Structural Basis of an ERAD Pathway Mediated by the ER-Resident Disulfide Reductase ERdj5

Endoplasmic reticulum-associated degradation (ERAD) is an ER quality-control process that eliminates terminally misfolded proteins. ERdj5 was discovered to be a key ER-resident enzyme that accelerates ERAD by reducing incorrect disulfide bonds in misfolded glycoproteins recognized by lectin-like protein EDEM1. In this study, we solved the crystal structure of full-length ERdj5, thereby revealing that ERdj5 contains the N-terminal J-domain and six tandem thioredoxin domains that can be divided into the N- and C-terminal clusters. Our *in vitro* and *in vivo* functional analyses clarified that it was the C-terminal cluster that forms a platform interacting with EDEM1 and reduces disulfide bonds in EDEM1-recruited substrates. This study presents a detailed molecular view of how ERdj5 mediates ERAD in concert with EDEM1.

In eukaryotic cells, the endoplasmic reticulum (ER) is the compartment where membrane and secretory proteins fold, assemble, and form disulfide bonds. The ER contains molecular chaperones that assist in protein folding, and also contains unique enzymes that catalyze co- and post-translational modifications, such as glycosylation and disulfide bond formation. In some cases, however, nascent polypeptide chains are terminally misfolded and retrotranslocated from the ER into the cytosol for degradation via the ubiquitin-proteasome system. This process is known as ER-associated degradation (ERAD).

Recently, we identified ERdj5, a member of the protein disulfide isomerase (PDI) family, as an ERAD enhancer that works in concert with EDEM1, ER-degradation enhancing a-mannosidase-like protein-1 and BiP, an ER-resident Hsp70 family chaperone [1]. ERdj5 most likely accelerates ERAD by reducing incorrect disulfide bonds of EDEM1-recruited misfolded proteins and transferring them to BiP. To gain further molecular insights into the ERdj5-mediated ERAD pathway, we here carried out crystallographic and structure-based biochemical studies on ERdj5 and its associated factors [2].

The 2.4-Å crystal structure of full-length ERdj5 from *M. musculus* revealed that the enzyme is composed of an N-terminal J-domain with BiP-binding ability and six tandem thioredoxin (Trx) domains, including two newly identified domains that lack a redox-active CXXC motif (referred to as Trxb1 and Trxb2). As illustrated in Fig. 1, these six Trx domains are contained in a single plane while the J-domain is slightly deviated from the plane and resides above the interface between Trx1 and Trx2. The overall structure of ERdj5 can be divided into two domain clusters, the N-terminal cluster consisting of J, Trx1, Trxb1, Trxb2 and Trx2, and the C-terminal cluster consisting of Trx3 and Trx4. The surface of the cleft formed between the two clusters is much narrower and less hydrophobic than that of PDI, suggesting that the cleft is not the primary site that interacts with misfolded proteins.

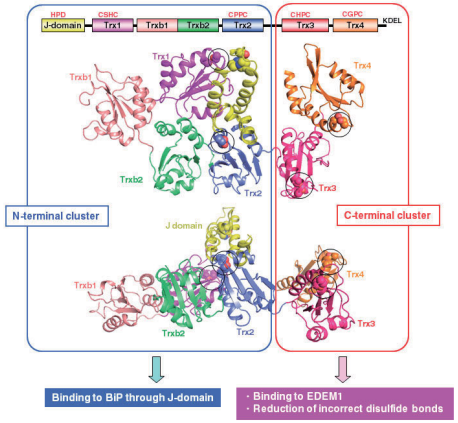

Figure 1
Overall structure of ERdj5 and functional roles of the N- and C-terminal clusters. The side chains of the redox active sites are shown in space-filling representation and encircled. The lower ribbon diagram illustrates the structure from the upper diagram rotated by 90° around the horizontal axis.

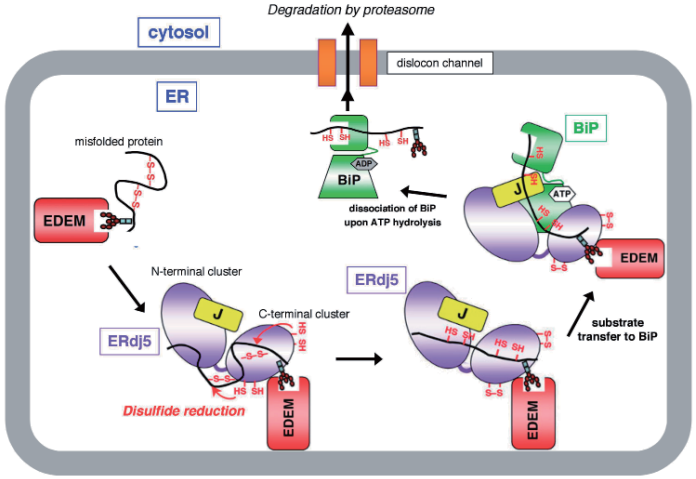

Figure 2
Plausible model of the ERdj5-mediated ERAD pathway.

On the basis of these structural insights, we performed systematic biochemical analyses. To explore which Trx domains in ERdj5 are primarily involved in ERAD acceleration, we constructed a set of mutants in which only one CXXC motif in ERdj5 is retained, while the remaining three CXXC motifs are substituted with AXXA. The pulse-chase experiment using the cell overexpressing each of the ERdj5 mutants demonstrated that a redox-active CXXC motif in the Trx3 or Trx4 domain was necessary and sufficient to accelerate ERAD. Consistently, our *in vitro* analysis characterized that the Trx3 and Trx4 domains had highly reducing redox potential and strong reductase activity against insulin.

Another central issue is which region of ERdj5 interacts with EDEM1. The co-immunoprecipitation experiments clarified that the C-terminal cluster alone was capable of binding EDEM1 like full-length ERdj5 while the removal of this cluster completely eliminated the ability of ERdj5 to associate with EDEM1. Importantly, the complex of EDEM1 and the ERdj5 C-terminal cluster contained a detectable level of ERAD substrate, suggesting that EDEM1 preferentially recruits the substrate to the C-terminal cluster of ERdj5 during the ERAD pathway. The actual involvement of BiP in the substrate transfer from ERdj5 to the retrotranslocon channel was also confirmed by our extensive pulse-chase experiment.

Taken together, we propose a model of the ERdj5-mediated ERAD pathway (Fig. 2). First, EDEM1 functions as a selection filter that exclusively recognizes a terminally misfolded glycoprotein released from the calnexin cycle. EDEM1 associates with the C-terminal cluster of ERdj5, which, in turn, engages in the disulfide bond reduction against substrate proteins recruited by EDEM1. At the later stage, BiP is likely involved in the substrate transfer to a retrotranslocation channel in an ATP-dependent manner. We thus established a structural and mechanistic basis of a sequential ERAD pathway that proceeds in mammalian cells.

REFERENCES

[1] R. Ushioda, J. Hoseki, K. Araki, G. Jansen, D.Y. Thomas and K. Nagata, *Science* **321** (2008) 569.
[2] M. Hagiwara, K Maegawa, M. Suzuki, R. Ushioda, K. Araki, Y. Matsumoto, J. Hoseki, K. Nagata and K. Inaba, *Mol. Cell* **41** (2011) 432.

BEAMLINE

5A

M. Hagiwara[1], K. Maegawa[2], M. Suzuki[3], K. Nagata[1] and K. Inaba[2] ([1]Kyoto Sangyo Univ., [2]Kyushu Univ., [3]Osaka Univ.)