Review

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 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 on how the Golgi stacking factors GRASP55 and GRASP65 generate the Golgi structure and how the conserved oligomeric Golgi complex maintains Golgi enzymes in different Golgi subcompartments by retrograde protein trafficking.

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 the 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, and 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 five to eight 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 and 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; (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 through a C–C bond [16]; and (4) glypiation, in which a glycan acts as a linker to bridge a protein to a glycosylphophatidylinositol 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 to the amide group of asparagine 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. The addition of GlcNAc on mannose allows for generating sugar branches in the medial Golgi. Decoration of galactose (Gal), sialic acid, and/or fucose 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 to the side chain of Ser/Thr and is then 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) in the early Golgi and then extended with repetitive disaccharide units, GlcNAc–glucuronic acid 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]. Rare *O*-glycans are found to be attached to epidermal growth factor–like repeats or thrombospondin repeats. 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 glycosylphophatidylinositol 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 (CDGs) are rare genetic diseases in which both *N*-glycan and *O*-glycan biosyntheses 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.