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Genetics

A pathway for disulfide bond formation *in vivo*

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ABSTRACT **Protein disulfide bond formation in *Escherichia coli* requires the periplasmic protein DsbA. We describe here mutations in the gene for a second protein, DsbB, which is also necessary for disulfide bond formation. Evidence suggests that DsbB may act by reoxidizing DsbA, thereby regenerating its ability to donate its disulfide bond to target proteins. We propose that DsbB, an integral membrane protein, may be involved in transducing redox potential across the cytoplasmic membrane.**

Although proteins can fold spontaneously *in vitro*, it has recently become clear that the process is assisted *in vivo* (1). Two classes of proteins have been implicated in assisting protein folding. One class, including folding catalysts such as protein disulfide isomerase and proline isomerase, is thought to act by accelerating rate-limiting steps *on* the folding pathway (2, 3). The second class consists of the molecular chaperones (2). These proteins are thought to act by inhibiting undesirable processes—such as aggregation, premature folding, and misfolding processes—that lead *off* the normal folding pathway.

Disulfide bonds between cysteines are crucial for the folding and stability of many proteins (4, 5). Since the studies of Anfinsen (6) on the folding of ribonuclease, it has been clear that disulfide bonds can form in a protein spontaneously in the presence of oxygen. Yet, the existence of certain enzymes, protein disulfide isomerase (7) and sulfhydryl oxidase (8), that catalyze the formation of disulfide bonds, has raised the possibility that *in vivo* this process does require accessory enzymes. Nevertheless, it has been thought that the important catalytic role for an enzyme may not be in the formation of disulfide bonds but rather in the rearrangement of these bonds to convert nonnative into native bonds.

We have recently presented evidence that, in the periplasmic space of *Escherichia coli*, a protein is required to *form* disulfide bonds, not just shuffle them. In strains mutant in a gene we call *dsbA* for its *d*isulfide *b*ond defect, disulfide bonds form in proteins at a severely reduced rate (9). The DsbA protein has been discovered independently in *E. coli* by another laboratory (10) and has also been found in the bacteria *Vibrio cholera* and *Hemophilus influenza* (11–13). We believe that the activity of this protein is probably required in all organisms and that in eukaryotic cells, either such a hitherto undiscovered activity exists or the previously characterized protein disulfide isomerase fulfills this role.

If DsbA acts by transferring its disulfide bond to other proteins, DsbA is reduced in the process. DsbA must be reoxidized to serve as a continual source of potential for forming disulfide bonds. We wondered how DsbA is recycled; it seemed likely that additional components, in either the periplasm or cytoplasmic membrane, were part of this process. To study this question, we have sought additional genes that code for components of the pathway for disulfide bond formation. In this paper, we describe a gene, *dsbB*, that codes for a cytoplasmic membrane protein that is required for oxidation of DsbA.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Genetic Manipulations, and Media. Bacterial strains are listed in Table 1. Plasmids used were as follows: p16-1 is a pBR322 clone of *dsbA* (9), pBJ41 is a DsbA expression plasmid (9), pDB147 is a pACYC184 derivative of pBJ41 derived by cloning a *Bam*HI–*Sca* I DsbA overexpression fragment from pBJ41 into *Bam*HI–*Eco*RV-cleaved pACYC184. p13-2 is a 4.2-kb *Bgl* II insert containing *dsbB*::kan5 and flanking chromosomal DNA in the vector pMT11. pSE114 is a pSC101 clone containing the *umuCD* and *dsbB* genes (16). p73-1 is a 1.4-kb *Nsi* I–*Hin*dIII fragment that contains *dsbB*, 23 bp of upstream flanking DNA, and 873 bp of downstream flanking DNA cloned into pSL1190 (Pharmacia). pGJ69 contains the insert from p73-1 cloned into pKK223-3 (Pharmacia), putting *dsbB* under control of the *tac* promotor. pGJ70-176 is a derivative of pGJ69 with the *E. coli* alkaline phosphatase gene fused to the last codon of *dsbB*, replacing the chromosomal DNA downstream of *dsbB*. pAID325.1 is a *camR lacI*^{q} plasmid (A. Derman, personal communication). Genetic manipulations were as described (9). NZY broth was used for growth of cells and for transformation and transduction experiments (14). NZY contains ≈0.3 mM cystine (Quest, Norwich, NJ). M63-glucose minimal medium (14) was supplemented with amino acids to 50 μg/ml, except that methionine was eliminated for labeling experiments, and cystine was added to the concentrations indicated.

Isolation of *dsbB* Mutants. We have developed a selection for *E. coli* mutants defective in disulfide bond formation (9). The selection exploits the specific properties of the MalF–βgal 102 fusion protein. This fusion lacks β-galactosidase activity in wild-type strains but becomes Lac$^+$ when the strain is defective in disulfide bond formation. We have isolated spontaneous Lac$^+$ derivatives from strain KM1086, which carries the MalF–βgal 102 fusion. *dsbA* mutations were previously isolated by using this selection (9). We reasoned that if there were additional components of the pathway leading to disulfide bond formation, mutants in genes for these components might be detected by more extensive use of the original selection. Spontaneous Lac$^+$ mutations were isolated and separated into three categories. Those that were linked to the gene fusion itself were detected by testing the supernatant of overnight cultures for spontaneously induced Lac$^+$ λ phage carrying the fusion. Those revertants due to mutations unlinked to the gene fusion fell into two classes. Class I (20 mutants) was composed of mutations tightly linked by P1 transduction to the *dsbA* gene

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Abbreviation: ORF, open reading frame; uPA, urokinase-type plasminogen activator.

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Table 1. *E. coli* strains

Strain	Genotype or phenotype
CAG18497	*fadR13*::Tn*10*
DB144	JCB570 pRDB8-A
DB145	JCB570 *dsbA*::kan1 pRDB8-A
DB146	JCB570 *dsbB*::kan5 pRDB8-A
JCB570	MC1000 *phoR zih12*::Tn*10*
JCB643	KM1086 *dsbA*::kan2
JCB656	KM1086 *dsbB*::kan5
JCB759	KM1086 *dsbA*::kan1
JCB771	JCB656 pBJ41, pAID325.1
JCB772	JCB570 pBR322
JCB773	JCB570 *dsbA*::kan1 pBR322
JCB775	JCB570 *dsbA*::kan1 *dsbB*::kan5 pBR322
JCB792	JCB570 λ102 *dsbB*::kan5 pBR322
KM1086	MC4100 *malT*^{c} (λ*malF–lacZ* 102)
MC1000	*araD139* Δ(*araABC–leu*)7679 *galU galK* Δ(*lac*)X74 *rpsL thi*
MC4100	*araD139* Δ(*argF–lac*)U169 *rpsL150* *relA1 flbB5301 deoC1 ptsF25 rbsR*

The source of all strains was this study, except for strains KM1086 and JCB570 from Bardwell *et al.* (9), MC1000 and MC4100 from Silhavy *et al.* (14), and CAG18497 from Singer *et al.* (15).

and complemented by clones of *dsbA*. The second class (five mutants) consisted of those linked neither to the gene fusion nor to *dsbA*.

The same genetic selection was done by using for mutagenesis the vector λ1316 carrying the mini-Tn*10*-kan transposon derivative 103, as described by Kleckner *et al.* (18). Such a selection would yield, for the most part, mutations only in nonessential genes (a null mutant of *dsbA* is viable). Again, mutations were classified according to whether or not they were linked to *dsbA*.

Cloning and Sequencing of *dsbB*. A class II mutation obtained by mini-Tn*10*-kan mutagenesis was cloned in vector pMT11 by selecting for Kan^R. The resulting clone, p13-2, was made radioactive by nick translation and hybridized to an ordered bacteriophage library of the *E. coli* chromosome (19). Phage 2A3 and 11G8 hybridized, suggesting a region of ≈25.5 min had been cloned. That the spontaneous class II mutations also mapped to this region was confirmed by transferring in *fadR13*::Tn*10*. All five spontaneous class II mutations were 98.6–100% linked to the *fadR*::Tn*10* by P1 cotransduction. The restriction map of this clone was then compared with the restriction map of *E. coli* (19), and a region very close to the *umuC, umuD* genes appeared to have been cloned. We then obtained a plasmid pSE114 that carried the *umuC, umuD* genes and adjacent regions (16) and showed that it would complement the mini-Kan insertion and all five spontaneous class II mutations. This located gene was named *dsbB*.

A subclone of plasmid pSE114 identified a region of 550 bases that complements the *dsbB*::kan5 mutation. This subcloned region was sequenced using Sequenase (United States Biochemical) and found to contain a single open reading frame (ORF). This ORF represents *dsbB* because the *dsbB*::kan5 insertion is located within this ORF and pGJ70.176, a clone containing this ORF and a total of only 23 bp of flanking DNA, complements the *dsbB*::kan5 mutation. The sequence of this region has been published by Pinner *et al.* (20), GenBank accession no. M83655. We find two differences between their sequence of this ORF and our determination: the published sequence reads 1960-GGCTTA-...GACTTG-2202, whereas our sequence reads GCGTTA-...GATTG. These errors lie within the *dsbB* ORF. This region has been sequenced independently by D. Missiakas and S. Raina, who agree with these revisions (S. Raina, personal communication). The corrected protein sequence is presented in Fig. 3; the corrections in nucleic acid sequence have been deposited in GenBank.

General Methods. Cell labeling, immunoprecipitation trypsin treatment, and PAGE were as described (9), except that disulfide bond formation was blocked by adding *N*-ethylmaleimide to 100 mM instead of iodoacetamide in labeling experiments. After PAGE the relative amounts of oxidized and reduced protein were measured by using a phosphoimager (Molecular Dynamics). Rabbit polyclonal antisera to purified DsbA was prepared by Immuno-Dynamics (La Jolla, CA).

Determination of Oxidation State of DsbA *in Vivo*. Iodoacetic acid was added to logarithmic-phase cultures grown in NZY broth to a final concentration of 100 mM to trap free thiols, and then periplasmic extracts were prepared by osmotic shock, as described (9), except that the spheroplast buffer was pH 7 and supplemented with 10 mM iodoacetic acid, and the solution used for osmotic shock was 3 mM iodoacetic acid/3 mM Mops, pH 7, instead of distilled water. Reduced and oxidized forms of DsbA were separated by native gel electrophoresis (17), and immunoblot analysis was done, as described (21).

Detection of Enzymatically Active Urokinase Plasmid in Bacterial Extracts. Strains were transformed with pRDB8-A, a murine urokinase-type plasminogen activator expression plasmid (uPA) in which the complete coding sequence (22) is constitutively expressed under control of a phage T4 gene 32 promoter cassette (22). Extracts of exponentially growing cultures were prepared, as described (23), and electrophoresed under nonreducing conditions. Active urokinase in the gels was revealed by zymography on casein–plasminogen–agar underlays: urokinase diffuses into the agar gel and converts plasminogen into plasmin, causing local caseinolysis. The transparent lytic bands appear dark under indirect illumination, whereas undigested casein in the opaque background is white (22).

RESULTS

Properties of *dsbB* Mutants. Previously we described a selection that yields mutations in a locus, called *dsbA*, required for disulfide bond formation. Using the same selection (as described in *Materials and Methods*), we have identified a second locus, called *dsbB*. To determine whether the *dsbB* mutants interfered with disulfide bond formation, we examined the state of the disulfides in the proteins OmpA and β-lactamase in *dsb* mutants. By pulse labeling the cells and running nonreducing SDS gels to distinguish oxidized vs. reduced cysteines, we showed that the *dsbB*::kan5 insertion was severely defective in disulfide bond formation for these proteins. The extent of the disulfide defect in the *dsb* mutants was analyzed by measuring the relative amounts of oxidized and reduced OmpA in pulse-chase experiments. Comparison of the relative rates of disulfide bond formation showed the *dsbB* mutation as slightly less defective than *dsbA* [in Fig. 1*A*, compare squares (*dsbB*$^-$) with triangles (*dsbA*$^-$)]. The *dsbA, dsbB* double mutants showed an identical defect to the *dsbA* single mutant [compare triangles (*dsbA*) with x's (*dsbAB*$^-$)]. OmpA is ideal for this analysis because it is stable *in vivo* in the absence of its disulfide bond, and protein folding does not appear to interfere with disulfide bond formation. In contrast, folding of β-lactamase appears to compete with disulfide bond formation; once folded into a protease-resistant form, disulfide bond formation in β-lactamase is arrested (9). β-Lactamase is rapidly oxidized in wild-type strains. In *dsbA*$^-$, *dsbB*$^-$, and *dsbA*$^-$, *dsbB*$^-$ strains 60–85% of β-lactamase was present in the reduced form immediately after synthesis. This reduced protein was not further oxidized with chase times of up to 20 min (ref. 9 and data not shown).

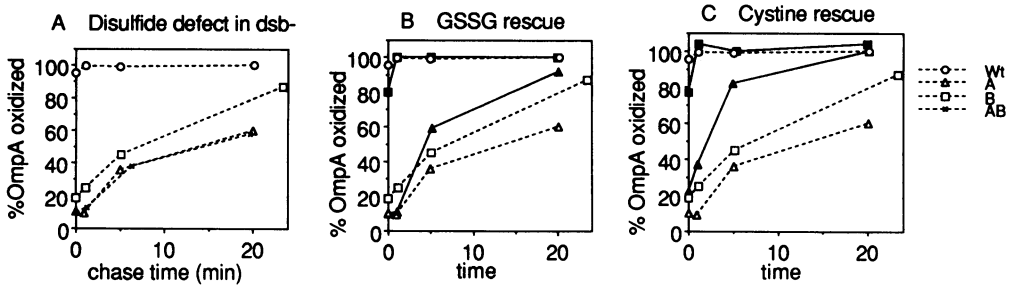

FIG. 1. Kinetics of disulfide bond formation in dsbA⁻ and dsbB⁻ and in *dsbA*⁻, *dsbB*⁻ strains. Wild type (○); dsbA⁻ (△); dsbB⁻ (□); and *dsbA*⁻, *dsbB*⁻ (x) strains were labeled with [^{35}S]methionine for either 20 sec or 20 sec followed by a various chase period with excess unlabeled methionine. Cells were lysed and immunoprecipitated with OmpA antiserum. PAGE under nonreducing conditions was done, and the proportion of oxidized OmpA was measured by using a phosphoimager. The samples were treated with trypsin at 10 μg/ml before immunoprecipitation. The reduced form of OmpA is trypsin sensitive (9). The strains used were JCB772 (wild type), JCB773 (*dsbA*::kan1), JCB792 (*dsbB*::kan5), and JCB775 (*dsbA*::kan1, *dsbB*::kan5). (*A*) Stains were grown in disulfide-free M63-glucose medium. (*B*) Effect of adding oxidized glutathione (GSSG; glutathione disulfide) to 6.5 mM (solid lines and filled symbols) is compared with disulfide-free medium (dotted lines and open symbols). (*C*) Effect of adding cystine to 0.33 mM (solid lines and filled symbols).

We also examined the formation of disulfide bonds in eukaryotic proteins expressed in *E. coli*. Both *dsbA*- and *dsbB*-containing strains were severely defective in production of active mouse uPA. uPA is a circulating serine protease with 12 disulfide bonds. Enzymatically active uPA was detected in bacterial extracts by using zymography (see *Materials and Methods*). The *dsbA* strains produce no measurable uPA; *dsbB* strains produce ≈100-fold less active uPA than do wild-type strains (see Fig. 2). Similar results were obtained with tissue-type plasminogen activator (tPA), another serine protease with 17 disulfide bonds (D.B., unpublished work).

DsbB Is an Inner-Membrane Protein. The *dsbB* sequence was determined, as described. A hydropathy plot of the DsbB sequence reveals several potential membrane-spanning sequences, suggesting that DsbB is localized to the cytoplasmic membrane. To study the location and topology of DsbB, we obtained a number of alkaline phosphatase fusions to the protein. The pattern of specific activities of these fusions strongly supports a cytoplasmic membrane location for this protein and a topological model that includes four membrane-spanning segments with both the N and C termini in the cytoplasm (Fig. 3 and G.J., unpublished work). Further, we studied the cellular fractionation properties of five of these fusion proteins and showed that they were localized to the cytoplasmic membrane fraction (G.J., unpublished work).

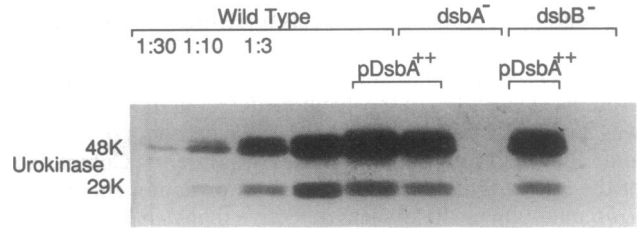

FIG. 2. Zymogram showing expression of recombinant murine urokinase (uPA) in *dsb* mutant strains. DsbA and DsbB are required for the activity of uPA, and overexpression of DsbA rescues the production of active urokinase in both *dsbA*⁻ and *dsbB*⁻ strains. Bacterial cultures were grown to logarithmic phase, induced with isopropyl β-D-thiogalactoside at 5 mM for 1 hr, and resuspended at 10 A_{600} units/ml; extracts were analyzed by zymography with plasminogen. The casein/agar underlays were incubated for 15 hr at 37°C. The sizes of two different urokinase forms [48 kDa (K) and 29 kDa] are indicated. Wild type is strain DB144, *dsbA*⁻ is strain DB145, and *dsbB*⁻ is strain DB146. The first three lanes contain serial dilutions of extract; all other lanes are undiluted. Where indicated by pDsbA⁺⁺, the strains contain pDB147, a derivative of pACYC184 that overexpresses DsbA; in all other lanes the strains contain only vector pACYC184.

Functions of DsbA and DsbB. Several lines of evidence contribute to understanding the functions of DsbA and DsbB and suggest that they are components of the same pathway. (*i*) The defects in disulfide bond formation seen in both types of mutants are very strong, indicating that DsbA and DsbB are both involved in the major pathway leading to disulfide bond formation. Disulfide bond formation is essentially complete for OmpA within the 20-sec pulse period in wild-type strains. In the *dsbA*:kan1 mutant JCB773 and the *dsbB*:kan5 mutant JCB792, disulfide bond formation is not complete even after a 20-min chase period. In addition, the double mutant JCB775, which lacks both DsbA and DsbB, is indistinguishable in its disulfide bond defect from the *dsbA* mutant (see Fig. 1*A*).

(*ii*) A role for DsbB in oxidation of DsbA is suggested by the ability of both cystine (at low concentrations) and oxidized glutathione to substitute for DsbB but not to substitute for DsbA. Cystine (0.6 mM) reversed the Lac⁺ phenotype of strain JCB656 (dsbB::kan5), carrying the λ 102MalF–βgal fusion, restoring low levels of β-galactosidase activity. In the accompanying paper, Dailey and Berg (24) show that DsbA and DsbB are required for motility and that the motility defect is rescued by 0.1 mM cystine in a *dsbB* mutant but is not rescued in a *dsbA* mutant (24).

We directly observed the effect of cystine on disulfide bond formation in the *dsbA* and *dsbB* mutants by examining the state of OmpA in pulse-chase experiments. The *dsbB* mutant cells were grown in M63-glucose minimal medium containing 0.33 mM cystine or 6.5 mM oxidized glutathione, and the proportion of OmpA that had formed its disulfide bond was determined. The disulfide defect of the *dsbB* mutant was almost completely rescued when either of these two disulfide-containing molecules was added (compare solid lines with filled squares to dotted line with open squares in Figs. 1 *B* and *C*). The effect was quite rapid with all OmpA found in the disulfide-bonded state at the 1-min time point. In contrast, in the same experiments with a *dsbA* mutant or a *dsbA, dsbB* double mutant, little oxidized OmpA was found after the

MLRFLNQCSQGRGAWLLMAFTALALELTALWFQH
VMLLKPCVLCIYERCALFGVLGAALIGAIAPKTPLR
YVAMVIWLYSAFRGVQLTYEHTMLQLYPSPFATCD
FMVRFPEWLPLDKWVPQVFVASGDCAERQWDFLG
LEMPQWLLGIFIAYLIVAVLVVISQPFKAKKRDLFGR

FIG. 3. Predicted amino acid sequence of DsbB. Possible transmembrane segments are underlined; positions of cysteines are in boldface type.

pulse, and disulfide bond formation was not complete even after long chase periods.

At higher concentrations, cystine could also reverse phenotypes of the *dsbA* mutant. The Lac$^+$ character of strain JCB643 (*dsbA*::kan2), carrying the λ102MalF–βgal fusion, was reversed by 8.6 mM cystine; the poor growth phenotype of *dsbA* strains on minimal medium was reversed, as well.

(*iii*) Examination of the oxido–reduction state of DsbA yielded information on its function and on the role of DsbB. Other periplasmic proteins, such as alkaline phosphatase and β-lactamase, in wild-type strains are in the fully oxidized state. However, a protein that must donate its disulfide bond to other proteins easily, such as DsbA, would be found in both an oxidized and reduced state, as is the case. Using immunoprecipitations to study the state of DsbA, we found a portion of DsbA was in the oxidized state and a portion was in the reduced state in a wild-type strain. However, in strains lacking DsbB, DsbA was in the fully reduced state (see Fig. 4). Oxidized DsbA was seen in the *dsbB* mutant JCB656 when the NZY growth medium was supplemented with 3.3 mM cystine (data not shown). The proportion of DsbA found in an oxidized form varied according to disulfide trapping conditions. The disulfide bond in DsbA is extremely labile and tends to act to oxidize free thiols both *in vivo* and *in vitro* (A. Zapun, J.C.A.B., and T. Creighton, unpublished work).

(*iv*) Although the multicopy *dsbB* plasmid p73-1 complemented only *dsbB* mutants, the *dsbA*-containing plasmid pDB147, surprisingly, complemented null mutations in *both* *dsbA* and *dsbB* genes for uPA activity (see Fig. 2) and β-galactosidase activity of MalF–βgal 102 (data not shown). Complementation of *dsbB*::kan5 by DsbA overproduction depended upon cystine addition to the medium. This complementation occurred in NZY medium (which contains ≈0.3 mM cystine) and in M63 minimal medium supplemented with cystine but did not occur in M63 minimal medium that lacked cystine. Apparently, the cystine level in NZY broth is sufficient to complement *dsbB*::kan5 only when DsbA is overproduced. In the *dsbB*::kan5, DsbA-overproducing strain, JBC771, grown in NZY broth a substantial proportion of DsbA was oxidized (data not shown). This result is consistent with the explanation that cystine suppression of *dsbB* mutants acts through DsbA oxidation. These results suggest that DsbB is necessary for oxidation of DsbA.

DISCUSSION

Our results suggest a pathway (Fig. 5) for the formation of disulfide bonds in *E. coli*. We have now identified two cellular components required for this process: one in the periplasm

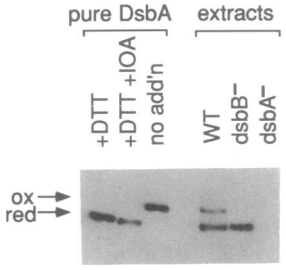

FIG. 4. Effect of *dsbB*::kan5 mutation on oxidation state of DsbA. *E. coli* cells were grown to logarithmic phase in NZY broth; free thiols were blocked with 100 mM iodoacetic acid (IOA), and periplasmic extracts were prepared. Wild-type (WT) and *dsbB*::kan5 (*dsbB*$^-$) and *dsbA*::kan1 (*dsbA*$^-$) strains were electrophoresed under native conditions. Purified oxidized (ox) DsbA and DsbA with 3 mM dithiothreitol (DTT) served as markers for oxidized and reduced DsbA (red). +IOA indicates that this pure DsbA sample was exposed to 10 mM iodoacetic acid for 10 min. The strains used were KM1086, JCB759 (*dsbA*::kan1), and JCB656 (*dsbB*::kan5).

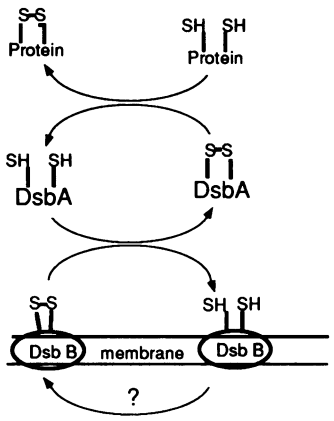

FIG. 5. Model for a pathway for disulfide bond formation.

and one in the cytoplasmic membrane. We propose that the DsbA protein is the direct source of oxidation for cysteines in proteins in which disulfide bonds are formed and that the role of DsbB is to continually reoxidize DsbA. This role for DsbB is supported by a number of lines of evidence.

(*i*) The two proteins would appear to be in the same pathway because mutants lacking either component have strong effects on disulfide bond formation and the double *dsbA*$^-$, *dsbB*$^-$ mutant does not have a more severe defect than the single *dsbA*::kan1 mutation. (*ii*) The disulfide-containing small molecules cystine and oxidized glutathione efficiently substitute for DsbB but only inefficiently substitute for DsbA. These molecules appear to do this by oxidizing DsbA, which, in turn, oxidizes proteins. High concentrations of cystine and GSSG may be able to directly oxidize proteins. (*iii*) Overproduction of DsbA suppresses a *dsbB* mutant, but the reverse is not true. We explain this effect of suppression by suggesting that increased amount of DsbA decreases the need for recycling (reoxidation) DsbA to the point that disulfide-containing small molecules, such as cystine present in rich medium, are sufficient for recycling DsbA.

(*iv*) In wild-type cells DsbA is a mixed population of oxidized and reduced forms; in a *dsbB* mutant, DsbA is fully reduced. This finding is consistent with the proposal that DsbB is necessary to oxidize DsbA but does not prove it. That DsbB is required for expression of DsbA seems unlikely; *dsbB* mutants produce normal amounts of DsbA (see Fig. 4). The transfer of oxidizing potential to DsbA might come ultimately from oxidation–reduction processes that occur in the cytoplasm or that involve membrane components interacting with the electron transport chain. Either way, a membrane component would seem necessary for the oxidation process; DsbB may be this membrane component. Consistent with the proposed role of DsbB in thiol–disulfide exchange, DsbB is cystine rich; five cystines occur in periplasmic domains, and one occurs in a cytoplasmic domain.

Our pathway is probably missing additional components. It is likely that we would not have detected mutations in the genes for essential proteins. There could be additional proteins in the periplasm. There are probably other proteins in the membrane or in the cytoplasm that connect the Dsb system directly to electron-transfer processes. At the present time little is known about the passage of any source of energy from the cytoplasm to the periplasm. It may be that quinones or certain membrane proteins capable of moving electrons through the membrane are needed to oxidize DsbB. Possibly mutations in these genes could have wider pleiotropic effects or could even be lethal.

The mechanism by which the final steps in cysteine oxidation occurs is unclear. DsbA could act directly on proteins, forming both correct and incorrect disulfide bonds that are subsequently shuffled. DsbA clearly does not act primarily as

a protein disulfide isomerase because *dsbA* mutant cells show a lack of disulfide bonds rather than the presence of incorrect ones. That high levels of the disulfide-containing molecules, cystine and oxidized glutathione, can substitute for DsbA and DsbB is further evidence that the Dsb system acts to oxidize the disulfide bonds in proteins rather than to isomerize them. The Dsb system appears remarkably general because it is required for the formation of disulfide bonds in not only a number of endogenous proteins but also in expressed recombinant proteins that have a complex pattern of multiple disulfide bonds. In the accompanying paper (24) Dailey and Berg have shown that *dsbA* and *dsbB* are required for motility and that the FlgI protein that constitutes the P ring of the flagellar motor is reduced in the *dsb* mutants and fails to assemble properly.

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