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Catalysis of disulfide bond formation and isomerization in Escherichia coli

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I. INTRODUCTION

The folding of proteins into their three-dimensional structure is essential for their biological function. For proteins that contain disulfide bonds, formation of these bonds is often an important step in the folding reaction. The presence of one or more disulfide bonds is crucial to the maintenance of the folded state of many secretory proteins. In contrast, cytosolic proteins form disulfide bonds only as part of their catalytic cycle, and are not stabilized by these bonds.

The classic experiments conducted by Anfinsen and co-workers proved that all the information for the three-dimensional structure of a protein is encoded by its amino acid sequence (Anfinsen, 1973) Bovine pancreatic RNase A, a 124-residue protein that contains four disulfide bonds in its native state, was used as a model protein (Sela et al., 1959; White, 1961). RNase A denatures readily and its disulfide bonds are reduced by incubation in urea and 2-mercaptoethanol. After removal of urea and 2-mercaptoethanol by dialysis, a very slow but nearly complete recovery of catalytic activity is observed. Thus, Anfinsen concluded that it is possible to refold denatured proteins into their active state in the test tube. Based on this observation, he further noted that “the information for the assumption of the native secondary and tertiary structure is contained in the amino acid sequence itself” (Anfinsen et al., 1961).
Native RNase A contains four disulfide bonds and the correct formation of these bonds is a key step during refolding of the protein (Anfinsen et al., 1961). Accordingly, if fully denatured and reduced RNase A is allowed to form disulfide bonds under denaturing conditions, e.g., in the presence of 8 M urea, a mixture of randomly oxidized RNase A molecules, called scrambled RNase, is obtained. Further, on removal of the denaturant, scrambled RNase is basically inactive, suggesting that incorrect disulfide bonds had "locked" the enzyme in numerous misfolded conformations. However, if scrambled RNase A is dialyzed against a buffer that contains a small amount of the reductant 2-mercaptoethanol, enzymatic activity is restored. This provided the first evidence that a low-molecular-weight reductant could reduce nonnative disulfide bonds and allow reformation of native disulfide bonds. Nevertheless, the ten hours needed for full restoration of RNase A activity under these conditions seemed much too long for efficient disulfide bond formation in vivo. It takes only two minutes for the cell to synthesize RNase A, and its folding is complete within minutes, not hours. This discrepancy led Anfinsen and co-workers to postulate and later to identify an enzymatic activity that greatly accelerates reactivation of RNase A in liver microsomes (Goldberger et al., 1963). The protein associated with this activity, protein disulfide isomerase (PDI), was the first protein folding catalyst found.

Recently, significant advances have been made in understanding how proteins fold in a cellular environment, and a large number of other proteins have been identified which assist the proper folding of proteins inside the cell. These proteins fall into two classes. First, there are true catalysts, such as PDI, which accelerate rate-limiting steps during protein folding. Such rate-limiting steps include the correct formation of disulfide bonds and the isomerization of proline residues in proteins. Molecular chaperones comprise a second class of proteins that assist the folding process. Chaperones prevent nonproductive reactions such as aggregation or premature folding of proteins, they also promote the folding and unfolding of proteins. The mechanisms of chaperone action will not be further discussed here, but excellent reviews are available in the literature (Beissinger and Buchner, 1998; Ellis and Hartl, 1999). This chapter deals with folding catalysts, in particular with catalysts that are essential for disulfide bond formation in Escherichia coli. Nevertheless, some of these catalysts contain chaperone activity, demonstrating that these two activities are sometimes found within the same protein molecule.

II. De Novo Formation of Disulfide Bonds in E. coli: The Discovery of DsbA

In 1991, Bardwell and co-workers reported the identification of DsbA, which they found to be involved in the formation of disulfide bonds in vivo (Bardwell et al., 1991). Mutants in the oxidative folding of several E. coli proteins showed DsbA was widely believed to be involved in the formation of a catalyst. Using DsbA as a model, concerning disulfide bond formation, it was expected that the oxidation of the multifunctional, multidomain 57-kDa protein has been made in analyzing its catalytic activity.

DsbA was identified by the use of MalF-β-galactosidase (Bardwell et al., 1991) β-galactosidase activity when present in that is competent in forming disulfide ligation by selecting for a Lac phenotype for the fusion protein but lacked the vation of β-galactosidase activity in the to reduction of cysteine residues of β a cytosolic protein that does not nor was suggested that on fusion with the portion of β-galactosidase is exported the enzyme then becomes sensitive to inactivation of β-galactosidase in a way ever, in a strain such as a dsbA null β-galactosidase remains reduced and a Loss of DsbA causes a severe defect in many secretory proteins including β-lactamase (Bardwell et al., 1991). Si a general lack of disulfide bonds included that DsbA is the major catalyst the periplasm. This key role of DsbA tants exhibit pleiotropic phenotypes. of dsbA null mutants are loss of motility benzylpenicillin, and metal ions such as 1993; Missiakas et al., 1993; Rensing). These phenotypes are due to the loss which results in a failure to form disto proteins. For instance, the loss of m disulfide bond in the P ring protein (1998). Another phenotype of dsbA n ria is attenuated virulence. Because s
disulfide bonds and the correct forming refolding of the protein (Anfinsen, 1973) denatured and reduced RNase A is available under denaturing conditions, e.g., in mixture of randomly oxidized RNase A ase, is obtained. Further, on removal of e is basically inactive, suggesting that in red” the enzyme in numerous misfolded ambled RNase A is dialyzed against a bit of the reductant 2-mercaptoethanol, is provided the first evidence that a lowerrd nonnative disulfide bonds and llide bonds. Nevertheless, the ten hours 2Nase A activity under these conditions lnt disulfide bond formation in vivo. It ll to synthesize RNase A, and its folding is ours. This discrepancy led Anfinsen and err to identify an enzymatic activity that of RNase A in liver microsomes (Goldin in association with this activity, protein he first protein folding catalyst found. have been made in understanding how rooment, and a large number of other rich assist the proper folding of proteins all into two classes. First, there are true cerate rate-limiting steps during prosteps include the correct formation of ization of proline residues in proteins. e a second class of proteins that assist s prevent nonproductive reactions such hilding of proteins, they also promote th. The mechanisms of chaperone action re, but excellent reviews are available inchner, 1998; Ellis and Hartl, 1999). This stts, in particular with catalysts that are ssion in Escherichia coli. Nevertheless, some none activity, demonstrating that these nd within the same protein molecule.

N OF DISULFIDE BONDS IN E. COLI:abby OF DSbA

in vivo (Bardwell et al., 1991). Mutants in dsbA exhibit a severe defect in the oxidative folding of several E. coli proteins. Prior to the discovery of DsbA, it was widely believed that the formation of disulfide bonds occurred spontaneously. However, the isolation of DsbA showed that disulfide bond formation in the cell depends on the presence of a catalyst. Using DsbA as a model catalyst, many crucial questions concerning disulfide bond formation were answered. Some advantages that make DsbA a good model protein are its small size (21 kDa) and its single function—the oxidation of disulfide bonds—in contrast to the multifunctional, multidomain 57-kDa PDI. For these reasons, rapid progress has been made in analyzing how oxidative folding of proteins is catalyzed within the cell.

DsbA was identified by the use of a disulfide indicator protein, MalF-β-galactosidase (Bardwell et al., 1991). This fusion protein lacks β-galactosidase activity when present in a wild-type E. coli background that is competent in forming disulfides. The dsbA null mutant was isolated by selecting for a Lac phenotype in a cell that expressed the gene for the fusion protein but lacked the wild-type lacZ gene. The restoration of β-galactosidase activity in the dsbA null mutant is most likely due to reduction of cysteine residues of β-galactosidase. β-Galactosidase is a cytosolic protein that does not normally contain disulfide bonds. It was suggested that on fusion with the inner membrane protein MalF, a portion of β-galactosidase is exported into the periplasm. This part of the enzyme then becomes sensitive to thiol oxidation, which leads to inactivation of β-galactosidase in a wild-type strain background. However, in a strain such as a dsbA null mutant where disulfides fail to form, β-galactosidase remains reduced and active.

Loss of DsbA causes a severe defect in the formation of disulfide bonds in many secretory proteins including OmpA, alkaline phosphatase, and β-lactamase (Bardwell et al., 1991). Since the absence of DsbA causes a general lack of disulfide bonds in periplasmic proteins, it was concluded that DsbA is the major catalyst of disulfide bond formation in the periplasm. This key role of DsbA also explains why dsbA null mutants exhibit pleiotropic phenotypes. Commonly observed phenotypes of dsbA null mutants are loss of motility and increased sensitivity to DTT, benzylpenicillin, and metal ions such as Hg²⁺ and Cd²⁺ (Dailey and Berg, 1993; Missiakas et al., 1993; Rensing et al., 1997; Stafford et al., 1999). These phenotypes are due to the loss of the oxidase activity of DsbA, which results in a failure to form disulfide bonds in many periplasmic proteins. For instance, the loss of motility is due to the absence of a disulfide bond in the P ring protein (Prg) of flagella (Dailey and Berg, 1993). Another phenotype of dsbA null mutants in pathogenic bacteria is attenuated virulence. Because so many virulence factors contain
disulfide bonds, DsbA is important for the disease-causing properties of enteropathogenic and uropathogenic *E. coli*, *Vibrio cholera*, and *Shigella flexneri* (Donnenberg *et al.*, 1997; Jacob-Dubuisson *et al.*, 1994; Peek and Taylor, 1992; Watarai *et al.*, 1995; Yu, 1998).

III. **DsbA Is the Most Oxidizing Disulfide Catalyst**

The 2.0-Å crystal structure revealed that DsbA contains a thioredoxin-like fold (Martin *et al.*, 1993). The thioredoxin fold includes a central β-sheet formed by four antiparallel β-strands. The central β-sheet is flanked by a perpendicular helix and two helices on the opposite side (Martin, 1995). Compared to thioredoxin, DsbA contains an additional β-strand in the central β-sheet and the insertion of a 65-residue helical domain (Fig. 1). Such insertions are commonly observed within the thioredoxin family (Martin, 1995; McCarthy *et al.*, 2000). Most members of the thioredoxin superfamily are involved in disulfide exchange reactions, and contain a redox-active CXXC motif in their active site. The CXXC motif participates in disulfide exchange reactions by going through reversible cycles of oxidation and reduction. In this motif, the

\[
K_{\text{ox}} = \frac{[\text{GSH}]^2[I]}{[\text{GSSG}][I]}
\]

From the equilibrium constant with glutathione, the potential of the GSSG/GSH pair, the calculated. The redox potential of DsbA oxidizing disulfide bond known. For of thioredoxin is −270 mV, and therefore the small equilibrium constant of indicates that the disulfide bond formed is highly stable of a particular disulfide bond core a protein is stabilized by this bond. In disulfide bond, the more stable the protein of DsbA, its unstable disulfide bond is protein conformation. This is indeed DsbA’s disulfide bond leads to stabilization by 4.5 kcal/mol (Zapun *et al.*, 1993). *I* bonds normally stabilize proteins. Yet, it function of DsbA as a donor of disulfide form of DsbA to be more stable than the oxidized DsbA, the N-terminal cysteine is very low \( K_s \) of ~3.5, in contrast to the monomer found in proteins (Nelson and Castricum, 1974). pH at which the group is half ionized. Ca
ant for the disease-causing properties of
ogenic E. coli, Vibrio cholera, and Shigella
7; Jacob-Dubuisson et al., 1994; Peek and
3; Yu, 1998).

**Oxidizing Disulfide Catalyst**

It is revealed that DsbA contains a thioredoxin-
type fold includes a central parallel β-sheet and
6 disulfide bonds. The central β-sheets are six and two helices on the opposite side
A CXXC motif in their active sites. As disulfide exchange reactions by going

N-terminal cysteine is solvent-exposed, making it the reactive species in
disulfide exchange reactions.

Despite their common structures, thioredoxin and DsbA fulfill different functions and exist in different cellular compartments. While thioredoxin acts as a reductant of disulfide bonds in the cytosol (for review see Rietsch and BeckWITH, 1998), DsbA introduces disulfide bonds into newly synthesized proteins during their translocation to the periplasm. Why does DsbA act as a donor of disulfide bonds? The disulfide bond formed by the CXXC motif of DsbA is highly reactive. Thus, oxidized DsbA will react rapidly with thiols, resulting in their oxidation. For example, DsbA reacts about a 1000-fold faster with reduced glutathione (GSH) than does a normal protein disulfide (ZAPUN et al., 1993). The extremely oxidizing nature of DsbA becomes evident from its equilibrium constant with glutathione ($\text{K}_{\text{ox}}$), which is very small, 0.1 mM, indicating that it will strongly tend to oxidize thiols. $\text{K}_{\text{ox}}$ is given by the following equation (WUNDERLICH and GLOCKSHUBER, 1993; ZAPUN et al., 1993):

$$
\text{K}_{\text{ox}} = \frac{[\text{GSH}]^2[\text{DsbA}^{\text{ox}}]}{[\text{GSSG}][\text{DsbA}^{\text{red}}]}
$$

From the equilibrium constant with glutathione and the standard redox potential of the GSSG/GSH pair, the redox potential of DsbA can be calculated. The redox potential of DsbA is -120 mV, making it the most oxidizing disulfide bond known. For comparison, the redox potential of thioredoxin is -270 mV, and therefore much more reducing.

The small equilibrium constant of DsbA with glutathione demonstrates that the disulfide bond formed by DsbA is highly unstable. The stability of a particular disulfide bond corresponds to the extent to which a protein is stabilized by this bond. In other words, the more stable the disulfide bond, the more stable the protein conformation. In the case of DsbA, its unstable disulfide bond should therefore destabilize the protein conformation. This is indeed observed, since the reduction of DsbA's disulfide bond leads to stabilization of its folded conformation by 4.5 kcal/mol (ZAPUN et al., 1993). This is unusual, since disulfide bonds normally stabilize proteins. Yet, it is in agreement with the in vivo function of DsbA as a donor of disulfide bonds. What causes the reduced form of DsbA to be more stable than its oxidized form? In the CXXC motif of DsbA, the N-terminal cysteine 30 is solvent-exposed and has a very low $pK_a$ of ~3.5, in contrast to the $pK_a$ of ~9.0 for cysteines commonly found in proteins (NELSON and CREIGHTON, 1994). The $pK_a$ is the pH at which the group is half ionized. Consequently, at physiologic pH,
cysteine 30 of DsbA is fully deprotonated and found as a thiolate anion carrying a negative charge. It is the stabilization of this negative charge that accounts for the difference in stability between the reduced and the oxidized form of DsbA (Grausakopf et al., 1995; Nelson and Creighton, 1994). This stabilization effect makes the reduction of DsbA very favorable and drives the reaction between oxidized DsbA and reduced substrate proteins.

The finding that cysteine 30 of DsbA has such a low pK<sub>a</sub> requires that its deprotonated form be stabilized by residues in the vicinity. For instance, histidine 32, which lies within the CXXC motif, plays an important role in determining the redox properties of DsbA (Grausakopf et al., 1995). Mutation of histidine 32 leads to a dramatic decrease of redox potential, making DsbA a less potent donor of disulfide bonds. Three crystal structures of DsbA histidine 32 mutants have been solved (Guddat et al., 1997a). The structures of these mutant proteins do not show any significant change in the overall fold, although their equilibrium constants with glutathione (K<sub>eq</sub>) are up to 1000-fold less oxidizing than wild-type DsbA. Apparently, these large differences in K<sub>eq</sub> cannot be explained by significant structural changes between the individual proteins. It was therefore suggested that electrostatic interactions, which are absent in the mutant proteins, stabilize the thiolate anion of cysteine 30, thus causing its extremely low pK<sub>a</sub>. Support for this model comes from comparison of the crystal structures of reduced and oxidized DsbA (Guddat et al., 1998). According to this study, the structure of reduced DsbA reveals potential hydrogen bonds between residues around cysteine 30, which are absent in the oxidized structure. This hydrogen bonding network includes the backbone amide of histidine 32, cysteine 33, and the side chains of cysteine 33. Further, histidine 32 moves toward cysteine 30 on the reduction of DsbA, bringing the residue within hydrogen bond distance. Since cysteine 30 is located at the N terminus of an α-helix, the thiolate of cysteine 30 is also stabilized by favorable interactions with the partial positive charge of the helix dipole. These data, taken together, suggest that DsbA’s highly oxidizing nature arises from a few electrostatic interactions that favor the very low pK<sub>a</sub> of cysteine 30.

Further insights into how DsbA’s redox properties are determined come from comparison between DsbA and thioredoxin. The redox potential of thioredoxin is approximately −270 mV as compared to −120 mV for DsbA. This makes thioredoxin a much more reducing catalyst than DsbA, and appears to suit the in vivo function of thioredoxin as a reductant of disulfide bonds in the cytosol. As in DsbA, the residues that lie within the CXXC motif of thioredoxin strongly influence the redox potential of the catalyst. For instance, if the active site of DsbA (CYPH tandem thioredoxin increases to −200 mV, et al., 1999). More support for the im CXXC motifs in determining the redox from in vivo studies with thioredoxin, as an export signal to the N terminus of the periplasm (Debarbieux and Beckwith, 2000). Thus, the DsbA-like catalyst by simply exchanging motif.

As the major oxidant in the periplasm into newly translocated protein competes with DsbA-mediated 10-25 times faster, thioredoxin could be involved in forming disulfide bonds, the by DsbA. Thus, DsbA must interact rapidly unfolded or only partially folded to residues before they get buried upon folding to propose that DsbA specifically interacts with substrate proteins. Indeed, there is good evidence (1) DsbA reacts about 10-25 times faster than it does with DTT (Wunderlich et al., 1995). (2) DsbA and a peptide derived from a DsbA (Frehl et al., 1996). These authors have shown that a DsbA variant lacking the second cysteine (Frehl et al., 1996). This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin. (3) Further, disulfic bond and DsbA occurs via a mixed d DsbA. This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin. (4) Colleagues reported the isolation of a DsbA variant lacking the second cysteine (Frehl et al., 1996). These authors have shown that a DsbA variant lacking the second cysteine (Frehl et al., 1996). This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin. (5) Further, disulfic bond and DsbA occurs via a mixed d DsbA. This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin. (6) Colleagues reported the isolation of a DsbA variant lacking the second cysteine (Frehl et al., 1996). These authors have shown that a DsbA variant lacking the second cysteine (Frehl et al., 1996). This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin. (7) Further, disulfic bond and DsbA occurs via a mixed d DsbA. This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin. (8) Colleagues reported the isolation of a DsbA variant lacking the second cysteine (Frehl et al., 1996). These authors have shown that a DsbA variant lacking the second cysteine (Frehl et al., 1996). This mixed disulfide bond is mobile between DsbA and glutathione, indicating the ability to interact with thioredoxin.
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αA's redox properties are determined n DsbA and thioredoxin. The redox mV as compared to -120 mV for DsbA. more reducing catalyst than DsbA, and tion of thioredoxin as a reductant of in DsbA, the residues that lie within the gly influence the redox potential of the catalyst. For instance, if the active site of thioredoxin (CGPC) is changed to match the sequence of DsbA (CPHC), the redox potential of the mutant thioredoxin increases to -200 mV, making it more oxidizing (Jonda et al., 1999). More support for the importance of central residues of CXXC motifs in determining the redox properties of the catalyst comes from in vivo studies with thioredoxin. Debarbieux and Beckwith fused an export signal to the N terminus of thioredoxin, causing its export to the periplasm (Debarbieux and Beckwith, 1998). Such a construct only partially promotes the formation of disulfide bonds of OmpA and alkaline phosphatase in a dshA null background. However, if the active site of this thioredoxin construct is mutated to resemble the active site of DsbA, thus making it more oxidizing, the folding yields of OmpA and alkaline phosphatase are nearly indistinguishable from a DshA⁺ strain (Debarbieux and Beckwith, 2000). Thus, thioredoxin can be turned into a DsbA-like catalyst by simply exchanging the two residues within its CXXC motif.

As the major oxidant in the periplasm, DsbA introduces disulfide bonds into newly translocated proteins. The folding of these proteins competes with DsbA-mediated formation of their disulfide bonds. Consequently, premature folding would mask cysteine residues normally involved in forming disulfide bonds, thereby preventing their oxidation by DsbA. Thus, DsbA must interact rapidly with proteins while they are still unfolded or only partially folded in order to gain access to cysteine residues before they get buried upon folding. It is therefore reasonable to propose that DsbA specifically interacts noncovalently with unfolded substrate proteins. Indeed, there is good experimental evidence that it does so. (1) DsbA reacts about 10–25 times faster with unfolded hirudin than it does with DTT (Wunderlich et al., 1993). (2) Disulfide exchange between DsbA and a peptide derived from residues 4–31 of BPTI occurs up to 1000-fold faster than between glutathione and DsbA (Darby and Creighton, 1995). (3) Further, disulfide exchange between the model peptide and DsbA occurs via a mixed disulfide between the peptide and DsbA. This mixed disulfide bond is more stable than the disulfide bond between DsbA and glutathione, indicating the presence of additional stabilizing interactions with the peptide. (4) Moreover, a study by Frech and colleagues reported the isolation of a mixed disulfide complex between a DsbA variant lacking the second cysteine and a ribonuclease T1 variant (Frech et al., 1996). These authors showed that the conformation of DsbA is stabilized by 4.7 kJ/mol in the mixed disulfide complex. This strongly suggests that DsbA interacts noncovalently with substrate proteins.

A potential substrate binding site of DsbA has been deduced from its crystal structure (Guddat et al., 1997b); however, no structure of a
complex between DsbA and a peptide has been solved to date. DsbA exhibits a potential hydrophobic peptide binding groove below the active site disulfide, which was modeled according to the NMR structure of a mixed disulfide complex of thioredoxin and a target peptide (Qin et al., 1995). According to this model, conserved uncharged residues around the active site of DsbA are involved in peptide binding. Another very recent approach to study the interaction of DsbA with peptides was carried out with a model peptide containing a bromine-substituted alanine (Couprie et al., 2000). DsbA was specifically crosslinked to this peptide via its reactive cysteine. Preliminary calorimetric and NMR analysis revealed stabilization of DsbA due to peptide binding, further supporting the importance of noncovalent interactions between DsbA and substrate proteins.

IV. DsbB Provides the Periplasm with Oxidizing Power

Following the transfer of disulfide bonds from DsbA to substrate proteins, the active site of DsbA must be reoxidized in order to go through another catalytic cycle. This is accomplished by the inner membrane protein DsbB, which is responsible for keeping the disulfide bond of DsbA in an oxidized state. The dsbB gene was originally identified by using the same selection that was used to isolate dsbA (Bardwell et al., 1993). Like dsbA mutants, dsbB mutants show a strong defect in the formation of disulfide bonds in periplasmic proteins such as OmpA, β-lactamase, and alkaline phosphatase. In an independent approach, the dsbB gene was isolated by a genetic screen for multicopy suppressors of DTT sensitivity (Masiakas et al., 1993). The rationale behind this latter screen is that a protein participating in an oxidative pathway should confer resistance to the reductant DTT. Such screens have been used by the same authors to isolate more genes belonging to the Dsb family (see below).

Early genetic evidence suggested that DsbA and DsbB participate in the same pathway (Bardwell et al., 1993), and that DsbB is responsible for the reoxidation of DsbA’s active site disulfide bond. For instance, dsbB null mutants accumulate DsbA in a reduced state in the periplasm, while DsbA is found in a mostly oxidized form in a wild-type background. This finding implies an important function for DsbB in reoxidizing DsbA. Evidence that DsbA and DsbB may interact directly comes from the isolation of a dsbA mutant that lacks cysteine 33 and therefore contains only the reactive cysteine 30 of its CXXC motif (Guilhot et al., 1995; Kishigami et al., 1995). This mutant dsbA C33Y, was identified by screening a library of randomly mutagenized DsbA-expression plasmids for a disulfide-negative phenotype in a DsbA+ expressed, dsbA C33Y displays a domino a severe defect in the formation of disul
date phenotype is suppressed when DsbA is expressed, dsbA C33Y displays a severe defect in the formation of disulfate-negative phenotype. This was
tion of a complex between DsbA C33Y
tive cysteine 30 of DsbA crosslinks to DsbA of a mixed disulfide complex between cannot resolve due to the lack of cyste
equence, DsbA C33Y inhibits DsbB.

Based on its sequence, DsbB was prec
protein, which was confirmed using the poroach (Jander et al., 1994). DsbB was s
brane domains, which are connected by
more, DsbB possesses four highly consen
for its activity in reoxidizing DsbA. On
CXXC motif in the first periplasmic loo
DsbB has no other similarity to thioredoxin
that DsbB belongs to the thioredoxin’s
rather than a CXXC motif.

The location of DsbB in the inner
ation that DsbB donates electrons gen
the respiratory chain (Bardwell
heme- or quinone-depleted cells accu
of the DsbB motif in a reduced e
Kobayashi et al., 1997). Nevertheless, t
power for oxidative protein folding re
anism whereby DsbB drives disulfide
has it been shown how DsbB interacts
DsbB oxidase, each of which acts as a
Ubiquinone was identified as an inter
chrome oxidases. Thus, DsbB cata
DsbA to ubiquinone, whence electron
zymes. The f. d. is that DsbB dire
explains why disulfide bond form
ence of molecular oxygen. Under th
replaced by menaquinones, which st
peptide has been solved to date. DsbA
sic peptide binding groove below the
odeled according to the NMR structure
thioredoxin and a target \( \beta \)-peptide (Qin
model, conserved uncharged residues
olved in peptide binding. Another
the interaction of DsbA with peptides
site containing a bromine-substituted
DsbA was specifically crosslinked to this
e. Preliminary calorimetric and NMR
DsB was due to peptide binding, further
oncovalent interactions between DsbA

**PERIPLASM WITH OXIDIZING POWER**

disulfide bonds from DsbA to substrate pro-
be reoxidized in order to go through
accomplished by the inner membrane
ible for keeping the disulfide bond of
\( \text{dsbB} \) gene was originally identified by us-
used to isolate \( \text{dsbA} \) (Bardwell et al.,
mutants show a strong defect in the
1 periplasmic proteins such as OmpA,
hatase. In an independent approach,
cenic screen for multicopy suppressors
(1, 1993). The rationale behind this
icipating in an oxidative pathway should
TT. Such screens have been used
genes belonging to the Dsb family
ated that DsbA and DsbB participate in
, 1993), and that DsbB is responsible for
site disulfide bond. For instance, \( \text{dsbB} \)
a reduced state in the periplasm, while
ld in a wild-type background. This
ction for DsbB in reoxidizing DsbA.
ay interact directly comes from the iso-
sks cysteine 33 and therefore contains
its CXXC motif (Guilhot et al., 1995;
U, \( \text{dsbA}, \) C33Y, was identified by screen-
enized DsbA-expression plasmids for a

disulfide-negative phenotype in a \( \text{DsbA}^+ \)-strain background. When over-
expressed, \( \text{dsbA} \) C33Y displays a dominant negative phenotype, causing a
severe defect in the formation of disulfide bonds. The dominant negative
phenotype is suppressed when DsbB is co-overexpressed. This sug-
ests that DsbA C33Y titrates out all the cellular DsbB, thus causing the
disulfide-negative phenotype. This was further supported by the isolation
of a complex between DsbA C33Y and DsbB. Apparently, the reactive
cysteine 30 of DsbA crosslinks to DsbB, thus leading to the formation
of a mixed disulfide complex between the two proteins. The complex
cannot resolve due to the lack of cysteine 33 in DsbA C33Y, and, as a
consequence, DsbA C33Y inhibits DsbB activity.

Based on its sequence, DsbB was predicted to be an inner membrane
protein, which was confirmed using the alkaline phosphatase fusion ap-
proach (Jander et al., 1994). DsbB was shown to contain four transmem-
brane domains, which are connected by two periplasmic loops. Further-
more, DsbB possesses four highly conserved cysteines, which are essential
for its activity in reoxidizing DsbA. One pair of cysteines is found as a
CXXC motif in the first periplasmic loop of DsbB. Apart from this motif,
DsbB has no other similarity to thioredoxin, making it rather unlikely
that DsbB belongs to the thioredoxin superfamily. The most important
criterion for a protein to be a member of this family is the presence of
a common fold rather than a CXXC motif (Martin, 1995).

The location of DsbB in the inner membrane led to the speculation
that DsbB donates electrons generated by disulfide bond forma-
tion to the respiratory chain (Bardwell, 1994). Consistent with this,
HEME- or quinone-depleted cells accumulate DsbA and the CXXC mo-
tif of the DsbB motif in a reduced state (Kobayashi and Ito, 1999;
Kobayashi et al., 1997). Nevertheless, the ultimate source of oxidizing
power for oxidative protein folding remained unclear, as did the mech-
anism whereby DsbB drives disulfide bond formation. Only recently
has it been shown how DsbB interacts with the electron transport sys-
tem (Bader et al., 1998, 1999, 2000). In a highly purified in vitro sys-
tem, DsbB activity requires the presence of either cytochrome \( \text{b} \) or
\( \text{bd} \) oxidase, each of which acts as a terminal step in the respiratory
chain by transferring electrons from ubiquinone to molecular oxygen.
Ubiquinone was identified as an intermediate between DsbB and the two
cytochrome oxidases. Thus, DsbB catalyzes the flow of electrons from
DsbA to ubiquinone, whence electrons are passed on to cytochrome
oxidases. The finding that DsbB directly interacts with quinones also
helps explain why disulfide bond formation is not impaired in the
absence of molecular oxygen. Under these conditions, ubiquinones get
replaced by menaquinones, which serve as mobile electron carriers
Fig. 2. The DsbA–DsbB pathway. DsbA is the immediate donor of disulfide bonds in the periplasm and cycles between its oxidized and reduced state. DsbB is directly involved in the reoxidation of DsbB. Electrons flow from DsbB via DsbB to ubiquinone and further on to respiratory complexes, which catalyze electron transfer to oxygen. Under anaerobic conditions, menaquinone serves as an electron carrier between DsbB and terminal complexes.

between complexes of the anaerobic electron transport chain (Wallace and Young, 1977). Biochemical and genetic evidence suggests that, under anaerobic conditions, DsbB switches to utilizing menaquinone as its immediate electron acceptor (Bader et al., 1999). This is supported by the observation that a ubiA–menA double mutant lacking both ubiquinones and menaquinones accumulates DsbA in a reduced form in vivo, while DsbA is mostly oxidized in a ubiA single mutant (Kobayashi et al., 1997). Electrons therefore flow from DsbB to menaquinones and further on to terminal complexes such as fumarate reductase. Taken together, the ability of DsbB to interact with either ubiquinones or menaquinones ensures efficient disulfide bond formation under nearly all growth conditions (Fig. 2).

V. CORRECTING WRONG DISULFIDE BONDS IN THE PERIPLASM: DISULFIDE BOND ISOMERIZATION BY DsbC

By screening for E. coli mutants that display a DTT-hypersensitive phenotype, Missiakas and co-workers identified three additional Dsb proteins: DsbC, DsbD, and DsbG (Missiakas et al., 1994, 1995). DsbC was also isolated from a multicopy plasmid library by its ability to confer resistance to high levels of DTT. Unlike dsbA and dsbB mutants, the effect of a dsbC null mutant on the growth of E. coli is not very strong (Rietsch et al., 1996). There is, however, good evidence that DsbC acts to isomerize incorrectly formed disulfid when eukaryotic proteins containing m geted to the E. coli periplasm (Rietsch yield of native urokinase, a enzyme holds, is undetectable in a dsbC null no folding yield of the E. coli alkaline phosphate disulfide bonds, is lowered by a mere 1. Most periplasmic E. coli proteins contain perhaps explaining why disulfide isomer for prokaryotes than it is in eukaryotes.

The crystal structure of DsbC, which of 1.9 Å (McCarthy et al., 2000), shows ben of two separate domains, an N-term a C-terminal thioredoxin domain (Fig. from each N-terminal domain interacts of the opposite molecule to form an o two C-terminal thioredoxin-like domain the V and include two redox active C² the V. As in DsbA, the N-terminal cyste is solvent-exposed, making it the reactive reactions. In addition, the disulfide bond equilibrium constant with glutathione is highly reactive and only slightly less oxi
sBA is the immediate donor of disulfide bonds its oxidized and reduced state. DsbB is directly electrons flow from DsbA via DsbB to ubiquinones, which catalyze electron transfer to oxygen. none serves as an electron carrier between DsbB and menaquinone (Wallace and genetic evidence suggests that, mutations to utilizing menaquinone as its quencher, 1999). This is supported by the double mutant lacking both ubiquinones s DsbA in a reduced form in vivo, while 4 single mutants (Kobayashi et al., 1997). sB to menaquinones and further on to urate reductase. Taken together, the ability of ubiquinones or menaquinones ensures ion under nearly all growth conditions robic electron transport chain (Wallace and genetic evidence suggests that, mutations to utilizing menaquinone as its quencher, 1999). This is supported by the double mutant lacking both ubiquinones s DsbA in a reduced form in vivo, while 4 single mutants (Kobayashi et al., 1997). sB to menaquinones and further on to urate reductase. Taken together, the ability of ubiquinones or menaquinones ensures ion under nearly all growth conditions.

**Disulfide Bonds in the Periplasm: Isomerization by DsbC**

ants that display a DTT-hypersensitive orkers identified three additional Dsb C (A. H. G. C. B. MII, 1994, 1995). DsbC copy plasmid library by its ability to contain DTT. Unlike dsbA and dsbB mutants, the growth of E. coli is not very strong however, good evidence that DsbC acts to isomerize incorrectly formed disulfide bonds. This becomes evident when eukaryotic proteins containing multiple disulfide bonds are targeted to the E. coli periplasm (Rietsch et al., 1996). For instance, the yield of native urokinase, a eukaryotic protein containing 12 disulfide bonds, is undetectable in a dsbC null mutant. On the other hand, the folding yield of the E. coli alkaline phosphatase, which contains only two disulfide bonds, is lowered by a mere 15% in a dsbC null background. Most periplasmic E. coli proteins contain only one or two disulfide bonds, perhaps explaining why disulfide isomerization appears less important for prokaryotes than it is in eukaryotes.

The crystal structure of DsbC, which has been solved to a resolution of 1.9 Å (McCarthy et al., 2000), shows DsbC to be a homodimer consisting of two separate domains, an N-terminal dimerization domain and a C-terminal thioredoxin domain (Fig. 3). In the dimer, two β-strands from each N-terminal domain interact with the corresponding strands of the opposite molecule to form an overall V-shaped structure. The two C-terminal thioredoxin-like domains form the bulk of the arms of the V and include two redox active CXXC motifs facing the inside of the V. As in DsbA, the N-terminal cysteine residue of the CXXC motif is solvent-exposed, making it the reactive species in disulfide exchange reactions. In addition, the disulfide bond formed by DsbC displays an equilibrium constant with glutathione (K_eq) of 0.12 mM, making DsbC highly reactive and only slightly less oxidizing than DsbA.

**FIG. 3.** The crystal structure of DsbC. DsbC forms a V-shaped homodimer. The monomer consists of a C-terminal thioredoxin domain and an N-terminal dimerization domain. The two domains are joined via a linker helix. The monomers interact via two consecutive β-strands, which form two extended β-sheets in the dimer.
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was identified by employing the same D
to identify DsbC (Andersen et al., 1997)
DsbG was identified by homology to D
DsbC, DsbG is a dimer and is kept in
an isomerase activity in vivo (Bessette et
In Figure 4, therefore, cysteine 101 might be important only for the resolution of kinetically trapped complexes between DsbC and target proteins. Indeed, such off-pathway intermediates seem to occur because mutations that alter cysteine 101 lead to a dramatic decrease in the folding yield of urokinase (Rietsch et al., 1996). The attack of cysteine 101 on such a trapped mixed disulfide leads to the oxidation of the CXXC motif of DsbC. In any case, only reduced DsbC is capable of attacking incorrect disulfide bonds. Therefore, there is a need to keep DsbC in a reduced state in vivo. This is accomplished by the inner membrane protein DsbD, which ensures a steady-state level of reduced DsbC in the cell (see below).

The mechanisms of DsbA and DsbC action are consistent with the observation that a mixed disulfide bond between DsbC and the model peptide BPTI 4-31 is 40- to 100-fold more stable than the corresponding complex between the model peptide and DsbA (Darby et al., 1998). The higher stability of DsbC in complex with peptides might explain why DsbC, but not DsbA, acts as an isomerase. As an isomerase, DsbC has to scan through many possible disulfide intermediates until a more native disulfide bond is formed in the substrate protein. It is therefore necessary that the mixed disulfide bond between DsbC and its substrate protein be more stable than the DsbA–protein complex. The latter has to be resolved rapidly to free DsbA for another cycle of disulfide bond formation after its oxidation by DsbB.

The higher stability of a mixed disulfide complex between peptide and DsbC is likely to result from enhanced peptide binding by DsbC. The inside of the V-like structure of DsbC is covered with uncharged and hydrophobic residues, forming a potential substrate binding surface (McCarthey et al., 2000). Further support for the hypothesis that the interior of DsbC is responsible for peptide binding by DsbC comes from the observation that the dimeric nature of DsbC is essential for its function as an isomerase in vitro (Sun and Wang, 2000). In contrast to wild-type DsbC, monomeric DsbC also lacks chaperone activity in vitro. Chaperones often interact nonspecifically with hydrophobic regions of a protein in order to prevent aggregation. Therefore, the loss of chaperone activity of the monomer is probably due to the destruction of the extended uncharged surface are of DsbC on monomerization.

A second disulfide isomerase called DsbG exists in the periplasm. DsbG was identified by employing the same DTT-hypersensitivity screen used to identify DsbC (Andersen et al., 1997). In an independent approach, DsbG was identified by homology to DsbC (Bessette et al., 1999). Like DsbC, DsbG is a dimer and is kept in a reduced state in vivo. DsbG has isomerase activity in vivo (Bessette et al., 1999). It is not clear what the
substrate specificities for DsbC and DsbG are. Do the two isomerases act on the same set of misoxidized protein substrates, or do they act on different, nonoverlapping sets of substrates? The redundancy of two isomerases in *E. coli* is surprising, since most secreted *E. coli* proteins possess just one or two disulfide bonds. On the other hand, the two proteins also act as chaperones *in vitro* and this activity might be an important part of their *in vivo* roles (Chen et al., 1999; Shao et al., 2000).

VI. DsbD Provides Reducing Equivalents in a Highly Oxidizing Environment

It is important for the cell to keep the two isomerases DsbC and DsbG in a reduced state since only their reduced forms are able to attack incorrect disulfide bonds. Reduction of DsbC and DsbG is carried out by an inner membrane protein called DsbD (Fig. 5) (Mistakas et al., 1995; Rietsch et al., 1996). Accordingly, a *dsbD* null mutant accumulates DsbC and DsbG in their oxidized forms, while the two isomerases are mainly found in their reduced forms in a wild-type background. The finding that DsbC and DsbG are kept reduced in the overall oxidizing environment of the periplasm requires the constant input from the periplasm. Genetic evidence suggests that the cytosolic thioredoxin system and the membrane via DsbD (Chung et al., 2002) was shown to consist of three domains: (α), a transmembrane domain (β), and domain (γ) (Fig. 5). Recently, a possible mechanism includes consecutive disulfide served cysteines, two of which are found in thioredoxin via the trans to the γ-domain and further on to the α-domain (Chung et al., 2002). According to this mechanism, mixed disulfide complex between DsbC and DsbG, which is transferred to the cytosol via the trans to the α-domain and further on to the periplasm, thus keeping DsbC and DsbG in a reduced state, a prerequisite for their isomerases.

![Diagram of the isomerization pathway](image)

**Fig. 5.** The *E. coli* isomerization pathway. DsbC and DsbG are kept in a reduced state in vivo by the inner membrane protein DsbD. DsbD provides reducing equivalents in the periplasm by transferring electrons from the cytosol across the membrane.

VII. Dsb Proteins and Cytosolic Cytochrome a Role Distinct from Disulfide Bond Isochymes.

By transferring reducing equivalents to a role distinct from disulfide bond isomerase, DsbB, was shown to disp. e-type cytochromes (Crooke and Cole, 1994). *E. coli* synthesizes one or more forms depending on the available electron acceptor. Maturation of e-type cytochromes is the transfer of heme moieties from the apo-protein via two residues that participate in such thioether heme attachment can occur (Fabian et al., 1994). Therefore, this is a reduced power to the periplasm. Reductive power is needed to inactivate cysteines in periplasmic disulfides by DsbA. To revert cysteine residues involved in heme binding, the CXCC motif of DsbE is kept. It was suggested that DsbE transfers directly or via an additional protein called DsbC (Reid et al., 1998). Thus, the active-site cysteines are kept in a reduced state before heme attachment.
C and DsbG are. Do the two isomerases oxidized protein substrates, or do they act sets of substrates? The redundancy of two sing, since most secreted *E. coli* proteins fide bonds. On the other hand, the two is, in *vitro* and this activity might be an roles (Chen et al., 1999; Shao et al., 2000).

**EDUCING EQUIVALENTS IN A HIGHLY OXIDIZING ENVIRONMENT**

keep the two isomerases DsbC and DsbG heir reduced forms are able to attack inction of DsbC and DsbG is carried out by sDsbD (Fig. 5) (Missiakas et al., 1995; gly, a *dsbD* null mutant accumulates DsbC ms, while the two isomerases are mainly wild-type background. The finding that ed in the overall oxidizing environment of the periplasm requires the constant flow of reducing equivalents to the periplasm. Genetic evidence suggests that reducing equivalents originate from the cytosolic thioredoxin system and are transferred across the membrane via DsbD (Chung et al., 2000; Stewart et al., 1999). DsbD was shown to consist of three domains: an N-terminal 16-kDa domain (α), a transmembrane domain (β), and the N-terminal thioredoxin domain (γ) (Fig. 5). Recently, a possible mechanism whereby DsbD transfers electrons through the membrane has been proposed. This mechanism includes consecutive disulfide exchange between six conserved cysteines, two of which are found in each domain of DsbD (Katzen and Beckwith, 2000). According to this model, electrons are transferred from cytosolic thioredoxin via the transmembrane domain (β) of DsbD to the γ-domain and further on to the α-domain. From there, electrons are finally passed on to DsbC, which was concluded from the isolation of a mixed disulfide complex between DsbC and the α-domain. Taken together, these data suggest that DsbD allows electron passage from the cytosol to the periplasm, thus keeping DsbC and DsbG in their reduced states, a prerequisite for their isomerase activities.

**VII. Dsb PROTEINS AND CYTOCHROME c MATURATION**

By transferring reducing equivalents to the periplasm, DsbD also plays a role distinct from disulfide bond isomerization. Mutants in *dsbD*, previously named *DipZ*, were shown to display a defect in the maturation of *e*-type cytochromes (Crooke and Cole, 1995). During anaerobic growth, *E. coli* synthesizes one or more of five different *e*-type cytochromes, depending on the available electron acceptor. An important step during the maturation of *e*-type cytochromes is the attachment of a covalently bound heme moiety to the apoprotein via a thioether linkage. The cysteine residues that participate in such thioether bonds have to be reduced before heme attachment can occur (Fabianek *et al.*, 2000). This poses a fundamental problem since cysteines in periplasmic proteins are efficiently oxidized to disulfides by DsbA. To reverse the DsbA-mediated oxidation of cysteine residues involved in heme binding, Dsb/DipZ provides reducing power to the periplasm. Reduction of *e*-type cytochromes requires the presence of yet another Dsb protein, which is called DsbE (CcmG) and acts in the same pathway with DsbD (Fabianek *et al.*, 1998). Accordingly, the CXXC motif of DsbE is kept in a reduced state by DsbD. It was suggested that DsbE transfers electrons to *e*-type cytochromes either directly or via an additional protein called CcmH (Fabianek *et al.*, 1999; Reid *et al.*, 1998). Thus, the active-site cysteines of *e*-type cytochromes are kept in a reduced state before heme attachment can occur.
VIII. Disulfide Bond Formation Does Not Interfere with Disulfide Isomerization

DsbD is central in providing the periplasm with reducing power from the cytosol, which is important for such different cellular processes as cytochrome c biogenesis and disulfide isomerization. In the periplasm, the isomerization pathway, which includes DsbC, DsbG, and DsbD (Fig. 5), reduces nonnative disulfide bonds formed by the DsbA–DsbB pathways. How are these two systems separated from each other? Any crosstalk between them would be destructive and result in futile cycles of mutual reduction and oxidation. Indeed, recent genetic evidence suggests that the isomerization pathway does not interfere with the oxidation pathway. This line of evidence is connected to the methods used in the isolation of the dsbD gene. DsbD mutants were identified by their ability to partially suppress the phenotypes associated with a dsbA null mutation (Missiakas et al., 1995). The suppression depends on the presence of the dsbC gene (Rietsch et al., 1996). Thus, in the dsbA–dsbD null mutant, DsbC accumulates in its oxidized state and can therefore serve as a net donor of disulfide bonds by rescuing some of the phenotypes associated with a dsbA null mutant. Although DsbC seems to play a role similar to DsbA in a dsbA–dsbD null background, it is important to note that its reoxidation under such conditions does not depend on the presence of DsbB, but rather on the oxidant cystine present in rich media. Based on this observation, Rietsch and colleagues (1998) concluded that DsbB is unable to reoxidize DsbC in vivo and thus discriminates DsbC from DsbA. As a consequence, the two pathways do not interfere with each other, allowing them to carry out their opposite functions of oxidizing and reducing disulfide bonds.

IX. Concluding Remarks

The past years have shown that disulfide bond formation is actively catalyzed in the cell. We now have a very detailed understanding of the different electron pathways during oxidative protein folding in prokaryotes. DsbB and DsbD are the central membrane components which provide the periplasm with oxidizing and reducing equivalents, respectively, by linking disulfide bond formation to cell metabolism. The soluble periplasmic proteins DsbA, DsbC, and DsbG interact with target proteins and function as disulfide catalysts. DsbA is part of the oxidative pathway, which is responsible for the net introduction of disulfide bonds. The reoxidation of DsbA is linked to electron transport by DsbB. On the other hand, DsbC and DsbG isomerize incorrect disulfide bonds and, in order to do so, have to be kept in a reduced state by DsbD. The coexistence of two distinct pathways in the environment that favors the correct form A pathway analogous to the DsbA–Dsl operate in the endoplasmic reticulum o 1998; 1999; Pollard et al., 1998). This pa bound protein ERO1, which keeps prote an oxidized state in vivo. Consequently, P to that of DsbA in catalyzing the folding of secreted proteins. The mecha folding in eukaryotes are described m this volume.

Although we now possess a broad pict ways during oxidative protein folding is seen how exactly electrons are passed proteins DsbB and DsbD. It is therefore anism of these two proteins in vivo. Kn between DsbB and its immediate electorate certainly increase our understanding of the electron transport chain. On the other hand, DsbD shuttles electrons through the mechanism factors that play a role in electron transport in periplasm might be necessary for DsbD.

Future work on the two disulfide iso reactors show how these proteins interact with It is of great interest to identify partial catalysts. The interaction of DsbC and D can then be studied in vivo and in vitro, an information, might lead to a better un that drive disulfide isomerization.

Ten years after the discovery of the fi has been made, but there is still much tein family. Multiple genetic, biochemi will be required to solve the remaining metabolism in prokaryotes.

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FORMATION DOES NOT INTERFERE WITH REDOX ISOMERIZATION

The periplasm with reducing power from for such different cellular processes as sulfide isomerization. In the periplasm, this includes DsbC, DsbG, and DsbD. Disulfide bonds formed by the DsbA-DsbB system are separated from each other. Any be destructive and result in futile cycles. Indeed, recent genetic evidence that a pathway does not interfere with the evidence is connected to the methods of gene. DsbD mutants were identified press the phenotypes associated with a et al., 1995). The suppression depends on the evidence. Rietisch et al., 1996). Thus, in the encompasses the oxidized state and can r of disulfide bonds by reducing some with dsbA null mutants. Although DsbC DsbA in a dsbA-dsbD null background, its reoxidation under such conditions is important, but rather on the oxidant. Based on this observation, Rietisch and that DsbB is unable to reoxidize DsbC DsbG from DsbA. As a consequence, the with each other, allowing them to carry oxidizing and reducing disulfide bonds.

CONCLUDING REMARKS

that disulfide bond formation is actively have a very detailed understanding of dys during oxidative protein folding in are the central membrane components with oxidizing and reducing equivalents, bond formation to cell metabolism. The sbA, DsbC, and DsbG interact with target fide catalysts. DsbA is part of the oxidable for the net introduction of disulfide A is linked to electron transport by DsbB. DsbG isomerize incorrect disulfide bonds be kept in a reduced state by DsbD. The coexistence of two distinct pathways in the periplasm guarantees a redox environment that favors the correct formation of disulfide bonds.

A pathway analogous to the DsbA-DsbB system was recently shown to operate in the endoplasmic reticulum of eukaryotes (Frand and Kaiser, 1998; 1999; Pollard et al., 1998). This pathway includes the membrane-bound protein ERO1, which keeps protein disulfide isomerase (PDI) in an oxidized state in vivo. Consequently, PDI plays a role somewhat similar to that of DsbA in catalyzing the formation of disulfide bonds during the folding of secreted proteins. The mechanisms driving oxidative protein folding in eukaryotes are described in more detail in another chapter in this volume.

Although we now possess a broad picture of the distinct electron pathways during oxidative protein folding in prokaryotes, it remains to be seen how exactly electrons are passed through the central membrane proteins DsbB and DsbD. It is therefore necessary to analyze the mechanism of these two proteins in vitro. Knowing the mode of interaction between DsbB and its immediate electron acceptor ubiquinone will certainly increase our understanding of how DsbB passes off electrons to the electron transport chain. On the other hand, the mechanism of how DsbD shuttles electrons through the membrane is less clear. Additional cofactors that play a role in electron transfer from the cytosol to the periplasm might be necessary for DsbD activity.

Future work on the two disulfide isomerases DsbC and DsbG might show how these proteins interact with their native substrate proteins. It is of great interest to identify particular in vivo substrates for these catalysts. The interaction of DsbC and DsbG with their native substrates can then be studied in vivo and in vitro, and in conjunction with structural information, might lead to a better understanding of the mechanisms that drive disulfide isomerization.

Ten years after the discovery of the first Dsb protein, much progress has been made, but there is still much to learn about the Dsb protein family. Multiple genetic, biochemical, and structural approaches will be required to solve the remaining questions concerning disulfide metabolism in prokaryotes.

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Catalysis of Disulfide Bond Formation and Isomerization


