FORMATION AND TRANSFER OF DISULPHIDE BONDS IN LIVING CELLS

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Protein disulphide bonds are formed in the endoplasmic reticulum of eukaryotic cells and the periplasmic space of prokaryotic cells. The main pathways that catalyse the formation of protein disulphide bonds in prokaryotes and eukaryotes are remarkably similar, and they share several mechanistic features. The recent identification of new redox-active proteins in humans and yeast that mechanistically parallel the more established redox-active enzymes indicates that there might be further uncharacterized redox pathways throughout the cell.

THIOL-REDOX REACTION
A reaction that involves the transfer of electrons from a donor molecule to an acceptor molecule if one of the molecules is a thiol-containing compound.

THIOL-DISULPHIDE EXCHANGE REACTION
A thiol-redox reaction that involves the exchange of electrons between a compound with free thiols and a disulphide-bonded molecule, which results in the transfer of a disulphide bond from one molecule to another.

GLUTATHIONE
A tripeptide — composed of glutamic acid, cysteine and glycine — that is the principal small thiol-containing molecule in the cell.

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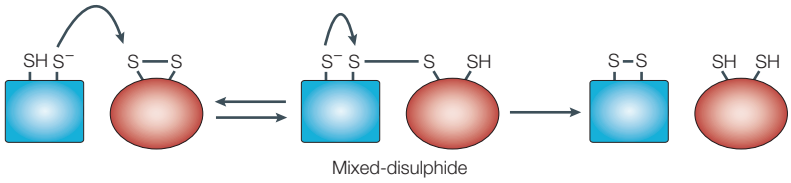
The formation of biosynthetic disulphide bonds is an important step in the maturation of the extracellular domains of both membrane and secreted proteins in eukaryotic and prokaryotic cells. Not only are disulphide bridges often vital for the stability of a final protein structure, the incorrect pairing of cysteine residues (hereafter referred to as cysteines or Cys) usually prevents the folding of a protein into its native conformation. THIOL-REDOX REACTIONS are also an essential part of the catalytic activity of several metabolic enzymes. For example, the cytoplasmic enzyme ribonucleotide reductase becomes oxidized during its catalytic cycle, and it must be recycled to its reduced form to be reactivated (reviewed in REF. 1). Protein activity can also be modulated by altering the redox state of cysteines. In plants, light-generated reducing equivalents are used to reduce the regulatory disulphide bonds in several photosynthetic enzymes, thereby inducing a switch from catabolic to anabolic respiration[2,3]. Under specific cellular conditions, several transcription factors, including the bacterial OxyR and Hsp33, also become activated by the oxidation of cysteines that form disulphide bonds[4,5].

Important progress has been made towards understanding how disulphide bonds are formed in cellular proteins. Core pathways that promote disulphide-bond formation have been delineated in both prokaryotes and eukaryotes. Recently, a new appreciation of how these pathways operate at the mechanistic level has been achieved. It is now apparent that the prokaryotic and eukaryotic pathways of disulphide-bond formation have remarkable similarities, which include their orchestration at the mechanistic level.

The chemistry of disulphide-bond formation is well established. The formation of a disulphide bond from two thiols (-SH) is a two-electron reaction that requires an oxidant or electron acceptor. Disulphide bonds can be formed spontaneously *in vitro* by the loss of electrons from two cysteine thiols coupled with the gain of electrons by an available acceptor, such as molecular oxygen. When molecular oxygen is used as an electron acceptor, an intermediary, such as a transition metal or flavin, is required to overcome the kinetically sluggish, yet thermodynamically favourable, association of oxygen with protein thiols.

In vivo, however, the most common mechanism for the formation of protein disulphide bonds is a THIOL-DISULPHIDE EXCHANGE REACTION of free thiols with an already disulphide-bonded species. A thiol-disulphide exchange reaction can occur between a protein and any sulfhydryl-containing substrate, including small thiol-containing compounds, such as GLUTATHIONE, or a protein containing a disulphide bond. Thiol-disulphide exchange reactions provide the cornerstone of catalysed protein disulphide-bond formation in all living organisms, from prokaryotes to eukaryotes (for details, see BOX 1).

Box 1 | **Thiol-disulphide exchange between proteins**



Thiol-disulphide exchange reactions are a key element in the process of cellular disulphide-bond formation. In an exchange reaction, a thiolate anion (-S⁻), which is formed by the deprotonation of a free thiol, displaces one sulphur of the disulphide bond in the oxidized species. This results in the formation of a transient mixed-disulphide bond between the two proteins, or between a protein and redox molecule (see figure). In a second exchange reaction, the remaining thiolate anion attacks the mixed-disulphide bond and resolves it. The net result of this thiol-disulphide exchange process is the oxidation of the originally reduced protein, and the concomitant reduction of the initially oxidized redox species. Such exchange reactions can also occur intramolecularly, leading to the rearrangement of disulphide bonds in a single protein. After the completion of a thiol-disulphide exchange reaction, the redox state of the active-site cysteines in either product can be regenerated for another catalytic cycle by another protein, or by a redox molecule such as glutathione.

Cellular enzymes known as thiol-disulphide oxidoreductases catalyse thiol-disulphide exchange reactions to promote the formation or reduction of protein disulphide bonds. The prototype of this group of enzymes is protein disulphide isomerase (PDI). The ability of PDI to catalyse the formation, reduction or isomerization of disulphide bonds illustrates the range of activities that can be carried out by thiol-disulphide oxidoreductases. If the active-site cysteines of PDI are in the oxidized (disulphide) form, the enzyme oxidizes protein dithiols, transferring disulphide bonds directly to substrate proteins (red protein in the figure). Conversely, when the active sites are in a reduced (dithiol) form, PDI can catalyse the reduction of mispaired thiol residues, functioning as a disulphide reductase or isomerase (blue protein in the figure).

A class of proteins commonly known as THIOL-DISULPHIDE OXIDOREDUCTASES catalyses thiol-disulphide exchange reactions *in vivo*. The activity of these proteins depends on a pair of cysteines that are often arranged in a Cys-X-X-Cys motif (where X is any amino acid). This motif is usually found embedded in a domain that shares structural homology with the small redox protein THIOREDOXIN. The presence of a Cys-X-X-Cys active-site motif has become the hallmark of proteins that are involved in forming or breaking disulphide bonds by an exchange reaction. PROTEIN DISULPHIDE ISOMERASE (PDI) was one of the first-identified thiol-disulphide oxidoreductases[6] and, consequently, its activities have been well characterized (reviewed recently in REFS 7–9). PDI is a remarkably versatile enzyme. Depending on the redox environment and the characteristics of the substrate proteins, PDI can catalyse the formation, reduction or isomerization of disulphide bonds[10]. Analysis of the REDOX POTENTIAL of PDI indicates that it is a mild oxidant with a redox potential of between –110 and –190 mV (REFS 11,12; BOX 2). However, it is not yet known whether the primary *in vivo* function of PDI is to reshuffle non-native disulphide bonds or to catalyse the formation of disulphide bonds at the outset.

In the cell, proteins that contain disulphide bonds are found primarily in relatively oxidizing environments. In eukaryotic cells, protein disulphide-bond formation proceeds predominantly in the lumen of the endoplasmic reticulum (ER), whereas in prokaryotic cells, most protein oxidation occurs in the periplasmic space. The oxidation of cysteines to form disulphide bonds is catalysed rapidly in both the ER and periplasmic space by several different thiol-disulphide oxidoreductases, including PDI. Thiol-disulphide oxidoreductases, such as thioredoxin, are also present in the relatively reducing environment of the cytoplasm, where they usually catalyse the reduction of protein disulphide bonds. However, the direction of the reaction catalysed by a thiol-disulphide oxidoreductase (reduction or oxidation) does not depend solely on the equilibrium redox potential of the compartment in which the enzyme resides. The redox potential of the thiol-disulphide oxidoreductase (BOX 2), its propensity to interact with other redox-active proteins and substrates, and the concentration of the substrate and product proteins all contribute to the nature of the *in vivo* enzyme activity.

The past few years have seen notable advances in our understanding of the pathways of protein disulphide-bond formation. It is now clear that protein oxidation is a catalysed process that requires many cellular thiol-disulphide oxidoreductases, as well as enzymes that couple the activity of the thiol-disulphide oxidoreductases to the redox chemistry of the cell. This review focuses on the emerging similarities between the prokaryotic and eukaryotic systems that catalyse the formation of structural disulphide bonds, and the general principles of disulphide-bond formation that can be deduced from the genetic, biochemical and structural studies of these systems.

Eukaryotic pathways for protein oxidation

Genetic and biochemical analysis of *Saccharomyces cerevisiae* has defined an essential pathway for protein disulphide-bond formation that involves two ER proteins: Ero1 (ER oxidoreductin) and PDI (FIG. 1). Ero1 is a glycosylated lumenal ER protein that is tightly associated with the ER membrane[13,14]. Ero1 is a component essential for the introduction of OXIDIZING EQUIVALENTS into the ER lumen. A conditional *ero1-1* mutant fails to provide the oxidizing equivalents that are necessary for protein disulphide-bond formation in the ER, and this results in the accumulation of misfolded proteins in the ER, the folding and transport of which are dependent on disulphide-bond formation[13]. The oxidizing capacity of the ER can be either increased or decreased by varying the cellular levels of active Ero1 (REFS 13,14).

Ero1 influences protein oxidation by transferring oxidizing equivalents directly to PDI, which, in turn, oxidizes the substrate proteins[15,16]. The transmission of oxidizing equivalents in this pathway occurs through a series of direct thiol-disulphide exchange reactions between the proteins[15]. Mutational analysis of Ero1 identified four cysteines that are essential for the oxidative activity of this protein[17]. The positions of these four cysteines indicate that Ero1 contains two active-site cysteine pairs: Cys100-Cys105 and Cys352-Cys355 (REF. 17). Mutation of any of these four essential cysteines disrupts the thiol-disulphide exchange between Ero1 and PDI, as assayed by the detection of Ero1–PDI MIXED-DISULPHIDES[17].

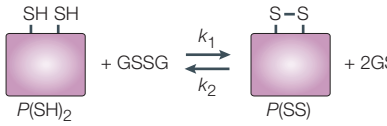
THIOL-DISULPHIDE OXIDOREDUCTASE
An enzyme that catalyses the transfer of electrons or hydrogen between molecules.

THIOREDOXIN
A ubiquitous small soluble protein with redox-active cysteines that catalyses thiol-disulphide exchange reactions.

PROTEIN DISULPHIDE ISOMERASE
A soluble protein with two thioredoxin-like domains that each contain a redox-active cysteine pair that donates disulphide bonds to newly synthesized proteins in the eukaryotic ER.

Box 2 | **Redox potential**

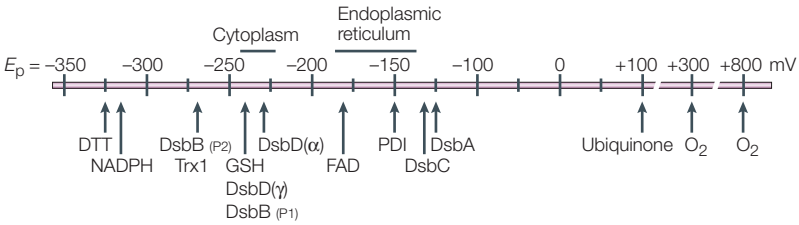
The propensity of a thiol-disulphide oxidoreductase to be in either a reduced or an oxidized state can be expressed in quantitative terms as its equilibrium redox potential. The redox potential is determined experimentally by measuring the relative amounts of oxidized and reduced protein species ($P(SS)$, $P(SH)_2$) in redox equilibrium with a compound of known redox potential, such as glutathione (reduced, GSH; oxidized, GSSG) (equations 1 and 2).



(1)

$$K_{eq} = k_1 k_2 = \frac{[P(SS)][GSH]^2}{[P(SH)_2][GSSG]} \quad (2)$$

$$E_p = \frac{RT}{nF} \ln K_{eq} + E_G \quad (3)$$



The redox potential of a protein is most often expressed as an electrochemical potential (E_p) in units of volts using the Nernst equation (equation 3), where R is the gas constant, T is the temperature, n is the number of electrons exchanged in the reaction (here $n = 2$) and F is the Faraday constant. E_G, the electrochemical potential of glutathione, is –240 mV (REF. 77). The scale shown in the figure depicts the measured redox potentials of several thiol-disulphide oxidoreductases and redox-active molecules[40,67,77–83]. The redox potentials of protein disulphide isomerase (PDI) and of the two cysteine pairs of DsbB (*P*1 and *P*2) are averages of the potentials that have been reported in REFS 11,12,84,85. The +300 and +800 mV redox potentials for molecular oxygen (O_2) correspond, respectively, to the O_2–H_2O_2 and O_2–H_2O redox pairs[83].

By comparing the relative redox potentials of proteins and other redox-active molecules, it is often possible to deduce the favoured pathways of disulphide-bond transfer. For example, a role for thioredoxin (Trx1) as a powerful cytosolic reductase is anticipated from the more reducing redox potential of the active site of thioredoxin relative to the cytoplasm.

Although the biological behaviour of a thiol-disulphide oxidoreductase often agrees well with its measured redox potential, there are notable exceptions. For example, in the bacterial periplasm, DsbA acts as an oxidant, whereas DsbC acts as a reductant or isomerase; nevertheless, the equilibrium redox potentials of the two proteins are similar when measured *in vitro*[80–82,86]. The recently determined redox potential of DsbB is also at odds with the *in vivo* role of DsbB as a carrier of electrons between DsbA and ubiquinone[84,85]. Moreover, in *Escherichia coli* cells that lack cytoplasmic thioredoxin reductase, thioredoxin can drive the oxidation of protein disulphide bonds in the cytosol despite its relatively negative redox potential[87]. The discrepancy between *in vitro* redox calculations and *in vivo* observations shows that a complete understanding of the *in vivo* biological function of a protein cannot be obtained solely from measurements of its equilibrium redox potential. In the cell, the biological function of a protein is influenced by its relative biochemical and kinetic preferences for reaction with the multitude of redox-active proteins and small molecules in the same cellular compartment.

REDOX (REDUCTION-OXIDATION) POTENTIAL
The propensity of a given protein (or molecule) to gain or donate electrons, which is usually expressed as an electrochemical potential in volts. A protein's redox potential can be measured by quantifying the steady-state ratios of the reduced and oxidized forms of this protein that are present in a buffer of defined redox composition. The term 'reduction potential' is often used instead.

OXIDIZING EQUIVALENTS
The loss of electrons by a molecule (this equals the gain of oxidizing equivalents).

MIXED-DISULPHIDE BOND
A disulphide bond that is formed between two proteins or redox molecules. These bonds are often transient and reflect an intermediate in the transfer of oxidizing equivalents between redox-active proteins and molecules.

An Ero1-dependent pathway for protein oxidation is also present in the mammalian ER. Two functional human homologues of yeast Ero1 have been identified, Ero1-Lα and Ero1-Lβ (Ero1-Like). Both Ero1-Lα and Ero1-Lα can complement the phenotypic defects associated with the yeast mutant *ero1-1* strain[18,19], although neither protein can complement the lethality that is associated with a complete disruption of yeast *ERO1* (REF. 20). The lack of complementation of an *ERO1* deletion in yeast by human *ERO1* has been attributed to the 127-residue carboxy-terminal domain of the yeast Ero1 protein, which is absent in the human proteins[20]. Ero1-Lα and Ero1-Lβ share a high degree of sequence similarity with each other and with yeast Ero1, but these mammalian proteins differ in their tissue distribution and transcriptional regulation[19]. Like yeast Ero1, both of the human Ero1 proteins facilitate disulphide-bond formation in substrate proteins, and Ero1–PDI mixed-disulphides have been isolated in mammalian cells[21,22].

Recently, a second pathway of disulphide-bond formation in the yeast ER has been identified, which involves a small ER oxidase known as Erv2 (FIG. 1). *ERV2* was identified as a gene that, when overexpressed, could restore viability to the mutant *ero1-1* strain[23]. *ERV2* encodes a 22-kDa membrane-associated ER protein with a lumenal domain that is non-covalently bound to flavin adenine dinucleotide (FAD)[23,24]. Using purified recombinant Erv2, it has been shown that this protein can catalyse the formation of disulphide bonds using molecular oxygen as an electron acceptor[23]. The *in vivo* activity of Erv2 is dependent on a pair of cysteines (Cys121 and Cys124) that are found in a Cys-X-X-Cys motif in a region of high sequence conservation among the Erv2 homologues, as well as on a second

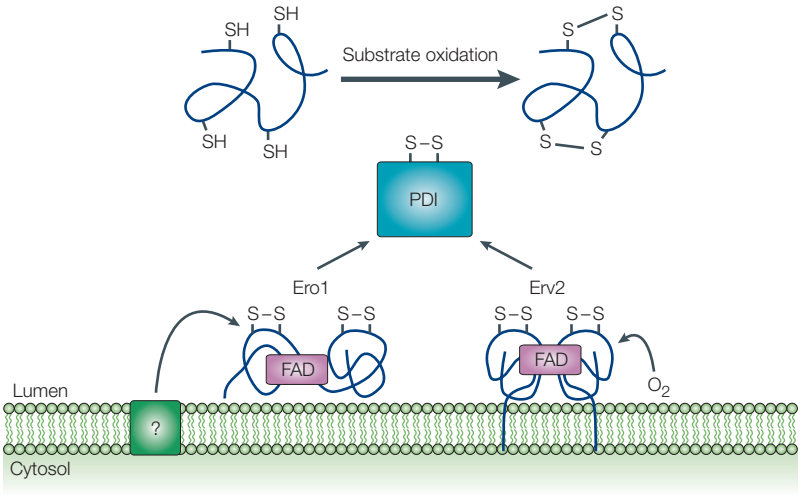


Figure 1 | **Pathways for protein oxidation in the endoplasmic reticulum of *Saccharomyces cerevisiae*.** Oxidizing equivalents for disulphide-bond formation are introduced into the endoplasmic reticulum (ER) by two parallel pathways. In the first pathway, oxidizing equivalents flow from Ero1 — or ER oxidoreductin — to the thiol-disulphide oxidoreductase protein disulphide isomerase (PDI), and from PDI to secretory proteins through a series of direct thiol-disulphide exchange reactions (thin arrows). Ero1 derives oxidizing equivalents from a flavin adenine dinucleotide (FAD) cofactor, but the ultimate source of oxidizing equivalents for Ero1 oxidation has not been identified. In the second pathway, the ER oxidase Erv2 transfers disulphide bonds to PDI before substrate oxidation. Erv2 obtains oxidizing equivalents directly from molecular oxygen through its flavin cofactor. In mammalian cells, two isoforms of Ero1 (Ero1-Lα and Ero1-Lβ) are present that transfer oxidizing equivalents to PDI. A mammalian, ER-localized Erv2-like protein has not been identified.

pair of cysteines (Cys176 and Cys178) that are present in a Cys-X-Cys arrangement in the carboxy-terminal portion of the protein[23,25]. Erv2 seems to drive the oxidation of substrate proteins *in vivo* as part of a cascade of disulphide-bond formation that involves PDI, as assayed by the capture of a mixed-disulphide intermediate of Erv2 and PDI[23]. However, *in vitro*, recombinant Erv2 is also able to oxidize substrate proteins directly[24]; the importance of this observation for Erv2 function *in vivo* is not clear. Erv2 is a member of a large family of thiol oxidases that are distributed widely among eukaryotic organisms and viruses (BOX 3; TABLE 1). The first member of this family to be identified and biochemically characterized was a thiol oxidase known as SOX, which was purified from avian egg whites[26–29].

The bacterial oxidation machinery

Many insights into the most fundamental aspects of protein disulphide-bond formation have come from studying the pathways of disulphide-bond formation in the periplasmic space of bacterial cells. In Gram-negative bacteria, the periplasmic space forms a compartment for the formation of disulphide bonds, which has similarities to the eukaryotic ER. Two proteins, the periplasmic thiol-disulphide oxidoreductase DsbA and the cytoplasmic inner-membrane protein DsbB, drive the formation of disulphide bonds in periplasmic proteins (reviewed recently in REFS 30–33) (FIG. 2). A disulphide bond that is formed between the active-site cysteines of DsbA is transferred directly to periplasmic substrate proteins, and the reduced form of DsbA is efficiently reoxidized by DsbB. The transfer of oxidizing equivalents between DsbB and DsbA occurs through direct protein-to-protein thiol-disulphide exchange, as shown by the capture of disulphide-linked complexes that contain DsbB and DsbA[34,35].

In addition to the DsbA–DsbB system for the formation of disulphide bonds, bacteria also contain a pathway that is dedicated to the isomerization of incorrectly paired cysteines. The two components of the isomerization pathway are the thiol-disulphide oxidoreductase DsbC and the cytoplasmic membrane protein DsbD (reviewed in REFS 30–33) (FIG. 2). DsbC catalyses disulphide reshuffling by reducing incorrectly paired disulphides. It is not known whether the complete reshuffling reaction can be carried out by DsbC alone, or whether a further oxidation step by DsbA is required. DsbC is maintained in a reduced active state by a continual flow of electrons from cytoplasmic thioredoxin to DsbC through the cytoplasmic membrane protein DsbD[36,37]. The transmission of a reducing potential across the cytoplasmic membrane is facilitated by a cascade of thiol-disulphide exchange reactions that take place between cysteines in the DsbD protein[38–40].

Comparing prokaryotic and eukaryotic pathways

There are many similarities between the key components of the prokaryotic and eukaryotic pathways. Most notable are the phenotypic parallels between mutants in these systems. Disruption of either DsbA or PDI interferes with the oxidation of secretory proteins[15,41]. The functional overlap between DsbA and PDI is evident with the ability of *dsbA* mutants to be complemented by the introduction of PDI into the bacterial periplasm[42]. Loss of functional Ero1 results in the accumulation of reduced PDI[15], and disrupting the function of DsbB causes defects in the reoxidation of DsbA[43]. Given the functional similarity between DsbB and Ero1, it is perhaps surprising that these proteins share no obvious sequence homology. The only apparent similarity between the primary sequence of these two proteins is the presence of two active-site cysteine pairs, which are essential for the function of either protein as a redox catalyst[17,44]. Alterations in the cysteines of DsbB result in phenotypes that are similar to those observed with Ero1 cysteine mutants[17,45]. It is interesting to note that, among the known bacterial DsbB homologues, the only amino acids that are strictly conserved are the two cysteine pairs and an arginine (Arg) at position 48 (REF. 46). Arg48 seems to assist in the interaction of DsbB with a QUINONE cofactor, which is necessary for DsbB oxidation[46].

A striking difference between the characterized prokaryotic and eukaryotic systems of disulphide-bond formation is the absence of an identified isomerization pathway in eukaryotes. At present, it is not known whether there is a reduction pathway that is analogous to the prokaryotic DsbC–DsbD system in eukaryotes.

Disulphide transfer in a single protein

All of the enzymatic pathways described above use a conserved thiol-disulphide exchange mechanism to transfer

QUINONES
A group of lipid-soluble compounds that function as electron carriers in the electron-transport chain reactions of cellular respiration.

Box 3 | **The Erv-like protein family**

The small oxidase Erv2 has recently been characterized as participating in disulphide-bond formation in the yeast endoplasmic reticulum (ER). Interestingly, a family of Erv-like sulfhydryl oxidases (see TABLE 1) is distributed widely among eukaryotic organisms and viruses. The members of the Erv-like family of proteins can be classified into two general types: proteins with Erv-like sequence homology, and proteins that contain both Erv-like and thioredoxin-like domains.

The first class of proteins includes small polypeptides (~20,000 Da) that contain a hydrophobic amino-terminal signal sequence or mitochondrial targeting signal. This group also includes several smaller viral proteins (~15,000 Da) that lack the hydrophobic amino terminus. Proteins in the second class share a conserved sequence organization that includes a hydrophobic signal sequence, an amino-terminal thioredoxin domain and a carboxy-terminal Erv-like domain, and an overall length of 400–600 amino acids. The members of this protein group that have been characterized are secreted into the extracellular space. The Erv-like sequence shared by both protein classes includes a highly conserved 100-residue core region containing a conserved Cys-X-X-Cys motif.

The characterized Erv-like oxidases use a common mechanism for disulphide transfer to protein substrates. The vaccinia virus E10R protein promotes disulphide-bond formation in cytoplasmic proteins through a virally encoded thioredoxin-like protein, G4L[70,71]. Similarly, Erv2 might operate in conjunction with protein disulphide isomerase (PDI) to oxidize cellular ER proteins[23]. For the subset of proteins that contain an Erv-like domain fused to a thioredoxin-like domain, it seems probable that oxidizing equivalents are transferred between these two domains.

The abundance of Erv-like proteins that are localized throughout the cell indicates that many new pathways for disulphide-bond formation outside the eukaryotic ER remain to be investigated. The secreted Erv-like proteins might affect the organization of the extracellular matrix. The secretion of quiescin correlates with the expression of several extracellular-matrix components known to contain structurally important disulphide bonds, which include four of the collagens and decorin[88]. The role of Erv-like proteins in mitochondrial function is not as readily apparent. However, the recent observation that Erv1 is necessary for iron–sulphur (Fe–S) protein maturation and the ability of the human protein known as augmenter of liver regeneration (ALR) to carry out the role of Erv1 in this process indicates a potential pathway that might contain redox-regulated steps[89].

disulphide bonds between separate components of the cellular redox systems. In addition to these inter-protein transfer events, it seems that the eukaryotic and prokaryotic pathways share a similar mechanism for disulphide-bond transfer between several pairs of cysteines in a single protein. The best-characterized example of such an intra-protein transfer event is the passage of electrons from the cytoplasm to the periplasm by DsbD. The mechanism of electron transfer by DsbD involves a cascade of disulphide-bond reduction events that take place between the three pairs of essential cysteines that are present in the DsbD protein[38–40,47–50].

It has been proposed that the activities of both DsbB and Ero1 rely on a similar disulphide shuttle between the two essential cysteine pairs in each protein[17,34,45]. For both DsbB and Ero1, one cysteine pair is thought to interact directly with its partner thiol-disulphide oxidoreductase (DsbA and PDI, respectively),

Table 1 | **Characterized Erv-like proteins**

Source	Protein	Size (kDa)	Cellular localization	Thioredoxin active site	Erv-like active site	Additional cysteine(s)	Additional features	References
H. sapiens	Quiescin Q6	50–80	ECM	$C_{70}GHC_{73}$	$C_{449}RDC_{452}$	4, 101, 110, 165, 237, 393, 405, 509*, 512*, 713	Induced during quiescence; secreted into medium in quiescent but not cycling cells	27,88,100, 101
	ALR	24	Mitochondria		$C_{141}EEC_{144}$	60, 70*, 73*, 94, 153,164, 170, 187, 203	Hepatic growth factor; expression rescues *Erv1*-null yeast	89,102–104
R. norvegicus	rSOX	64	ND	$C_{73}GHC_{76}$	$C_{452}RDC_{455}$	4, 104, 113, 168, 240, 396, 408, 512*, 515*	Inhibits papain activity; glycosylated	105
C. porcellus	SOx-3	68	ND	$C_{71}GHC_{74}$	$C_{450}RDC_{453}$	4, 102, 111, 166, 238, 394, 406, 510*, 513*	Induced by serum depletion; levels vary during oestrus cycle	106
S. cerevisiae	Erv1	22	Mitochondria		$C_{130}NWC_{133}$	30*, 33*, 159, 176	Essential for viability; role in Fe–S protein biogenesis	89,107–109
	Erv2	22	ER		$C_{121}GEC_{124}$	150, 167, 176*, 178*	Drives ER protein oxidation; directly oxidizes PDI	23,24
Vaccinia virus	E10R	12	Cytoplasm		$C_{43}PAC_{46}$	30	Role in virion morphogenesis; promotes viral protein oxidation	70,71,110

*Cysteine residues that form a CXXC or CXC motif. ALR, augmenter of liver regeneration; *C. porcellus*, *Cavia porcellus*; ECM, extracellular matrix; ER, endoplasmic reticulum; *E. coli*, *Escherichia coli*; *H. sapiens*, *Homo sapiens*; ND, not determined; PDI, protein disulphide isomerase; *R. norvegicus*, *Rattus norvegicus*; *S. cerevisiae*, *Saccharomyces cerevisiae*.

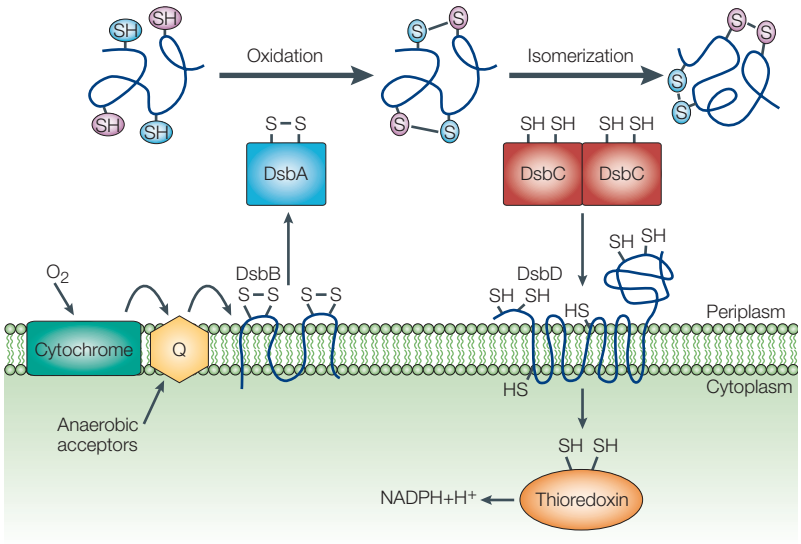


Figure 2 | **Periplasmic pathways for protein oxidation and isomerization in *Escherichia coli*.** Oxidizing equivalents are introduced into substrate proteins in the periplasmic space through a thiol-disulphide exchange reaction (thin arrows) with the soluble thiol-disulphide oxidoreductase DsbA. DsbA is reoxidized by the cytoplasmic membrane protein DsbB, which, in turn, obtains oxidizing equivalents from a quinone cofactor (Q). Electrons are ultimately transferred from quinone to oxygen under aerobic conditions, or to nitrate or fumarate under anaerobic conditions, by a cytochrome oxidase. Disulphide-bond rearrangement is catalysed by the thiol-disulphide oxidoreductase DsbC, which is maintained in a reduced state by the cytoplasmic membrane protein DsbD. DsbD is kept reduced by cytoplasmic thioredoxin, which ultimately obtains electrons from NADPH through thioredoxin reductase (not shown).

and this thiol pair is thought to be reoxidized by the internal transfer of oxidizing equivalents from the second cysteine pair. A direct interaction between the two cysteine pairs of DsbB was confirmed recently by the detection of a transient disulphide bond between the two active-site cysteine pairs[51]. A similar transfer of oxidizing equivalents between cysteine pairs in a single molecule has also been indicated for Erv2 (REF. 25). Here, a cysteine pair in a flexible tail region of the protein has been proposed to accept electrons from target proteins, and to shuttle these electrons to the FAD-proximal Cys-X-X-Cys cysteine pair[25]. The conservation of both pairs of cysteines in all of the Erv2 homologues (TABLE 1) lends support to such a transfer model. The only exception is the viral protein E10R, which contains a single Cys-X-X-Cys active site[52]. However, E10R associates with another viral protein, A2.5L, that contains a cysteine pair in a Cys-X-X-X-Cys motif[53].

Intriguingly, the structural analysis of several of the cellular thiol-disulphide oxidoreductases indicates that the relay of oxidizing equivalents might follow an alternating pattern of transfer between thioredoxin-like domains and non-thioredoxin-like domains. Thioredoxin-like domains adopt a characteristic structure formed by α helices and β sheets with the overall fold βαβαβαββα (REF. 54). The active-site Cys-X-X-Cys motif is found in an exposed turn that links β2 to α2 (REF. 54). In the DsbC–DsbD system, electrons are transferred from the cytoplasmic thioredoxin, to cysteines in the non-thioredoxin-like transmembrane domain of DsbD, then to cysteines in a thioredoxin fold in the carboxy-terminal periplasmic domain of DsbD, on to the non-thioredoxin amino-terminal DsbD domain, and finally to the thioredoxin family member DsbC[38,55,56]. Although the FAD-proximal Cys-X-X-Cys cysteine pair of Erv2 is not in a thioredoxin fold, the structural environment of this Cys-X-X-Cys pair is similar to that of the DsbA and thioredoxin active sites[25,57]. The cysteine pair in the non-thioredoxin-like tail region of Erv2 might serve as a disulphide-bond shuttle between thioredoxin-like proteins, such as PDI, and the thioredoxin-like environment of the FAD-proximal cysteine pair. The alternation of disulphide-bond transfer between regions with different protein folds, which is observed with DsbD and Erv2, might reflect a fundamental regulatory mechanism that allows transfer only between thioredoxin-like and non-thioredoxin-like domains. These structural constraints might function to direct the flow of electrons along specific pathways.

Specificity of transfer

Several thioredoxin-like proteins have been identified in the ER and the periplasmic space (see TABLE 2 and BOX 4 for a discussion of eukaryotic homologues). A central question that remains unanswered, however, is which functions each of the thioredoxin-like proteins have in disulphide-bond formation. In yeast, it has been speculated that Ero1 and Erv2 have different preferences for each of the PDI homologues, and perhaps act on different substrate proteins. Ero1 transfers oxidizing equivalents to PDI and Mpd2 (REF. 15), and perhaps Mpd1 (REF. 58), whereas, so far, Erv2 has only been shown to associate with PDI[23]. The interaction of mammalian Ero1-Lα with PDI and ERp44, but not with the homologous protein ERp57, might also reflect the presence of distinct oxidation pathways in the mammalian ER[21,59]. The idea of several oxidizing or reducing pathways that are designed for distinct substrates is borne out in the prokaryotic system in which DsbD promotes disulphide-bond isomerization (by DsbC) as well as cytochrome *c* maturation (by CcmG) (reviewed in REFS 31,32,60).

The simultaneous operation of several protein-oxidation pathways within the ER and periplasmic space would require a way to ensure the specificity of disulphide transfer to appropriate substrates. So far, it is not clear how such specificity is achieved. Perhaps the flexible carboxy-terminal region of Erv2 (discussed above) is designed to interact specifically with a particular PDI-like partner molecule? It is interesting to note that all the ERV-LIKE FAMILY members contain a Cys-X-Cys or Cys-X-X-Cys cysteine pair in addition to the Cys-X-X-Cys pair found in the 100-residue conserved core domain, but the position of these cysteines relative to the core domain Cys-X-X-Cys pair varies between the family members (TABLE 1). The protein context of the second pair of cysteines might direct the interaction of each Erv-like protein with a unique thioredoxin-like partner. A DsbD homologue from *Rhodobacter capsulatus* — CdcA — contains a cysteine pair in a DsbD-like hydrophobic domain, but lacks the two extra active-site

ERV-LIKE PROTEIN FAMILY
A family of flavoprotein thiol-oxidases — named after their homology to the yeast protein Erv1 — that couples the oxidation of free thiols with the reduction of molecular oxygen to hydrogen peroxide.

Table 2 | **Eukaryotic PDI-like proteins**

Protein (synonyms)	Size* (kDa)	Thioredoxin motifs	Calsequestrin motif	Acidic domain	Active-site sequences	ER-localization motif	Mixed-disulphide with Ero1‡	Unique features	References
Mammalian homologues									
PDI (P4HB, P55)	55	2	1	1	CGHC	KDEL	Ero1-L	General peptide-binding site	8,21,111, 112
ERp57 (GRP58, P58, ERp60, ERp61)	54	2	-	-	CGHC	QDEL	ND	Interacts with amino-glycosylated proteins through calnexin/calreticulin	8,21,92, 93,113–115
ERp72 (CaBP2, ERP70)	71	3	1	1	CGHC	KEEL	ND	-	8,21, 116–118
ERp44	44	1	1	-	CRFS	RDEL	Ero1-L	-	59
P5 (ERp5, CaBP1)	46	2	-	1	CGHC	KDEL	ND	-	8,21,118, 119
PDIp (PDIP)	55	2	-	-	CGHC, CTHC	KEEL	NR	Pancreas-specific expression	8,94,120
PDIr (PDIR)	57	3	-	-	CSMC, CGHC, CPHC	KEEL	NR	-	8,121
S. cerevisiae *homologues*									
Pdi1 (Trg1)	58	2	-	1	CGHC	HDEL	Ero1	Essential for viability	15,122–124
Eps1	81	1	-	-	CPHC	KKXXX	NR	Transmembrane domain	125
Eug1	58	2	-	-	CLHS, CIHS	HDEL	NR	-	126
Mpd1	36	1	-	-	CGHC	HDEL	ND	-	15,127
Mpd2	32	1	-	-	CQHC	HDEL	Ero1	-	15,128

*Sizes for the yeast proteins refer to the predicted molecular weights of each gene product based on the DNA sequence and do not reflect any post-translational modifications. ‡The interaction detected under experimental conditions as described in the cited literature. CaBP, calcium-binding protein; ND, no interaction detected under the experimental conditions as described in the cited literature; NR, an attempt to detect an interaction between the PDI-like protein and Ero1 has not been reported in the literature; PDI, protein disulphide isomerase; PDIP, a PDI homologue that is expressed in pancreatic tissue; PDIR, PDI-related protein; *S. cerevisiae*, *Saccharomyces cerevisiae*; X, any amino acid.

cysteine pairs that are found in the periplasmic domains of DsbD. Interestingly, a recent comparison of the functional domains of *Escherichia coli* DsbD and *R. capsulatus* CdcA indicates that the extra thiol-containing domains of DsbD might expand the substrate range of DsbD relative to CcdA[61]. Perhaps other redox-active proteins also contain cysteine pairs in unique structural motifs that promote interactions with specific substrate thiol-disulphide oxidoreductases?

The coincident operation of oxidation and isomerization pathways in the periplasmic space poses a similar problem for specific electron transfer. For proteins of the oxidizing pathway to be able to carry out their function, their active sites must be in an oxidized state. Similarly, proteins that function to reduce or isomerize substrates must achieve a reduced state to fulfil their roles (see, for example, REF. 62). The accidental transfer of oxidizing equivalents into the isomerization pathway, or of reducing equivalents into the oxidizing pathway, would inactivate the enzymes in either pathway and result in the incapacitation of either system. Indeed, little cross-talk in the form of disulphide-bond transfer between the pathways for oxidation and reduction/isomerization is evident: *in vitro*, DsbB oxidizes only DsbA and not DsbC, despite the fact that both proteins have similar redox potentials[63]. A recent analysis of *dsbC* mutants that can complement a *dsbA*-null strain indicates that dimerization of the DsbC isomerase/reductase enzyme might normally block its active sites from recognition by DsbB, and prevent misoxidation of DsbC by DsbB[64].

Coupling disulphide bonds and small molecules

Glutathione and the ER redox potential. To fully understand the process of cellular protein oxidation, it is necessary to determine the ultimate origin of the oxidative power for disulphide-bond formation. Several small thiol-containing molecules, such as cystamine[65], vitamin K epoxide[66] and glutathione[67], have been proposed to contribute to the oxidation of proteins in the ER lumen. Of these molecules, oxidized glutathione has attracted the most attention and, until recently, it was widely considered to be the prime candidate for the source of the oxidizing equivalents that are necessary to generate protein disulphide bonds. The most compelling evidence in support of a role for glutathione in protein oxidation came from the observation that a higher ratio of oxidized to reduced glutathione is present in the ER relative to the cytosol[67]. The mixture of oxidized and reduced glutathione detected in the ER was similar to that found in redox buffers that afford optimal rates of protein oxidation *in vitro*[68].

However, a direct experimental test in *S. cerevisiae* showed that, despite the abundance of oxidized glutathione in the ER lumen, glutathione is not required for oxidative protein folding in the eukaryotic ER[13]. Moreover, the *in vitro* oxidation of RNase A by purified

Box 4 | **Functions of diverse PDI homologues**

Human and yeast cells both contain several protein disulphide isomerase (PDI) homologues in the endoplasmic reticulum (ER) (TABLE 2). The PDI homologues are characterized by the presence of one or more domains with sequence homology to thioredoxin, a signal sequence and a (K/H)DEL LOCALIZATION SIGNAL. In *Saccharomyces cerevisiae*, the complete genome sequence encodes four PDI-like proteins, whereas at least six mammalian PDI-like homologues have been identified. The presence of so many thioredoxin-like proteins in the ER raises questions about whether these proteins have redundant or distinct functions.

Distinct roles for the characterized PDI homologues have been indicated by variations in the ability of the PDI homologues to ensure viability of *S. cerevisiae* when PDI function is compromised[58,90]. The inability of ERp57 to replace mammalian PDI as a subunit for prolyl-4-hydroxylase (P4H) also attests to a lack of functional conservation among homologues[91]. Several observations indicate that individual PDI homologues facilitate the maturation of discrete sets of proteins. ERp57 acts in a unique pathway, together with the CHAPERONES calnexin and calreticulin, to assist the maturation of a class of glycoproteins[92,93]. The restricted exocrine pancreas-tissue expression of the PDI homologue PDIp indicates that PDIp contributes to the folding of a subset of pancreatic enzymes[94].

Individual PDI homologues might also differ in their redox activity in the cell. Mammalian and yeast PDI can reduce, isomerize or oxidize, depending on the redox environment[10]. An intriguing possibility is that different PDI homologues take on distinct redox activities. The oxidation and reduction activities of PDI seem to have distinct structural requirements[95,96]. On the basis of the observed structural constraints, it has been speculated that the PDI homologues P5 or Mpd2 are dedicated to oxidizing proteins, whereas other homologues, such as ERp57 and Eug1, are isomerases[8,9]. The presence of a Cys-X-X-Ser active-site motif, rather than the typical Cys-X-X-Cys motif, is thought to be a potential indicator of isomerase activity[97,98]. Two PDI-like proteins, human ERp44 and yeast Eug1, both contain Cys-X-X-Ser motifs. However, mutation of the Eug1 active sites to Cys-X-X-Cys sequences creates a mutant enzyme that, *in vitro*, has not only a better oxidative refolding activity, but also a better isomerase activity than wild-type Eug1. This implies that the unusual nature of the Eug1 active site did not evolve to optimize isomerase activity[99].

Ero1 and PDI does not require oxidized or reduced glutathione[16]. Interestingly, the disulphide-bond formation that is driven by the *in vitro* Ero1–PDI system can proceed in the presence of a vast excess of reduced glutathione[16]. Likewise, the cytoplasmic vaccinia virus protein-oxidation pathway can operate in the presence of the excess of reduced glutathione that is found in the cytoplasm[69–71]. These observations have led to a revised view that protein-oxidation pathways proceed by the direct transfer of oxidizing equivalents between enzymes and do not rely on oxidizing equivalents provided by glutathione.

Further experiments have indicated that *in vivo* glutathione might compete with proteins for oxidizing equivalents. In *ero1-1* mutants, the reduction of the intracellular glutathione level, by disruption of the *GSH1* gene (which encodes the enzyme that catalyses the first and rate-limiting step in glutathione synthesis), actually restores disulphide-bond formation activity to the compromised protein-oxidation system[72]. So, glutathione acts as a net reductant in the ER that counteracts the oxidizing activity of the Ero1 pathway. The production of oxidized glutathione could result from the reduction of a protein disulphide bond in any component of the eukaryotic protein-oxidation pathway: Ero1, PDI or secretory proteins. Recent experiments have begun to narrow down the potential source of glutathione oxidation in the ER. The *in vitro* characterization of Ero1 and Erv2 has shown that neither protein directly oxidizes glutathione[16,23]. The complete reconstitution of the Ero1–PDI pathway for protein oxidation *in vitro* indicates that glutathione oxidation is driven by Ero1-derived disulphide bonds in PDI and/or substrate proteins[16].

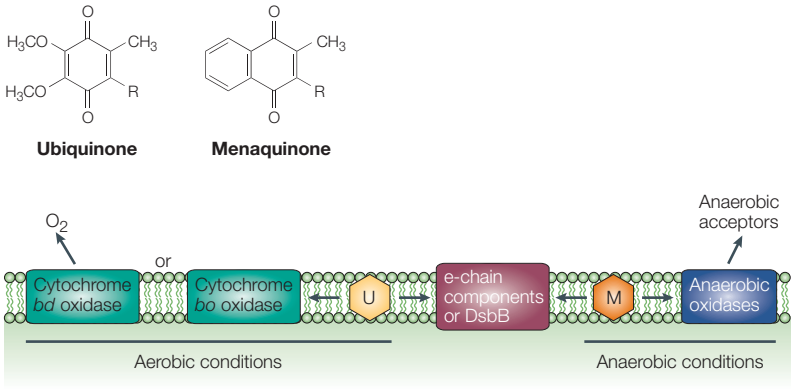
These observations raise the question of why the ER maintains two seemingly competing pathways: a glutathione-based pathway that introduces reducing equivalents and a protein-oxidation pathway that is driven by the enzymatic transfer of oxidizing equivalents. The importance of a proper ratio of reducing and oxidizing equivalents for *in vitro* refolding reactions has been shown repeatedly. An attractive possibility is that glutathione functions as a buffer for the ER redox environment. Under hyperoxidizing conditions, the reducing equivalents from glutathione might be used to reduce improperly paired cysteines, facilitating the correct folding of proteins. Instead of interacting directly with substrate proteins, glutathione could also reduce the normally oxidized PDI, shifting PDI activity from oxidation to isomerization. Glutathione might also counteract oxidative stress simply by consuming excess oxidizing equivalents during the conversion of reduced glutathione to oxidized glutathione. A role for glutathione in counteracting oxidative stress is supported by the observation that oxidative protein folding is more readily compromised by the addition of the oxidant diamide in a *gsh1* mutant strain[72].

Flavins and eukaryotic disulphide bonds. As glutathione seems to provide reducing, rather than oxidizing, equivalents in the ER, a renewed search has begun for the oxidative source for the ER. Recent experiments indicate that flavin moieties provide a source of oxidizing equivalents for both the Ero1 and Erv2 pathways of disulphide oxidation. *In vivo*, the depletion of riboflavin, and therefore its flavin derivatives, including FAD, inhibits disulphide-bond formation and results in the accumulation of reduced Ero1[16]. The *in vitro* oxidative folding of reduced RNase A that is catalysed by purified Ero1 and

(K/H)DEL SIGNAL
An ER-localization motif for soluble lumenal proteins that includes the short carboxy-terminal sequence Lys/His-Asp-Glu-Leu.

CHAPERONE
A protein that catalyses the correct folding of newly synthesized or denatured proteins into their native conformations.

Box 5 | **The respiratory electron-transport chain**



The cellular respiratory electron-transport chain includes a series of intermediate electron carriers that facilitate the transfer of electrons, which are produced by the oxidation of substrate molecules, to molecular oxygen or some other inorganic compound or ion. The transfer of electrons between the components of the electron-transport chain gives rise to energy that is used for various cellular processes, including ATP synthesis.

The respiratory electron-transport chain of eukaryotes is located in the mitochondrial inner membrane. In bacteria, an analogous respiratory chain is found in the cell membrane. Both pathways use similar proteins and small-molecule redox carriers. The main protein components of the electron-transport chain are flavoproteins, iron–sulphur (Fe–S) proteins and cytochromes. Each protein component has a redox-active small molecule or metal cofactor that can accept or donate electrons: flavoproteins contain a flavin cofactor, iron–sulphur proteins carry an equivalent number of iron and sulphur atoms, whereas cytochromes bind iron-containing haem rings. The electron-transport system also includes a group of non-protein, lipid-soluble electron carriers called quinones. Quinones promote the transfer of electrons between the protein components of the electron-transport chain — a process that is facilitated by their ability to move in the lipid bilayer. Two types of quinone can be found in the cell: ubiquinones and menaquinones (see figure). Ubiquinones are derivatives of benzoquinone (coenzyme Q) with a variable-length isoprenoid chain attached to each C6 group (denoted as R). Menaquinone groups are derivatives of naphthoquinone (vitamin K); they are also attached to an isoprenoid chain.

As discussed in the main text, the DsbA–DsbB pathway for biosynthetic disulphide-bond formation derives oxidizing equivalents from the terminal steps of the bacterial electron-transport chain (see figure; arrows represent the flow of electrons). In the terminal portion of the bacterial electron-transport pathway, electrons are shuttled from ubiquinone (U) or menaquinone (M) carriers to molecular oxygen or anaerobic acceptors by protein complexes. DsbB taps into this pathway by donating electrons directly to quinones that feed into the terminal steps of the bacterial electron-transport chain.

PDI also seems to rely on the oxidizing equivalents that are provided by the addition of FAD[16]. However, the ultimate oxidizing source for FAD and Ero1 remains elusive. During a catalytic cycle of the Ero1 system, FAD will become reduced to $FADH_2$ on the transfer of oxidizing equivalents to Ero1. In the *in vitro* pathway, the requirement for a stoichiometric excess of FAD indicates that Ero1 might exchange the reduced $FADH_2$ for oxidized flavin, FAD, from solution. Although Ero1 might exchange $FADH_2$ for free FAD *in vivo*, it seems unlikely that such an exchange is the normal physiological mechanism for Ero1 oxidation. Most flavoproteins tightly bind their cofactors, which would impede a catalytic exchange mechanism. In addition, the concentration of FAD in yeast cells is much lower than the levels required for the *in vitro* Ero1 oxidation reaction[73].

Although the identity of the oxidant for Ero1 and its FAD cofactor remains elusive, physiological experiments give us some clues about the types of oxidation process that are possible. The *ero1-1* mutant is not viable at high temperatures, either in the presence or the absence of oxygen, which indicates that Ero1 is an essential part of the oxidation pathway under aerobic and anaerobic conditions. Although Ero1 might use molecular oxygen as an electron acceptor during aerobic growth, the ability of Ero1 to operate under conditions in which oxygen is limited indicates that there must be a physiological electron acceptor for Ero1 that is not molecular oxygen and does not depend on oxygen for its generation. Conversely, molecular oxygen functions as the obligate electron acceptor for the second ER pathway that is driven by Erv2 (REF. 23). In the Erv2 pathway, the flavin cofactor of Erv2 interacts directly with molecular oxygen to contribute the oxidizing equivalents that are necessary for disulphide-bond formation.

Quinones as prokaryotic electron carriers. In prokaryotes, a more complete understanding of how the oxidation of protein thiols is integrated into the redox chemistry of the cell has been achieved. Experiments in *E. coli* have shown that the RESPIRATORY ELECTRON-TRANSPORT CHAIN (BOX 5) is necessary for the complete oxidation of DsbB. Disruption of the respiratory chain, by depletion of the intracellular pools of haem or ubiquinone and menaquinone, impedes the flow of oxidizing equivalents into the DsbA–DsbB system[74]. Under these depletion conditions, DsbA accumulates in its reduced form[74]. The recent reconstitution of the DsbA–DsbB system has established that DsbB uses a small electron carrier, a quinone cofactor, to transfer electrons to the terminal oxidases of the electron transport chain and then to either molecular oxygen or other electron acceptors[63,75]. Under conditions of aerobic growth, electrons flow from DsbB directly to ubiquinone that is associated with cytochrome *bd* or *bo* oxidase, and then to molecular oxygen. During anaerobic growth, DsbB uses menaquinone as an electron carrier that transfers electrons to alternative acceptors such as fumarate and nitrate, rather than oxygen[75]. Alleles of *dsbB* that encode single amino-acid substitutions for Arg48 show a greater defect in the use of menaquinone than of ubiquinone[46]. Consistent with the role of menaquinone as the anaerobic electron acceptor for DsbB, these mutants show the greatest defect in protein oxidation under anaerobic growth conditions.

Future directions and implications

The past few years have seen significant advances in our understanding of the pathways of protein disulphide-bond formation in the periplasm of bacteria and the ER of eukaryotic cells. This review has concentrated on the emerging similarities between the prokaryotic and eukaryotic systems. Both pathways include a conserved thiol-disulphide exchange mechanism that transfers disulphide bonds between the enzymatic components of the pathways of disulphide-bond formation. In addition, new mechanistic insights into the functions of several

RESPIRATORY ELECTRON-TRANSPORT CHAIN
A series of redox-active membrane proteins and small molecules in either the bacterial plasma membrane or the mitochondrial inner membrane that carry out the step-by-step transfer of electrons from NADH and $FADH_2$ to O_2 with the concomitant generation of a membrane proton potential.

redox-active proteins show that cellular redox pathways often rely on the relay of electrons between pairs of cysteines in a single protein. The cellular oxidation pathways seem to be controlled by the specificity of intra-protein and inter-protein interactions. The work that was discussed here also introduced a new family of eukaryotic and viral thiol-oxidases, the Erv-like family, whose role in disulphide-bond formation was identified recently. Notably, the initial characterization of members of the Erv family shows that many of the same characteristics are shared between the more established ER and periplasmic pathways.

The studies reviewed here provide solid groundwork for future studies of protein disulphide-bond formation. It will be of interest to understand the biological significance and division of labour among the various homologues that are implicated in disulphide-bond formation in mammalian and yeast cells. Similarly, the diversity and ubiquity of the Erv family of proteins indicate that it might be possible to extend our understanding of how oxidizing equivalents can be transferred specifically from one protein to another, and to other compartments, such as the mitochondria, cytosol and extracellular space.

The early focus on the identification and initial characterization of the pathways of protein oxidation and/or reduction in prokaryotes and eukaryotes has clearly shifted during the past few years towards understanding the mechanistic and structural details of these pathways. Now, the structural data on the amino-terminal domain of DsbD[56], the flavoprotein-oxidase Erv2 (REF. 25) and the bacterial thioredoxin-like proteins DsbA and DsbC[55,57], along with the recent ability to reconstitute the Ero1–PDI[16], DsbB–DsbA[63,76] and DsbD–DsbC[40] pathways *in vitro*, have set the stage for a detailed structural picture of the pathways of disulphide-bond formation. The tools available allow rational mutagenesis, domain swapping and biochemical studies, to test the current models that are designed to explain the specificity observed in the electron transfer in and between proteins. Clearly, the structural analysis of Ero1, as well as of complexes between Ero1 and PDI, is a crucial goal for understanding the structural basis of selectivity in eukaryotic disulphide-bond formation.

1. Jordan, A. & Reichard, P. Ribonucleotide reductases. *Annu. Rev. Biochem.* **67**, 71–98 (1998).
2. Dai, S., Schwendtmayer, C., Schurmann, P., Ramaswamy, S. & Eklund, H. Redox signaling in chloroplasts: cleavage of disulfides by an iron–sulfur cluster. *Science* **287**, 655–658 (2000).
3. Buchanan, B. B. Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective on its discovery, present status, and future development. *Arch. Biochem. Biophys.* **288**, 1–9 (1991).
4. Zheng, M., Åslund, F. & Storz, G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**, 1718–1721 (1998).
5. Jakob, U., Muse, W., Eser, M. & Bardwell, J. C. Chaperone activity with a redox switch. *Cell* **96**, 341–352 (1999).
6. Golberger, R. F., Epstein, C. J. & Anfinsen, C. B. Acceleration of reactivation of reduced bovine pancreatic ribonuclease by a microsomal system from rat liver. *J. Biol. Chem.* **238**, 628–635 (1963).
7. Noiva, R. Protein disulfide isomerase: the multifunctional redox chaperone of the endoplasmic reticulum. *Semin. Cell Dev. Biol.* **10**, 481–493 (1999).
8. Ferrari, D. M. & Soling, H. D. The protein disulphide-isomerase family: unravelling a string of folds. *Biochem. J.* **339**, 1–10 (1999).
9. Freedman, R. B., Klappa, P. & Ruddock, L. W. Protein disulfide isomerases exploit synergy between catalytic and specific binding domains. *EMBO Rep.* **3**, 136–140 (2002).
10. Gilbert, H. F. in *Mechanisms of Protein Folding* (ed. Pain, R. H.) 104–135 (Oxford Univ. Press, Oxford, UK, 1994).
11. Hawkins, H. C., de Nardi, M. & Freedman, R. B. Redox properties and cross-linking of the dithiol/disulphide active sites of mammalian protein disulphide-isomerase. *Biochem. J.* **275**, 341–348 (1991).
12. Lundstrom, J. & Holmgren, A. Determination of the reduction–oxidation potential of the thioredoxin-like domains of protein disulfide-isomerase from the equilibrium with glutathione and thioredoxin. *Biochemistry* **32**, 6649–6655 (1993).
13. Frand, A. R. & Kaiser, C. A. The *ERO1* gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum. *Mol. Cell* **1**, 161–170 (1998).
14. Pollard, M. G., Travers, K. J. & Weissman, J. S. Ero1p: a novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. *Mol. Cell* **1**, 171–182 (1998).
15. Frand, A. R. & Kaiser, C. A. Ero1p oxidizes protein disulfide isomerase in a pathway for disulfide bond formation in the endoplasmic reticulum. *Mol. Cell* **4**, 469–477 (1999).
This paper presents the first evidence for the direct flow of oxidizing equivalents from Ero1 to substrate proteins by PDI.
16. Tu, B. P., Ho-Schleyer, S. C., Travers, K. J. & Weissman, J. S. Biochemical basis of oxidative protein folding in the endoplasmic reticulum. *Science* **290**, 1571–1574 (2000).
This paper shows that oxidative protein folding in yeast is dependent on FAD levels and provides evidence that Ero1 binds FAD. The reconstitution of the eukaryotic ER pathway for protein disulphide-bond formation *in vitro* using FAD, Ero1 and PDI is also described.
17. Frand, A. R. & Kaiser, C. A. Two pairs of conserved cysteines are required for the oxidative activity of Ero1p in protein disulfide bond formation in the endoplasmic reticulum. *Mol. Biol. Cell* **11**, 2833–2843 (2000).
18. Cabibbo, A. *et al.* ERO1-L, a human protein that favors disulfide bond formation in the endoplasmic reticulum. *J. Biol. Chem.* **275**, 4827–4833 (2000).
19. Pagani, M. *et al.* Endoplasmic reticulum oxidoreductin 1-Lβ (ERO1-Lβ), a human gene induced in the course of the unfolded protein response. *J. Biol. Chem.* **275**, 23685–23692 (2000).
20. Pagani, M., Pilati, S., Bertoli, G., Valsasina, B. & Sitia, R. The C-terminal domain of yeast Ero1p mediates membrane localization and is essential for function. *FEBS Lett.* **508**, 117–120 (2001).
21. Mezghrani, A. *et al.* Manipulation of oxidative protein folding and PDI redox state in mammalian cells. *EMBO J.* **20**, 6288–6296 (2001).
This article reports that mammalian Ero1 selectively oxidizes PDI, which, in turn, catalyses the formation of disulphide bonds in immunoglobulin subunits. The interesting observation that exogenous expression of active-site mutants of Ero1-Lα limits substrate protein oxidation is also presented.
22. Benham, A. M. *et al.* The CXXCXXC motif determines the folding, structure and stability of human Ero1-Lα. *EMBO J.* **19**, 4493–4502 (2000).
23. Sevier, C. S., Cuozzo, J. W., Vala, A., Åslund, F. & Kaiser, C. A. A flavoprotein oxidase defines a new endoplasmic reticulum pathway for biosynthetic disulphide bond formation. *Nature Cell Biol.* **3**, 874–882 (2001).
This paper presents evidence for a second, Ero1-independent oxidation pathway in yeast ER that uses the small ER oxidase Erv2 and PDI. This and reference 24 show that Erv2 is a flavoenzyme that uses the oxidizing potential of molecular oxygen to drive the formation of disulphide bonds.
24. Gerber, J., Muhlenhoff, U., Hofhaus, G., Lill, R. & Lisowsky, T. Yeast ERV2p is the first microsomal FAD-linked sulfhydryl oxidase of the Erv1p/Alrp protein family. *J. Biol. Chem.* **276**, 23486–23491 (2001).
25. Gross, E., Sevier, C. S., Vala, A., Kaiser, C. A. & Fass, D. A new FAD-binding fold and intersubunit disulfide shuttle in the thiol oxidase Erv2p. *Nature Struct. Biol.* **9**, 61–67 (2002).
This paper presents the structural analysis of yeast Erv2. Structural and biochemical analysis indicates that Erv2 might use an internal thiol-transfer relay between two pairs of cysteines to facilitate the transfer of disulphide bonds to substrate proteins.
26. Hoober, K. L., Joneja, B., White, H. B. & Thorpe, C. A sulfhydryl oxidase from chicken egg white. *J. Biol. Chem.* **271**, 30510–30516 (1996).
27. Hoober, K. L., Glynn, N. M., Burnside, J., Coppock, D. L. & Thorpe, C. Homology between egg white sulfhydryl oxidase and quiescin Q6 defines a new class of flavin-linked sulfhydryl oxidases. *J. Biol. Chem.* **274**, 31759–31762 (1999).
This article shows that the flavin-dependent sulfhydryl oxidase from chicken egg white is homologous to the human protein quiescin Q6, thereby identifying a new family of cellular oxidases. See references 26, 28 and 29 for the biochemical characterization of the avian sulfhydryl oxidase.
28. Hoober, K. L., Sheasley, S. L., Gilbert, H. F. & Thorpe, C. Sulfhydryl oxidase from egg white. A facile catalyst for disulfide bond formation in proteins and peptides. *J. Biol. Chem.* **274**, 22147–22150 (1999).
29. Hoober, K. L. & Thorpe, C. Egg white sulfhydryl oxidase: kinetic mechanism of the catalysis of disulfide bond formation. *Biochemistry* **38**, 3211–3217 (1999).
30. Collet, J. F. & Bardwell, J. C. Oxidative protein folding in bacteria. *Mol. Microbiol.* **44**, 1–8 (2002).
This paper and reference 31 provide the most recent reviews focused on the bacterial disulphide-bond formation and isomerization pathways. See the references in these reviews for a more detailed discussion of the history of the identification of the bacterial enzymes for disulphide-bond formation.
31. Ritz, D. & Beckwith, J. Roles of thiol-redox pathways in bacteria. *Annu. Rev. Microbiol.* **55**, 21–48 (2001).
32. Fabianek, R. A., Hennecke, H. & Thöny-Meyer, L. Periplasmic protein thiol:disulfide oxidoreductases of *Escherichia coli*. *FEMS Microbiol. Rev.* **24**, 303–316 (2000).
33. Debarbieux, L. & Beckwith, J. Electron avenue: pathways of disulfide bond formation and isomerization. *Cell* **99**, 117–119 (1999).
34. Guilhot, C., Jander, G., Martin, N. L. & Beckwith, J. Evidence that the pathway of disulfide bond formation in *Escherichia coli* involves interactions between the cysteines of DsbB and DsbA. *Proc. Natl Acad. Sci. USA* **92**, 9895–9899 (1995).
35. Kishigami, S., Akiyama, Y. & Ito, K. Redox states of DsbA in the periplasm of *Escherichia coli*. *FEBS Lett.* **364**, 55–58 (1995).
36. Missiakas, D., Schwager, F. & Raina, S. Identification and characterization of a new disulfide isomerase-like protein (DsbD) in *Escherichia coli*. *EMBO J.* **14**, 3415–3424 (1995).

37. Rietsch, A., Bessette, P., Georgiou, G. & Beckwith, J. Reduction of the periplasmic disulfide bond isomerase, DsbC, occurs by passage of electrons from cytoplasmic thioredoxin. *J. Bacteriol.* **179**, 6602–6608 (1997).
38. Katzen, F. & Beckwith, J. Transmembrane electron transfer by the membrane protein DsbD occurs via a disulfide bond cascade. *Cell* **103**, 769–779 (2000).
This article probes the mechanism of the thiol-disulphide oxidoreductase DsbD and provides an elegant example of the intra-protein transfer of disulphide bonds that seems be important in the function of several proteins involved in cellular protein oxidation and reduction.
39. Krupp, R., Chan, C. & Missiakas, D. DsbD-catalyzed transport of electrons across the membrane of *Escherichia coli*. *J. Biol. Chem.* **276**, 3696–3701 (2001).
40. Collet, J. F., Riemer, J., Bader, M. W. & Bardwell, J. C. Reconstitution of a disulfide isomerization system. *J. Biol. Chem.* **277**, 26886–26892 (2002).
41. Bardwell, J. C., McGovern, K. & Beckwith, J. Identification of a protein required for disulfide bond formation *in vivo*. *Cell* **67**, 581–589 (1991).
This paper reports the initial identification of DsbA, providing the first evidence that the formation of protein disulphide bonds in bacteria is a catalysed process.
42. Humphreys, D. P., Weir, N., Mountain, A. & Lund, P. A. Human protein disulfide isomerase functionally complements a dsbA mutation and enhances the yield of pectate lyase C in *Escherichia coli*. *J. Biol. Chem.* **270**, 28210–28215 (1995).
43. Bardwell, J. C. *et al.* A pathway for disulfide bond formation *in vivo*. *Proc. Natl Acad. Sci. USA* **90**, 1038–1042 (1993).
44. Jander, G., Martin, N. L. & Beckwith, J. Two cysteines in each periplasmic domain of the membrane protein DsbB are required for its function in protein disulfide bond formation. *EMBO J.* **13**, 5121–5127 (1994).
45. Kishigami, S. & Ito, K. Roles of cysteine residues of DsbB in its activity to reoxidize DsbA, the protein disulphide bond catalyst of *Escherichia coli*. *Genes Cells* **1**, 201–208 (1996).
46. Kadokura, H., Bader, M., Tian, H., Bardwell, J. C. & Beckwith, J. Roles of a conserved arginine residue of DsbB in linking protein disulfide-bond-formation pathway to the respiratory chain of *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **97**, 10884–10889 (2000).
47. Stewart, E. J., Katzen, F. & Beckwith, J. Six conserved cysteines of the membrane protein DsbD are required for the transfer of electrons from the cytoplasm to the periplasm of *Escherichia coli*. *EMBO J.* **18**, 5963–5971 (1999).
48. Chung, J., Chen, T. & Missiakas, D. Transfer of electrons across the cytoplasmic membrane by DsbD, a membrane protein involved in thiol-disulphide exchange and protein folding in the bacterial periplasm. *Mol. Microbiol.* **35**, 1099–1109 (2000).
49. Gordon, E. H., Page, M. D., Willis, A. C. & Ferguson, S. J. *Escherichia coli* DipZ: anatomy of a transmembrane protein disulphide reductase in which three pairs of cysteine residues, one in each of three domains, contribute differentially to function. *Mol. Microbiol.* **35**, 1360–1374 (2000).
50. Goldstone, D. *et al.* DsbC activation by the N-terminal domain of DsbD. *Proc. Natl Acad. Sci. USA* **98**, 9551–9556 (2001).
51. Kadokura, H. & Beckwith, J. Four cysteines of the membrane protein DsbB act in concert to oxidize its substrate DsbA. *EMBO J.* **21**, 2354–2363 (2002).
This study shows the direct flow of electrons between the two pairs of cysteines in DsbB, and between the cysteines in DsbB and DsbA. See also references 34 and 35 for the original observations of a direct interaction between DsbA and DsbB, and the recent references 84 and 85, which report the redox potentials for the DsbB cysteine pairs.
52. Senkevich, T. G., Koonin, E. V., Bugert, J. J., Darai, G. & Moss, B. The genome of *Molluscum contagiosum* virus: analysis and comparison with other poxviruses. *Virology* **233**, 19–42 (1997).
53. Senkevich, T. G., White, C. L., Koonin, E. V. & Moss, B. Complete pathway for protein disulfide bond formation encoded by poxviruses. *Proc. Natl Acad. Sci. USA* **99**, 6667–6672 (2002).
This paper shows that three cytoplasmic thiol-disulphide oxidoreductases (E10R, A2.5L and G4L) encoded by vaccinia virus cooperate in a cytosolic pathway for the formation of disulphide bonds in virion proteins. See also references 52, 70, 71 and 110.
54. Martin, J. L. Thioredoxin—a fold for all reasons. *Structure* **3**, 245–250 (1995).
55. McCarthy, A. A. *et al.* Crystal structure of the protein disulfide bond isomerase, DsbC, from *Escherichia coli*. *Nature Struct. Biol.* **7**, 196–199 (2000).
56. Goulding, C. W. *et al.* Thiol-disulfide exchange in an immunoglobulin-like fold: structure of the N-terminal domain of DsbD. *Biochemistry* **41**, 6920–6927 (2002).
57. Martin, J. L., Bardwell, J. C. & Kuriyan, J. Crystal structure of the DsbA protein required for disulphide bond formation *in vivo*. *Nature* **365**, 464–468 (1993).
58. Nørgaard, P. *et al.* Functional differences in yeast protein disulfide isomerases. *J. Cell Biol.* **152**, 553–562 (2001).
This study addresses the functional differences between yeast PDI and the four yeast PDI homologues, and illustrates some of the difficulties in sorting out the overlapping functions of the PDI homologues.
59. Anelli, T. *et al.* ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. *EMBO J.* **21**, 835–844 (2002).
60. Thöny-Meyer, L. Cytochrome *c* maturation: a complex pathway for a simple task? *Biochem. Soc. Trans.* **30**, 633–638 (2002).
61. Katzen, F., Deshmukh, M., Daldal, F. & Beckwith, J. Evolutionary domain fusion expanded the substrate specificity of the transmembrane electron transporter DsbD. *EMBO J.* **21**, 3960–3969 (2002).
62. Joly, J. C. & Swartz, J. R. *In vitro* and *in vivo* redox states of the *Escherichia coli* periplasmic oxidoreductases DsbA and DsbC. *Biochemistry* **36**, 10067–10072 (1997).
63. Bader, M. W., Xie, T., Yu, C. A. & Bardwell, J. C. Disulfide bonds are generated by quinone reduction. *J. Biol. Chem.* **275**, 26082–26088 (2000).
64. Bader, M. W. *et al.* Turning a disulfide isomerase into an oxidase: DsbC mutants that imitate DsbA. *EMBO J.* **20**, 1555–1562 (2001).
This article proposes that the lack of cross-talk between the bacterial oxidation and isomerization pathways is a result of the dimerization of the DsbC isomerase/reductase enzyme. The dimerization of DsbC is suggested to insulate the active sites from recognition by DsbB, preventing misoxidation of DsbC by DsbB.
65. Ziegler, D. M., Duffel, M. W. & Poulsen, L. L. Studies on the nature and regulation of the cellular thio:disulphide potential. *Ciba Found. Symp.* **72**, 191–204 (1979).
66. Soute, B. A., Groenen-van Dooren, M. M., Holmgren, A., Lundstrom, J. & Vermeer, C. Stimulation of the dithiol-dependent reductases in the vitamin K cycle by the thioredoxin system. Strong synergistic effects with protein disulphide-isomerase. *Biochem. J.* **281**, 255–259 (1992).
67. Hwang, C., Sinskey, A. J. & Lodish, H. F. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* **257**, 1496–1502 (1992).
68. Creighton, T. E. *et al.* On the biosynthesis of bovine pancreatic trypsin inhibitor (BPTI). Structure, processing, folding and disulphide bond formation of the precursor *in vitro* and in microsomes. *J. Mol. Biol.* **232**, 1176–1196 (1993).
69. Locker, J. K. & Griffiths, G. An unconventional role for cytoplasmic disulfide bonds in vaccinia virus proteins. *J. Cell Biol.* **144**, 267–279 (1999).
70. Senkevich, T. G., White, C. L., Koonin, E. V. & Moss, B. A viral member of the ERV1/ALR protein family participates in a cytoplasmic pathway of disulfide bond formation. *Proc. Natl Acad. Sci. USA* **97**, 12068–12073 (2000).
71. White, C. L., Senkevich, T. G. & Moss, B. Vaccinia virus G4L glutaredoxin is an essential intermediate of a cytoplasmic disulfide bond pathway required for virion assembly. *J. Virol.* **76**, 467–472 (2002).
72. Cuozzo, J. W. & Kaiser, C. A. Competition between glutathione and protein thiols for disulphide-bond formation. *Nature Cell Biol.* **1**, 130–135 (1999).
This study shows that glutathione is not the predominant source of oxidizing equivalents for the formation of disulphide bonds in proteins in the ER. Instead, it indicates that cellular glutathione might function as a net reductant of the ER, and might protect the ER against transient hyperoxidizing conditions.
73. Gliszczynska, A. & Koziolowa, A. Chromatographic determination of flavin derivatives in baker's yeast. *J. Chromatogr. A* **822**, 59–66 (1998).
74. Kobayashi, T. *et al.* Respiratory chain is required to maintain oxidized states of the DsbA–DsbB disulfide bond formation system in aerobically growing *Escherichia coli* cells. *Proc. Natl Acad. Sci. USA* **94**, 11857–11862 (1997).
75. Bader, M., Muse, W., Ballou, D. P., Gassner, C. & Bardwell, J. C. Oxidative protein folding is driven by the electron transport system. *Cell* **98**, 217–227 (1999).
This paper, along with references 63 and 74, shows that the bacterial periplasmic disulphide-bond formation pathway derives oxidizing equivalents from the cellular electron-transport system.
76. Bader, M., Muse, W., Zander, T. & Bardwell, J. Reconstitution of a protein disulfide catalytic system. *J. Biol. Chem.* **273**, 10302–10307 (1998).
77. Rost, J. & Rapoport, S. Reduction potential of glutathione. *Nature* **201**, 185–187 (1964).
78. Szajewski, R. P. & Whitesides, G. M. Rate constants and equilibrium constants for thiol-disulfide interchange reactions involving oxidized glutathione. *J. Am. Chem. Soc.* **102**, 2011–2025 (1980).
79. Krause, G., Lundstrom, J., Barea, J. L., Pueyo de la Cuesta, C. & Holmgren, A. Mimicking the active site of protein disulfide-isomerase by substitution of proline 34 in *Escherichia coli* thioredoxin. *J. Biol. Chem.* **266**, 9494–9500 (1991).
80. Zapun, A., Missiakas, D., Raina, S. & Creighton, T. E. Structural and functional characterization of DsbC, a protein involved in disulfide bond formation in *Escherichia coli*. *Biochemistry* **34**, 5075–5089 (1995).
81. Wunderlich, M. & Glockshuber, R. Redox properties of protein disulfide isomerase (DsbA) from *Escherichia coli*. *Protein Sci.* **2**, 717–726 (1993).
82. Zapun, A., Bardwell, J. C. & Creighton, T. E. The reactive and destabilizing disulfide bond of DsbA, a protein required for protein disulfide bond formation *in vivo*. *Biochemistry* **32**, 5083–5092 (1993).
83. Segel, I. H. *Biochemical Calculations* 2nd edn 414–415 (Wiley, New York, 1976).
84. Inaba, K. & Ito, K. Paradoxical redox properties of DsbB and DsbA in the protein disulfide-introducing reaction cascade. *EMBO J.* **21**, 2646–2654 (2002).
85. Regeimbal, J. M. & Bardwell, J. C. DsbB catalyzes disulfide bond formation *de novo*. *J. Biol. Chem.* **277**, 32706–32713 (2002).
86. Rietsch, A., Belin, D., Martin, N. & Beckwith, J. An *in vivo* pathway for disulfide bond isomerization in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **93**, 13048–13053 (1996).
87. Stewart, E. J., Åslund, F. & Beckwith, J. Disulfide bond formation in the *Escherichia coli* cytoplasm: an *in vivo* role reversal for the thioredoxins. *EMBO J.* **17**, 5543–5550 (1998).
88. Coppock, D., Kopman, C., Gudas, J. & Cina-Poppe, D. A. Regulation of the quiescence-induced genes: quiescin Q6, decorin, and ribosomal protein S29. *Biochem. Biophys. Res. Commun.* **269**, 604–610 (2000).
89. Lange, H. *et al.* An essential function of the mitochondrial sulfhydryl oxidase Erv1p/ALR in the maturation of cytosolic Fe/S proteins. *EMBO Rep.* **2**, 715–720 (2001).
90. Günther, R. *et al.* Functional replacement of the *Saccharomyces cerevisiae* Trg1/Pdi1 protein by members of the mammalian protein disulfide isomerase family. *J. Biol. Chem.* **268**, 7728–7732 (1993).
91. Koivunen, P. *et al.* ERp60 does not substitute for protein disulphide isomerase as the β-subunit of prolyl 4-hydroxylase. *Biochem. J.* **316**, 599–605 (1996).
92. Oliver, J. D., van der Wal, F. J., Bulleid, N. J. & High, S. Interaction of the thiol-dependent reductase ERp57 with nascent glycoproteins. *Science* **275**, 86–88 (1997).
93. Molinari, M. & Helenius, A. Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells. *Nature* **402**, 90–93 (1999).
94. Desilva, M. G., Notkins, A. L. & Lan, M. S. Molecular characterization of a pancreas-specific protein disulfide isomerase, PDIp. *DNA Cell Biol.* **16**, 269–274 (1997).
95. Darby, N. J. & Creighton, T. E. Characterization of the active site cysteine residues of the thioredoxin-like domains of protein disulfide isomerase. *Biochemistry* **34**, 16770–16780 (1995).
96. Darby, N. J., Penka, E. & Vincentelli, R. The multi-domain structure of protein disulfide isomerase is essential for high catalytic efficiency. *J. Mol. Biol.* **276**, 239–247 (1998).
97. Laboissiere, M. C., Sturley, S. L. & Raines, R. T. The essential function of protein-disulfide isomerase is to unscramble non-native disulfide bonds. *J. Biol. Chem.* **270**, 28006–28009 (1995).
98. Walker, K. W., Lyles, M. M. & Gilbert, H. F. Catalysis of oxidative protein folding by mutants of protein disulfide isomerase with a single active-site cysteine. *Biochemistry* **35**, 1972–1980 (1996).
99. Nørgaard, P. & Winther, J. R. Mutation of yeast Eug1p CXXS active sites to CXXC results in a dramatic increase in protein disulphide isomerase activity. *Biochem. J.* **358**, 269–274 (2001).
100. Coppock, D. L., Cina-Poppe, D. & Gilleran, S. The quiescin Q6 gene (*QSCN6*) is a fusion of two ancient gene families: thioredoxin and ERV1. *Genomics* **54**, 460–468 (1998).
101. Coppock, D. L., Kopman, C., Scandalis, S. & Gilleran, S. Preferential gene expression in quiescent human lung fibroblasts. *Cell Growth Differ.* **4**, 483–493 (1993).
102. Francavilla, A. *et al.* Augmenter of liver regeneration: its place in the universe of hepatic growth factors. *Hepatology* **20**, 747–757 (1994).

103. Hagiya, M. *et al.* Cloning and sequence analysis of the rat augmenter of liver regeneration (*ALR*) gene: expression of biologically active recombinant ALR and demonstration of tissue distribution. *Proc. Natl Acad. Sci. USA* **91**, 8142–8146 (1994).
104. Lisowsky, T., Lee, J. E., Polimeno, L., Francavilla, A. & Hofhaus, G. Mammalian augmenter of liver regeneration protein is a sulfhydryl oxidase. *Dig. Liver Dis.* **33**, 173–180 (2001).
105. Benayoun, B., Esnard-Feve, A., Castella, S., Courty, Y. & Esnard, F. Rat seminal vesicle FAD-dependent sulfhydryl oxidase. Biochemical characterization and molecular cloning of a member of the new sulfhydryl oxidase/quiescin Q6 gene family. *J. Biol. Chem.* **276**, 13830–13837 (2001).
106. Musard, J. F. *et al.* Identification and expression of a new sulfhydryl oxidase SOx-3 during the cell cycle and the estrus cycle in uterine cells. *Biochem. Biophys. Res. Commun.* **287**, 83–91 (2001).
107. Lee, J., Hofhaus, G. & Lisowsky, T. Erv1p from *Saccharomyces cerevisiae* is a FAD-linked sulfhydryl oxidase. *FEBS Lett.* **477**, 62–66 (2000).
108. Lisowsky, T. Dual function of a new nuclear gene for oxidative phosphorylation and vegetative growth in yeast. *Mol. Gen. Genet.* **232**, 58–64 (1992).
109. Hofhaus, G., Stein, G., Polimeno, L., Francavilla, A. & Lisowsky, T. Highly divergent amino termini of the homologous human ALR and yeast scERV1 gene products define species specific differences in cellular localization. *Eur. J. Cell Biol.* **78**, 349–356 (1999).
110. Senkevich, T. G., Weisberg, A. S. & Moss, B. Vaccinia virus E10R protein is associated with the membranes of intracellular mature virions and has a role in morphogenesis. *Virology* **278**, 244–252 (2000).
111. Edman, J. C., Ellis, L., Blacher, R. W., Roth, R. A. & Rutter, W. J. Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin. *Nature* **317**, 267–270 (1985).
112. Klappa, P., Ruddock, L. W., Darby, N. J. & Freedman, R. B. The b′ domain provides the principal peptide-binding site of protein disulfide isomerase but all domains contribute to binding of misfolded proteins. *EMBO J.* **17**, 927–935 (1998).
113. Hirano, N. *et al.* Molecular cloning of the human glucose-regulated protein ERp57/GRP58, a thiol-dependent reductase. Identification of its secretory form and inducible expression by the oncogenic transformation. *Eur. J. Biochem.* **234**, 336–342 (1995).
114. Frickel, E. M. *et al.* TROSY-NMR reveals interaction between ERp57 and the tip of the calreticulin P-domain. *Proc. Natl Acad. Sci. USA* **99**, 1954–1959 (2002).
115. Elliott, J. G., Oliver, J. D. & High, S. The thiol-dependent reductase ERp57 interacts specifically with N-glycosylated integral membrane proteins. *J. Biol. Chem.* **272**, 13849–13855 (1997).
116. Mazzarella, R. A., Srinivasan, M., Haugejorden, S. M. & Green, M. ERp72, an abundant luminal endoplasmic reticulum protein, contains three copies of the active site sequences of protein disulfide isomerase. *J. Biol. Chem.* **265**, 1094–1101 (1990).
117. Nigam, S. K. *et al.* A set of endoplasmic reticulum proteins possessing properties of molecular chaperones includes Ca^{2+}-binding proteins and members of the thioredoxin superfamily. *J. Biol. Chem.* **269**, 1744–1749 (1994).
118. Lundstrom-Ljung, J., Birnbach, U., Rupp, K., Soling, H. D. & Holmgren, A. Two resident ER-proteins, CaBP1 and CaBP2, with thioredoxin domains, are substrates for thioredoxin reductase: comparison with protein disulfide isomerase. *FEBS Lett.* **357**, 305–308 (1995).
119. Fullekrug, J. *et al.* CaBP1, a calcium binding protein of the thioredoxin family, is a resident KDEL protein of the ER and not of the intermediate compartment. *J. Cell Sci.* **107**, 2719–2727 (1994).
120. Desilva, M. G. *et al.* Characterization and chromosomal localization of a new protein disulfide isomerase, PDIp, highly expressed in human pancreas. *DNA Cell Biol.* **15**, 9–16 (1996).
121. Hayano, T. & Kikuchi, M. Molecular cloning of the cDNA encoding a novel protein disulfide isomerase-related protein (PDIR). *FEBS Lett.* **372**, 210–214 (1995).
122. Scherens, B., Dubois, E. & Messenguy, F. Determination of the sequence of the yeast *YCL313* gene localized on chromosome III. Homology with the protein disulfide isomerase (PDI gene product) of other organisms. *Yeast* **7**, 185–193 (1991).
123. Gunther, R. *et al.* The *Saccharomyces cerevisiae TRG1* gene is essential for growth and encodes a lumenal endoplasmic reticulum glycoprotein involved in the maturation of vacuolar carboxypeptidase. *J. Biol. Chem.* **266**, 24557–24563 (1991).
124. LaMantia, M. L. & Lennarz, W. J. The essential function of yeast protein disulfide isomerase does not reside in its isomerase activity. *Cell* **74**, 899–908 (1993).
125. Wang, Q. & Chang, A. Eps1, a novel PDI-related protein involved in ER quality control in yeast. *EMBO J.* **18**, 5972–5982 (1999).
126. Tachibana, C. & Stevens, T. H. The yeast *EUG1* gene encodes an endoplasmic reticulum protein that is functionally related to protein disulfide isomerase. *Mol. Cell. Biol.* **12**, 4601–4611 (1992).
127. Tachikawa, H. *et al.* Isolation and characterization of a yeast gene, *MPD1*, the overexpression of which suppresses inviability caused by protein disulfide isomerase depletion. *FEBS Lett.* **369**, 212–216 (1995).
128. Tachikawa, H. *et al.* Overproduction of Mpd2p suppresses the lethality of protein disulfide isomerase depletion in a CXXC sequence dependent manner. *Biochem. Biophys. Res. Commun.* **239**, 710–714 (1997).

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