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Protein Folding and Quality Control in the ER

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The endoplasmic reticulum (ER) uses an elaborate surveillance system called the ER quality control (ERQC) system. The ERQC facilitates folding and modification of secretory and membrane proteins and eliminates terminally misfolded polypeptides through ER-associated degradation (ERAD) or autophagic degradation. This mechanism of ER protein surveillance is closely linked to redox and calcium homeostasis in the ER, whose balance is presumed to be regulated by a specific cellular compartment. The potential to modulate proteostasis and metabolism with chemical compounds or targeted siRNAs may offer an ideal option for the treatment of disease.

The endoplasmic reticulum (ER) serves as a protein-folding factory where elaborate quality and quantity control systems monitor an efficient and accurate production of secretory and membrane proteins, and constantly maintain proper physiological homeostasis in the ER including redox state and calcium balance. In this article, we present an overview the recent progress on the ER quality control system, mainly focusing on the mammalian system.

TRANSLATION OF ER-TARGETED PROTEINS

Most mammalian secretory and membrane proteins are cotranslationally imported into the ER (Fig. 1A). Signal sequence on the newly synthesized polypeptides are caught by the signal-recognition particle (SRP), whose binding slows protein synthesis in a process known as elongation arrest, and directs polypeptides to the translocon, composed of the Sec61αβγ complex in the ER membrane (Johnson and Van Waes 1999; Saraogi and Shan 2011). After arriving at the translocon, translation resumes in a process called cotranslational translocation (Hegde and Kang 2008; Zimmermann et al. 2010). Numerous ER-resident chaperones and enzymes aid in structural and conformational maturation necessary for proper protein folding, including signal-peptide cleavage, N-linked glycosylation, disulfide bond formation, and glycophosphatidylinositol (GPI)-anchor addition (Ellgaard and Helenius 2003).

Recently, the synthesis of posttranslationally inserted proteins, known as tail-anchored (TA) proteins, was elucidated (Fig. 1B) (Rabu et al. 2009; Brodsky 2010; Borgese and Fasana 2011). Tail-anchored (TA) proteins are translated in the cytosol, and the carboxy-terminal single *trans*-membrane domain (TMD) is recognized by the cytoplasmic chaperones TRC40 (Get3) together with the three-protein complex

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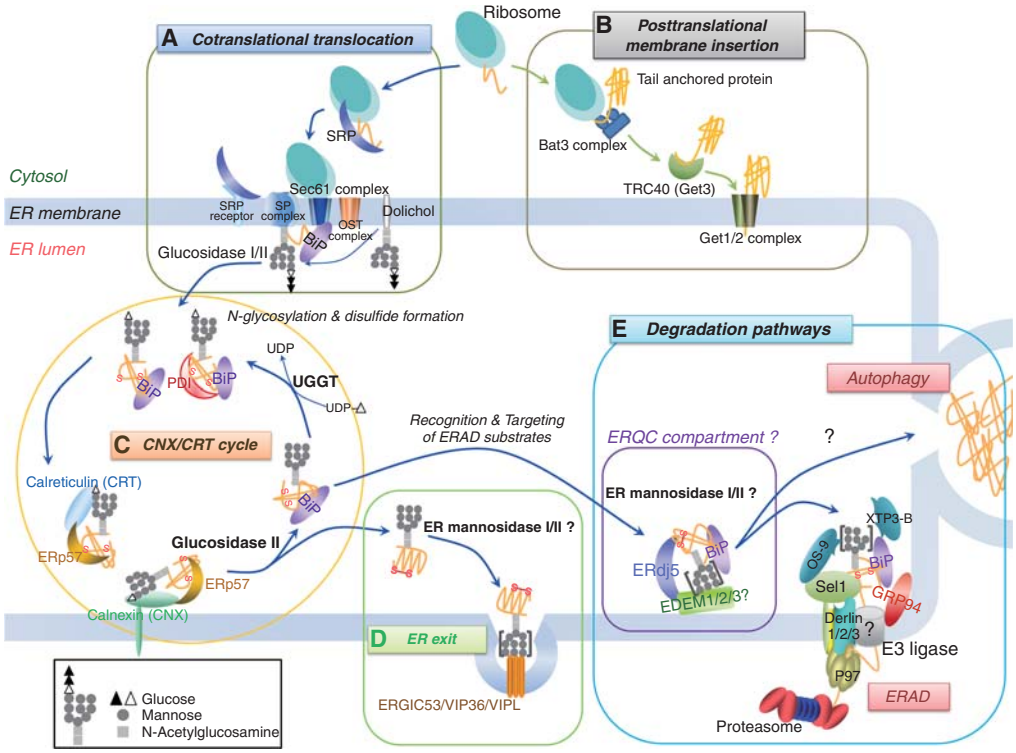

Figure 1. Schematic model of the ER quality control system. Selected components are depicted in this model. (*A*) Cotranslational translation: The ER signal sequence of a newly synthesized polypeptide is bound by a signal-recognition particle (SRP). The SRP–ribosome complex is guided to the Sec complex by an ER membrane-localized SRP receptor. After release of the SRP and SRP receptor, the polypeptide begins translocation. Subsequently, the signal sequence is processed by the signal peptidase (SP) complex (Paetzel et al. 2002). Transfer of oligosaccharides is catalyzed by the oligosaccharyl transferase (OST) complex, and the two outermost glucose residues are sequentially removed by glucosidases I and II. (*B*) Posttranslational membrane insertion: a tail-anchored (TA) protein is posttranslationally inserted into the ER membrane. The carboxy-terminal single *trans*-membrane domain of the TA protein is recognized by the Bat3 complex and transferred to the cytoplasmic chaperone TRC40 for targeting to the ER-membrane localized Get1/2 receptor. (*C*) CNX/CRT cycle: a monoglucosylated N-glycan of the polypeptide recruits the ER lectin chaperones, calnexin (CNX) and/or calreticulin (CRT), which promote proper folding by preventing aggregation and premature export from the ER. ERp57, a CNX/CRT-bound oxidoreductase, catalyzes disulfide formation. Trimming of the innermost glucose residue by glucosidase II then releases the polypeptide from CNX/CRT. UDP-glucose/glycoprotein glucosyl transferase (UGGT) monitors the folding state of released glycoprotein and, if the correct conformation has not been achieved, UGGT reglucosylates it to be reengaged by CNX/CRT. (*D*) ER exit: The natively folded protein is released from the CNX/CRT cycle and transported to its destination. In the early secretory pathway, lectins (ERGIC53, VIP36, and VIPL) support the sorting or trafficking of glycosylated proteins from the ER to the Golgi (Kamiya et al. 2008; Dancourt and Barlowe 2010). "[ ]" indicates that there are several possibilities for N-glycan formation. (*E*) Degradation pathways: Terminally misfolded proteins are degraded primarily through ER-associated degradation (ERAD) or autophagic degradation. Before degradation, N-glycans on ERAD substrates are extensively trimmed for efficient degradation, possibly in a specific compartment within the ER known as the ER quality control (ERQC) compartment, where ERAD machineries such as ER mannosidase I and EDEM family proteins are enriched. Subsequently, ERAD substrates are retrotranslocated into the cytosol, possibly through an E3 ligase complex, and finally degraded by the ubiquitin proteasome pathway. A recent report suggests that misfolded TA proteins are also degraded by the ERAD pathway (Claessen et al. 2010). Autophagy also degrades some ERAD substrates, but its recognition mechanism is not well understood.

Bat3 (Sgt2 in yeast), TRC35 (Get4), and Ubl4A (Get5). The proteins guide and facilitate the insertion of the TA protein into the ER membrane with the aid of the ER-membrane localized Get1/2 receptor in a Sec61-independent manner (Mateja et al. 2009; Mariappan et al. 2010; Wang et al. 2010a).

PROTEIN FOLDING AND POSTTRANSLATIONAL MODIFICATION

Glycosylation

The covalent addition of N-linked glycans to proteins is one of the major biosynthetic functions of the ER and occurs in 90% of all glycoproteins (Helenius 1994). Polypeptides entering the ER lumen are covalently modified by attachment of the preformed oligosaccharide $Glc_3Man_9GlcNAc_2$ (Glc: glucose, Man: mannose, GlcNAc: N-acetylglucosamine) to asparagine side chains in Asn-Xxx-Ser/Thr sequons, catalyzed by oligosaccharyltransferase (OST), a multisubunit enzyme associated with the translocon complex (Fig. 1A) (Shibatani et al. 2005; Ruiz-Canada et al. 2009). The transfer of N-glycans occurs cotranslocationally in a single enzymatic step, and immediately the two outermost glucose residues of the N-glycans are sequentially removed by glucosidases I and II, thereby generating monoglucosylated oligosaccharides ($GlcMan_9GlcNAc_2$) (Grinna and Robbins 1979). These N-glycans are recognized by ER lectin-like chaperones calnexin (CNX) and/or calreticulin (CRT), which promote proper folding by preventing aggregation and premature export from the ER (Fig. 1C) (Williams 2006; Rutkevich and Williams 2010). Trimming of the innermost glucose residue by glucosidase II releases those polypeptides from CNX/CRT. UDP-glucose/glycoprotein glucosyl transferase (UGGT) senses the folding state of released glycoproteins and, if the correct conformation has not been achieved, UGGT reglucosylates the N-glycan again to be reengaged by CNX/CRT (Solda et al. 2007; D'Alessio et al. 2010). In this way, the structure of the N-glycan codes the mandatory information for folding state of the glycoproteins (Hebert et al. 2005; Aebi et al. 2010). Correctly folded proteins are released from this cycle and transported to their destinations (Fig. 1D) (Molinari 2007; Lederkremer 2009).

Folding by Chaperones and Co-Chaperones

In addition to the CNX/CRT complex, the other major chaperone system in the ER is the BiP/GRP78 or Hsp70 system (Hendershot 2004; Dudek et al. 2009; Otero et al. 2010). BiP (binding immunoglobulin protein) is one of the most abundant ER chaperones and serves multiple roles in the ER ranging from productive folding to ERAD. BiP is composed of two domains; an ATPase domain and a substrate-binding domain (SBD) that contains a hydrophobic region suitable for binding to unfolded substrates. After the hydrolysis of ATP, ADP-bound BiP acquires a high affinity for substrates in which the hydrophobic region is closed. By binding to substrates with high affinity, BiP prevents unfolded proteins from forming aggregates. As such, BiP recognizes and helps to assemble unfolded or misfolded regions of the polypeptide (Hendershot 2004).

To date, seven BiP cochaperones, known as DnaJ/Hsp40 family members (ERdj1–7), have been identified in the ER. They contain a J-domain with a His-Pro-Asp (HPD) motif required for the binding with Hsp70 or BiP. These cochaperones play a decisive and critical role not only in stimulating ATP hydrolysis of BiP, but also in regulating its various activities. The ADP form of BiP is converted to the ATP form by nucleotide exchange factors (NEFs) including GRP170 and Sil1/BAP, which direct BiP to the open and accessible form for the substrates (Chung et al. 2002; Kampinga and Craig 2010). These hydrolytic cycles of BiP regulated by the DnaJ family cochaperones and NEFs ensure the solubility of nascent and misfolded proteins in the ER by preventing their aggregation.

Of the Hsp40 family proteins, ERdj1, ERdj2, ERdj4, and ERdj7 have *trans*-membrane domains, whereas the others are ER luminal proteins. ERdj3–6 have been reported to be up-regulated by ER stresses, whereas ERdj1 and 2 are not. ERdj1 and 2 (homologs of yeast

Sec63) presumably recruit BiP to the translocon gate to facilitate the translocation of newly synthesized polypeptides into the ER, and also close the translocon channel to maintain the ER environment. ERdj3 was identified in canine pancreatic microsomes as a component of a multiprotein complex that directly binds to immunoglobulin G during folding in the ER (Meunier et al. 2002; Shen and Hendershot 2005; Jin et al. 2008). ERdj4 and ERdj5 are reported to enhance the ERAD of misfolded proteins. ERdj5 was shown to interact with EDEM (ER degradation-enhancing α-mannosidase-like protein), and overexpressed ERdj4 and ERdj5 interact with p97, a component of the ERAD machinery (Dong et al. 2008; Ushioda et al. 2008; Ushioda and Nagata 2011) (see also later section). ERdj6, designated $p58^{IPK}$, was initially reported to negatively regulate PKR and PERK phosphorylation in the cytosol (Gale et al. 1998; Yan et al. 2002). However, it was later determined that ERdj6 is also localized to the ER by an ER-targeting signal and that ERdj6 binds to BiP through its J-domain or the tetratricopeptide (TPR) repeat domain and functions as a cochaperone (Rutkowski et al. 2007; Petrova et al. 2008). ERdj6 is most probably involved in the productive folding of newly synthesized proteins in the ER lumen. Proteomics analysis with canine pancreatic microsomes revealed another Hsp40 family protein ERdj7, which possesses a *trans*-membrane and luminal domain (Zahedi et al. 2009). As such, ER resident-Hsp40 family proteins cooperatively regulate a wide spectrum of BiP functions.

Recently, a novel ER membrane cochaperone called DNAJB12 was identified. It contains a cytosolic J-domain that interacts with cytosolic Hsp70 and plays a role in the degradation of membrane proteins, including CFTR and TCRα, which suggests that it functions in ERAD on the cytosolic side (Grove et al. 2010; Yamamoto et al. 2010).

Disulfide Bond Formation

Another important maturation step in the ER is the formation of disulfide bonds, which are crucial for protein function and stability (Appenzeller-Herzog 2011). The oxidative environment of the ER is suitable for the oxidation of free sulfhydryl (SH) groups on cysteines to form disulfide (S–S) bonds. Oxidoreductases, called PDI family proteins, catalyze these reactions by acting as an oxidase and isomerase, thereby promoting the formation of native disulfides. PDI family members are defined by the presence of at least one thioredoxin (Trx)-like domain containing Cys-X-X-Cys (CXXC) motifs in the active site. To date, approximately 20 PDI family members have been identified, including soluble and *trans*-membrane-containing proteins, most of which are ubiquitously expressed (Ellgaard and Ruddock 2005). PDI is a canonical member of the PDI family and also functions as a molecular chaperone (Hatahet et al. 2009). PDI is composed of two active Trx-like domains (called the a and a′ domains) that are linked by two inactive Trx-like domains (called the b and b′ domains), and its primary substrate binding site is located in the b′ domain. ERp57, the other member, stably binds to CNX or CRT and acts as an oxidoreductase, especially for glycoproteins (Oliver et al. 1999). ERp57 also acts as a thiol oxidoreductase of heavy chain (HC) oxidation in MHC class I biogenesis, and as a structural component of the peptide loading complex (PLC), which consists of the HC-β2m heterodimer, CRT (or CNX), and the additional components tapasin, TAP and Bap31 (Chapman and Williams 2010). PDI may also come into play and reoxidize HC (Park et al. 2006). Other ER proteins, such as ERp44, which is localized to the ER-Golgi intermediate compartment (ERGIC), engages in the folding/oligomerization or retention of some proteins in the ER (Anelli and Sitia 2008; Cortini and Sitia 2010). ERdj5 participates in ERAD as a reductase (Ushioda et al. 2008; Hagiwara et al. 2011) (see later section). The ER also contains a number of selenoproteins. One of these is Sep15, which binds to UGGT and presumably works as a reductase, as suggested by the reducing potential of selenocysteine (Korotkov et al. 2001).

Why are there so many oxidoreductases in the ER? The reason is not entirely clear, but

some oxidoreductases appear to have a specific function, whereas others have redundant functions (Feige and Hendershot 2010; Rutkevich et al. 2010). Specific functions or redundancies are often inferred from their direct or indirect binding partners (e.g., ERp57-CNX/CRT, BiP-P5, ERdj5-BiP-EDEM, ERp44-ERGIC53, etc.), which define substrate specificities and specific cellular compartments in which the oxidoreductases localize (Jessop et al. 2009). Knockout mice models also elucidate specific and redundant functions of ER oxidoreductases. For example, ERp57 knockout mice showed embryonic lethality, suggesting that ERp57 has a specific role in early development (Garbi et al. 2006; Coe et al. 2010). Knockout mice of ERdj5, Prdx4, and Ero1α/β (see later section) showed less severe phenotypes, which suggests that their roles can be compensated for by other factors (Iuchi et al. 2009; Hosoda et al. 2010; Zito et al. 2010a). In addition, some ER proteins appear to possess functions outside the ER, such as in mitochondria or nucleus (P5 and ERp57) (Coppari et al. 2002; Kimura et al. 2008). Clarifying their detailed roles and regulatory mechanisms will be an exciting topic for future work (Appenzeller-Herzog and Ellgaard 2008).

Other Specific Chaperones

A number of substrate-specific chaperones have been reported, among which collagen-specific or related chaperones have been well illustrated. HSP47/SERPINH1 specifically and transiently binds to various types of collagen in the ER and is believed to facilitate the triple-helical structure of collagen (Nagata 2003). The P4H complex, consisting of α2β2 tetramers, in which the β-subunits are identical to PDI, and the P3H complex, containing cartilage-associated protein (CRTAP), prolyl 3-hydroxylase 1 (P3H1), and cyclophilin B, are also known to be essential assembling factors and collagen chaperones (Ishikawa et al. 2009; Gorres and Raines 2010; Morello and Rauch 2010). Mutation or knockout of these factors results in embryonic lethality or osteogenesis imperfecta, which clearly shows their importance for productive procollagen folding and maturation (Nagai et al. 2000; Morello et al. 2006; Cabral et al. 2007; Choi et al. 2009). Microsomal triglyceride transfer protein (MTP) plays a pivotal role in lipoprotein assembly, and receptor-associated protein (RAP) participates in the maturation of several membrane receptor proteins, such as low-density lipoprotein receptor-related protein (LRP) and lipoprotein receptor 11 (SorLA/LR11) (Orlando 2004; Blasiole et al. 2007).

ERAD

Terminally misfolded or unassembled proteins that are unable to acquire their native structure must be degraded to prevent fruitless folding attempts and the accumulation of misfolded polypeptides in the ER (Fig. 1E). This degradation process is known as ER-associated degradation (ERAD), which occurs in three primary steps: (1) recognition and targeting (substrate recognition within the ER and targeting to the retrotranslocon), (2) retrotranslocation (substrate delivery from the ER to the cytosol), and (3) degradation (ubiquitin–proteasome dependent degradation) (Hegde and Ploegh 2010).

Recognition and Targeting

The mechanism by which ERAD substrates are recognized and distinguished from properly folded proteins or those that are in the process of being correctly folded remains largely unknown. A large portion (around 75%) of proteins such as CFTR, apolipoprotein A, or the erythropoietin receptor, which are difficult to fold properly, are degraded even under normal conditions (Kopito 1999; Sanders and Myers 2004). On the other hand, influenza HA protein folds with near 100% efficiency (Braakman et al. 1991). A recently proposed model suggests that the efficiency of productive folding and trafficking cannot be defined by a single feature, but rather by the combination of multiple factors, including protein stability, folding rate, enzyme distribution, and metabolism (redox state, calcium flux, etc.), collectively

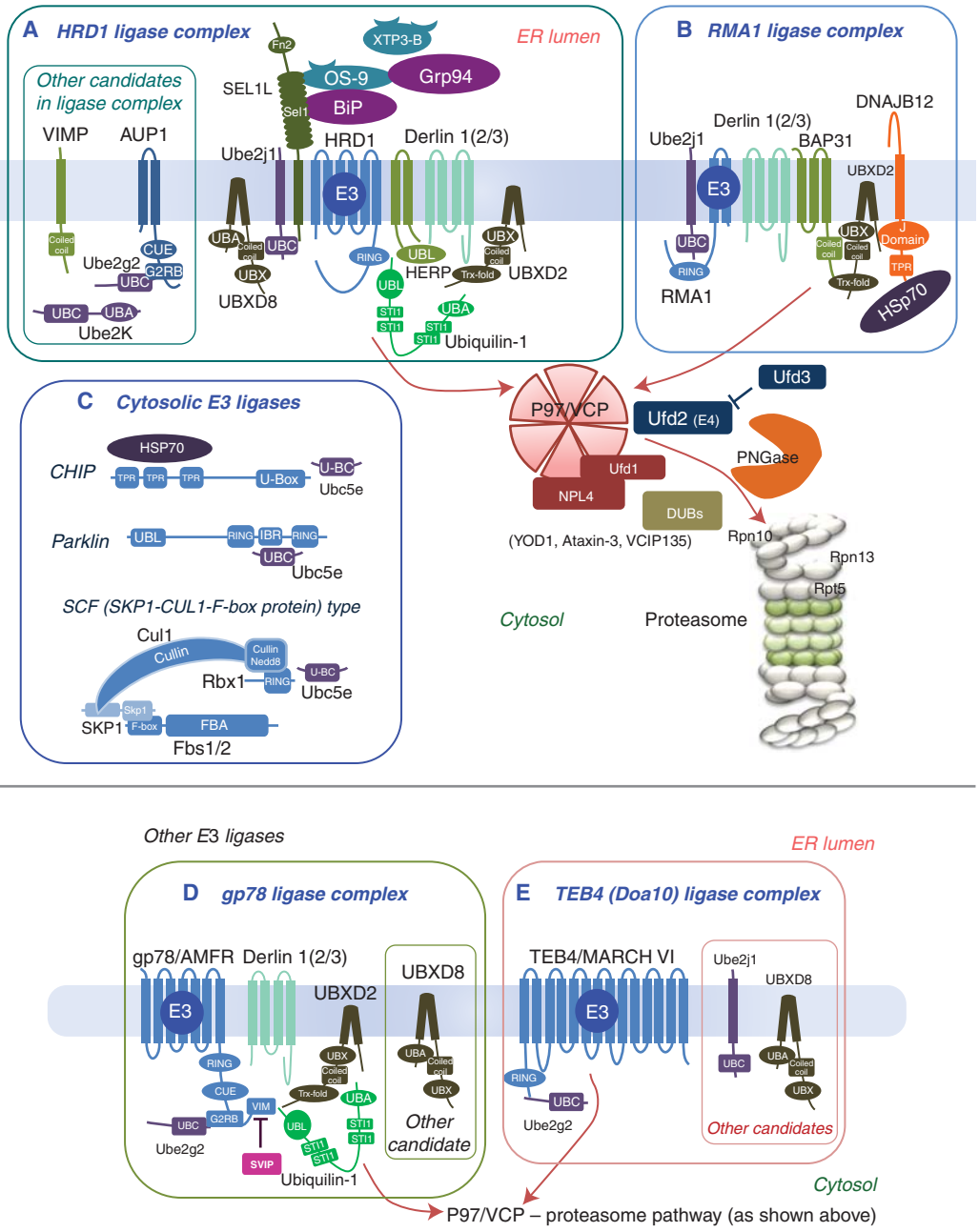

Figure 2. Schematic models of mammalian E3 ligase complexes. Selected components are depicted in each model (Tsai and Weissman 2010). The majority of motif annotations are taken from the Pfam and SMART databases (see Tables 1 and 2; Letunic et al. 2009; Finn et al. 2010). (*A*) HRD1 ligase complex: This complex is a well-illustrated E3 complex that primarily targets proteins for ERAD-L. OS-9 and XTP3-B recognize aberrant nonglycosylated or glycosylated proteins in the ER lumen. Both associate with the HRD1 complex through SEL1L in a mutually exclusive manner. BiP and GRP94 presumably cooperate to regulate the assembly/disassembly of the HRD1 complex and sequester misfolded proteins to prevent other interactions until retrotranslocation. Derlin family proteins (Derlin 1, 2, or 3) might participate in substrate retrotranslocation from the ER lumen into the cytosol. (*See facing page for legend.*)

called ERAF (ER-associated folding) (Sekijima et al. 2005). Therefore, protein quality control, now conceptualized as proteostasis (protein homeostasis), is maintained by the teleological relationship between conformational maturation (ERAF) and recognition for disposal in the ER lumen (ERAD), and thereby determines the probability that newly synthesized proteins will acquire their native structure (Kowalski et al. 1998; Kjeldsen et al. 2002; Brodsky 2007; Wiseman et al. 2007; Hutt and Balch 2010).

Because different quality control (QC) machineries detect structurally different defects in different environments, three subdivisions of the ERAD pathway have been proposed in budding yeast dependent on the site of the defect, ERAD-C (cytosol), ERAD-M (membrane), and ERAD-L (lumen) (Taxis et al. 2003; Vashist and Ng 2004; Carvalho et al. 2006), although this classification might be oversimplified (Goeckeler and Brodsky 2010).

ERAD machineries often organize higher molecular weight complexes around E3 ligase (Fig. 2) (Kawaguchi and Ng 2007). Yeast has two major membrane-associated E3 ligases containing RING domain, Doa10p (degradation of Mat-α2-10 protein) and Hrd1p (HMG-CoA reductase degradation 1 protein) (Carvalho et al. 2006; Denic et al. 2006). Doa10p works on substrates with misfolded

Figure 2. (*Continued*) UBXD8 and UBXD2 bind to p97/VCP through their UBX domain and accelerate the degradation of ERAD substrates. E2 ubiquitin-conjugating enzymes (Ube2j1, Ube2k) mediate substrate ubiquitination (Burr et al. 2011), whereas the p97/VCP hexamer promotes substrate extraction into the cytosol. Ubiquilin-1 is suggested to act as a ubiquitin–proteasome shuttle protein. Other ubiquitin-chain modifiers may also come into play, such as E4 ubiquitin-conjugating enzyme (Ufd2), Ufd2 inhibitor (Ufd3), and deubiquitinases (Ataxin-3, YOD1, VCIP135) (Rumpf and Jentsch 2006). The deglycosylating enzyme PNGase releases N-linked glycan chains from the glycopeptide (Tanabe et al. 2006). The proteasome then captures the polyubiquitin chains on the substrate through specific subunits (Rpn10/13, Rpt5) and degrades it. (*B*) RMA1 ligase complex: RMA1/RNF5 associates with Derlin-1 and E2 Ube2j1. BAP31, known to be an ER sorting factor of diverse membrane proteins, interacts with RMA1 as well as components of the Sec61 pore, which suggests that BAP31 might recruit the ERAD complex to the translocon channel to clear newly synthesized misfolded membrane proteins from the channel. In addition, DNAJB12, which contains the cytosolic J-domain, may also participate in the degradation of membrane proteins together with HSP70. All of these factors have been reported to play a role in the degradation of cystic fibrosis *trans*-membrane conductance regulator (CFTR) and its mutant (CFTRΔ508) during the early steps of translation (Younger et al. 2006; Morito et al. 2008). (*C*) Cytosolic E3 ligases: CHIP (carboxyl terminus of Hsp70-interacting protein) possesses a U-box domain, which has a structure similar to the RING-finger domain, and a tetratricopeptide repeat domain (TPR) that interacts with Hsp70 and Hsp90. In contrast to RMA1, CHIP posttranslationally monitors the folding of CFTR or CFTRΔ508. Parkin, which is responsible for autosomal recessive juvenile Parkinsonism, targets several proteins, such as O-glycosylated α-synuclein, the Pael receptor (Pael-R), Synphilin-1, and Tau. Regarding Pael-R, Parkin and CHIP act together to enhance its ubiquitination and inhibit cell death induced by accumulated unfolded Pael-R. Parkin also works with the E2 proteins Ube2j1 and Ube2g2, which suggests that it is involved in ERAD. The SCF (SKP1-CUL1-F-box protein) is composed of three proteins (Cullin1, Skp1, and RING finger protein Rbx1) and one F-box protein (Fbs1 or Fbs2). Fbs1 and Fbs2 are novel F-box proteins that recognize sugar chains in N-linked glycoproteins and show a chaperone-like activity to prevent their aggregation (Yoshida and Tanaka 2010). (*D*) gp78 ligase complex: The carboxyl terminus of gp78 is composed of four motifs: RING, CUE, G2BR (Ube2g2 binding region), and VIM (p97/VCP-interacting motif). The gp78 ligase complex usually consists of Ube2g2, Derlin-1, p97/VCP, and UBXD2 or UBXD8. gp78 mediates several ERAD substrates, including T-cell receptor subunits (CD3-δ and TCR-α), apolipoprotein B-100, Insig-1, and HMG-CoA reductase. The latter two substrates suggest that gp78 is involved in cholesterol metabolism (Jo and Debose-Boyd 2010). gp78 also cooperates with RMA1 to degrade CFTRΔ508. SVIP is reported to inhibit the assembly of the gp78 ligase complex (Derlin-1, gp78, and p97), which suggests an inhibitory effect on ERAD (Ballar et al. 2007). (*E*) TEB4 (Doa10) ligase complex: TEB4 is known to be a mammalian homolog of yeast Doa10. Together with Ube2g2, TEB4 is implicated in the degradation of ER resident type 2 iodothyronine deiodinase (Zavacki et al. 2009). Based on the homology of the yeast Doa10, TEB4 might also interact with Ube2j1 (Ubc6) and UBXD8 (Ubx2).

Table 1. Selected ERAD-related proteins

Human	Other name	Localization	Main motifs	Yeast homolog
Processing and targeting				
ER ManI		Membrane	Glyco_hydro_47	Mns1
EDEM1-3		Possibly ER or ER membrane	Glyco_hydro_47	Htm1(Mnl1)
PDI		ER	Trx	Pdi
BiP	GRP78	ER	SBD, NBD	Kar2
GRP94		ER	SBD, NBD	—
ERdj4		Membrane	DnaJ	—
ERdj5	JPDI	ER	DnaJ, Trx	—
ERFAD	FOXRED2	ER	FAD/NADPH binding motif	—
CyPB	Cyclophilin B	ER	Pro_isomerase	Cpr2
OS-9		ER	MRH	Yos9
XTP3-B		ER	MRH	Yos9
SEL1L		Membrane	SEL1, Fn	Hrd3
Possible retrotranslocation channel				
Sec61 complex		Membrane	Sec motifs	Sec61, Ssh1 complex
Derlin1-3		Membrane	DER1	Dfm1, Der1
Other possible component or regulator				
HERP	Mif1	Membrane	UBL	Usa1
VIMP	ERASIN, SelS	Membrane	SelS motif	—
BAP31		Membrane	—	Yet3
JAMP		Membrane	—	—
DNAJB12		Membrane	DnaJ, TPR	Hlj1
SPP	HM13	Membrane	Peptidase_A22B	—
TRAP complex		Membrane	TRAP motifs	—
TRAM		Membrane	TRAM motif	—
AUP1		Membrane	CUE	—
SVIP		Cytosol	VIM	—
E2 ubiquitin-conjugating enzyme				
UBE2K	UBE2D1	Cytosol	UBA, UBC	Ubc1
UbcH5	Ubc5a	Cytosol	UBC	Ubc5
Ube2j1/2	NCUBE-1/-2	ER membrane	UBC	Ubc6
Ube2g1/2		Cytosol	UBC	Ubc7
Ubc13	UBE2N	Cytosol	UBC	Ubc13
E3 ubiquitin-protein ligase enzyme				
HECT (homologous to E6-AP carboxyl terminus) type E3				
NEDD4-2		Cytosol	HECT, C2, WW	Rsp5
RING (really interesting new gene)-finger type E3				
Prakin	PARK2	Cytosol	RING, UBL, IBR	—
RNF5	RMA1	Membrane	RING	—
RNF45	AMFR, gp78	Membrane	RING, CUE, VIM	—
HRD1	Synoviolin	Membrane	RING	Hrd1/Der3
TEB4	MARCH VI	Membrane	RING	Doa10
RNF139	TRC8	Membrane	RING	—

Continued

Table 1. *Continued*

Human	Other name	Localization	Main motifs	Yeast homolog
RNF77	RFP2	Membrane	RING, BBOX	—
RNF103	Kf-1	Membrane	RING	—
RNF19	Dorfin	Membrane	RING, IBR	—
RNF121		Membrane	RING	—
UBOX type E				
CHIP	STUB1	Cytosol	U-box, TPR	—
SKP1-CUL1-F-box (SCF) E3 (F-box protein component)				
SKP1		Cytosol	Skp1	skp1
CUL1		Cytosol	Cullin	Cdc53
Fbs1	FBXO2	Possibly membrane associated or cytosol	FBA, F-box	—
Fbs2	FBXO6	Possibly membrane associated or cytosol	F-box, SBD	—
Rbx1		Cytosol	RING	Hrt1
E4 ubiquitin-conjugating enzyme				
Ufd2	Ube4	Cytosol	U-box, UFD2 core motif	Ufd2
Ufd2 inhibitor				
Ufd3	PLAA	Cytosol	PFU, PUL, WD40	Doa1
Substrate extraction and recruiting				
p97	VCP	Possibly membrane associated or cytosol	BS1 box	Cdc48
UFD1		Cytosol	UFD1 motif	Ufd1
NPL4		Cytosol	NPL4 motifs	Npl4
UBXD family protein				
Ubxd1	Ubxn6	Cytosol	UBX, PUB	—
Ubxd2	Erasin	Membrane associated	UBX, Trx-fold	Ubx7
Ubxd7		Cytosol	UBX, UBA, UIM, UAS	Ubx5
Ubxd8	ETEA	Membrane associated	UBX, UBA	Ubx2
Ubxd10	SAKS1	Cytosol	UBX	—
Deglycosylating enzyme				
PNGase		Cytosol	PUB	Png1
DUB (deubiquitylation)				
VCIP135		Cytosol	OTU motif	—
YOD1	Otu1	Cytosol	OTU motif	Otu1
Ataxin-3		Cytosol	UIM, Josephin	—
USP19		Membrane	UCH, MYND finger	—
Shuttle protein				
Ubiquilin-1	Dsk2, UBQLN1,	Possibly membrane associated or cytosol	UBL, UBA, STI1	Dsk2
HR23A/B	RAD23A/B	Cytosol	UBL, UBA, STI1	Rad23
Ubiquitin receptor				
Rpn10	S5a	Cytosol	VWA, UIM	Rpn10
Rpt5	S6′	Cytosol	AAA	Rpt5
Rpn13	ADRM1	Cytosol	PH	Rpn13

Table 2. Motif names[a]

Motif name	Pfam annotation or other comment
AAA	ATPase family associated with various cellular activities
BBOX	B-Box-type zinc finger
BS1 box	BS1 (binding site 1) is a p97-interacting domain
C2	Ca^{2+}-dependent membrane-targeting module
CUE	CUE domains may be involved in binding ubiquitin-conjugating enzymes (UBCs) or ubiquitin.
Cullin	Cullins are a family of hydrophobic proteins that act as scaffolds for ubiquitin ligases (E3).
DER1	Der1-like family
DnaJ	DnaJ domain
FBA	F-box associated region
F-box	The F-box domain has a role in mediating protein–protein interactions.
Fn2	Fibronectin type 2 domain
Glyco_hydro_47	Glycosyl hydrolase family 47
HECT	The name HECT-domain (ubiquitin-transferase) comes from Homologous to the E6-AP Carboxyl Terminus.
IBR	The IBR (in between ring fingers) domain is often found to occur between pairs of ring finger.
Josephin	The name Josephin comes from Machado–Joseph Disease protein MJD.
MRH	Mannose 6-phosphate receptor homology domain
MYND finger	The MYND (myeloid, Nervy, and DEAF-1) zinc finger domain might be involved in protein–protein interactions.
NBD	Nucleotide binding domain.
OTU	The ovarian tumor (OTU) -like cysteine protease
Peptidase_A22B	Signal peptide peptidase domain
PFU	PFU is the ubiquitin binding domain of Doa1
PH	PH (pleckstrin homology) is involved in intracellular signaling or as constituents of the cytoskeleton.
Pro_isomerase	Cyclophilin type peptidyl-prolyl *cis-trans* isomerase
PUB	The PUB (also known as PUG) domain is found in peptide N-glycanase where it functions as a AAA ATPase binding domain.
PUL	The PUL (PLAP, Ufd3p and Lub1p) domain is a novel α-helical Ub-associated domain; it directly binds to Cdc48.
RING	RING finger is a cysteine-rich domain of 40 to 60 residues that coordinates two zinc ions and plays a key role in the ubiquitination pathway.
SBD	Substrate binding domain
SEL1	This short repeat is found in the Sel1 protein; it is related to TPR repeats.
Skp1	SKP1 (together with SKP2) was found to bind several F-box containing proteins (e.g., Cdc4, Skp2, cyclin F) and to be involved in the ubiquitin protein degradation pathway.
STI1	Heat shock chaperonin-binding motif is found in the stress-inducible phosphoprotein STI1.
TPR	The tetratrico peptide repeat (TPR) mediates protein–protein interactions and the assembly of multiprotein complexes.
Trx	Thioredoxin domain
UAS	UAS is a domain of unknown function found in FAF1 proteins (FAS-associated factor 1) and in other proteins.
UBA	Ubiquitin associated domain
UBC	Ubiquitin-conjugating enzyme (E2) catalytic domain
UBL	Ubiquitin-like (UBL) domain
U-box	U-box has a similar structure to the RING-finger domain and bears ligase activity.

Continued

Table 2. *Continued*

Motif name	Pfam annotation or other comment
UBX	UBX domain is present in ubiquitin-regulatory proteins and is a general Cdc48-interacting module.
UCH	Ubiquitin carboxy-terminal hydrolase
UIM	Ubiquitin interacting motif (UIM) containing domains all interact with ubiquitin.
VIM	p97/VCP-interacting motif
VWA	The von Willebrand factor is a large multimeric glycoprotein found in blood plasma.
WD40	Repeated WD40 motifs act as a site for protein–protein interaction.
WW	The WW domain is a protein module with two highly conserved tryptophans that binds proline-rich peptide motifs in vitro.

The majority of the annotations are taken from the Pfam and SMART databases.
[a]Even if the human gene lacks a yeast homolog, another functionally relevant gene might exit.

regions on the cytosolic side (ERAD-C), whereas the Hrd1p complex, comprised of the ubiquitin ligases Hrd1p, Hrd3p, Usa1p, and Der1p, acts on substrates with defects in the luminal region (ERAD-L). ERAD-M requires a subset of these components required for ERAD-L (Bordallo et al. 1998; Bays et al. 2001a; Swanson et al. 2001). Following polyubiquitination, these pathways merge at an ATPase complex consisting of the ATPase Cdc48p and two cofactors, Ufd1p and Npl4p (Bays et al. 2001b; Braun et al. 2002; Jarosch 2002; Rabinovich et al. 2002; Zito et al. 2010b). In contrast to yeast, mammalian ERAD E3 ligases are more diverse (HRD1, RMA1, Parkin, CHIP, gp78, TEB4, TRC8, etc.) and additional ERAD E3 ligases continue to be identified, which indicates that various complexes are needed to monitor different classes of substrates (Hirsch et al. 2009; Tsai and Weissman 2010; Neutzner et al. 2011).

In mammalian ERAD, the degradation of N-glycosylated proteins is well-characterized. As mentioned, the structure of the N-glycan codes the mandatory information on the state of protein folding. CNX/CRT recognizes monoglucosylated oligosaccharides ($GlcMan_9GlcNAc_2$) and the CNX/CRT cycle enhances productive folding and protects immature glycopolypeptides from ERAD. Proteins that will be terminally misfolded are retained longer in this cycle, which may raise the probability of mannose trimming of the polypeptide-bound N-glycans by ER-mannosidase I, a process known as the mannose timer model (Helenius 1994). These mannose trimmed structures are recognized by ERAD factors, most likely EDEM family proteins (Molinari 2007; Hosokawa et al. 2010a).

EDEM family proteins (EDEM1, EDEM2, and EDEM3) have α-mannosidase-like domains with conserved catalytic residues for glycolytic activity (Kanehara et al. 2007). EDEM1 presumably possesses mannosidase activity that trims the C branch of N-glycans on misfolded proteins; however, that activity is apparently not required for ERAD acceleration because mutant EDEM1 that lacks the putative active site for mannosidase is still able to accelerate ERAD (Hosokawa et al. 2001, 2006, 2010b; Molinari et al. 2003; Oda et al. 2003; Olivari et al. 2006). EDEM2 also promotes ERAD, even though it has no enzymatic activity (Mast et al. 2005; Olivari et al. 2005). In contrast, ERAD-acceleration by EDEM3 (Htm1p/Mnl1p in yeast) is dependent on its mannosidase activity (Hirao et al. 2006; Clerc et al. 2009). Therefore, EDEM family proteins may not be functionally redundant, but they all contribute to ERAD acceleration.

Extensive trimming of N-glycans would lead to the increased hydrophobicity of misfolded proteins, and the Man7GlcNAc2 form of N-glycans is recognized by the lectins OS9 and perhaps XTP3-B, which contain one and two MRH (mannose 6-phosphate receptor homology) domains, respectively (Fig. 2A) (Hosokawa et al. 2010a; Satoh et al. 2010).

Although OS-9 and XTP3-B do not interact with each other, both associate with the HRD1 E3 ubiquitin ligase complex through SELIL, a multiply glycosylated type I ER membrane protein. OS-9 and XTP3-B recognize aberrant nonglycosylated or glycosylated proteins even when their MRH domains are mutated (Christianson et al. 2008; Hosokawa et al. 2008). OS-9 also associates with BiP/GRP94 (an ER-resident Hsp90 homolog) and SELIL in a mutually exclusive manner, where BiP and GRP94 presumably contribute to regulate the assembly/disassembly of the HRD1 complex and sequester misfolded proteins to prevent other interactions until retrotranslocation (Eletto et al. 2010). Consistent with ERAD in yeast, mammalian HRD1 complexes are required for ERAD- L but are not necessary for ERAD-M (Bernasconi et al. 2010).

Our laboratory recently identified ERdj5 as an EDEM1-binding protein that can accelerate ERAD by reducing the incorrect formation of disulfide bonds in misfolded glycoproteins (Hoseki et al. 2010). Based on its crystal structure, ERdj5 contains a J-domain and six tandem thioredoxin domains, two of which do not contain redox active CXXC motif (Hagiwara et al. 2011). ERdj5 can be structurally divided into two clusters, called the N- and C-clusters. The N-cluster contains the J-domain, and the C-cluster interacts with EDEM1 and can efficiently reduce the disulfide bonds of recruited substrates. ERAD substrates are sequentially transferred from calnexin to the EDEM1-ERdj5 complex, and subsequently to BiP, which tightly binds substrates in a dislocation-competent state until retrotranslocation after stimulation of its ATPase activity by ERdj5. However, we have yet to identify the reductive source of ERdj5. One potential candidate is the recently reported flavoprotein, ERFAD, which interacts with SEL1L and OS9, and might provide reducing equivalents to ERdj5 (Riemer et al. 2009).

For nonglycosylated proteins, the recognition mechanism is apparently somewhat distinct from that of glycosylated proteins. The unfolded regions of nonglycosylated proteins are recognized by ER chaperones, mostly by BiP. The DnaJ family proteins, cochaperones of BiP, play a crucial role in regulating its various activities. Accumulated evidence suggests that ERdj3 and ERdj6 are primarily involved in productive-folding (ERAF), whereas ERdj4 and ERdj5 predominantly affect ERAD (Otero et al. 2010). The process of transition from folding to degradation is far from clear. Presumably, the retention time of a substrate by BiP and its cofactors (e.g., ERdj3/6) may determine its fate. Prolonged retention would recruit other cofactors involved in ERAD, such as ERdj4/5 and p97/VCP (Otero et al. 2010). HERP, a membrane-bound ubiquitin (Ub)-like protein, has been implicated in the efficient delivery of nonglycosylated substrates to the proteasome (Okuda-Shimizu and Hendershot 2007).

Retrotranslocation and Degradation

After recognition and targeting of the substrates, they must be dislocated/retrotranslocated into the cytosol for proteolysis by 26S proteasomes. Although the identities of the components that comprise the retrotranslocation channel remain unclear, Sec61 complex and Derlin-1 are possible candidates (Knop et al. 1996; Wiertz 1996; Pilon et al. 1997; Willer et al. 2008; Schafer and Wolf 2009). Sec61 complex has been reported to interact with several ERAD substrates and ERAD machineries including proteasome, TRAP complex, SPP (signal peptide peptidase), PDI, and BAP31 (Wiertz et al. 1996; Pilon et al. 1997; Loureiro et al. 2006; Nagasawa et al. 2007; Ng et al. 2007; Wang et al. 2008; Lee et al. 2010). Derlin-1 was initially reported to be important for the extraction of MHC class I molecules from the ER membrane in cytomegalovirus-infected cells (Lilley and Ploegh 2004; Ye et al. 2004). Reconstitution assay using a fluorescently labeled substrate also showed that Derlin-1 is involved in substrate dislocation, which is independent of Sec61α (Wahlman 2007). The yeast Hrd1p E3 ligase, which interacts with the ERAD substrate via its *trans*-membrane region, is also a possible candidate (Carvalho et al. 2010). Thus far, E3 ligase complexes are the predominant candidates because they constitute large protein

complexes containing multispanning membrane proteins such as HRD1 and Derlin-1, which can efficiently recognize, target, retrotranslocate, and ubiquitinate ERAD substrates within the organized complexes (Fig. 2) (Bagola et al. 2011).

Ubiquitination takes place in the cytosol, which ensures efficient delivery of the substrates to the proteasome. ERAD substrates are ubiquitinated at serine/threonine residues and less frequently at lysine residues (Shimizu et al. 2010). This process is apparently different from that of other cytosolic ubiquitination mechanisms (Wang et al. 2009; Ishikura et al. 2010). At the cytosolic face of the ERAD complex, the AAA+ ATPase p97/VCP (Cdc48 in yeast) hexamer directs substrate to be drawn into the cytosol (Chapman et al. 2010). p97 binds to several ERAD components, including Derlin-1, VIMP (SelS), UBXD2 (Erasin), and UBXD8, and recruits several ubiquitin-chain modifiers including E3 ligases (gp78, HRD1, etc.), chain elongation factors (Ufd2, E4 ubiquitin ligase), and deubiquitinases (YOD1, Ataxin-3, VCIP135) (Liang et al. 2006; Mueller et al. 2008; Schuberth and Buchberger 2008; Ernst et al. 2009). Ubiquitinated substrates are transferred to the proteasome by shuttle proteins, known as HR23A/B or Ubiquilin-1 (Rad23 and Dsk2 in yeast), which contain ubiquitin-associated (UBA) and ubiquitin-like (UBL) domains that bind to polyubiquitin chains and the proteasome subunits (Rpn10/13, Rpt5), respectively (Deveraux et al. 1994; Lam et al. 2002; Raasi and Wolf 2007; Husnjak et al. 2008; Finley 2009; Lim et al. 2009).

Other Degradation Pathways

Cells possess additional degradation pathways to cope with a variety of situations based on the characteristics of the misfolded proteins (Fu and Sztul 2009; Kroeger et al. 2009; Wong and Cuervo 2010). We have reported previously that the aggregated or insoluble form of type I collagen in the ER is degraded by autophagy-mediated lysosomal degradation, whereas nonaggregated forms are subject to ERAD (Fig. 1E) (Ishida et al. 2009). When ERAF/ERAD or ubiquitin proteasome activities are compromised, autophagy is triggered via signaling pathways that usually involve unfolded protein response (UPR)-dependent elements (Ogata et al. 2006; Fujita et al. 2007; Hosokawa et al. 2007; Kouroku et al. 2007; Yorimitsu and Klionsky 2007b). Additionally, the ER membrane itself may be a source of lipids for autophagosome formation (Hayashi-Nishino et al. 2009; Yla-Anttila et al. 2009). Hence, the ER and the process of autophagy would certainly have a tight physiological relationship. Organelles are also selectively delivered to lysosomes by macroautophagy, known as ERphagy (reticulophagy), mitophagy, or pexophagy (Kim et al. 2007; Yorimitsu and Klionsky 2007a; Ding and Yin 2008; Todde et al. 2009; Manjithaya et al. 2010).

Intriguingly, endogenous EDEM1, a major component of ERAD, is constitutively degraded via an autophagic-like pathway (Cali et al. 2008). In support of this, electron microscopy showed that EDEM1 is primarily localized in double-membrane buds that form outside canonical ER exit sites, known as EDEMosomes (Zuber et al. 2007; Le Fourn et al. 2009; Reggiori et al. 2010). At steady state, short-living ERAD components like EDEM1 and OS-9 appeared to be engulfed in these buds in a COPII-independent manner and degraded without the attachment of nonlipidated LC3. This turnover of ERAD factors, known as ERAD tuning, maintains an extra capacity of ERAD factors at steady state by EDEMosome-linked degradation (Bernasconi and Molinari 2011). It is hypothesized that this makes it possible for the ER to respond quickly to sudden changes without waiting for transcriptional UPR responses. It is still unclear whether EDEM1 is an intrinsic component of the EDEMosome and the mechanism by which these buds are created.

ER HOMEOSTASIS

As discussed above, the ER is the primary site of secretory and membrane protein production. The proteostatic balance is intimately associated with ER redox homeostasis and calcium balance (Fig. 3).

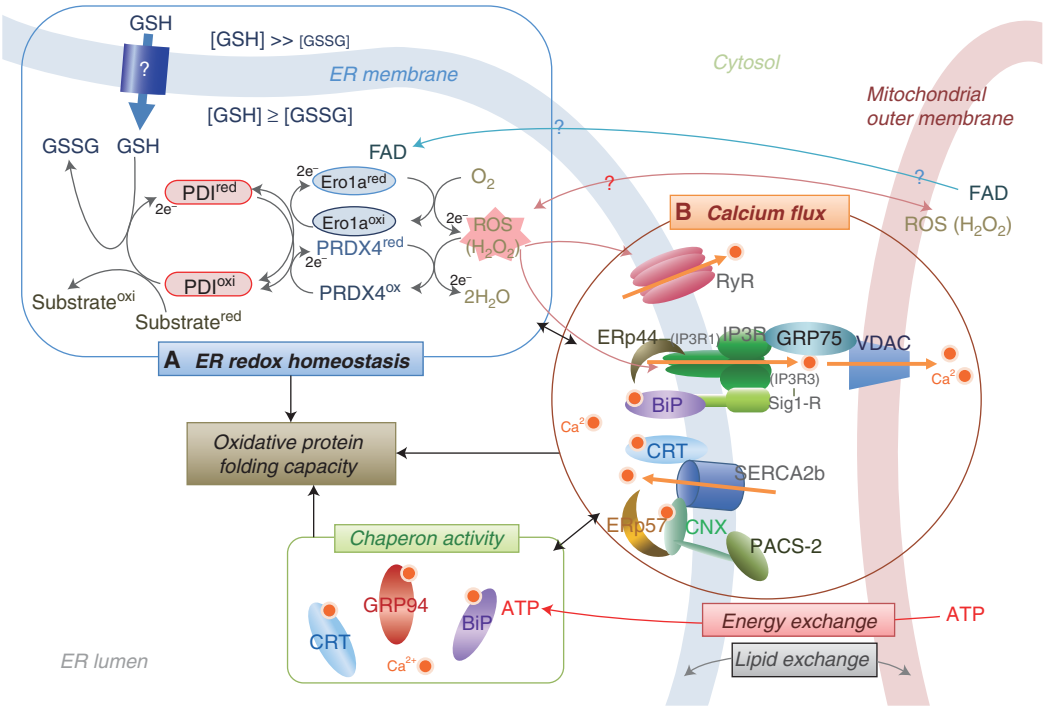

Figure 3. ER redox and calcium homeostasis on the MAM. On the mitochondria-associated membrane (MAM), ER chaperones (CNX, BiP), oxidoreductases (Ero1α, ERp44), and Ca^{2+} channels/pumps (IP3R3, SERCAs, and Sig-1R) are enriched, thereby creating an ideal environment for oxidative protein folding, as well as the regulation of Ca^{2+} flux. (*A*) ER redox homeostasis: Ero1α serves as the primary oxidase of PDI. Concomitantly, hydrogen peroxide (H_2O_2) is thought to be produced from oxygen as an electron acceptor. Peroxiredoxin IV (PRDX4) is thought to work as a H_2O_2 reducer. PRDX4 can also oxidize some PDI family members. Oxidized PDI family members drive oxidative protein folding, as well as oxidize GSH into GSSG, thereby generating an oxidative environment. The FAD, cofactor of Ero1α, may be delivered from mitochondria via unknown transporters. (*B*) Calcium flux: Mammalian cells contain two main channels responsible for Ca^{2+} efflux from the ER, inositol 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptors (RyRs), and one pump responsible for Ca^{2+} influx into the ER, sarcoplasmic reticulum Ca^{2+}-ATPase (SERCAs). ERp44 interacts with the luminal loop of IP3R type 1 (IP3R1) and inhibits its activity. ERp57 oxidizes the luminal thiols of SERCA2b in a Ca^{2+}-dependent manner (Li and Camacho 2004). By-product of oxidative protein folding (i.e., ROS) affects the redox states of the channels (RyR and IP3R1) and changes their activities. In this way redox homeostasis and Ca^{2+} flux are interrelated. Sigma-1 receptor (Sig-1R) works as a Ca^{2+} sensor and interacts with BiP. On Ca^{2+} depletion from the ER via IP3R, BiP dissociates from Sig-1R, interacts with IP3R, and protects intrinsically unstable IP3R from degradation. Cytosolic sorting protein PACS-2 recruits CNX to the MAM (Myhill et al. 2008). Both CRT and CNX interact with SERCA2b and inhibit Ca^{2+} oscillations (John et al. 1998). The MAM is also the place where energy (ATP) and lipids are exchanged between ER and mitochondria. Ca^{2+} and ATP levels affect the activities of ER chaperones and foldases. Thus, redox homeostasis, Ca^{2+} flux and the activities of ER foldases integrally affect the oxidative protein folding capacity in the ER.

ER Redox Homeostasis

Conditions in the ER are more oxidizing than those of the cytosol to favor the oxidative protein folding. This oxidative environment was long thought to be maintained by preferential GSSG influx into the ER lumen, but the discovery of an ER-resident flavoprotein, Ero1p (ER oxidoreductin 1) in yeast, changed this concept (Fig. 3A) (Hwang et al. 1992; Frand and Kaiser 1998; Pollard et al. 1998). Ero1, which has two orthologs (Ero1α and Ero1β) in higher

eukaryotes, is now thought to be the oxidative engine that serves as the primary oxidase of PDI (Appenzeller-Herzog et al. 2010). Concomitantly, hydrogen peroxide (H_2O_2) is thought to be produced from oxygen as an electron acceptor (Enyedi et al. 2010). Ero1α is expressed broadly in multiple human tissue-types, whereas Ero1β is well-expressed only in certain tissue-types, such as the pancreas (Dias-Gunasekara et al. 2005). Interestingly, Ero1α activity is regulated by the isomerization/reduction of intramolecular disulfide bonds that are exerted by PDI monitoring the redox state of the ER, whereas Ero1β seems to be less tightly regulated and shows higher oxidase activity in vitro (Inaba et al. 2010; Tavender and Bulleid 2010a; Wang et al. 2011). Surprisingly, a mutant mouse lacking both isoforms of intact Ero1 is viable, even though Ero1 genes in both *Saccharomyces cerevisiae* and *Drosophila* are essential (Frand and Kaiser 1998; Tien et al. 2008; Zito et al. 2010a). Recent studies revealed an alternate cascade centered on peroxiredoxin IV (PRDX4), which is thought to work as a H_2O_2 reducer (Tavender and Bulleid 2010b). PRDX4 can oxidize several different PDI family members, and H_2O_2 itself may also directly oxidize PDI family members (Karala et al. 2009; Margittai and Banhegyi 2010; Tavender et al. 2010; Zito et al. 2010b). These alternative pathways would serve to alleviate excess ROS production in the ER and suggest the existence of diversified and intricate oxidative cascades (Csala et al. 2010).

ER Calcium Balance

The ER plays a major role in Ca^{2+} homeostasis and signaling, and contains a total Ca^{2+} concentration of 1–3 mM and a free Ca^{2+} concentration of 60–400 μM (Bygrave and Benedetti 1996). A number of Ca^{2+}-binding proteins reside in the ER, including calreticulin, calnexin, BiP, GRP94, calumenin, and the reticulocalbins (Coe and Michalak 2009; Michalak et al. 2009). Their functions are facilitated by high Ca^{2+} concentrations, whereas Ca^{2+} depletion with agents such as thapsigargin results in the deterioration of the proteins and triggers the UPR. It has been speculated that the higher Ca^{2+} concentration in the lumen mimics the effect of extracellular Ca^{2+} and helps proteins adopt a stable conformation for secretion.

Accumulating evidence shows that the ER calcium flux is linked with the luminal redox condition (Fig. 3B) (Gorlach et al. 2006). The ryanodine receptor is a redox sensitive Ca^{2+} channel in the membrane of the sarcoplasmic reticulum (Zable et al. 1997; Feng et al. 2000). ERp44, which senses the redox state as well as luminal pH and Ca^{2+} concentration, interacts with the luminal loop of IP3R type 1 (IP3R1) and directly inhibits its activity (Higo et al. 2005). ERp57, another ER oxidoreductase, has been reported to oxidize the luminal thiols of SERCA2b in a Ca^{2+}-dependent manner, thereby reducing the frequency of SERCA2b-dependent Ca^{2+} oscillations (Li and Camacho 2004). These interactions maintain ER calcium concentration and play an antiapoptotic role during cellular stress responses (Rizzuto et al. 2009). On the other hand, the ER-resident oxidase Ero1α has a proapoptotic role. Ero1α is induced by the CHOP-dependent stress response and hyperoxidizes the ER environment, which indirectly activates IP3R1 and releases Ca^{2+} into the cytosol (Li et al. 2009). The released Ca^{2+} activates calcium/calmodulin-dependent protein kinase II (CaMKII), which triggers apoptosis through both mitochondrial pathways and death receptor (Timmins et al. 2009). Thus, redox and Ca^{2+} homeostasis are interrelated in both a physical and physiological context.

STIM 1 and 2 (stromal-interacting molecules 1 and 2) were recently discovered to be ER Ca^{2+} sensors that, under depletion of Ca^{2+} stores, signal to the outer plasma membrane to activate store-operated Ca^{2+} channels (e.g., Orai1, Orai2, and Orai3) and inhibit voltage-gated Ca^{2+} channels (e.g., $Ca_V1.2$ channel) (Liou et al. 2005; Park et al. 2010; Wang et al. 2010b). Sigma-1 receptor (Sig-1R), which has a BiP-interacting lumenal domain and is primarily localized to the mitochondria-associated membrane (MAM), also works as a Ca^{2+} sensor. On Ca^{2+} depletion from the ER via IP3R type 3 (IP3R3), BiP dissociates from Sig-1R,

associates with IP3R3, and protects intrinsically unstable IP3R3 from degradation (Hayashi and Su 2007). Thereby, Ca^{2+} signaling to the mitochondria is stabilized, which potentially contributes to antiapoptotic regulation.

SPECIALIZED COMPARTMENTS WITHIN THE ER

Although the ER is composed of a continuous and interconnected tubular membrane network, it performs diversified and sometimes apparently opposing functions, such as cotranslation translocation of nascent polypeptides and retrotranslocation of misfolded proteins into the cytosol, or oxidation and reduction of cysteine residues of secretory and membrane proteins. To perform these various functions, the ER maintains morphologically and functionally different subdomains. Structurally, the rough ER (ribosome-attached), smooth ER (ribosome-free), and nuclear envelope are clearly distinguished (Voeltz et al. 2002). Constituents and contents of the ER, such as Ca^{2+} levels and lipid compositions, are also heterogeneous throughout (Rizzuto and Pozzan 2006; Pani and Singh 2009). Therefore, specialized regions will exist, such as the ER quality control (ERQC) compartment where ERAD machineries are enriched and N-glycans of misfolded glycoproteins are trimmed extensively for efficient degradation (Avezov et al. 2008; Lederkremer 2009). These specialized regions are also linked to other organelles, including mitochondria, the plasma membrane, peroxisomes, lysosomes, etc. (Lebiedzinska et al. 2009). In particular, a tight interaction between the ER and the mitochondria-associated membrane (MAM) is now emerging as a major cellular signaling hub (Fig. 3B)(Hayashi et al. 2009; Simmen et al. 2010). On the MAM, ER chaperones (CNX, BiP), oxidoreductases (ERp44, Ero1α), and Ca^{2+} channels/pumps (IP3R3, SERCA2b, and Sig-1R) are enriched, thereby creating an ideal environment for oxidative protein folding, as well as the regulation of Ca^{2+} flux (Gilady et al. 2010). On the MAM, IP3Rs, SERCA2b, and Sig-1R are regulated by ER oxidoreductases and/or chaperones, and ROS are produced as a by-product of oxidative protein folding. Both Ca^{2+} and ROS often function as signaling molecules. Thus, the MAM appears to be a processing hub that integrates signaling information derived from redox, Ca^{2+}, and protein homeostasis. PAM (plasma membrane-associated membranes) is another example, albeit less well-characterized, in which the ER is located in the proximity of the plasma membrane (Lebiedzinska et al. 2009). This region is involved in cellular Ca^{2+} homeostasis, particularly capacitative Ca^{2+} entry (CCE), by facilitating interactions between STIMs and Orais. Elucidating the functions and mechanisms of these specialized compartments will broaden and integrate our understanding of proteostatic and metabolic regulation in the ER.

THERAPEUTIC PERSPECTIVES

Protein density in the ER is extremely high, around 100 mg/ml, together with concomitant protein manufacturing (Stevens and Argon 1999). Therefore, the elaborate proteostasis network described above is vital. Its absence inevitably leads to aberrant folding, degradation defects, and pathological consequences. For instance, the amounts and activities of ER chaperones and foldases decrease with age, resulting in a reduction in basal metabolism, which is responsible for a number of maladies, including neurodegenerative diseases, diabetes, cancer, and obesity (Douglas and Dillin 2010; Naidoo 2009). In addition, there are numerous inherited loss-of-function disorders caused by the mutation of specific genes related to ER homeostasis such as the cystic fibrosis *trans*-membrane conductance regulator (CFTR) and α 1-antitrypsin Z (ATZ) (Hebert and Molinari 2007).

For therapeutic purposes, chemical chaperones that stabilize mutant proteins and help polypeptides to achieve native structure have been extensively investigated (Lawrence et al. 2011; McLaughlin and Vandenbroeck 2010). 4-phenyl butyric acid (4-PBA) is a successful low-molecular weight compound that has been shown to have a beneficial effect on several misfolding-related diseases, including cystic

fibrosis, α-1-antitrypsin (α1AT) deficiency, and type 2 diabetes mellitus (Ozcan et al. 2006; Hutt et al. 2009). The precise role of 4-PBA is not yet clear, but it has been determined that it has chaperone-like activities and may inhibit histone deacetylases (HDACs) at high concentrations (Powers et al. 2009). Boosting the capacity of the proteostasis network is another promising approach for therapy. For example, increasing calcium levels in the ER enhances the ER chaperone capacities and increases the production of misfolding-prone enzymes such as mutant variant of glucocerebrosidase (Ong et al. 2010). Also in the cytosol, the induction of Hsp70 or chaperonin CCT/TRiC may inhibit the formation of toxic oligomers, thereby preventing the onset of protein-folding diseases such as Huntington's disease (Behrends et al. 2006; Kitamura et al. 2006; Tam et al. 2006). On the other hand, inhibition of specific chaperones is also useful for the treatment of some diseases. Inhibitors of PDI were reported to suppress the toxicity of misfolded huntingtin in rat neuronal cells, presumably through inhibiting the proapoptotic function of PDI on the MAM (i.e., ROS production) (Hoffstrom et al. 2010). When it comes to in vivo case, vitamin A–coupled liposomes, which deliver small interfering RNA (siRNA) against collagen specific chaperone HSP47 to the hepatic stellate (HS) cell, showed the favorable therapeutic potential for suppressing the liver cirrhosis by reducing the accumulation of insoluble collagen in HS cells (Sato et al. 2008). In addition, stimulating the appropriate degradation of pathogenic proteins will also be beneficial for some cases. For example, the drug carbamazepine has been used to enhance the autophagic pathway, which results in a reduction of the hepatic load of ATZ (Hidvegi et al. 2010). Combination approach also could be used to synergize these effects (Mu et al. 2008).

CONCLUDING REMARKS

In the ER, protein homeostasis, redox, and calcium balances appear to be closely related to each other. We need to have a broader understanding on these processes, especially for complicated biological phenomena such as aging, disease chronicity, and neoplasm. The state-of-the-art "omics" technologies would be helpful to capture the holistic biological points of view (Chen et al. 2010; Churchman and Weissman 2011; Olzscha et al. 2011). On the other hand, established reductionistic approaches are also continuously needed for acquiring knowledge on fundamental biological processes such as the identification of components of the ERAD complex. The knowledge gained from these studies can be applied for the treatment of diseases and improving our health.

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