Catalysts of *De Novo* Disulfide Bond Formation

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Disulfide bonds are crucial for the folding and stability of many cell-surface and secreted proteins. In addition, disulfides can be used for redox control of protein activity. *De novo* disulfide bond formation by enzymes such as those that drive oxidative protein folding requires transfer of electrons from dithiols to non-thiol electron acceptors. We have determined the structures of representatives of the two known eukaryotic enzyme families that catalyze this process. These enzymes, Ero1 and Erv2, are flavoproteins that share similar structural and mechanistic features despite a lack of sequence similarity. In particular, our crystallographic and enzymological studies suggest that both enzymes operate by a "disulfide shuttle" mechanism, in which a dithiol motif on a mobile segment of each enzyme transfers electrons from cysteines in substrate proteins to the rigid active-site disulfide. In both enzyme families, the shuttle disulfide determines the substrate specificity. Furthermore, both the Ero1 and Erv2 families can transfer electrons to a similar panel of electron acceptors. One striking difference between the two enzymes, however, is that Ero1 displays kinetic complexities not shared by Erv2. This difference will be discussed in light of the structures, a mechanistic model will be presented, and a possible physiological role for the complex behavior of Ero1 will be suggested.

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