Update

Research Focus

# Sweet bays of ERAD

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**Proteins that improperly mature in the endoplasmic reticulum (ER) are dislocated to the cytoplasm for proteasome-mediated destruction. A recent study provides insight into the incompletely understood processes for selection and targeting of aberrant proteins for ER-associated protein degradation. The identification of the ER chaperones GRP94 and BiP as binding partners for the mannose-binding proteins OS-9 and XTP3-B, indicates that these protein complexes bind to aberrant proteins and direct them to the Hrd1 dislocation and ubiquitylation complex in the ER membrane.**

## ERAD substrate selection and delivery

Protein maturation in the cell can be an inefficient process. For secretory cargo that matures in the endoplasmic reticulum (ER), a quality control process selects terminally misfolded substrates for degradation by cytosolic proteasomes through the ER-associated protein degradation (ERAD) pathway. This process requires dislocation of the defective protein to the cytoplasm for ubiquitylation and subsequent proteasomal degradation. The degradation of aberrant secretory cargo prevents potential blocks within the secretory pathway, creates antigens and provides a mechanism for destroying toxic substrates. Asparagine-linked glycans (N-linked glycans) have a central role in this selection and targeting process [1,2]. A recent study provides important insight into the nature of the ER protein machinery involved in the selection and delivery of aberrant secretory cargo to ER-membrane dislocation sites [3]. The abundant ER chaperones, GRP94 (94-kDa glucose-regulated protein, an ER Hsp90 family member) and BiP (immunoglobulin heavy chain-binding protein, an ER Hsp70 family member), were found in a complex with the carbohydrate-binding quality control receptors Osteosarcoma 9 (OS-9) and XTP3-transactivated gene B precursor (XTP3-B, also known as Erlectin). This finding indicates a role for these protein complexes in the ERAD selection and targeting processes. Interestingly, the OS-9 and XTP3-B carbohydrate-binding properties do not seem to be involved in cargo selection as previously proposed; rather, they are probably used to deliver these complexes to the ER membrane to promote the dislocation and subsequent cytoplasmic proteasomal degradation of the defective cargo.

## The role of N-linked glycans in quality control

N-linked glycans are added co-translationally and co-translocationally, as the nascent chain emerges into the ER lumen. These modifications function as dynamic maturation and quality control tags that help to direct secretory pathway traffic [2]. The glucose-containing branch of the carbohydrate dictates interactions with the carbohydrate-binding (lectin) molecular chaperones, calnexin and calreticulin. These interactions retain non-native substrates in an environment optimized for folding, thereby protecting them from destruction or premature anterograde exit to the Golgi. Glycoproteins are initially inspected by the UDP-glucose:glycoprotein glucosyltransferase, which re-glucosylates misfolded or unassembled proteins to support their re-entry into the calnexin-binding cycle [4]. Properly folded and assembled proteins can advance through the secretory pathway following the trimming of a few terminal mannose residues by ER mannosidase I. Mannose trimming also has an important role in sorting aberrant cargo to the ERAD pathway. Several newly identified ER-resident mannose-trimming or mannose-binding proteins seem to help select or target defective cargo for destruction by the ERAD pathway [5,6].

Pharmacological inhibition of mannose trimming was first shown to stabilize defective secretory cargo 15 years ago [7]. More recent studies using both pharmacological and genetic inhibition of mannose trimming demonstrate that mannose trimming participates in the dislocation of non-native proteins to the cytoplasm for proteasomal destruction [8–10]. These observations led to the hypothesis that slow trimming of mannose residues on aberrant glycoproteins by ER mannosidases helps to promote their extraction from the calnexin-binding cycle and marks them for destruction by the ERAD pathway [11]. The proposed use of mannose trimming as a degradation signal seems to require at least three components: (i) trimmed mannose-containing glycans that serve as the degradation signal; (ii) mannosidases that create this signal on defective proteins; and (iii) mannose-binding proteins that recognize the signal and sort the defective protein for dislocation from the ER for ubiquitylation and degradation by the proteasome in the cytoplasm.

The identity of the carbohydrate ERAD signal remains controversial. Initially, it was proposed that an N-linked glycan structure containing eight mannose residues (Man8) served as the degradation signal in yeast [8]. Additional studies uncovered a role for more extensive trimming to Man5–6 as the degradation signal in mammalian cells [12]. The identity of the glycosidases involved in de-mannosylation remains unknown. The ER contains several members of the glycosylhydrolase 47 family, including ER mannosidase I and the ER-degradation enhancing α-mannosidase-like (EDEM) family (EDEM1–3). These proteins seem to have crucial roles in the preparation or selection of aberrant cargo for destruction because their overexpression accelerates the degradation

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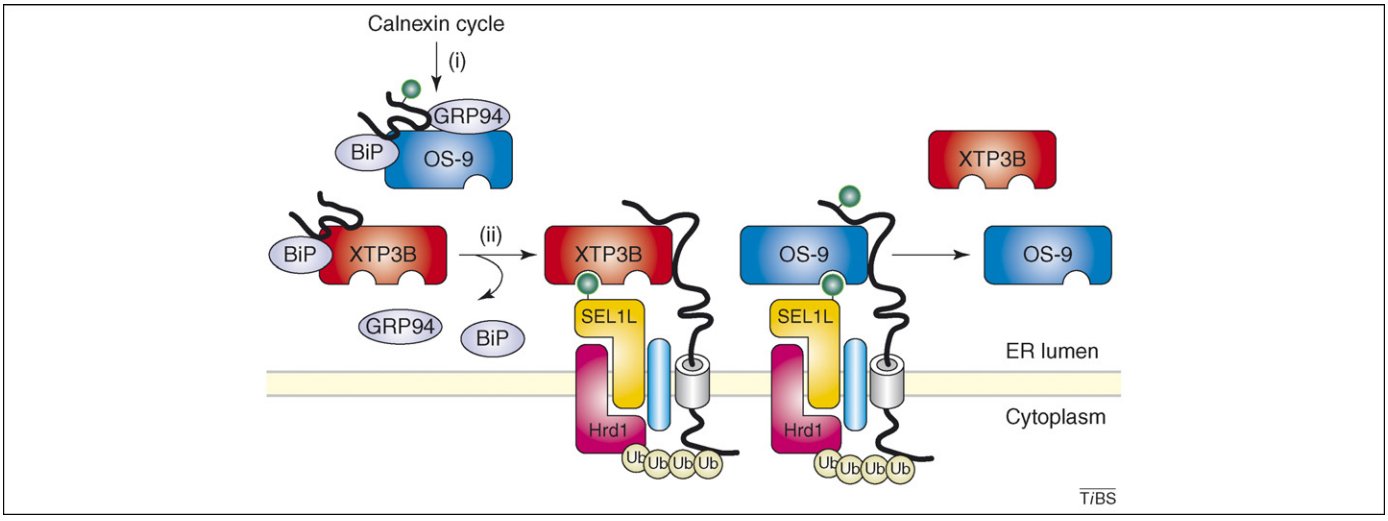


**Figure 1**. OS-9 and XTP3-B select and target ERAD substrates. Terminally misfolded proteins (thick black line) that fail quality control inspection in the ER have mannoses trimmed from their N-linked glycans (Man5–8; green sphere). This process decreases calnexin or calreticulin binding, thereby enabling the transfer of the misfolded protein to OS-9 (blue)–BiP (pale purple)–GRP94 (pale purple) or XTP3-B (red)–BiP complexes (i). The non-native protein is then delivered to the Hrd1 complex (pink) in the ER membrane (ii) for dislocation through a channel (gray) to the cytoplasm for ubiquitylation (Ub) and subsequent proteasomal degradation. The open half circles on OS-9 and XTP3-B represent MRH domains that are proposed to mediate binding of SEL1L (yellow) glycans (green).

of glycosylated ERAD substrates; however, knowledge of their precise role in the ERAD pathway is incomplete [13–16].

## OS-9 and XTP3-B show them the way

Studies in yeast have provided valuable insight into the identification of the machinery involved in targeting aberrant cargo for dislocation and destruction. Yos9p (the yeast OS-9 orthologue) has been implicated as a key factor in the degradation of misfolded glycoproteins in *Saccharomyces cerevisiae* [17–23]. Yos9p was first identified through a genomic library screen that searched for factors involved in ERAD substrate clearance [17]. A soluble ER-resident protein, it possesses a C-terminal His-Asp-Glu-Leu ER-retention sequence and a single mannose-6 phosphate receptor homology (MRH) domain, which is indicative of its role in mannose recognition. Yos9p preferentially associates with the model glycosylated yeast ERAD substrate CPY* (a mutant form of carboxypeptidase Y), which contains both Man8 and Man5 structures (Man5–8) [20]. CPY* degradation is delayed by an amino acid substitution in the MRH domain that disrupts carbohydrate binding [19]. Yos9p assists in the degradation of glycoproteins that possess lumenal- or membrane-exposed defects, but not cytosolic lesions [18]. Interestingly, Yos9p interacts with the Hrd1 putative dislocation and ubiquitylation complex [21–23], which includes Hrd1p (HMG-CoA reductase degradation protein 1, an ER membrane-associated E3 ubiquitin ligase), Hrd3p (a membrane integrated adaptor), BiP and Der1p (degradation in the ER protein 1, a putative protein dislocation channel). These results indicate that Yos9p links the recognition of defective proteins in the lumen with their delivery to the ER membrane for dislocation into the cytoplasm.

A recent study from Kopito and colleagues has extended this understanding to mammalian cells, and provides additional insight into the mechanisms for the selection and targeting of defective proteins for ERAD [3]. OS-9 and XTP3-B are soluble Yos9p orthologues that contain N-terminal signal sequences and one or two MRH domains, respectively. Initial experiments established that both proteins are *bona fide* lumenal ER-resident proteins. OS-9 and XTP3-B lack C-terminal ER-retention motifs, indicating that they are retained in the ER through interactions with other ER factors. Affinity pull-down assays demonstrated that OS-9 and XTP3-B both associate with SEL1L (suppressor of lin-12-like protein, the mammalian Hrd3p orthologue), a multiply glycosylated type-I membrane protein that is a component of the mammalian Hrd1 complex (Figure 1). SEL1L depletion by shRNA knockdown inhibited the association of OS-9 and XTP3-B with the Hrd1 complex, indicating that SEL1L could serve as a membrane adaptor that links OS-9 and XTP3-B to the membrane components of the Hrd1 complex.

An unbiased mass-spectrometry search for proteins that interact with S-tagged versions of OS-9 and XTP3-B identified an efficient association between BiP and both MRH-containing proteins [3]. Surprisingly, GRP94 was the most prominent OS-9-interacting protein identified. This interaction persisted in the absence of SEL1L or Hrd1, indicating that GRP94 might interact directly with OS-9 and also that this interaction is positioned upstream of its interaction with the Hrd1 complex. Although GRP94 is one of the most abundant ER-resident proteins, a clear understanding of its function has remained elusive. Hsp90 family members work in concert with distinct groups of co-chaperones to provide a large range of functions throughout the cell. These functions include the post-translational targeting of substrates for translocation and the folding of signal-transduction proteins [24]. This new finding establishes a previously unidentified role for GRP94 in protein quality control and degradation. Indeed, shRNA-mediated GRP94 knockdown resulted in the stabilization of the well-characterized soluble glycosylated ERAD substrate *null* Hong Kong mutant of α1-antitrypsin (NHK A1AT) [3].

The OS-9 and XTP3-B MRH domains were expected to participate in the recognition of mannose-trimmed glycans

on defective substrates. However, amino acid substitutions within the MRH domain that should impair the OS-9 and XTP3-B sugar-binding pockets disrupted SEL1L, but not NHK A1AT, binding [3]. Thus, the MRH domains are not required for OS-9 and XTP3-B to interact with defective substrates; rather, they seem to be crucial for associations with their downstream target, SEL1L. The dispensability of the MRH domains for substrate binding was further demonstrated by the ability of XTP3-B to bind to mutant, but not wild-type, non-glycosylated transthyretin. These results are supported by earlier studies in yeast that found that an amino acid substitution within the Yos9p MRH domain increased CPY* binding, but disrupted its rapid turnover [19]. Together, these results indicate that mannose binding can be used to target quality control sorting receptors to the ER membrane ubiquitin ligase–translocon complex rather than simply for substrate recognition (Figure 1). However, this conclusion is in conflict with additional studies from yeast that demonstrate that the Yos9p MRH domain is not required for its association with the Hrd3p ER membrane complex [21,23].

## Concluding remarks and future perspectives

These recent findings by Christianson *et al.* [3] establish a new role for GRP94 in protein quality control and challenge the current thinking about how mannose trimming directs the trafficking of defective proteins to the ERAD pathway. However, many questions remain unanswered. Is substrate selection determined by the OS-9- and XTP3-B-associated chaperones BiP and GRP94, or can OS-9 and XTP3-B recognize non-native substrates directly? Does OS-9 associate with both GRP94 and BiP simultaneously? Why does XTP3-B have two MRH domains? How are these complexes targeted to the membrane for the delivery and passage of aberrant substrates to the ubiquitin ligase complex? Future studies will be required to understand the complete mechanism of ERAD-substrate selection and targeting.

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