Surface Proteins of Gram-Positive Bacteria and How They Get There

June R. Scott and Timothy C. Barnett

Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322; email: scott@microbio.emory.edu, barnett@microbio.emory.edu

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Abstract

Surface proteins are critical in determining the identifying characteristics of individual bacteria and their interaction with the environment. Because the structure of the cell surface is the major characteristic that distinguishes gram-positive from gram-negative bacteria, the processes used to transport and attach these proteins show significant differences between these bacterial classes. This review is intended to highlight these differences and to focus attention on areas that are ripe for further investigation.

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INTRODUCTION

The first impressions life forms give are very important. For a bacterium, the "face" it presents to the world is determined in part by the proteins on its surface. Because proteins show greater variability among different types of bacteria than other surface components, surface proteins help to define both the unique aspects by which each strain is recognized and the ability of each strain to colonize and interact with its own special environmental niche. Thus, surface proteins are critically important in determining the success of a bacterial strain in its competition for survival in the world.

The functions of bacterial surface proteins are varied. Some protect bacteria from environmental challenges, including toxic conditions or host immune defense system proteins [e.g., surface (S)-layer proteins and antiphagocytic molecules, respectively]; some allow bacteria to use nutrients from their environment (see Cellulosomes, below); some allow bacteria to attach to specific environmental components (e.g., adhesins, including pili); some allow bacteria to interact with each other (e.g., biofilm formation in *Staphylococcus epidermidis*; reviewed in Reference 53); some facilitate competition with other bacteria for specific niches (lytic enzymes such as staphylolysin, see below); and some, which are essential proteins, are required for bacterial growth, cell wall (cw) maintenance, and cell division (the autolysins, see below).

The two major classes of bacteria, gram-positive and gram-negative, are differentiated primarily because of differences in their surfaces. Gram-positive bacteria have much thicker peptidoglycan cw layers and no outer membrane external to this structure. Thus, gram-positive bacteria also lack a morphologically distinct periplasmic space, which is usually defined as the region between the cytoplasmic membrane (cm) and outer membrane. Because of these major differences in surface structure from the better-studied gram-negative bacteria, secretion, folding, and

attachment of proteins to the envelope must differ in fundamental ways between the two bacterial groups.

This review does not attempt to summarize or list surface proteins in different gram-positive bacteria because the increased use of proteomic analyses would cause any such list to be outdated by the time it is published. We hope that a mechanistically focused analysis of surface localization of gram-positive bacterial proteins is more interesting and more valuable. Thus, this review tries to summarize the ways in which proteins reach the surface of gram-positive bacteria and the mechanisms by which such proteins are attached to the bacterial cell envelope. We focus on the newer aspects not previously reviewed and refer to previous reviews for other cases. We have found reviewing this literature to be exciting and hope we can convey that to the reader. We hope that this review helps to identify the areas in which further research is badly needed. Although we have tried to cite appropriate references and/or reviews that include primary citations, we apologize for inadvertent omissions that occur herein.

CELL WALL STRUCTURE

For gram-positive bacteria, a single cm surrounded by a thick layer of peptidoglycan provides both a physical barrier for protection from the environment and a scaffold for the attachment of secondary cw polymers (including teichoic and teichuronic acids) and surface proteins (**Figure 1** shows the structure of a typical gram-positive cw). Cross-linking of the linear glycan strands by short peptides results in a single huge molecule 15 to 30 nm thick that completely surrounds the cell. Teichoic acids may be attached either to a glycolipid associated with the cm (lipoteichoic acids, LTAs) or to the cw (wall teichoic acids, WTAs) (2) (**Figure 1**). Together with peptidoglycan, teichoic acids form a polyanionic matrix that has a variety of functions, including cation homeostasis, trafficking of various ions and nutrients, and the display of surface proteins. Excellent reviews provide more information on the structure and function of the cw (13, 117) and on secondary cw polymers (96).

TRANSPORT THROUGH THE CYTOPLASMIC MEMBRANE

The first step in the localization of proteins to the cell surface is transport through the cm. The cm must maintain integrity of the cytoplasmic contents while allowing passage of molecules, some of which are finally found attached to the membrane itself. Most bacterial proteins are transported unfolded, which allows them to proceed through a small gated translocation channel. The most common mechanism for this is a process called the general secretory (Sec) pathway, which is dependent on the Sec system. Some folded proteins can be exported by the twin arginine or TAT pathway (reviewed in Reference 50a). In addition, there are several specialized paths for secretion of specific proteins. The following is a brief discussion of the Sec pathway that highlights its main differences in gram-positive versus gram-negative bacteria. For further information and original references, the reader is referred to several excellent recent reviews (36, 38, 91, 134, 136). This is followed by a discussion of a newly recognized specific secretion system encoded in genomes of several gram-positive bacteria. The wide array of different ABC transporter proteins dedicated to secretion of limited substrates through the cm is not discussed in this review.

The Sec Pathway

Most of our knowledge of this pathway comes from studies of *Escherichia coli* and *Bacillus subtilis*. In Sec-dependent secretion, the membrane channel is formed by the highly conserved proteins SecY and SecE interacting with the cm protein SecG. Recent work suggests that SecY forms the plug to gate the channel (126). In addition, the channel may also include SecD, SecF, and YajC, although

Cellulosome: large structure composed of an ordered array of enzymes involved in cellulose breakdown attached noncovalently to the cw by SLH domains

cw: cell wall

cm: cytoplasmic membrane

LTA: lipoteichoic acid

WTA: wall teichoic acid

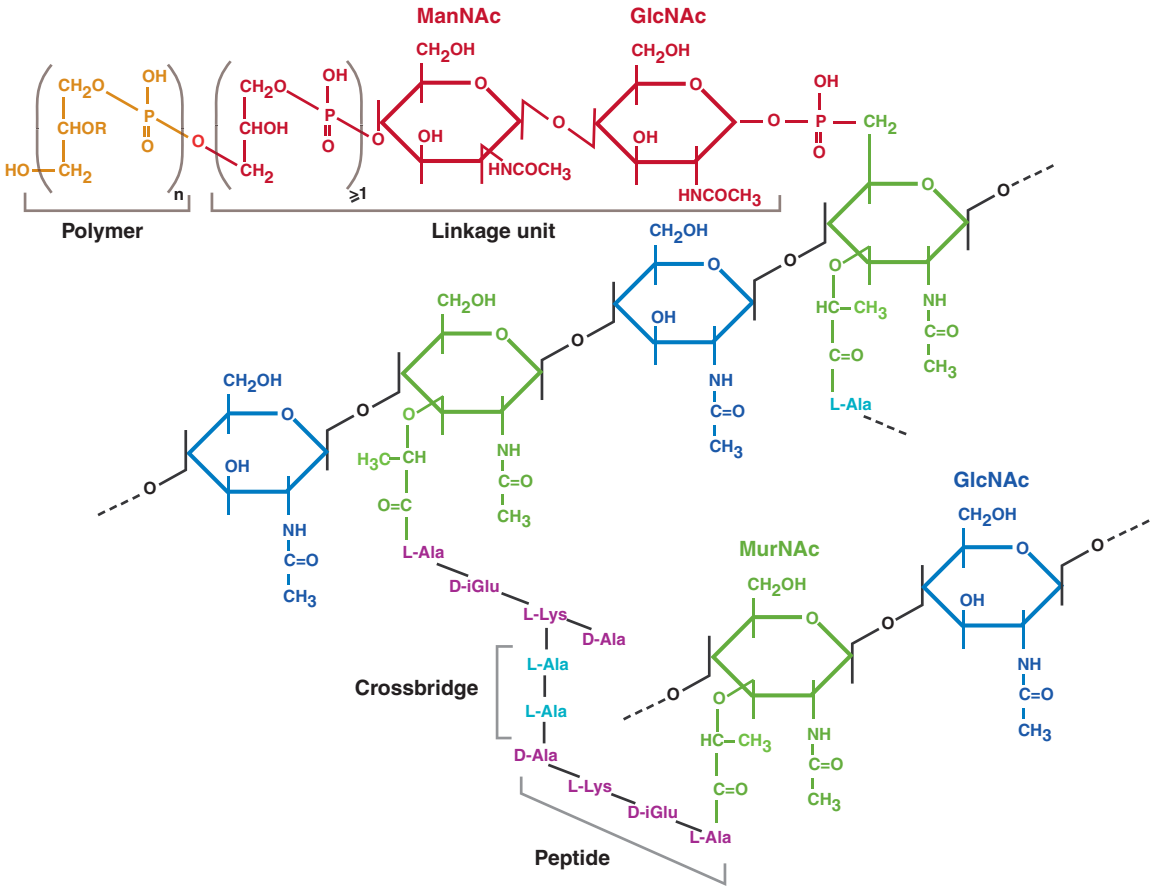

Figure 1

Structure of the *Streptococcus pyogenes* cw peptidoglycan with teichoic acid. Peptidoglycan is a repeating disaccharide of *N*-acetylmuramic acid (MurNAc; *green*) linked to *N*-acetylglucosamine (GlcNAc; *dark blue*) by a β 1–4 glycosidic bond. These strands are cross-linked by a cw peptide (*purple*) and a peptide crossbridge (*light blue*). The composition of both the cw peptide and peptide crossbridge varies with the organism (117). The wall teichoic acid is a polymer of glycerol phosphate (*orange*) that is attached to peptidoglycan via a linkage unit (*red*) (2, 120). In *Bacillus subtilis*, teichoic acids, which are phosphate-free polymers of glucuronic acid and GlcNAc, may replace wall teichoic acid under phosphate-limiting conditions.

Translocon: channel composed of specific proteins located in the cm through which secreted proteins with Sec signals at their N terminus are transported

homologs of SecD and SecF are absent from gram-positive genomes. SecA, often called the motor, is the ATPase that enables ATP to be used as an energy source for this process when it associates with the other Sec proteins that constitute the translocon. In *B. subtilis*, SecA appears also to act as an export-specific chaperone to help prevent folding of proteins prior to their translocation. It has been proposed (109, 110) that in gram-positive bacteria translocons are clustered at restricted (bacilli) or unique (cocci) sites of the cm termed the ExPortal (see below).

In *Streptococcus gordonii*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, there are two pairs of SecA and SecY proteins, one of which is essential and the other of which is required for export of a single, large cw-anchored protein. In *S. gordonii*, this protein is an adhesin encoded in an operon with the SecA and SecY

that transport it (10). In *Mycobacterium tuberculosis* (21, 22) and *Listeria monocytogenes* (76, 77), one of the two *secA* genes is also not essential. SecA2 seems to aid in export of a subset of proteins, some of which lack recognizable Sec sequences and are not expected to be secreted (including proteins needed for transcription and translation). In *L. monocytogenes*, SecA2 increases the amount of several autolysins on the exterior of the cw, which raises the concern that the presence of stable abundant cytoplasmic proteins in the "secretome" may result from autolysis.

In gram-negative bacteria, targeting of proteins to the translocon channel may occur by one of two mechanisms: the SecB pathway or the signal recognition particle (SRP) pathway. SecB binds to proteins that have an N-terminal signal sequence of at least 20 residues and targets these proteins to the Sec apparatus. This process is largely posttranslational. SecB also acts as a secretion-specific chaperone to prevent folding prior to export. However, genomes of gram-positive bacteria whose sequence is available do not encode orthologs of SecB. Thus, it is not clear how proteins are chaperoned to the translocon in gram-positive bacteria. Several secretion-specific chaperones that may play a role similar to that of SecB have been identified or suggested in some gram-positive bacteria, including CsaA in *B. subtilis* (79, 92, 93), which has orthologs in some (66) but not all gram-positive bacteria.

Signal Recognition Particle

The other pathway known to target proteins to the translocon channel uses the SRP, which is composed of a small cytoplasmic RNA (scRNA) (4.5S in *E. coli*) and a GTPase, encoded by *ffh* (54 homolog) (61, 108), which binds the signal sequence of the preprotein. This pathway is found in the genomes of all organisms whose complete sequence is available, including bacteria, archaea, and eukaryotes (29). In addition to *ffh* and the scRNA, in *B. subtilis*, HBSu, a histone-like protein, also binds the SRP. In gram-negative bacteria, the SRP pathway appears to be used for most proteins whose final localization is the inner membrane as well as for some secreted proteins. In gram-positive bacteria, because of the absence of a SecB homolog, the SRP pathway is the only mechanism known for targeting proteins to the cm and to the translocon channel. However, the SRP pathway is dispensable for growth under nonstress conditions in streptococci (34), although eliminating both YidC2 and the Srp pathway severely inhibits growth (57). This suggests the existence of an alternative to the SRP pathway that requires YidC2.

During protein synthesis, Ffh of the SRP competes with trigger factor, a ribosome-associated chaperone, for binding to nascent protein chains. Thus, the nascent protein becomes associated with the SRP. The SRP is targeted to the membrane by association with FtsY, another GTPase, which is an ortholog of the SRP receptor in eukaryotes. The SRP has been shown by cross-linking analysis (in vitro in *B. subtilis*) (24) to interact with SecA and thus target the newly synthesized protein to the translocon.

Recently, elegant structural analyses showed that the active site for GTP hydrolysis in the translocon channel is formed by the Ffh-FtsY heterodimer when it associates with the membrane (43). This heterodimer, which has been called a molecular latch, dissociates when the GTP is hydrolyzed, releasing the SRP constituents to recycle.

Signal Peptidases

Secreted proteins that use the Sec pathway are recognized by the presence of an N-terminal signal sequence, which is cleaved by a type 1 signal peptidase during the process of secretion through the cm (for a review see Reference 135). This signal sequence is much longer in gram-positive bacteria than in gram-negative bacteria.

Many gram-positive bacteria encode more than one type 1 signal peptidase. In these

ExPortal: cell surface site at which Sec translocons are found

SRP: signal recognition particle

scRNA: small cytoplasmic RNA constituting part of the SRP

Snm: secretion in *Mycobacteria* (specific non-Sec-dependent system)

cases, their functions may be completely or partially redundant or they may have unique specificities. In *B. subtilis*, there are five signal peptidases, three of which are essential. In *Streptomyces lividans*, type 1 signal peptidases also show different specificities (99). In most other gram-positive bacteria, functional analyses of signal peptidases have not yet been performed.

Type 2 signal peptidases recognize diacylglyceryl-modified preproteins at the "lipobox" (LXXC motif, gram-positive lipobox motif) (125) and covalently attach them by an N-terminal cysteine residue to a lipid component of the cm. The resulting lipoprotein is thus retained at the cw-cm interface. These peptidases always have four transmembrane domains and include Asp at their active site, which is located outside the cm.

Specific Secretion Systems: Snm

Among the secretion systems responsible for export of specific proteins that lack an N-terminal Sec signal and that are not processed during secretion is the recently described Snm pathway (secretion in *Mycobacteria*). The nomenclature for the components of this pathway has not been standardized and the mechanism of transport is not yet completely understood. We hope the description below encourages additional investigators to study this system.

The Snm pathway was first identified in *M. tuberculosis* by bioinformatic analyses (32, 127) and as a virulence factor by signature-tagged mutagenesis (123). This region of the *M. tuberculosis* genome, known as RD1 (for region of difference 1 between the BCG vaccine strain and virulent strains), is present in all virulent strains and absent from all avirulent strains of *M. tuberculosis* (9, 52). The genes *snm1*, *snm2*, and *snm4* (RV3870, RV3871, and RV3877, respectively) encode proteins required for secretion of ESAT-6 (early secreted antigen target-6) and a related protein, CFP-10, whose genes (RV3875 and RV3874, respectively) they flank. ESAT-6 and CFP-10 are small proteins that serve as major T cell antigens (54, 123) and they are required for virulence.

Snm1 and Snm2 have homology with AAA ATPases, each has several transmembrane domains, and they belong to the Fsd (FtsK/SpoIIIE domain) class of proteins involved in chromosome partitioning and translocation. Snm4 is predicted to have 12 transmembrane domains. In a yeast two-hybrid system, Snm1 and Snm2 interact with each other and with their substrate proteins (107, 123). Although additional proteins may be involved in transport through this pathway, it seems likely that the energy for transport is derived from ATP using Snm1 and Snm2 and that Snm4 is located in the membrane and participates in formation of a channel (123). Very recently, Snm9 and Snm10, encoded outside the RD1 locus of *M. tuberculosis*, were identified to be required for CFP-10 and ESAT-6 secretion as well (83).

The Snm pathway is required for replication of *Mycobacteria* in macrophages and is an important virulence factor for *M. tuberculosis*. Homologs of the *snm* genes have been identified in *Mycobacterium smegmatis*, *Mycobacterium leprae* (which has a minimal genome), and many other G+C gram-positive bacteria, including both pathogens and nonpathogens (100). The conservation of this secretion system among organisms with such divergent lifestyles might indicate that substrates in addition to ESAT-6 and CFP-10 orthologs are exported by this system or that the *snm* homologs in other bacteria are sufficiently different to serve other functions (25). In *M. smegmatis*, whose RD1 region was shown by complementation to be functionally equivalent to that of *M. tuberculosis*, the Snm pathway also regulates conjugative transfer of DNA (42). Thus, even in *M. tuberculosis*, the Snm system serves several functions.

In *S. aureus*, two ESAT-6-like proteins, EsxA and EsxB, are also secreted with intact N termini, and this requires the FSD (similar to Snm1 and Snm2, see above) protein

EssC and two other membrane proteins (EssA and EssB) (25). Although the precise function of this system has not yet been determined, it makes a significant contribution to abscess formation in mice. Thus, like the Snm system in *M. tuberculosis*, it is important for virulence. It appears at this time that the Snm system may be important in many different gram-positive bacteria, and further studies leading to a greater understanding of its mechanism of protein transport, substrate specificity, and role in the lifestyle of different organisms are eagerly awaited.

COVALENT ATTACHMENT TO CELL WALL PEPTIDOGLYCAN: SORTASES

Mechanism of Attachment by SrtA and its Substrates

In gram-positive bacteria, surface proteins that are covalently attached to peptidoglycan are anchored through their carboxy terminus by transpeptidases called sortases (Srt). The majority of these covalently attached surface proteins are anchored by a single enzyme, the "housekeeping" sortase, usually designated SrtA. Originally identified in *S. aureus* (87), SrtA homologs have now been characterized experimentally from a wide variety of gram-positive bacteria (see Reference 128 and 84a for reviews), including *L. monocytogenes* (15, 46), *Bacillus anthracis* (50), and several streptococcal species (6, 19, 67, 73, 75, 98). The number and types of proteins anchored by SrtA are predicted, on the basis of genome sequence analyses, to vary among organisms, from 1 or 2 in *Tropheryma whipplei* and *B. subtilis*, respectively, to up to 43 in *L. monocytogenes* (17). Because most bacteria require SrtA for attachment of more than one protein to the cell surface, *srt*A mutants often have pleiotropic effects, including alterations in bacterial virulence and in attachment to various surfaces. In pathogenic bacteria, SrtA is also considered a possible target for development of therapeutic agents.

Sortase-attached surface proteins can be recognized by the presence of a conserved C-terminal cw-anchoring domain that consists of a sortase-recognition sequence (usually LPXTG) followed by a hydrophobic stretch of amino acids and a positively charged tail (41). Current evidence supports a four-step anchoring mechanism that results in covalent attachment via a peptide bond between the threonine residue of the LPXTG motif and the peptide crossbridge of the cw (**Figure 2**) (for recent reviews see References 84a, 128, and 103).

Sortase (Srt): transpeptidase that cleaves a specific motif in a protein substrate and links it covalently to the cw peptide crossbridge

Some heterologous proteins can be anchored by SrtA if they are fused to the C-terminal cw-anchoring domain of a naturally SrtA-dependent surface protein such as *S. aureus* protein A. However, it appears that information from the N terminus of a protein may also be required because there are exceptions that cannot be anchored when fused to an anchoring motif (5, 118).

Multiple Sortases Encoded in the Genomes of Gram-Positive Bacteria

Sortase genes have been identified in silico by database homology searches (17, 33, 101) and on the basis of several criteria including the presence of a conserved signature motif (TLXTC) that contains the catalytic cysteine residue (130). Sortase genes are present in the genomes of all gram-positive bacteria that have been examined to date, as well as in at least two Archaea (*Methanobacterium thermoautotrophicum* and *Methanopyrus kandleri*) and a few gram-negative Proteobacteria (*Colwellia psychrerythraea*, *Microbulbifer degradans*, *Bradyrhizobium japonicum*, *Shewanella putrefaciens*, and *Shewanella oneidensis*) (33).

A surprising result of the database searches was the finding that most gram-positive bacteria encode one or more sortases in addition to SrtA. Most protein substrates for the "accessory" sortases that have been characterized experimentally are encoded near or adjacent to the sortase that anchors them. Such substrate proteins include those required for

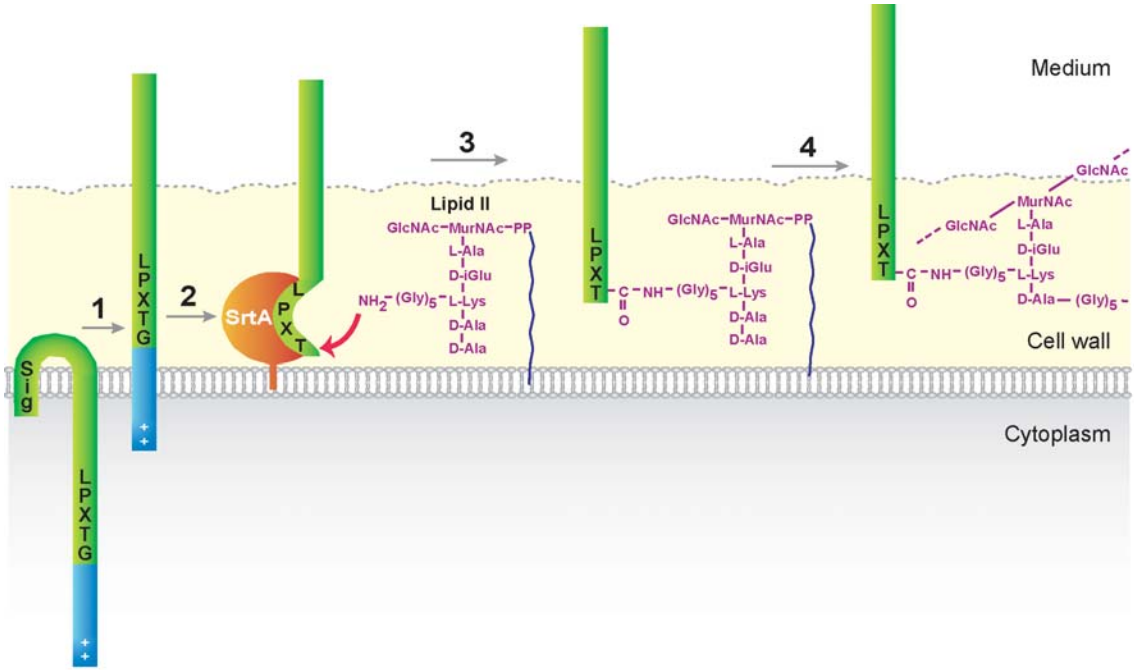

Figure 2

Covalent attachment of proteins to the peptidoglycan by *S. aureus* SrtA. (Step 1) Surface proteins are first secreted through the membrane via the Sec pathway and the N-terminal signal sequence (Sig) is removed. The C-terminal hydrophobic domain and positively charged tail (*blue*) retain the protein in the membrane and allow the LPXTG motif to be recognized by the membrane-bound sortase enzyme (*orange*) (86, 119). (Step 2) Sortase then cleaves the LPXTG motif between the threonine and glycine residues (95) by a reverse-protonation catalytic mechanism (44), which releases the C-terminal hydrophobic region and charged tail. The N-terminal portion of the cleaved protein forms an acyl-enzyme intermediate with sortase. (Step 3) This is resolved following a nucleophilic attack (*red arrow*) by an amine group on the cw precursor, lipid II (104, 112). (Step 4) The protein-linked lipid II molecule is then incorporated into the mature cw by the transglycosylation and transpeptidation reactions of bacterial peptidoglycan synthesis.

iron acquisition in *S. aureus* (88, 89) and homologous proteins in *L. monocytogenes* (14), a trypsin-resistant protein (T6) in *Streptococcus pyogenes* (6) recently shown to be incorporated into pili (90), and other proteins required for the production of pili in several organisms (see below).

The mechanism of action of the accessory sortases is believed to be similar to that of SrtA; however, their protein substrates may have distinct recognition sequences, such as NPQTN (*S. aureus* SrtB) (89) and QVPTGV (*S. pyogenes* SrtC2) (5). Other accessory sortases, including some required for the formation of pili (see below), recognize an LPXTG motif as does SrtA but act on a different subset of proteins to form pilin polymers (see below). The mechanism by which substrate proteins are identified by a sortase has not yet been investigated.

Accessory sortases may anchor their substrates to different components of the peptidoglycan than does SrtA. This appears to be true for SrtB of *S. aureus*, whose substrate is IsdC. Unlike SrtA-anchored proteins, cw-anchored IsdC is not accessible to digestion with trypsin. In addition, the cw fragments to which IsdC is attached display significantly

less cross-linking than the fragment to which SrtA-anchored proteins are linked (85). No other proteins that require accessory sortases have been studied in enough detail to determine whether most accessory sortases attach their substrates to unique peptidoglycan locations.

Another Sortase Function: Polymerization into Pili

Perhaps the most unexpected role for a sortase homolog is in the formation of pili in gram-positive bacteria such as *Actinomycetes naeslundii* (147) and *Corynebacterium diphtheriae* (84a, 131, 132, 142). Gram-positive pili appear to be composed of a string of covalently attached protein subunits that are not dissociated by boiling in sodium dodecyl sulfate (SDS) or other harsh chemical treatments. Yeung et al. (147) proposed that the subunits of *A. naeslundii* pili may be covalently attached to each other by a sortase-like mechanism. This proposal was based on the finding that the subunits in assembled pili lack the C-terminal hydrophobic region and charged tail that follows the LPXTG motif, although these regions are present when the protein is synthesized in *E. coli*. In further support of this idea, the gene immediately downstream of that encoding *A. naeslundii* pilin that is required for pilus assembly (147) was later identified as being homologous to SrtA in *S. aureus* (131).

Pili in *C. diphtheriae* also require a sortase for assembly and attachment to the cw (131). Ton-That & Schneewind (131) suggested that the pilin subunits would be cleaved at the LPXTG motif and linked to a free amine group of a lysine residue in successive monomers. Further N-terminal in the pilin protein, they identified a conserved "pilin motif" by aligning the sequence of pilin proteins from SpaA, SpaD, and SpaH pili of *C. diphtheriae* and FimA and FimP pili of *A. naeslundii*. Changing the conserved lysine in this motif (WxxxVxVYPK) to arginine or alanine prevented or reduced *C. diphtheriae* pilus assembly (131). Thus, the pilin motif is thought to anchor the N-terminal end of the pilin protein to the next subunit. In some cases, a minor pilin may have a different motif.

The same pilin motif was also identified in genes of *Clostridium perfringens*, *Enterococcus faecalis*, *Streptococcus agalactiae*, and *S. pneumoniae* (131), suggesting the possibility of a similar mechanism for pilus assembly in these bacteria, although pili have not yet been demonstrated in all these organisms. The similarity of the mechanism for pilus assembly in several gram-positive bacteria (for a review see References 84a, 119a, 132) was demonstrated by the ability of *C. diphtheriae* to assemble the major pilin subunit from *A. naeslundii* (FimA) into short pili (129). Surprisingly, however, this heterologous assembly of FimA requires a different sortase homolog than that used to polymerize SpaA pili, although both require an LPXTG anchor domain for pilus assembly.

Recently, thin pili were identified on the surface of *S. agalactiae* (Group B *Streptococcus*) following genome screening for predicted surface proteins (with LPXTG motives) that might serve as vaccine candidates (74). These pili are composed of a protein that includes the pilin motif of Ton-That and Schneewind (see above), which is encoded in an operon containing two sortase-related genes. This finding suggests that a similar sortase-dependent mechanism may be involved in their formation.

Some strains of *S. pyogenes* have chromosomal regions that encode one or two sortase homologs in addition to proteins with C-terminal anchoring domains that include sequences related to LPXTG (e.g., the "FCT" region) (5, 12). In cw extracts from a strain of *S. pyogenes*, a protein substrate (*orf*100) for one of these sortases (SrtC2) produces a "laddering" pattern of successive multimers on SDS-PAGE (5). Recently, electron microscopic analyses demonstrated that these FCT-encoded proteins are subunits of pili (90), although they do not include the pilin motif defined for *C. diphtheriae* and *A. naeslundii*. Thus, it appears that pili may be much

more common in gram-positive bacteria than previously believed and that their subunits may be covalently linked by sortase homologs.

The sortase homolog encoded in the pilin gene cluster is not sufficient to anchor the pili to the cw in the only cases in which this has been investigated, SpaA pili of *C. diphtheriae* (131) and T6 pili of *S. pyogenes* (D. Zähner & J. R. Scott, manuscript submitted). Instead cw attachment requires the housekeeping sortase. Therefore, the transpeptidase encoded in the pilin gene cluster does not have a sorting function, so it should be considered a transpeptidase (or pilin polymerase) rather than a sortase (119a).

The specific sortase required for pilus polymerization has been defined by mutagenesis in the case of A. *naeslundii* (147), *C. diphtheriae* SpaA pili (131), and the *S. pyogenes* FCT region proteins T6 (6) and Orf100 (5). In other pili, some of which are encoded in loci containing more than one sortase, it is not clear which sortase(s) is needed for pilin polymerization or attachment of the pili to the bacterial cw. Furthermore, the mechanisms by which the minor pilin subunits are added, when they are present, have not yet been clarified. Thus, although a great deal has been learned about gram-positive pili in a short time, much more remains to be discovered.

Nomenclature

While the rapid identification and characterization of sortases from different gram-positive bacteria has provided a wealth of information about the substrates of these enzymes in diverse organisms, the result has been a nomenclature that is somewhat confusing. Recently, schemes have been proposed to classify sortases into four (39) or five (33) groups on the basis of phylogenetic (cluster) analysis. Dramsi et al. (39) suggest that sortases that share extensive sequence similarity have similar functions and should be named accordingly. However, the data currently available already indicate this may not always be true. For example, while the protein anchored by SrtB of *S. aureus* is required for iron acquisition (89), the sortase from *S. pyogenes* that belongs to this class (called SrtC2) covalently links pilin subunits to form the pilus structure (6, 90). In addition, some bacteria encode several sortases of a single class with potentially different roles. In the scheme suggested, these sortases would all receive the same name, which might lead to additional confusion. At this time, therefore, it seems appropriate to name sortases with successive letters in a given organism in order of discovery or, if they are identified in silico, in order around a genome. Further experimental characterization is needed to determine which members of a phylogenetic cluster are functionally similar. When functions are defined, it may be useful to rename some transpeptidases that polymerize pilin subunits to distinguish them from sorting transpeptidases and to group the others into classes given new designations.

NONCOVALENT ATTACHMENT TO THE GRAM-POSITIVE SURFACE

Teichoic Acid Binding Surface Proteins

Overview. In several gram-positive bacteria, proteins exposed on the cell surface are associated with the TA present in the cm, cw, or both. This association usually occurs through repeats present in the C-terminal domain of the surface protein. In the few bacterial species containing choline in their LTA (*S. pneumoniae* and some *Clostridia*), these repeats bind choline (see below). This can be demonstrated by the release of such proteins from the bacterial cell surface upon the addition of choline to whole bacteria. Choline binding proteins can be purified on choline-affinity columns, which has been important historically for their identification.

For bacteria lacking choline in their TA, the ligand associated with the cell surface

protein has not been identified. For the best studied of these LTA binding proteins, the C-terminal repeats required for attachment (GW repeat modules) are longer than choline binding repeats and the two types of repeats show no homology, except that both begin with the residues GW (glycine tryptophan).

Most proteins attached to LTA contain N-terminal Sec secretion signals and can associate with the cell surface from either within or outside the cell. Many of these proteins are amidases, which hydrolyze the cw of the bacterium that synthesizes them and/or of another related species. Such cw hydrolases or autolytic enzymes are necessary for insertion of new peptidoglycan material as cells grow, and they are often localized to specific sites of the cell surface, such as the septum and poles (see below). These amidases are often essential proteins, e.g., the choline-binding protein LytA of *S. pneumoniae*, presumably because they are required for cell growth and division.

Choline binding proteins of *S. pneumoniae* and other bacteria. The best-studied example of a bacterial choline binding protein is probably PspA, an important virulence factor and vaccine target found on the surface of *S. pneumoniae* (23). Washing the cells with Na_2CO_3, pH 11.2, releases peripheral membrane proteins but does not release PspA. The C-terminal region of PspA contains a large proline-rich domain that includes 10 highly conserved 20-amino-acid repeat units and a short C terminus that is only weakly hydrophobic and poorly charged (148). These repeats begin with the amino acids GW. Whether produced from within the *S. pneumoniae* cell or added externally to cells lacking *psp*A, the PspA protein associates with the cell surface in a process that requires at least five of the C-terminal repeat units (149). Yother & White (149) elegantly showed that the surface attachment of PspA is due to its interaction with the choline present in the LTA of *S. pneumoniae* and that the addition of a high concentration of choline (2%) releases the protein from the cell.

Other *S. pneumoniae* proteins also associate with the cell surface by binding choline. These include LytA (60), an essential autolytic amidase. Unlike most LTA binding proteins, LytA does not have an N-terminal signal sequence. Several additional amidases have been identified in *S. pneumoniae* by the use of choline-affinity columns and some of these are phage-encoded lytic enzymes (47–49, 81).

Other bacteria that contain choline in their cell envelope are members of the heterogeneous genus *Clostridium* (105). CspA of *Clostridium acetobutylicum*, which is probably the best-studied protein of this group, is found both on the cell surface and, as expected from the presence of an N-terminal signal sequence, in the culture medium, where it is the predominant protein (113). At its C terminus, CspA has choline binding repeats beginning with GW that resemble the consensus for choline binding repeats of pneumococcal cw hydrolases (49). The function of CspA has not yet been investigated.

GW repeat modules for noncovalent attachment to LTA. In studying surface proteins of *L. monocytogenes* that lack LPXTG-type surface anchoring domains (26), Cossart and colleagues (45) identified InlB, which is required for invasion of host cells by this human pathogen. The C-terminal region of InlB is necessary and sufficient for surface anchoring. This region contains modules termed GW repeats, because each of these 80- to 90-residue tandem repeats begins with glycine-tryptophan (20). The GW repeats are longer than choline binding repeats and share no further homology with them.

Like PspA of *S. pneumoniae*, the InlB protein of *L. monocytogenes*, which contains an N-terminal secretion signal recognized by the Sec system, associates with the bacterial surface whether it is produced from within the cell or added externally (20). It cannot be released efficiently from the surface of *L. monocytogenes* by a muramidase (65, 94) because it is attached to the LTA in the cell membrane (65). Although the importance of the GW

residues at the beginning of each repeat module for attachment was not addressed, other proteins with homologous tandem GW repeat modules are also surface associated. Such proteins in *L. monocytogenes* include Ami, an amidase that can lyse both *Listeria* and other gram-positive species (20), and Auto, an autolysin (27). Additional proteins with GW repeat modules in different positions are also encoded in the *L. monocytogenes* genome sequence, and most of these contain an amidase domain similar to Ami (26).

Similar GW repeat modules are required for surface targeting of several staphylococcal surface autolysins (4) including AtlC from *Staphylococcus caprae* (1), AtlE from *Staphylococcus epidermidis* (58), and Aas from *Staphylococcus saprophyticus* (59). An unusual case is that of the *Staphylococcus simulans* protein lysostaphin, in which the GW modules at the C terminus are also responsible for noncovalent association of this secreted bacteriolytic enzyme with the cell surface. However, the *S. simulans* enzyme does not lyse *S. simulans* but rather recognizes peptidoglycan crossbridges of *S. aureus* (3). Thus, lysostaphin enables *S. simulans* to lyse *S. aureus* and can be viewed as an example of chemical warfare used by bacteria to gain predominance in colonizing a specific niche.

Surface (S) layer: composed of semicrystalline-ordered array of a noncovalently associated protein

SLH: S-layer homology

S-Layer Proteins

Most genera of eubacteria and archaea include strains that produce surface (S) layers that appear crystalline. S layers are composed of many copies of a protein linked noncovalently to produce planar arrays. When present, S-layer proteins usually constitute the most abundant protein of the cell. In spite of their abundance and striking appearance as obvious cell surface structures, there seem to be few general rules about their function or how they reach or are anchored to the cell envelope. Furthermore, when bacteria are grown under optimal laboratory conditions they often lack the S layers that may be present in more natural growth situations.

S layers may serve several functions in the same cell. These include acting as molecular sieves, protective coats, structures involved in specific adhesion and surface recognition, attachment sites for exoenzymes, ion traps, and templates for fine-grain mineralization. In pathogens, S layers are often virulence factors, presumably because they protect the bacteria from the immune system. In addition, alternative alleles for S layers are generated by recombination, which causes antigenic variation that leads to immune evasion.

Most, but not all, S-layer proteins have N-terminal signal sequences. They are usually acidic (pI 4–6) and are often glycosylated. These proteins usually, but not always, contain S-layer homologous (SLH) motifs of 50 to 60 amino acids at their N terminus that are apparently involved in their noncovalent attachment to the cell surface. S-layer proteins that have been investigated in detail (e.g., *Bacillus anthracis*, *B. stearothermophilus*, *Clostridium thermocellum*, and *B. sphaericus*) do not appear to be attached directly to the primary wall polymer. Instead, most are attached to secondary polymers, like teichoic or teichuronic acid, or to polymers that are further modified, e.g., by the addition of pyruvic acid units. For excellent recent reviews, the reader is referred to References 114, 115, and 121.

Cellulosome

Another surface structure attached to the bacterial cw by SLH domains of component proteins is the cellulosome. These structures are present on anaerobic bacteria that break down and utilize cellulose including several species of *Clostridia* and *Ruminococcus* as well as some gram-negative species. Cellulosomes are large (about 18 nm in diameter) complexes of enzymes and structural proteins (scaffolding proteins, dockerins, and cohesins) organized in a chain-like array that facilitates attachment to and utilization of cellulose. They are found outside the cell surface where they can be visualized as polycellulosome structures. Surface localization of discretely

organized enzymes in cellulosomes is likely to significantly improve the efficiency of cellulose digestion, especially in the challenging anaerobic environment, by bacteria responsible for breaking down decaying plant matter (in compost and sewage) and by bacteria that assist higher animals (ruminants) with the conversion of plant material into energy. Thus these structures are likely to play an important role in the global carbon cycle. For a more detailed understanding of these structures the reader is referred to References 8 and 37.

The LysM Domain

Cell wall attachment of many bacterial proteins in gram-positive and gram-negative organisms occurs through the lysin motif (LysM) domain, often repeated several times in the protein sequence. The name results from the original identification of this motif in bacterial lysins (16). Many LysM proteins are cw hydrolases (see Asymmetric Surface Localization, below), although other proteins, including spore-specific proteins in *B. subtilis*, also use this domain for surface attachment. This approximately 40-residue domain in the membrane-bound lytic murein transglycosylase D (MltD) protein of *E. coli* was found to assume a β−α−α−β structure (7). Within the LysM domain a carbohydrate binding YG motif (YXXXXGXX-Hyd) was recently identified, although its function has not been determined (133). LysM domains are found in glucosyl transferases and glucan binding proteins, as well as in other types of proteins.

Proteins with LysM domains attach to the gram-positive cell surface not only when they are synthesized by the bacterium to which they attach, but also when they are added to the culture. Using AcmA (major autolysin) of *Lactococcus lactis*, Steen et al. (124) showed that the C-terminal region, which contains six LysM domains, is necessary for attachment to the *L. lactis* surface. When added externally to the bacteria, this domain is also sufficient to anchor a heterologous protein fragment to the surface of *L. lactis* as well as to the surface of many other gram-positive bacteria (124). The LysM-containing fragment binds to lactococcal and *B. subtilis* cell walls even when they have been treated with SDS to remove cw-associated proteins or with TCA, which is believed to remove carbohydrates and (lipo) teichoic acids (124). This suggests that the LysM domain mediates binding to the peptidoglycan. Furthermore, purified peptidoglycan (see Cell Wall Structure, above) (**Figure 1**) binds the LysM domain fragment. The immunofluorescence pattern of the bound LysM fragment on several gram-positive bacterial cells indicates nonrandom localization in many organisms. Because treatment with TCA, which should remove LTA, resulted in more uniform distribution, it was suggested that surface components attached to the cw can interfere with either LysM protein binding or its detection with antibodies (124).

Lysin motif (LysM) domain: domain found in some surface proteins that serves to link them noncovalently to the cw

ASYMMETRIC SURFACE LOCALIZATION

Autolysins Localized to Poles and Septum

In gram-positive bacteria, some surface proteins are distributed around the entire cell periphery and others appear to be localized to specific regions, usually the cell poles, septa, or both. The mechanisms of their localization are just beginning to be investigated. A large class of proteins in gram-positive bacteria localized to the cell division plane comprises a subset of autolysins. These autolysins are thought to be required for the separation of dividing cells, so their localization is critical for bacterial multiplication (51). The cw hydrolases LytE and LytF of *B. subtilis* are probably the best-studied examples. These proteins have N-terminal LysM motifs that are presumably important for attachment to the peptidoglycan (see above). Because *lyt*F mutants grow in long chains (97), and *lyt*E mutants are longer than wild-type cells (63), these enzymes seem to be involved in separating daughter cells after division. Using

tagged proteins and immunofluorescence microscopy, Yamamoto et al. (146) showed that LytE and LytF are localized to the cell division site, even in minicells. In vegetatively growing cells they are also found at one pole, and in the absence of the cell surface protease WprA and the extracellular protease Epr, they can be detected at both poles. This finding was interpreted to mean that the proteases are responsible for removing the remaining LytE and LytF hydrolases from division sites (new poles) after the cells divide. In contrast, LytC, another major autolysin whose cw binding domain does not include LysM motifs and which is not required for cell separation (72, 84), was uniformly distributed around the entire periphery of *B. subtilis* cells (146).

Another case in which an autolysin is localized to sites of cell division was first described for *S. aureus* by Yamada et al. (145). The bifunctional Atl enzyme contains an acetylglucosaminidase and an acetylmuramyl-alanine amidase domain, which are separated by proteolytic processing. Using immunogold labeling and protein A, Yamada et al. showed by scanning, backscattering, and transmission electron microscopy that each constituent enzyme is located in equatorial rings that separate as the cell divides. The amazing pictures (**Figure 3**) look as though the *Staphylococcus* is being "cut on the dotted line" during binary fission! Although Baba & Schneewind (4) have since shown that three repeat domains at the center of pro-Atl are necessary and sufficient for localization and that this occurs prior to processing into two separate enzymes, the cw constituent to which Atl is attached has not yet been identified. From their micrographs, Yamada et al. (145) suggest that the attachment is not to peptidoglycan, but to a fibrous material extending 20 to 50 nm from the cell surface, which they suggest might be LTA.

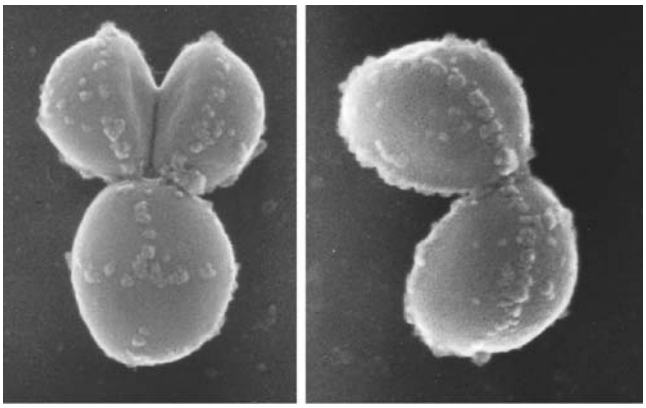

Figure 3

Scanning electron micrograph showing localization of Atl on the surface of *S. aureus* during the division cycle (reproduced, with permission, from Reference 145). The cells were reacted with anti-Atl IgG and then with colloidal gold-labeled protein A.

Other Surface-Localized Proteins

Localization to specific sites on the gram-positive bacterial surface has been observed also for some proteins that are not involved in cell division. ActA of the intracellular pathogen *L. monocytogenes* (68) is one protein in which asymmetric surface localization is critical for function. ActA is associated to the cm by its hydrophobic C terminus (118). It is absent from one pole (the pole formed during the previous division) and present in increasing concentration toward the other. The recently proposed mechanism for this localization suggests that polarized distribution is a direct consequence of differential cw growth along the bacterium (105a). Because ActA serves as the receptor on which actin filaments polymerize in the host cell, the unidirectional movement of *L. monocytogenes* from one host cell to the next, which is required for its spread in the human host, depends on this localization (122).

Localization of the Sec Translocon: The ExPortal

Recently, there have been several reports of localization of the Sec translocon to discrete sites on gram-positive bacterial surfaces, which results in localization of proteins secreted through this channel. In the coccoid gram-positive bacterium *S. pyogenes*, Rosch & Caparon (109) showed by immunogold

electron microscopy that SecA, the ATPase that reacts with the SecYEG translocon in the membrane, is found only at a single cell surface site. They observed that SpeB, a cysteine protease secreted by *S. pyogenes*, is transiently localized to a single nonpolar site on the bacterial surface, and that SpeB and SecA colocalize. A similar single localization site was observed for PhoZ, a foreign protein, when it was overproduced artificially from a plasmid in the same streptococcus (109). Rosch & Caparon believe that the single bacterial surface location of these two secreted proteins and SecA represents a specialized cm site to which the sec translocons are restricted and they named this the ExPortal.

Secreted proteins are only transiently associated with the ExPortal, and other factors determine their final location. The M protein, a sortase-anchored cw protein, is covalently linked at the newly growing area of the cw (119) and then becomes uniformly distributed over the entire cell surface (31). In contrast, some membrane proteins such as HtrA are retained at the ExPortal (110). Rosch & Caparon also suggest that the transient colocalization at the ExPortal of HtrA, which is required for maturation of the secreted protein SpeB, facilitates the processing necessary for activation of this enzyme. Thus, colocalization of secreted proteins to the unique ExPortal of the cell may play a major role in protein biogenesis, similar to that of the periplasm of a gram-negative cell.

Recently, the localization of SecA and SecY was also investigated on the surface of the rod-shaped gram-positive bacterium *B. subtilis* (28). The Errington group found foci at poles, septa, and intermediate positions to which GFP fusions to SecA and SecY localized. Results from deconvoluting microscopy could be interpreted to suggest that SecA-GFP formed two spiral-like structures around the cell, while SecY-GFP formed arcs, not continuous helices. As a control, Campo et al. (28) used SpoIVFB, a membrane protein, which they found to be distributed randomly around the cell surface, as expected. Freeze fracture microscopy showed that SecY appeared to be located on both sides of the cm, unlike the control SpoIVFB protein. In addition, Campo et al. (28) showed colocalization of SecY with SecA-GFP under conditions in which translocation through the cm appeared to be limiting. SecA localized to the same sites in all phases of growth but, surprisingly, in stationary phase SecY became delocalized and was found in the cytoplasm. Thus, it appears that secretion of proteins through the Sec translocon may be limited to discrete sites, which differ in location in cocci and rod-shaped gram-positive bacteria. The mechanisms by which the translocon is localized are just beginning to be investigated.

PROTEIN FOLDING

Proteins are transported through the translocon channel in the cell membrane in an unfolded state, but most surface and secreted proteins assume folded tertiary configurations that protect them from active "quality control" surface proteases. In gram-negative bacteria, this occurs in the periplasm, which provides an oxidizing atmosphere. However, the lack of an outer membrane in gram-positive bacteria means there is no comparable structurally defined compartment. It has been argued that in gram-positive bacteria the region either between the cm and cw, or external to the cm but including at least part of the cw, functions similarly to a gram-negative periplasm. It has also been pointed out that in gram-positive bacteria, homologs of gram-negative bacterial periplasmic proteins are often lipid modified, which keeps them tethered to the cm (116). Thus, the membrane-cw interface may function as a periplasm for gram-positive organisms where the polyanionic D-alanylated teichoic acids (LTA and WTA) are located. More recently, maturation of SpeB was demonstrated to require the colocalization of HtrA at the ExPortal in *S. pyogenes* (110). Therefore, these authors suggested that the ExPortal may play an important role in protein biogenesis because it provides a site at

which substrate proteins are colocalized with proteins that process them.

Specific Foldases

Several proteins of gram-positive bacteria function in posttranslocational folding of secreted proteins. Some of these have specific substrates encoded by the adjacent gene. One example of this is PrtM of *Lactcoccus* spp., which is a cm-associated lipoprotein (56) and a member of the family of peptidyl-prolyl isomerases (PPIases) (106, 111). PrtM is a dedicated foldase for PrtP. PrtP is a secreted serine protease necessary for growth of these bacteria in milk because it enables them to break down casein and use it as a source of amino acids (55, 56, 139). The genes encoding PrtM and PrtP are always found adjacent to each other and are transcribed in opposite directions. They are both plasmid located and often constitute the only conserved regions in the plasmids on which they are found (55). Although the N terminus of immature PrtP, like many proteases, has a "pro" sequence that is expected to act as a chaperone, PrtM is still required for proper folding and export of PrtP.

PrsA

The best-studied cell surface "foldase" whose substrate specificity is not limited to a single protein is PrsA of *B. subtilis* (69–71, 116). PrsA is important for extracytoplasmic folding of model proteins both with and without pro sequences (64). At least one homolog of this protein is found in all gram-positive species, and in strains with more than one PrsA-type gene where function has been studied, the second appears to interact specifically with the protein encoded immediately adjacent to its gene, e.g., PrtM and PrtP of *L. lactis* (40).

PrsA is a lipoprotein. It appears to be anchored to the outer surface of the cm because when protoplasts are produced, it becomes accessible to trypsin (78). Using the secretion reporter α amylase (AmyQ) of *Bacillus amyloliquefaciens*, a linear relation between the rate of secretion and the amount of PrsA protein in the *B. subtilis* cells was established (138). Furthermore, depleting the amount of PrsA protein leads to a deficit of several exoproteins in the culture medium (137). The converse is also true: Overproduction of PrsA leads to a dramatic increase in the secretion of reporter proteins in *Bacillus* spp., indicating that PrsA is a bottleneck for protein secretion (71, 143). While this is important for the industrial production of foreign proteins from *Bacillus*, it is not clear whether PrsA is limiting for folding and secretion of native proteins.

Although PrsA is required for secretion and folding of AmyQ from intact *B. subtilis* cells, it is not required for these functions in protoplasts (140). Furthermore, purified AmyQ refolds rapidly in vitro after denaturation, indicating that the folding delay observed in intact cells is related to the presence of the cw (140).

In *B. subtilis*, PrsA is an essential protein (138), suggesting that it assists the folding of at least one extracytoplasmic protein required for cell survival and/or that accumulation of misfolded protein is itself lethal. Because PrsA depletion in *B. subtilis* results in morphological changes that resemble those seen when synthesis of cw polymers is deficient, PrsA may be needed for folding of one or more cw synthetic enzymes (138). In contrast, depletion of the PrsA homolog of *L. lactis*, PspA, has no obvious effect on growth (40).

The abundance of PrsA indicates that it is in great excess over the number of translocon channels, which suggests a chaperone-like rather than an enzymatic function (138). PrsA appears to act as a chaperone or foldase to facilitate posttranslocational folding and/or to retard misfolding. Either function would reduce the misfolding of the secreted protein, which would target it for degradation by proteases in the cw or membrane-cw interface (35, 144).

Sequence analysis indicates that PrsA is a member of the parvulin group of PPIases (106). However, the PPIase motif is absent from PmpA, the *L. lactis* homolog with a

function similar to that of PrsA, and from the PrsA homologs of streptococci (40). Furthermore, AmyQ has no *cis*-prolines, so the PPIase enzymatic activity should not be necessary for processing this protein. Recently, site-directed mutagenesis of residues in the PPIase domain of PrsA showed that although the domain is required, the residues essential for enzymatic PPIase activity in vitro are not required for the essential role of PrsA in folding of AmyQ in vivo (137). This again suggests that the role of PrsA and its homologs in secretion from gram-positive cells results from its chaperone activity.

OTHER SURFACE PROTEINS

In several gram-positive pathogens, proteins that lack both an N-terminal Sec signal for transport through the cm and a known cell surface–anchoring motif have been identified on the external bacterial surface. The first of these, glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) of *S. pyogenes*, was simultaneously reported to be surface located by Lottenberg et al. (82) and Pancholi & Fischetti (102), who found it to be the major protein detectable in SDS-PAGE analysis of a surface extract. On intact bacteria, GAP-DH was sensitive to trypsin and resistant to removal with 2 M NACl or 2% SDS, suggesting that it is tightly attached to the outer bacterial surface. Since its discovery, surface localization of GAP-DH has been demonstrated in many other bacteria, both gram-positive and gram-negative.

More recently, proteomic analyses and biotinylation of intact bacteria followed by two-dimensional gel analyses led to the identification of many other metabolic enzymes as surface proteins in *S. pyogenes*, *S. agalactiae*, and *L. monocytogenes* (30, 62, 76, 80). Since *S. pyogenes* has only one gene for GAP-DH, the process of surface localization of GAP-DH and probably of the other glycolytic enzymes found on the *S. pyogenes* surface must be sufficiently inefficient to allow adequate protein to remain within the cytoplasm for metabolic purposes. It is generally believed that when these enzymes are surface localized, they have additional nonenzymatic functions, such as adherence to host molecules. For example, GAP-DH binds plasminogen, and several anchorless surface proteins in streptococci bind fibrinogen.

GAP-DH: glyceraldehyde-3-phosphate dehydrogenase

In the absence of an N-terminal signal sequence, it is possible that, instead of secretion through the cm, these proteins are released by autolysis into the medium and attached from outside (but also see the discussion of SecA2 in The Sec Pathway, above). The enolase of *S. pneumoniae* is capable of attachment when added to bacterial cells, so the authors believe it is released by autolysis and then associated with the cell surface (11). In other organisms, the mode of secretion through the cm and attachment to the cw of these proteins have not been investigated, most likely because they are essential enzymes; therefore deletion mutants are not expected to be viable. Recently, a clue about surface attachment was provided by Pancholi's group (18), who reported that addition of a 12-residue hydrophobic tail to the C terminus of *S. pyogenes* GAP-DH prevents its surface localization. Little tagged protein is found in the supernatant and, perhaps surprisingly, most appears to be in the cytoplasm and not in the cm fraction. Because of the prevalence of such molecules on the surface of important gram-positive pathogens, where they represent vaccine and/or drug targets, the mechanism of their surface localization is a critically important area for further study.

CONCLUSIONS AND FUTURE DIRECTIONS

From this overview, it should be clear that there are major differences between gram-positive and gram-negative bacteria in transport and attachment of cell surface proteins. With the wealth of genomic information for gram-positive organisms and the new availability of the needed genetic tools, the processes unique to this important group of bacteria have become attractive for further study.

Key questions that require further investigation in gram-positive bacteria include the following: What substitutes for SecB to chaperone secreted proteins to the translocon? How do accessory sortases function in attaching their substrates to the cw? What role do sortase homologs play in pilus morphogenesis and what other enzymes may be involved in this process? How are SLH proteins associated with the bacterial cell surface? How do LysM domains attach to the cw and to which moiety do they attach? Finally, as in gram-negative bacteria, the questions of how proteins become localized to restricted sites on the cell surface remains open. We hope that this review stimulates additional investigations of these and other questions. A greater understanding of the mechanisms of protein attachment to gram-positive bacteria would be helpful in developing tools to combat human pathogens and in improving the efficiency of bacteria in the energy cycle and of bioremediation of toxic waste.

SUMMARY POINTS

1. Gram-positive bacteria lack homologs of SecB, the chaperone that targets proteins to the Sec translocon for passage through the cm.
2. Covalent attachment of a protein to the gram-positive cw usually requires a transpeptidase called a sortase. Most proteins are attached by the housekeeping SrtA, but some are attached by a specific sortase, usually encoded in the vicinity of the gene encoding the surface protein. Some sortase homologs covalently link subunits of pili in gram-positive bacteria, and these transpeptidases may not be required for linkage of proteins to the peptidoglycan.
3. Some proteins can associate noncovalently with the gram-positive surface either when transported through the cm or when added from outside the bacterium. Motifs that determine such association with teichoic acids include GW repeat modules and choline binding motifs, both of which are usually at the C terminus of the protein. In contrast, LysM domains, which also often occur as repeats, appear to mediate association with peptidoglycan.
4. S-layer proteins and cellulosomes are large surface structures noncovalently attached through SLH motifs to a component of the cw. They are important for the lifestyle of the bacteria that synthesize them.
5. Asymmetric surface localization of proteins (including autolysins) is important for cell division and other critical gram-positive cell functions. Mechanisms for this are not yet understood.
6. Since proteins are transported through the cm in an unfolded state, gram-positive bacteria, which lack a structurally distinct periplasm, face the problem of correct folding of surface proteins. PrsA appears to be essential for this, although its mechanism of action has not yet been described. In addition, a unique ExPortal, composed of the Sec translocons at a single cm site in streptococci, may also retain chaperones important for folding of surface proteins.

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