Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 88

This document contains 4 pathways and 3 reactions (see Table of Contents)
N-linked glycosylation is the most important form of post-translational modification for proteins synthesized and folded in the Endoplasmic Reticulum (Stanley et al. 2009). An early study in 1999 revealed that about 50% of the proteins in the Swiss-Prot database at the time were N-glycosylated (Apweiler et al. 1999). It is now established that the majority of the proteins in the secretory pathway require glycosylation in order to achieve proper folding. The addition of an N-glycan to a protein can have several roles (Shental-Bechor & Levy 2009). First, glycans enhance the solubility and stability of the proteins in the ER, the golgi and on the outside of the cell membrane, where the composition of the medium is strongly hydrophilic and where proteins, that are mostly hydrophobic, have difficulty folding properly. Second, N-glycans are used as signal molecules during the folding and transport process of the protein: they have the role of labels to determine when a protein must interact with a chaperon, be transported to the golgi, or targeted for degradation in case of major folding defects. Third, and most importantly, N-glycans on completely folded proteins are involved in a wide range of processes: they help determine the specificity of membrane receptors in innate immunity or in cell-to-cell interactions, they can change the properties of hormones and secreted proteins, or of the proteins in the vesicular system inside the cell.

All N-linked glycans are derived from a common 14-sugar oligosaccharide synthesized in the ER, which is attached co-translationally to a protein while this is being translated inside the reticulum. The process of the synthesis of this glycan, known as Synthesis of the N-glycan precursor or LLO, constitutes one of the most conserved pathways in eukaryotes, and has been also observed in some eubacteria. The attachment usually happens on an asparagine residue within the consensus sequence asparagine-X-threonine by an complex called oligosaccharyl transferase (OST). After being attached to an unfolded protein, the glycan is used as a label molecule in the folding process (also known as Calnexin/Calreticulin cycle) (Lederkremer 2009). The majority of the glycoproteins in the ER require at least one glycosylated residue in order to achieve proper folding, even if it has been shown that a smaller portion of the proteins in the ER can be folded without this modification. Once the glycoprotein has achieved proper folding, it is transported via the cis-Golgi through all the Golgi compartments, where the glycan is further modified according to the properties of the glycoprotein. This process involves relatively few enzymes but due to its combinatorial nature, can lead to several millions of different possible modifications. The exact topography of this network of reactions has not been established yet, representing one of the major challenges after the sequencing of the human genome (Hossler et al. 2006).

Since N-glycosylation is involved in a great number of different processes, from cell-cell interaction to folding control, mutations in one of the genes involved in glycan assembly and/or modification can lead to severe development problems (often affecting the central nervous system). All the diseases in genes involved in glycosylation are collectively known as Congenital Disorders of Glycosylation (CDG) (Sparks et al. 2003), and classified as CDG type I for the genes in the LLO synthesis pathway, and CDG type II for the others.

**Literature references**


### Editions

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Biosynthesis of the N-glycan precursor (dolichol lipid-linked oligosaccharide, LLO) and transfer to a nascent protein ➔

Location: Asparagine N-linked glycosylation

Stable identifier: R-HSA-446193

N-linked glycosylation commences with the 14-step synthesis of a dolichol lipid-linked oligosaccharide (LLO) consisting of 14 sugars (2 core GlcNAcs, 9 mannoses and 3 terminal GlcNAcs). This pathway is highly conserved in eukaryotes, and a closely related pathway is found in many eubacteria and Archaea. Mutations in the genes associated with N-glycan precursor synthesis lead to a diverse group of disorders collectively known as Congenital Disorders of Glycosylation (type I and II) (Sparks et al. 1993). The phenotypes of these disorders reflect the important role that N-glycosylation has during development, controlling the folding and the properties of proteins in the secretory pathway, and proteins that mediate cell-to-cell interactions or timing of development.

Literature references


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Transfer of N-glycan to the protein

Location: Asparagine N-linked glycosylation

Stable identifier: R-HSA-446209

Type: transition

Compartments: endoplasmic reticulum membrane

The 14-sugar N-glycan precursor (aka lipid-linked oligosaccharide, LLO), synthesized in the previous reactions, is attached in a single step to a nascent protein, releasing the dolichyl phosphate anchor and the as-yet unfolded glycoprotein. The reaction occurs cotranslationally as the growing peptide chain leaves a ribosome associated with the ER membrane and enters the ER lumen. This reaction is catalyzed by the oligosaccharyltransferase (OST) complex, comprising at least seven proteins; DAD1 (Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1), DDOST (OST48 in yeast), RPN1 (ribophorin 1), RPN2 (ribophorin 2), OST4, TUSC3 (N33), MAGT1 (magnesium transporter protein 1) and either STT3A or STT3B (Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A and B), which contain the catalytic domain (Kelleher & Gilmore 2006). A mutation in RPN2 is associated with CDG-Ix (Vleugels et al. 2009). The signal for glycosylation is the consensus sequence Asn - X - Thr/Ser, where the first amino acid is always Asn, the second can be any amino acid except for Pro, and the third position may be Thr, Ser or Cys, with a preference for the first (Breuer et al. 2001). Not all Asn - X - Thr/Ser sites are modified in vivo (Petrescu et al. 2004).

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N-glycan trimming in the ER and Calnexin/Calreticulin cycle

Location: Asparagine N-linked glycosylation

Stable identifier: R-HSA-532668

After being synthesized in the ER membrane the 14-sugars lipid-linked oligosaccharide is co-translationally transferred to an unfolded protein, as described in the previous steps. After this point the N-glycan is progressively trimmed of the three glucose and some of the mannoses before the protein is transported to the cis-Golgi. The role of these trimming reactions is that the N-glycan attached to an unfolded glycoprotein in the ER assume the role of ‘tags’ that direct the interactions of the glycoprotein with different elements that mediate its folding. The removal of the two outer glucose leads to an N-glycan with only one glucose, which is a signal for the binding of either one of two chaperone proteins, calnexin (CNX) and calreticulin (CRT). These chaperones provide an environment where the protein can fold more easily. The interaction with these proteins is not transient and is terminated by the trimming of the last remaining glucose, after which the glycoprotein is released from CNX or CRT and directed to the ER Quality Control compartment (ERQC) if it still has folding defects, or transported to the Golgi if the folding is correct. The involvement of N-glycans in the folding quality control of proteins in the ER explains why this form of glycosylation is so important, and why defects in the enzymes involved in these reactions are frequently associated with congenital diseases. However, there are many unknown points in this process, as it is known that even proteins without N-glycosylation sites can be folded properly (Caramelo JJ and Parodi AJ, 2008).

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At least two mechanisms of transport of proteins from the ER to the Golgi have been described. One is a general flow requiring no export signals (Wieland et al, 1987; Martinez-Menarguez et al, 1999). The other is mediated by LMAN1/MCFD2, mannose-binding lectins that recognize a glycan signal (Zhang B et al, 2003).

**Literature references**


Asialoglycoprotein receptors 1 and 2 (ASGR1 and ASGR2) mediate the endocytosis of plasma glycoproteins whose terminal sialic acid residues on their complex carbohydrate moieties have been removed. They can bind each other to form at least a heterodimeric complex (Bischoff et al. 1988). The resultant ligand:receptor complex is internalised and transported to a sorting organelle, where receptor and ligand are disassociated. The receptor then cycles back to the cell membrane surface. Palmitoylation of ASGR1 (Cys36) and ASGR2 (Cys54, 58) is essential for efficient endocytosis of ligand by the clathrin-dependent endocytic pathway and especially for the proper dissociation and delivery of ligand to lysosomes (Saxena et al. 2002, Yik et al. 2002).

Literature references


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B4GALNT2 transfers GalNAc from UDP-GalNAc to Sial-Gal-GlcNAc-Gal to form the Sd(a) antigen on UMOD

**Location:** Asparagine N-linked glycosylation

**Stable identifier:** R-HSA-8855954

**Type:** transition

**Compartments:** Golgi lumen, Golgi membrane

The histo-blood group antigen Sda was discovered to be a dominant character found in more than 90% of Caucasian red blood cells. In addition to erythrocytes, the Sda antigen is also found in other tissues and body fluids, particularly in urine of humans and other mammals. The Sda antigen shares a common minimal saccharide structure (GalNAc-beta1-4[Neu5Ac-alpha2-3]Gal-beta-) with the Cad antigen. Both antigens contain a pentasaccharide structure, the Sda antigen’s structure being GalNAc-beta1-4[Neu5Ac-alpha2-3]Gal-beta1-4GlcNAc-beta1-3Gal. The last step in the biosynthesis of both Sda and Cad antigens is catalysed by beta-1,4 N-acetylgalactosaminyltransferase 2 (B4GALNT2), a Golgi membrane protein that transfers N-acetylgalactosamine (GalNAc) from UDP-GalNAc to position C4 of the Gal residue of the Neu5Ac-alpha2-3Gal-beta1 sequence (Montiel et al. 2003, Lo Presti et al. 2003, Dall’Olio et al. 2014). Both antigens can be expressed by N- or O-linked chains of glycoproteins. Tamm–Horsfall glycoprotein (THGP, aka UMOD) is a major carrier of the Sda antigen in urine (Soh et al. 1980, Serafini-Cessi & Conte 1982). After proteolytic cleavage, UMOD is secreted into the urine where it may play roles in colloid osmotic pressure, retarding passage of positively charged electrolytes, preventing urinary tract infection and modulating formation of supersaturated salts and their crystals (Schmid et al. 2010).

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