ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 5 & 6, 2006

Forum Review

Role of Membrane-Bound Thiol–Disulfide Oxidoreductases in Endospore-Forming Bacteria

MIRJA MÖLLER and LARS HEDERSTEDT

ABSTRACT

Thiol–disulfide oxidoreductases catalyze formation, disruption, or isomerization of disulfide bonds between cysteine residues in proteins. Much is known about the functional roles and properties of this class of redox enzymes in vegetative bacterial cells but their involvement in sporulation has remained unknown until recently. Two membrane-embedded thiol–disulfide oxidoreductases, CcdA and StoA/SpoIVH, conditionally required for efficient production of *Bacillus subtilis* heat-resistant endospores, have now been identified. Properties of mutant cells lacking the two enzymes indicate new aspects in the molecular details of endospore envelope development. This mini-review presents an overview of membrane-bound thiol–disulfide oxidoreductases in the Gram-positive bacterium *B. subtilis* and endospore synthesis. Accumulated experimental findings on CcdA and StoA/SpoIVH are reviewed. A model for the role of these proteins in endospore cortex biogenesis in presented. *Antioxid. Redox Signal.* 8, 823–834.

INTRODUCTION

Bacterial endospores are remarkable forms of life. In the state of an endospore, a bacterium can survive for many thousands of years and can stand conditions that kill most organisms. Endospores endure heat (boiling), desiccation, radiation, high hydrostatic pressure, organic solvents, and antibiotic agents to a much higher extent than vegetative bacteria (44). One should note that bacteria of only a few genera can form endospores and that only one spore is produced by each bacterial cell. The process of endospore formation, generally called sporulation, is a cell differentiation process induced under nutrient deprivation. When conditions again become favorable, the endospore can germinate into a vegetative cell that can grow and multiply (46). For recent reviews on endospores and sporulation see Refs. 6, 23, 28, 44, 48, 49, and 62. The biology and properties of endospores are of considerable interest from both the basic and applied science perspectives (43). Depending on the circumstances, endospores can cause, or be used to solve, practical problems. *Bacillus anthracis* endospores have again recently attracted much attention as a dangerous contagious agent. Endospores of bacterial species, such as *Clostridium botulinum, Clostridium tetani,* and *Bacillus cereus*, cause considerable problems within the food and health sectors of society. On the beneficial side, endospores are used, for example, as pesticides, in vaccine production, and as probiotic agents (30, 43, 56). Bacteria of the genera *Bacillus* and *Clostridium* are, like most other endospore-formers, Gram-positive bacteria. The vegetative cell of these bacteria has outside the cytoplasmic membrane a thick cell wall consisting mainly of peptidoglycan and teichoic acids. Gram-negative bacteria, such as *Escherichia coli*, have in addition an outer membrane that together with the cytoplasmic membrane enclose a defined compartment called the periplasm which contains the cell wall and a large number of proteins that function specifically in this subcellular compartment. A periplasm including the outer surface region of the cytoplasmic membrane, the cell wall, and a special repertoire of proteins is present in Gram-positive bacteria as well but its outmost border is not clearly defined (37, 40).

Bacillus subtilis is a rod-shaped, nonpathogenic, facultative aerobic bacterium commonly found in soil. It can develop natural competence which is the ability to take up naked DNA from the environment and by recombination in-

Department of Cell and Organism Biology, Lund University, Lund, Sweden.

corporate it into its own genetic material (17). A wealth of data is available on the genetics of sporulation, endospore structure and chemistry, and endospore maturation and germination of *B. subtilis* (2, 15, 16, 23, 28, 46, 49, 50).

Bacterial sporulation has for many years been used as a relatively simple but powerful model system to understand principles of intra- and intercellular signaling and molecular mechanisms of cell differentiation. Endospore maturation occurs inside the bacterial cell, called the mother cell. The process takes about 7 h to complete at 37°C and involves several morphological stages (called stage I to VII) (48). More than 400 genes of the total 4,100 protein coding genes in *B. subtilis* strain 168 are of importance for endospore biogenesis (35, 49). Recently it was discovered that membrane-bound thiol–disulfide oxidoreductases (TDORs) participate in endospore maturation in *B. subtilis* (22, 31, 58). Although the importance of disulfide bonds in proteins is well understood (9, 32, 55) and disulfide linkages between spore envelope proteins were indicated many years ago (2), little is known about protein thiol redox processes in sporulation. In this mini-review we present the newly identified *B. subtilis* TDORs important for sporulation. The possible role of the enzymes in spore synthesis in *B. subtilis* and other endospore-forming bacteria is discussed. TDORs constitute a potential drug target to inhibit endospore synthesis in bacteria and knowledge about the role of these proteins in sporulation provides further insight into the fascinating biological process of endospore maturation. To provide a background before we go into the details of endospore morphology and biogenesis, we briefly describe the general properties of TDORs and provide an overview on membrane bound TDORs in *B. subtilis*.

THIOL–DISULFIDE OXIDOREDUCTASES

TDORs catalyze the formation and breakage of disulfide bonds in proteins in cells. Disulfide bonds in proteins are under oxidative conditions formed without the involvement of TDORs but the rate of formation is usually too slow to be compatible with cell physiology (32, 57). Disulfide bonds play important roles in proteins; they help stabilize the tertiary or quaternary structure and they are part of catalytic or regulatory sites (55). Stable (permanent) disulfide bonds are not found in proteins in the cytoplasm of mesophilic organisms (grow at ≤50°C) nor in some thermophilic bacteria (3) because of the presence of reducing TDORs that break disulfide bonds (32). In mesophilic bacteria stable disulfide bonds in proteins are almost exclusively found on the outer side of the cytoplasmic membrane, in proteins associated with the cell envelope or excreted from the cell (9, 55). Cysteine residues in polypeptides secreted unfolded through the cytoplasmic membrane by the Sec translocon are in the reduced state during transport. Bacteria contain TDORs on the outer side of the cytoplasmic membrane (soluble in the periplasm or tethered to the membrane) that catalyze disulfide bond formation in newly secreted polypeptides.

TDORs contain one pair of cysteine residues in the active site. These two cysteinyls are either located in a sequence motif, –CysXaaYaaCys–, called a thioredoxin-like motif, or are far apart in the primary structure of the polypeptide to become close in the fully folded polypeptide. TDORs generally lack overall sequence similarity (55) but have in many cases a common three-dimensional fold called the thioredoxin-like fold consisting of a five stranded β-sheet surrounded by four α-helices (29). To catalyze the breakage of a disulfide bond the two active site cysteine residues in the TDOR must be in the reduced state. The thiolate group of one of the cysteine residues makes a nucleophilic attack on the disulfide bond in the substrate protein resulting in an intermolecular disulfide bond between the two proteins. A nucleophilic attack by the second thiolate in the TDOR breaks the intermolecular disulfide bond resulting in a disulfide bond in the TDOR and a dithiol (reduced) substrate protein. The disulfide in the TDOR must then be reduced by some means for the enzyme to perform another round of catalysis. Whether a TDOR has a reducing or an oxidizing function is determined in part by the redox potential of the active site, which is determined mainly by the pK_a value of the most exposed thiol (42). The N-terminal cysteinyl in the –CysXaaYaaCys– motif is generally more exposed to the surface of the protein and therefore is more nucleophilic than the second cysteinyl and has a lower pK_a value.

OVERVIEW OF MEMBRANE-BOUND THIOL–DISULFIDE OXIDOREDUCTASES IN *B. SUBTILIS*

Eight membrane-bound TDORs have thus far been identified in *B. subtilis* (Fig. 1). These proteins function in widely different cellular processes; synthesis of cytochrome *c* (20, 59), development of competence (21, 39), production of an antibiotic (13), and endospore maturation (22, 31, 58). Common to the eight proteins is that they function in thiol–disulfide oxidoreductase reactions on the outer side of the cytoplasmic membrane. The BdbA, BdbD, ResA, StoA, and YneN proteins constitute a family of bitopic membrane-bound TDORs (5, 20–22, 31, 39). They are anchored to the membrane by one N-terminal transmembrane α-helical segment and have a thioredoxin-like ecto-domain of 100–150 residues (including a –CysXaaYaaCys– sequence motif) exposed on the outer side of the membrane. The BdbB, BdbC, and CcdA proteins in contrast, are polytopic membrane proteins that oxidize or reduce other TDORs. BdbB and BdbC are paralogs having four transmembrane α-helical segments and a –CysXxaYaaCys– motif in the loop connecting transmembrane segments I and II exposed on the outer side of the membrane (Fig. 1). CcdA has six transmembrane segments and lacks a thioredoxin-like sequence motif (12, 60). A physiological function has been assigned to all the identified *B. subtilis* TDORs except for the YneN protein. An *yneN* knockout mutant is normal in sporulation (22).

OXIDIZING TDORS IN *B. SUBTILIS* MEMBRANES

BdbC together with BdbD constitutes a system that, with apparently broad substrate specificity, efficiently catalyzes the

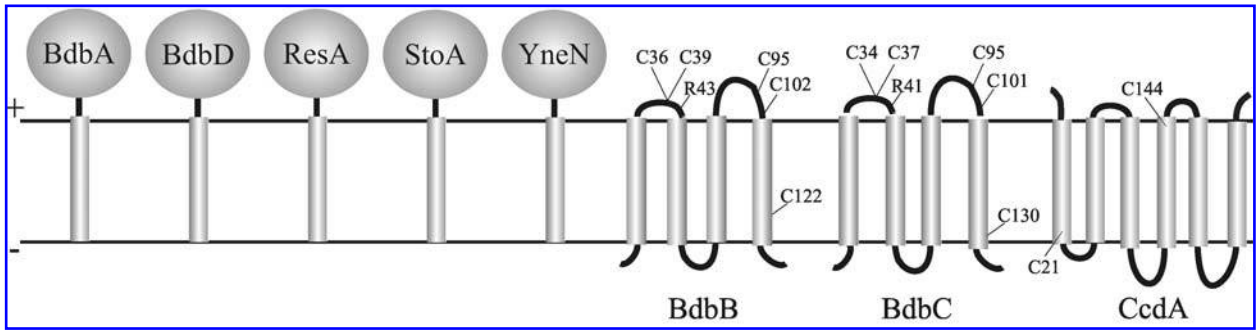

FIG. 1. Membrane-bound thiol–disulfide oxidoreductases identified in *B. subtilis*. Five similar bitopic thiol–disulfide oxidoreductases with a thioredoxin-like membrane-extrinsic domain containing a -CysXaaYaaCys- sequence motif are anchored to the membrane by one N-terminal transmembrane helix. Among the three polytopic proteins, BdbB and BdbC are closely related. Positions of conserved cysteine and arginine residues in the polytopic proteins are indicated. The *outer* and *inner sides* of the cytoplasmic membrane are indicated + and −, respectively.

formation of disulfide bonds in a variety of proteins on the outer side of the cytoplasmic membrane. Stable disulfide bonds have so far only been found in four *B. subtilis* proteins; three Com proteins and the lantibiotic Sublancin 168 that is secreted by vegetative cells. ComGC, ComGG, and ComEC function in the uptake of DNA from the environment (14, 17). The former two proteins are located in the cell wall of the bacterium. ComGC forms a pseudopilus complex, whereas ComEC forms a DNA uptake pore in the cytoplasmic membrane. Functional ComGC and ComEC each have one intramolecular disulfide bond. ComGG is a homodimer with one intermolecular disulfide bond. Sublancin 168 has two disulfide bonds and one thioether lanthionine bond (47). Disulfide bond formation in the Com proteins is catalyzed by BdbD on the outer side of the cytoplasmic membrane. Consequently, BdbD-deficient mutants are defective in competence (21, 39). BdbD apparently also catalyzes disulfide bond formation in apo-cytochrome *c* polypeptides and, as will be presented later in this mini-review, in not yet identified component(s) important for spore cortex formation. The gene for Sublancin 168, *sunA*, is encoded by the prophage SPβ which is found in the chromosome of many *B. subtilis* strains. Clustered with *sunA* on the prophage genome are the *bdbA* and *bdbB* genes which encode a BdbD and a BdbC paralog, respectively. BdbA and BdbB probably function together to catalyze disulfide bond formation in the Sublancin 168 polypeptide (13).

The sequence of the membrane-extrinsic domain of BdbD is similar to bacterial thioredoxin and contains a –CysProSer-Cys– active site sequence (21, 39). *B. subtilis* BdbD is an analog of the well-characterized water-soluble *E. coli* DsbA protein that functions as a general disulfide oxidase in the periplasm (32, 55). Reduced DsbA is oxidized by DsbB which is located in the *E. coli* cytoplasmic membrane. DsbB is in turn oxidized by the reduction of quinone (32). *B. subtilis* BdbB and BdbC are both *E. coli* DsbB homologs and the available experimental data suggests that they function to oxidize BdbA and BdbD, respectively (21, 39). Four cysteine residues in the two extracytoplasmic domains and an arginine residue are conserved in these proteins (Fig. 1). Two of the cysteine residues in BdbB/BdbC are located in a thioredoxin-like motif. The conserved arginine residue is located close to this motif and, based on the similarity to *E. coli* DsbB, is probably important for the interaction of these TDORs with menaquinone in the *B. subtilis* membrane.

REDUCING TDORS IN *B. SUBTILIS* MEMBRANES

CcdA, ResA, and StoA are *in vivo* thought to break or isomerize disulfide bonds. The CcdA protein is similar to the central part (the β-domain) of *E. coli* DsbD and has two conserved cysteine residues both in a –ProCys– motif (Fig. 2). One such motif is located towards the cytoplasmic side in transmembrane segment I and the other is in transmembrane segment IV, close to the extracytoplasmic side. How these two cysteine residues, that are required for the function of CcdA and DsbD (12), form an active site is unknown. *E. coli* DsbD is much larger than CcdA. DsbD has an N-terminal α-domain with an immunoglobulin-like fold, and a C-terminal γ-domain with a thioredoxin-like fold and a –CysXaaYaa-Cys– motif (Fig. 2). *E. coli* DsbD functions as a transmembrane thiol disulfide transporter and reduces TDORs in the periplasm, the water-soluble DsbC and DsbG and the membrane-bound CcmG (32). Electrons donated by thioredoxin in the cytoplasm are transported across the membrane via the β-domain to the γ-domain and finally to the α-domain which is the electron donor to the periplasmic substrates of DsbD (33). It has been demonstrated with recombinant DsbD that the three domains, when synthesised individually, assemble into a functional enzyme in the membrane. *B. subtilis* CcdA can be viewed as a naturally occurring isolated β-domain and an evolutionary precursor to the DsbD-like proteins (34).

Based on the similarities to the well-characterized *E. coli* DsbD protein (34), *B. subtilis* CcdA most likely accepts electrons from thioredoxin (TrxA) in the cytoplasm and transfers them to TDORs on the outer side of the membrane to reduce disulfide bonds (Fig. 2). The ResA protein is thought to be reduced by CcdA and functions in cytochrome *c* synthesis (20). The membrane extrinsic domain of ResA has a thioredoxin-like fold (10). *B. subtilis* contains four *c*-type cytochromes that all are membrane bound (63). On the outer side of the cytoplasmic membrane ResA is supposed to keep the two cys-

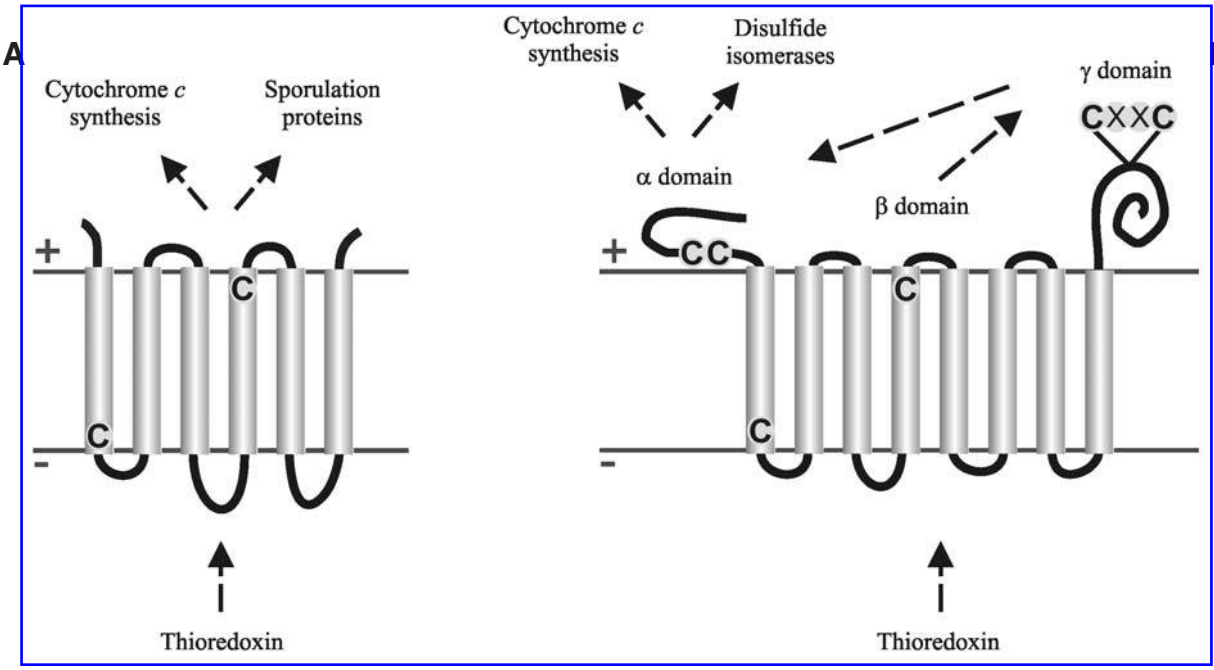

FIG. 2. Comparison of the overall structure and function of (A) *B. subtilis* CcdA and (B) *E. coli* DsbD. The approximate positions of conserved cysteine residues are indicated. The direction and pathway of electron flow is indicated by *arrows*. The *outer* and *inner sides* of the cytoplasmic membrane are indicated + and −, respectively. The figure is based on a drawing published by Katzen *et al.* (34).

teine residues in the heme binding site (–CysXaaYaa-CysHis–) of apocytochrome *c* reduced to allow heme to become covalently bound (10, 20). ResA can be regarded as corresponding to the γ-domain of DsbD and might form a stable functional complex with CcdA in the membrane. A *B. subtilis* protein corresponding to the α-domain of DsbD has not been found. CcdA-like proteins are also found in chloroplasts (45) and some bacteria, such as *Rhodobacter capsulatus*, contain both a CcdA and a DsbD (12).

CcdA was the first membrane-bound TDOR to be identified in a Gram-positive bacterium (60). The *ccdA* gene was found mutated in *B. subtilis* isolates defective in cytochrome *c* synthesis. Subsequent studies showed that CcdA-deficient strains are also defective, but not completely blocked, in synthesis of heat-resistant endospores (58). This defect is not due to cytochrome *c* deficiency and indicated multiple roles of CcdA (58). As a result of systematic inactivation of *B. subtilis* genes of unknown function and those encoding putative TDORs in *B. subtilis*, the StoA protein was found to function in endospore synthesis (22, 31). Endospores produced by CcdA- and StoA-deficient strains carry envelope defects. Due to its involvement in sporulation, the StoA protein is also called SpoIVH (31). The remaining part of this mini-review concerns the role of CcdA and StoA/SpoIVH in endospore synthesis in *B. subtilis* and related Gram-positive bacteria.

ENDOSPORE STRUCTURE AND COMPOSITION

Endospores have several layers, or shells, that protect the central spore core that harbors the chromosome and corresponds to the cytoplasm of the vegetative cell (Fig. 3, left hand panel). A membrane, analogous to the cytoplasmic membrane of the vegetative cell, encloses the core. A thick peptidoglycan layer, called the cortex, is located outside this membrane. The cortex layer is missing in the heat-sensitive spores produced by CcdA- and StoA-defective mutants. The next layer of the endospore is the coat which exhibits an outer and an inner coat layer. The coat is a major protectant of the spore against damaging chemicals and enzymes (15). The cortex and the coat layers are needed for two apparently opposing functions; in addition of being protective structures essential for resistance and dormancy of the endospore they are critical for the response of spores to molecules that trigger the germination process (15, 46). Endospores of some bacteria (e.g., *B. anthracis*) have an additional outermost layer called exosporium. The coat contains at least 40 different proteins, many of which are rich in cysteine residues. Coat proteins in the mature endospore are to some extent locked together by different types of covalent linkages. Some of these links appear to be disulfide bridges (2, 36). The formation of these putative bridges is expected to be catalyzed by TDORs but there is no evidence indicating that CcdA or StoA is involved in that process.

ENDOSPORE BIOGENESIS

Sporulation is an irreversible process. Once initiated the cell cannot turn back to the vegetative state unless sporulation is completed. The onset of sporulation in a bacterial culture is therefore a critical decisive step. To access more nutrients and eventually delay initiation of sporulation some cells in the population cannibalize other bacteria (27). Spo0A is

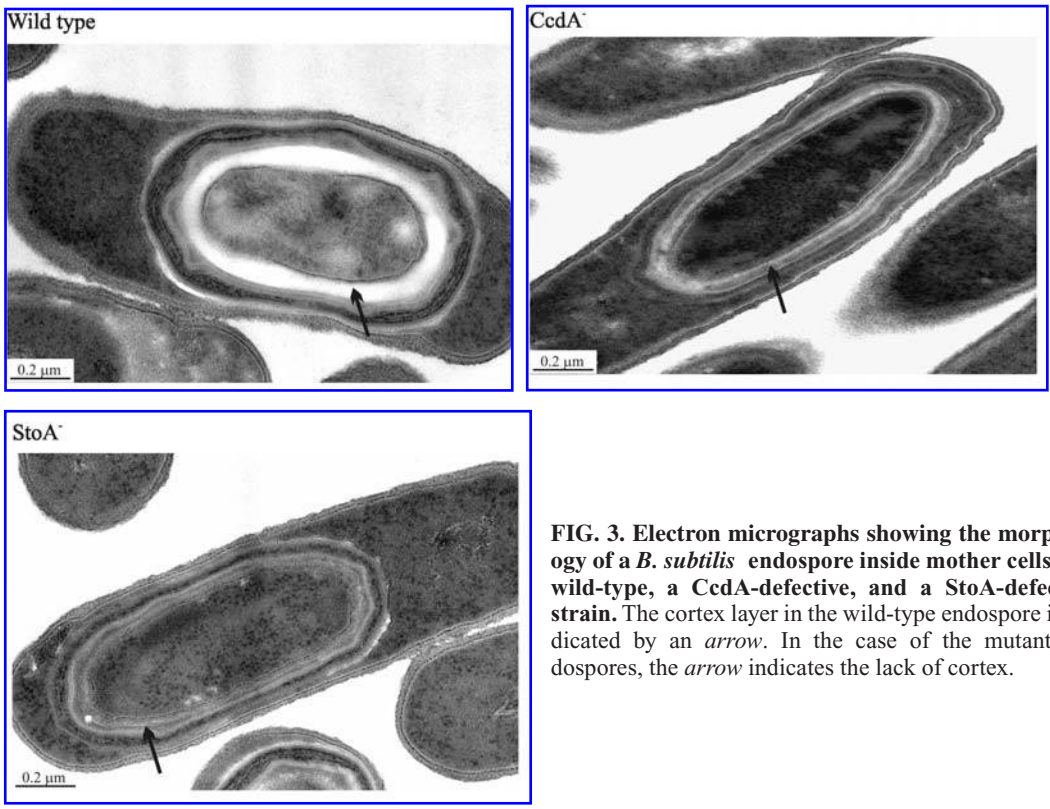

FIG. 3. Electron micrographs showing the morphology of a *B. subtilis* endospore inside mother cells of a wild-type, a CcdA-defective, and a StoA-defective strain. The cortex layer in the wild-type endospore is indicated by an *arrow*. In the case of the mutant endospores, the *arrow* indicates the lack of cortex.

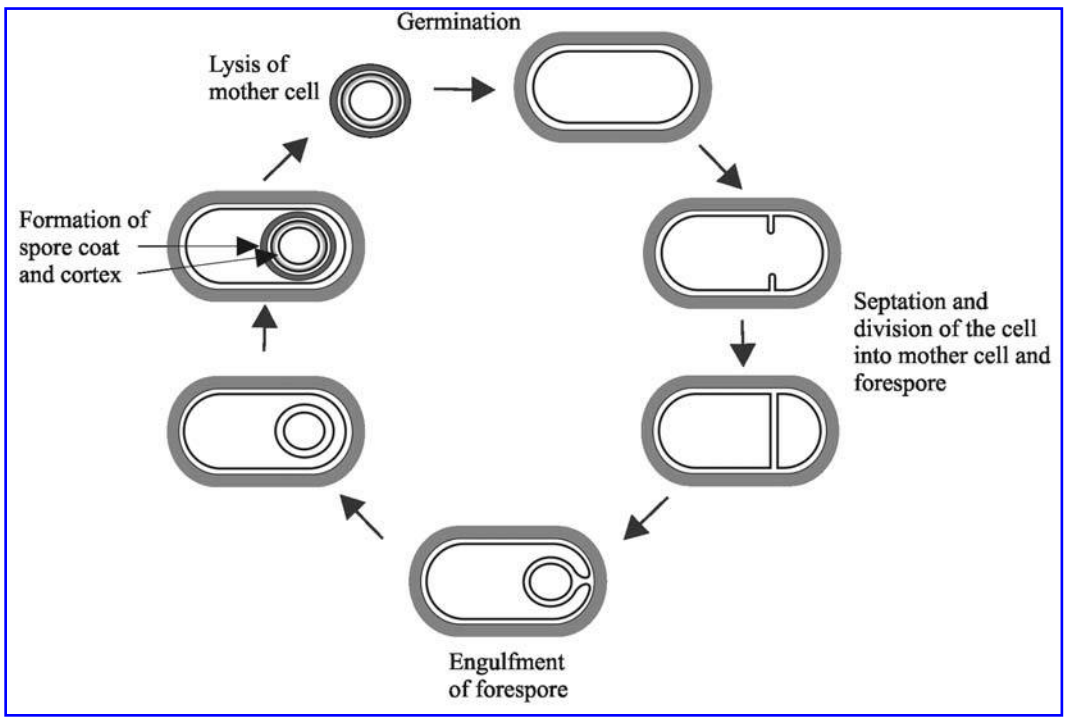

FIG. 4. Schematic drawing of endospore morphogenesis in bacteria. Early after initiation of sporulation in a vegetative cell an asymmetric septum is formed. The forespore receives a copy of the chromosome and is then engulfed by the mother cell resulting in a "cell within a cell." The endospore protective layers, cortex, and coat consisting mainly of peptidoglycan and protein, respectively, are then assembled. The mature endospore, which is metabolically dormant, is finally released by lysis of the mother cell. By the process of germination and outgrowth the endospore is transformed to a vegetative cell.

the master transcription regulator for initiation of sporulation. It is activated by phosphorylation on an aspartate residue, an event that in turn is controlled by a protein phosphorelay that integrates physiological and environmental signals (23, 49). Active Spo0A triggers asymmetric division of the cell and induce key transcription regulators. Asymmetrical cell division early during sporulation produces two cells with different fates; the mother cell and the smaller forespore (Fig. 4). The forespore develops into an endospore while the mother cell lyses after spore formation is complete. Until then the mother cell is necessary for the maturation of the spore. Approximately 1 h after the asymmetric division of the cell, the forespore is engulfed by the mother cell. This results in an organelle (the forespore) enclosed by two membranes within the mother cell cytoplasm. The cortex is deposited between these two membranes, whereas the coat is assembled on the outer side of the outer forespore membrane. Endospore maturation requires communication between the forespore and the mother cell as well as directed transport of biosynthetic material from the mother cell to the forespore.

Sporulation includes a cascade of sigma factors that mediate coordinated temporal expression of genes in the mother cell and the forespore (28, 48, 49). σ^F and σ^E are produced in inactive form in the predivisional cell. After division of the cell into mother cell and forespore, σ^F is by a transmembrane mechanism activated in the forespore. σ^F in turn controls activation of σ^E in the mother cell. After engulfment of the forespore, σ^G is activated in the forespore. Activation of σ^G is under the dual control of events in the forespore and in the mother cell by the actions of σ^F and σ^E. Active σ^G is needed for the activation of the mother cell specific sigma factor σ^K whose activation also is under the control of σ^E. The main vegetative sigma factor, σ^A, continues to be active in the mother cell and the forespore both before and after engulfment.

The σ^F regulon is relatively small but includes genes for many important regulatory factors. σ^E directs the transcription of an unusually large regulon comprising at least 262 genes (18). Among these are genes encoding proteins that function in mother cell metabolism, synthesis of spore cortex, and early stage coat assembly. The DNA-binding protein SpoIIID is under the control of σ^E and regulates, positively or negatively, the transcription of many other σ^E-dependent genes (18). The σ^G regulon comprises genes for proteins that accumulate in the forespore and protect the genome of the mature endospore from damage and are needed for germination. Expression of most genes for spore coat proteins is directed by σ^K. In general, protein components of the developing spore are synthesised in the compartment where they function (48). However, those active in the forespore intermembrane space are exceptional because they are entirely or in part exported from the mother cell cytoplasm or the forespore core compartment.

CORTEX STRUCTURE AND SYNTHESIS

The spore cortex is like the cell wall of vegetative cells composed of peptidoglycan (24, 38). Peptidoglycan determines cell shape and provides resistance against the turgor of the cell cytoplasm. The basic structure of peptidoglycan is long glycan strands, made up of alternating disaccharide residues of *N*-acetyl-glucosamine (NAG) and *N*-acetyl-muramic acid (NAM), cross-linked via peptide side chains (Fig. 5). Each NAM residue carries a peptide side chain of which approximately 40% are involved in interstrand peptide cross-linking to form a network of peptidoglycan chains. The peptidoglycan of the endospore is composed of two adjacent structures; a thin inner layer called the germ cell wall and a thicker outer layer which is the cortex. Germ cell wall peptidoglycan is very similar to that of the vegetative cell wall and serves as primer for cell wall synthesis during spore outgrowth (38, 51, 53). Cortex peptidoglycan differs from peptidoglycan of vegetative cells by the absence of teichoic acids and fewer peptide side chains and less cross-linking of the glycan strands. Furthermore, about half of the NAM residues in the cortex lacks a peptide side chain and are ring closed to form muramic-δ-lactam (MAL)

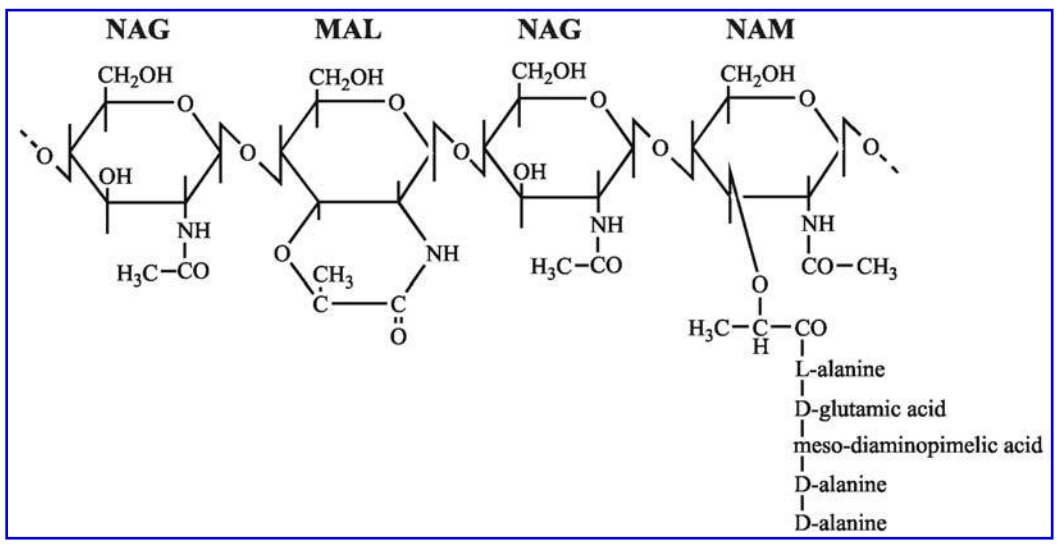

FIG. 5. Unit of glycan strand in endospore cortex peptidoglycan. Alternating residues of *N*-acetyl-glucosamine (NAG), muramic-δ-lactam (MAL), and *N*-acetyl-muramic acid (NAM) are β 1–4 linked to form a linear polymer. Different glycan strands are cross-linked via peptide chains of NAM residues.

(52, 53). During spore germination the cortex is rapidly degraded by autolysins (4, 25, 41). Absence of cortex structure in endospores of mutants as determined by electron microscopy thus can be the result of defective cortex synthesis or premature hydrolysis of cortex or both.

Synthesis of spore peptidoglycan starts about 3 h after the onset of sporulation and occurs in the space between the inner and outer membranes of the forespore (50). Cell wall precursors, in the form of NAG–NAM–pentapeptides, are made in the cytoplasm of the mother cell, and to some extent probably also in the forespore, and are transported to the intermembrane space linked to Lipid II. How cortex synthesis is accomplished from these precursors is not understood in any detail and little information is available about proteins present in the intermembrane compartment during cortex synthesis (16, 50, 61). Understanding the roles of CcdA and StoA in endospore synthesis would lead to better knowledge about cortex biogenesis. The spore germ cell wall seems synthesized by proteins on the surface of the inner forespore membrane (26). Cortex is thought to be synthesized with germ cell wall as template by proteins present on the surface of the outer forespore membrane (50). Based on knowledge available on vegetative cell wall synthesis morphogenic proteins, such as SpoVE (a RodA paralog), most likely dictate the formation of the cortex peptidoglycan three-dimensional architecture and activities of various penicillin-binding proteins that are glycosyl transferases, transpeptidases, and peptidases in peptidoglycan synthesis (8). A handful of different penicillin-binding proteins have been found associated with *B. subtilis* spores. Several of these, for example PBP1 (PonA), PBP2a (PbpA), and PBP3 (PbpC), apparently function both in vegetative cell wall synthesis and spore cell wall synthesis (7). Others, such as SpoVD (a PBP3 paralog), PBP5* (DacB), and DacF, are specific for cortex synthesis (11, 16). Notably, membrane proteins like SpoVE and SpoVD contain cysteine residues that are conserved in orthologous proteins in other spore-forming bacteria. The localization of individual membrane-bound proteins to the inner or outer forespore membrane can be deduced from the sigma-factor dependency of the corresponding genes. For example, expression of the *spoVE, spoVD,* and *dacB* genes is dependent on σ^E activity and therefore SpoVE, SpoVD, and DacB are most likely synthesized in the mother cell and inserted into the outer forespore membrane. DacF is probably located in the inner forespore membrane since the *dacF* gene is under σ^F control.

WHAT ARE THE ROLES OF SToA AND CcdA IN ENDOSPORE MATURATION?

CcdA- and StoA-negative mutants produce heat-sensitive endospores lacking cortex as determined by electron microscopy (22, 31) (Fig. 3). The *ccdA* gene is transcribed from a relatively weak σ^A-promoter, whose activity increases in early stationary phase (58). The amount of membrane bound CcdA polypeptide peaks about 1 h into stationary phase (i. e., at about the onset of sporulation) (58). Synthesis of CcdA and its subcellular localization during sporulation has not been investigated. It seems likely that CcdA is present in one or perhaps both forespore membranes. In the outer forespore membrane CcdA could transport electrons from thioredoxin in the mother cell to components in the forespore intermembrane space.

The *stoA* gene is located in a di-cistronic operon together with the *ykvU* gene (Fig. 6). YkvU is predicted to be a membrane protein with twelve transmembrane segments and, interestingly, shows sequence similarity to the SpoVB protein that is required for cortex formation (54). YkvU contains four cysteine residues, one of which seems conserved in SpoVB, and localizes to the sporulation septum and is later found in the outer forespore membrane (19). These facts combined suggest that StoA and YkvU functionally interact. However, as determined from the properties of knockout mutants, YkvU is not very important for synthesis of heat-resistant spores (22, 31). Recently it was shown that YkvU functions in germination but the precise role of the protein is unclear (1).

The *ykvU–stoA* operon has two promoters, one upstream of *ykvU* and one internal promoter upstream of *stoA* (Fig. 6) (31). The *ykvU* promoter is under the control of σ^E and is negatively regulated by the SpoIIID DNA-binding protein. The *stoA* promoter is under the control of σ^G. This transcriptional organization suggests that during endospore development *stoA* is expressed both in the mother cell and in the forespore, whereas *ykvU* only is expressed in the mother cell. Moreover *ykvU–stoA* di-cistronic mRNA is probably produced only for a short period as its expression is repressed by the SpoIIID protein in the mother cell compartment. StoA protein synthesized in the mother cell would be incorporated into both the cytoplasmic membrane of the mother cell and the outer membrane of the forespore if there is no mechanism of directed membrane protein insertion. When synthesized in the forespore, StoA will only be localized to the inner forespore membrane. Irrespective of where StoA is synthesized the thiol–disulfide catalytic domain will be located in the intermembrane space of the forespore. Imamura *et al.* (31) demonstrated that if *stoA* is expressed from the σ^E promoter alone (StoA only produced in the mother cell), heat-resistant endospores are formed at a very low efficiency. If the gene is expressed from a σ^G promoter (StoA produced in the forespore), near wild-type amounts of heat-resistant endospores are formed. Thus, for efficient cortex synthesis it seems more important to have StoA close to the inner than to the outer forespore membrane. Imamura *et al.* also showed that StoA is

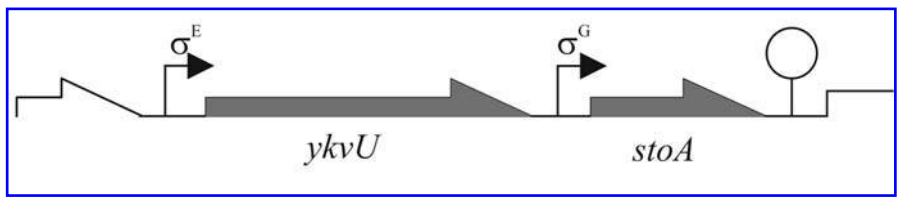

FIG. 6. The *ykvU–stoA* operon in the *B. subtilis* chromosome. *Bent arrows* denote promoters dependent on the indicated sigma factors for activity. A transcription terminator is indicated after *stoA*. Alternative names for the *stoA* gene is *ykvV* and *spoIVH*.

functional in the intermembrane space also without its membrane anchor (31).

The endospore developmental program in CcdA- and StoA-deficient cells is essentially normal as judged from analysis of the sigma-factor cascade (22, 58) except perhaps for σ^K dependent genes (31, 58). Endospore maturation in the absence of CcdA results in 1%–5% of the normal yield of heat-resistant spores produced from a culture. Lack of StoA brings this number down to 0.05%. A CcdA StoA double-deficient mutant shows an approximately cumulative defect producing about 0.006% heat resistant endospores compared to wild type. The heat-sensitive endospores of the respective single mutant and the double mutant lack visible cortex (22), but the germ cell wall seems intact (unpublished data) as determined by electron microscopy of negatively stained spores. The morphological data suggest that StoA and CcdA are involved in the same process in endospore maturation.

BdbC and BdbD are not required for efficient production of normal endospores in *B. subtilis*. However, BdbD- or BdbC-deficiency suppresses the sporulation defect of CcdA- and StoA-negative mutants (21, 22). In other words, the requirement of CcdA and StoA for efficient synthesis of heat-resistant spores is conditional. This finding together with the assumed disulfide-reductive properties of CcdA and StoA, indicate that functional BdbCD is present in the intermembrane space of the forespore and there catalyze disulfide bond formation in components important for cortex synthesis. CcdA and StoA apparently counteract BdbCD activity and break these bonds. The much stronger effect on cortex synthesis by StoA-deficiency compared to CcdA-deficiency indicates that CcdA has a more peripheral role. The leaky phenoptype of CcdA- and StoA-deficient cells with the production of a low amount of heat-resistant endospores, probably result from the fact that the BdbCD system is not 100% effective in introducing disulfide bonds in target proteins and that disulfide bonds can be broken in the forespore envelope by CcdA- and StoA-independent factors or proteins that to some degree substitute for the function of CcdA or StoA. We have not detected a CcdA paralog in *B. subtilis* and only found suppressor mutations in *bdbC* or *bdbD*. Furthermore, ResA cannot complement for StoA deficiency (22).

MEMBRANE-BOUND THIOL–DISULFIDE OXIDOREDUCTASES IN ENDOSPORE-FORMING BACTERIA OTHER THAN *B. SUBTILIS*

Based on the physiological role of StoA one would expect to find this protein only in endospore-forming bacteria that contain BdbD or a similar oxidizing TDOR. Except for the case of *Clostridium acetobutylicum*, all available complete genome sequence of endospore forming Gram-positive bacteria (TIGR database; June 2005) contain genes for membrane-bound TDORs similar to one or several of those (Fig. 1) present in *B. subtilis*. *Bacillus* species contain at least one gene encoding a protein with similarity to either *B. subtilis* BdbB or BdbC and located just upstream of a gene for a BdbA or BdbD homolog. Interestingly, all *Bacillus* species, except *B. subtilis* and *B. licheniformis,* have a *bdbA*-like gene adjacent to a *bdbC*-like gene. In *B. cereus* and *B. anthracis,* the *bdbD*-like gene is located downstream of a gene for a putative penicillin-binding protein (a PBP1/PBP2/PBP4 ortholog), which, however, contains no cysteine residues. Proteins similar to BdbB and BdbC are not found in *Clostridium* species.

For the TDORs with a single transmembrane segment (those similar to *B. subtilis* ResA, StoA, BdbA, BdbD, or YneN), sequence comparisons provide in most cases little information about the specific function. However, the function can in some cases be determined from the gene context. For example, the *resA* gene is only found in aerobic cytochrome *c*-containing bacteria and in a *resA–resB–resC–resD–resE* gene cluster. Genes encoding proteins with sequence similarity to *B. subtilis* StoA and YneN are widespread among endospore-formers but only in the case of *B. licheniformis* can *stoA* be identified from the gene context (colocalization with *ykvU*). In the other cases one can only speculate whether the encoded protein corresponds to YneN, StoA, or to a TDOR with some other function. Genes encoding YneN-like proteins are found in one of three alternative contexts. In some bacteria the gene is located downstream of the *citB* gene (encodes aconitase). This is the case in *B. subtilis*, *B. licheniformis,* and *Oceanobacillus iheyensis*. In other bacteria, such as *B. cereus, B. anthracis, Geobacillus kaustophilus,* and *C. tetani*, the gene is located adjacent to a gene for a CcdA-like protein. *G. kaustophilus* has two variants of genes encoding StoA/YneN-like proteins. In one case the gene is cotranscribed with the *ccdA*-like gene and in the other case it is transcribed in the opposite direction of another *ccdA*-like gene. An extreme variant is found in *Clostridium perfringens* which apparently contains a CcdA-YneN/StoA fusion protein. The third variant is bacteria in which the gene for YneN shows a random localization on the chromosome.

Two chromosomal genes encoding proteins similar to *B. subtilis* CcdA seem to be a common feature of endospore forming bacteria and is found in for example *B. licheniformis, B. cereus,* and *B. anthracis*. Notably, *G. kaustophilus* contains three such genes. In *Bacillus* species one of the genes is located in the same context as the single *B. subtilis ccdA* gene, whereas the second gene is linked to a gene for an YneN-like protein as was mentioned before. The CcdA- and YneN-like proteins encoded by the same operon most likely function as a pair; the CcdA ortholog would be the electron donor to the thioredoxin domain of the YneN ortholog.

FUNCTIONAL MODEL FOR SToA AND CcdA IN ENDOSPORE SYNTHESIS

During endospore maturation, the forespore can be regarded as an intracellular organelle. Membrane proteins in the two membranes of the forespore are presumably in a more reducing environment compared to those in the cytoplasmic membrane of the mother cell or the vegetative *B. subtilis* cell. This difference is due to the reducing environment of the mother cell cytoplasm that completely surrounds the forespore. Another notable aspect is that substrates for biosynthetic purposes and energy metabolism in the forespore must be transported from the mother cell cytoplasm to the forespore. A multitude of enzyme activities and transport func-

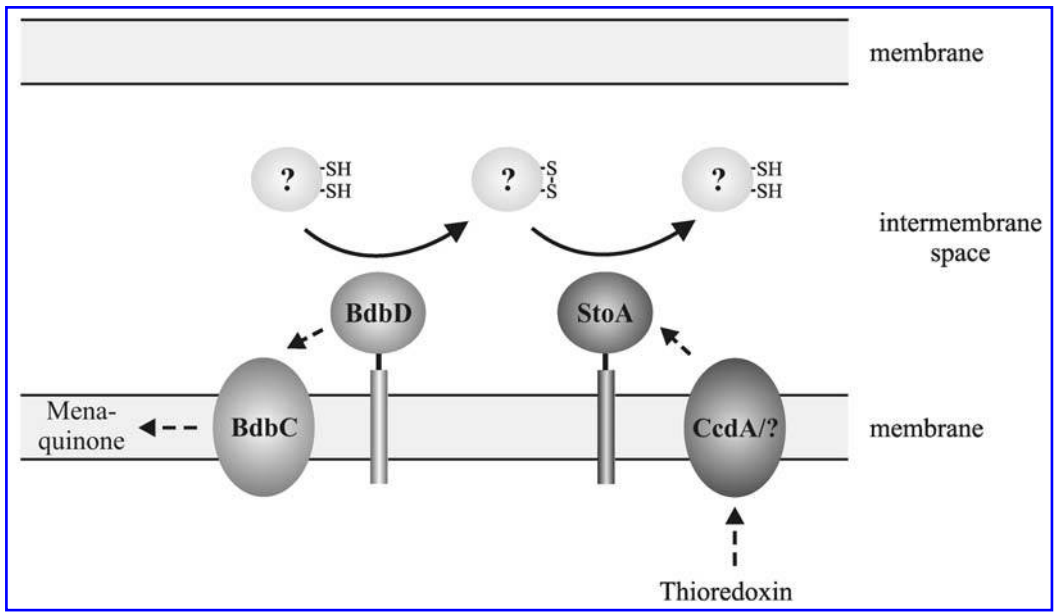

FIG. 7. Topology and suggested function of CcdA and StoA in the envelope of the forespore during endospore maturation. Not yet identified StoA substrate proteins in the intermembrane space are indicated by *question marks* (CcdA is a postulated StoA electron donor). *Arrows with interrupted lines* indicate the direction of electron flow. Disulfide bonds in (or between) proteins are formed by the action of BdbD and this prevents formation of the cortex resulting in heat-sensitive endospores. StoA restores cortex synthesis by breaking protein disulfide bonds. See text for further details.

tions are therefore probably required for cortex synthesis in the intermembrane space.

Taken together, the available data on StoA strongly indicate that its oxidoreductase domain is located in the forespore intermembrane compartment where peptidoglycan is polymerized to form the thick cortex layer of the mature endospore (Fig. 7). StoA most likely catalyzes the breakage of disulfide bonds in (or between) proteins that might function in transport, in assembly of protein complexes, in regulation of cortex synthesis, or directly in cortex synthesis. Disulfide bonds in these proteins are formed by the action of the BdbCD system and somehow block or interfere with their function. In the absence of a thiol-oxidizing system StoA plays no critical role for normal endospore synthesis. The disulfide bonds formed by the action of BdbCD and broken by StoA are presumably located in protein domains exposed to the intermembrane space of the forespore. Proteins such as SpoVD and SpoVE have like StoA a sporulation-specific and important role in cortex peptidoglycan synthesis. SpoVD and SpoVE contain cysteine residues predicted to be exposed to the inter-membrane space of the forespore. The importance of these cysteine residues for function are not known.

In order for StoA to act as an efficient reducing TDOR in the intermembrane space of the forespore there must be an electron donor protein (Fig. 7). CcdA possibly has this function. Maturation of endospores to a heat-resistant state occurs at 1%–5% efficiency compared to normal in the absence of CcdA. In the absence of StoA the efficiency is 100-fold lower and in a CcdA StoA double-deficient mutant it is 1000-fold lower. These relative numbers are reasonable if StoA directly interacts with the target proteins to function as a specific disulfide reductase. In the absence of CcdA, the StoA protein would still be active but not be efficiently re-reduced. This would result in a less accentuated phenotype compared to that resulting from StoA-deficiency. The challenge is now to identify the cysteine-containing proteins that in the intermembrane compartment of the forespore are kept reduced by StoA and demonstrate thiol–disulfide exchange *in vivo* between StoA and CcdA and between StoA and these proteins.

ACKNOWLEDGMENTS

The authors thank Lydur Erlendsson and Lise Peterson for many discussions and input. Work done in our laboratory was supported by the Swedish National Research School in Genomics and Bioinformatics and grants 621–2001–3125 and 621-2004-2762 from the Swedish Science Research Council.

ABBREVIATIONS

MAL, muramic-σ-lactam; NAG, *N*-acetyl-glucosamine; NAM, *N*-acetyl-muramic acid; TDOR, thiol–disulfide oxidoreductase.

REFERENCES

1. Aertsen A, Opstal IV, Vanmuysen SC, Wuytack EY, and Michiels CW. Screening for *Bacillus subtilis* mutants deficient in pressure induced spore germination: identification of *ykvU* as a novel germination gene. *FEMS Microbiol Lett* 243: 385–391, 2005.
2. Aronson AI and Fitz-James P. Structure and morphogenesis of the bacterial spore coat. *Bacteriol Rev* 40: 360–402, 1976.

3. Beeby M, Connor BDO, Ryttersgaard C, Boutz DR, Perry LJ, and Yeates TO. The genomics of disulfide bonding and protein stablization in thermophiles. *PLOS Biology* 3:e309, 2005.
4. Boland FM, Atrih A, Chirakkal H, Foster SJ, and Moir A. Complete spore cortex hydrolysis during germination of *Bacillus subtilis* 168 requires SleB and YpeB. *Microbiology* 146: 57–64, 2000.
5. Bolhuis A, Venema G, Quax WJ, Bron S, and van Dijl JM. Functional analysis of paralogous thiol–disulfide oxidoreductases in *Bacillus subtilis*. *J Biol Chem* 274: 24531–24538, 1999.
6. Brun YV and Shimkets LJ. *Prokaryotic Development*. Washington, DC. American Society for Microbiology Press, 2000.
7. Buchanan CE and Neyman SL. Correlation of penicillin-binding protein composition with different functions of two membranes in *Bacillus subtilis* forespores. *J Bacteriol* 165: 498–503, 1986.
8. Cabeen MT and Jacobs-Wagner C. Bacterial cell shape. *Nature Rev Microbiol* 3: 601–610, 2005
9. Creighton TE, Zapun A, and Darby NJ. Mechanisms and catalysts of disulfide bond formation in proteins. *Trends Biotechnol* 13: 18–23, 1995.
10. Crow A, Acheson RM, Le Brun NE, and Oubrie A. Structural basis of redox-coupled protein substrate selection by the cytochrome *c* biosynthesis protein ResA. *J Biol Chem* 279: 23654–23660, 2004.
11. Daniel RA, Drake S, Buchanan CE, Scholle R, and Errington J. The *Bacillus subtilis spoVD* gene encodes a mother-cell-specific penicillin-binding protein required for spore morphogenesis. *J Mol Biol* 235: 209–220, 1994.
12. Deshmukh M, Brasseur G, and Daldal F. Novel *Rhodobacter capsulatus* genes required for the biogenesis of various *c*-type cytochromes. *Mol Microbiol* 35: 123–138, 2000.
13. Dorenbos R, Stein T, Kabel J, Bruand C, Bolhuis A, Bron S, and Quax WJ. Thiol–disulfide oxidoreductases are essential for the production of the lantibiotic sublancin 168. *J Biol Chem* 277: 16682–16688, 2002.
14. Draskovic I and Dubnau D. Biogenesis of a putative channel protein, ComEC, required for DNA uptake: membrane topology, oligomerization and formation of disulphide bonds. *Mol Microbiol* 55: 881–896, 2004.
15. Driks A. Proteins of the spore core and coat. In: *Bacillus subtilis and its closests relatives*. *From genes to cells*. Hoch JA, Sonenshein AL, and Losick R. (eds.). Washington DC: American Society for Microbiology Press, 2000, pp. 527–535.
16. Driks A and Setlow P. Morphogenesis and properties of the bacterial spore. In: *Prokaryotic development*, Brun YV and Shimkets LJ (eds.). Washington DC: American Society for Microbiology Press, 2000. pp. 191–218.
17. Dubnau D and Lovett J. Transformation and recombination. In: *Bacillus subtilis and its closests relatives. From genes to cells*. Hoch JA, Sonenshein AL, and Losick R (eds.). Washington DC: American Society for Microbiology Press, 2002, pp. 453–471.
18. Eichenberger P, Fujita M, Jensen ST, Conlon EM, Rudner DZ, Wang ST, Ferguson C, Haga K, Sato T, Liu JS, and Losick R. The program of gene transcription for a single differentiating cell type during sporulation in *Bacillus subtilis*. *PLoS Biol* 2:e328, 2004.
19. Eichenberger P, Jensen ST, Conlon EM, van Ooij C, Silvaggi J, Gonzales-Pastor J-E, Fujita M, Ben-Yehuda S, Stragier P, Liu JS, and Losick R. The sigma E regulon and the identification of additional sporulation genes in *Bacillus subtilis*. *J Mol Biol* 327: 945–972, 2003.
20. Erlendsson LS, Acheson R, Hederstedt L, and Le Brun NE. *Bacillus subtilis* ResA is a thiol–disulfide oxidoreductase involved in cytochrome *c* synthesis. *J Biol Chem* 278: 17852–17858, 2003.
21. Erlendsson LS and Hederstedt L. Mutations in the thiol–disulfide oxidoreductases BdbC and BdbD can supress cytochrome *c* deficiency of CcdA-defective *Bacillus subtilis* cells. *J Bacteriol* 184: 1423–1429, 2002.
22. Erlendsson LS, Möller M, and Hederstedt L. *Bacillus subtilis* StoA is a thiol–disulfide oxidoreductase important for spore cortex synthesis. *J Bacteriol* 186: 6230–6238, 2004.
23. Errington J. Regulation of endospore formation in *Bacillus subtilis*. *Nature Rev Microbiol* 1: 117–126, 2003.
24. Foster SJ and Popham DL. Structure and synthesis of cell wall, spore cortex, teichoic acids, S-layers and capsules. In: *Bacillus subtilis and its closest relatives. From genes to cells*, Hoch JA, Sonenshein AL, and Losick R (eds.). Washington DC: American Society for Microbiology Press, 2002, pp. 21–41.
25. Fukushima T, Yamamoto HT, Atrih A, Foster SJ, and Sekiguchi J. A polysaccharide deacetylase gene (*pdaA*) is required for germination and for production of muramic delta-lactam residues in the spore cortex of *Bacillus subtilis*. *J Bacteriol* 184: 6007–6015, 2002.
26. Gilmore ME, Bandyopadhyay D, Dean AM, Linnstaedt SD, and Popham DL. Production of muramic d-lactam in *Bacillus subtilis* spore peptidoglycan. *J Bacteriol* 186: 80–89, 2004.
27. Gonzalez-Pastor JE, Hobbs EC, and Losick R. Cannibalism by sporulating bacteria. *Science* 301: 510–513, 2003.
28. Hilbert DW and Piggot PJ. Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol Mol Biol Rev* 68: 234–262, 2004.
29. Holmgren A. Thioredoxin. *Annu Rev Biochem* 54: 237–271, 1985.
30. Hong HA, Duc LH, and Cutting SM. The use of bacterial spore formers as probiotics. *FEMS Microbiol Rev* 29: 813–835, 2005.
31. Imamura D, Kobayashi K, Sekiguchi J, Ogasawara N, Takeuchi M, and Sato T. *spoIVH* (*ykvV*), a requisite cortex formation gene, is expressed in both sporulating compartments of *Bacillus subtilis*. *J Bacteriol* 186: 5450–5459, 2004.
32. Kadokura H, Katzen F, and Beckwith J. Protein disulfide bond formation in prokaryotes. *Ann Rev Biochem* 72: 111–135, 2003.
33. Katzen F, and Beckwith J. Transmembrane electron transfer by the membrane protein DsbD occurs via a disulfide bond cascade. *Cell* 103: 769–779, 2000.
34. Katzen F, Deshmukh M, Daldal F, and Beckwith J. Evolutionary domain fusion expanded the substrate specificity of the transmembrane electron transporter DsbD. *EMBO J* 21: 3960–3969, 2002.
35. Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azvedo V, *et al*. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* 390: 249–256, 1997.

36. Lai EM, Phadke ND, Kachman MT, Giorno R, Vazquez S, Vazquez JA, Maddock JR, and Driks A. Proteomic analysis of the spore coats of *Bacillus subtilis* and *Bacillus anthracis*. *J Bacteriol* 185: 1443–1454, 2003.
37. Matias VRF and Beveridge TJ. Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. *Mol Microbiol* 56: 240–251, 2005.
38. Meador-Parton J and Popham DL. Structural analysis of *Bacillus subtilis* spore peptidoglycan during sporulation. *J Bacteriol* 182: 4491–4499, 2000.
39. Meima R, Eschevins C, Fillinger S, Bolhuis A, Hamoen LW, Dorebos R, Quax WJ, van Dijl JM, Provvedi R, Chen I, Dubnau D, and Bron S. The *bdbDC* operon of *Bacillus subtilis* encodes thiol-disulfide oxidoreductases required for competence development. *J Biol Chem* 277: 6994– 7001, 2002.
40. Merchante R, Pooley HM, and Karamata D. A periplasm in *Bacillus subtilis*. *J Bacteriol* 177: 6176–6183, 1995.
41. Moriyama R, Fukuoka H, Miyata S, Hattori A, Kozuka S, Yasuda Y, Tochikubo K, and Makino S. Expression of a germination-specific amidase, SleB, of bacilli in the forespore compartment of sporulating cells and its localization on the exterior side of the cortex in dormant spores. *J Bacteriol* 181: 2373–2378, 1999.
42. Moutevelis E and Warwicker J. Prediction of pK_a and redox properties in the thioredoxin superfamily. *Protein Sc*i 13: 2744–2752, 2004.
43. Nicholson WN. Roles of *Bacillus* endospores in the environment. *Cell Mol Life Sci* 59: 410–416, 2002.
44. Nicholson WN, Munakata N, Horneck G, Melosh HJ, and Setlow P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol Mol Biol Rev* 64: 548–572, 2000.
45. Page MLD, Hamel PP, Gabilly ST, Zwegzouti H, Perea JV, Alonso JM, Ecker JR, Theg SM, Christensen SK, and Merchant S. A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the cytochrome b_6f complex in *Arabidopsis* chloroplasts. *J Biol Chem* 279: 32474–32482, 2004.
46. Paidhungat M and Setlow P. Spore germination and outgrowth. In: *Bacillus subtilis and its closest relatives. From genes to cells*, Hoch JA. Sonenshein AL, and Losick RL (eds.). Washington DC: American Society for Microbiology Press, 2002, pp. 537–548.
47. Paik SH, Chakicherla A, and Hansen JN. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of, Sublancin 168, a novel lantibiotic produced by *Bacillus subtilis* 168. *J Biol Chem* 273: 23134–23142, 1998.
48. Piggot PJ and Hilbert DW. Sporulation of *Bacillus subtilis*. *Curr Opin Microbiol* 7: 579–586, 2004.
49. Piggot PJ and Losick R. Sporulation genes and intercompartemental regulation. In: *Bacillus subtilis and its closest relatives. From genes to cells*, Hoch JA, Sonenshein AL, and Losick R (eds.), Washington DC: .American Society for Microbiology Press, 2002, pp. 483–517.
50. Popham DL. Specialized peptidoglycan of the bacterial endospore: the inner wall of the lockbox. *Cell Mol Life Sci* 59: 426–433, 2002.
51. Popham DL, Gilmore ME, and Setlow P. Roles of low-molecular-weight penicillin-binding proteins in *Bacillus subtilis* spore peptidoglycan synthesis and spore properties. *J Bacterio*l 181: 126–132, 1999.
52. Popham DL, Helin J, Costello CE, and Setlow P. Analysis of the peptidoglycan structure of *Bacillus subtilis* endospores. *J Bacteriol* 178: 6451–6458, 1996.
53. Popham DL, Helin J, Costello CE, and Setlow P. Muramic lactam in peptidoglycan of *Bacillus subtilis* spores is required for spore outgrowth but not for spore dehydration or heat resistance. *Proc Natl Acad Sci USA* 93: 15405–15410, 1996.
54. Popham DL and Stragier P. Cloning, characterization, and expression of the *spoVB* gene of *Bacillus subtilis*. *J Bacteriol* 173: 7942–7949, 1991.
55. Raina S and Missiakas D. Making and breaking disulfide bonds. *Ann Rev Microbiol* 51: 179–202, 1997.
56. Ricca E, Henriques AO, and Cutting SM. *Bacterial spore formers. Probiotics and emerging applications*. Wymondham: Horizon Bioscience, 2004.
57. Rietsch A, Bessette P, Georgiou G, and Beckwith J. Reduction of the periplasmic disulfide bond isomerase, DsbC, occurs by passage of electrons from cytoplasmic thioredoxin. *J Bacteriol* 179: 6602–6608, 1997.
58. Schiött T and Hederstedt L. Efficient spore synthesis in *Bacillus subtilis* depends on the CcdA protein. *J Bacteriol* 182: 2845–2854, 2000.
59. Schiött T, Throne-Holst M, and Hederstedt L. *Bacillus subtilis* CcdA defective mutants are blocked in a late step of cytochrome *c* biogenesis. *J Bacteriol* 179: 4523–4529, 1997.
60. Schiött T, von Wachenfeldt C, and Hederstedt L. Identification and characterization of the *ccdA* gene, required for cytochrome *c* synthesis in *Bacillus subtilis*. *J Bacteriol* 179: 1962–1973, 1997.
61. Silvaggi JM, Popham DL, Driks A, Eichenberger P, and Losick R. Unmasking novel sporulation genes in *Bacillus subtilis*. J Bacteriol 186: 8089–8095, 2004.
62. Sonenshein AL. Endospore-forming bacteria: An overview. In: *Prokaryotic development*, Brun YV and Shimkets LJ (eds.). Washington DC: American Society for Microbiology Press, 2000, pp. 133–150.
63. von Wachenfeldt C and Hederstedt L. Respiratory cytochromes, other heme proteins, and heme biosynthesis. In: *Bacillus subtilis and its closest relatives: from genes to cells*, Sonenshein AL, Hoch JA, and Losick R (eds.). Washington DC: American Society for Microbiology Press, 2001, pp. 163–179.

Address reprint requests to:
Professor Lars Hederstedt
Department of Cell and Organism Biology
Biology Building
Lund University
Sölvegatan 35
SE-22362 Lund, Sweden

E-mail: lars.hederstedt@cob.lu.se

Date of first submission to ARS Central, October 31, 2005; date of acceptance, December 19, 2005.

This article has been cited by:

1. Géraldine Bonnard , Vincent Corvest , Etienne H. Meyer , Patrice P. Hamel . 2010. Redox Processes Controlling the Biogenesis of c-Type Cytochromes. *Antioxidants & Redox Signaling* **13**:9, 1385-1401. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. D. Michelon, S. Abraham, B. Ebel, J. De Coninck, F. Husson, G. Feron, P. Gervais, R. Cachon. 2010. Contribution of exofacial thiol groups in the reducing activity of Lactococcus lactis. *FEBS Journal* **277**:10, 2282-2290. [CrossRef]
3. Yiming Liu, Mirja Carlsson MÃ¶ller, Lise Petersen, Christopher A. G. SÃ¶derberg, Lars Hederstedt. 2010. Penicillin-binding protein SpoVD disulphide is a target for StoA in Bacillus subtilis forespores. *Molecular Microbiology* **75**:1, 46-60. [CrossRef]
4. Thijs R. H. M. Kouwen, Jan Maarten van Dijl. 2009. Applications of thiol-disulfide oxidoreductases for optimized in vivo production of functionally active proteins in Bacillus. *Applied Microbiology and Biotechnology* **85**:1, 45-52. [CrossRef]
5. Umesh Ahuja, Peter Kjelgaard, Benjamin L. Schulz, Linda Thöny-Meyer, Lars Hederstedt. 2009. Haem-delivery proteins in cytochrome c maturation System II. *Molecular Microbiology* **73**:6, 1058-1071. [CrossRef]
6. Thijs R.H.M. Kouwen, Jan Maarten van Dijl. 2009. Interchangeable modules in bacterial thiol-disulfide exchange pathways. *Trends in Microbiology* **17**:1, 6-12. [CrossRef]
7. Anna M. #asica, El#bieta K. Jagusztyn-Krynicka. 2007. The role of Dsb proteins of Gram-negative bacteria in the process of pathogenesis. *FEMS Microbiology Reviews* **31**:5, 626-636. [CrossRef]
8. Hiroshi Kadokura . 2006. Oxidative Protein Folding: Many Different Ways to Introduce Disulfide Bonds. *Antioxidants & Redox Signaling* **8**:5-6, 731-733. [Citation] [Full Text PDF] [Full Text PDF with Links]