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A Novel Role for *N*-Glycans in the ERAD System

Yukiko Yoshida*

Laboratory of Frontier Science, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613; and CREST, Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012

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The endoplasmic reticulum (ER) provides a quality-control system for newly synthesized secretory and membrane proteins. Any improperly folded or incompletely assembled oligomers are retained in the ER, and they are retro-translocated into the cytosol when misfolding persists, where they are destroyed by the proteasome through ubiquitylation. This disposal process is called ER-associated degradation (ERAD). Although much is known about the fate of ERAD substrates near the point of degradation, little information is available about how these proteins are recognized, retained, and targeted for translocation and ubiquitylation machinery. Recent studies indicate that N-linked oligosaccharides attached to nascent proteins function as tags for several processes of a quality-control system, such as individual steps of ER-retention, selection for ERAD substrates, and ubiquitylation. In this review, I describe recent advances in the molecular basis of the ERAD system, particularly those mediated by *N*-glycan recognition molecules.

Key words: endoplasmic reticulum-associated degradation (ERAD), F-box protein, *N*-glycan, quality control, ubiquitin ligase.

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GlcNAc, N-acetyl glucosamine; PNGase, peptide: *N*-glycanase; TCRα, T-cell receptor α chain; UGGT, UDP-glucose:glycoprotein glucosyltransferase.

Proteins destined for the secretory pathway first pass the membrane of the endoplasmic reticulum (ER) through a translocation channel termed translocon (*1*). These proteins are translocated in an unfolded state, so that assembly into their native conformations occurs as the next step in the ER. In the ER lumen, molecular chaperones (thereafter refereed simply chaperones) and folding enzymes reside at high concentrations, and assist in the folding and assembly of proteins. While the ER is optimised for efficient protein maturation, not all proteins mature correctly. An estimated one third of all synthesized proteins are degraded immediately after translation (*2*). To monitor the integrity of the maturation process and prevent terminally misfolded or unassembled non-functional proteins from being deployed throughout the cell, the cell possesses a quality control system that sorts aberrant proteins for destruction (*3*). It is now clear that this degradation process does not occur in the ER, and that the 26S cytosolic proteasome is involved in the degradation of the aberrant proteins (*4*). Therefore, a retrograde protein translocation process toward the cytosol, referred to retro-translocation or dislocation, is required for the ERAD pathway. In addition, the ERAD pathway also plays a role in the regulation of native proteins involved in cholesterol synthesis and lipoprotein metabolism (*5*).

Most proteins in the secretory pathway are glycosylated, and the quality control system of the ER employs oligosaccharides as tags to mediate chaperone interactions involved in ER-retention and targeting of glycoproteins to the ERAD pathway (*6*). It has been shown that several ERAD substrates are deglycosylated when proteasomal proteolysis is blocked (*7–10*), indicating that peptide: *N*-glycanase (PNGase) in the cytosol acts on substrates prior to destruction by the proteasome. It is not clear whether the *N*-glycans play any role in the cytosol, but deglycosylation precedes, follows or occurs simultaneously with ubiquitylation. The recent finding that an E3 ubiquitin-ligase that recognizes *N*-glycan in the cytosol provides the missing links between glycosylation and ubiquitylation (*11*).

N-glycan synthesis and processing in the ER

Eukaryotic cells have an abundant and diverse repertoire of N-linked oligosaccharide structures (*12*). N-linked oligosaccharides expressed on the surface of most cells and secreted proteins contribute to their multiple biological functions (*13*). The structural diversification of *N*-glycans is introduced by a series of glycosyltransferases in the Golgi complex. In the ER, *N*-glycan represents a conserved structure in evolution from fungal to mammalian cells, so that the glycans provide a common role in assisting protein folding, quality control, and transport (*6*).

N-linked oligosaccharide synthesis starts on the cytosolic surface of the ER membrane by the addition of sugars to dolichylphosphate (dolichol-P-P) (Fig. 1). When $Man_5GlcNAc_2$ oligosaccharide is formed, the oligosaccharide is flipped to the luminal side of the membrane, probably by the protein Rtf1 (*14*). Once inside the ER, this oligosaccharide is further modified with four more mannose and three glucose residues. The preassembled oligo-

*To whom correspondence should be addressed. Tel: +81-3-3823-2105, Fax: +81-3-3823-2965, E-mail: yyosida@rinshoken.or.jp

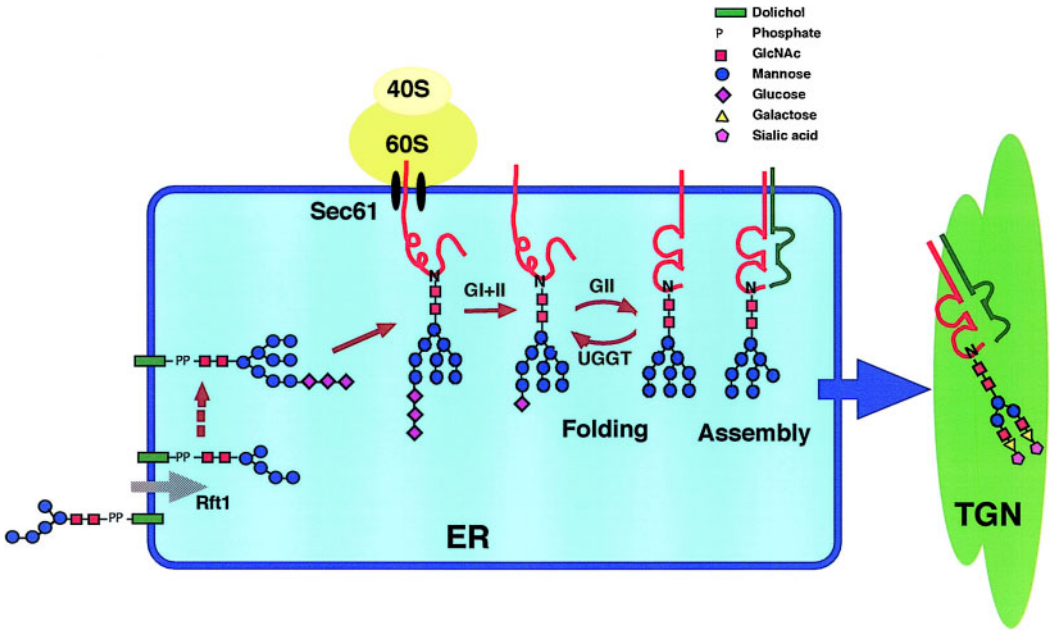

Fig. 1. **Biosynthesis of the N-linked oligosaccharide in the ER.** Synthesis starts with the sequential addition of seven monosaccharides (GlcNAc-phosphate, GlcNAc, and five mannose residues) to a dolichylmonophosphate on the cytosolic surface of the ER membrane. This lipid-linked oligosaccharide is flipped into the luminal side of the membrane, and four mannose and three glucose residues are added. Then, the core oligosaccharide is transferred to the asparagines residues of nascent growing polypeptides mediated by the oligosaccharyltransferase complex. The three glucose residues are trimmed away by glucosidase I and II (GI and II). The concerted action of GII and UGGT constitutes a cycle that deglucosylates and reglucosylates the oligosaccharide on the folding peptide. After the protein has folded and assembled in the ER, the peptide moves to the Golgi complex and *N*-glycan receive further modification.

saccharide (14-saccharide: $Glc_3Man_9GlcNAc_2$) is transferred *en bloc* from dolichol-P-P to the nascent polypeptide chain, and is coupled through an *N*-glycosidic bond to the nitorogen of the amido group in the side chain of asparagines in the target proteins in the ER. The oligosaccharyltransferase responsible for this transfer recognizes a specific sequence Asn-X-Ser/Thr in the nascent polypeptide chain slipping out the translocon. These bulky, hydrophilic and flexible modifications act as tags within the lumen of the secretory pathway to recruit chaperones and maturation factors. After coupling to the polypeptide chain, terminal three glucose residues are trimmed away by glucosidase I and II, and terminal mannose residues by one or more different ER mannosidases. The ER also contains a deglucosylation-reglucosylation cycle, which consists of glucosydase II and glucosyltransferase that reglucosylate $Man_9GlcNAc_2$ (Fig. 1). After the glycoprotein acquires the correct fold and moves to the Golgi complex, the *N*-glycans receive further processing of mannose residues and adding terminal glycosylation to produce complex glycans.

Although the *N*-glycan structures in the ER are homogeneous and simple, the number of enzymes that are needed to synthesize and trim N-linked glycans in the ER is large, and the pathway for *N*-glycan synthesis seems to be ineffective. However, it might be reasonable that this wasteful pathway produces individual intermediates that are involved in the protein quality control as a tag.

Roles of *N*-glycans in protein folding and quality control

Several co-translational and post-translational modifications occur in the ER, such as *N*-glycosylation, disulphide-bond formation, and GPI-anchor addition. These modifications are important for correct protein folding. A strict quality control in the ER is essential in order to prevent incompletely folded molecules from transporting downstream organelles in the secretory pathway. Therefore, the quality control system is a retention system in which the incompletely folded conformers and unassembled oligomers are recognized and selectively retained. ER-resident chaperones and folding enzymes participate in protein folding, oligomer assembly, and the retention. The molecular chaperones and folding sensors include BiP, calnexin, calreticulin, glucose-regulated protein (GRP) 94 thiol-disulphide oxidoreductases protein disulphide isomerase (PDI) and ERp57 (*15*). Among these, the calnexin-calreticulin cycle has been extensively examined and characterized (Fig. 2). Calnexin (transmembrane) and calreticulin (soluble) are homologous ER lectins specific for monoglucosylated high-mannose oligosaccharides (*16*). These lectins are highly asymmetric molecules with two distinctly different domains. The interaction with monovalent glycan occurs through a binding site in the globular lectin domain, which is structurally related to legume lectins (*17*). The long extended arm most likely interacts with other proteins PDI ortholog ERp57 (*18*). Association of calnexin with the unfolded glycoprotein may be mediated only by the glycan (lectin only model), with the substrate polypeptide interacting directly only with ERp57 (*18*). Alternatively,

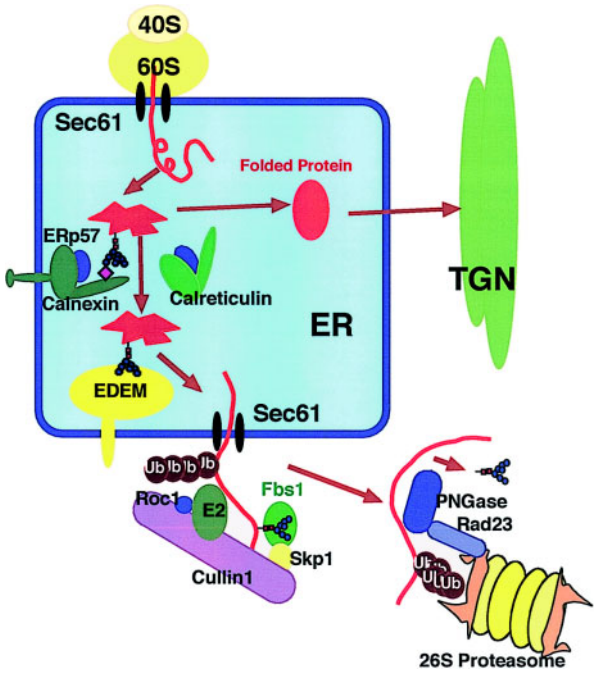

Fig. 2. **The glycoprotein quality control system mediated by *N*-glycan.** Calnexin and calreticulin, ER lectin-type chaperones specific for monoglucosylated high-mannose oligosaccharide, assist the folding of newly synthesized glycoproteins. Both chaperones associate with ERp57 through an extended arm-like domain. Correctly folded glycoproteins can exit the ER to Golgi complex. Proteins that fail to fold properly or assemble with their partners are retained in the ER and become a substrate of ER mannosidase I after a certain lag time. Such proteins are recognized by EDEM, which probably targets glycoproteins for ERAD, extracted from the ER and ubiquitiylated by the E3 such as SCF^{Fbs1}. The 26S proteasome degrades the glycoprotein after its de-glycosylation by PNGase.

binding of the glycan may be a prerequisite for interactions between the polypeptide moiety of the glycoprotein and calnexin, ERp57, or both (lectin first model) (*19*).

Two functionally opposite ER enzymes mediate the on/off-cycle in the calnexin-calreticulin system. Since glucosidase II removes the glucose from the monoglucosylated high-mannose glycans, it is responsible for dissociating the substrate glycoprotein from the lectins. On the other hand, UDP-glucose:glycoprotein glucosyltransferase (UGGT) is responsible for reglucosylation of the substrate for reassociation with the lectins. It is well known that UGGT acts on only the incompletely folded proteins and serves as the folding sensor (*20*). *In vitro* studies have shown that UGGT also preferentially reglucosylates glycoproteins in partially folded, molten globule conformations (*21*), and that an important feature for recognition is the exposure of hydrophobic clusters and innermost GlcNAc residue (*22*).

Repeated rounds of reglucosylation lead to prolonged association of the lectins with the misfolded protein until the glycoprotein either reaches its native conformation or is targeted for degradation. Therefore, glucose acts as a selective tag for incompletely folded proteins.

Roles of *N*-glycans in targeting glycoproteins to ERAD pathway

When the improperly folded or incompletely assembled proteins fail to restore their functional states, they are degraded by the ERAD system, which involves retrograde transfer of proteins from the ER to the cytosol followed by ubiquitin-mediated degradation by the proteasome. Because inhibition of α-mannosidase I by treatment with kifnensine, a specific inhibitor, or gene disruption stabilizes misfolded glycoproteins, the timer seems to be provided by the α-mannosidase I, which removes the α1,2-linked mannose residue from the α1,3-branch of high-mannose oligosaccharide, producing $Man_8GlcNAc_2$ (B isoform; Man8B) (*23*, *24*). Since the mannosidase reaction is slow, the mannose residues of only misfolded proteins that spend an excessive amount of time in the ER will be trimmed (*25*). The presence of a Man8B-binding lectin, which targets misfolded glycoproteins for degradation, has been postulated, and it has been recently demonstrated that mammalian and yeast homologs (EDEM in mammalian, and Htm1 or Mnl1 in yeast) of an inactive form of ER mannosidase I is involved in the ERAD pathway (Fig. 2) (*26–28*). EDEM overexpression accelerates degradation of the misfolded glycoprotein, and deletion of the Htm1/Mnl1 reduces the rate of degradation of ERAD substrate glycoproteins. The binding activities of the mannosidase-like proteins to Man8B remain elusive, however, as expected, EDEM has been shown to function in the ERAD pathway by accepting substrate glycoproteins from the calnexin cycle, thereby accelerates its degradation (*29*, *30*). Indeed, EDEM interacts with calnexin through their transmembrane domains (*29*). However, in some cases, mannosidase-dependent degradation of some ERAD substrate glycoproteins occurs without involvement of either calnexin or UGGT (*31*, *32*).

Quality control in the Golgi complex

A remarkable advance was provided by the recent finding that the Golgi complex can take part in the quality control system. While we might assume that the quality control occurs in the ER, several lines of evidence have suggested that cycling between the ER and Golgi complex is necessary for the degradation of several misfolded proteins. The retrieval pathway involves COPI-coated vesicles and is dependent on BiP, a KDEL protein (*33*). In the case of T-cell receptor α chain (TCRα), known as a typical ERAD substrate in mammals, disruption of the ligand-binding function of the KDEL receptor releases TCRα to the cell surface, so that TCRα is no longer subject to degradation. In yeast, mutations in the components involved in the vesicular transport to and from the Golgi complex, such as Sec12p, Sec18p, Erv29, or Bst1, slowed the degradation of proteins (*34*, *35*). Although the main ubiquitylation system involved in ERAD is the HRD/DER pathway (see below), the retrieval substrates exploit another ubiquitin ligase, Rsp5p (Fig. 3) (*36*).

Recently, a Golgi complex resident transmembrane-ubiquitin ligase, Tul1, was identified as an enzyme that recognizes polar residues in the transmembrane region of membrane cargo proteins such as carboxypeptidase S (*37*). It is possible that Tul1 contributes to the quality control, by identifying the misfolded membrane proteins and marking them for transport to endosomes.

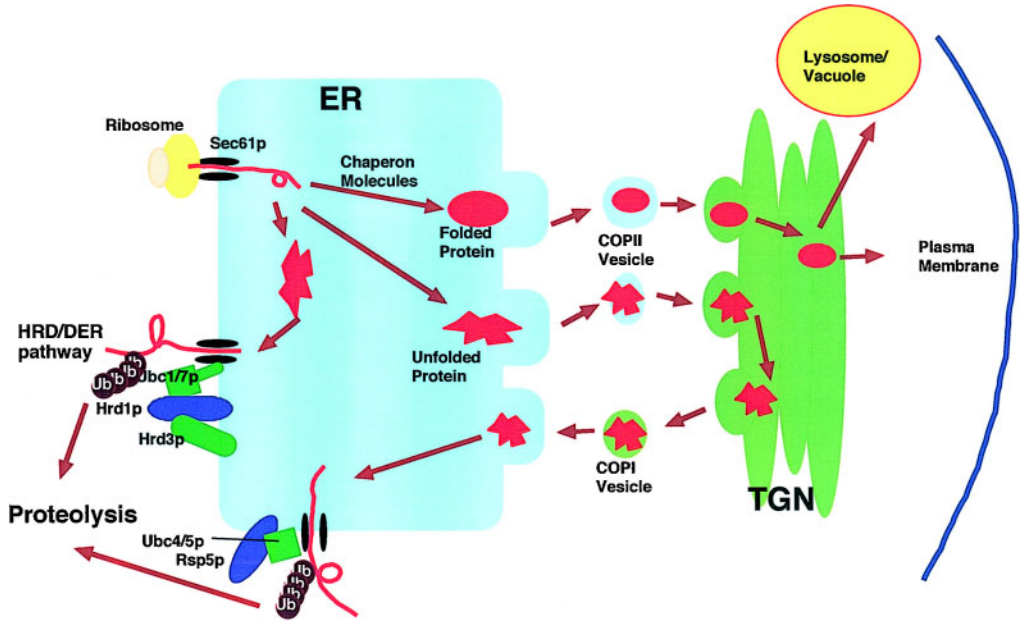

Fig. 3. **Ubiquitylation system in the ERAD in yeast.** The misfolded proteins are sorted for the retention pathway or retrieval pathway. In the retrieval pathway, proteins are packaged into COPII vesicles, transported to the *cis*-Golgi, and retrieved through the retrograde transport pathway. While the retained substrates are thought to be ubiquitylated by HRD/DRE pathway, the retrieved substrates seem to be ubiquitylated by the ubiquitin ligase Rsp5p. In latter pathway, the exact site(s) where Rsp5 ubiquitylates the substrates is unknown at present.

Ubiquitylation system in the ERAD

Most of ERAD substrates undergo polyubiquitylation, a repetitive chain of ubiquitin molecules conjugated to lysine residues in the target protein, during their export from the ER into the cytosol, and this modification might be required for not only degradation but also retro-translocation. The retro-translocation of ERAD substrates requires a pushing or pulling driving force to ensure unidirectional translocation into the cytosol. While the mechanism of retro-translocation is unclear, polyubiquitylation at the cytosolic side or the ER membrane seems to assist in the process (*38*). One possibility is that the proteasome pulls polyubiquitin chains out of the membrane. However, mutation of the 19S regulatory particle, which recognizes polyubiquitin chains, does not prevent retro-translocation of a substrate from the yeast ER. Several experiments have shown that the AAA family of ATPase, Cdc48 in yeast and p97 in mammals, is involved in extracting polypeptides from the ER membrane (*38*). Cdc48/p97 (also called VCP) interacts with Ufd1 and Npl4 that recognize polyubiquitin chains in lysine 48-linkage by a novel ubiquitin-binding domain in Ufd1. In yeast mutants of *cdc48*, *ufd1*, or *npl4*, the degradation of the ERAD substrates is retarded, and most misfolded proteins accumulate inside the ER.

Ubiquitin is covalently attached to target proteins by a cascade system consisting of E1 (ubiquitin-activating), E2 (ubiquitin-conjugating), and E3 (ubiquitin-ligating) enzymes. In the first step, which is catalysed by an E1, the COOH-terminus of ubiquitin is activated in an ATP-dependent step and covalently linked through a thioester bond to an active-site cystein in the E1. In the second step, ubiquitin is transferred from the E1 to an active-site cystein in one of the at least 13 known E2 in mammal. In the final step, which is accomplished by the action of an E3 ubiqutin-ligase, ubiquitin is covalently linked through an isopeptide bond to a lysine in the target protein or to another ubiquitin that has been linked to the target protein. In the ubiquitin pathway, E3 plays an important role in the selection of target proteins for degradation, because each distinct E3 usually binds a protein substrate with a degree of selectivity for ubiquitylation. E3s are thought to exist as molecules with a large diversity, presumably in more than hundreds species, which are classified into many subfamilies (*39*).

The components of the ubiquitylation system involved in ERAD have been identified in yeast, termed as the HRD/DER pathway or the HRD complex (Fig. 3). The HRD/DER pathway consists of Hrd1p/Der3p, Hrd3p, Der1p, and Ubc7p and Ubc1p as the E2 (*40–42*). Hrd1p/Der3p, a multi-spanning ER membrane protein, is the E3 ubiquitin-ligase whose cytosolically positioned RING-H2 domain, which binds Ubc1p or Ubc7p, mediates the specific covalent attachment of ubiquitin to the target substrate (*43*). The RING-H2 motif is characteristic of a group of ubiquitin ligase. Hrd3 stabilizes Hrd1p (*44*), whereas the function of Der1p remains unknown at present (*41*).

Other E3 ubiquitin ligases involved in the ERAD pathway have also been identified. In yeast, it has been reported that Doa10, a RING finger E3 that targets the soluble transcription factor Matα2, has an overlapping role with Hrd1p/Der3p in promoting ERAD (*45*). Doa10 functions with two E2s, Ubc6, and Ubc7. Rsp5p is a HECT type E3 ubiquitin-ligase, and functions in an HRD/DER-independent pathway withUbc4p and Ubc5p (Fig. 3). In this HRD/DER-independent degradation system, ER-Golgi trafficking and Rsp5p-dependent ubiquit-

ylation are required before the degradation by the proteasome. In mammals, gp78, CHIP, Parkin, and SCF^{Fbx2} have been characterized as the ERAD E3s. gp78, known as the tumour autocrine motility factor, is a multi-spanning glycoprotein, and was first identified as a RING-type E3 implicated in degradation of CD3-δ, a well-characterized ERAD substrate in mammals (*46*). CHIP (C-terminus of Hsc70-interaction protein) is a U-box type E3, another group of ubiquitin ligase that HECT and RING-finger families, and binds to the chaperon molecules Hsp90 or Hsc70 through tetratricopeptide repeat domains in the cytosol. It has been shown that CHIP can ubiquitylate the substrate in the presence of Hsp90 or Hsc70 and Hsp40 in addition to E1 and E2 (Ubc4 or UbcH5c) *in vitro* only after the substrate is denatured, suggesting that CHIP is a new category of E3 responsible for the quality control (*47*). Moreover, it has been reported that CHIP along with Hsc70 targets the immature CFTR, a typical ERAD substrate in mammal, for proteasomal degradation (*48*). Parkin, a gene product responsible for autosomal recessive juvenile Parkinsonism (AR-JP), is a RING-type E3 ubiquitin-ligase that targets several proteins, such as *O*-glycosylated α-synuclein, Pael receptor, CDCrel-1, Synphilin-1, CyclinE, and Tau. With regard to the Pael receptor, a putative G protein-coupled transmembrane polypeptide, the ERAD pathway mediated by Parkin may play a role in the prevention of accumulation of the unfolded Pael receptor involved in the dopaminergic neuronal death in AR-JP (*49*). Since Parkin is upregulated during UPR and binds to ER-associated UBC6 and UBC7, it is likely to be involved in ERAD. Interestingly, the insoluble Pael receptor by overexpression leads to unfolded protein-induced cell death, and overexpression of Parkin confers resistance to ER stress induced by the unfolded protein in dopaminergic neuroblastoma cells.

Hrd1/Der3 is thought to be a common ubiquitin ligase responsible for the ERAD pathway, and other ubiquitin ligases in mammal play a role in the degradation of certain substrates. Recent studies identified a human homolog of yeast Hrd1 and indicated its upregulation by ER stress via IRE1 and ATF6, ER stress transducers (*50*). In addition, the SCF^{Fbx2} ubiquitin ligase specific for proteins carrying high-mannose oligosaccharides, which are added in the ER, have been found in adult mouse brain (*11*). Since it recognizes N-linked high-mannose in cytosol, it might function against ubiquitous glycoproteins.

E3 ubiquitin-ligases that recognize sugar chains

The SCF is composed of three constant proteins, Cullin1/Cdc53, Skp1, and RING finger protein Roc1/Rbx1/Hrt1, and one member of a large family of F-box proteins. F-box proteins typically have a bipartite structure with a N-terminal ~40 amino acid F-box motif that is responsible for binding to Skp1, and a C-terminal region that interacts with the substrate, and their function is to trap target proteins (*51*, *52*). To date, at least 50 different members of this family of proteins have been identified. Accumulating evidence suggests that phosphorylation of target proteins is a prerequisite for their recognition by SCF complexes. In addition, it has been shown that proline hydroxylation of the transcription factor HIF1α serves as a signal for ubiquitylation by the SCF-like Cullin2-based VBC ubiquitin-ligase.

Our screening for proteins bound to various sugar probes led to the identification of a F-box protein, Fbs1 (F-box protein that recognizes sugar chains; equivalent to Fbx2 or NFB42 [neural F-box 42 kDa (*53*)], which is the substrate-recognition component of SCF-type ubiquitin-ligases, from mouse brain extract. The reconstituted system using recombinant proteins produced by baculovirus had the ability to ubiquitylate the GlcNAc-terminated fetuin (*11*). Fbs1 is a novel example of F-box proteins that has evolved to recognize sugar chains in N-linked glycoproteins. Fbs1 could interact with glycoproteins containing high-mannose oligosaccharides through its substrate-binding domain *in vivo*. Because the protein modification by high-mannose oligosaccharides occurs in the ER, it seems likely that SCF^{Fbs1} is important in the ER quality control and functions to ubiquitylate ERAD substrates (Fig. 2). Indeed, one of the identified Fbs1-substrate proteins was pre-integrin β1, which was modified with high-mannose oligosaccharides, and their interactions occurred in the cytosol. It is known that the pre-integrin β1 chain is present in the ER and is subjected to the ER protein quality control (*54*). Integrin β1 is a type-1 membrane glycoprotein with small cytoplasmic and large extracellular domains. The β1 subunit can dimerise with at least twelve α subunits; most cells express several αβ1 integrins, and β1-chains are expressed in excess over α-chains in different types of cells (*55*, *56*). It has been shown that excess non-complexed β1 is either immediately degraded in a compartment prior to the Golgi or that some of it remains in the ER by the calnexin ready to associate with freshly synthesized α-chains (*54*, *56*). The physical association of Fbs1 with pre-integrin β1 in the cytosol was shown only in the presence of the proteasome inhibitor, suggesting that SCF^{Fbs1} is responsible for the ubiquitylation of N-linked glycoproteins including β1-chains through the ERAD pathway. Supporting this contention, overexpression of Fbs1ΔF, a dominant-negative form of Fbs1 lacking the F-box domain essential for the formation of the SCF complex, appreciably blocked the degradation of typical substrates, such as the mutant form of the cystic fibrosis transmembrane conductance regulator (CFTR) lacking phenylalanine 508 (*10*) and the TCRα (*57*).

Pull-down analysis using various oligosaccharides revealed that $Man_{3\text{-}9}GlcNAc_2$ glycans were required for efficient Fbs1 binding while the deletion of inner GlcNAc reduced the Fbs1 binding, suggesting that it recognize especially the inner chitobiose structure in *N*-glycan (*11*). In many native glycoproteins, the internal diacetylchitobiose is not accessible to macromolecules such as peptide *N*-glycanase (PNGase), and cleavage of oligosaccharides requires denaturation of the glycoproteins *in vitro*. Therefore, the inner chitobiose residues of the target glycoproteins for Fbs1 may be exposed to the outside of molecules upon protein denaturation. It has been suggested that UGGT, which serves as a sensor for misfolded proteins in the ER, recognizes both the innermost GlcNAc unit of the oligosaccharide and protein domains with hydrophobic patches exposed in the substrates (*20*). Thus, it is possible that Fbs1 recognizes the GlcNAc unit in a manner similar to PNGase or UGGT, in order to dis-

criminate between non-native and folded glycoproteins. However, the molecular mechanisms of the recognition of *N*-glycan by PNGase and UGGT, are not clear at present. Most recently, our coordinated X-ray crystallographic and NMR studies of the substrates-binding domain of Fbs1 with diacetylchitobiose provided the structural basis for the substrate recognition by SCF^{Fbs1}, in that Fbs1 recognizes the inner chitobiose of N-linked high-mannose oligosaccharides by a small hydrophobic pocket located at the top of the β-barrel (unpublished data). This mechanism is different from that of sugar-recognition by most lectins that uptake non-reducing terminal sugar in the concave surface, which consists of several strands of β-sheets.

Since Fbs1 is expressed mainly in neuronal cells in the adult brain (*53*), it is likely that the SCF^{Fbs1}-mediated ERAD pathway specifically contributes to the rapid elimination of glycoproteins present in neurons. There is ample evidence at present to suggest that intracellular accumulation of aggregated proteins is linked to many conditions including aging-related neurodegeneration (*58*). Aggresomes are frequently found around the centrosomes in cells when the amount of misfolded proteins increases under stress conditions or when heterooligomeric proteins are not successfully assembled in the ER. Although the precise molecular mechanism involved in the formation of aggresomes in cells remains elusive, it has been postulated that when the retrograde transport rates are greater than those of degradation, the accumulated proteins of abnormal structures aggregate to form the inclusion bodies. Aggresome formation seems to be an *in vitro* model system for analysing the process of inclusion body formation found in neuronal diseases. Interestingly, the forced expression of Fbs1 interfered with the formation of aggresomes, which are intracellular inclusion bodies that contain misfolded CFTR. Thus, we hypothesize that SCF^{Fbs1}-mediated ubiquitylation plays a role in inhibiting neuronal inclusion body formation, thereby preventing neurodegenerative diseases.

Another F-box protein Fbs2 that recognizes high-mannose oligosaccharides was recently identified (unpublished data). Fbs2 is widely expressed in various tissues in contrast to the limited expression of Fbs1 in the brain. In addition, the affinity for oligosaccharides and the preference for substrates are different between Fbs1 and Fbs2, suggesting that these Fbs proteins have distinct substrates or roles *in vivo*.

In the ERAD pathway, *N*-glycans seem to be removed from the substrate glycoproteins, a process mediated by the cytosolic PNGase, prior to destruction by the proteasome (*7–10*). The gene encoding PNGase (Png1p) has been identified in the yeast and shown to bind to the 26S proteasome through its interaction with a component of the DNA repair system, Rad23p (*59*). The formation of a Rad23p-Png1p complex was found to be distinct form the Rad23p-Rad4p complex required for DNA repair, and both complexes were found to involve binding to the C-terminal domain of Rad23p. Orthologues of yeast Png1p were found widely distributed in higher eukaryotes. The mouse PNGase, which has an extended N-terminal domain not found in yeast, was found to bind not only to the Rad23 but also various proteins related to the ubiquitin/proteasome pathway through PUB/PUG domain in the extended N-terminal domain (*60*). Taken together, *N*-glycan serves as a simultaneous signal for ubiquitin-attachment and recruitment for the proteasome.

All eukaryotic cells possess the *N*-glycosylation system and the ER quality control system including the ERAD. Indeed, some *N*-glycan recognition molecules, such as calnexin, EDEM/Htm1/Mnl1, and cytosolic PNGase, are conserved from yeast to mammals. However, in yeast, the UGGT pathway and Fbs-type ubiquitin ligases have not been found, and they might be created for evolutionary development of the ER quality control system. Unlike Fbs2, Fbs1 is especially expressed in adult neuronal cells, and the development of quality control system seems to be a strategy for maintenance neuronal cells that are able to proliferate.

Conclusions and perspectives

The biosynthesis pathway of *N*-glycans is a costly system with respect to the number of enzymes that are involved in the synthesis and trimming of *N*-glycans. While the structural diversity of *N*-glycans in mature proteins in the cell surface is introduced by glycosyltransferases in the Golgi complex, the core glycans in the ER are universal from yeast to mammal and have been considered as intermediates. However, further recognition of intracellular lectin molecules indicates that the *N*-glycans of glycoproteins function as universal tags for the protein quality control system, i.e., folding and degradation of aberrant glycoproteins. Most proteins in the ER possess N-linked high-mannose oligosaccharides, and thus the mechanisms of distinction of folding state arbitrated by *N*-glycans are not clear at present. Analysis of the molecular mechanism of the recognition of *N*-glycan by intracellular lectin molecules and screening for novel sugar recognition molecules such as lectin and enzymes should enhance our understanding of the ER quality control system.

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