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Forum Editorial

Oxidative Protein Folding: Recent Advances and Some Remaining Challenges

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Of all the 20 amino acids commonly found in proteins, the cysteine residue arguably has the most to answer for. In fact, if it wasn't for cysteine, none of the reviews and articles in this edition on oxidative protein folding could have been written. What is it about cysteine that makes it so special and has caused it to occupy the thoughts of so many biologists? The unique property that marks cysteine out for attention is that, in the context of a polypeptide chain, the sulfhydryl group can exist in a reduced form (-SH) or, when meeting a like-minded partner, an oxidized form: the disulfide bond (–S-S-). The oxidation of cysteine is a covalent modification that can be either *intra*molecular or *inter*molecular, and it adds an extra dimension of complexity to protein structure and function. This complexity gives the protein new opportunities, in terms of the chemistry of an active site, for example, or the potential for regulated interaction with partner proteins. But it comes at a cost. The oxidation of cysteine must be controlled, and it is now apparent that all organisms devote considerable resources to forming, maintaining, and exchanging disulfide bonds. The seemingly simple process of protein oxidation does not occur spontaneously in the cell and is carefully regulated.

This forum edition examines oxidative protein folding from a number of different perspectives. The scope of the topic is illustrated by the fact that disulfide bond formation occurs and is regulated in all organisms, including bacteria, viruses, and eukaryotes (9). Despite their obvious differences, these organisms have evolved very similar ways of controlling disulfide bond formation, using protein oxidants and small-molecular-weight electron shuttles and cofactors. However, the genomics approach of Fomenko and Gladyshev indicates that there are still exciting things to discover about oxidative protein folding, particularly in higher eukaryotes (9). Uncovering the precise role of endoplasmic reticulum (ER) resident glutathione peroxidases, selenoproteins, and other thioredoxin domain-containing proteins of the ER will be challenging, but should be very informative.

The most common compartments in which oxidative protein folding occurs are the bacterial periplasm and the eukaryotic ER. The Dsb proteins of the bacterial periplasm have fascinated researchers since 1991, when it was first shown that a mutation in *E. coli dsbA* could prevent the secretion of various oxidized proteins (4, 11). Some beautiful biochemical experiments have followed, and the Dsb story is reviewed here by Ortenberg and Beckwith (16). It is sobering to note that the predicted properties of the Dsb proteins do not always correspond to their true redox function, and this is one of many lessons from bacteria that are pertinent to the study of eukaryotes. The chemical and protein microenvironments have a big say in whether a particular Dsb will act as an oxidant or reductant, and one of the current challenges is how to study the chemistry of these proteins in their "natural surroundings." Nevertheless, *in vitro* analysis of protein function can still be informative, as Blank *et al.* show in their article on the DsbA protein of *Vibrio cholerae* (5). These authors used mutational and enzymatic studies to investigate the role of amino acid residues surrounding the DsbA active site, comparing the *V. cholerae* enzyme to that of *E. coli*. In particular, Blank *et al.* propose that mutating His94 can increase the activity of DsbA.

The knowledge gained from the bacterial Dsb system was valuable in 1997–98, when two groups showed that, in yeast, an *ero1–1* mutant also failed to secrete disulfide bonded proteins (10, 17). The mutated gene, ERO1, is found in all eukaryotic organisms thus far sequenced, and work from our group describes the initial characterization of these proteins in plants (8). Other studies have shown that, in the ER, Ero1p oxidizes protein disulfide isomerase (PDI) in a partnership reminiscent of DsbA/DsbB. The job of DsbA and PDI is to oxidize a substrate protein in the prokaryote and eukaryote, respectively. PDI has many functional strings to its bow, and this class of proteins has attracted interest from biotechnologists eager to harness its oxidizing power to develop protein-oxidizing drugs. These drugs could be used to catalyze the oxidation of recombinant proteins more efficiently *in vitro*, for example. Here, Kersteen and Raines discuss the oxidative role of PDI and describe the progress that has been made toward developing small-molecular-weight PDI mimics (14).

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One problem that dogs the PDI field is that, despite years of effort, there is no crystal structure of this extremely flexible protein (or any of its close homologues). Our structural knowledge is restricted to NMR studies of individual PDI domains (12, 13). Often, the exact domain boundaries are unclear because of limited homology between PDI family members in these regions. Ruddock and colleagues have taken on the challenge of expressing in bacteria almost every domain from all the characterized PDI family members. They have combined their results with a multiple alignment strategy to define the domain boundaries of the PDI proteins more closely (1). PDI is just one of a handful of thioredoxin domain-containing ER resident proteins. One of its cousins, Erp57, has a particular role in the biochemistry of antigen presentation, and is found in the ER in a complex with the major histocompatibility complex (MHC) class I molecule, a protein that presents antigenic viral peptides to cytotoxic T cells (6). The initial thinking was that Erp57 acted as a calnexin-binding oxidase to introduce disulfide bonds into the MHC class I heavy chain as it folded. However, the picture is complicated. Erp57 might indirectly oxidize MHC class I via a bridging molecule called tapasin (7). It may also be that Erp57 can act as a reductase to unfold surplus MHC class I heavy chains and target them for ER-associated degradation (3). The difficulties in defining whether Erp57 is an oxidant, reductant, or both *in vivo* bring to mind the conundrum of the bacterial Dsb proteins. An article published in this issue from Antoniou and Powis adds to the mix by using thiol-trapping experiments. They show that Erp57, by virtue of having two oxidizable WCGHC motifs, has the potential to engage in redox reactions while covalently linked to tapasin (2).

Oxidative protein folding, by its very nature, produces oxidants and reductants that could be very damaging to the cell if left uncontrolled. One challenge is to discover how the ER interprets this oxidative stress, and how these messages are integrated into other related signaling pathways. Van der Vlies *et al.* provide some interesting data using acetylTyrFluo, a fluorescent probe of oxidative stress, which labels a high proportion of ER proteins when cells are subjected to peroxide (18). Their experiments demonstrate that ER protein folding can be influenced by oxidative stress, and they provide an experimental approach by which the relationship between oxidative protein folding and oxidative stress can be studied (19).

When oxidative protein folding is discussed by biochemists, most attention traditionally focuses on the ER and the bacterial periplasm. The inherently oxidizing environment of these compartments means that they are often regarded as the only places where disulfide bond formation occurs. Recent work illustrates that this is not the case. Disulfide bonds can be formed and regulated in proteins that function in the cytosol and in the nucleus. The prototypical examples are the molecular chaperone Hsp33, and the transcription factors Yap1 and OxyR. Linke and Jakob discuss the remarkable way in which these proteins are regulated by reversible disulfide bond formation in this issue (15). Their review also draws our attention to the fact that redox regulation can even occur at the plasma membrane and in the extracellular space. The regulation of disulfide switching at the cell surface, as well as the implications for the activity of receptors and signaling molecules, is an expanding area of research, providing the biochemical community with another challenge for the future. Thus, despite the large and ever-growing experimental interest in oxidative protein folding, there are many challenges and questions waiting to be answered. The redox chemistry of the simple cysteine residue should keep researchers busy for some time to come.

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ABBREVIATIONS

ER, endoplasmic reticulum; MHC, major histocompatibility complex; PDI, protein disulfide isomerase.

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