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Review

# Glycan regulation of ER-associated degradation through compartmentalization

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ABSTRACT

The internal environment of the eukaryotic cell is divided by membranes into various organelles, containing diverse functional subcompartments, which allow complex cellular life. The quality control of newly made secretory proteins relies on the ability of the endoplasmic reticulum (ER) to segregate and compartmentalize molecules at different folding states. These folding states are communicated by *N*-glycans present on most secretory proteins. In ER-associated degradation (ERAD), protein molecules that have been identified as terminally misfolded are sent for degradation at the cytosolic proteasomes after being dislocated from the ER to the cytosol. This review will focus on how misfolded glycoprotein molecules are segregated from their properly folded counterparts and targeted to ERAD. The pathway involves compartmentalization, which is intimately linked to differential *N*-glycan processing. Recent data suggests that these processes are very dynamic, and include transient assembly of ERAD machinery complexes.



**Contents**



*Abbreviations:* Bap31, B-cell receptor associated protein 31; CNX, calnexin; CRT, calreticulin; ER, Endoplasmic reticulum; ERAD, ER associated degradation; RER, rough ER; ERESs, ER exit sites; ERGIC, ER to Golgi intermediate compartment; ERManI, α1,2 ER mannosidase 1; ERQC, ER-derived quality control compartment; GI, glucosidase 1; GII, glucosidase 2; HERP, homocysteine induced ER protein; MAMs, mitochondrial associated membranes; PNGase, peptide:N-glycanase; QCVs, quality control vesicles; SER, smooth ER; UGGT, UDP-glucose:glycoprotein glucosyltransferase; VIP36, are vesicular integral membrane protein of 36Kda; VIPL, Vip36 like.

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## 1. Translocation/glycosylation

Nascent proteins that are destined for secretion, for the plasma membrane or for the secretory and endocytic compartments undergo processes of maturation and quality control in the secretory pathway, and must first be translocated or integrated into the ER. Translocation into the ER starts the journey of secretory proteins that are compartmentalized and thus segregated from the cytosol into specialized compartments. In the mammalian cell, protein translocation occurs mostly co-translationally. The targeting of the ribosomal-polypeptide complex to the rough ER (RER) membrane is mainly carried out by recognition of an N-terminal signal sequence by the signal recognition particle [1]. However, there is new evidence for alternative recognition pathways [2]. The translocation machinery utilizes the energy of the translation elongation process for transporting the polypeptide chain across the ER membrane [3].

The mammalian translocation apparatus is composed of several complexes, primarily the Sec61 complex that forms the translocation channel. It is comprised of 3 subunits: The α subunit that spans the membrane 10 times and creates the hydrophilic pore of the channel, and the β and γ subunits [4]. Most of the newly synthesized polypeptides emerging at the luminal site of the translocon channel gain one or more 14-residue *N*-glycans. This *N*-glycosylation event is the most common post-translational modification for proteins synthesized in the ER. It is carried out by the translocon-associated multi-subunit complex oligosaccharyltransferase, which catalyzes the covalent linkage of a pre-assembled core oligosaccharide onto asparagines within the acceptor sequence N-X (any amino acid except proline)-S/T [5,6]. The conserved Glucose3-mannose9-*N*-acetylglucosamine2 ($Glc_3Man_9GlcNAc_2$) oligosaccharide is of great importance for the folding and maturation of the glycoprotein. Not only does it stabilize the nascent oligopeptide and promote folding of the newly-made glycoprotein by altering its intrinsic physical properties, it also serves as the ligand for chaperones and other folding agents assisting the glycoprotein in gaining its proper conformation. Most *N*-glycosylation is cotranslational, but some glycosylation occurs posttranslationally, directed by a specialized oligosaccharyltransferase catalytic subunit, STT3B, with the aid of accessory proteins [7]. Differential processing of the precursor oligosaccharide creates tags recognized by the quality control and ERAD machineries, which direct the nascent glycoprotein to different subcompartments of the ER and early secretory pathway [8]. As will be discussed later, the oligosaccharide structures reflect the folding status of the glycoprotein allowing the quality control machinery to differentiate between properly folded, unfolded or misfolded glycoproteins [9–11].

## 2. Calnexin and other lectins in the early secretory pathway

### 2.1. Malectin, calnexin and calreticulin

Almost immediately following *N*-glycosylation, the $Glc_3Man_9GlcNAc_2$ oligosaccharide begins to undergo processing (Fig. 1). The first modification to occur is the trimming of the terminal α1,2-linked glucose residue by the ER transmembrane glucosidase 1 enzyme (GI). This trimming results in a di-glucosylated $Glc_2Man_9GlcNAc_2$ oligosaccharide which is identified by an ER transmembrane lectin, malectin. This interaction may serve to facilitate the ER retention of nascent glycoproteins and to modulate further processing of the oligosaccharide [12,13]. The second modification is the removal of the now exposed α1,3-linked glucose residue by the soluble ER enzyme glucosidase 2 (GII). This trimming step results in a monoglucosylated $Glc_1Man_9GlcNAc_2$ oligosaccharide that can be identified by the ER transmembrane lectin-chaperone calnexin (CNX) or by its soluble homolog calreticulin (CRT), in a manner which is not dependent upon the folding state of the glycoprotein [14]. These initial processing steps and lectin association take place in the rough ER (RER), near the translocation sites [15]. CNX is a type I transmembrane ER protein that relies on an arginine rich domain for its ER retention, whereas CRT possesses a KDEL sequence. The association of an unfolded glycoprotein with CNX or CRT allows time for folding, keeping the glycoprotein sequestered from its surroundings, thus preventing aggregation. At this point the lectins and associated client glycoproteins traffic to the ER-derived quality control compartment (ERQC), which will be described later, helping to segregate unfolded and misfolded proteins from translocating nascent proteins (Fig. 2). CNX and CRT may function in the shuttling of their client glycoproteins from the rough ER to the ERQC. CNX recruits the thiol disulfide oxidoreductase ERp57, which promotes the formation of proper disulfide bonds in the substrate glycoprotein [16]. The dissociation of a substrate glycoprotein from CNX and ERp57 follows dynamics that are dependent upon its folding state, as a misfolded glycoprotein will quickly dissociate from ERp57 while maintaining its association with CNX for longer periods. This mechanism implicates CNX in a first step of targeting to ERAD of misfolded glycoproteins [17]. CNX will dissociate at some point from its client, regardless of whether or not proper folding has been achieved. This dissociation appears to be regulated by palmitoylation of CNX, which targets it from the ERQC to a new destination, the mitochondria-associated membranes (MAMs) [18]. Whereas non-palmitoylated CNX has a central role in glycoprotein folding and quality control, after palmitoylation it functions in ER-mitochondrial crosstalk. CNX dissociation allows GII to trim the final α1,3-linked glucose residue from substrate glycoproteins, producing a non glucosylated $Man_9GlcNAc_2$ oligosaccharide [19]. At this point, two types of glycoproteins must be discriminated-correctly folded versus unfolded or misfolded glycoproteins, a distinction which is made by the folding sensor UDPGlc: glycoprotein glucosyltransferase (UGGT) [20,21]. UGGT recognizes the core pentasaccharide ($Man_3GlcNAc_2$) of the *N*-glycan and probes the polypeptide for exposed hydrophobic tracts in order to assess the folding state of the glycoprotein [22,23]. If the glycoprotein is correctly folded, it will no longer be a substrate of UGGT and it will continue its maturation through the secretory pathway. However, if it is still unfolded or misfolded, UGGT will reattach a glucose residue to the acceptor mannose on its *N*-glycans, allowing reassociation with CNX for further folding attempts. The cyclic processes of CNX association, glucose trimming, examination by UGGT, reglucosylation and renewed CNX association are commonly known as the CNX cycle [11,24]. In S. *cerevisiae*, the CNX cycle does not occur, not for lack of CNX (although it is quite different from its mammalian counterpart) but rather for lack of UGGT [20]. CNX also interacts with B-cell receptor associated protein 31 (BAP31), both of which cycle between the ER, the ERQC and the MAMs [25–27] and both might play a role in the signaling between ER subcompartments [8]. BAP31 association with CNX induces BAP31 cleavage to produce a pro-apoptotic fragment at the MAMs [28,29]. BAP31 is also involved in the export of transmembrane proteins from the ER to the Golgi [30,31].

### 2.2. Lectins from the ER to the Golgi

While the lectins Malectin, CNX and CRT and the glucosidases GI and GII identify nascent and still-folding glycoproteins in the early stages of their synthesis and maturation, other lectins are specific to later stages. These lectins identify and help properly folded glycoproteins in their export from the ER. The transmembrane lectin ERGIC-53 cycles between the ER, the ER to Golgi intermediate compartment (ERGIC) and the Golgi, binding and releasing



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