Identification and characterization of the *Escherichia coli* gene *dsbB*, whose product is involved in the formation of disulfide bonds *in vivo*

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**ABSTRACT** We have identified and characterized the *Escherichia coli* gene *dsbB*, whose product is required for disulfide bond formation of periplasmic proteins, by using two different approaches: (i) screening of a multicopy plasmid library for clones which protect *E. coli* from the lethal effects of dithiothreitol (DTT), and (ii) screening of insertion libraries of *E. coli* for DTT-sensitive mutants. Mapping and characterization of mutations conferring a DTT-sensitive phenotype also identified the *dsbA*, *trxA*, and *trxB* genes, whose products are involved in different oxidation–reduction pathways. Null mutations in *dsbB* conferred pleiotropic phenotypes such as sensitivity to benzylpenicillin and inability to support plaque formation of filamentous phages, and they were shown to severely affect disulfide bond oxidation of secreted proteins such as OmpA and β-lactamase. These phenotypes resemble the phenotype of bacteria carrying either a null mutation in the *dsbA* gene or the double mutation *dsbA dsbB*. Sequencing and expression of the *dsbB* gene revealed that it encodes a 20-kDa protein predicted to possess an "exchangeable" disulfide bond in -Cys-Val-Leu-Cys-. The *dsbB* gene maps at 26.5 min on the genetic map of the *E. coli* chromosome, and its transcription is directed from two promoters, neither of which resembles the canonical Eσ-recognized promoter.

Relatively little is understood thus far regarding the process of folding and assembly of secreted proteins in prokaryotes. Exported proteins face a harsher environment in the periplasm, since this space is more vulnerable to environmental stresses than is the cytosol (1). It is thus reasonable to expect that in *Escherichia coli* there may be periplasmic chaperones whose function is to ensure the proper folding and maintenance of periplasmic proteins. Another expected class of proteins is enzymes that can either directly catalyze disulfide bond formation or maintain an appropriate oxidized environment in the periplasm. It is indeed well established that the vast majority of disulfide bond-containing proteins are either secreted or integral membrane proteins, since the bacterial cytoplasm is a comparatively reducing environment (2). Thus it is quite important that in *E. coli* a periplasmic protein, designated DsbA, was recently discovered and shown to be involved in disulfide bond formation (3). DsbA has been characterized *in vivo* as well as *in vitro* (3–5). It has been shown that in *dsbA* mutants a variety of secreted proteins lack disulfide bonds (3, 4).

In an attempt to identify genes whose products are involved in the stability or assembly of proteins in the periplasmic space, we initiated a series of experiments utilizing various genetic approaches. In the present study, we took advantage of the phenotypes of either resistance or sensitivity to strong reducing agents such as dithiothreitol (DTT) to characterize gene products involved in the oxidation and/or reduction pathways. In one approach, a multicopy *E. coli* genomic library was used to identify those proteins whose overproduction would protect bacteria against the lethal reducing effects of DTT. In a complementary approach, insertional mutants that exhibited hypersensitivity to sublethal concentrations of DTT were isolated. Both studies identified the existence of a gene designated *dsbB*. § A null mutation in *dsbB* leads to the pleiotropic phenotype, similar to the one exhibited by *dsbA* null mutants, of deficiency in disulfide bond formation of secreted proteins such as OmpA and β-lactamase. These results are consistent with independent studies (6, 7) also reporting the identification of the *dsbB* gene. The insertional mutational approach for DTT sensitivity also resulted in the isolation of mutations in the known genes *trxA* (encoding thioredoxin), *trxB* (encoding thioredoxin reductase), and the recently described *dsbA* (3, 4, 8).

**MATERIALS AND METHODS**

Selection Strategies and Cloning of *dsbB*. Chromosomal DNA isolated from *E. coli* wild-type strain MC4100 was subjected to partial digestion with *Sau*3A to produce DNA fragments 2–6 kb in length. These DNA fragments were gel-purified and ligated into the BamHI site of the p15A-based vector pOK12. A library of at least 20,000 independent recombinant clones was made, and DNA from such a pool was used to transform various wild-type *E. coli* strains. Twenty-four plasmid clones whose presence conferred resistance to otherwise lethal DTT levels (20 mM) were thus selected. The restriction enzyme digestion pattern of these 24 plasmids showed that they all carried the same chromosomal insert. Plasmid clones pDM243 and pDM244, which carry the same 1.5-kb *Sau*3A fragment but in opposite orientations with respect to the T7 promoter of the pOK12 vector, were retained and shown to contain *dsbB*. Subcloning of the 950-bp *Kpn* 1–*Sau*3A fragment, which contains the minimal *dsbB* gene, resulted in plasmid pDM353 (Table 1).

The overexpression of DsbB was achieved by first amplifying the *dsbB* gene minimal coding region by the PCR method (14), using primers 5′-GGAGCGCGCAATGGATC-CCGGACGCAA-3′ and 5′-TATTGCGAGCATAGGATA-TGTTG-3′ and cloning in the T7 promoter expression vector PET3a (pDM507). The *dsbB* gene (1.8-kb *Sau*3A DNA fragment) was cloned, using the genomic library described above (pSR1865), by selecting for its ability to complement SR1790 (*dsbA43::TnlO*) and DM547 (*dsbA43::TnlO*) mutant bacteria and was verified by its ability to recombine with various *TnlO* insertions within the *dsbA* gene.

Abbreviations: DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Kan, kanamycin resistance.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L03721).
Table 1. E. coli strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genetic markers</th>
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<tbody>
<tr>
<td>CA8000</td>
<td>Wild type (9)</td>
</tr>
<tr>
<td>DM215</td>
<td>MC4100 fhaH::Tn10</td>
</tr>
<tr>
<td>DM391</td>
<td>CA8000 dsbB36::Tn10</td>
</tr>
<tr>
<td>DM547</td>
<td>CA8000 dsbA43::Tn10</td>
</tr>
<tr>
<td>SR688</td>
<td>CA8000 zbh::Tn5</td>
</tr>
<tr>
<td>SR1748</td>
<td>CA8000 trxB::Tn10</td>
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<tr>
<td>SR1753</td>
<td>CA8000 dsbB65::Tn10</td>
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<tr>
<td>SR1789</td>
<td>CA8000 trxA::Tn10 Kan'</td>
</tr>
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<td>SR1790</td>
<td>CA8000 dsbA101::Tn10 Kan'</td>
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<tr>
<td>SR1855</td>
<td>CA8000 dsbA101::Tn10 Kan' dsbB36::Tn10</td>
</tr>
<tr>
<td>CAG18599</td>
<td>MG1655 ilv::Tn10 Kan'</td>
</tr>
<tr>
<td>GW2100</td>
<td>AB1157 umuC122::TnS (10)</td>
</tr>
<tr>
<td>RP6866</td>
<td>(motA-motB) DEM12-13 his-4/pJ120 carrying in-frame TnphoA at codon 84 of motB (11)</td>
</tr>
</tbody>
</table>

Plasmids

- pOK12: p15A-based cloning vector used to construct genomic library
- pET-3a: T7 expression vector (12)
- pHF45: ColE1-based vector conferring resistance to chloramphenicol and ampicillin (13)
- pDM243/244: pOK12 carrying 1.5-kb Sau3A dsbB+ fragment
- pDM353: pOK12 carrying 950-bp Kpn1–Sau3A dsbB+ fragment in orientation with T7 promoter of the vector
- pDM507: pET-3a carrying 536-bp minimal coding region of the dsbB+ fragment cloned in NdeI– BamHI sites
- pSR1865: pOK12 carrying 1.5-kb Sau3A dsbA+ fragment

Kan', kanamycin resistance.

To isolate chromosomal mutations which render E. coli sensitive to DTT, pools of approximately 5 × 10^8 independent insertional events of either ΔTn10 (tetracycline resistance) or ΔTn10 (Kan') (15) were constructed in wild-type E. coli strain CA8000. These bacterial pools were subsequently screened for sensitivity to otherwise nonlethal concentrations of DTT (7 mM). Seventy-eight such independently isolated mutants were mapped by hybridizing the complementing clones to the ordered E. coli genomic library (16) and were confirmed by linkage to nearby known genetic markers. The assignment of mutations to the dsbA locus was done by using fhaH::Tn10 from DM215 (Table 1) as the linked marker; the dsbB insertions were mapped by using the umuC122::TnS (10) linked marker; the trxB::Tn10 insertions were mapped by confirming their linkage to the Kan' marker of SR688, which carries a Tn5 insertion located 115 nt downstream of the trxB termination codon. ilv::Tn10 Kan' marker from strain CAG18599 (17) was used to assign the trxA::Tn10 insertions.

**DNA Sequence Analysis.** The 1.5-kb Sau3A fragment of pDM243 (dsbB+) or its derived subclones such as pDM353 were sequenced by using the United States Biochemical Sequenase kit. To locate the exact positions of the Tn10 insertions in the dsbB gene, these insertions were first recombined onto plasmid pDM243. The junction between Tn10 and the chromosomal DNA was determined by using the synthetic 24-mer oligonucleotide primer 5'-ATTGGATCATATGACAAAGATGGT-3', which directs DNA replication away from the ends of the Tn10 insertion element. The sequence of the dsbB gene along with the flanking regions has been deposited in GenBank.5

**RNA Isolation, Northern Blotting, and Mapping of 5' Terminal of dsbB.** Total cellular RNA was isolated by using the hot SDS/phenol extraction procedure (18). Approximately 5 μg of each RNA sample was analyzed by the Northern technique (18). To probe for dsbB message, 100 ng of the 280-bp Nsi I–Pvu I 1 DNA fragment (internal to the dsbB gene) was isolated from pDM353 and 32P-labeled with [α-32P]dATP (3000 Ci/mmol; 1 Ci = 37 GBq) using the nick-translation procedure (18). To define the transcriptional start sites, ~10 ng of the oligonucleotide probe 5'-ACTGCTCTGGCAGTGGGAACGTG-3', which is complementary to nucleotide positions 66–86 of the dsbB transcript, was annealed with 10 μg of total cellular RNA. The annealed primer was extended by reverse transcriptase from avian myeloblastosis virus, essentially as described (9). The primer extension products were electrophoresed on the same gel as the dideoxy sequencing reaction products, using the same primer.

**Labeling, Fractionation, and Immunoprecipitation.** For labeling of proteins, cells were grown in M9 minimal medium (18), supplemented with each of the amino acids (except methionine and cysteine) at 20 μg/ml and 0.4% glucose. Bacterial cultures were pulse-labeled with [35S]methionine at 100 μCi/ml for 45 sec followed by a 1000-fold excess of unlabeled methionine. Disulfide bond formation in OmpA and β-lactamase was assessed by the method described by Pollitt and Zalkin (19). The anti-OmpA antiserum was a kind gift of S. Mizushima, and anti-β-lactamase antibody was purchased from 5 Prime → 3 Prime, Inc.

**Results**

**Cloning of dsbB.** In an attempt to identify genes whose products are involved in protein stabilization, particularly in the periplasm, we selected for tolerance to otherwise lethal levels of DTT, a reducing agent that has been shown to reduce a variety of proteins, including protein disulfide isomerase, and thioredoxin. Thus, it is expected that the presence of high amounts of DTT will result in the misfolding of many proteins which require disulfide bond(s) to achieve their functional native form. Overexpression of either a chaperone or a cellular component able to catalyze or participate in disulfide exchange reactions may relieve the cells of DTT's toxic effect. A library of E. coli chromosomal DNA prepared in the p15A-based vector pOK12 was used to select for clones which enable wild-type E. coli to form colonies on L agar (18) supplemented with 20 mM DTT, a concentration at which wild-type bacteria do not normally grow. Twenty-four large colonyforming units were thus isolated and subsequently shown to share an identical 1.5-kb Sau3A DNA fragment (pDM243 and pDM244). Further subcloning experiments showed that DNA sequences required to confer resistance to DTT were present on a 950-bp Kpn1–Sau3A fragment (pDM353), defining the dsbB gene.

**Mapping of dsbB.** We hybridized the 32P-labeled 1.5-kb Sau3A fragment derived from plasmid pDM243 (dsbB+) to the E. coli DNA library in bacteriophage λ (16). Hybridization was observed with bacteriophages A243 (2A3) and A244 (11G8), which carry the overlapping region corresponding to kbp 1235–1245 of the E. coli physical map. Further comparisons of the restriction maps of the dsbB+ subclones containing the 950-bp Kpn1–Sau3A fragment with the restriction map of E. coli covering this region confirmed that dsbB is located in the 1238–kbp region, corresponding to the 26.5-min region of the recalibrated E. coli genomic map (20). Since the umuC gene, whose product is required for inducible mutagenesis, also maps in this region, we used plasmids pSE114 and pSE116 (10) to verify our results. We found that, whereas both plasmids carry the umuCD genes, pSE114 also contains the dsbB gene; thus, we were able to place dsbB immediately upstream of umuC. The position of dsbB was also verified genetically by using the umuC122::Tn5 as a marker for bacteriophage P1 transduction experiments with our dsbB::Tn10 isolates.
Sequencing of dsbB and Identification of Its Product. The nucleotide sequence determination of the 1.5-kb Sau3A DNA fragment revealed a 534-nucleotide open reading frame (ORF). This sequence encodes a predicted 178 amino acid residue polypeptide of 20,329 Da, with a CXXC motif located at position 43–46 (Fig. 1). The predicted sequence of the DsbB protein also shows at least three potential transmembrane domains (Fig. 1), indicating a potential cytoplasmic membrane location. Upstream of the dsbB ORF we found a truncated ORF that corresponds to the end of the nhaB gene, encoding the Na\(^+\)/H\(^+\) antiporter (21). The recently reported nhaB sequence includes part of the dsbB ORF (21). However, this sequence differs from ours by an extra base at position 794, leading to a frameshift in the dsbB sequence, and at position 479 (G vs C), leading to amino acid change from glycine to alanine (amino acid residue 52) of the DsbB protein (Fig. 1).

The positions of two of the six dsbB::Tn10 insertions, able to recombine with the dsbB\(^+\)-carrying plasmid pDM243, were determined by sequencing the DNA junction between Tn10 and the dsbB gene, using primer reading out of the Tn10 ends. The sites of the mini-Tet::Tn10 insertions in DM391 and SR1753 were found to be within the deduced dsbB gene at nucleotide positions 36 and 65, respectively, from the putative ATG initiation codon (Fig. 1).

To identify the dsbB gene product we used \(^{35}\)S-labeled methionine to label BL21(DE3) cells carrying either plasmid pDM353 or pDM507, which contain the dsbB gene under the exclusive T7 promoter expression system (12). When the T7 promoter was induced, a simultaneous induction of an \(\approx 20\)-kDa protein was observed, consistent with the predicted size of DsbB protein (Fig. 2).

Phenotypic Characterization and Mapping of DTT-Sensitive Mutants. Seventy-eight DTT-sensitive Tn10 (Kan\(^\)') or Tn10 (Tet\(^\)') insertions were isolated as described in Materials and Methods. Of these, six mutations were shown to map in the dsbB gene and two in the dsbA gene. Among the other genes identified in this screen were the trxA (SR1789) and trxB (SR1748) genes (Table 1). The trxA and trxB genes have been shown to encode thioredoxin and thioredoxin reductase, respectively (8). The majority of the DTT-sensitive mutants were found to share the following phenotypes: (i) hypersensitivity to benzylpenicillin, especially the dsbB and dsbA null mutant bacteria, with dsbB being more sensitive (15 \(\mu\)g/ml); (ii) reduced overall expression of alkaline phosphatase [this phenotype was assayed by first transducing these mutations, by using bacteriophage P1, into strain RP6866, which contains plasmid pJJ20 carrying an in-frame TnphoA fusion at codon 84 of the motB gene (11)]; (iii) inability to support plaque formation of filamentous bacteriophages such as M13 or f1. These pleiotropic phenotypes suggested a generalized defect in the maturation of translated proteins. Since the DsbB protein is predicted to lie in the membrane and to possess a disulfide bond active site, we tested whether these defects could be due to the accumulation of reduced forms of periplasmic proteins. Hence, we performed a quantitative analysis of periplasmic fractions prepared by osmotic shock (22) from DTT-sensitive mutant bacteria; the analysis used Ellman’s reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (23). Interestingly, the incubation of the periplasmic fraction with an excess of DTNB (0.8 mM) resulted in a significant increase in absorbance at 412 nm due to formation of the 5-thio-2-nitrobenzoate ion, as compared with the absorbance observed with the extracts prepared from wild-type bacteria (Table 2).

Oxidized DTT Can Suppress Phenotypic Defects Exhibited by dsbB Mutant Bacteria. We further confirmed that the phenotypes observed with dsbB null mutant bacteria were due to defects in disulfide bond formation by testing the ability of oxidized DTT to revert phenotypes such as hypersensitivity to benzylpenicillin. It has been shown that the penicillin-binding protein 4 (PBP4) has two disulfide bridges, which are predicted to be essential for its enzymatic activity (deacylation of benzylpenicillin) (24). Thus, we reasoned that hypersensitivity of dsbB mutant bacteria to benzylpenicillin could be due to the
Osmotic shock fluids (22) were prepared from the three isogenic strains harvested at OD595 = 0.4 in 50 mM sodium phosphate buffer, pH 7.5, containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride. DTTB prepared in the same buffer was added to each sample to a final concentration of 0.8 mM. The reaction at equilibrium was assessed by measuring the absorbance at 412 nm.

The lack of disulfide bridges in PBP4. In agreement with this assumption, we observed that the addition of submillimolar amounts of oxidized DTT (0.2 mM) to the growth medium restored the colony-forming ability of all six dsbB::Tnl0 mutant bacteria to the wild-type level on L-agar plates supplemented with benzylpenicillin (50 μg/ml). A similar phenotypic suppression by supplementation with oxidized DTT was obtained with bacteria carrying either the dsbA10::Tnl0 or the dsbA43::Tnl0 mutation. Taken together, these results suggest an important role of the DsbB protein in disulfide bond formation of a variety of proteins such as PBP4.

Disulfide Bond Formation of OmpA and β-Lactamase Is Altered in a dsbB Null Mutant. More direct evidence that DsbB can facilitate disulfide bond formation in vivo was sought by using two different secreted proteins as substrates, OmpA and β-lactamase. Proteins were labeled by pulse-chase and then directly treated with iodoacetamide (modifying cysteine residues) or pretreated with DTT in the control experiments, as described by Pollitt and Zalkin (19). The different forms of immunoprecipitated OmpA and β-lactamase were separated by SDS/PAGE. Fig. 3 shows the pattern of migration of reduced and oxidized proteins, corresponding respectively to retarded and faster-migrating species. Immediately after the chase (time 0 min), β-lactamase (Fig. 3A) or OmpA (Fig. 3B) was found to be mostly oxidized in wild-type bacterial backgrounds. In contrast, these proteins were found primarily in a reduced state in the null mutant strain dsbB− (DM391) (Fig. 3) and remained so, even after 10 min of chase. Similar results were obtained with bacteria carrying null mutations either in dsbA (SR1790) or in both dsbA and dsbB (SR1855).

Table 2. Accumulation of proteins with reduced cysteines as judged by reaction with Ellman’s reagent

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A412</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.081</td>
</tr>
<tr>
<td>dsbA10::Tnl10</td>
<td>0.331</td>
</tr>
<tr>
<td>dsbB36::Tnl10</td>
<td>0.305</td>
</tr>
</tbody>
</table>

| Osmotic shock fluids (22) were prepared from the three isogenic strains harvested at OD595 = 0.4 in 50 mM sodium phosphate buffer, pH 7.5, containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride. DTTB prepared in the same buffer was added to each sample to a final concentration of 0.8 mM. The reaction at equilibrium was assessed by measuring the absorbance at 412 nm.

Fig. 3. dsbB null mutants are defective in disulfide bond formation. Bacterial cultures CA8000 (dsbA+ dsbB+), SR1790 (dsbA10:: Tnl0), DM391 (dsbB36::Tnl0), and SR1855 (dsbA− dsbB−) were grown in M9 medium and either labeled with [35S]methionine for 45 or labeled for 45 sec followed by a 10-min chase with excess unlabeled methionine. Cells were lysed and immunoprecipitated with antisera to either β-lactamase (A) or OmpA (B). Autoradiograms of gel electrophoresis performed under nonreducing conditions are shown. The positions of oxidized (ox) and reduced (red) forms are indicated by arrows. To assay for β-lactamase, the four strains were first transformed with pHIP45 plasmid, which carries resistances to chloramphenicol and ampicillin, and then selected only for resistance to chloramphenicol.

Fig. 4. Northern analysis of dsbB transcripts. RNA was extracted from dsbB+ bacteria grown at 30°C or shifted to 42°C. Approximately 5 µg of total RNA was analyzed by the Northern blot technique, using as a probe the 32P-labeled 250-bp Nsi I–Hpa I DNA fragment (100 ng), which contains the amino end of the dsbB coding sequence.

Transcriptional Regulation of dsbB. Northern analysis of dsbB transcripts showed that the gene is transcribed as a monocistronic message of ~650 nt. As seen in Fig. 4, the dsbB mRNA levels did not substantially change after a shift up in temperature from 30°C to 42°C. Thus the dsbB gene does not appear to be regulated by heat shock.

Primer extension analysis was used to identify the 5′ termini of dsbB-specific mRNA species. It was found that dsbB transcription is initiated from two sites, designated as P1 and P2, located at 237 and 40 nt upstream of the putative ATG initiation codon (Fig. 5). Interestingly, the P1 transcriptional start site is located within the coding region of the nhaB gene. The -10 and -35 regions corresponding to the two transcriptional start sites do not resemble canonical Eσ70-transcribed promoters or those recognized by RNA polymerase coupled with any other known σ factors. Comparison of efficiency of promoter usage in different genetic backgrounds clearly shows that the more distal promoter P1 is more frequently utilized in wild-type bacteria, as judged by the relative amounts of transcripts. In contrast, transcripts initiated from the P2 start site were found to be more abundant when RNA was analyzed from isogenic bacterial strains carrying a null mutation in the rpoH (25), katF (26), or lrp (27) gene, encoding σ32, σA, and leucine response regulatory proteins, respectively. The efficiency of the P2 promoter usage in rpoHΔ (σ32) strains is higher than that found for the wild type or katF or lrp mutant bacteria (Fig. 5).

Fig. 5. Mapping of 5′ termini of dsbB transcripts: Primer extension reactions of total cellular RNA hybridized to a 32P-ended-labeled DNA oligonucleotide probe complementary to the sequence from nucleotide 64 to nucleotide 86 of the dsbB coding region. RNA was extracted from dsbB+ bacteria grown at 30°C, from wild type or from isogenic strains carrying null mutations in one of the genes rpoH, katF, and lrp. Lanes labeled G, A, T, and C correspond to the dideoxy sequencing reactions carried out, using the same oligonucleotide as primer. The asterisk and the arrow indicate the P1 and P2 transcriptional start sites, respectively.
The first approach was to select for genes whose presence on a multicopy plasmid enabled E. coli to survive the otherwise lethal effect of DTT (20 mM). All 24 clones isolated in this screen carried the dsbB gene (although in reconstruction experiments, we showed that the dsbA gene on the same multicopy plasmid enabled E. coli to form colonies, although smaller, in the presence of DTT). The second approach was to screen for insertional mutations which result in increased sensitivity to 7 mM DTT, a concentration that wild-type cells can tolerate. One of the major targets of insertional mutations was the dsbB gene. We found that inactivation of the trxA and trxB genes (8) and the dsbA gene (3, 4) also resulted in hypersensitivity to DTT. Thus, it appears that mutations in genes whose products are involved in maintaining an oxidation-reduction balance, either in the periplasm or in the cytosol, lead to DTT sensitivity.

In this work, we have presented different lines of evidence which demonstrate that in vivo there is a strong requirement for DsbA, DsbB, or both, to oxidize disulfide bonds of variety of proteins. Consequently, null mutations in the dsbB gene were shown to confer a highly pleiotropic phenotype similar to that observed in dsbA mutants, such as (i) inability to support plaque-forming ability of filamentous bacteriophages such as M13 [such a phenotype may be due to lack of F pili, as has been reported for dsbA mutants (3)]; (ii) reduced expression of periplasmic secreted proteins such as alkaline phosphatase; (iii) accumulation of reduced forms of the secreted proteins OmpA and β-lactamase in the periplasm, as judged by direct comparison of amounts of oxidized versus reduced forms of these proteins; (iv) sensitivity to drugs such as benzylpenicillin whose target is penicillin-binding protein 4, which has two disulfides bridged as part of its active site; and (v) overall accumulation of proteins with reduced cysteines in the periplasm as assayed with DTNB. The fact that these phenotypic defects could be overcome by supplementation with submillimolar amounts of oxidized DTT clearly showed that DsbB is involved in the oxidation of disulfide bonds of substrate proteins.

Since null mutations in either dsbA or dsbB alone or in both dsbA and dsbB exhibit identical phenotypes, most likely DsbA and DsbB are part of the same periplasmic pathway of disulfide oxidation-reduction. DsbA has been shown to be a disulfide oxidase capable of oxidizing substrate proteins, such as alkaline phosphatase and ribonuclease A (5), or reducing oxidized insulin (3); hence, it is likely that the role of DsbB is to oxidize reduced forms of DsbA, thus allowing recycling of active DsbA. It is also possible that DsbB is able to directly oxidize other periplasmic proteins in a substrate-specific or nonspecific manner. In this case, DsbA would be simply another substrate of DsbB. The independent study from Bardwell et al. (6) favors the model where DsbB preferentially oxidizes DsbA. Examination of the DsbB sequence leads to the prediction that it is an integral membrane protein with a CXXC motif in the periplasmic space, which is consistent with such a disulfide oxidase role for the protein. The putative membrane localization of DsbB raises the possibility of its coupling to a membrane electron transport system, thus enabling its reoxidation and recycling. If DsbA and DsbB proteins are indeed part of the same pathway, it is very likely that genes homologous to dsbB will be found in other organisms, as is the case for the dsbA gene (28–30).

An interesting point concerns the type of transcriptional regulation to which the dsbB gene is subjected. Although two promoters were found, neither was related to any known consensus promoter sequence. The P2 promoter was activated in the absence of gene products, such as σ32, σ4, or the leucine-responsive regulatory protein, involved in the transcription of groups of genes under special growth conditions. Perhaps, the P2 promoter is responsible for ensuring continuous transcription of the dsbB gene under a variety of stresses since, under the same conditions, transcription from the P1 promoter is drastically diminished.

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