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Updates on "Endoplasmic Reticulum Redox"

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PROBABLY THE MOST IMPORTANT MESSAGE that a Forum on the reduction–oxidation (redox) environment in the endoplasmic reticulum (ER) must nowadays convey is that an "ER redox state" as such does not exist. The ER rather features a variety of redox systems, which—in many cases—react independently with incoming substrates and xenobiotics. Thus, any statement claiming ER hyper- or hypo-oxidation under given experimental conditions potentially lacks specificity, because it is not clear which ones of the many redox couples in the ER are in fact being affected. Prominent redox couples abundantly present in the ER include, but are not limited to, reduced polypeptide–disulfide-linked polypeptide, glutathione (GSH)–glutathione disulfide (GSSG), nicotinamide adenine dinucleotide $(NAD)^+$–NADH, NAD phosphate $(NADP)^+$–NADPH, ascorbate (vitamin C)–dehydroascorbic acid (DHA), water–hydrogen peroxide (H_2O_2), and H_2O_2–molecular oxygen (O_2). None of these redox couples are in thermodynamic equilibrium with each other in the ER—a feature that generally applies to all living systems.

In their review article to this effect, Bánhegyi *et al*. elaborate that the presence or—alternatively—the specific absence of a funneled (*i.e.*, catalyzed) crosstalk between different ER redox couples can be essential to maintain the functionality of the ER (4). The article focuses on the three low-molecular-weight redox couples: GSH–GSSG, $NAD(P)^+$–NAD(P)H, and ascorbate–DHA. While the interrelationships between these electron carriers have largely remained an orphan field in ER biology that certainly will deserve increased attention in the future, the authors still draw some interesting and important conclusions. For instance, the remarkable coexistence of the predominantly reduced NAD(P) pool, which bears enormous reducing power, with the relatively oxidizing thiol-disulfide pool (*e.g.*, represented by the redox state of GSH–GSSG) is made possible by tight kinetic barriers in between the two redox systems (12). By contrast, DHA is an excellent oxidant of protein thiols in ER folding substrates (13), although a physiological proof of this pathway for disulfide-bond formation will yet depend on the successful translation of *in vitro* information into a living cell system.

Another ER luminal oxidant that has recently moved into the focus of investigations is H_2O_2. We now know that this peroxide is being metabolized to water in the ER through the action of dedicated peroxidases, which are reviewed by Roberto Sitia and colleagues (9). Two types of ER-resident peroxidases exist: Peroxiredoxin 4 (Prx4) is a typical 2-cysteine peroxiredoxin (15), and glutathione peroxidases 7 and 8 (GPx7 and GPx8) belong to the non-selenium thioredoxin GPx-like peroxidase family (11). All three ER peroxidases catalyze the transfer of electrons from protein disulfide isomerase (PDI)-like oxidoreductases (PDIs) to H_2O_2. A dual significance in ER homeostasis is ascribed to this type of redox reaction: First, it removes H_2O_2 from the ER lumen. As the use of O_2 as terminal electron acceptor in disulfide-bond formation (see below) implies the production of stoichiometric amounts of H_2O_2, the catalyzed detoxification of this reactive oxygen species on the spot is probably fundamental (2). Second, it constitutes one of the pathways that form an active-site disulfide in PDIs. Oxidized PDIs—as discussed further below—fulfill critical roles in the oxidative folding of secretory and membrane proteins.

Indeed, the oxidative process of disulfide-bond formation is of central importance for ER redox. For instance, the high concentration of GSSG, which, together with GSH, forms a thiol-disulfide buffering system in the ER (2), is a consequence of the local machinery for disulfide production (4). Still, although a number of enzymes with disulfide-catalyzing activity are known by now, their precise *in vivo* roles are controversial and remain to be settled. Prominent examples are the quiescin sulfhydryl oxidases and their smaller sister enzymes in fungi, the Erv2-like oxidases. The members of both flavoprotein families harbor a so-called Erv/ALR domain that can produce active-site disulfides at the expense of O_2, but only in the former, this domain is fused to additional redox-active thioredoxin-type domains. Carolyn Sevier surveys both the enzymological details and the proposed functions of these proteins, which are associated with the secretory pathway and the extracellular space (14). She concludes that knowledge on how the sulfhydryl oxidase activity of these enzymes integrates with cellular physiology is still largely missing. In equal measure, the fundamental role of the ER-resident sulfhydryl oxidases of the Ero1 family in disulfide-bond formation (that has almost universally been adopted as of the time of their discovery) has recently been called into question. This was based on the observation that mice (almost) exclusively expressing truncated, nonfunctional Ero1 proteins are perfectly viable (18). In this Forum, the structure, mechanism, and evolution of Ero1 family members is discussed and compared to that of other redox enzymes by Araki and Inaba (3). Among many other interesting features, their article explains in atomic resolution the crucial ability of human Ero1α to transfer its product—a disulfide bond—specifically to PDI.

PDI is a disulfide-carrying protein that shuttles disulfide bonds from, for example, Ero1, Prx4, or GSSG, to cysteine pairs in nascent polypeptide chains. Equally important, PDIs

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can also act (i) as disulfide reductants, which is often essential for the exchange of non-native disulfide bonds in folding proteins with the correct ones, and (ii) as molecular chaperones that facilitate the solubility and folding attempts of substrate proteins. At least in the case of PDI itself, this molecular chaperone function is redox regulated (17). Here, a review by Adam Benham assembles the existing links between PDI family proteins and human diseases (5). Interestingly, not all of these links take place in PDIs' cognate organelle, the ER, but also at the cell surface and—possibly—in the cytosol. The pathologies associated with the PDI family include Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, infertility, and various types of infectious diseases. A subset of the latter are mechanistically related to the endogenous process of ER-associated degradation (ERAD). This catabolic pathway includes the recognition of terminally misfolded proteins in the ER, their retrotranslocation to the cytosol, ubiquitination, and proteasomal proteolysis. As summarized by Billy Tsai and colleagues (16), PDIs are known to contribute to the substrate recognition step through both their oxidoreductase and their redox-regulated chaperone activities. In some ERAD substrates, disulfide bonds need to be resolved by PDIs before membrane translocation. Similarly, a set of bacterial toxins and viruses (ab)use the same route and machinery to penetrate the cytosol through the ER membrane. An example is simian virus 40. Upon reaching the ER lumen, the disulfide pattern in the shell of this nonenveloped virus is rearranged by the PDI ERp57. This leads to the exposure of a previously buried hydrophobic peptide that subsequently interacts with ERAD factors in the ER membrane and triggers membrane penetration and infection (8).

What's next in ER redox? Without intending to be exhaustive, three eminent questions shall be highlighted. (i) Is there—in light of the reports on Ero1-independent organisms—something like a major pathway for disulfide-bond generation in the ER, or do multiple pathways form an insusceptible oxidative network that guarantees thiol-disulfide homeostasis in the ER? These questions are addressed in several contributions to this Forum (3, 4, 9). (ii) With regard to the many structural and functional ER subcompartments known to date (7, 10), are the redox states of different redox couples compartmentalized in the ER (as should probably be expected)? (iii) Recent observations strongly suggest a tight coupling of the calcium concentration and the redox state (as measured by a H_2O_2-responsive sensor) in the ER (6). What are the mechanisms of this coupling? Do they include the regulatory function that Ero1α performs in the process of inositol 1,4,5-trisphosphate-induced calcium release from the ER (1)? Future studies are warranted to discover more redox connections between substrate proteins, resident enzymes, and low-molecular-weight electron carriers in the ER, many of which are likely fulfilling as yet uncharacterized tasks.

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References

1. Anelli T, Bergamelli L, Margittai E, Rimessi A, Fagioli C, Malgaroli A, Pinton P, Ripamonti M, Rizzuto R, and Sitia R. Ero1alpha regulates Ca(2+) fluxes at the endoplasmic reticulum-mitochondria interface (MAM). *Antioxid Redox Signal* 2011 [E-pub ahead of print]; DOI: 10.1089/ars.2011.4004.
2. Appenzeller-Herzog C. Glutathione- and non-glutathione-based oxidant control in the endoplasmic reticulum. *J Cell Sci* 124: 847–855, 2011.
3. Araki K and Inaba K. Structure, mechanism and evolution of Ero1 family enzymes. *Antioxid Redox Signal* 16: 790–799, 2012.
4. Bánhegyi G, Margittai E, Szarka A, Mandl J, and Csala M. Crosstalk and barriers between the electron carriers of the endoplasmic reticulum. *Antioxid Redox Signal* 16: 772–780, 2012.
5. Benham A. The protein disulfide isomerase (PDI) family: key players in health and disease. *Antioxid Redox Signal* 16: 781–789, 2012.
6. Enyedi B, Varnai P, and Geiszt M. Redox state of the endoplasmic reticulum is controlled by Ero1L-alpha and intraluminal calcium. *Antioxid Redox Signal* 13: 721–729, 2010.
7. Friedman JR and Voeltz GK. The ER in 3D: a multifunctional dynamic membrane network. *Trends Cell Biol* 21: 709–717, 2011.
8. Geiger R, Andritschke D, Friebe S, Herzog F, Luisoni S, Heger T, and Helenius A. BAP31 and BiP are essential for dislocation of SV40 from the endoplasmic reticulum to the cytosol. *Nat Cell Biol* 13: 1305–1314, 2011.
9. Kakihana T, Nagata K, and Sitia R. Peroxides and peroxidases in the endoplasmic reticulum: integrating redox homeostasis and oxidative folding. *Antioxid Redox Signal* 16: 763–771, 2012.
10. Lynes EM and Simmen T. Urban planning of the endoplasmic reticulum (ER): how diverse mechanisms segregate the many functions of the ER. *Biochim Biophys Acta* 1813: 1893–1905, 2011.
11. Nguyen VD, Saaranen MJ, Karala AR, Lappi AK, Wang L, Raykhel IB, Alanen HI, Salo KE, Wang CC, and Ruddock LW. Two endoplasmic reticulum PDI peroxidases increase the efficiency of the use of peroxide during disulfide bond formation. *J Mol Biol* 406: 503–515, 2011.
12. Piccirella S, Czegle I, Lizak B, Margittai E, Senesi S, Papp E, Csala M, Fulceri R, Csermely P, Mandl J, Benedetti A, and Banhegyi G. Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment. *J Biol Chem* 281: 4671–4677, 2006.
13. Saaranen MJ, Karala AR, Lappi AK, and Ruddock LW. The role of dehydroascorbate in disulfide bond formation. *Antioxid Redox Signal* 12: 15–25, 2010.
14. Sevier CS. Erv2 and quiescin sulfhydryl oxidases: Erv-domain enzymes associated with the secretory pathway. *Antioxid Redox Signal* 16: 800–808, 2012.
15. Tavender TJ, Sheppard AM, and Bulleid NJ. Peroxiredoxin IV is an endoplasmic reticulum-localized enzyme forming oligomeric complexes in human cells. *Biochem J* 411: 191–199, 2008.
16. Walczak CP, Bernardi KM, and Tsai B. Endoplasmic reticulum-dependent redox reactions control ER-associated degradation and pathogen entry. *Antioxid Redox Signal* 16: 809–818, 2012.
17. Wang C, Yu J, Huo L, Wang L, Feng W, and Wang CC. Human protein disulfide isomerase is a redox-regulated

chaperone activated by oxidation of domain a'. *J Biol Chem* 2011 [Epub ahead of print]; DOI: 10.1074/jbc.M111.303149

18. Zito E, Chin KT, Blais J, Harding HP, and Ron D. ERO1-beta, a pancreas-specific disulfide oxidase, promotes insulin biogenesis and glucose homeostasis. *J Cell Biol* 188: 821–832, 2010.

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Abbreviations Used

DHA = dehydroascorbic acid
ER = endoplasmic reticulum
ERAD = ER-associated degradation
GPx = glutathione peroxidase
GSH = glutathione
GSSG = glutathione disulfide
H_2O_2 = hydrogen peroxide
NAD = nicotinamide adenine dinucleotide
PDI = protein disulfide isomerase
Prx4 = peroxiredoxin 4

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