

New insights into oxidative folding

Carolyn S. Sevier

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

The oxidoreductase ERO1 (endoplasmic reticulum [ER] oxidoreductin 1) is thought to be crucial for disulfide bond formation in the ER. In this issue, Zito et al. (2010. *J. Cell Biol.* doi:10.1083/jcb.200911086) examine the division of labor between the two mammalian isoforms of ERO1 (ERO1- α and - β) in oxidative folding. Their analysis reveals a selective role for ERO1- β in insulin production and a surprisingly minor contribution for either ERO1 isoform on immunoglobulin folding and secretion.

In eukaryotic cells, protein disulfide bond formation occurs in the lumen of the ER as part of the folding and assembly process for newly synthesized secretory proteins. A pathway formed by the oxidoreductases ERO1 (ER oxidoreductin 1) and protein disulfide isomerase (PDI) drives the thiol–disulfide equilibrium in the ER toward disulfide bond formation (Tu and Weissman, 2004; Sevier and Kaiser, 2008). PDI directly catalyzes the formation of disulfides in secretory proteins, accepting electrons from thiols in folding nascent chains. To sustain additional rounds of protein disulfide formation, PDI transfers these electrons to ERO1. In turn, ERO1 passes its electrons to molecular oxygen and/or alternate acceptors in the cell.

Despite an overall conservation of the ERO1–PDI pathway among eukaryotes, important differences exist between organisms. Although many simple eukaryotes like *Saccharomyces cerevisiae* and *Caenorhabditis elegans* encode a single ERO1, mammals contain two ERO1 isoforms, ERO1- α and - β . At present, no major differences in redox activities or substrate interactions have been identified between the characterized human paralogues. Continuing this trend, in this issue, Zito et al. show that the two mouse ERO1 proteins have similar in vitro biochemical activities. Distinct tissue distribution and transcriptional regulation for the ERO1 isoforms has suggested the potential for unique contributions toward disulfide bond formation in the cell (Pagani et al., 2000; Dias-Gunasekara et al., 2005). However, the actual redundancy and/or specificity for the ERO1 isoforms in vivo has remained an open question. Zito et al. (2010) begin to address this issue by focusing on the roles of ERO1- α and - β in mice. Their experiments suggest a selective, nonredundant function for ERO1- β in oxidative protein folding in insulin-producing cells. Their work also provides the first indication as to how ERO1 activity impacts the development of individual tissues, advancing our knowledge of the role for ERO1 in oxidative folding in the animal.

The work of Zito et al. (2010) primarily focuses on ERO1- β , which has been the lesser characterized of the two isoforms. Using antisera specific for each of the mouse ERO1 isoforms, they observe tissue-specific expression of the mouse isoforms similar to that previously reported for the human ERO1s (Pagani et al., 2000; Dias-Gunasekara et al., 2005). Most striking was a strong and selective staining for ERO1- β in the pancreas. In contrast, ERO1- α was detected in all tissues. To study the cellular activity of ERO1- β , Zito et al. (2010) developed a mouse model homozygous for an insertion in intron 14 of the *Ero1b* locus that compromised ERO1- β expression in the pancreas. Disruption of ERO1- β resulted in defective oxidative folding in the ER; in particular, homozygous mutant mice showed a kinetic delay in the processing of disulfide-bonded proinsulin to insulin. As might have been anticipated by this delay in the folding of disulfide-linked insulin, loss of ERO1- β function adversely impacted insulin biogenesis and glycemic control in mice. By 3 mo of age, the majority of homozygous mutant mice showed a stable diabetic phenotype.

A surprise came upon further characterization of the residual oxidative folding observed in the homozygous ERO1- β mutant, which might have been expected to be a product of the activity from the remaining ERO1 isoform. Remarkably, the residual disulfide-linked folding of insulin does not depend on ERO1- α ; no enhancement in phenotype was observed with concomitant disruption of both the *Ero1l* and *Ero1b* loci. Considering that the ERO1 genes in both *S. cerevisiae* and *C. elegans* are essential for viability, the viability of the mutant mouse lacking both isoforms of ERO1 is quite surprising. These observations suggest that ERO1- β serves as an islet-selective isoform of ERO1 that enhances the oxidative folding capacity of insulin-producing cells. At least in the pancreas, the two ERO1 isoforms appear nonredundant. An additional unexpected result came with the analysis of the oxidative folding for immunoglobulin-producing cells in the double mutant mouse that revealed only a modest delay in the oxidative folding of IgM. As the authors propose, this provides strong evidence for an important ERO1-independent mechanism for generating disulfide bonds in mammals. What accounts for the residual activity remains an open question.

Only three classes of enzymes that can couple small molecule redox chemistry to the de novo formation of disulfides have been identified in the ER: ERO1, VKOR, and the ERV/QSOX superfamily. VKOR catalyzes the reduction of vitamin K, which

© 2010 Sevier This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons License [Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>].

Correspondence to Carolyn S. Sevier: csevier@mit.edu

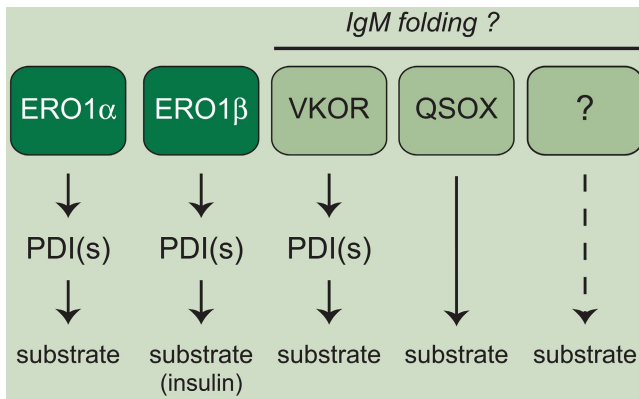


Figure 1. **Pathways for oxidative folding in the ER of *Mus musculus*.** Mice contain two ERO1 isoforms (α and β) that use PDI and/or PDI-like proteins (PDI(s)) to oxidize substrates. ERO1- β has a specific impact on the oxidative folding of insulin in the mouse pancreas (Zito et al., 2010). VKOR and QSOX proteins may also facilitate oxidative folding in the ER, although their connections to folding in the ER lumen are less well established. VKOR, QSOX, or alternative unidentified pathways in the ER, including small molecules, may allow for the oxidative folding of IgM in the absence of ERO1 activity.

is ultimately required for proper blood coagulation. It has been suggested that the catalytic cycle of VKOR is coupled with oxidative folding in the ER via PDI (Wajih et al., 2007). The ERV/QSOX superfamily consists of disulfide bond-forming catalysts that share a common flavin-binding domain that is found either alone (ERVs; Fass, 2008) or fused with a PDI-like domain (QSOX family; Thorpe and Kodali, 2010). Erv2 is the only ER-localized ERV protein and is a fungal-specific enzyme that can operate parallel to ERO1 in the yeast ER to introduce disulfide bonds into substrate proteins (Sevier et al., 2001). Erv2 is not present in mice. However, proteins of the QSOX family are present in vertebrates, plants, and protists. Mammalian QSOX enzymes were first characterized as extracellular proteins; however, recently, QSOX isoforms have been localized to the ER/Golgi region (Tury et al., 2004; Chakravarthi et al., 2007). It will be interesting to determine whether QSOX or VKOR enzymes account for the oxidizing power remaining in the ER of mammalian cells lacking ERO1 (Fig. 1). Intriguingly, genetic data in flies suggest that a member of the QSOX family may contribute to disulfide bond formation when the function of the single *Ero11* gene in the fly is compromised (Tien et al., 2008). Future efforts in the field will surely focus on the further characterization of alternate (and perhaps novel) oxidase systems beyond ERO1 in the ER lumen and how these additional pathways relate to the ERO1-PDI pathway.

At a broader level, the work of Zito et al. (2010) also highlights the challenge in reconciling an understanding of redox homeostasis in cultured cells with the additional complexities present in a whole animal. Alteration of ERO1 activity has been shown to help cells cope with redox imbalances in the ER of yeast and worms (Harding et al., 2003; Haynes et al., 2004; Marciniak et al., 2004). In keeping with these prior studies, Zito et al. (2010) observed that knockdown of ERO1- β in cultured Min6 cells strongly protected cells from toxicity of an insulin mutant defective for oxidative folding (proinsulin^{Akita}). However, the benefits caused by a decrease in ERO1 activity in cultured cells were not observed in pancreatic cells of live mice; the diabetic phenotype of the proinsulin^{Akita}

mutation was accentuated by genomic disruption of ERO1- β expression. The authors put forth several distinctions between cultured cells relative to tissues in the animal that may contribute to the differences observed in cell culture relative to the whole animal, including differences in oxygen levels and/or developmental programs. Alternatively, these disparities may relate to experimental differences: a stable disruption of ERO1- β in the mouse (disrupted throughout development) relative to a transient RNAi knockdown in the cultured system. Importantly, elucidating the contributing factors toward the different phenotypic outcomes of the loss of ERO1 activity will be an important step toward understanding how redox imbalances are offset in the whole organism.

Submitted: 19 February 2010

Accepted: 1 March 2010

References

- Chakravarthi, S., C.E. Jessop, M. Willer, C.J. Stirling, and N.J. Bulleid. 2007. Intracellular catalysis of disulfide bond formation by the human sulfhydryl oxidase, QSOX1. *Biochem. J.* 404:403–411. doi:10.1042/BJ20061510
- Dias-Gunasekara, S., J. Gubbens, M. van Lith, C. Dunne, J.A. Williams, R. Katak, D. Scoones, A. Laphorn, N.J. Bulleid, and A.M. Benham. 2005. Tissue-specific expression and dimerization of the endoplasmic reticulum oxidoreductase Erolbeta. *J. Biol. Chem.* 280:33066–33075. doi:10.1074/jbc.M505023200
- Fass, D. 2008. The Erv family of sulfhydryl oxidases. *Biochem. Biophys. Acta.* 1783:557–566. doi:10.1016/j.bbamer.2007.11.009
- Harding, H.P., Y. Zhang, H. Zeng, I. Novoa, P.D. Lu, M. Calfon, N. Sadri, C. Yun, B. Popko, R. Paules, et al. 2003. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell.* 11:619–633. doi:10.1016/S1097-2765(03)00105-9
- Haynes, C.M., E.A. Titus, and A.A. Cooper. 2004. Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death. *Mol. Cell.* 15:767–776. doi:10.1016/j.molcel.2004.08.025
- Marciniak, S.J., C.Y. Yun, S. Oyadomari, I. Novoa, Y. Zhang, R. Jungreis, K. Nagata, H.P. Harding, and D. Ron. 2004. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* 18:3066–3077. doi:10.1101/gad.1250704
- Pagani, M., M. Fabbri, C. Benedetti, A. Fassio, S. Pilati, N.J. Bulleid, A. Cabibbo, and R. Sitia. 2000. Endoplasmic reticulum oxidoreductin 1-beta (ERO1-Lbeta), a human gene induced in the course of the unfolded protein response. *J. Biol. Chem.* 275:23685–23692. doi:10.1074/jbc.M003061200
- Sevier, C.S., and C.A. Kaiser. 2008. Ero1 and redox homeostasis in the endoplasmic reticulum. *Biochim. Biophys. Acta.* 1783:549–556. doi:10.1016/j.bbamer.2007.12.011
- Sevier, C.S., J.W. Cuozzo, A. Vala, F. Aslund, and C.A. Kaiser. 2001. A flavoprotein oxidase defines a new endoplasmic reticulum pathway for biosynthetic disulfide bond formation. *Nat. Cell Biol.* 3:874–882. doi:10.1038/ncb1001-874
- Thorpe, C., and V.K. Kodali. 2010. Oxidative protein folding and the quiescin-sulfhydryl oxidase family of flavoproteins. *Antioxid. Redox Signal.* doi:10.1089/ars.2010.3098.
- Tien, A.C., A. Rajan, K.L. Schulze, H.D. Ryoo, M. Acar, H. Steller, and H.J. Bellen. 2008. Ero1L, a thiol oxidase, is required for Notch signaling through cysteine bridge formation of the Lin12-Notch repeats in *Drosophila melanogaster*. *J. Cell Biol.* 182:1113–1125. doi:10.1083/jcb.200805001
- Tu, B.P., and J.S. Weissman. 2004. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J. Cell Biol.* 164:341–346. doi:10.1083/jcb.200311055
- Tury, A., G. Mairet-Coello, F. Poncet, C. Jacquemard, P.Y. Risold, D. Fellmann, and B. Griffond. 2004. QSOX sulfhydryl oxidase in rat adenohypophysis: localization and regulation by estrogens. *J. Endocrinol.* 183:353–363. doi:10.1677/joe.1.05842
- Wajih, N., S.M. Hutson, and R. Wallin. 2007. Disulfide-dependent protein folding is linked to operation of the vitamin K cycle in the endoplasmic reticulum. A protein disulfide isomerase-VKORC1 redox enzyme complex appears to be responsible for vitamin K1 2,3-epoxide reduction. *J. Biol. Chem.* 282:2626–2635. doi:10.1074/jbc.M608954200
- Zito, E., K.-T. Chin, J. Blais, H.P. Harding, and D. Ron. 2010. ERO1- β , a pancreas-specific disulfide oxidase, promotes insulin biogenesis and glucose homeostasis. *J. Cell Biol.* 188:821–832. doi:10.1083/jcb.200911086