Chapter 71
Endoplasmic Reticulum-Associated Degradation (ERAD) of Misfolded Glycoproteins and Mutant P23H Rhodopsin in Photoreceptor Cells

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71.1 The Endoplasmic Reticulum: Protein Folding and Quality Control

ER-resident chaperones are among the first proteins that interact with a nascent polypeptide chain. For instance, BiP/Grp78, an Hsp70 orthologue, detects and binds unfolded hydrophobic regions of a nascent polypeptide chain in an ATP-dependent process (Hendershot et al. 1995). ER-resident J-domain co-chaperones, ERdj1 and ERdj2, regulate the interaction between BiP/Grp78 and the nascent peptide (Blond-Elguindi et al. 1993).

The initial step of ER glycoprotein modification involves attachment of a Glc3Man9GlcNAc2-core glycan onto a nascent polypeptide chain that is further processed by activities of Glucosidase I and II (Aebi et al. 2009). On removal of glucose residues, the monoglycosylated N-glycan becomes a substrate for ER-resident lectins, calreticulin (CRT) and calnexin (CNX). CRT and CNX both require Ca^{2+} for their activities; CNX is ER membrane-bound, while CRT is soluble in the ER lumen (Wada et al. 1991; Peterson et al. 1995). Both lectins promote protein folding by stabilizing folding sequences, preventing aggregation of unfolded proteins, and facilitating disulfide-bond formation through association with ER oxidoreductase, ERp57, a protein disulfide isomerase (PDI) homologue (Oliver et al. 1999; Ellgaard 2004).

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Glycoproteins that fail to fold correctly are subject to a quality control process (Trombetta and Parodi 2003). A key quality control sensor of the ER is UGGT1, which recognizes structural formation of misfolded proteins and alters their glycosylation stage to regenerate monoglycosylated glycans, which subsequently renews binding to CNX and CRT and reentrance into the ER protein-folding cycle (Trombetta and Helenius 2000). This cycle continues until the native conformation of the protein is achieved, or failing this, until the protein is targeted for disposal by endoplasmic reticulum-associated degradation (ERAD) (Lippincott-Schwartz et al. 1988). Some ER-retained proteins can also be modified by mannosidases, which may act as a timer for glycoprotein degradation and thus prevent glycoproteins from becoming permanently trapped in the reglucosylation/folding cycle (Fagioli and Sitia 2001).

71.2 Recognition Misfolded Proteins in the ER

The complete mechanism for recognizing misfolded ER proteins is poorly understood. One step in directing glycoprotein substrates to the ERAD machinery is the formation of the Man_7 N-glycan with a 1,6-linked mannose (Hosokawa et al. 2010a). Various ER-resident enzymes are able to trim mannose residues, such as ER mannosidase I, a member of the glycosyl hydrolase 47 family, which also includes the ER degradation enhancing α-mannosidase-like proteins 1–3 (EDEM1–3) and Golgi mannosidases (Aebi et al. 2009). EDEM1 enhances ERAD through its ability to extract misfolded glycoproteins from the CNX/CRT cycle (Molinari et al. 2003; Oda et al. 2003). EDEM1 and EDEM3 also trim mannose residues from N-glycan (Hirao et al. 2006; Hosokawa et al. 2010b). By contrast, EDEM2 has no enzymatic activity, but still increases turnover of misfolded proteins in the ER and likely plays nonenzymatic roles in ERAD (Mast et al. 2005). The mammalian PDI orthologue, ERdj5, is a cochaperone of EDEM1 and BiP/Grp78. ERdj5 recognizes misfolded proteins and reduces disulfide bonds via its reductase activity, which is important for protein dislocation (Ushioda et al. 2008).

71.3 From Quality Control to Dislocation for ERAD

How are misfolded proteins targeted for dislocation from the ER to the cytosol? OS-9 and XTP3-B are ER lectin-like proteins that contain mannose 6-phosphate receptor homology domains and N-linked glycosylation sites. OS-9 and XTP3-B may recognize and transfer misfolded proteins to an ER membrane-bound dislocation complex (Christianson et al. 2008; Hosokawa et al. 2008; Bernasconi et al. 2010). OS-9 interacts further with the ER-luminal Hsp90 homologue, 94 kDa glucose-regulated protein (Grp94), to deliver ERAD substrates to the dislocation complex. The complex contains the E3 ubiquitin ligase (HRD), the membrane adaptor

protein (Sel1L), and a membrane-embedded pore that forms the dislocation channel (Christianson et al. 2008; Hosokawa et al. 2008; Mueller et al. 2008). Sel1L is a type I transmembrane glycoprotein, which interacts with the ERAD components HRD1, Derlin1, and Derlin2 as well as with the cytoplasmic protein p97/VCP (valosin-containing protein) (Lilley and Ploegh 2005). OS-9 and XTP3-B associate with the HRD1-Sel1L ubiquitin ligase complex and XTP3-B is able to recognize both glycosylated and nonglycosylated ERAD substrates and facilitate their degradation (Hosokawa et al. 2008). Additional proteins and regulatory steps are likely to be involved in determining how misfolded proteins are selected for ERAD and delivered to the ER dislocation channel.

71.4 Cytosolic Events of ERAD

The dislocation and translocation of an ERAD substrate from the ER to cytosol requires activity of AAA-ATPases such as p97/VCP (Ye et al. 2001; Jarosch et al. 2002). p97/VCP forms homohexamers, which associate with the cofactors Ufd1 (ubiquitin fusion degradation 1) and Npl4 (nuclear protein localization 4) to extract substrates from the ER membrane (Bays et al. 2001; Ye et al. 2001; Braun et al. 2002) using energy provided by ATP hydrolysis (Zhang et al. 2000).

ERAD substrates are further ubiquitinated once in the cytosol through a process that requires three cytosolic enzymes. E1 activates ubiquitin in an ATP-dependent manner; E2 then conjugates activated ubiquitin through a thiol-ester bond to its essential cysteine residue, and the E3 ligase transfers ubiquitin onto one or more lysine residues or the N-terminus of the target proteins (Weissman 2001). The E4 ubiquitin-chain-extension enzyme is also shown to be involved in the ERAD degradation pathway (Richly et al. 2005).

The ubiquitinated substrate is ultimately degraded by the proteasome. The 26S proteasome is a large cytosolic protease complex, consisting of a 20S core particle that is capped by the 19S regulatory particle (Finley 2009). Four heptameric rings, two outer α subunits, and two inner β subunits form a barrel-shaped structure with proteolytic activity in the central cavity (Groll et al. 1997). The core particle entrance is very narrow and requires partial unfolding of the substrate for entrance (Finley 2009). The regulatory particle contains ATPase subunits and plays an important role in substrate recognition, unfolding, and translocation of target proteins into the core particle (Finley 2009). Proteins that target polyubiquitinated substrates to the proteasome include: Rad23 (radiation sensitive 23); Dsk2 (dominant suppressor of Kar1); Rpn10 (regulatory particle non-ATPase10); and Rpn13 (Finley 2009). Before proteolysis, proteasome-associated deubiquitin (DUBs) enzymes cleave and shorten the ubiquitin chain of target proteins resulting in the insertion of the substrate into the proteasome. Human proteasomes have three distinct DUB's, RPN11, UCH37 and USP14, which are associated with the regulatory particle (Finley 2009). Deubiquitin hydrolases remove the polyubiquitin chain, and ubiquitin proteins are recycled. Additionally, cytosolic N-glycanase removes oligosaccharides from

ERAD substrates to allow translocation into the proteasome (Blom et al. 2004; Misaghi et al. 2004). N-glycanase interacts with other ERAD components and Rad23 (Suzuki et al. 2001). The regulatory particle then unfolds the substrate and translocates it to the core particle for degradation.

71.5 ERAD in Retinitis Pigmentosa

In retinitis pigmentosa arising from the P23H rhodopsin (Rho) mutation, P23H Rho proteins are misfolded in the ER/Golgi and associate with CNX, BiP/Grp78 and Grp94 (Fig. 71.1a) (Anukanth and Khorana 1994; Noorwez et al. 2009). Recent studies implicate ERAD in the removal of misfolded P23H Rho. EDEM1 recognizes mutant Rho in the ER lumen and targets it for ERAD (Fig. 71.1b) (Kang and Ryoo 2009; Kosmaoglou et al. 2009). The complete mechanism of how mutant

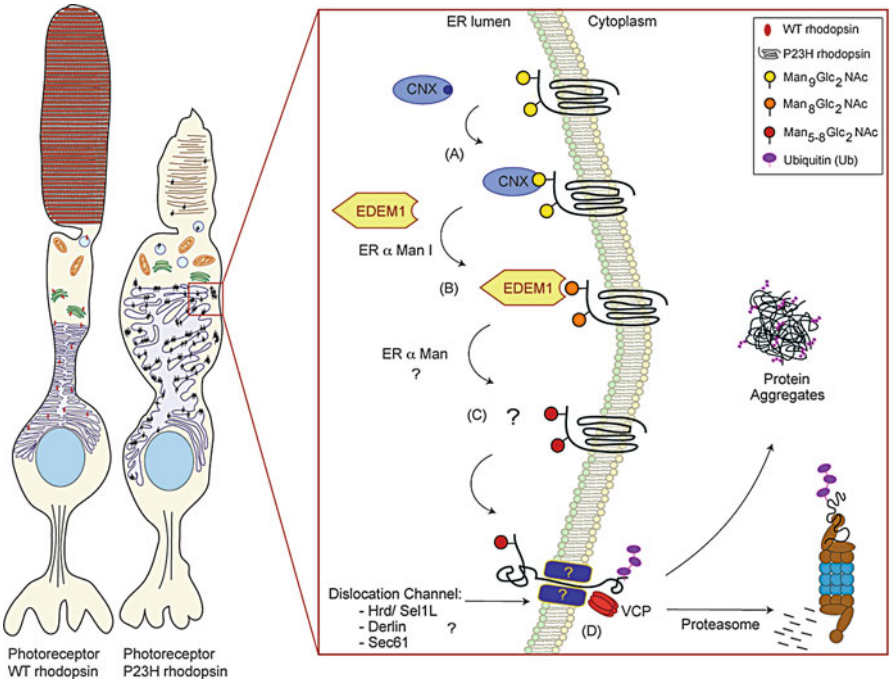

Fig. 71.1 Model of P23H rhodopsin clearance in photoreceptors by ERAD (**a**). Misfolded P23H rhodopsin (Rho) is a glycoprotein that interacts with calnexin (CNX) during folding. (**b**) Misfolded P23H Rho is trapped in the quality control/folding cycle and becomes a target for ER α-mannosidase I. After removal of mannose residues, mutant Rho is recognized by EDEM1. (**c**) Once associated to EDEM1, P23H Rho may be further demannosylated and modified by other ER-resident chaperones, which also promote the delivery of P23H Rho to the membrane-bound dislocation channel. (**d**) p97/VCP extracts P23H Rho through the channel into the cytosol, where it will be degraded by the proteasome or form aggregates

Rho is dislocated from the ER membrane to the cytosol is unknown, but the AAA-ATPase p97/VCP is one factor in the dislocation and delivery of P23H Rho to the proteasome (Fig. 71.1d) (Griciuc et al. 2010a, b). In vitro studies have shown that misfolded P23H Rho is ubiquitinated and targeted for proteasomal degradation (Sung et al. 1991; Illing et al. 2002; Saliba et al. 2002). Many other ERAD components are likely to be involved in the identification and delivery of P23H Rho to ERAD (Fig. 71.1c).

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