Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2024 25(3):212-232
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2300403

Advances in the study of protein folding and endoplasmic reticulum-associated degradation in mammal cells

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Abstract: The endoplasmic reticulum is a key site for protein production and quality control. More than one-third of proteins are synthesized and folded into the correct three-dimensional conformation in the endoplasmic reticulum. However, during protein folding, unfolded and/or misfolded proteins are prone to occur, which may lead to endoplasmic reticulum stress. Organisms can monitor the quality of the proteins produced by endoplasmic reticulum quality control (ERQC) and endoplasmic reticulum-associated degradation (ERAD), which maintain endoplasmic reticulum protein homeostasis by degrading abnormally folded proteins. The underlying mechanisms of protein folding and ERAD in mammals have not yet been fully explored. Therefore, this paper reviews the process and function of protein folding and ERAD in mammalian cells, in order to help clinicians better understand the mechanism of ERAD and to provide a scientific reference for the treatment of diseases caused by abnormal ERAD.

Key words: Endoplasmic reticulum-associated degradation (ERAD); Protein folding; Ubiquitination; Retrotranslocation

1 Introduction

The endoplasmic reticulum (ER), as a continuous membrane system in eukaryotic cells, is an important organelle responsible for protein, lipid synthesis, and calcium storage. Analysis of the human genome revealed that more than one-third of proteins undergo post-translational modifications and fold in ER, which depends on the enrichment of protein modification-related enzymes, protein-folding-related molecular chaperones, and catalysts in the ER (Braakman and Bulleid, 2011). However, the process of protein folding is inherently error-prone as there are several unnatural intermediate states in peptide folding (Jahn and Radford, 2005). The probability of protein misfolding increases under the stimulation of hypoxia, starvation, toxicity, and the accumulation of reactive oxygen species (ROS) (Iurlaro and Muñoz-Pinedo, 2016). The accumulation of misfolded or unfolded proteins disrupts ER homeostasis and leads to ER stress. Excessive ER stress, in turn, impairs ER function, which poses a serious threat to cell autophagy, and apoptosis appears (Walter and Ron, 2011).

Endoplasmic reticulum quality control (ERQC) has evolved in the ER to monitor the protein-folding process and reduce misfolded proteins, whereas misfolded and unfolded proteins can undergo additional folding cycles to refold in the context of the substrate. On the other hand, misfolded proteins can also be reversely transported into cells for degradation through endoplasmic reticulum-associated degradation (ERAD) (McCracken and Brodsky, 1996). ERAD was first discovered in the late 1980s. These studies identified a pre-Golgi proteolysis pathway that targets misfolded proteins where degradation sites are associated with the ER (Lippincott-Schwartz et al., 1988; Bonifacino et al., 1990). Subsequently, this degradation method in the ER by the ubiquitin-protease degradation system was named ERAD (McCracken and Brodsky, 1996).

ERAD is a pathway that recognizes irreversibly misfolded and unfolded proteins, retrogradely transports them to the cytoplasm, and degrades them via the ubiquitin-proteasome system (UPS) in the ER (Smith

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Received June 11, 2023; Revision accepted Aug. 3, 2023; Crosschecked Jan. 15, 2024

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et al., 2011). The ERAD pathway, which is widespread in eukaryotes, can degrade misfolded and unfolded proteins, maintain protein quality, and balance the number of ER-resident proteins (Printsev et al., 2017). Accumulating evidence suggests that both musculoskeletal and neurological diseases are associated with the accumulation of misfolded proteins in the ER lumen, such as Paget's bone disease (Fernández-Sáiz and Buchberger, 2010), rheumatoid arthritis (RA) (Xu and Fang, 2020), Alzheimer's disease (AD) (Nowakowska-Gołacka et al., 2021), and amyotrophic lateral sclerosis (ALS) (Shahheydari et al., 2017). In addition, knockdown of ERAD components can also lead to the occurrence of various diseases and even embryonic lethality (Francisco et al., 2010; Eura et al., 2012). This paper reviews the process and function of protein folding and ERAD in mammalian cells, and further elaborates the basic content and research status of ERAD, which will help us to better understand the pathogenesis of related diseases and develop effective clinical strategies.

2 Process of protein folding

Ribosome-catalyzed protein biosynthesis (translation) is the process of using messenger RNA (mRNA) as a template upon which to form a polypeptide chain by combining amino acids of a specific sequence through peptide bonds by interpreting the genetic code. Protein translation is one of the most delicate and critical life activities in cells. To obtain the normal function of the protein, the peptide chain must be folded quickly and correctly after translation, to obtain a three-dimensional structure in a correct conformation. The ER plays an important role in the maturation of proteins as a site for the folding and modification of one-third of proteins (Braakman and Bulleid, 2011). The presence of large amounts of lectins and chaperones in the ER leads to the high efficiency of protein folding (Fig. 1) (Määttänen et al., 2010).

2.1 Lectin—calnexin/calreticulin cycle

Calcium-binding proteins calnexin (CNX) and calreticulin (CRT) are typical representatives of lectins in the ER. The CNX/CRT cycle is responsible for the folding of glycoproteins in the ER. About 70% of the proteins synthesized in the ER are glycoproteins that require *N*-link glycosylation. This sugar chain tag plays a role in the process of protein folding, protein maturation, and being transported to the right place (Hebert et al., 2014). *N*-linked glycosylation is the process of an oligosaccharide chain (Glc3Man9GlcNAc2) transferred onto the asparagine (Asn, N) acceptor site (N-X-S/T) of the protein by the oligosaccharyltransferase (OST) (Khoury et al., 2011). In order to enter the CNX/CRT cycle, glucosidase I (GCS I) and GCS II trim the glucose residues of Glc3Man9GlcNAc2 to form Glc1Man9NAc2, which can be recognized and bound by CNX and CRT. The CNX/CRT binding relationship facilitates protein folding by exposing proteins to protein disulfide isomerases (PDIs). PDI promotes the formation of protein disulfide bonds during ongoing folding, accelerating protein folding (Zapun et al., 1998), which can prevent aggregation and its exit from the ER of the unfolded protein. Subsequently, the last glucose of the oligosaccharides is further removed by GCS II to form Man9GlcNAc2. The oligosaccharides without glucose will separate from the CNX/CRT, at which point the protein faces three possible scenarios (Helenius and Aebi, 2004). (1) If folded correctly, proteins are transferred from the ER to the Golgi complex, where *N*-linked glycans are further trimmed and modified. (2) If folded incompletely, the incompletely folded glycoprotein can be recognized by the molecular chaperone uridine diphosphate (UDP)-glycoprotein glycosyltransferase (UGT) and catalyze its re-glycosylation to form Glc1Man9NAc2 again, which can be re-recognized and refolded by CNX/CRT and PDI. In this process, the glycoprotein remains in CNX/CRT circulation until it is properly folded or degraded, which is also known as the CNX/CRT cycle. Almost all newly synthesized glycoproteins have a transient binding to CNX/CRT. (3) If incorrectly folded, the endoplasmic reticulum degradation-enhanced mannosidase (EDEM) will bind to mannose in misfolded glycoprotein, causing demannosidation of the substrate. The demannosylated substrate is subsequently degraded by ERAD.

The CNX/CRT cycle is a critical step in glycoprotein folding within the ER. The presence of UGT and EDEM provides sufficient time for protein folding to avoid the degradation of folding intermediates, thereby maintaining normal intra-ER protein turnover.

2.2 Molecular chaperone—BiP ATPase cycle

Chaperones are another key component within ER involved in protein folding. Heat shock proteins

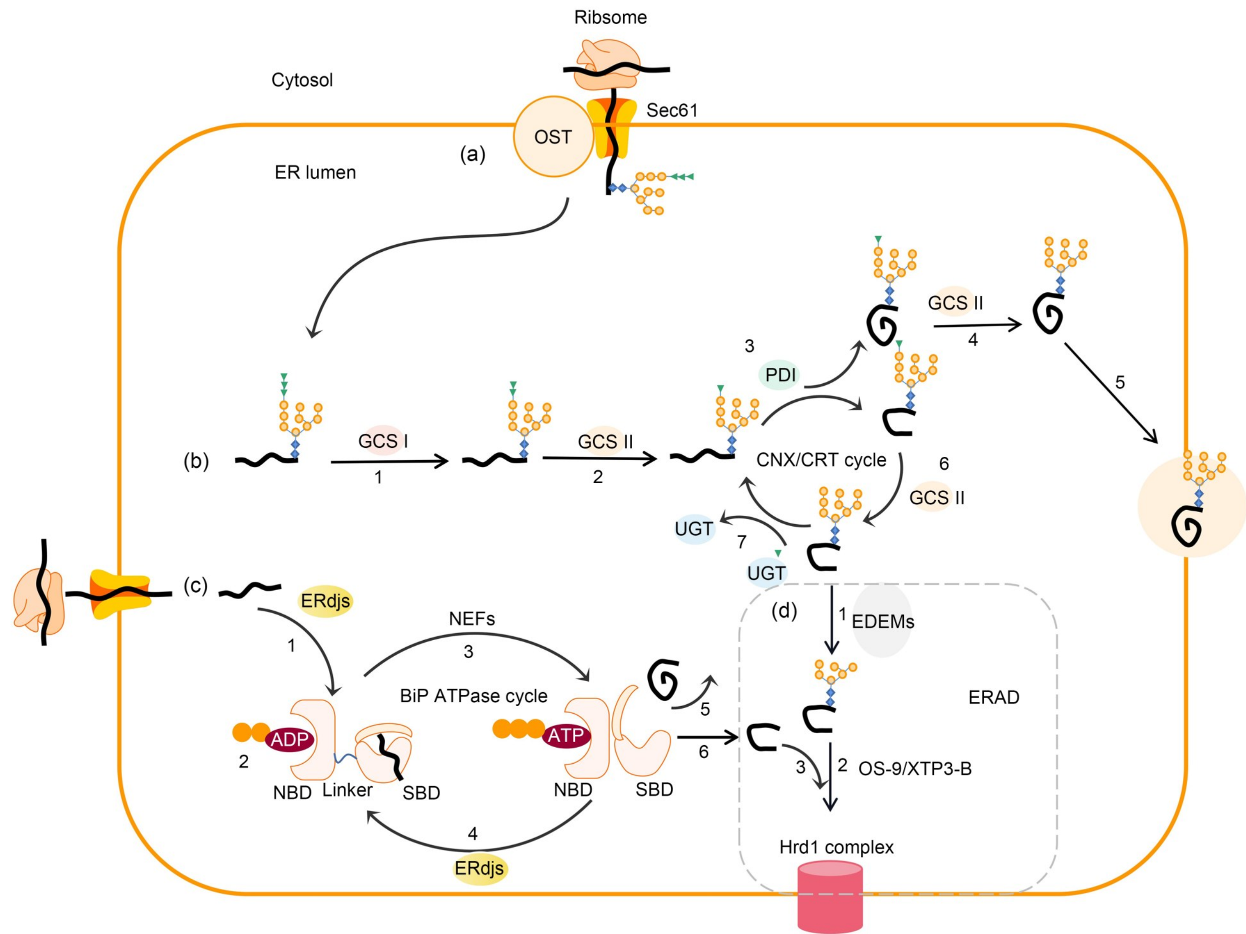

Fig. 1 Process of protein folding: calnexin/calreticulin (CNX/CRT) cycle and BiP adenosine triphosphatase (ATPase) cycle. (a) Ribosomes catalyze protein translation to generate polypeptide chains, which enter the endoplasmic reticulum (ER) lumen through the Sec61 channel and complete *N*-linked glycosylation modifications catalyzed by oligosaccharyltransferase (OST). (b) Glycoproteins enter the CNX/CRT cycle to complete protein folding: (b1) The oligosaccharide unit (Glc3Man9GlcNAc2) was trimmed by glucosidase I (GCS I) to Glc2Man9NAc2; (b2) Glc2Man9NAc2 was trimmed by GCS II to Glc1Man9NAc2; (b3) Glc1Man9NAc2 is recognized by CNX/CRT, and protein folding is completed under the action of protein disulfide isomerase (PDI); (b4) Correctly folded—GCS II keeps trimming Glc1Man9NAc2 to Man9GlcNAc2; (b5) Correctly folded—the substrate separated from CNX/CRT and left the ER; (b6) Incorrectly folded—GCS II keeps trimming Glc1Man9NAc2 to Man9GlcNAc2; (b7) Incorrectly folded—the substrate will be recognized by UGT and UGT catalyzes the substrate re-glycosylation to form Glc1Man9NAc2 and to re-enter the CNX/CRT cycle. (c) Non-glycoproteins enter the BiP ATPase cycle to complete protein folding: (c1) The unfolded protein enters the ER lumen, interacts with ER-localized DnaJ family members (ERdjs), and is transferred to BiP by ERdjs; (c2) BiP promotes protein binding and folding by hydrolyzing adenosine triphosphate (ATP); (c3) Under the action of nucleotide-exchange factors (NEFs), the N-terminal nucleotide-binding domain (NBD) releases adenosine diphosphate (ADP) and rebinds to ATP, which promotes substrate-binding domain (SBD) to release protein substrates; (c4) The unfolded protein interacts with ERdjs and re-enters the BiP ATPase cycle; (c5) The folded protein is released from BiP; (c6) The unfolded proteins enter the endoplasmic reticulum-associated degradation (ERAD) pathway to be degraded. (d) The unfolded proteins enter ERAD: (d1) Endoplasmic reticulum degradation-enhanced mannosidase (EDEM) trims the misfolded glycoproteins, thus exposing mannose residues on glycoproteins; (d2) Osteosarcoma-9 (OS-9) and XTP3-transactivated gene B (XTP3-B) recognize mannose residues exposed by glycoproteins, and initiate ERAD; (d3) BiP transfers the unfolded protein into ERAD. UGT: uridine diphosphate (UDP)-glycoprotein glycosyltransferase; Hrd1: 3-hydroxy-3-methylglutaryl coenzyme A reductase degradation protein 1.

(Hsps) can act as chaperones that assist in the folding of both glycoproteins and non-glycoproteins. Hsps can also promote the degradation of misfolded and unfolded proteins through ERAD, thus playing an important role in protein maturation and quality control. BiP, a member of the Hsp70 family, is an adenosine

triphosphate (ATP)-dependent chaperone protein. BiP has a highly conserved N-terminal nucleotide-binding domain (NBD) and a substrate-binding domain (SBD) with a lid. NBD can combine with ATP and promote its hydrolysis. When NBD hydrolyzes ATP, the energy from ATP hydrolysis closes the lid of SBD, greatly increasing the affinity between SBD and the protein, and promoting protein binding and folding. Subsequently, NBD releases adenosine diphosphate (ADP) and binds ATP under the action of nucleotide-exchange factors (NEFs). In the NBD-ATP-binding form, the lid of SBD keeps open, making the protein affinity of the SBD low and promoting the release of the folded substrate. With NEFs, BiP adenosine triphosphatase (ATPase) can use its SBD to bind, fold, and release proteins, which is also known as the BiP ATPase cycle. In addition, this cycle is regulated by ER-localized DnaJ family members (ERdjs). ERdjs can interact directly with unfolded proteins and transfer the substrate to BiP while triggering NBD-bound ATP hydrolysis (Kityk et al., 2018). Moreover, BiP has another special identity, the allosteric effector of the Sec61 complex (Sicking et al., 2021). The Sec61 channel is a conserved protein conduction channel that mediates some secreted proteins and membrane proteins translocated across or inserted into the ER membrane (Pfeffer et al., 2015). BiP can open the Sec61 complex channels to facilitate the entry of proteins responsible for ribosome synthesis into the ER. The proteins entering the ER are tagged with sugar chains according to their functional conditions and are then folded through the CNX/CRT cycle. Furthermore, the protein can also directly interact with ERdjs to enter the BiP ATPase cycle to start the folding process.

The BiP ATPase cycle and the CNX/CRT cycle are important steps in the ER for folding glycoproteins and non-glycoproteins. Any disorder of the two cycles will lead to the misfolding of proteins, resulting in the aggregation of misfolded proteins. In this regard, the ERAD is used to degrade the misfolded protein to avoid protein aggregation in the ER.

3 ERAD

An overview of ERAD is illustrated in Fig. 2. (1) Unfolded and misfolded proteins are recognized by chaperones and chaperone-like lectins in ER (Fig. 2a). (2) The recognized substrate is transported onto the ERAD complex and is retrotranslocated to the cytoplasm by the ERAD complex and p97 (Fig. 2b). (3) Once the substrate enters the cytoplasm, it is ubiquitinated immediately (Fig. 2c). (4) The ubiquitinated substrate is degraded by the 26S proteasome (Fig. 2d). The main components in the four process are shown in Table S1.

3.1 Substrate recognition

3.1.1 Sugar chain recognition

The key component in the recognition of misfolded glycoproteins is EDEM. EDEM is a mannose enzyme that continuously trims *N*-glycans (Man9GlcNAc2→Man8GlcNAc2→Man7GlcNAc2). The mannose residues (Man5–7GlcNAc2) expose α-1,6-mannose residues on the C branch, which are recognized by ER lectin (osteosarcoma-9 (OS-9)/XTP3-transactivated gene B (XTP3-B)) to initiate glycoprotein ERAD (gpERAD). The slow process of mannose trimming allows substrate proteins a certain amount of time for re-folding (Helenius and Aebi, 2004). This period is known as "mannose timing." When the "timing" is complete, the exposed α-1,6-mannose residues of unfolded substrate can be recognized by ER-lectins OS-9 and XTP3-B, and are recruited to the ERAD complex, which is eventually degraded (Christianson et al., 2008; Alcock and Swanton, 2009). OS-9 and XTP3-B are ER-resident lectins that act as the "gatekeeper" of the ERAD, ensuring that protein substrates undergo repeated folding attempts before the substrate enters ERAD. OS-9 and XTP3-B can interact with suppressor/enhancer of lin-12-like (SEL1L) through the mannose 6-phosphate receptor homologous (MRH) domain (Christianson et al., 2008). SEL1L is an ER resident adaptor protein of the E3 ubiquitin ligase 3-hydroxy-3-methylglutaryl coenzyme A reductase degradation protein 1 (Hrd1), which is involved in the formation of the Hrd1 complex on the ER membrane. Hrd1 complex is a conserved member of the ERAD complex in mammals, and is responsible for the ubiquitination and degradation of misfolded proteins within the ER (Mueller et al., 2008). Briefly, OS-9 and XTP3-B first recognize misfolded glycoproteins and subsequently transfer substrates to the SEL1L (Hrd1 complex component) via the MRH domains of OS-9 and XTP3-B, ultimately initiating gpERAD (Christianson et al., 2008) (Fig. 2a1).

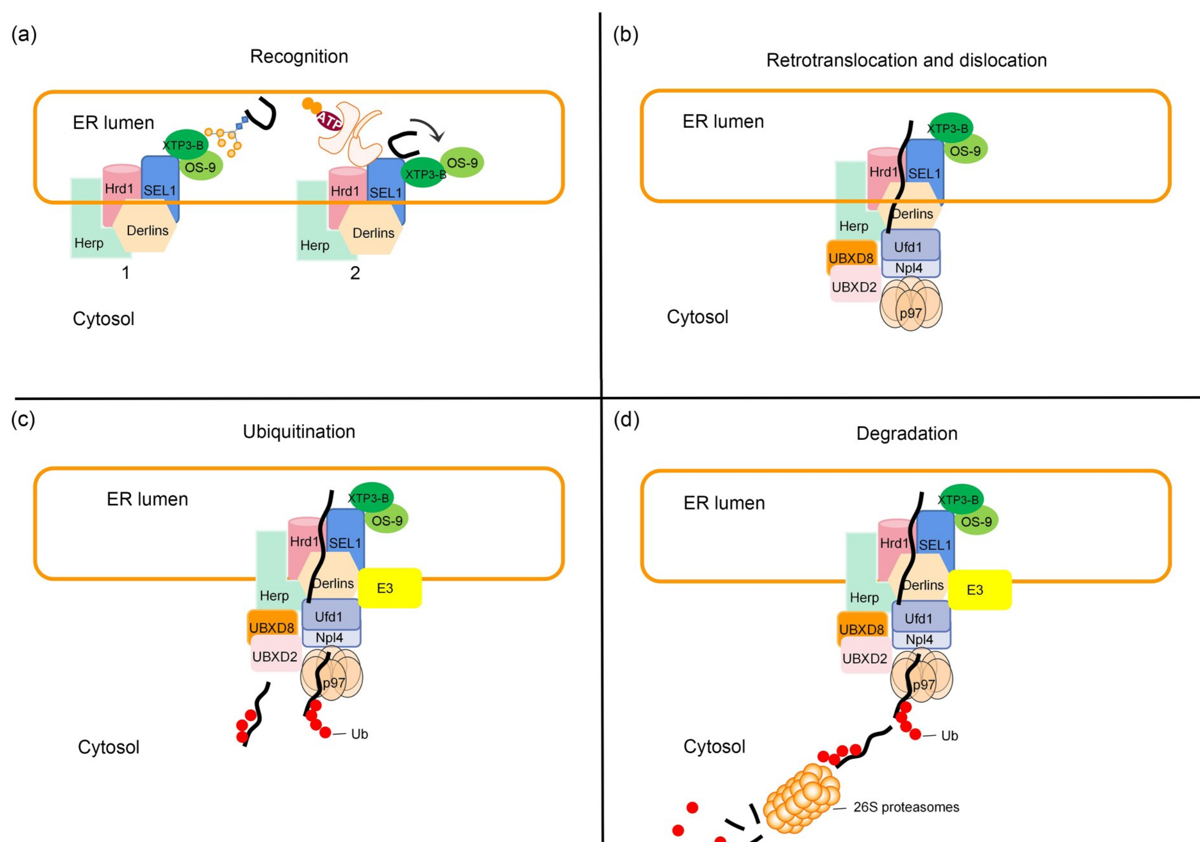

Fig. 2 Brief overview of the endoplasmic reticulum-associated degradation (ERAD) process. (a) Unfolded and misfolded proteins are recognized by chaperones and chaperone-like lectins in endoplasmic reticulum (ER): (a1) Sugar chain recognition; (a2) Hydrophobic amino acid residue recognition. (b) Unfolded and misfolded proteins are localized in the ERAD complex and are retrotranslocated to the cytoplasm driven by the energy from the p97 complex. (c) Once the substrate enters the cytoplasm, it is ubiquitinated. (d) The ubiquitinated substrate is degraded by the 26S proteasome. Herp: homocysteine-induced endoplasmic reticulum protein; Hrd1: 3-hydroxy-3-methylglutaryl coenzyme A reductase degradation protein 1; SEL1: suppressor/enhancer of lin-12-like; XTP3-B: XTP3-transactivated gene B; OS-9: osteosarcoma-9; ATP: adenosine triphosphate; Derlins: Der-like domain-containing family proteins; UBXD2: ubiquitin regulatory X domain protein 2; Ufd1: ubiquitin-fusion degradation protein 1; Npl4: nucleoprotein locates protein 4; E3: E3 ubiquitin ligase; Ub: ubiquitin.

3.1.2 Hydrophobic amino acid residue recognition

Hydrophobic amino acid residues are usually buried deep inside the soluble protein to keep the energy state to a minimum in the natural conformation of proteins (Jahn and Radford, 2005). However, hydrophobic amino acid residues in misfolded and unfolded proteins are exposed to the outside. BiP can bind to hydrophobic short polypeptides to identify exposed hydrophobic regions of misfolded and unfolded proteins or integral membrane proteins (Feige and Hendershot, 2013). With the participation of ERdjs and NEFs, the special domain of BiP (SBD domain) briefly catches unfolded peptide chains and folds the substrate. However, the long-term binding of BiP to the substrate can serve as a signal for initiating ERAD (Määttänen et al., 2010). Previous studies have found that BiP can form an ERQC scaffold complex by combining with XTP3-B and Hrd1 complex, providing a platform for the recognition, and later ERAD, of misfolded glycoprotein and non-glycoprotein (Hosokawa et al., 2008). Furthermore, BiP-bound unfolded and misfolded substrates are transferred to SEL1L and retrotranslocate to cytoplasm by the Hrd1 complex (Ushioda et al., 2013; Peters et al., 2016) (Fig. 2a2).

3.2 Retrotranslocation and dislocation

Substrates are transported to the Hrd1 complex after the recognition of the ER lectin and chaperone. Subsequently, substrates enter the cytoplasm from the

ER (in contrast to the protein secretion pathway) through the protein channel of the Hrd1 complex, driven by the energy from the p97 complex (Fig. 2b). This step is critical because the proteasome is in the cytoplasm, and all substrates in the ERAD need to be retrotranslocated from the ER into the cytoplasm before they are finally degraded by the UPS.

3.2.1 Hrd1 complex

Whether gpERAD or non-gpERAD, substrates are recognized and recruited onto the Hrd1 complex on the ER for reverse transport. The Hrd1 complex in yeast is mainly composed of Hrd1, Hrd3, Der1, Yos9, and Usa1, while the Hrd1 complex in mammals is mainly composed of its homologues: Hrd1, SEL1L, Der-like domain-containing family protein 1–3 (Derlin-1–3), OS-9, and homocysteine-induced endoplasmic reticulum protein (Herp) (Schulze et al., 2005; Carvalho et al., 2006; Oda et al., 2006; Mueller et al., 2008; Preston and Brodsky, 2017). Hrd1, as a core member of the Hrd1 complex, can combine with SEL1L on the ER lumen side (Yagishita et al., 2005). SEL1L binds OS-9/XTP3-B through the MRH domain for targeting substrate onto the Hrd1 complex (Christianson et al., 2008). SEL1L is also the core protein of the Hrd1 complex, and its deficiency directly leads to ER stress and embryonic lethality (Francisco et al., 2010). SEL1L and Hrd1 can maintain the stability of each other and jointly regulate the normal function of the Hrd1 complex (Iida et al., 2011; Christianson et al., 2012). SEL1L is involved in the identification and ubiquitination of most ER luminal substrates by its ER transmembrane domain (Bernasconi et al., 2010; Horimoto et al., 2013; Hosokawa and Wada, 2016). Hrd1 binds to the Hrd1 complex component—Herp on its cytoplasmic side. Herp, a bi-transmembrane protein, acts as a bridge linking the Hrd1 complex and the proteasome, which is essential for the ERAD of non-glycoprotein substrates (Okuda-Shimizu and Hendershot, 2007). Furthermore, Herp can recruit the transmembrane protein Derlin-1 and link Derlin-1 to Hrd1, and then form the complete Hrd1 complex (Eura et al., 2020). Derlins belong to the rhomboid family and consist of Derlin-1, Derlin-2, and Derlin-3. Previous studies have found that Derlin-2 and Derlin-3 are mainly involved in the gpERAD (Oda et al., 2006), while Derlin-1 is mainly responsible for the degradation of non-glyco substrates together with Herp (Okuda-Shimizu and Hendershot, 2007). A recent study revealed that Derlin-2 is located in the curled membrane of viral replicating organelles, which allows Derlin-2 to participate in the ERAD process of regulating the viral non-structural protein in combination with the Hrd1 complex (Tabata et al., 2021), indicating that ERAD plays a crucial role in viral infections.

3.2.2 p97 complex

The driving force for the reverse transport of substrates is mainly provided by the hydrolysis of ATP by the p97 complex (Vasic et al., 2020). After entering the hydrophilic channel, the substrate protein can slide toward the cytoplasm or the ER lumen without directionality. Once the substrate contacts with the cytoplasm, it is ubiquitinated immediately. The addition of ubiquitin chains hinders the sliding of the substrate protein towards the ER lumen and increases the affinity of the substrate protein, which then binds to the recruited p97 complex and drags the protein into the cytoplasm with energy from ATP hydrolysis (Vasic et al., 2020; Zhou et al., 2020).

p97, belonging to the AAA family, is a homohexameric ATP hydrolase located in the cytoplasm. p97 consists of one N-terminal domain and two ATPase domains (D1 and D2). The D1 domain is mainly responsible for ATP binding and p97 hexamer formation, while the D2 domain is mainly responsible for promoting the hydrolysis of ATP (Chou et al., 2014). p97 can act as a segregase to promote substrate delocalization, which is a process that requires the involvement of cofactors as well as the Hrd1 complex. Ubiquitin recognition factor in ER-associated degradation 1 (Ufd1) and nucleoprotein locates protein 4 (Npl4) are p97 cofactors that form a heterodimer and recruit p97 to assemble the p97 complex, thereby mediating the extraction of the protein from the ER membrane into the cytoplasm (Ye et al., 2001; Tsai et al., 2002; Alzayady et al., 2005). Cofactors ubiquitin regulatory X (UBX) domain protein 2 (UBXD2) and UBXD8 can also bind to p97 through the UBX domain. Subsequently, the p97 complex is indirectly recruited to the Hrd1 complex (Ye et al., 2001; Alzayady et al., 2005; Schuberth and Buchberger, 2008; Suzuki et al., 2012). Furthermore, key components of Hrd1 complex (Hrd1, Derlins, and Herp) directly recruit p97 to the dislocation site, facilitating p97 to efficiently capture substrates emerging from the Hrd1 channel (Lilley and Ploegh, 2005; Ye et al., 2005; Carvalho et al., 2010;

Greenblatt et al., 2011). In addition, E3 enzyme glycoprotein 78 (gp78) can recruit p97 complexes by interacting directly with p97 or binding to cofactor Ufd1 through its valosin-containing protein (VCP) interaction motif (VIM) domain (Zhong et al., 2004; Ballar et al., 2006; Cao et al., 2007). Subsequently, the misfolded protein is delocalized and unfolded into the cytoplasmic matrix via the p97 hexameric central channel (Meyer et al., 2012; Blythe et al., 2017; van den Boom and Meyer, 2018).

In summary, the p97 complex is essential for the delocalization of retrotranslocated proteins into the cytoplasm in mammals. The deletion of p97 results in embryonic lethality in mice (Müller et al., 2007), demonstrating that the deletion of important components of ERAD may cause abnormal development in mouse embryos. Multiple cofactors are key components of the body to ensure effective recruitment of p97 for the successful delocalization and degradation of misfolded proteins.

3.3 Ubiquitination

The substrate is ubiquitinated immediately after delocalization into the cytoplasm (Fig. 2c), and the detailed process of ubiquitination is shown in Fig. 3. Ubiquitin (Ub) is a small protein composed of 76 amino acids. The ubiquitination means that ubiquitin can be covalently bound to the lysine (mostly) or serine, threonine, and cysteine (in some cases) of proteins as monoubiquitination or chains (polyubiquitination) (Wang et al., 2007; Ishikura et al., 2010; Shimizu et al., 2010). In the late 1970s, studies found that ubiquitination is important for labelling protein degradation. However, ubiquitin contains seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) and different ubiquitin sites responsible for different functions (Pickart and Fushman, 2004). For example, K11 Ub-linked chains can target ERAD substrates for proteasomal degradation (Xu et al., 2009), while K63-linked ubiquitin chains are usually involved in DNA repair and inflammatory signaling (Pickart and Fushman, 2004).

During ERAD, ubiquitinated substrates facilitate the delocalization of substrates into the cytoplasm by recruiting the p97 complex (Preston and Brodsky, 2017; Zhou et al., 2020). Subsequently, ubiquitinated proteins are specifically recognized and degraded by 26S proteasome. Ubiquitination is critical for ERAD by preventing misfolded proteins from being exported

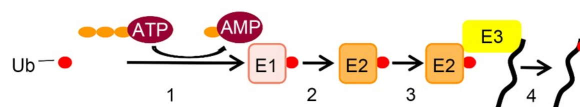

Fig. 3 Ubiquitination process. (1) E1 enzymes activate ubiquitin molecules in an ATP-dependent manner. (2) E1 enzyme transfers ubiquitin to the active cysteine of E2 ubiquitin-conjugated enzyme. (3) E2 enzyme and its bound ubiquitin bind to E3 enzyme. (4) E3 enzyme binds to substrates and transfers ubiquitin to the substrate. Ub: ubiquitin; ATP: adenosine triphosphate; AMP: adenosine monophosphate.

by the ER. When ubiquitination is impaired, the misfolded substrate is exported by ER, which is detrimental to the degradation of the misfolded protein and leads to organismal dysfunction (Sun et al., 2021). Therefore, substrate ubiquitination is an important step in the removal of unfolded and misfolded proteins.

The ubiquitination requires three enzymes—E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugated enzyme, and E3 ubiquitin ligase enzyme. Recent studies have found that ubiquitin chain elongation factor E4 also plays a role in the process of protein ubiquitination. The specific ubiquitination steps are as follows (Preston and Brodsky, 2017). (1) E1 enzymes activate ubiquitin molecules in an ATP-dependent manner. ATP hydrolysis and adenosine monophosphate (AMP) derivative formation lead to the formation of a thioester bond between the E1-activating enzyme and the C-terminus of ubiquitin, which activates ubiquitin. (2) E1 enzyme transfers ubiquitin to the active cysteine of E2 ubiquitin-conjugated enzyme via a trans-thio reaction. (3) E2 enzyme and its bound ubiquitin bind to E3 enzyme. (4) E3 enzyme binds to substrates and promotes the formation of an isopeptide bond between ubiquitin and the lysine residue on the substrate. This process can only add one ubiquitin molecule on the substrate. (5) The ubiquitin chain on the ERAD substrate can be further extended by ubiquitin chain elongation factor E4 or E2 enzyme.

3.3.1 E2 ubiquitin-conjugated enzyme

In mammals, ubiquitin-like modifier activating enzyme 1 (UBA1) is the predominant E1 ubiquitin-activating enzyme responsible for initiating more than 99% of the ubiquitination cascade reactions (Barghout and Schimmer, 2021; Borgo et al., 2022). Currently, a small number of ERAD-related E2 ubiquitin-conjugating enzymes have been identified, including

ubiquitin-conjugating enzyme E2 J1 (UBE2J1)/UBC6e, UBE2J2 (Wang et al., 2009; Hagiwara et al., 2016), UBE2D1/UBCH5a (Nadav et al., 2003; Younger et al., 2004; Kaneko et al., 2010), UBE2G2 (Chakrabarti et al., 2017), and UBE2K (Flierman et al., 2006).

E2 enzyme is the key enzyme that links ubiquitin to E3 enzyme after ubiquitin activation, which is an important step in ERAD. Recent studies have shown that certain E2 enzymes can be involved in the degradation of ERAD components, thus providing quality control of ERAD. Therefore, E2 enzymes are an important component in maintaining protein turnover within the ER.

3.3.2 E3 ubiquitin ligase enzyme

Up to 20 ERAD-related E3 ubiquitin ligases have been identified in mammals. Among them, the most studied are the really interesting new gene (RING) finger domain-containing E3 ubiquitin ligases: Hrd1 (Kaneko et al., 2002), gp78 (Fang et al., 2001), membrane-associated RING-CH-type finger VI (MARCH VI, also called TEB4) (Bartee et al., 2004), and carboxyl terminus of heat-shock cognate protein 70 (Hsc70)-interacting protein (CHIP) (Jiang et al., 2001).

Hrd1, also known as Synoviolin, is the first E3 enzyme identified in mammals that is involved in the ERAD process. Studies have found that Hrd1 is a joint pathogenic factor associated with RA (Amano et al., 2003). Hrd1 forms a ubiquitin ligase complex with SEL1L, Derlins, Herp, and OS-9. Hrd1 complex plays an important role in the identification and reverse transport of substrates, and ubiquitination. Ubiquitination targets of Hrd1 include lipid metabolizing enzyme ATP citrate lyase (ACLY) (Li et al., 2021), cholesterol synthesis key enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMF-CoA) reductase (Hampton et al., 1996), unfolded protein response (UPR) sensor inositol-requiring enzyme 1α (IRE1α) (Sun et al., 2015), liver transcription factor cyclic AMP (cAMP)-responsive element-binding protein H (CREBH) (Wei et al., 2018), bone calcium homeostasis-related factor sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2) (Wei et al., 2022), T cell receptor α (TCR-α), and cluster of differentiation 3-δ (CD3-δ) (Kikkert et al., 2004). The RING finger domain in the C-terminal cytoplasmic tail of Hrd1 is a key structure for the E3 enzyme function. Hrd1 binds to the E2 enzyme by virtue of this structural domain, and transfers activated ubiquitin from E2 to the substrate, finally completing ubiquitination (Miyamoto et al., 2019). In general, Hrd1 is the core protein of the Hrd1 complex, dominating the reverse translocation and ubiquitination of substrates.

Transmembrane protein gp78 is a core mediator in ERAD. gp78, also known as autocrine motility factor receptor (AMFR), was originally identified as an AMFR-mediating cancer cell motility and metastasis (Nabi and Raz, 1987). It was found that gp78 is also a RING finger depending on E3 ubiquitin-linked enzyme (Fang et al., 2001). Cumulative evidence suggests that common targets of gp78 include CD3-δ (Fang et al., 2001), HMF-CoA reductase (Song et al., 2005), insulin-induced gene 1 (Insig-1) (Song et al., 2005; Lee et al., 2006), pigment protein P450 (Kwon et al., 2019), and apolipoprotein B100 (apoB100) (Liang et al., 2003), autocrine motility factor (Wang et al., 2014), deletion of phenylalanine 508 of cystic fibrosis transmembrane conductance regulator (CFTR) (Vij et al., 2006), and virus-induced-signaling adapter (VISA) protein (Luo et al., 2017). The domains of gp78 are complex, including RING domain, coupling of ubiquitin conjugation to endoplasmic reticulum degradation (CUE) domain, and UBE2G2-binding region (G2BR) domain. The CUE domain, as a conserved region with about 40 amino acids, is a UBD responsible for E2–E3 interaction (Ponting, 2000; Bagola et al., 2013). gp78 specifically binds substrates in a CUE domain-dependent manner. The CUE domain can also drive the assembly of polyubiquitin chains at the active site of UBE2G2, facilitating further extension of the ubiquitin chain (Liu WX et al., 2014). In addition, G2BR domain confers a unique mechanism by which gp78 binds to the E2 enzyme UBE2G2. The G2BR domain can bind specifically to UBE2G2 in a "back-to-back" form, which can change UBE2G2 structure, dramatically increasing the affinity between E2 and RING finger domains (Das et al., 2009). Moreover, the G2BR domain can also promote ubiquitin transfer from E2 by binding to the donor UBE2G2-Ub and by participating in ubiquitin chain synthesis (Liu WX et al., 2014). Therefore, all three domains of gp78 are indispensable. Disruption of either domain results in abnormal ubiquitination and the degradation of substrate proteins mediated by gp78 (Chen et al., 2006).

3.4 Proteasome degradation

After ubiquitination labeling in the cytoplasm, the substrate will undergo several processes such as deglycosylation and deubiquitination. Then substrate is finally bound by the ubiquitin-binding protein and presented to the 26S proteasome for degradation (Fig. 2d). Proteasomes can rely on the myristoylation of regulatory particle AAA-ATPase 2 (Rpt2) to anchor on ER membranes in order to better perform the function of UPS (Zhang et al., 2023).

The proteasome is a multisubunit complex, also known as the 26S proteasome because of its 26S sedimentation coefficient, consisting mainly of the 20S core particle (CP), and the 19S regulatory particles (RPs). The 20S CP is a barrel-shaped structure with an internal chamber for protein hydrolysis sites with caspase-like, trypsin-like, and chymotrypsin-like activity. This chamber is responsible for the degradation of proteins entering from the 19S RP translocation into 3 to 22 polypeptides (Tanaka, 2009). The 19S RP is located above the 20S CP and is essential for the proteasome to recognize polyubiquitinated proteins, unfold them, and control their entry into the 20S chamber. 19S RP contains six regulatory particle AAA-ATPases (Rpt1–6) and 13 non-ATPase subunits (regulatory particle non-ATPases, Rpns) (Eisele et al., 2018). The proteasome binds ubiquitinated substrates through the key subunit Rpn10 as well as other ubiquitin-binding proteins. Then, the bounded substrate is subsequently carried to the AAA-ATPase on 19S RP by binding to proteasome subunits such as Rpn1 (Elsasser et al., 2002). The six AAA-ATPase subunits of 19S RP form a heterohexameric ring overlying 20S. Once the ubiquitin chain is near the six AAA-ATPase ring pores, Rpn11 immediately translocates to complete the trimming of the chain to allow the protein to pass through the pore. As the protein passes through the narrow pore, the ATPase can also unfold the protein by hydrolyzing ATP, promoting proteins entering into the 20S hydrolysis lumen to complete hydrolysis (Davis et al., 2021).

In brief, the process of proteasomal degradation of ubiquitination is divided into two main stages: substrate binding (substrate recognition, deubiquitination, and unfolding) and substrate degradation. After completing a round of degradation, the proteasome may return to a quiescent state and wait for a new round of substrate binding and degradation. Proteasomal degradation is the final step in the ERAD process, and its efficient degradation properties can effectively remove misfolded and unfolded proteins, and maintain intracellular protein and amino acid homeostasis.

3.4.1 Deglycosylation

After the substrate protein enters the cytoplasm and is ubiquitinated, it is then transported to the 26S proteasome for degradation. For misfolded glycoprotein, deglycosylation is needed after ubiquitination and then enters the proteasome to complete degradation (Hirsch et al., 2003). The cytosolic peptide: *N*-glycanase (PNGase) is in charge of the deglycosylation process for the glyco-substrate. *N*-Glycanase 1 (NGLY1), a PNGase in mammals, is the most important PNGase for misfolded glycoprotein. NGLY1 is located in the cytoplasm and possesses three important domains: PNGase and ubiquitin (PUB), PNGases and other worm (PAW), and transglutaminase (TGase) domains. The PAW domain can specifically bind high mannose glycans of unfolded or misfolded glycoproteins. The TGase domain can cleave β-aspartyl-glucosamine bonds in glycoproteins to release free Man5–9GlcNAc2 and deglycosylated proteins. PUB domain is a UBD that can form a ternary complex of p97 complex, gp78, and proteasome. This ternary complex can promote substrate protein dislocate into cytoplasm and then progressively complete ubiquitination, deglycosylation, and proteasome degradation (Li et al., 2005, 2006).

PNGase can act as a bridge linking the p97 complex, E3 enzyme, and proteasome, allowing the glycoprotein substrate to be delocalized into the cytoplasm, and is immediately ubiquitinated and recognized by the proteasome. In addition, PNGase can also maintain proteasome homeostasis by balancing the stability of nuclear respiratory factor 1 (NRF1) that is a key regulator of 26S proteasome formation (Yoshida et al., 2021). In conclusion, PNGase plays a facilitating role in the ERAD process of misfolded, unfolded protein substrates in multiple ways.

3.4.2 Deubiquitination

When the substrate completes polyubiquitination, it will carry long ubiquitin chains. These too-long ubiquitin chains restrict the substrate from entering the proteasome, so some deubiquitinases (DUBs) are needed to trim the ubiquitin chains for substrate degradation by the proteasome. DUBs are proteases

that cleave ubiquitin or ubiquitin-like proteins. Human DUBs are divided into five families with different domains: ubiquitin-specific protease (USP), JAB1/MPN/Mov34 metalloenzyme (JAMM), ovarian tumor (OUT), Josephin, and ubiquitin C-terminal hydrolase (UCH) families (Reyes-Turcu et al., 2009). DUBs can bind directly or indirectly to the important ERAD components p97, Hrd1, gp78, etc. and play an important regulatory role in the ERAD process (Sowa et al., 2009). Rpn11, the subunit on the proteasome, is the JAMM domain DUBs and is the key to the degradation of ubiquitinated proteins into the proteasome. Rpn11 can trim from the base of the chain of proteasome-bounded ubiquitinated proteins, facilitating the unfolding of the protein and transferring through a narrow constriction into the proteasome to complete degradation (Yao and Cohen, 2002; Lee et al., 2011).

However, the other DUBs may perform deubiquitination before the substrate makes contact with the proteasome. For example, USP14 and UCH37 are trimmed from the distal end of the ubiquitin chain, and the breaking of the ubiquitin chain promotes the detachment of the substrate from the proteasome and exerts antagonistic proteasomal degradation (Lee et al., 2011). This deubiquitination can exert quality control over the amount of ubiquitinated protein to avoid incorrect ubiquitination and to avoid proteasome functional overload as well.

In mammals, multiple DUBs act in concert to regulate the ubiquitination status of the substrate to balance intracellular protein turnover. It also promotes intracellular ubiquitin recycling and avoids cytotoxicity due to ubiquitin depletion (Hanna et al., 2003). DUBs perform quality control of ubiquitinated substrates for ER delocalization, trimming ubiquitin chains on proteins that should not be ubiquitously labeled to ensure ERAD efficiency (Jung et al., 2015). On the other hand, the ubiquitinated substrates trimmed by different DUBs have different outcomes with the proteasome (release or bind), which balance each other to avoid the accumulation of excessive ubiquitinated substrates and the impairment of proteasome function. In addition, some DUBs can also associate with p97 and participate in the retrotranslocation of substrate.

3.4.3 Ubiquitin-binding proteins

Ubiquitin-binding proteins play a key role in the binding of ubiquitinated substrate for recognition by the proteasome. Rpn10, also known as S5a, is a ubiquitin-binding protein located in the proteasome (van Nocker et al., 1996). Rpn10 contains two ubiquitin-interacting motifs (UIMs), which are mainly responsible for binding ubiquitin chains. In addition to Rpn10, there are also ubiquitin-binding proteins that are not located on the proteasome. These proteins shuttle between ubiquitinated proteins and the proteasome to promote proteasomal degradation of ubiquitinated substrates. These conserved proteins are known as the shuttle family, also called the ubiquitin-like (UBL)-ubiquitin-associated (UBA) family because of their specific domain characteristics. The UBL-UBA ubiquitin receptor family contains a unique UBA domain that binds ubiquitin, and a UBL domain that binds proteasomes (Wilkinson et al., 2001; Elsasser et al., 2002). Yeast Rad23 (human hHR23a/b), yeast Dsk2 (human hPLIC-1/2), and yeast Ddi1 (human DDI2) all belong to the UBL-UBA family (Kleijnen et al., 2000; Chen and Madura, 2002).

The ubiquitin-binding proteins promote substrate proteasomal degradation by binding ubiquitinated proteins and transferring them to the proteasome. Multiple ubiquitin-binding proteins may play upstream and downstream or fulfil parallel roles acting as a linking bridge between ubiquitinated substrates and the proteasome to ensure proper intracellular turnover of ubiquitinated substrates. Among them, DDI2 can play a special role other than binding ubiquitin. DDI2 exerts protein hydrolase activity to assist the proteasome in the degradation of intracellular ubiquitinated substrates, and also enhances proteasome activity by cleaving NRF1 when proteasome function is impaired.

4 ERAD quality control

4.1 ERAD tuning

ERAD is a critical process for maintaining protein quality within the ER. Any abnormalities in ERAD components can lead to the disruption of intracellular protein homeostasis, which in turn can lead to the development of related diseases. In response, the body derived ERAD tuning—without stress stimuli, important components of the ERAD are selectively degraded. ERAD tuning can maintain low levels of ERAD capacity to protect the normal folding of non-native folding intermediates and avoid premature

interruption of the ongoing folding process (Calì et al., 2008). Many of the important components of ERAD are short-lived proteins in the normal unstimulated state, and some of them are also substrates of ERAD. UBE2J1, Hrd1, gp78, Smad ubiquitylation regulatory factor 1 (SMURF1), ataxin-3, and OS-9 can enter the ubiquitin-proteasome degradation system by self-ubiquitination or by ubiquitination thorough E3 enzymes (Hassink et al., 2005; Ying et al., 2009; Ballar et al., 2010; Xie et al., 2013; Chen et al., 2016; Ye et al., 2018; Peterson et al., 2019). The degradation of SEL1L and Herp is also proteasome-dependent (Hori et al., 2004; Mueller et al., 2006). In addition, key chaperones that recognize unfolded and misfolded proteins, such as EDEM1 and OS-9, are delivered to the lysosomal compartment for degradation via ER-derived vesicles, known as EDEMosomes (Reggiori et al., 2010).

ERAD tuning is a way for cells to modulate the activity of ERAD components in unstimulated situations, which can provide sufficient time for protein folding and avoid excessive ERAD. This is not set in stone either. ERAD function can be enhanced by UPR regulation when cells are under stress with a large accumulation of unfolded or misfolded proteins. In stress situations, ERAD tuning is turned off until the problem is resolved (Rutkowski and Hegde, 2010; Bernasconi and Molinari, 2011).

4.2 ERAD component-mediated regulation of ERAD

ERAD tuning occurs mainly through the degradation of ERAD components to maintain the capacity of ERAD in its physiological state. ERAD tuning can avoid excessive ERAD leading to massive degradation of proteins in the folded intermediate state within the ER, resulting in the disruption of proteostasis. ERAD is an extremely intelligent process. In addition to tuning to reduce ERAD levels, it is also possible to adapt to the body's demand for ERAD levels through dynamic self-regulatory effects between components.

4.2.1 EDEM1-mediated control of ERAD homeostasis

EDEM1 can combine with OS-9, SEL1L, and Hrd1 to form a dynamic self-regulatory complex (Chiritoiu et al., 2020). When SEL1L is silenced, EDEM1 and OS-9 levels can be autoregulated to be dynamically elevated, while XTP3-B and Hrd1 expression is dynamically reduced to maintain the normalization of ERAD function mediated by the complex. Of particular note, EDEM1 plays a key role in the recognition and targeting of Hrd1 complexes by glycoproteins and non-glycoproteins. EDEM1 can also promote ER autophagy to degrade the massive accumulation of protein aggregates within the ER and maintain ER homeostasis when proteasome function is severely impaired.

4.2.2 E2 enzyme UBE2J1-mediated control of ERAD homeostasis

Under normal conditions, ERAD enhancers EDEM1, EDEM3, OS-9, and SEL1L are short-lived proteins that are controlled by E3 enzyme-mediated proteasomal and EDEMosome-mediated lysosomal degradation. However, Hagiwara et al. (2016) found that the E2 enzyme UBE2J1 can also play a role in the homeostatic regulation of ERAD. UBE2J1 can form supramolecular complexes with Derlin-2 to target ERAD enhancers EDEM1, OS-9, and SEL1L for degradation under physiological conditions, thereby maintaining endogenous levels of ERAD. Knockdown of UBE2J1 enhances the level of ERAD components in a UPR-independent manner, thereby promoting ERAD function.

ERAD components (EDEM1 and UBE2J1) can not only play an enhancer role in the ERAD process but also act as functional molecules involved in regulating the level of ERAD, maintaining the protein turnover and homeostasis within the ER.

5 ERAD in common diseases

ERAD dysfunction can lead to the disruption of ER protein homeostasis. The dysfunction of some key molecules in ERAD will specifically affect the occurrence and progression of certain diseases such as multiple sclerosis (MS), AD, Huntington's disease (HD), and ALS.

5.1 Immune inflammatory diseases

5.1.1 Rheumatoid arthritis

RA is a chronic autoimmune disease characterized primarily by synovial cell infiltration and hyperplasia, and diffuse inflammation in the joint. Researchers believe that RA is a hyper-ERAD disease

(Yamasaki et al., 2005). As a key molecule in ERAD, Hrd1 was first revealed as a joint pathogenic factor associated with RA (Amano et al., 2003). Hrd1 overexpression in mice will lead to the development of spontaneous arthritis, while knockout of Hrd1 protects mice from RA. Further research found that Hrd1 can act as an E3 enzyme to mediate the ubiquitination degradation of IRE1α and p53 to antagonize ER stress-induced cell death within RA, leading to synovial cell overgrowth (Yamasaki et al., 2007; Gao et al., 2008). RA is characterized by the excessive proliferation and anti-apoptosis of synovial cells. The survival of synovial cells depends on the continuous removal of proteins by the UPS degradation pathway. The excessive proliferation and anti-apoptosis of RA synovial cells can be alleviated by inhibiting the proteasome (van der Heijden et al., 2009; Connor et al., 2012).

In conclusion, Hrd1 and proteasome dysfunction can regulate the proliferation of synovial cells and the production of inflammatory factors, thereby affecting the disease progression of RA.

5.1.2 Multiple sclerosis

MS is a chronic autoimmune inflammatory disease characterized by leukotic demyelinating lesions of the central nervous system.

Hrd1 and its adaptor SEL1L also play a key role in MS. First, it was found that the expression of Hrd1 in the T-cells of MS patients was higher than that of healthy individuals. Then, the knockdown of Hrd1 inhibits T-cell proliferation and the activation and differentiation of T helper 1 (Th1) and Th17 cells. These protect mice from damage from experimental autoimmune encephalomyelitis (EAE) (Xu et al., 2016). Hrd1 is a positive regulator of T-cell immunity, but SEL1L acts slightly differently. The study found that the knockout of T-cell-specific SEL1L promoted Th1/Th17-cell differentiation, making it more vulnerable to EAE (Yao et al., 2022). In this way, SEL1L may be a negative regulator of T-cell immunity. Continuous research is needed to refine the understanding of the regulatory differences between Hrd1 and SEL1L on T-cell immunity.

5.2 Neurodegenerative diseases

The causes of neurodegenerative diseases, such as AD, HD, and ALS, are related to the accumulation of degenerative proteins in the brain. As an important mechanism for the degradation of misfolded proteins in the body, ERAD impairment is one of the causes of such diseases.

5.2.1 Alzheimer's disease

AD is a common degenerative central nervous system disorder in older people, characterized by the formation of *β*-amyloid (Aβ) plaques. The large accumulation of Aβ can lead to abnormal phosphorylation of tau protein, forming highly phosphorylated tau tangles. These two large accumulations together cause neurotoxicity, impaired blood flow in the brain, and cognitive destruction (Noble et al., 2013; Albrecht et al., 2020).

Aβ is produced by amyloid precursor peptide (APP) by degradation of α-, β-, and γ-secretases. ERAD can affect the occurrence and development of AD by participating in the degradation of APP. Hrd1 and EDEM1 could specifically recognize APP and promote its ERAD degradation, thereby inhibiting the generation and aggregation of Aβ (Kaneko et al., 2010; Nowakowska-Gołacka et al., 2021). In addition, the degradation of full-length APP can be inhibited after inhibition of proteasomes (Jung et al., 2015). The level of Hrd1 protein in the cerebral cortex of AD patients is significantly reduced. The loss of Hrd1 also leads to the accumulation of APP and the production of Aβ (Kaneko et al., 2010). In addition, there are other components of ERAD that are also involved in regulating Aβ production. ER protein membralin is also a component of ERAD, which can bind and promote the ERAD of nicastrin, a key component of γ-secretase (Zhu et al., 2017), thereby inhibiting γ-secretase activity and indirectly affecting the production of Aβ and the development of AD (Zhu et al., 2017). ERAD is also regulated by tau protein, which affects the development of AD. On the one hand, a large amount of accumulated tau proteins can interact abnormally with p97 and Hrd1, thereby damaging ERAD and causing ER stress and cell death (Abisambra et al., 2013). On the other hand, the impaired function of ERAD will lead to abnormal metabolism of APP, resulting in the formation of Aβ plaques. Aβ plaques further promote the formation of highly phosphorylated tau tangles, exacerbate neurotoxicity, and further worsen the course of AD.

In conclusion, ERAD can reduce the formation of Aβ plaques by regulating the degradation of APP,

thereby reducing phosphorylated tau tangles and comprehensively regulating the disease process of AD.

5.2.2 Huntington's disease

HD is an inherited neurodegenerative disease that is mainly caused by neuronal toxicity caused by polyglutamine (polyQ) amplification in huntingtin protein (HTT). Oxidative stress, transcriptional dysregulation, mitochondrial dysfunction, and ERAD dysfunction can all lead to abnormal amplification of polyQ (Roze et al., 2008; Tydlacka et al., 2008). Physiologically, HTT can complete ERAD through colocalization of UPS and inclusions. However, polyQ abnormal amplification protein can capture ERAD component proteins Ufd1, Npl4, and p97. This will inhibit the normal progression of ERAD, leading to the further accumulation of mutant HTT to accelerate the development of HD disease. In addition, HTT can also hinder the degradation of HTT by ERAD by inhibiting the binding of gp78 to ubiquitin and p97.

In conclusion, ERAD can degrade polyQ abnormally-amplified HTT, thereby regulating the course of HD. However, denatured HTT also inhibits the normal progression of ERAD and avoids its own degradation.

5.2.3 Amyotrophic lateral sclerosis

ALS is a motor neuron degeneration disease affecting upper and lower motor neurons in the brain stem, cortex, and spinal cord (Geevasinga et al., 2016). There are many pathogenic factors of ALS, including protein misfolding and aggregation, ER stress, mitochondrial dysfunction, ubiquitin proteasome dysfunction, and genetic mutations (Peters et al., 2015). The intracellular, insoluble inclusions composed of misfolded proteins are hallmarks of early ALS pathology (Kanning et al., 2010). Ubiquilin 2-positive inclusions are widely found in ALS patients (Zhang et al., 2014). Ubiquilin 2 (*UBQLN2*) is one of the common mutated genes in the ALS family. Ubiquilin 2 is a member of the ubiquitin-like protein family that mediates the degradation of ubiquitin protein delivery proteasomes and plays a key role in ERAD. However, ALS-associated ubiquilin 2 inhibits the entry of ERAD substrates into the proteasome by binding to the p97 cofactor UBXD8, thereby exacerbating the accumulation of misfolded proteins (Xia et al., 2014). Cu/Zn-superoxide dismutase 1 (*SOD1*) is one of the common mutated genes of family ALS. Mutated *SOD1* inhibits ERAD function by specifically interacting with Derlin-1, leading to ER stress and cell death (Nishitoh et al., 2008). *VCP*/*p97* is also one of the common mutated genes of the ALS family (Johnson et al., 2010). p97 has also been shown to regulate ERAD retrotranslocation. The mutant *p97* will definitely destroy the ERAD process.

Genetic mutations are a common cause of familial ALS. After gene mutations, some genes such as *SOD1* and *p97* can impair ERAD function, resulting in further accumulation of misfolded proteins that aggravate ALS disease.

5.3 Cardiovascular diseases

The dynamic balance of protein turnover is required for cardiac homeostasis. The heart undergoes remodeling (cardiac hypertrophy) in response to changes in physiological demand or disease state, where increased protein synthesis is inevitable. Therefore, for protein quality control purposes, the demand for ERAD of misfolded and unfolded proteins is increased in cardiac hypertrophy. The impaired ERAD function will contribute to cardiovascular diseases such as myocardial hypertrophy, heart failure, and long QT syndrome (LQTS).

5.3.1 Myocardial hypertrophy and heart failure

Myocardial hypertrophy is classified as either physiological hypertrophy or pathological hypertrophy. Here, we focus on the pathological hypertrophy. While myocardial hypertrophy has a beneficial effect in the short term to balance the wall stress, long-term myocardial hypertrophy can lead to heart failure. High protein turnover is needed in myocardial hypertrophy, and high ERAD function is also needed to maintain the quality of protein.

The loss-of-function of ERAD element Hrd1 will decrease the level of ERAD and exacerbate pathological cardiac hypertrophy in response to pressure overload (Doroudgar et al., 2015). However, researchers found that canonical ERAD must not always be directly related to alleviating pathological cardiac hypertrophy. The ERAD-out is a new noncanonical form of ERAD that can also mediate the level of cardiac hypertrophy (Blackwood et al., 2023). Pharmacological inhibition of ERAD constituents (proteasome activity) can either prevent or induce hypertrophy according to

the dose, duration, and type of inhibitors. Short term inhibition may mediate a protective effect in the heart, whereas sustained inhibition may be toxic and deteriorate hypertrophy (Pagan et al., 2013).

Heart failure is the dysfunction of the heart due to systolic and/or diastolic function, and when the heart is unable to pump enough blood and oxygen to support the body's organs. Long-term myocardial hypertrophy can lead to heart failure. Therefore, the disease mechanism of heart failure is also closely related to ERAD. A large number of studies have found that there is insufficient ERAD in heart failure, resulting in abnormal aggregation of misfolded proteins. The application of bortezomib (proteasome inhibition) results in heart failure and death in mice induced by transverse aortic constriction (TAC) (Carrier, 2010). The above studies support the concept that defective ERAD contributes to heart failure (Predmore et al., 2010; Day, 2013). On the other hand, pharmacological enhancement of the ERAD component function (proteasome function) of cardiomyocytes can effectively reduce the accumulation of ubiquitinated proteins and abnormal protein aggregation in the heart, thereby slowing down heart failure progression (Ranek et al., 2013).

In conclusion, ERAD function is highly related to myocardial hypertrophy and heart failure. Treatments targeting ERAD to relieve disease progression need to be studied in the future.

5.3.2 Long QT syndrome

LQTS is a heart signaling disorder characterized by prolongation of the Q-T interval on electrocardiogram (ECG) and ventricular arrhythmias (Crotti et al., 2008). Human ether-a-go-go-related gene (*hERG*, also known as *KCNH2*) encodes the voltage-gated K (Kv) channel α-subunit Kv11.1. The mutation or loss of function of *hERG* decreases rapid delayed rectifier potassium current (I_{Kr}), which leads to type2 LQTS (LQT2) (Curran et al., 1995). *hERG* mutation will decrease the folding efficiency of Kv11.1 proteins, and the misfolded Kv11.1 proteins are retained in the ER until they are degraded in the ERAD. CNX and CRT have an association with hERG mutant protein and contribute to their degradation in ERAD pathway (Wang et al., 2012). In addition, Hsp70 (BiP) and Hsp90 can interact directly with the core-glycosylated form of unfolded hERG proteins and increase their degradation in the ERAD (Smith et al., 2016). Hsp70 NEF Bag1 promotes hERG degradation by the ERAD pathway. Meanwhile, E3 enzyme translocation in renal carcinoma on chromosome 8 protein (TRC8) acts as Hsp70-independent E3 ligase for hERG ERAD (Hantouche et al., 2017). A recent study showed that, in post-ER compartments, the misfolded hERG can also be rapidly endocytosed from the plasma membrane via a ubiquitin-independent mechanism and be rapidly targeted for lysosomal degradation (Foo et al., 2019).

In conclusion, the abnormal trafficking or degradation of misfolded hERG is the primary mechanism of LQT2, and CNX, CRT, Hsp70, Hsp90, and TRC8 are closely related with misfolded hERG degradation in the ERAD pathway.

6 Concluding remarks

The ER is a key site for protein production and quality control in vivo. The degradation of abnormally folded proteins by the ER is an important mechanism for maintaining protein homeostasis in the ER. However, in the process of life, cells are inevitably exposed to some adverse environments. Hypoxia, toxic stimuli, and the accumulation of large amounts of ROS can lead to the accumulation of large amounts of abnormally folded proteins. The accumulation of a large number of abnormally folded proteins in the ER leads to increased ER stress. In response, the ER can enhance ERAD function through UPR to eliminate excess protein accumulation. ERAD is a key program for maintaining proteostasis within the ER. So far, the detailed description of the mechanism of ERAD in mammals is not comprehensive enough. This is also the original intention of this article. It is hoped that by describing the detailed mechanism of ERAD in mammals, scholars can better understand the basic content and research status of ERAD, the abnormal protein folding problem, and the pathogenesis of related diseases, in order to provide therapeutic targets for subsequent clinical treatment. The description of key ERAD molecules in this paper is still only based on existing studies, and the role of some key ERAD components in ERAD needs to be continuously explored in the future.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 82071762), the Shanghai Key Lab of Human Performance (Shanghai University of Sport) (No. 11DZ2261100), and the 2021 Capacity Building of Shanghai Universities (No. 21010503600), China.

Author contributions

Hong CAO performed the conceptualization, validation, and writing – original draft preparation; Xuchang ZHOU contributed to the methodology; Jianming GUO and Nan LI performed the investigation; Miao WANG contributed to the resources; Han HU, Bowen XU, and Yuwei MA performed the writing – review and editing; Jun ZOU and Nan LI contributed to the funding acquisition. All authors have read and approved the final version of the manuscript.

Compliance with ethics guidelines

Hong CAO, Xuchang ZHOU, Bowen XU, Han HU, Jianming GUO, Yuwei MA, Miao WANG, Nan LI, and Jun ZOU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Table S1