restrict the activity of glycosylation enzymes to their target Golgi compartment . However, this might not be the complete explanation considering the broad distribution of pH optima and the small differences in absolute pH between the cisternae. Instead, or additionally, the pH-sensitive binding and release to cargo adapters might ensure correct enzyme localization to the target Golgi compartment. Several models for the trafficking routes in the Golgi exist, but the most favorable model of membrane traffic within the Golgi is the cisternal maturation model. Cisternal maturation is the gradual conversion of a Golgi compartment by the delivery of proteins and lipids from more mature Golgi compartments concomitant with the removal of Golgi proteins and lipids from previous Golgi compartments by coatomer (coat protein complex I; COPI)-mediated retrograde membrane trafficking. Before membrane fusion of these COPI vesicles, a set of molecular instruments orchestrates correct vesicle targeting to and within the Golgi. An important group of such trafficking factors is the Golgin family, which consist of large coiled-coil proteins that associate with the Golgi membrane. Golgins form a tentacular web in the cytosol that efficiently and selectively tethers cargo vesicles. Concurrently, Golgins can act as scaffolding proteins for small Rab or Arf GTPases. At the Golgi, Rab6 and Rab30 can recruit effectors, such as the cytoskeletal motor protein myosin II, for vesicle trafficking. Completing the ensemble is the conserved oligomeric Golgi (COG) tethering complex, a hetero-octameric protein complex bridging the Golgi membrane and COPI vesicles. Finally, when the Golgi membrane and the uncoated COPI vesicle are in close enough proximity, membrane fusion occurs. Membrane fusion is performed by soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. As glycosylation is such an extensive process with a multitude of different factors that operate together for sequential remodeling of glycan moieties, only slight disturbances can have major implications on glycosylation. As such, over 100 monogenic diseases have been identified characterized by dysfunctional glycosylation, which form a group collectively known as congenital disorders of glycosylation (CDG). A large subset of these includes genetic variants in the prior mentioned trafficking proteins, but also in subunits of the vacuolar H^+-ATPase and its assembly factors. Recent technological advances in CDG diagnostics have enabled more comprehensive analysis of glycosylation disorders. Novel mass spectrometric methods to detect changes in glycosylation together with next-generation sequencing to detect novel genomic mutations are a powerful combination for the interrogation of membrane trafficking components in CDGs. This review serves to provide a comprehensive overview of trafficking-related CDGs and to form a detailed understanding of how Golgi trafficking influences glycosylation.

Peter T. A Linders

Tumor Immunology Lab, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands