ENDOPLASMIC RETICULUM ASSOCIATED DEGRADATION OF GLYCOPROTEINS IN *CAENORHABDITIS ELEGANS*

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The endoplasmic reticulum (ER) is an essential cellular compartment for protein synthesis and maturation. To prevent accumulation of toxic proteins in ER, misfolded or unassembled subunits of multimeric proteins that do not pass the quality control are eliminated by ER-associated degradation (ERAD). ERAD targets are transported from ER to the cytosol by a process termed translocation and are destroyed by the cytoplasmic ubiquitin-proteasome machinery. This review summarizes the advances in elucidation of ERAD machinery in the nematode *Caenorhabditis elegans*.

Key words: endoplasmic reticulum, glycoproteins, ER degradation, proteasome.

INTRODUCTION

Glycosylation is one of the most common post-translational protein modifications in eukaryotic cells. Glycoconjugates are involved in multiple biological processes such as protein folding and oligomerization, in the ER-based quality control system, targeting to the lysosome (1), and act as markers that mediate cell-cell and cell-matrix recognition events such as differentiation, embryogenesis, inflammation, cancer and metastasis (2-5). Recently, using lectin affinity chromatography 304 N-glycoproteins have been identified in *C. elegans* (6, 7). Many of them are extracellular matrix components that have been implicated in cell adhesion or are components of basement membranes (8).

To maintain protein homeostasis in secretory compartments, eukaryotic cells harbor a quality control system that monitors protein folding and protein complex assembly in the endoplasmic reticulum (ER). Glycoproteins with a native conformation exit ER and continue their maturation through the secretory pathway. Misfolded proteins or those that have failed to become post-translationally modified, together with unassembled members of multiprotein complexes are

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discarded by ER-association degradation (ERAD) (9). This process involves recognition and targeting of the substrate by specific quality control factors followed by polyubiquitination and retrotranslocation through the translocation channel to the cytosol where it is degraded by the proteasome. The factors involved in these processes in *C. elegans* are summarized in Table 1.

Table 1

Components required for ERAD

Component	***C. elegans***	**Yeast**	**Mammals**
Recognition			
Calnexin	cnx-1	Cne1	calnexin
Calreticulin	crt-1	Unknown	calreticulin
α-Mannosidase-like	C47E12.3	Htm1	EDEM1
	F10C2.5	Unknown	EDEM2
	ZC506.1	Unknown	EDEM3
Mannose-6-phosphate receptor-like	Y105E8A.2	YOS9	OS9; XTP-3B
Retrotranslocation			
Sec61 complex	Y57G11C.15	Sec61 complex and Ssh1 complex	Sec61 complex
Derlins	cup-2 R151.6 Unknown	Dfm1 and Der1 Unknown Unknown	Derlin-1 Derlin-2 Derlin–3
Ubiquitylation			
E1 ubiquitin-activating enzyme	uba-1	Uba1	UBE1
E2 ubiquitin-conjugating enzyme	ubc-15 ubc-14 ubc-20	Ubc6 Ubc7 Ubc1	UBC6e UBC7 UBC1
E3 ubiquitin ligase	HRD-1-SEL-1 complex MARC-6	Hrd1/Der3–Hrd3 complex Doa10	HRD1-SEL1L complex TEB4 (MARCH IV)
Cdc48 complex	Cdc-48.1/CDC-48.2–Ufd1–Npl4	Cdc48–Ufd1–Npl4	p97–UFD1–NPL4
Proteasomal targeting			
Ubiquitin receptor	RPN-1 Unknown RPT-5	Rpn10 Rpn13 Rpt5	RPN10 (S5a) RPN13 RPT5 (TBP1 or S6)

N-LINKED GLYCANS

In eukaryotic cells, nearly all secreted proteins enter the ER where they undergo specific posttranslational modification. The yeast and mammalian

posttranslational modifications of glycoproteins have been elucidated in great detail (10, 11) and the initial steps of this pathway are conserved in most eukaryotes. The *C. elegans* genome contains more than 150 genes that are orthologues of mammalian genes involved in the assembly, processing and modification of the complex carbohydrates (12), suggesting the existence of a similar post-translational machinery.

An oligosaccharide composed of 14 sugars $Glc_3Man_9GlcNAc_2$ (of which GlcNAc is N-acetylglucosamine, Man is mannose and Glc is glucose) is transferred *en bloc* to the Asn residue of the nascent polypeptide chain by oligosaccharyl-transferase (OST). In mammalian cells, the sole consensus amino acid sequence needed for the transfer of *N*-glycans is Asn-X-Ser/Thr (where X is any amino acid except Pro or Asp). Interestingly, *C. elegans* has an additional Asn-X-Cys consensus sequence (13).

Although much of the *N*-glycan biosynthesis in *C. elegans* is conserved with those of mammals, their terminal structures are rather different. Thus, the most abundant *N*-glycans are those of the high mannose-type, but there are also small amounts of hybrid or complex-type in addition to fuco-paucimannosidic, and phosphorylcholine glycans. The high mannose, complex, and hybrid glycans show a high degree of conservation with those of mammals, whereas the last two are unique to *C. elegans* (14–16).

ER QUALITY CONTROL

Transfer of $Glc_3Man_9GlcNAc_2$ to Asn is followed by glucose trimming by glucosidases and folding assisted by the lectin-like chaperones calnexin and calreticulin. Subsequent cycles of glucose re-addition by UDP-glucose: glycoprotein glucosyltransferase (UGGT) and removal participate in quality control of protein folding. Terminally misfolded or unassembled glycoproteins are recognized by ER degradation-enhancing α-mannosidase-like proteins (EDEMs), which might prevent glycoproteins from becoming permanently trapped in a re-glucosylation and folding cycle (17). Other factors that recognize glycoproteins are the lectins that contain mannose-6-phosphate receptor-like domains, such as Yos9 (in yeast) and OS9 and XTP-3B (in mammals). It is thought that these factors deliver ERAD substrates to the retrotranslocation channel (18, 19).

So far, little is known about the factors that are required for ERAD substrate targeting in *C. elegans*. However, based on phylogenetic analyses and the existence of mannan Man3–9GlcNAc2 series (20), it can be speculated that *C. elegans* also possesses enzymes with an identical function to mammalian EDEM 1–3. Indeed, a search of *C. elegans* genome identifies the mammalian EDEM 1–3 orthologues, which share 46, 39, and 41% amino acid sequence identity, respectively, and similar domain architecture with their human counterparts. It will be interesting to

determine whether and what degree of functional redundancy or competition the *C. elegans* EDEMs exhibit on the glycoproteins to be discarded.

The *C. elegans* Y105E8A.2 is the closest phylogenetic orthologue of the human XTP-3B, and although it shares only 10% sequence identity with XTP-3B it possesses a similar domain architecture. The low sequence identity might be explained by the fact that the two proteins recognize glycoproteins with a different glycan structure; therefore, similar function in ERAD is expected.

In yeasts, the position of the misfolded domain in a substrate has led to the subdivision of ERAD into three pathways ERAD-cytosolic (ERAD-C), ERAD-lumenal (ERAD-L), and ERAD-membrane (ERAD-M), respectively. Proteins with misfolded lumenal or membrane domains (ERAD-L and ERAD-M substrates, respectively) use the ubiquitin ligase Hrd1p, whereas membrane proteins with misfolded cytosolic domains (ERAD-C substrates) use the ligase Doa10p.

The yeast HRD1 complex is composed of ubiquitin ligase Hrd1/Der3 and its partner Hrd3, the cytoplasmic ubiquitin-conjugating enzyme Ubc7 bound to Cue1, transmembrane Der1 and its recruitment factor Usa1. Based on genetic evidence, it has been postulated that in yeasts, the ER protein import channel Sec61 also participate in retro-translocation (21). However, in mammalian system, Derlin-1 is essential for the extraction and degradation of class I MHC molecules, suggesting that Derlin-1 might be part of the retro-translocation pore (22–24). Further, Derlin-1 binds to misfolded and ubiquitinated proteins, and RNA interference (RNAi) of Derlin-1 in *C. elegans* evokes ER stress, suggesting an essential role of Derlin-1 in ERAD (23). However, as in yeasts, the *C. elegans* Derlin-1 is not essential for viability. Depletion of Derlin-2 by RNAi in *C. elegans* did not elicit the UPR, suggesting that it may not be involved in retro-translocation or may be required for the degradation of a restricted subset of substrates (23).

UBIQUITINATION AND RETROTRANSLOCATION

As proteins exit the retro-translocon they are polyubiquitylated. Ubiquitination is a covalent modification by which a highly conserved polypeptide chain of minimum four ubiquitin moieties is attached to a terminally misfolded protein (25). The attachment of ubiquitin to a protein functions as a degradation signal and is mediated by three enzymes: the E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligase. As the other eukaryotes, the *C. elegans* possess a single gene encoding the E1-activating enzyme (26).

The major yeast E2 for Hrd1/Der3-catalyzed polyubiquitylation is Ubc7, although Ubc1 is also utilized. The other ERAD E2, Ubc6, is part of the Doa10 complex (27). In *C. elegans*, only the orthologue of Ubc7 (*ubc-14*) is essential for embryonic viability, and Ubc1 (*ubc-20*) exhibit L3 & L4 larval arrest by RNAi.

Inhibition by RNAi of Ubc6 orthologue (*ubc-15*) was not associated with any apparent defects (28).

While in yeasts there are two E3 ubiquitin ligases Hrd1p and Doa10p that tag proteins for degradation (27, 29, 30), in mammals, along with the yeasts orthologues of Hrd1p, Hrd1 and TEB4/ MARCH VI (31, 32), there are many other E3 ubiquitin ligases, some with a role in the quality control of disease-related proteins (33–36). The *C. elegans* orthologues of HRD1 (HRD-1), gp78 (HRDL-1) and MARCH VI (MARC-6) ubiquitin ligases have been described in some detail (37). They are multi-spanning membrane proteins and contain catalytic RING domains. Yeasts cells lacking both Hrd1 and Doa10 exhibit a strong Unfolded Protein Response (UPR), while loss of only one E3 results in a modest induction (30). Similarly, although each *C. elegans* single mutant did not exhibit growth defects, the simultaneous depletion of all three E3 ligases (HRD-1, HRDL-1 and MARCH VI) caused an extremely delayed growth. The *C. elegans* possess two orthologues of the ER chaperone BiP (HSP-3 and HSP-3). Interestingly, HRD-1 and HSP-3 play important roles in the developmental growth and function of intestinal cells, while HRD-1 and HSP-4 in the gonad formation. This reflects a convergent function of the two BiP chaperones that exhibit different substrate specificities toward the HRD-1 complex (37).

The *C. elegans sel-1* orthologue of *HRD3* was identified as an extragenic suppressor of the egg-laying defect caused by a temperature sensitive hypomorph of *lin-12/*Notch activity (38). Inactivation of *sel-1* gene does not exhibit an obvious phenotype except for an elevated level of *lin-12* hypomorph activity (39, 40), indicating that the SEL-1 might be involved in the degradation of the LIN-12. RNAi of the *sel-1* gene resulted in up-regulation of the chaperone *hsp-4*, which is an ER-stress indicator. A role of *sel-1* in ERAD is also suggested by the fact that combined inactivation of *sel-1* and *abu-1* (*A*ctivated in *B*locked *U*nfolded protein response) increased the lethality of the nematode, most probably because *sel-1* and *abu-1* perform partially redundant functions in the unfolded protein response (41). The human SEL1 seems to have a more complex role, since downregulation of human Sel1L in epithelial cells has been associated with the development of primary breast carcinomas and pancreatic adenocarcinomas (42).

The HRD1 complex associates with the Cdc48 complex (43) composed of a homohexamer of the AAA-ATPase Cdc48 and its co-factors Npl4 and Ufd1 (44–46). A main function of CDC-48/p97 complex is to segregate ubiquitylated proteins from their binding partners and to regulate the length of the attached polyubiquitin chain before they are translocated across the ER membrane in the cytosol where they are degraded by the 26S proteasome (47, 48). In contrast to other organisms, *C. elegans* has two CDC-48/p97 orthologues, CDC-48.1 and CDC-48.2, which share 88% amino acids identity. Only simultaneous depletion of

both isoforms by RNAi resulted in ER stress, indicating a redundant function in ERAD (37, 49, 50). Both CDC-48.1 and CDC-48.2 form a complex related to yeast $CDC48^{UFD1/NPL4}$ or mammalian $p97^{UFD1/NPL4}$ complexes and can bind to each other (49).

In an elegant study, Janiesch revealed an unexpected function for CDC-48.1 in the correct folding and assembly of myosin during the formation of muscle thick filaments, both in *C. elegans* and humans (51). CDC-48.1, but not CDC-48.2, assembles into a complex together with UFD-2 and CHN-1 to target the myosin assembly chaperone UNC-45 for degradation in a similar manner to the yeast escort pathway described for yeast CDC48p and Ufd2p. Moreover, mutations in human p97, known to cause hereditary inclusion-body myopathy, abrogate UNC-45 degradation leading to protein aggregation and inclusion-body formation in human skeletal muscle (51–53). Beside its function in the ERAD pathway, cdc-48 is required for DNA replication during cell cycle progression (54, 55).

PROTEASOMAL DEGRADATION

Once a polyubiquitinated substrate is displaced into the cytoplasm, it is recognized by the 26S proteasome (56, 57). The 26S proteasome is formed by the association of two subcomplexes: a 20S particle that constitutes the proteolytic core, and a regulatory 19S particle that caps the 20S at both ends. The 19S particle recognizes and unfolds the substrate, then translocates the substrate into the 20S core (58). *C. elegans* has 14 conserved subunits that comprise the 20S core, as well as 18 conserved 19S components (59). One of the best-characterized functions for ubiquitination and proteasomal degradation in *C. elegans* is the coordination of early events of embryogenesis as cell cycle progression, cytoskeletal regulation and cell fate determination. RNAi depletion of proteasome components during larval stages produces larval arrest and lethality (60).

CONCLUDING REMARKS

Recent studies provided a more coherent and detailed picture of the mechanisms that are involved in ERAD. However, many unanswered questions remain, especially what is the precise role of ERAD in neurodegenerative diseases that are associated with the formation of large protein aggregates. The availability of well-established *C. elegans* model organism coupled with the sophisticated genetics and molecular biology tools available for study in this organism, should allow detailed and systematic dissection of the ERAD process.

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