New insights into oxidative folding

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New insights into oxidative folding

Carolyn S. Sevier

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 Erol1b 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 Erol1 and Erol1b 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
Cells of live mice; the diabetic phenotype of the proinsulin Akita
ERO1 activity in cultured cells were not observed in pancreatic
is ultimately required for proper blood coagulation. It has been
an increased stress response regulates amino acid metabolism and resistance to oxidative stress.
In keeping with these prior studies, Zito et al. (2010) observed that
equivalent to ERO1 in the yeast ER to introduce disulfide bonds into
the catalytic cycle of VKOR is coupled with oxidative
doing their part in folding and helping cells cope with redox imbalances in the
Processing of insulin in the mouse pancreas (Zito et al., 2010). VKOR and QSOX
The authors put forth several distinctions between cul-
ture of the unfolded protein response.
whether VKOR or QSOX enzymes account for the oxidizing
protein synthesis and oxidation in the stressed endoplasmic reticulum. 
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 Ero1l 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, including small molecules, may allow for the oxidative folding of IgM in the absence of ERO1 activity.

**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.