trap to ClpA and GFP–SsrA, the trap successfully captured unfolded GFP–SsrA generated by the action of ClpA. This result shows that ClpA alters the conformation of this substrate, converting it to a non-native state. Although binding to GroEL and loss of fluorescence both indicate that GFP–SsrA is significantly unfolded by ClpA, the authors confirmed this conclusion with an additional experiment. Using deuterium–hydrogen (^{2}H–^{1}H) exchange, they showed that ClpA causes exposure of the entire polypeptide chain in GFP–SsrA to water, including regions of the GFP that are normally buried (and, hence, protected from solvent) by the three-dimensional state of the folded protein. Thus, ClpA has the capacity to globally unfold substrate proteins.

Global unfolding is likely to be a general feature during translocation of a substrate protein from ClpA into the proteolytic chamber of ClpP. Protein unfolding could also account for the roles of the Clp/Hsp100 proteins in other biological processes. However, it remains to be seen whether proteins that are remodelled or disassembled, rather than being degraded, are subject to such global unfolding. Moreover, ClpAP is a prototype for many proteolytic enzymes that have similar protein architectures, with ATPase subunits flanking a cylindrical proteolytic chamber. The 26S proteasome, for example — the machine responsible for nearly all the protein degradation in eukaryotic cells — falls into this class. Perhaps the ATPases that make up the proteasome's 19S cap have a similar unfolding function to ClpA. The use of GroEL traps, and glowing substrates such as GFP, should facilitate the mechanistic exploration of these more complex protein machines. ■

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Protein folding

Where do the electrons go?

Rudi Glockshuber

The biologically active, three-dimensional structure of a protein is its energetically most favourable conformation, and is encoded by its amino-acid sequence. Many proteins can fold correctly on their own (or with the help of molecular chaperones). But things are different for proteins containing disulphide bonds. Formation of a disulphide bond from the thiol (S–H) groups of two cysteine residues generates two protons and two electrons and changes the covalent structure of the polypeptide chain (Fig. 1). Consequently, these bonds cannot form spontaneously unless the protein interacts with an oxidant that accepts the electrons[1]. Where do these electrons then go? Reporting in *Cell*, James Bardwell and colleagues[2] provide compelling experimental evidence that, in bacteria, oxidative protein folding is coupled to the electron-transport chain.

Why has it been so difficult to answer such an important question? The formation of disulphide bonds was originally thought to proceed directly from oxygen. But the pathway of electron flow that allows oxidative protein folding *in vivo* soon proved to be complex, involving a series of specialized bacterial enzymes. Several years ago, Bardwell and others[3] found that formation of disulphide bonds in the bacterial periplasm (the oxidizing compartment between the inner and outer bacterial membranes) requires an enzyme called DsbA. This enzyme randomly transfers its extremely reactive catalytic disulphide bond to folding proteins, and is itself recovered as an oxidant by disulphide exchange with a protein in the inner membrane called DsbB[4] (Fig. 2). Then, last year, molecular oxygen was found[5] to act as the final electron acceptor for DsbB-catalysed protein folding *in vitro*.

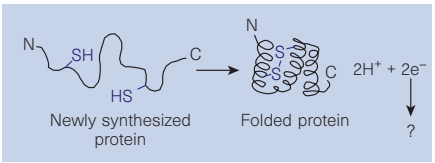

Figure 1 **Formation of disulphide bonds during protein folding. Disulphide bonds form between sulphur atoms of the amino acid cysteine. They cannot form spontaneously unless the polypeptide chain interacts with an oxidant, which accepts the two electrons that are generated by the formation of each disulphide bond from two free thiol (S–H) groups.**

Can DsbB — which lacks cofactors such as haems or flavins — be oxidized directly by oxygen? The answer came from an intriguing observation. When a DsbB preparation was purified from 97% to more than 99% purity, the enzyme lost its ability to catalyse the oxidation of DsbA by molecular oxygen *in vitro*. But this pure DsbB then provided an assay for identifying the crucial components of the inner membrane that had been removed during the final purification step. Using this assay, Bardwell and co-workers[2] first found that the missing component is the haem-containing cytochrome *bd* oxidase complex — a hint that protein folding is linked to the electron-transport chain. Yet disulphide bonds still form normally in a cytochrome *bd* oxidase-deficient strain of *Escherichia coli*. So, the authors used membrane fractions of this strain to identify the cytochrome *bo* oxidase complex as an alternative component that can restore DsbB activity. These results mean that both terminal oxidases of the electron-transport chain in *E. coli* can independently transfer electrons from DsbB to molecular oxygen via their haem groups. When the authors analysed the membrane fractions from a strain deficient in both terminal oxidases they found that DsbB was not oxidized, showing that no other proteins can catalyse the flow of electrons from DsbB to oxygen.

Do both of these terminal oxidases interact directly with DsbB? Both are known to catalyse the flow of electrons from the ubiquinone pool in the inner membrane to molecular oxygen, suggesting that electrons might flow from DsbB to ubiquinone, then from ubiquinone, via the terminal oxidases, to oxygen[6]. Indeed, Bardwell and colleagues found that, *in vitro*, pure DsbB catalyses the oxidation of DsbA by ubiquinone derivatives extremely efficiently. The catalytic power of the purified terminal oxidases is also striking. In a completely reconstituted *in vitro* redox system, both were catalytically active at concentrations 1,000-fold lower than that of DsbA[2].

But this is still not the end of the story. Bardwell and co-workers[2] also found the pathway of electron flow that guarantees the formation of disulphide bonds in the absence of oxygen (Fig. 2). The existence of this pathway became evident when a strain of *E. coli* lacking both terminal oxidases and grown in the absence of oxygen showed no defects in disulphide-bond formation. It turns out that oxidized menaquinone — which can replace ubiquinone under anaerobic conditions and transports electrons to alternative electron acceptors such as fumarate or nitrate — can oxidize DsbB directly, albeit with a lower efficiency than ubiquinone[2]. This menaquinone pathway also allows disulphide bonds to be formed

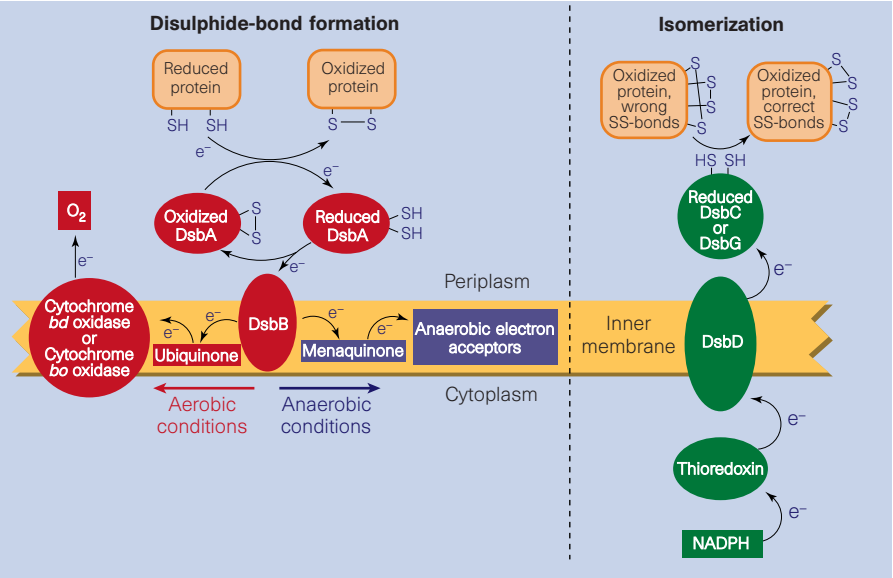

Figure 2 **Electron-flow pathways for oxidative protein folding under aerobic and anaerobic conditions, as described by Bardwell and colleagues[2]. An independent electron-flow pathway (right) catalyses isomerization of wrongly formed disulphide bonds in the periplasm. Electrons from NADPH in the cytosol keep the disulphide isomerases DsbC and DsbG in their catalytically active, reduced form through thioredoxin and DsbD. The reactions of DsbA, DsbC and DsbG with the folding polypeptide involve two electrons and occur via disulphide interchange reactions. The same applies to the oxidation of DsbA by DsbB, and the reduction of DsbC and DsbG by DsbD.**

in strains that lack ubiquinone. But ubiquinone and menaquinone are probably the only oxidants in the *E. coli* inner membrane that can directly oxidize DsbB. Strains lacking ubiquinone and menaquinone show a strong defect in disulphide-bond formation[6], and the same is true for an anaerobically grown strain in which fumarate cannot act as electron acceptor owing to the lack of fumarate reductase[2].

Bardwell and colleagues' study[2] answers many questions about how disulphide bonds form in bacteria. The pathway — in particular, the direct interaction between DsbB and quinones — is unexpected. Another surprise has been the recently discovered mechanism by which the DsbC or DsbG proteins catalyse isomerization of wrongly formed disulphide bonds in the periplasm[7,8] (Fig. 2). These proteins must be kept reduced to be catalytically active as disulphide isomerases. This is accomplished by an inverse electron flow *towards* the periplasm, from NADPH in the cytosol, via thioredoxin to DsbD, and via DsbD across the inner membrane to DsbC and DsbG[9].

That's all fine for bacteria, but how does oxidative protein folding occur in eukaryotic cells? Disulphide-bonded proteins generally cannot fold in reducing cellular compartments such as the cytoplasm. Instead, they fold in an oxidizing compartment, the endoplasmic reticulum. Oxidized glutathione has long been thought to act as the electron acceptor that reoxidizes protein disulphide isomerase — the main catalyst of oxidative protein folding in the endoplasmic reticulum. But yeast cells deficient in glutathione biosynthesis have no defect in disulphide-bond formation, and reduced glutathione can even compete with reduced proteins for oxidizing equivalents[10,11]. A protein called Ero1p, on the other hand, is associated with the membrane surrounding the endoplasmic reticulum, and is essential for the formation of disulphide bonds in yeast[10,12]. So, Ero1p may be functionally equivalent to DsbB. However, the real source of oxidizing equivalents in the endoplasmic reticulum has not yet been identified, and additional components may also be required.

The extraordinary work by Bardwell and colleagues teaches us an important lesson. They were able to reconstitute the pathway of disulphide-bond formation by a single prerequisite — a pure protein preparation of DsbB. Or, as my first scientific mentor Wolfram Schäfer used to say, by "the secret of the pure substance". ■

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Earth science

Does global cooling reduce relief?

Eric Small

The nature of the interactions between climate and tectonic processes is strongly debated. It has been proposed that a shift towards a cooler and more erosive climate would enhance topographic relief in mountain ranges[1]. The isostatic response to this change in relief (whereby parts of the Earth's lithosphere rise if material is removed and sink if it is deposited) could raise mountain crests even higher, driving climate to an even more erosive state and forming a positive feedback loop. On page 39 of this issue, Whipple *et al.*[2] present an alternative hypothesis — that the change to a more erosive climate would lead to either a decrease or no change in relief in active mountain ranges, indicating a negative or non-existent coupling between cooling and mountain uplift.

Sorting out the connection between climate and tectonics is important for understanding why the Earth has become progressively cooler over the past 50 million years[3]. It has been proposed that uplift of the Himalayas, Tibetan plateau and other mountain ranges during the Cenozoic era (from 64 million years ago) would result in cooler global temperatures in three ways. First, persistent snow cover at higher altitudes would enhance the Earth's reflectivity. Second, increased uplift at mid-latitudes would dramatically alter atmospheric circulation, as demonstrated by global-climate models[4]. And third, enhanced weathering in mountainous regions would weaken the natural greenhouse effect, because weathering of silicate rocks consumes atmospheric CO_2.

Of course, uplift is only a reasonable source of Cenozoic cooling if it preceded the observed climate change. It is not disputed that tectonic forces uplifted the Himalayas and Tibetan plateau at some point during the Cenozoic. But much of the evidence for Cenozoic uplift in other parts of the world is ambiguous and may instead reflect global cooling[1]. Greater erosion in mountainous