Protein oxidation: prime suspect found 'not guilty'

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Glutathione has long been suspected to be the primary source of oxidative power for protein folding. It has now been shown to be just the opposite, namely a source of reductants. The ultimate origin of oxidants has become even more of a mystery.

A prime suspect in the oxidation of proteins has now been declared to be an innocent victim by Cuozzo and Kaiser on page 130 of this issue of *Nature Cell Biology*[1].

Most proteins that are destined for secretion from the eukaryotic cell are tied by disulphide bonds while passing through the endoplasmic reticulum (ER), one of the organelles that secretory proteins must journey through on their way to the cell surface. Intramolecular disulphides, which act to crosslink cysteine residues, are important for the folding and function of secreted proteins. Indeed, the cell is dependent on disulphide-bond formation for viability. Furthermore, some cell types have a daily secretory output that exceeds their own mass — so their disulphide-bond-tying systems have to be extremely efficient. Protein disulphide isomerase (PDI) and its structural relatives have been shown to catalyse both the isomerization and the formation of disulphide bonds. Oxidized glutathione was suspected of being the ultimate source of these disulphides and the oxidizing equivalents needed to generate disulphide bonds. But now it appears that oxidized glutathione (GSSG) is itself generated from reduced glutathione (GSH) by the oxidizing environment of the ER[1]. (GSSG consists of two GSH molecules joined together by a disulphide bond.) Instead of supplying oxidizing power, glutathione seems instead to provide reducing equivalents to the ER. Thus GSSG appears to be a victim, rather than the cause, of the oxidizing conditions in the ER.

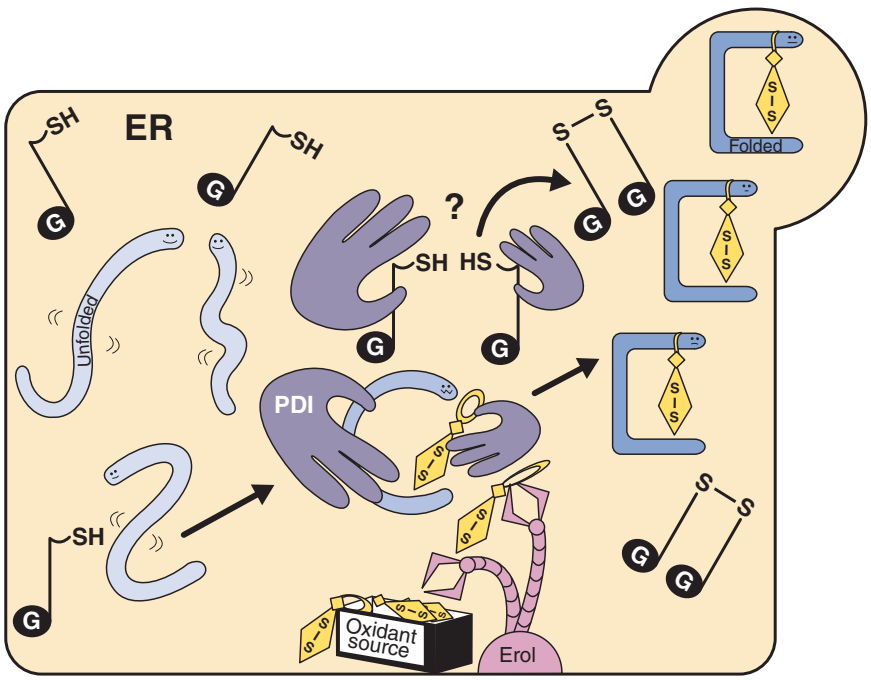

Figure 1 **Crime scene: endoplasmic reticulum (ER). The crime: proteins are forced to wear disulphide ties. This folds them properly and allows them to enter the work force. The mystery: where do these disulphide bonds come from? The Ero1 and protein disulphide isomerase (PDI) proteins are implicated as working to tie the proteins with disulphides. Ero1 is needed to mobilize the disulphide ties, and PDI makes sure that all the knots are tied correctly. Proteins containing disulphides are stable and active. Proteins without ties are flexible, free to take on many different conformations, but cannot do any useful work. Oxidized glutathione (GSSG) was accused of providing the disulphides but is now declared to be an innocent victim, created from reduced glutathione (GSH) by the oxidizing conditions in the ER. GSSG is created in an Ero1-dependent reaction, possibly by PDI. The ultimate source of the disulphides themselves has become a mysterious black box.**

Previously, there was a strong circumstantial case implicating glutathione in the oxidation of proteins in the ER[2]. High levels of GSSG were detected within the ER, and careful measurements could detect no other small-molecule redox component that was capable of crosslinking to a cysteine-containing peptide. Also, a GSH/GSSG mixture is known to be effective in the oxidative folding of proteins *in vitro*[3]. Thus, GSSG was capable, abundant and apparently alone at the scene of the crime.

Evidence has, however, recently been collected against another suspect, Ero1, which is implicated in mobilizing the oxidizing equivalents used in the formation of protein disulphide bonds[4,5]. Cuozzo and Kaiser[1] now implicate Ero1 as the oxidant of glutathione as well.

Rather than being an intermediate in the pathway for the formation of protein disulphides, glutathione now appears to be in competition with proteins for oxidizing equivalents generated by Ero1. This conclusion is mainly based on the surprising observation that mutations in the glutathione-biosynthesis pathway are capable of suppressing mutations in the *ero1* gene in yeast. Yeast strains that fail to make glutathione allow the growth of an *ero1* temperature-sensitive mutant at an otherwise restrictive temperature. In the single mutant *ero1* strain, the folding of a disulphide-bond-containing marker protein is impaired, but this folding is restored in the double mutant, indicating that GSH may normally compete with proteins for the disulphide-bond-formation

machinery (Fig. 1). Here it is important to note that an environment that is too oxidizing may be deleterious to efficient folding, as free sulphydryls (such as those of GSH) are necessary so that incorrect protein disulphide bonds can be untied, using the reductive power of the free sulphydryls, and correct bonds formed. GSH may therefore provide an important reductive force.

Cuozzo and Kaiser's key observation, that disulphide-bond formation proceeds efficiently in strains that are simultaneously defective in both glutathione biosynthesis and Ero1 function, is still rather odd. Why would the cell have an Ero1-dependent system to make the ER an oxidizing environment, and a glutathione-dependent system that makes the ER less oxidizing, if the cell can get along just fine without either system? Why does the double mutant strain *ero1*$^-$ *gsh1*$^-$ form disulphides normally? One solution to this paradox is to postulate the existence of a second, yet-to-be-discovered, disulphide-bond-forming system that is capable of oxidizing proteins but only in the absence of the load that reduced glutathione imposes on the system.

With the elimination of GSSG as a suspect, the ultimate source of oxidizing equivalents needed for folding of disulphide-containing proteins is now a complete mystery. This is very surprising given that PDI has been studied for over 35 years[6]. Why has it proven to be so hard to work out the pathway of disulphide-bond formation? First of all, the system studied is difficult to quantify because of the intrinsically complicated nature of the substrate (newly synthesized proteins about to fold). Second, the number of electrons flowing in this pathway is minimal as compared with other oxidative systems in the cell. Tracing the flow of these electrons against the huge background of electron transport and other redox reactions taking place in the cell is not trivial. For instance, it is not clear to what degree depletion of glutathione in the ER affects the redox potential. Thus, the need for methods to measure this parameter in the cell by non-invasive means becomes increasingly acute.

The mechanisms of disulphide-bond formation have proven to be conserved in evolution — PDI and Ero1 are found in many eukaryotic species[3–5]. Many of the proteins involved in disulphide formation and isomerization contain a motif known as the 'thioredoxin fold'. Such proteins include PDI and its homologues in eukaryotes, and DsbA, DsbC, DsbD DsbE and DsbG in prokaryotes[7]. The DsbA protein is the direct oxidant of secreted proteins in the *Escherichia coli* periplasm; DsbA receives its oxidizing equivalents from DsbB[8], making DsbB a possible analogue of Ero1. Whereas in eukaryotes the ultimate source of disulphides is a mysterious 'black box', in prokaryotes it has recently been found that molecular oxygen can serve as the ultimate oxidant source[9]. *In vitro* reconstitution of the prokaryotic oxidative catalytic system is likely to be key in helping us to solve the mystery of how disulphides are generated in the cell.

In the prokaryotic periplasm, the disulphide oxido-reductases (DsbC and DsbD) are maintained in a reduced state. This allows DsbC to carry out disulphide shuffling[10]. Here again, it is tempting to see the analogy between the *E. coli* periplasm and the yeast ER. In the ER, glutathione might act as a small-molecule equivalent of DsbC, providing reductive power.

Given these evolutionary parallels, the lessons learned in bacteria may help us to solve the long-standing mystery of how oxidizing equivalents flow in yeast and higher eukaryotes. □

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'Syniping' away at glucose transport

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Insight into the mechanism by which insulin promotes glucose uptake by muscle and adipose cells has been provided by the discovery and characterization of synip, an insulin-regulated protein involved in recruiting glucose transporters to the cell surface.

Pancreatic β-cells respond to increases in circulating glucose levels by secreting insulin. Insulin, in turn, promotes the uptake of glucose by peripheral tissues, primarily muscle and adipose tissues, and so maintains glucose homeostasis. The cellular mechanisms responsible for insulin-regulated glucose transport have attracted considerable attention because deficiencies in this process are among the defects underlying type II diabetes. An intriguing report by Min *et al.* in *Molecular Cell*[1] now introduces us to synip, a potentially key protein in the glucose-uptake story.

We know that the insulin-induced potentiation of glucose uptake across the plasma membrane of muscle and adipose cells is principally due to the recruitment of the GLUT-4 glucose transporter from a specialized intracellular storage compartment to the cell surface[2,3]. This process — often referred to as GLUT-4 translocation — occurs through the regulated fusion of GLUT4-containing vesicles with the plasma membrane. Substantial insight into the molecular mechanisms that mediate insulin-stimulated glucose uptake has been provided in recent years by two fundamental strategies: working 'downstream' from the insulin receptor on the surface of muscle and adipose cells, through its intracellular signal-transduction pathways[4], and working 'upstream' from the GLUT4-containing vesicle, through proteins that regulate membrane-trafficking events[3]. These approaches have yet to converge, but synip[1] represents the strongest candidate yet for mediating insulin's regulatory effects on the translocation of GLUT4-containing vesicles.

The molecular machinery responsible for the delivery and fusion of intracellular transport vesicles at each stage along the secretory and endocytic pathways is highly conserved[5]. At the heart of this machinery are the proteins that form SNARE complexes, including vesicle-associated (v-) SNAREs belonging to the VAMP family and target-membrane-associated (t-) SNAREs belonging to the syntaxin and SNAP-25