ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 3 & 4, 2006

Forum Editorial

The Diversity of Oxidative Protein Folding

ADAM M. BENHAM[1] and ROBERTO SITIA[2]

THIS FORUM CELEBRATES the richness and diversity of research into oxidative protein folding. Essentially, oxidative protein folding involves the formation of disulfide bonds between two cysteine residues in a protein, a process that is usually catalytically controlled in the endoplasmic reticulum (ER), but that can occur in other cellular compartments. Our understanding of oxidative protein folding has advanced considerably in the last decade, but many crucial aspects remain unanswered.

One of the most important recent advances in this area was the discovery of the endoplasmic reticulum oxidoreductases (EROs) and the demonstration that these proteins are required for viability and oxidative protein secretion from yeast (Ero1p) (8, 20) to man (Ero1α and Ero1β) (4, 18). EROs work as electron acceptors (and hence disulfide donors) for Protein Disulfide Isomerase (PDI). EROs reoxidize PDI, recharging this catalyst so that it can oxidize (and isomerize) substrate proteins in the ER. Without their appropriate disulfides in place, the secretory pathway proteins that require oxidation misfold, and are retained in the ER or are targeted for ER-associated degradation (ERAD). The EROs are unusual ER residents because neither the yeast nor mammalian versions contain an obvious ER retention/retrieval signal such as a KDEL motif. The long nonconserved tail of the *S. cerevisiae* Ero1p is at least partly responsible for Ero1ps residence in the ER (19). Mammalian EROs lack this tail, and are held in the ER by other protein–protein interactions. Work in this issue shows that both Ero1α and Ero1β are retained in the ER by interactions with both PDI and the PDI homolog Erp44 (17). This retention mechanism can be saturated, since over expression of EROs leads to part of this protein pool getting secreted. It will be interesting to determine whether regulation of ERO retention and secretion occurs, perhaps by controlling relative ERO expression levels *in vivo*. This might allow oxidoreductases to function in the extracellular space. Evidence is continuing to emerge for the active reshuffling of disulfide bonds at the cell surface, with a clear example being in platelets. Jordan and Gibbins address the topic in this issue, exploring the role of PDI family members in regulating cell surface thiols. The PDI homolog P5 has been subject to less attention than its more famous cousin, but it seems likely that P5, despite having an ER retrieval sequence, plays a role in modulating disulfide bonds at the platelet cell surface (10). Platelets are required for blood clotting and the structural status of their receptors and adhesion proteins at the plasma membrane is likely to be vital for their activity. Given that lymphocytes can also actively change the disulfide chemistry of the CD4 protein at the cell surface during HIV infection (13), it will be interesting to discover just how widespread the regulation of cell surface thiols becomes. As Linke and Jakob wrote in our last Forum on this topic: "Not every disulfide lasts forever" (12).

P5 is just one of an expanding number of PDI family members that populate the early secretory pathway and whose exact function(s) remain unknown or incompletely understood. There are surprisingly few studies in which the properties of the different PDI homologs have been directly compared. Alanen et al. have set out to address this issue by expressing six recombinant PDI homologs (PDI, PDIp, Erp57, Erp72, P5 and PDIr) in bacteria, and analyzing the pH dependence of their catalytic activities (1). The authors find that the catalytic activities of the different PDI proteins are similar when using model peptide substrates. This work suggests that other factors, such as substrate binding/selection and regulation/specificity of expression, are important in defining the individual roles of the PDI protein family, rather than redox activity *per se*. Specificity in substrate binding may be crucial to construct an efficient network of protein-based relays within the ER lumen, and allow for the regulated execution of competing redox reactions (7, 24).

The PDI proteins listed above all have a WCGHC motif in their thioredoxin-like active sites. The spacing of the two cysteine residues, and the presence of the histidine, are important for the lability of this intramolecular disulfide, and mean

[1]Department of Biological and Biomedical Sciences, University of Durham, Durham, United Kingdom.
[2]Università Vita-Salute San Raffaele, DiBiT, San Raffaele Scientific Institute, Milano, Italy.

that a PDI protein is ready to "donate" this reactive disulfide to an incoming substrate. The PDI homolog ERp29 is atypical in that it does not have a CGHC motif and is unable to act as a disulfide donor. Study of this protein is important because it may illustrate how PDI proteins have evolved. In their review, Mkrtchian and Sandalova explore what is known about the tissue expression, biochemistry and structure of ERp29, and suggest that this unusual PDI homolog is likely to be an escort protein with broad substrate specificity (14). Investigating ERp29 should help us find out how the chaperone activities and substrate specificity of the PDI family contribute to function in the absence of redox activity.

Of course, there is more to oxidative protein folding than the PDI family, and one set of proteins that is deservedly beginning to receive more attention is the QSOX (quiescin-sulfhydryl oxidase) family, reviewed here by Coppock and Thorpe (5). QSOX proteins use molecular oxygen to generate a disulfide bond from two free sulfhydryls, creating hydrogen peroxide in the process. Like the ERO proteins, QSOX are flavin dependent. They are strongly expressed in secretory tissues, particularly those that need to produce large amounts of disulfide-containing polypeptides. The reactions catalyzed by QSOX proteins are now quite well characterized *in vitro*, but their precise roles *in vivo* have remained elusive. In this regard, it is interesting to note that these enzymes generate hydrogen peroxide, and may therefore be important in oxidative stress and redox-based signaling pathways (25).

Another type of protein of emerging relevance to oxidative protein folding is represented by the Vitamin K epoxide reductase (VKOR) family. Johannes Oldenburg describes the recent characterization of VKORC1, an ER resident enzyme that recycles reduced vitamin K (15). The recent identification of this elusive enzyme by two groups of investigators (11, 22) is a hugely important step, not only for our understanding of blood clotting and hematological disease, but also for furthering our understanding of how this new family of redox proteins operates in the ER of other organisms (9).

As each year passes, our understanding of the machinery of disulfide bond formation grows. One reason why we need a thorough knowledge of the pathways and consequences of protein oxidation is because disease can result when oxidative protein folding goes wrong. Two articles in this edition serve to illustrate the point by focusing on fibrillin-1 misfolding (26) and MHC class I misfolding (23), which can result in Marfan syndrome (a connective tissue disorder) and ankylosing spondylitis (a rheumatoid disorder) respectively. Penny Handford's review explains how the structure of fibrillin-1 is particularly disulfide rich, being composed of EGF-like (Epidermal Growth Factor) domains and TB-like (Transforming Growth factor Beta Binding Protein) domains (26). Marfan syndrome occurs when the large fibrillin-1 protein misfolds or mistrafficks, giving rise to characteristically tall, thin individuals with spinal deformity and heart problems. Interestingly, patients with another disease, homocystinuria, have skeletal defects that resemble those of Marfan patients. Homocystinuria is caused by defective methionine metabolism, and results in high levels of circulating homocysteine in the blood. It has been proposed that the increased levels of homocysteine react with and reduce the disulfide bonds present in fibrillin-1 (and probably other proteins) resulting in a secondary effect on the structure of the extracellular matrix.

In contrast to fibrillin-1, MHC class I molecules are involved in immune defense, protecting cells from intracellular pathogens (e.g., viruses) by picking up peptide fragments from viral proteins within the infected cell and displaying them at the cell surface. In this way, the infected cell can be removed by cytotoxic T cells before the infection has a chance to spread too far. MHC class I molecules fold in the ER, where they acquire antigenic peptides. Human beings express many different MHC class I alleles to cope with the huge diversity of potential antigenic peptides. One of these alleles, HLA-B27, has a protein product with a reactive unpaired cysteine at position 67, causing it to misfold and form homodimers which can be found both at the cell surface and in the ER (2). HLA-B27 is expressed by 95% of all patients with ankylosing spondylitis, so there has been considerable interest in uncovering exactly why HLA-B27 makes people so prone to this autoimmune condition (21). In this issue, Saleki et al. have examined the oxidation of HLA-B2705 and its cysteine mutants in transfected cells, as well as the behaviour of HLA-B2704 and HLA-B2705 in lymphocytes (23). Despite the fact that HLA-B2704 and B2705 differ by just three amino acids, they have redox-dependent conformational differences when probed with MHC class I-specific antibodies. The redox state of HLA-B molecules can also be manipulated by BMC, a redox active drug, suggesting that in the future, the properties of MHC class I molecules could be controlled through the use of refined redox drugs.

The therapeutic use of redox reagents to target specific proteins for specific diseases remains some way off, in part because of the inability to restrict the targeting of such reagents. Until recently, it has also been almost impossible to accurately monitor and report on the redox state of the living cell because of the lack of suitable, nonperturbing reporters. In the last few years, much progress has been made in this area through the development of redox reporters based on green fluorescent protein (GFP). Engineering a disulfide bond into GFP results in a protein whose fluorescent properties change depending on whether it is reduced or oxidized. This approach has been used to evaluate the redox state of living cells by two groups (6, 16). Here, Jakob Winther and colleagues critically assess the current state of play, explaining the biochemical properties, applications, and limitations of these novel fluorescent reporters (3).

Armed with new reporters, new proteins and a growing list of diseases related to oxidative protein misfolding, researchers in this field still have a lot of exciting research ahead as we move into 2006 and beyond.

ABBREVIATIONS

BMC, 1,2-bis(2-mercaptoacetamido)cyclohexane; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERAD, ER associated degradation; ERO, endoplasmic reticulum oxidoreductases; ERP, ER protein; GFP, green fluorescent protein; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; MHC, major histocompatibility complex; PDI, protein disulfide isomerase; QSOX, quiescin-sulfhydryl

oxidase; TB, transforming growth factor beta binding protein; VKOR, vitamin K epoxide reductase.

REFERENCES

1. Alanen HI, Salo KEH, Pirneskoski A, and Ruddock LW. pH dependence of the peptide thiol-disulfide oxidase activity of six members of the human protein disulfide isomerase family. *Antioxid Redox Signal* 8: 283–291, 2006.
2. Antoniou A and Powis SJ. Characterisation of the ERp57-tapasin complex by rapid cellular acidification and thiol modification. *Antioxid Redox Signal* 5: 375–380, 2003.
3. Björnberg, O, Østergaard H, and Winther JR. Measuring intracellular redox conditions using GFP-based sensors. *Antioxid Redox Signal* 8: 354–361, 2006.
4. Cabibbo A, Pagani M, Fabbri M, Rocchi M, Farmery MR, Bulleid NJ, and Sitia R. ERO1-L, a human protein that favors disulfide bond formation in the endoplasmic reticulum. *J Biol Chem* 275: 4873–4833, 2000.
5. Coppock DL and Thorpe C. Multidomain flavin-dependent sulfhydryl oxidases. *Antioxid Redox Signal* 8: 300–311, 2006.
6. Dooley CT, Dore TM, Hanson GT, Jackson WC, Remington SJ, and Tsien RY. Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. *J Biol Chem* 279: 22284–22293, 2004.
7. Fassio A and Sitia R. Formation, isomerisation and reduction of disulphide bonds during protein quality control in the endoplasmic reticulum. *Histochem Cell Biol* 117: 151–157, 2002.
8. Frand AR and Kaiser CA. The ERO1 gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum. *Mol Cell* 1: 161–170, 1998.
9. Goodstadt L and Ponting CP. Vitamin K epoxide reductase: homology, active site and catalytic mechanism. *Trends Biochem Sci* 29: 289–292, 2004.
10. Jordan PA and Gibbins JM. Extracellular disulfide exchange and the regulation of cellular function. *Antioxid Redox Signal* 8: 312–324, 2006.
11. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, and Stafford DW. Identification of the gene for vitamin K epoxide reductase. *Nature* 427: 541–554, 2004.
12. Linke K and Jakob U. Not every disulfide lasts forever: disulfide bond formation as a redox switch. *Antioxid Redox Signal* 5: 425–434, 2003.
13. Matthias LJ, Yam PT, Jiang XM, Vandegraaff N, Li P, Poumbourios P, Donoghue N, and Hogg PJ. Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. *Nat Immunol* 3: 727–732, 2002.
14. Mkrtchian S and Sandalova T. ERp29, an unusual redox-inactive member of the thioredoxin family. *Antioxid Redox Signal* 8: 325–337, 2006.
15. Oldenburg J, Bevans CG, Müller RC, and Watzka M. Vitamin K epoxide reductase complex subunit 1 (VKORC1): the key protein of the vitamin K cycle. *Antioxid Redox Signal* 8: 347–353, 2006.
16. Ostergaard H, Henriksen A, Hansen FG, and Winther JR. Shedding light on disulfide bond formation: engineering a redox switch in green fluorescent protein. *EMBO J* 20: 5853–5862, 2001.
17. Otsu M, Bertoli G, Fagioli C, Guerini-Rocco E, Nerini-Molteni S, Ruffato E, and Sitia R. Dynamic retention of Ero1α and Ero1β in the endoplasmic reticulum by interactions with PDI and Erp44. *Antioxid Redox Signal* 8: 274–282, 2006.
18. Pagani M, Fabbri M, Benedetti C, Fassio A, Pilati S, Bulleid NJ, Cabibbo A, and Sitia R. Endoplasmic reticulum oxidoreductin 1-lbeta (ERO1-Lbeta), a human gene induced in the course of the unfolded protein response. *J Biol Chem* 275: 23685–23692, 2000.
19. Pagani M, Pilati S, Bertoli G, Valsasina B, and Sitia R. The C-terminal domain of yeast Ero1p mediates membrane localization and is essential for function. *FEBS Lett* 508: 117–120, 2001.
20. Pollard MG, Travers KJ, and Weissman JS. Ero1p: a novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. *Mol Cell* 1: 171–182, 1998.
21. Ramos M and de Castro JAL. HLA-B27 and the pathogenesis of spondyloarthritis. *Tissue Antigens* 60: 191–205, 2002.
22. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG, Muller CR, Strom TM, and Oldenburg J. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 427: 537–541, 2004.
23. Saleki K, Hartigan N, van Lith M, Bulleid N, and Benham AM. Differential oxidation of HLA-B2704 and HLA-B2705 in lymphoblastoid and transfected adherent cells. *Antioxid Redox Signal* 8: 292–299, 2006.
24. Sitia R and Braakman I. Quality control in the endoplasmic reticulum protein factory. *Nature* 426: 891–894, 2003.
25. Sitia R and Nerini-Molteni S. Stress, protein (mis)folding, and signaling: the redox connection. *Sci STKE* 239: pe27, 2004.
26. Whiteman P, Hutchinson S, and Handford PA. Fibrillin-1 misfolding and disease. *Antioxid Redox Signal* 8: X-X, 2006.

Address reprint requests to:
Dr. Adam M. Benham
Department of Biological and Biomedical Sciences
University of Durham
South Road
Durham, DH1 3LE, United Kingdom

E-mail: adam.benham@durham.ac.uk

Received for publication September 12, 2005; accepted September 12, 2005.

This article has been cited by:

1. Simone Cenci, Roberto Sitia. 2007. Managing and exploiting stress in the antibody factory. *FEBS Letters* **581**:19, 3652-3657. [CrossRef]