Seminars in Cell & Developmental Biology 21 (2010) 526–532

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

ERAD substrates: Which way out?

Daniel N. Hebert[a,**], Riccardo Bernasconi[b], Maurizio Molinari[b,c,*]

[a] Department of Biochemistry and Molecular Biology, Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003, USA
[b] Institute for Research in Biomedicine, Protein Folding and Quality Control, CH-6500 Bellinzona, Switzerland
[c] Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, CH-1015 Lausanne, Switzerland

ARTICLE INFO

Article history:
Available online 22 December 2009

Keywords:
Endoplasmic reticulum
Protein folding
Protein quality control
ER-associated degradation
Molecular chaperones
Folding enzymes
De-mannosylation
E3 ubiquitin ligases
Dislocons

ABSTRACT

Global folding of polypeptides entering the endoplasmic reticulum (ER) starts as soon as they emerge from the narrow Sec61 translocon. Attainment of the native structure can take from several minutes to hours, depending on the gene product. Until then, non-native folding intermediates must be protected from molecular chaperones that recognize misfolded determinants and could prematurely interrupt folding programs by re-directing them to disposal pathways. On the other hand, futile folding attempts must actively be stopped to prevent intraluminal accumulation of defective cargo. This review describes recent advances in understanding how terminally misfolded polypeptides are extracted from the folding environment and directed to specific dislocons within the ER membrane for transfer to the cytoplasm for proteasome-mediated degradation.

Contents

1. Folding intermediates *versus* terminally misfolded polypeptides, a matter of time

Nascent polypeptide chains co-translationally enter the mammalian endoplasmic reticulum (ER) through the Sec61αβγ complex [1]. The translocation channel formed by the α-subunit can accommodate α-helical structures but probably not larger structures. As a consequence, even the small, 17-kDa Semliki Forest virus capsid protein (Cp) cannot fold in the translocation

* Corresponding author at: Institute for Research in Biomedicine, Protein Folding and Quality Control, CH-6500 Bellinzona, Switzerland. Tel.: +41 91 8200319.
** Corresponding author.
E-mail addresses: dhebert@biochem.umass.edu (D.N. Hebert), maurizio.molinari@irb.unisi.ch (M. Molinari).

1084-9521/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2009.12.007

pore. Rather, its native structure is attained only after exiting the translocon [2], where crowded conditions within the lumen appear unfavorable for protein maturation (~300 mg/mL of protein). This is not a problem for Cp, which is an unconventionally fast and efficient folder that spontaneously reaches its native structure in ~50 ms [3]. However, for the vast majority of gene products expressed in mammalian cells, conformational maturation is much slower and non-native, aggregation-prone conformers can remain in the crowded luminal environment for several minutes to hours as is the case for complex proteins such as the coagulation factors V and VIII [4]. During this lapse of time, immature polypeptides are vulnerable since they expose misfolded determinants that will eventually become buried in the core of the native molecule later in the maturation process. Maintenance of ER homeostasis requires preservation of non-native intermediates of ongoing folding programs until complete maturation, and efficient clearance of terminally misfolded polypeptides. This must be fine tuned to avoid premature interruption of protein folding, or unwanted accumulation of misfolded polypeptides. In light of these requirements, some of the questions of interest are: What are the mechanisms operating in the ER lumen that help sort misfolded polypeptides for destruction from folding intermediates to be protected? Is this process based on time spent in unproductive folding attempts? In this review, we will discuss how progressive processing of oligosaccharides displayed on newly synthesized polypeptides allows retention in the folding environment of on-pathway intermediates to complete maturation while also acting as a molecular timer to eventually interrupt futile folding attempts and re-direct off-pathway intermediates to the ER-associated degradation (ERAD). Current models describing the dislocation of terminally misfolded polypeptides across the mammalian ER membrane for disposal by cytosolic proteasomes will also be presented.

2. Regulation of protein folding attempts

2.1. Prolongation of folding attempts and release of native polypeptides from the calnexin cycle

The best characterized protein quality control system operating in Eukarya is based on the "glycan-code" of the ER [5]. Most of the polypeptides entering the ER lumen are covalently modified with pre-assembled glucose$_3$-mannose$_9$-N-acetylglucosamine$_2$-oligosaccharides added onto asparagine side chains in Asn-Xxx-Ser/Thr sequons (Fig. 1). Rapid removal of the two outermost glucose residues by sequential intervention of glucosidases I and II, generates a mono-glucosylated intermediate. Mono-glucosylated N-linked glycans recruit the ER lectins calnexin and/or calreticulin and their associated oxidoreductase ERp57. ERp57 can catalyze the formation of disulfide bonds, a rate limiting reaction of polypeptide maturation, by acting as an oxidizing agent. Removal of the innermost glucose residue by the glucosidase II prevents re-association with the lectin chaperones for those polypeptides that collapse into the native conformation upon release from calnexin/calreticulin. These native polypeptides rapidly exit the ER for transport to their final destinations.

A fraction of the polypeptides released from the lectin chaperones and de-glucosylated by the glucosidase II collapses into non-native structures. The quality control machinery decides whether these misfolded molecules should be given another chance to attain their native structure, or if they should be extracted from the folding environment and degraded (see Section 2.2). A polypeptide that has been subjected to a single round of association with calnexin/calreticulin is likely to expose a high-mannose oligosaccharide tree comprising 9 mannose residues. The folding sensor UDP-glucose:glycoprotein glucosyl transferase (UGT1)

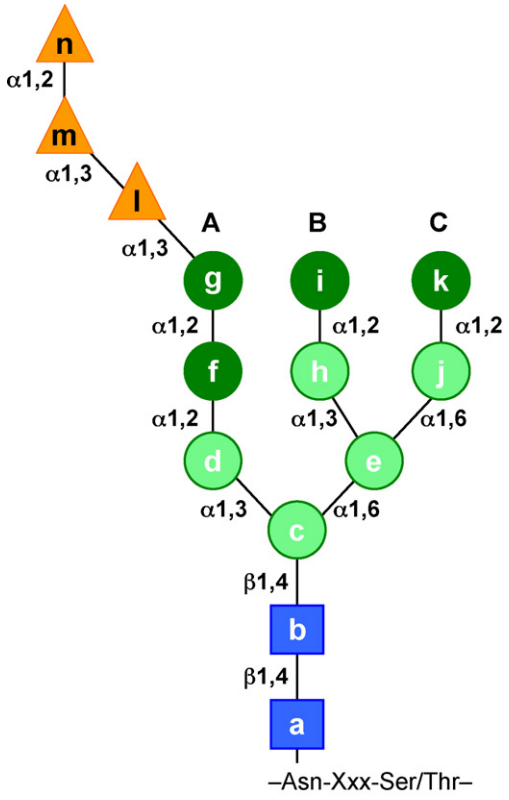

Fig. 1. Structure of an N-linked glycan. The asparagine-linked core oligosaccharide is composed of two N-acetylglucosamine (blue squares), nine mannose (green circles, dark green for the cleavable α1,2-bonded mannose residues) and three glucose residues (orange triangles). Letters a–n are assigned to each residue and the type of linkage is designated between the saccharides. A–C define the tree antenna of the protein-bound oligosaccharide.

readily re-glucosylates the terminal α1,2-bonded mannose on branch A (Fig. 1) of high-mannose oligosaccharides displayed on non-native polypeptides through its recognition of exposed hydrophobic regions [6–9]. This supports substrate re-association with calnexin/calreticulin [9,10]. Lectin chaperone binding can direct the maturation pathway by slowing the folding process, inhibiting oligomerization and aggregation, and retaining non-native substrates in the ER [11–14]. Furthermore, rebinding to calnexin and calreticulin can promote the reshuffling of non-native disulfide bonds, which has been reported to be the major *in vivo* activity of ERp57 [15]. One or more cycles of release/re-association might be required to eventually attain the unique native architecture of the mature polypeptide chain for proper secretion [16,17].

2.2. Extraction of terminally misfolded polypeptides from the calnexin cycle

2.2.1. Consequences of extensive de-mannosylation of terminally misfolded polypeptides

Mechanisms are in place to avoid the risk that folding-defective polypeptides are unduly retained by the folding machinery. Both the structure of the polypeptide chain and the composition of the N-linked oligosaccharides play a crucial role in the interruption of futile folding attempts. First, the UGT1, whose action is required to retain non-native polypeptides in the calnexin chaperone system, specifically recognizes near native or pseudo native conformers [8,18] and is proposed to ignore extensively misfolded polypeptides. Second, persistent failure to attain a native structure exposes N-glycans to ER-resident exo-mannosidases that progressively remove terminal α1,2-bonded mannose residues (dark green

in Fig. 1) thereby reducing the reglucosylation capacity of UGT1 (100% for Man_9 oligosaccharides, 50% for Man_8, and 15% for Man_7 [19]). Removal of the terminal A-branch mannose residue (mannose g in Fig. 1), which is the acceptor for UGT1-reglucosylation eventually results in the irreversible extraction of the terminally misfolded polypeptide from the calnexin binding cycle.

The trimming of A-branch mannose residues appears to be crucial for protein disposal from the mammalian ER. In fact, pharmacologic inhibition of α1,2-mannosidases not only prevents glycoprotein degradation in wild type cells but also in cell lines that transfer truncated oligosaccharides (e.g. $Glc_3Man_5GlcNAc_2$ (B3F7 cells) or $Man_5GlcNAc_2$ (MadIA214 cells)). In these cells, the only cleavable α1,2-bonded mannose residues are those on the A-branch, whose removal is thus required to elicit disposal [20–22]. In keeping with the important role of substrate de-mannosylation in preventing prolonged retention of folding-defective polypeptides in the calnexin chaperone system, inactivation of α1,2 mannosidases or deletion of EDEM1 substantially delays release of ERAD substrates from calnexin [23,24]. Mannose trimming has two additional consequences: it might generate an oligosaccharide, which serves as a ligand for the ERAD lectins OS-9 and XTP3-B (see below) and it might facilitate dislocation across the ER membrane by decreasing the size of the bulky, highly hydrophilic oligosaccharides displayed on terminally misfolded polypeptides.

2.2.2. Members of the glycosyl hydrolase family 47 as ERAD regulators

In the budding yeast, substrate de-mannosylation is operated by two distinct α1,2 mannosidases, namely Mns1p (an ortholog of the mammalian ER mannosidase I, ERManI) and Htm1p (an ortholog of EDEM) [25]. A degradation code is generated that appears to consist of an exposed terminal α1,6-bonded mannose residue, which supports the recruitment of ERAD sorting lectins [26].

For mammalian cells, it remains unclear whether extensive de-mannosylation is mediated by one or by more members of the glycosyl hydrolase family 47 (GH47) of α1,2 mannosidases. This family comprises the ERManI [27], EDEM family members (EDEM1,−2 and −3 [28]) and a collection of Golgi-resident enzymes [29]. It has been claimed that in Mammalia, the ERManI might be sufficient to extensively de-mannosylate folding defective polypeptides (reviewed in [30,31]). However, *in vitro* experiments showed that this enzyme efficiently removes the *i* mannose from Man_9 oligosaccharides (Fig. 1) to generate Man_{8B}, but it fails to further process the oligosaccharide to any significant extent even after 24 h of incubation at non-physiologic concentrations of enzyme [32]. Moreover, ERManI overexpression in mammalian cells results in accumulation of Man_8 species [33]. These findings suggest that like yeast, the extensive de-mannosylation of ERAD substrates observed in mammalian cells requires intervention by other members of the GH47 family. One possibility is that EDEM family members contribute to substrate de-mannosylation. Initially, EDEM proteins were described as mannosidase-like lectins rather than active mannosidases since they lack two cysteine residues thought to be conserved in active mannosidases [34–36]. Moreover, it had been shown that deletion of Mns1p was sufficient to abolish substrate de-mannosylation in *Saccharomyces cerevisiae*, thus identifying Mns1p as the sole active mannosidase in the budding yeast and implying that Htm1p, the yeast ortholog of EDEM, had no mannosidase activity [35]. Both these observations leading to conclude that mammalian EDEM proteins must be enzymatically inactive lectins appear to be incorrect. First, it appears that the two cysteine residues lacking in EDEM proteins are actually not conserved amongst α1,2-mannosidases and that their presence is dispensable for glycanase activity [37]. Second, it was shown that removal of mannose *i* by Mns1p is a pre-requisite for further oligosaccharide processing by Htm1p in *S. cerevisiae*. Thus, lack of protein de-mannosylation upon Mns1p inactivation was not due to the absence of other α1,2-mannosidases in yeast, but to the failure to generate the sugar structure that serves as a substrate for other ER-resident α1,2-mannosidases, namely Htm1p [25]. Hence, at least in the budding yeast, two distinct α1,2-mannosidases appear to co-operate in the extensive de-mannosylation of misfolded polypeptides that is required for their re-routing to the ERAD pathway [25].

Attempts to monitor the glycanase activity of mammalian EDEM proteins *in vitro* have failed thus far. However, EDEM proteins conserve the sequence and the 3D structure of the catalytic site of ERManI and also conserve all the residues required to bind kifunensine, a specific inhibitor of α1,2-mannosidases [29,38]. It is possible that EDEM proteins facilitate ERAD by acting as mannose-binding lectins that bind misfolded proteins or components of dislocation complexes (see Section 3). However, data showing that overexpression of EDEM1 [20,39] or EDEM3 [33] enhances de-mannosylation of folding-defective polypeptides in mammalian cells unless conserved residues of the catalytic site are mutated are consistent with a direct role of EDEM proteins acting as α1,2-mannosidases that generate the glycan code for ERAD (discussed in [30]).

3. Re-routing of folding-defective polypeptides to ER membrane dislocons

The exposure of oligosaccharides lacking terminal α1,2-bonded mannoses distinguishes non-native polypeptides to be re-routed into the ERAD pathways from newly synthesized polypeptides to be retained in the folding machinery for completion of the folding program. In mammalian cells, removal of mannose *i* from the oligosaccharide B branch might be a pre-requisite for further mannose processing assuming that the mechanisms operating in *S. cerevisiae* [25,26] are conserved in higher Eukarya. Removal of mannose *g* from the oligosaccharide branch A (Fig. 1) might only be critical for those organisms equipped with the calnexin chaperone system because it prevents UGT1-operated substrate re-glucosylation thus irreversibly extracting non-native polypeptides from the calnexin cycle. Finally, removal of mannose *k* exposes a terminal α1,6-bonded mannose residue to generate a carbohydrate signal that is decoded by a series of ERAD lectins containing mannose 6-phosphate receptor homology domains (MRH), namely the OS-9 and the XTP3-B splice variants [40]. Interestingly, both of these proteins can also specifically bind misfolded non-glycosylated structures [40–44] or glycosylated substrates when their MRH domain is mutated [42,44] suggesting that they also possess the ability to directly recognize aberrant proteins. Moreover, it has recently been shown that their intervention is crucial for disposal of soluble, misfolded polypeptides, but it is dispensable for disposal of the same polypeptides when anchored at the ER membrane [44a].

OS-9 and XTP3-B are inducible genes whose expression is regulated by the ER-stress activated transcription factor Xbp1 [42]. Their splice variants play a role in retention-based ER quality control and ERAD [40,42–45]. Current models propose that OS-9 and XTP3-B shuttle misfolded polypeptides to retrotranslocation (dislocation) complexes in the ER membrane containing an E3 ubiquitin ligase (HRD1), a membrane adapter protein (SEL1L) and an elusive membrane-embedded pore that serves as the conduit for the dislocation of misfolded proteins to the cytoplasm (discussed in Section 4, Fig. 2 and [42–45]). OS-9 and XTP3-B deliver misfolded polypeptides to the adapter protein SEL1L, which helps in the recruitment of lectin receptors to the ERAD membrane complex [42–45].

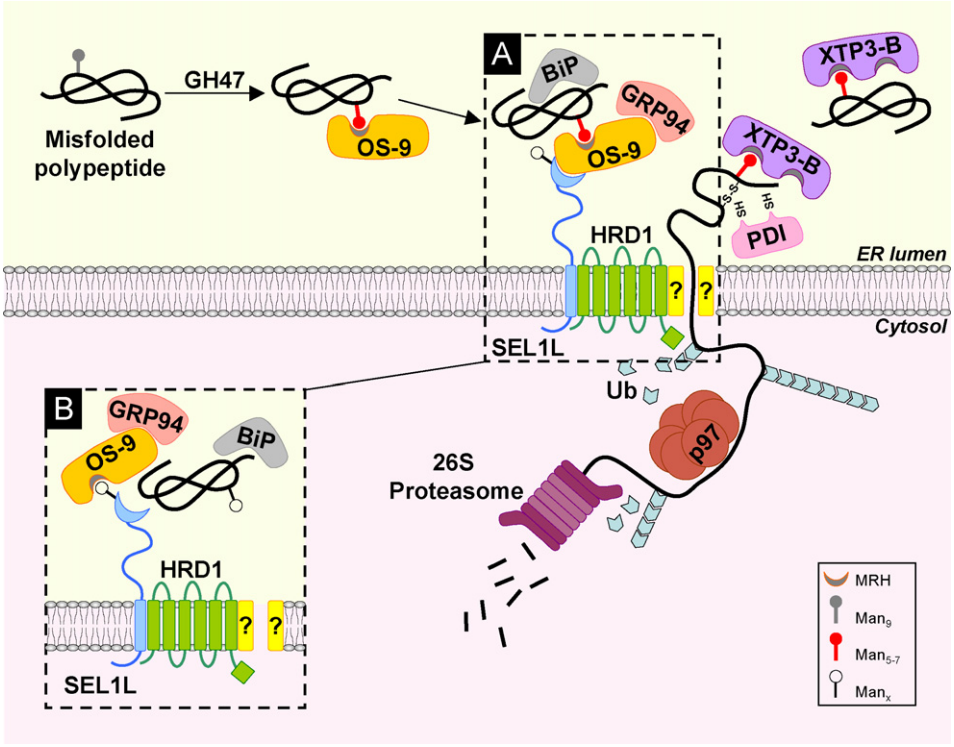

Fig. 2. Models for ERAD substrate targeting to ER membrane dislocons. (A) *Oligosaccharides act as signals for ERAD*. Polypeptides that cannot reach their native structures are subjected to extensive de-mannosylation by GH47 family members. The generated Mannose$_{5-7}$ oligosaccharide is then bound by the lectins ERAD receptors OS-9 and XTP3-B, which then direct the misfolded polypeptide to the HRD1 dislocation complex through binding to the adaptor protein SEL1L. The polypeptide is unfolded, reduced by PDI (or another reductase), polyubiquitylated by HRD1 and retrotranslocated into the cytoplasm as driven by the action of p97. The poly-ubiquitylated polypeptide is destroyed by the 26S proteasome in the cytoplasm. (B) *Oligosaccharides as a link to the dislocation machinery*. Alternatively, OS-9 and XTP3-B bind misfolded polypeptides independent of the displayed N-glycan and through its lectin site (MRH domain) they bind the N-glycans of SEL1L. The different mannose composition of the N-glycan is depicted in color code: gray, oligosaccharide with nine mannose (Man$_9$); red, five to seven mannose residues (Man$_{5-7}$); white, oligosaccharide with an undefined number of mannose residues (Man$_x$).

The role of de-mannosylated oligosaccharides as a signal for polypeptide disposal decoded by ERAD lectins is widely accepted [30,31,46]. However, one report showed that the OS-9 MRH domain supports the binding to oligosaccharides displayed on SEL1L, thus with the dislocation machinery rather than with ERAD substrates [44] (a finding that failed to be confirmed by others [43]) and a second one showed that EDEM1 as well can use its lectin site to form a functional complex with SEL1L [39]. These data are puzzling because they seem to question the actual role of protein-bound oligosaccharides as ERAD signals. EDEM1, OS-9 and XTP3-B have all been shown to bind misfolded substrates irrespective of their glycosylation status [39–44]. Moreover, the active-site directed inhibitor kifunensine reduces association of EDEM1 with SEL1L, leaving substrate binding unaffected [39]. EDEM1 is also thought to deliver ERAD substrates to Derlin-2 and Derlin-3, or to a quality control compartment in which the highly enriched ERManI would extensively de-mannosylate ERAD substrates [47]. Altogether, these results suggest that bipartite signals may be involved in ERAD substrate selection involving both misfolded and glycan signals, and similar forces may be used in the recruitment of ERAD machinery complexes.

In addition to OS-9 and XTP3-B, other ER-resident chaperones have also been reported to act as quality control receptors that shuttle ERAD substrates for delivery to a mammalian ERAD dislocation complex. BiP and PDI have been shown to associate with ERAD substrates just before dislocation [24] for delivery to SEL1L [43]. Finally, GRP94 has also been reported to deliver ERAD substrates to SEL1L possibly upon formation of transient complexes with OS-9 [44]. Therefore, a number of ERAD receptors appear to be involved in the selection of ERAD substrates and their delivery to ERAD exit sites.

4. Dislocation across the ER membrane and poly-ubiquitylation

Aberrant proteins identified by quality control receptors are targeted to ERAD complexes that contain a retrotranslocation membrane channel that provides a route for the displacement of the ERAD substrate to the cytoplasm for proteasomal degradation. Translocons involved in the anterograde translocation of proteins have been extensively studied and are made up of integral membrane proteins that contain multiple membrane spans creating a gated aqueous pore within the membrane [1,48]. The identity of the retrotranslocation channel(s)/dislocon(s) remains controversial as a number of ER membrane proteins have been proposed to serve this function for the ERAD pathway including Sec61, Derlin and E3 ubiquitin ligase family members [49]. All in all, dislocons support the emergence of the ERAD substrates in cytoplasm for poly-ubiquitylation and subsequent proteasomal degradation.

4.1. ERAD substrates dislocation, the identity of the channel

4.1.1. Sec61

Sec61 is the translocon or the portal for entrance of nascent chains into the ER or the secretory pathway [1,48]. Sec61 has also been implicated in supporting dislocation of ERAD substrates to the cytoplasm. The analysis of yeast strains containing mutations in Sec61p has uncovered strains that are anterograde translocation proficient but deficient in retrotranslocation suggestive of a role

for Sec61 in the dislocation of ERAD substrates to the cytoplasm [50–52]. Furthermore, ERAD substrates including class I heavy chain directed to ERAD by the cytomegalovirus gene products US2/US11, the yeast mating pheromone pαF, the rapidly turned-over protein Deg1:Sec62^{ProtA}, or the catalytic subunits of bacterial toxins to be dislocated from the ER lumen into the cytosol have been cross-linked or co-precipitated with Sec61 [50,53–55]. The inactivation or depletion of Sec61 prevented dislocation of the A1 subunit of cholera toxin into the cytosol [53]. Also, proteasomes associate with Sec61 [56] and the co-translocational degradation of ApoB utilizes Sec61 [57] and the Sec61-associated co-chaperone p58, which is hypothesized to convert the translocon complex into a dislocation machinery [58]. While ample evidence supports Sec61 acting as the retrotranslocation channel, the crystallographic structure of archaeal homologue of Sec61, SecY, suggests that the pore size is small (~3 Å [59]), making it difficult to envision how this translocon could support the dislocation of ERAD substrates that, even when fully unfolded, might display oligosaccharides moieties extending for about 30 Å from the polypeptide chain.

4.1.2. Derlins

Additional ER polytopic membrane proteins have also been proposed to play a role in ERAD substrate dislocation. The human cytomegalovirus (US2/US11)-induced dislocation of class I heavy chain has been reported to depend on Derlin proteins, rather than Sec61 [60,61]. Furthermore, the translocation of pαF from mammalian microsomes using a real-time translocation assay was unaffected by antibodies to Sec61, but blocked by Derlin-1 antibodies [62]. A yeast homologue of Derlin-1 termed Der1p for 'degradation in the ER' was first identified through a screen that produced strains characterized by stabilization and ER retention of yeast ERAD substrates [63]. Derlin-1 has two additional family members in mammalian cells called Derlin-2 and Derlin-3. The overexpression of Derlin-1, -2 and -3 accelerates the degradation of ERAD substrates, and their knockdown can block degradation [60,61,64]. As for Sec61, a role of Derlins in dislocation across the ER membrane of viruses [65,66] and of bacterial products [67] has been reported. The Derlins are also associated with luminal, membrane-embedded and cytosolic components required for protein disposal from the ER such as EDEM1, PDI, HRD1, SEL1L, and VIMP, which recruits the p97 ATPase and its cofactors to aid in the ERAD substrates extraction process [44,60,61,64,67] (Fig. 2). However, it is possible that Derlins participate in a complex that contain the dislocation channel, rather than forming the channel themselves. Additional studies will be required to determine the precise role of the Derlin proteins in ERAD.

4.1.3. E3 ubiquitin ligases

Cumulating data show that dislocation of ERAD substrates across the ER membrane follows specific pathways regulated by luminal, transmembrane, and cytosolic complexes built around E3 ubiquitin ligases in the ER membrane [49,68–70]. In *S. cerevisiae*, selection of the E3 ligases regulating polyubiquitylation and dislocation of misfolded polypeptides is at least in part determined by the localization of the folding defect. Transmembrane proteins with cytosolic defects exploit the E3 ligase Doa10p and associated factors (ERAD-C pathway). Transmembrane polypeptides with transmembrane lesions (ERAD-M substrates) as well as polypeptides with lesions in the ER lumen (ERAD-L substrates) are cleared from the ER upon intervention of the E3 ligase Hrd1p and the several proteins interacting with it [71–77]. In mammalian cells, the presence/absence of a transmembrane anchor might play a crucial role in selection of the E3 ubiquitin ligase complex assisting dislocation/poly-ubiquitylation of a folding-defective polypeptide [44a].

The mammalian ER membrane contains several poorly characterized E3 ubiquitin ligases (e.g. RNF5/RMA1, TEB4, TRC8, RFP2) and others such as Synoviolin/HRD1 and GP78, which have been better characterized. The interacting partners of both are known to some extent and their involvement in disposal of numerous ERAD substrates has been clearly established [49,68–70]. The HRD1 complex comprises several luminal and membrane-bound proteins such as OS-9, XTP3-B, EDEM1, BiP or GRP94 (Fig. 2) that do not participate in the GP78 dislocation complex [39,41,43–45,78,79]. Certainly, the different composition of the complexes might determine substrate selection. Further work will allow characterization of substrate specificity and to predict which pathway will be used by a given folding-defective polypeptide for efficient clearance from the mammalian ER [44a].

5. Conclusions and Perspectives

Maintenance of cells, tissues and eventually organisms homeostasis relies on the capacity to translate the information stored in the DNA in the language of proteins with high efficiency and fidelity. Work performed by many groups in the last fifteen years shed light on the important role of N-linked oligosaccharides and their processing in regulation of protein biogenesis, quality control and degradation. Rapid removal of terminal glucoses, cycles of removal and re-addition of a single glucose residue, slow and irreversible trimming of α1,2-bonded mannose residues generate an array of signals displayed on immature, native or terminally misfolded conformers that eventually determine a protein's fate. About one third of the genome products mature in the ER. An apparent small number of quality control and ERAD factors are responsible to survey their conformation. While the number of quality control and ERAD factors appears to be slightly expanded in higher organisms, a fundamental selection process is still needed to properly survey such a large array of cargo that traffic through the secretory pathway. Further studies are required to determine how aberrant protein structures are recognized and encoded using a carbohydrate code that can be recognized by ERAD receptors. The ERAD receptor–substrate complex is then targeted to a distinct number of ER membrane ubiquitylation/translocation complexes responsible for the dislocation/polyubiquitylation of misfolded proteins to the cytoplasm for degradation. The importance in understanding these events is underscored by the hundreds of human diseases caused by inefficient protein folding, premature disposal or defective removal of aberrant by-products of protein synthesis. The precise characterization of the mechanisms regulating protein folding, quality control and degradation is crucial to develop therapeutic intervention to contrast conformational diseases.

Acknowledgements

M.M. is supported by grants from the Foundation for Research on Neurodegenerative Diseases, ONELIFE, the Fondazione San Salvatore, the Swiss National Center of Competence in Research on Neural Plasticity and Repair, the Swiss National Science Foundation, the Synapsis Foundation and the Bangerter-Rhyner Foundation. D.N.H. is support by US Public Health grants GM086874 and AI078142.

References

[1] Rapoport TA. Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. Nature 2007;450:663.
[2] Kowarik M, Kung S, Martoglio B, Helenius A. Protein folding during cotranslational translocation in the endoplasmic reticulum. Mol Cell 2002;10:769.
[3] Sanchez IE, Morillas M, Zobeley E, Kiefhaber T, Glockshuber R. Fast folding of the two-domain semliki forest virus capsid protein explains co-translational proteolytic activity. J Mol Biol 2004;338:159.

[4] Pittman DD, Tomkinson KN, Kaufman RJ. Post-translational requirements for functional factor V and factor VIII secretion in mammalian cells. J Biol Chem 1994;269:17329.
[5] Hebert DN, Garman SC, Molinari M. The glycan code of the ER: asparagine-linked carbohydrates as protein maturation and quality-control tags. Trends Cell Biol 2005;15:364.
[6] Sousa M, Parodi AJ. The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. EMBO J 1995;14:4196.
[7] Parodi AJ, Mendelzon DH, Lederkremer GZ, Martin-Barrientos J. Evidence that transient glucosylation of protein-linked Man9GlcNAc2, Man8GlcNAc2, and Man7GlcNAc2 occurs in rat liver and Phaseolus vulgaris cells. J Biol Chem 1984;259:6351.
[8] Caramelo JJ, Castro OA, de Prat-Gay G, Parodi AJ. The endoplasmic reticulum glucosyltransferase recognizes nearly native glycoprotein folding intermediates. J Biol Chem 2004;279:46280.
[9] Hammond C, Braakman I, Helenius A. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. Proc Natl Acad Sci USA 1994;91:913.
[10] Hebert DN, Foellmer B, Helenius A. Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. Cell 1995;81:425.
[11] Daniels R, Kurowski B, Johnson AE, Hebert DN. N-linked glycans direct the cotranslational folding pathway of influenza hemagglutinin. Mol Cell 2003;11:79.
[12] Vassilakos A, Cohen-Doyle MF, Peterson PA, Jackson MR, Williams DB. The molecular chaperone calnexin facilitates folding and assembly of class I histocompatibility molecules. EMBO J 1996;15:1495.
[13] Hebert DN, Foellmer B, Helenius A. Calnexin and calreticulin promote folding, delay oligomerization and suppress degradation of influenza hemagglutinin in microsomes. EMBO J 1996;15:2961.
[14] Rajagopalan S, Brenner MB. Calnexin retains unassembled major histocmpatibility complex class I free heavy chains in the endoplasmic reticulum. J Exp Med 1994;180:407.
[15] Soldà T, Garbi N, Hammerling GJ, Molinari M. Consequences of ERp57 deletion on oxidative folding of obligate and facultative clients of the calnexin cycle. J Biol Chem 2006;281:6219.
[16] Soldà T, Galli C, Kaufman RJ, Molinari M. Substrate-specific requirements for UGT1-dependent release from calnexin. Mol Cell 2007;27:238.
[17] Fanchiotti S, Fernandez F, D'Alessio C, Parodi AJ. The UDP-Glc:glycoprotein glucosyltransferase is essential for Schizosaccharomyces pombe viability under conditions of extreme endoplasmic reticulum stress. J Cell Biol 1998;143:625.
[18] Pearse BR, Gabriel L, Wang N, Hebert DN. A cell-based reglucosylation assay demonstrates the role of GT1 in the quality control of a maturing glycoprotein. J Cell Biol 2008;181:309.
[19] Sousa MC, Ferrero-Garcia MA, Parodi AJ. Recognition of the oligosaccharide and protein moieties of glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. Biochemistry 1992;31:97.
[20] Olivari S, Cali T, Salo KE, Paganetti P, Ruddock LW, Molinari M. EDEM1 regulates ER-associated degradation by accelerating de-mannosylation of folding-defective polypeptides and by inhibiting their covalent aggregation. Biochem Biophys Res Commun 2006;349:1278.
[21] Ermonval M, Kitzmuller C, Mir AM, Cacan R, Ivessa NE. N-glycan structure of a short-lived variant of ribophorin I expressed in the MadIA214 glycosylation-defective cell line reveals the role of a mannosidase that is not ER mannosidase I in the process of glycoprotein degradation. Glycobiology 2001;11:565.
[22] Ruddock LW, Molinari M. N-glycan processing in ER quality control. J Cell Sci 2006;119:4373.
[23] Molinari M, Calanca V, Galli C, Lucca P, Paganetti P. Role of EDEM in the release of misfolded glycoproteins from the calnexin cycle. Science 2003;299:1397.
[24] Molinari M, Galli C, Piccaluga V, Pieren M, Paganetti P. Sequential assistance of molecular chaperones and transient formation of covalent complexes during protein degradation from the ER. J Cell Biol 2002;158:247.
[25] Clerc S, Hirsch C, Oggier DM, Deprez P, Jakob C, Sommer T, et al. Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. J Cell Biol 2009;184:159.
[26] Quan EM, Kamiya Y, Kamiya D, Denic V, Weibezahn J, Kato K, et al. Defining the glycan destruction signal for endoplasmic reticulum-associated degradation. Mol Cell 2008;32:870.
[27] Cabral CM, Liu Y, Sifers RN. Dissecting glycoprotein quality control in the secretory pathway. Trends Biochem Sci 2001;26:619.
[28] Olivari S, Molinari M. Glycoprotein folding and the role of EDEM1, EDEM2 and EDEM3 in degradation of folding-defective glycoproteins. FEBS Lett 2007;581:3658.
[29] Moremen KW, Molinari M. N-linked glycan recognition and processing: the molecular basis of endoplasmic reticulum quality control. Curr Opin Struct Biol 2006;592.
[30] Aebi M, Bernasconi R, Clerc S, Molinari M. N-glycan structures: recognition and processing in the ER. Trends Biochem Sci 2010;35:74.
[31] Lederkremer GZ. Glycoprotein folding, quality control and ER-associated degradation. Curr Opin Struct Biol 2009;19:1.
[32] Herscovics A, Romero PA, Tremblay LO. The specificity of the yeast and human class I ER alpha 1,2-mannosidases involved in ER quality control is not as strict previously reported. Glycobiology 2002;12:14G.
[33] Hirao K, Natsuka Y, Tamura T, Wada I, Morito D, Natsuka S, et al. EDEM3, a soluble EDEM homolog, enhances glycoprotein ERAD and mannose trimming. J Biol Chem 2006;281:9650.
[34] Lipari F, Herscovics A. Role of the cysteine residues in the alpha1,2-mannosidase involved in N-glycan biosynthesis in *Saccharomyces cerevisiae*. The conserved Cys340 and Cys385 residues form an essential disulfide bond. J Biol Chem 1996;271:27615.
[35] Jakob CA, Bodmer D, Spirig U, Battig P, Marcil A, Dignard D, et al. Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep 2001;2:423.
[36] Hosokawa N, Wada I, Hasegawa K, Yorihuzi T, Tremblay LO, Herscovics A, et al. A novel ER {alpha}-mannosidase-like protein accelerates ER-associated degradation. EMBO Rep 2001;2:415.
[37] Tatara Y, Yoshida T, Ichishima E. A single free cysteine residue and disulfide bond contribute to the thermostability of Aspergillus saitoi 1,2-alpha-mannosidase. Biosci Biotechnol Biochem 2005;69:2101.
[38] Karaveg K, Siriwardena A, Tempel W, Liu ZJ, Glushka J, Wang BC, et al. Mechanism of class 1 (glycosylhydrolase family 47) {alpha}-mannosidases involved in N-glycan processing and endoplasmic reticulum quality control. J Biol Chem 2005;280:16197.
[39] Cormier JH, Tamura T, Sunryd JC, Hebert DN. EDEM1 recognition and delivery of misfolded proteins to the SEL1L-containing ERAD complex. Mol Cell 2009;34:627.
[40] Hosokawa N, Kamiya Y, Kamiya D, Kato K, Nagata K. Human OS-9, a lectin required for glycoprotein endoplasmic reticulum-associated degradation, recognizes mannose-trimmed N-glycans. J Biol Chem 2009;284:17061.
[41] Alcock F, Swanton E. Mammalian OS-9 is upregulated in response to endoplasmic reticulum stress and facilitates ubiquitination of misfolded glycoproteins. J Mol Biol 2009;385:1032.
[42] Bernasconi R, Pertel T, Luban J, Molinari M. A dual task for the Xbp1-responsive OS-9 variants in the mammalian endoplasmic reticulum: inhibiting secretion of misfolded protein conformers and enhancing their disposal. J Biol Chem 2008;283:16446.
[43] Hosokawa N, Wada I, Nagasawa K, Moriyama T, Okawa K, Nagata K. Human XTP3-B forms an endoplasmic reticulum quality control scaffold with the HRD1-SEL1L ubiquitin ligase complex and BiP. J Biol Chem 2008;283:20914.
[44] Christianson JC, Shaler TA, Tyler RE, Kopito RR. OS-9 and GRP94 deliver mutant alpha1-antitrypsin to the Hrd1/SEL1L ubiquitin ligase complex for ERAD. Nat Cell Biol 2008;10:272.
[44a] Bernasconi R, Galli C, Calanca V, Nakajima T, Molinari M. Stringent requirement for HRD1, SEL1L and OS-9/XTP3-B for disposal of ERAD-LS substrates. J Cell Biol 2010;188:223.
[45] Mueller B, Klemm EJ, Spooner E, Claessen JH, Ploegh HL. SEL1L nucleates a protein complex required for dislocation of misfolded glycoproteins. Proc Natl Acad Sci USA 2008;105:12325.
[46] Hebert DN, Molinari M. In and out of the ER: protein folding, quality control, degradation, and related human diseases. Physiol Rev 2007;87:1377.
[47] Avezov E, Frenkel Z, Ehrlich M, Herscovics A, Lederkremer GZ. Endoplasmic reticulum (ER) mannosidase I is compartmentalized and required for N-glycan trimming to Man5 6GlcNAc2 in glycoprotein ER-associated degradation. Mol Biol Cell 2008;19:216.
[48] Johnson AE, Haigh NG. The ER translocon and retrotranslocation: is the shift into reverse manual or automatic? Cell 2000;102:709.
[49] Nakatsukasa K, Brodsky JL. The recognition and retrotranslocation of misfolded proteins from the endoplasmic reticulum. Traffic 2008;9:861.
[50] Scott DC, Schekman R. Role of Sec61p in the ER-associated degradation of short-lived transmembrane proteins. J Cell Biol 2008;181:1095.
[51] Plemper RK, Egner R, Kuchler K, Wolf DH. Endoplasmic reticulum degradation of a mutated ATP-binding cassette transporter Pdr5 proceeds in a concerted action of Sec61 and the proteasome. J Biol Chem 1998;273:32848.
[52] Plemper RK, Bohmler S, Bordallo J, Sommer T, Wolf DH. Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. Nature 1997;388:891.
[53] Schmitz A, Herrgen H, Winkeler A, Herzog V. Cholera toxin is exported from microsomes by the Sec61p complex. J Cell Biol 2000;148:1203.
[54] Wiertz EJ, Tortorella D, Bogyo M, Yu J, Mothes W, Jones TR, et al. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 1996;384:432.
[55] Pilon M, Schekman R, Romisch K. Sec61p mediates export of a misfolded secretory protein from the endoplasmic reticulum to the cytosol for degradation. EMBO J 1997;16:4540.
[56] Kalies KU, Allan S, Sergeyenko T, Kroger H, Romisch K. The protein translocation channel binds proteasomes to the endoplasmic reticulum membrane. EMBO J 2005;24:2284.
[57] Liang S, Wu X, Fisher EA, Ginsberg HN. The amino-terminal domain of apolipoprotein B does not undergo retrograde translocation from the endoplasmic reticulum to the cytosol. Proteasomal degradation of nascent apolipoprotein B begins at the carboxyl terminus of the protein, while apolipoprotein B is still in its original translocon. J Biol Chem 2000;275:32003.
[58] Oyadomari S, Yun C, Fisher EA, Kreglinger N, Kreibich G, Oyadomari M, et al. Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. Cell 2006;126:727.
[59] Van den Berg B, Clemons Jr WM, Collinson I, Modis Y, Hartmann E, Harrison SC, et al. X-ray structure of a protein-conducting channel. Nature 2004;427:36.

[60] Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. Nature 2004;429:834.
[61] Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature 2004;429:841.
[62] Wahlman J, DeMartino GN, Skach WR, Bulleid NJ, Brodsky JL, Johnson AE. Real-time fluorescence detection of ERAD substrate retrotranslocation in a mammalian in vitro system. Cell 2007;129:943.
[63] Knop M, Finger A, Braun T, Hellmuth K, Wolf DH. Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast. EMBO J 1996;15:753.
[64] Oda Y, Okada T, Yoshida H, Kaufman RJ, Nagata K, Mori K. Derlin-2 and Derlin-3 are regulated by the mammalian unfolded protein response and are required for ER-associated degradation. J Cell Biol 2006;172:383.
[65] Lilley BN, Gilbert JM, Ploegh HL, Benjamin TL. Murine polyomavirus requires the endoplasmic reticulum protein Derlin-2 to initiate infection. J Virol 2006;80:8739.
[66] Schelhaas M, Malmstrom J, Pelkmans L, Haugstetter J, Ellgaard L, Grunewald K, et al. Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. Cell 2007;131:516.
[67] Bernardi KM, Forster ML, Lencer WI, Tsai B. Derlin-1 facilitates the retro-translocation of cholera toxin. Mol Biol Cell 2008;19:877.
[68] Hirsch C, Gauss R, Horn SC, Neuber O, Sommer T. The ubiquitylation machinery of the endoplasmic reticulum. Nature 2009;458:453.
[69] Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin–proteasome system. Nat Rev Mol Cell Biol 2008;9:679.
[70] Kostova Z, Tsai YC, Weissman AM. Ubiquitin ligases, critical mediators of endoplasmic reticulum-associated degradation. Semin Cell Dev Biol 2007;18:770.
[71] Vashist S, Ng DT. Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. J Cell Biol 2004;165:41.
[72] Denic V, Quan EM, Weissman JS. A luminal surveillance complex that selects misfolded glycoproteins for ER-associated degradation. Cell 2006;126:349.
[73] Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin–ligase complexes define convergent pathways for the degradation of ER proteins. Cell 2006;126:361.
[74] Gauss R, Sommer T, Jarosch E. The Hrd1p ligase complex forms a linchpin between ER-lumenal substrate selection and Cdc48p recruitment. EMBO J 2006;25:1827.
[75] Huyer G, Piluek WF, Fansler Z, Kreft SG, Hochstrasser M, Brodsky JL, et al. Distinct machinery is required in *Saccharomyces cerevisiae* for the endoplasmic reticulum-associated degradation of a multispanning membrane protein and a soluble luminal protein. J Biol Chem 2004;279:38369.
[76] Taxis C, Hitt R, Park SH, Deak PM, Kostova Z, Wolf DH. Use of modular substrates demonstrates mechanistic diversity and reveals differences in chaperone requirement of ERAD. J Biol Chem 2003;278:35903.
[77] Willer M, Forte GM, Stirling CJ. Sec61p is required for ERAD-L: genetic dissection of the translocation and ERAD-L functions of Sec61P using novel derivatives of CPY. J Biol Chem 2008;283:33883.
[78] Mueller B, Lilley BN, Ploegh HL. SEL1L, the homologue of yeast Hrd3p, is involved in protein dislocation from the mammalian ER. J Cell Biol 2006;175:261.
[79] Morito D, Hirao K, Oda Y, Hosokawa N, Tokunaga F, Cyr DM, et al. Gp78 Cooperates with RMA1 in endoplasmic reticulum-associated degradation of CFTR{Delta}F508. Mol Biol Cell 2008;19:1328.