Trends in Microbiology

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Review

Thiol-Disulfide Exchange in Gram-Positive Firmicutes

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Extracytoplasmic thiol-disulfide oxidoreductases (TDORs) catalyze the oxidation, reduction, and isomerization of protein disulfide bonds. Although these processes have been characterized in Gram-negative bacteria, the majority of Gram-positive TDORs have only recently been discovered. Results from recent studies have revealed distinct trends in the types of TDOR used by different groups of Gram-positive bacteria, and in their biological functions. Actinobacteria TDORs can be essential for viability, while Firmicute TDORs influence various physiological processes, including protein stability, oxidative stress resistance, bacteriocin production, and virulence. In this review we discuss the diverse extracytoplasmic TDORs used by Gram-positive bacteria, with a focus on Gram-positive Firmicutes.

Thiol-Disulfide Exchange in the Bacterial Cell Envelope

Protein disulfide bonds, which are covalent bonds formed between the sulfur atoms of cysteine residues, contribute to the folding of extracytoplasmic proteins. Disulfide bonds are formed by an oxidation reaction that involves the loss of two electrons; conversely, the bonds are broken by a reduction reaction in which cysteines gain electrons. TDORs catalyze the oxidation, reduction, and isomerization (rearrangement) of protein disulfide bonds. These enzymes typically have an active site with two cysteines separated by two amino acids (CXXC) and work by coupling the oxidation or reduction of their own active site cysteines with those in their substrates in a thiol-disulfide exchange reaction. While cytoplasmic TDORs, such as thioredoxin, maintain a reducing environment inside the cell, extracytoplasmic TDORs in the cell envelope can participate in a variety of physiological processes, including disulfide bond formation [1], oxidative stress resistance [2], energy generation [3], sporulation [4], and in many bacterial pathogens they are essential for virulence [5].

Recent investigations have revealed distinct trends in the extracytoplasmic TDORs used by different groups of bacteria. The best-characterized enzymes belong to the Dsb pathway of the Gram-negative bacterium *Escherichia coli*, which includes the well known disulfide catalyst DsbA (Box 1). In Gram-negative bacteria, disulfide bond formation occurs in the periplasm [6]. Gram-positive bacteria, in contrast, lack a periplasmic compartment and instead use TDORs located at the cell wall [7]. Possibly as a result of these fundamental differences, the TDORs of Gram-positive bacteria are not as well studied, and many have been identified only within the last 5 years (Table 1). As more information emerges, it is clear that there is considerable diversity in the enzymes used by the two Gram-positive phyla, Actinobacteria and Firmicutes. Here we review recent discoveries in thiol-disulfide exchange in the Gram-positive cell envelope, with a focus on one of the most diverse and least understood groups, the Gram-positive Firmicutes.

Trends in Thiol-Disulfide Exchange in Gram-Positive Bacteria

General trends in the distribution of Gram-positive TDORs can be visualized using a sequence similarity network. The network display nodes (sequences) connected by edges (lines) that

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At one time little was known about thiol-disulfide oxidoreductases (TDORs) in Gram-positive bacteria, but recent results revealed that these enzymes play important roles in diverse Gram-positive species.

Actinobacteria have TDORs that are essential for growth. Aerobic Firmicutes have similar TDORs, but they have few associated phenotypes.

Anaerobic Firmicutes use specialized TDORs to form disulfide bonds in bacteriocins.

Streptococcus gordonii SdbA catalyzes disulfide bonds, and mutants have a pleiotropic phenotype.

CcdA proteins and their associated TDORs contribute to virulence in *Bacillus* and *Streptococcus*.

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Box 1. Thiol-Disulfide Exchange in the *Escherichia coli* Cell Envelope

Gram-negative bacteria carry out a range of redox reactions in the periplasmic compartment between the inner and outer cell membranes. The best characterized TDORs in the cell envelope belong to the Dsb pathway of *E. coli* K12, which has become the archetype of oxidative protein folding (reviewed in [6,88]). DsbA and DsbB act as partners to catalyze disulfide bond formation, while DsbC and DsbD catalyze disulfide bond isomerization.

Disulfide bond formation is catalyzed by DsbA (Figure IA), a soluble periplasmic protein that introduces disulfide bonds into hundreds of substrates. The catalytic mechanism is a redox reaction that involves the transfer of two electrons from the substrate to DsbA. DsbA has a Cys30-Pro31-His32-Cys30 active site motif that contains a disulfide bond between Cys30 and Cys33. When DsbA encounters a substrate, it forms a transient intermolecular disulfide bond between its active site cysteine (C30) and the substrate. Following the reaction with DsbA, the substrate acquires a new disulfide bond and the active site of DsbA is reduced. DsbA is then reoxidized by its partner, the integral membrane protein DsbB. Under aerobic conditions, DsbB passes electrons to quinones and into the electron transport chain, while alternative electron acceptors, such a fumarate, are used under anaerobic conditions [6]. Upon transferring electrons to DsbB, DsbA is ready for another catalytic cycle.

DsbA is both highly reactive and nonspecific, and has a tendency to introduce incorrect disulfide bonds. These bonds are repaired by DsbC (Figure IB), a disulfide isomerase that rearranges disulfide bonds in proteins with multiple cysteines [6]. DsbC is maintained in a reduced state by its redox partner DsbD. DsbD plays a key role in all of the reducing (Figure IC) pathways of *E. coli* by passing electrons from the cytoplasm to the periplasm. In addition to DsbC, DsbD also donates electrons to a sulfenic acid reductase DsbG, and the cytochrome *c* maturation protein CcmG [65].

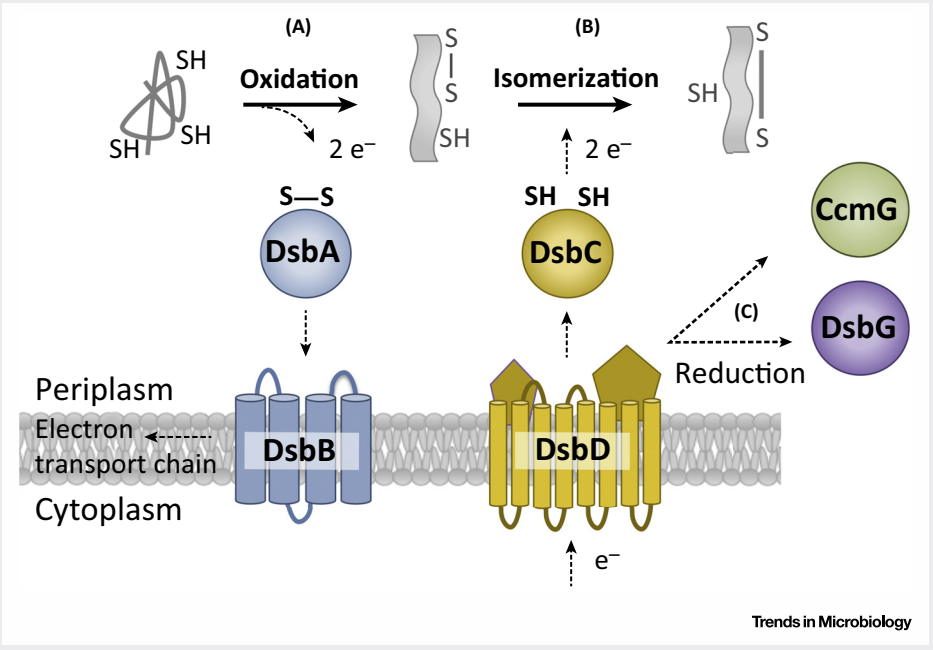

Figure I. Thiol-Disulfide Exchange in the *Escherichia coli* Cell Envelope. (A) Disulfide bond formation. (B) Repair of disulfide bonds.

reflect pairwise similarity determined by BLAST [8–10]. A sequence similarity network revealed that Gram-positive TDORs form functionally distinct clusters corresponding to their Pfam families [11]: DsbA-like TDORs (PF13462, thioredoxin_4), bacteriocin-related TDORs (PF00085, thioredoxin), CcdA enzymes involved in reducing pathways (PF02683, DsbD), and a diverse group that fall into the AhpC/TSA family (PF00578) (Figure 1, Key Figure).

Generally, the TDORs in each cluster of the sequence similarity network catalyze the same type of reaction (Figure 1B). For example, the DsbA-like TDORs catalyze disulfide bond formation [12,13]. Several bacteriocin-associated TDORs are also thought to catalyze disulfide bond