07 *ABSTRACT*

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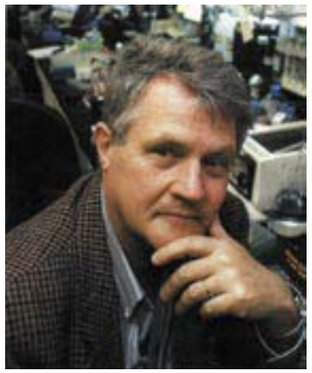
The Quality Control of Glycoprotein Folding in the Endoplasmic Reticulum



profile

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For many years I have been studying the mechanism by which oligosaccharides are added to proteins in the lumen of the endoplasmic reticulum and the effect that such bulky, highly hydrophilic structures have on the acquisition by glycoproteins of their proper tertiary structures. I described that in mammalian cells an oligosaccharide is transferred "en bloc" from a lipid (dolichol) pyrophosphate derivative to asparagine units in proteins and obtained hints that this compound was further processed once transferred. I conducted then further studies that revealed that the same glycosylation mechanism is operative in the common yeast *Saccharomyces cerevisiae*, in which it may lead to synthesis of mannan, a cell wall polysaccharide. Studies on protein glycosylation conducted in my laboratory showed, first in trypanosomatid protozoa and then in mammalian cells, that protein-linked saccharides are transiently glucosylated in the endoplasmic reticulum. The enzyme responsible for glucosylation (UDP-Glc:glycoprotein glucosyltransferase) appeared to behave as a sensor of glycoprotein conformations as it only glucosylates saccharides linked to improperly folded protein moieties. The glucosyltransferase is a key element in the endoplasmic reticulum retention of malfolded conformers and in the mechanism by which glycoproteins acquire their native three-dimensional structures.

Key words Endoplasamic, reticulum/Oligosaccharide, processing/Glycoprotein, folding/Calnexin/Glucosyltransferase

Proteins entering the secretory pathway acquire their proper tertiary and in certain cases also quaternary structures in the endoplasmic reticulum (ER). Incompletely folded species are prevented from transit to the Golgi apparatus and eventually degraded by the proteasome. The emerging principles by which *N*-glycan processing in the ER participates in the quality control process will be dealt with in the talk. Monoglucosylated glycans formed by glucosidase I and II (GII)-catalyzed deglucosylation of the oligosaccharide transferred from lipid (dolichol) pyrophosphate derivatives to proteins ($Glc_3Man_9GlcNAc_2$) mediate the binding of glycoproteins to two ER resident lectins, calnexin (CNX), a transmembrane protein, and its soluble homolog, calreticulin (CRT). Further deglucosylation of glycans by GII liberates glycoproteins from CNX/CRT. Glycans may be then reglucosylated by the UDP-Glc:glycoprotein glucosyltransferase (GT), and thus recognized again by CNX/CRT, only when linked to incompletely folded protein moieties. The reglucosylaging enzyme behaves as a sensor of glycoprotein conformations. Deglucosylation-reglucosylation cycles catalyzed by the opposing activities of GII and GT stop when proper folding is achieved as glycoproteins become then substrates for GII but not for GT. Permanent liberation from CNX/CRT allows further glycoprotein transit through the secretory pathway (Fig. 1). The CNX/CRT-monoglucosylated glycan interaction is one of the mechanisms by which cells retain incompletely folded glycoproteins in the ER and, in addition, it enhances folding efficiency by preventing protein aggregation and allowing intervention of additional ER chaperones and folding facilitating proteins (1). A still controversial protein-protein interaction between folding species and CNX/CRT might further assist the folding process. In addition, there is evidence, suggesting that Man removal, mediated by ER mannosidases might act as a timer mechanism for the disposal of incompletely folded glycoproteins bound for proteasome degradation. Synthesis of mRNAs encoding both unconventional chaperones (CNX and CRT) and the sensor of glycoprotein conformations (GT) have been shown to be induced under stress conditions that promote ER accumulation of misfolded glycoproteins. Moreover, interfering with monoglucosylated glycan formation elicits the unfolded protein response as shown by the upregulation of the main ER chaperone (BiP) (2-4).

The mechanism described constitutes a novel system, different from those of classical molecular chaperones, for retaining non-native conformers and facilitating protein folding and oligomerization. CNX and CRT are unconventional chaperones that apparently do not directly sense the folding status of the substrate proteins as classical chaperones do. This task is reserved to an enzyme (GT) that introduces a covalent modification on glycoproteins lacking their native conformations. This covalent carbohydrate modification is the element recognized by this new kind of chaperones. Although the main features of this system are increasingly clear, there are several aspects that remain obscure. More detailed biochemical and structural studies are needed to understand the recognition of non-native structures by GT, as well as CNX/CRT-ligand interaction. The controversial protein-protein interaction between CNX/CRT and folding glycoproteins and the role of glycoprotein reglucosylation in multicellular organisms needs to be established. The involvement of Man removal in the disposal of permanently misfolded species has to be further substantiated, and the interplay between the components of the CNX/CRT pathway and other folding factors in the ER should be determined to advance our understanding of the quality control mechanisms.

GT is the key element of the quality control of glycoprotein folding in the ER as the enzyme only glucosylates high mannose-type oligosaccharides linked to incompletely folded protein moieties. Two structural elements are recognized by GT in non-native conformers: the innermost GlcNAc residue in $Man_9GlcNAc_2$, which in native conformers is buried within the protein scaffold, and a protein determinant. To characterize protein structures recognized by GT in misfolded conformers we synthesized neoglycoproteins by chemical coupling $Man_9GlcNAc_2$-Asn to fragments of a modified (L8C) chymotrypsin inhibitor II (CI-2) having 25, 40, 54 or 64 (full length) amino acid residues. We found that the 1-54 fragment had the highest glucose acceptor capacity (Km= 60 micromolar), followed by the 1-40 fragment (Km= 170 micromolar). The 1-25 and full-length (1-64) fragments only had residual acceptor capacity. Structural analysis showed that the 1-54 neoglycoprotein had a molten globule conformation, whereas the 1-40 and 1-25 fragments lacked structure. The full length CI-2 had a properly folded glycoprotein conformation. The main difference between fragments 1-25 and 1-40 resides in a stretch of hydrophobic amino acids occurring between amino acids 27 and 40 that also display a putative BiP binding capacity. Both the 1-40 and 1-54 fragments expose those hydrophobic amino acids, as revealed by the lack of tertiary structure of the former and the capacity of ANS (8-anilinonaphtalene-1-sulphonate) binding of the latter. It was concluded that GT recognizes hydrophobic amino acids but preferentially those in patches exposed in molten globule conformers. Moreover, BiP binds preferentially to the 1-40 fragment (Km of fragment = 70 micromolar) than to the molten globule structure (Km higher than 500 micromolar) as revealed by enhancement of ATPase activity and, simultaneously, preferentially inhibits GT when 1-40 fragment is used as acceptor substrate. It was concluded that BiP regulates GT activity by hiding hydrophobic amino acid residues exposed in acceptor glycoproteins still in extended conformations.

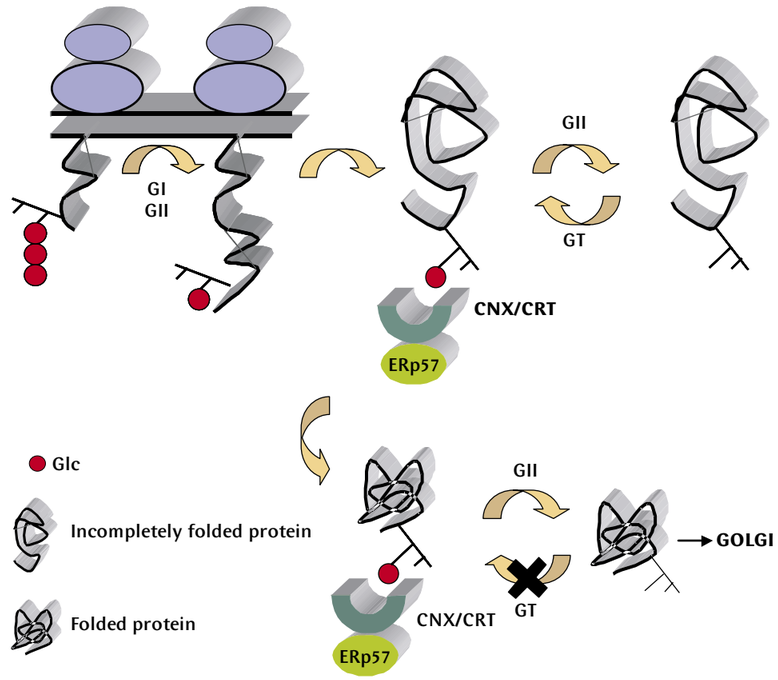

Figure 1. The model proposed for the quality control of glycoprotein folding.
Protein-linked $Glc_3Man_9GlcNAc_2$ is partially deglucosylated to the monoglucosylated derivative by GI and GII and this structure is recognized by CNX/CRT. $Man_9GlcNAc_2$ is glucosylated by GT if complete deglucosylation occurs before lectin binding. The glycoprotein is liberated from the CNX/CRT anchor by GII and reglucosylated by GT only if not properly folded. Upon adoption of the native tertiary structure, the glycoprotein is released from CNX/CRT by GII and not reglucosylated by GT.

References

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