【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Biology)

Title of Project : Mechanism of the maintenance of ER homeostasis by redox regulation

Kazuhiro Nagata
(Kyoto Sangyo University, Faculty of Life Sciences, Professor)

Research Area : Biology・Biological Science・Cell Biology
Keyword : Protein degradation, ER

【Purpose and Background of the Research】

Membrane and secretory proteins misfolded in the endoplasmic reticulum (ER) are degraded by so-called ER-associated degradation (ERAD) by ubiquitin-proteasome system after retrogradely translocated from the ER to the cytosol. We have found two critical factors involved in the ERAD, EDEM and ERdj5. ERdj5 reduces and cleaves the disulfide bonds in misfolded proteins to facilitate the retrotranslocation. Mammalian ER contains more than 20 oxidoreductases that are important not only for productive folding of nascent proteins but also for their degradation. In the ER, the maintenance of protein homeostasis, redox homeostasis and calcium homeostasis are indispensable for cell survival. In this study, we reveal the mechanism of the maintenance of ER homeostasis by redox regulation and analyze the crosstalk between the homeostasis at the molecular level.

【Research Methods】

(1) Maintenance of protein homeostasis by quality control (ERAD) in the ER

We have revealed that ERdj5 reduces and cleaves disulfide bonds in misfolded proteins in the ER to facilitate the retrotranslocation for degradation. However, it remains to be addressed how ERdj5 obtain reducing power in the oxidative conditions in the ER. Even if disulfide bonds are reduced, they would be expected to be oxidized rapidly. We will focus how reduced forms of cysteins are maintained until they reach the retrotranslocation channel by exploring the binding or escort proteins for ERdj5.

(2) Maintenance of redox homeostasis in the ER

As the ER is the major organelle for protein synthesis, more than 20 oxidoreductases exist in the mammalian ER in addition to the molecular chaperones and folding enzymes. However, little is known on how so many oxidoreductases are necessary in mammalian cells, and how they exert oxidative or reductive forces for the client proteins through their networks or cascades. We focus our effort on two representative oxidases, Ero1a and PDI, both of which make a regulatory hub complex. We reveal the entity of cascade of oxidative reaction by this hub complex on other oxidoreductases in the ER, and also reveal the intramolecular and intermolecular electron transfer pathways by biochemical approaches.

(3) Maintenance of calcium homeostasis in the ER

ER is known as the main organelle for calcium storage as well as protein synthesis. Calcium homeostasis is regulated by two calcium pumps, IP3 receptor and SERCA2 for influx and efflux, respectively. Both of which are known to be regulated by the redox conditions in the ER. Thus, we will identify which oxidoreductases are involved in the regulation of these calcium pumps.

【Expected Research Achievements and Scientific Significance】

The maintenance of three major homeostasis in the ER, including protein, redox and calcium homeostasis, are requisite for cell survival. Among them, regulation of calcium in the ER is would be the central issue of this project. Our study will reveal the molecular mechanism of the crosstalk between the homeostasis and would shed light on the indispensible role of the ER.

【Publications Relevant to the Project】

Usioda, R. et al., ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. ***Science*** **321**; 569-572 (2008)
Hagiwara, M. et al., Structural bases of an ERAD pathway mediated by the ER-resident protein disulfide reductase ERdj5. ***Mol. Cell*** **41**; 432-444 (2011)

【Term of Project】 FY2012-2016

【Budget Allocation】 167,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.cc.kyoto-su.ac.jp/~nagata/index-e.html