Published in final edited form as:

Microbiol Spectr. 2019 July ; 7(4): . doi:10.1128/microbiolspec.GPP3-0070-2019.

Staphylococcal Protein Secretion and Envelope Assembly

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Abstract

The highly cross-linked peptidoglycan represents the rigid layer of the bacterial envelope and protects bacteria from osmotic lysis. In Gram-positive bacteria, peptidoglycan also functions as a scaffold for the immobilization of capsular polysaccharide, wall teichoic acid (WTA), and surface proteins. This chapter captures recent development on the assembly of the envelope of *Staphylococcus aureus* including mechanisms accounting for immobilization of molecules to peptidoglycan as well as hydrolysis of peptidoglycan for the specific release of bound molecules, facilitation of protein secretion across the envelope and cell division. Peptidoglycan, WTA and capsular polysaccharide are directly synthesized onto undecaprenol. Surface proteins are anchored by Sortase A, a membrane-embedded transpeptidase that scans secreted polypeptides for the C-terminal LPXTG motif of sorting signals. The resulting acyl enzyme intermediate is resolved by lipid II, the undecaprenol-bound peptidoglycan precursor. While these pathways share membrane diffusible undecaprenol, assembly of these molecules occurs either at the cross-walls or the cell poles. In *S. aureus*, the cross-wall represents the site of *de novo* peptidoglycan synthesis which is eventually split to complete the cell cycle yielding newly divided daughter cells. Peptidoglycan synthesized at the cross-wall is initially devoid of WTA. Conversely, lipoteichoic acid (LTA) synthesis which does not require bactoprenol is seemingly restricted to septal membranes. Similarly, *S. aureus* distinguishes two types of surface protein precursors. Polypeptides with canonical signal peptides are deposited at the cell poles, whereas precursors with conserved YSIRK-GXXS motif signal peptides traffic to the cross-wall. A model for protein trafficking in the envelope and uneven distribution of teichoic acids is discussed.

The cell wall envelope of *Staphylococcus aureus* is a surface organelle with anchored proteins, teichoic acids, and polysaccharides that enable bacteria to promote specific interactions with their environment (1). During colonization and invasion of their mammalian hosts, secreted and cell wall-anchored surface proteins fulfill a wide spectrum of functions, including bacterial adhesion to host cells or tissues, evasion of innate host defenses, and diversions of host adaptive immune responses (2). Secreted proteins trafficking in and out of the cell wall envelope promote both the persistent colonization of mammalian hosts and the pathogenesis of specific disease states that are characteristically associated with *S. aureus* (2).

CELL WALL SYNTHESIS AND ENVELOPE ASSEMBLY

When analyzed by electron microscopy of thin-sectioned bacteria, staphylococci are spherical cells whose envelope is composed of a plasma membrane and surrounding cell wall layer (20 to 40 nm in diameter) (3). Cell walls are isolated by physical disruption of staphylococci and chemical treatments that remove secondary polymers, including wall teichoic acids (WTAs), polysaccharides, and proteins (4). The remaining structures, designated murein sacculi, retain the spherical shape of the bacteria and are composed of peptidoglycan, including glycan strands with a repeating disaccharide, $\rightarrow 4(N$ -acetylmuramoyl- β -(1 $\rightarrow$ 4)-*N*-acetylglucosaminyl- β -(1 $\rightarrow$), abbreviated (MurNAc-GlcNAc)_n, and cross-linked wall peptides, L-alanyl-D-isoglutaminyl-(pentaglycyl)L-lysyl-D-alanyl_n, abbreviated [Ala-iGln-(Gly₅)Lys-Ala]_n (5, 6). Wall peptides are tethered to glycan chains via amide bonds formed by the amino-group of L-alanyl and the carboxyl-group of MurNAc (6, 7). Wall peptides of neighboring peptidoglycan strands are cross-linked by amide bonds formed from the amino group of pentaglycyl and the carboxyl-group of D-alanyl (8). During peptidoglycan synthesis, the bactoprenol-linked intermediate, lipid II [C₅₅-(PO₄)₂-MurNAc(-Ala-iGln-(NH₂-Gly₅)Lys-Ala-Ala-COOH)-GlcNAc], is translocated across the staphylococcal plasma membrane (9, 10). Penicillin binding protein 2 polymerizes lipid II substrate to generate glycan strands and cross-linked wall peptides via hydrolysis of its phosphodiester and Ala-Ala bonds (11–13).

WTAs and capsular polysaccharide are also synthesized as bactoprenol-linked intermediates (14, 15). Once translocated across the membrane, WTAs and capsular polysaccharides are anchored to the peptidoglycan scaffold (16, 17). Staphylococci replicate by forming cell division septa at midcell and by synthesizing peptidoglycan in the cross-wall compartment, which is bounded by the septal membranes of newly dividing staphylococci (3, 18). Once completed, cross-wall peptidoglycan is split to separate the dividing cells (3). Staphylococcal murein sacculi are not permeable for proteins and other large macromolecules. Cell division, assembly of secondary polymers, and the secretion of proteins across the bacterial cell wall requires specific murein hydrolases that modify murein sacculi, thereby enabling protein secretion across the cell wall envelope and the anchoring of secondary cell wall polymers to peptidoglycan (19, 20).

TEICHOIC ACID SYNTHESIS AND ENVELOPE ASSEMBLY

The envelope of *S. aureus* encompasses both polyribitol-phosphate and polyglycerol-phosphate polymers (21, 22). Polyribitol-phosphate [(Rib-PO₄)_n], also designated WTA, is synthesized in the bacterial cytoplasm from CDP-ribitol and tethered to its bactoprenol-linked carrier, thereby generating lipid III [C₅₅-(PO₄)₂-GlcNAc-ManNAc-(Gro-PO₄)₂-(Rib-PO₄)_{11–40}] (23, 24). Rib-PO₄ is polymerized via phosphodiester bonds engaging ribitol hydroxyls at O1 and O5; the remaining hydroxyl moieties (O2, O3, and O4) are variably substituted with α - or β -linked GlcNAc (25–27). Following translocation of lipid III across the membrane, LCP enzymes tether WTA to peptidoglycan, generating phosphodiester bonds between the O1 of GlcNAc and the O6 of MurNAc (28). The Rib-PO₄ polymer of anchored WTA is further modified via esterification with D-alanyl (29, 30). Peptidoglycan synthesized at the cross-wall is initially devoid of WTA (31) (Fig. 1). Upon cross-wall

splitting and cell separation, cross-wall peptidoglycan is modified with WTA (31). LtaS polymerizes polyglycerol-phosphate [(Gro-PO₄)_n], also designated lipoteichoic acid (LTA), from phosphatidylglycerol, transferring the assembled Gro-PO₄ polymer onto its glycolipid anchor gentiobiosyldiacylglycerol: Glc-β-(1→6)-Glc-β-(1→3)-diacylglycerol [Glc₂-DAG] (32–34). The O₂ hydroxyl of Gro-PO₄ is variably substituted with esterified D-alanyl (30, 35). LtaS and LTA synthesis are restricted to septal membranes and are required for staphylococcal cell division and growth (36) (Fig. 1). Thus, assembly sites of WTA and LTA synthesis are unevenly distributed in the envelope of dividing staphylococci.

PROTEIN SECRETION

The genome of *S. aureus* carries more than 1,300 genes with products encompassing predicted N-terminal signal peptides (37). When subjected to proteomic analysis, most of these gene products can be identified in the filtered supernatant of centrifuged *S. aureus* cultures (38). Genetic and biochemical experiments characterized the Sec secretion pathway in the model organism *Escherichia coli* (39–41). Elements of this pathway are conserved among all bacteria, including staphylococci (42). Some of the *sec* genes have also been characterized in *S. aureus*. Signal peptide-bearing precursors are translocated across the plasma membrane through the Sec translocon, a complex formed from three membrane proteins, SecY, SecE, and SecG (43). Protein translocation requires SecA, a cytoplasmic ATPase that pushes unfolded precursors through the translocon (44, 45). SecDF, a chaperone whose activity is sustained by the proton motive force, assists in the folding of translocated proteins (46, 47). *Similar to E. coli*, *S. aureus secA* is absolutely essential for bacterial growth and protein secretion, whereas *secDF* is not essential, providing auxiliary functions to protein secretion (48–50). Translocated precursors are cleaved by signal peptidase I, LepB in *E. coli* (51). *S. aureus* carries two signal peptidase I genes, *spsA* and *spsB* (52). SpsB, but not SpsA (it lacks a critical catalytic residue), can substitute for *E. coli* LepB, and expression of *spsB* is required for staphylococcal growth and the cleavage of secreted precursors in *S. aureus* (52). Suppressor mutations that induce expression of a putative ABC transporter (ABC1–3) can restore both growth and precursor secretion in *S. aureus* *spsB* mutants (53, 54). A physiological role has not yet been assigned to the putative ABC1–3 transporter. *SagB* glucosaminidase cleaves staphylococcal peptidoglycan to generate short glycan strands with variable lengths (MurNAc-GlcNAc)_{3–10}. *S. aureus sagB* mutants exhibit diminished growth, cross-wall separation defects during cell division, and reduced secretion of proteins into the extracellular medium (19). Thus, the generation of short glycan strands is a prerequisite for the transport of protein across the cell wall peptidoglycan. Three other murein hydrolases—autolysin (Atl amidase and glucosaminidase), Sle1, and LytN (CHAP domain amidases and endopeptidases)—contribute to cell division, cross-wall separation, and likely also to protein secretion across the staphylococcal cell wall envelope (55–59).

PROTEIN ANCHORING TO THE CELL WALL

Surface proteins of staphylococci are covalently linked to peptidoglycan (60). Following translocation of signal peptide-bearing precursors across the plasma membrane, the C-terminal end of surface proteins is cleaved between the threonyl (T) and the glycyl (G) of their LPXTG motif sorting signal by sortase A, which generates a thioester-linked acyl-

enzyme intermediate (61–64) (Fig. 2). Nucleophilic attack of the peptidoglycan biosynthetic intermediate, i.e., of the amino-group of pentaglycyl within lipid II, resolves the sortase A acyl-enzyme, synthesizing an amide linkage between the carboxyl group of the C-terminal threonyl of surface proteins and the amino-group of pentaglycyl (65, 66). The surface protein-lipid II intermediate is subsequently incorporated into the peptidoglycan via penicillin binding protein 2-catalyzed transglycosylation and transpeptidation reactions (67) (Fig. 2). Sortase A and surface protein anchoring to the cell wall envelope occur both within the cross-wall compartment and within polar peptidoglycan (68, 69) (Fig. 1). Genome sequences of thousands of isolates reveal between 18 and 23 genes for sortase A-anchored surface proteins in *S. aureus* (70).

PROTEIN SECRETION INTO THE CROSS-WALL COMPARTMENT

Thirteen *S. aureus* surface protein genes encode precursors with YSIRK-GXXS signal peptides, and their products are secreted across septal membranes into the cross-wall compartment (69, 71). Septal secretion promotes abundant deposition and broad distribution of these proteins over the staphylococcal surface (68). In contrast, surface proteins secreted via canonical signal peptides are deposited with low abundance into polar peptidoglycan (69). In addition to sortase A-anchored surface proteins, four other staphylococcal precursors carry YSIRK-GXXS signal peptides: the LytN murein hydrolase (55), the giant protein Ebh (72), and two glycerol-ester hydrolases, GehA and GehB (73). Septal secretion of LytN into the cross-wall promotes cleavage of peptidoglycan and the separation of cells that have completed the division process (55). The 10,421-residue Ebh precursor comprises an N-terminal YSIRK-GXXS signal peptide, a hyperosmolarity resistance domain (residues 179 to 2530), 7 repeats of the 54-residue FIVAR domain (possibly associated with polysaccharide binding), 50 repeats of the 123-residue FIVAR-GA domain, 7 repeats of DUF1542 (a 72-residue domain of unknown function), a putative SMC domain (structural maintenance of chromosomes; residues 8976 to 9576), a transmembrane domain (residues 10227 to 10247), and a positively charged cytoplasmic domain (72). Mutations that abolish *ebh* expression increase staphylococcal cell size and perturb the integrity of the cell wall envelope, suggesting that Ebh is a size determinant for the staphylococcal murein sacculus (72). GehA and GehB are secreted as 72-kDa pro-proteins with 250-residue N-terminal domains that are thought to assist in the folding of C-terminal glycerol-ester hydrolase domains (74). GehA cleaves the ester bonds of shortchain fatty acids within acyl-glycerol, whereas GehB hydrolyzes preferentially long-chain fatty acid esters (75–77). Following cell division, GehB pro-protein is released into the extracellular medium and cleaved by aureolysin, a cysteine protease that is secreted via the canonical Sec pathway (75). A specific role for GehA and GehB during staphylococcal cell division has thus far not been revealed.

During secretion, the precursor of SpA (staphylococcal protein A) is cleaved at Gly¹³, i.e., between the conserved (underlined) elements of its YSIRKLG¹³VGIAS motif (50). After translocation across the plasma membrane, SpsB signal peptidase cleaves SpA at Ala³⁷, thereby removing the N-terminal hydrophobic signal peptide (78). Translocated SpA is retained within the secretory pathway by its C-terminal sorting signal and is subsequently anchored to the cell wall envelope (79). Amino acid substitutions in the YSIRK-GXXS

motif perturb both the proteolysis and the secretion of SpA precursors, suggesting that cleavage of the YSIRK-GXXS motif is a prerequisite for septal secretion (50). SecA ATPase and SecDF foldase promote the secretion of precursor proteins at septal and polar membranes (50). In contrast, LtaS and LTA synthesis are required for septal, but not polar, secretion in *S. aureus* (50). Thus, synthesis of LTA within septal membranes represents a unique trait of staphylococcal biology that directs precursors with YSIRK-GXXS signal peptides into the cross-wall compartment (Fig. 1). It is not yet clear, however, whether the abundance of LTA, the depletion of phosphatidylglycerol, i.e., the lipid substrate of LtaS, or the accumulation of di-acylglycerol, i.e., the byproduct of LTA synthesis, make specific contributions toward septal secretion. It is also conceivable that septal secretion of GehA and GehB may contribute to the specific features of septal membranes that support trafficking of YSIRK-GXXS precursors into the cross-wall compartment.

RELEASE OF SURFACE PROTEINS FROM THE STAPHYLOCOCCAL ENVELOPE

During staphylococcal growth, cell wall-anchored surface proteins are released from the staphylococcal envelope into the extracellular milieu, where they fulfill specific functions (80). For example, release of SpA requires LytN CHAP amidase and LytM endopeptidase, releasing surface protein with murein tetrapeptide-tetraglycyl [_L-Ala-D-iGln-(SpA-Gly₅)-Lys-D-Ala-Gly₄] linked to the C-terminal threonyl (81) (Fig. 2). Surface proteins of staphylococci fulfill complex biological functions both on the bacterial surface and following their release from the peptidoglycan scaffold by murein hydrolases (82).

STAPHYLOCOCCAL PROTEIN A

All clinical isolates of *S. aureus* harbor the *spa* gene for staphylococcal protein A (SpA), which generates a precursor product composed of an N-terminal YSIRK/GXXS signal peptide followed by 4 or 5 immunoglobulinbinding domains (IgBDs), region X repeats (Xr), an LysM domain, and an LPXTG sorting signal (70, 83, 84). Following septal secretion and anchoring to cross-wall peptidoglycan, SpA is displayed on the bacterial surface (68). The IgBDs of SpA bind to the Fc γ domain of human and animal IgG, effectively blocking effector functions of antibodies, i.e., the binding of C1q complement or the engagement of Fc receptors, thereby inhibiting opsonophagocytic killing of staphylococci (85–87). SpA released from the bacterial envelope crosslinks V_H3 idiotype IgM B cell receptors and promotes the nonproductive proliferation of B cells and secretion of V_H3 idiotype antibodies that do not recognize staphylococcal antigens (88, 89). This B cell superantigen activity requires endocytosis of SpA crosslinked to B cell receptors, signaling via RIPK2 kinase, and CD4⁺ T helper cell functions (90). The B cell superantigen activity of SpA effectively blocks host adaptive immune responses and the establishment of protective immunity against *S. aureus* (85).

SURFACE PROTEINS AND HEME-IRON TRANSPORT

S. aureus encodes a second sortase gene, designated sortase B (*srtB*) (91). Sortase B cleaves the NPQTN-motif sorting signal of IsdC (iron-regulated surface determinant C) (92). Unlike

sortase A, the sortase B acyl-enzyme intermediate is resolved by nucleophilic attack of the amino group of pentaglycyl within polymerized peptidoglycan, thereby anchoring of IsdC to peptidoglycan in the vicinity of the staphylococcal membrane (92). The sortase B anchoring mechanism enables *S. aureus* surface proteins to promote heme-iron scavenging from host hemoglobin and haptoglobin (93, 94). Initially, sortase A-anchored IsdB and IsdH retrieve heme-iron from host hemoproteins (95, 96). IsdA, another sortase A-anchored surface protein, captures the heme-iron from IsdB and IsdH for passage of the nutrient across the staphylococcal cell wall envelope and transfer to IsdC (97). IsdE lipoprotein and IsdF transporter are thought to capture heme-iron from IsdC for import into the bacterial cytoplasm (97, 98). IsdG and IsdI cleave the tetrapyrrol ring of heme to generate staphylobilin and iron for the assembly of iron-sulfur cluster proteins (99, 100). NEAT domains (near-iron transporter domains about 120 residues in length) promote the heme-iron binding and transfer reactions and are found in variable numbers within IsdA, IsdB, IsdC, IsdE, and IsdH (93, 101, 102).

REFERENCES

1. Schneewind O, Missiakas D. 2014 Sec-secretion and sortase-mediated anchoring of proteins in Gram-positive bacteria. *Biochim Biophys Acta* 1843:1687–1697 10.1016/j.bbamcr.2013.11.009. [PubMed: 24269844]
2. Thammavongsa V, Kim HK, Missiakas D, Schneewind O. 2015 Staphylococcal manipulation of host immune responses. *Nat Rev Microbiol* 13:529–543 10.1038/nrmicro3521. [PubMed: 26272408]
3. Giesbrecht P, Wecke J, Reinicke B. 1976 On the morphogenesis of the cell wall of staphylococci. *Int Rev Cytol* 44:225–318 10.1016/S0074-7696(08)61651-4. [PubMed: 770370]
4. de Jonge BLM, Chang YS, Gage D, Tomasz A. 1992 Peptidoglycan composition of a highly methicillin-resistant *Staphylococcus aureus* strain. The role of penicillin binding protein 2A. *J Biol Chem* 267:11248–11254. [PubMed: 1597460]
5. Ghuysen J-M, Strominger JL. 1963 Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. II. Separation and structure of the disaccharides. *Biochemistry* 2:1119–1125 10.1021/bi00905a036. [PubMed: 14087370]
6. Ghuysen J-M, Tipper DJ, Birge CH, Strominger JL. 1965 Structure of the cell wall of *Staphylococcus aureus* strain Copenhagen. VI. The soluble glycopeptide and its sequential degradation by peptidases. *Biochemistry* 4:2245–2254 10.1021/bi00886a043.
7. Tipper DJ, Ghuysen J-M, Strominger JL. 1965 Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. III. Further studies of the disaccharides. *Biochemistry* 4:468–473 10.1021/bi00879a015.
8. Tipper DJ, Strominger JL. 1965 Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. *Proc Natl Acad Sci USA* 54:1133–1141 10.1073/pnas.54.4.1133. [PubMed: 5219821]
9. Anderson JS, Matsushashi M, Haskin MA, Strominger JL. 1965 Lipidphosphoacetylmuramyl-pentapeptide and lipid-phosphodisaccharidepentapeptide: presumed membrane transport intermediates in cell wall synthesis. *Proc Natl Acad Sci USA* 53:881–889 10.1073/pnas.53.4.881. [PubMed: 14324547]
10. Higashi Y, Strominger JL, Sweeley CC. 1967 Structure of a lipid intermediate in cell wall peptidoglycan synthesis: a derivative of a C55 isoprenoid alcohol. *Proc Natl Acad Sci USA* 57:1878–1884 10.1073/pnas.57.6.1878. [PubMed: 5231417]
11. Leski TA, Tomasz A. 2005 Role of penicillin-binding protein 2 (PBP2) in the antibiotic susceptibility and cell wall cross-linking of *Staphylococcus aureus*: evidence for the cooperative functioning of PBP2, PBP4, and PBP2A. *J Bacteriol* 187:1815–1824 10.1128/JB.187.5.1815-1824.2005. [PubMed: 15716453]

12. Pinho MG, Errington J. 2005 Recruitment of penicillin-binding protein PBP2 to the division site of *Staphylococcus aureus* is dependent on its transpeptidation substrates. *Mol Microbiol* 55:799–807 10.1111/j.1365-2958.2004.04420.x. [PubMed: 15661005]
13. Reed P, Atilano ML, Alves R, Hoiczky E, Sher X, Reichmann NT, Pereira PM, Roemer T, Filipe SR, Pereira-Leal JB, Ligoxygakis P, Pinho MG. 2015 *Staphylococcus aureus* survives with a minimal peptidoglycan synthesis machine but sacrifices virulence and antibiotic resistance. *PLoS Pathog* 11:e1004891 10.1371/journal.ppat.1004891. [PubMed: 25951442]
14. Brown S, Zhang YH, Walker S. 2008 A revised pathway proposed for *Staphylococcus aureus* wall teichoic acid biosynthesis based on *in vitro* reconstitution of the intracellular steps. *Chem Biol* 15:12–21 10.1016/j.chembiol.2007.11.011. [PubMed: 18215769]
15. O’Riordan K, Lee JC. 2004 *Staphylococcus aureus* capsular polysaccharides. *Clin Microbiol Rev* 17:218–234 10.1128/CMR.17.1.218-234.2004. [PubMed: 14726462]
16. Chan YG, Kim HK, Schneewind O, Missiakas D. 2014 The capsular polysaccharide of *Staphylococcus aureus* is attached to peptidoglycan by the LytR-CpsA-Psr (LCP) family of enzymes. *J Biol Chem* 289:15680–15690 10.1074/jbc.M114.567669. [PubMed: 24753256]
17. Chan YGY, Frankel MB, Dengler V, Schneewind O, Missiakas D. 2013 *Staphylococcus aureus* mutants lacking the LytR-CpsA-Psr family of enzymes release cell wall teichoic acids into the extracellular medium. *J Bacteriol* 195:4650–4659 10.1128/JB.00544-13. [PubMed: 23935043]
18. Tzagoloff H, Novick R. 1977 Geometry of cell division in *Staphylococcus aureus*. *J Bacteriol* 129:343–350. [PubMed: 830642]
19. Chan YG, Frankel MB, Missiakas D, Schneewind O. 2016 SagB glucosaminidase is a determinant of *Staphylococcus aureus* glycan chain length, antibiotic susceptibility, and protein secretion. *J Bacteriol* 198:1123–1136 10.1128/JB.00983-15. [PubMed: 26811319]
20. Wheeler R, Turner RD, Bailey RG, Salamaga B, Mesnage S, Mohamad SA, Hayhurst EJ, Horsburgh M, Hobbs JK, Foster SJ. 2015 Bacterial cell enlargement requires control of cell wall stiffness mediated by peptidoglycan hydrolases. *MBio* 6:e00660 10.1128/mBio.00660-15. [PubMed: 26220963]
21. Archibald AR, Baddiley J, Heckels JE. 1973 Molecular arrangement of teichoic acid in the cell wall of *Staphylococcus lactis*. *Nat New Biol* 241:29–31 10.1038/newbio241029a0. [PubMed: 4512329]
22. Armstrong JJ, Baddiley J, Buchanan JG, Davison AL, Kelemen MV, Neuhaus FC. 1959 Composition of teichoic acids from a number of bacterial walls. *Nature* 184:247–248 10.1038/184247a0. [PubMed: 13794315]
23. McArthur HA, Roberts FM, Hancock IC, Baddiley J. 1978 Lipid intermediates in the biosynthesis of the linkage unit between teichoic acids and peptidoglycan. *FEBS Lett* 86:193–200 10.1016/0014-5793(78)80561-4. [PubMed: 624402]
24. Rajbhandary UL, Baddiley J. 1963 The intracellular teichoic acid from *Staphylococcus aureus* H. *Biochem J* 87:429–435 10.1042/bj0870429. [PubMed: 13990553]
25. Baddiley J. 1968 Teichoic acids in walls and the molecular structure of bacterial walls. *Proc R Soc Lond B Biol Sci* 170:331–348. [PubMed: 4385583]
26. Brown S, Xia G, Luhachack LG, Campbell J, Meredith TC, Chen C, Winstel V, Gekeler C, Irazoqui JE, Peschel A, Walker S. 2012 Methicillin resistance in *Staphylococcus aureus* requires glycosylated wall teichoic acids. *Proc Natl Acad Sci USA* 109:18909–18914 10.1073/pnas.1209126109. [PubMed: 23027967]
27. Xia G, Maier L, Sanchez-Carballo P, Li M, Otto M, Holst O, Peschel A. 2010 Glycosylation of wall teichoic acid in *Staphylococcus aureus* by TarM. *J Biol Chem* 285:13405–13415 10.1074/jbc.M109.096172. [PubMed: 20185825]
28. Kawai Y, Marles-Wright J, Cleverley RM, Emmins R, Ishikawa S, Kuwano M, Heinz N, Bui NK, Hoyland CN, Ogasawara N, Lewis RJ, Vollmer W, Daniel RA, Errington J. 2011 A widespread family of bacterial cell wall assembly proteins. *EMBO J* 30:4931–4941 10.1038/emboj.2011.358. [PubMed: 21964069]
29. Neuhaus FC, Baddiley J. 2003 A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in Gram-positive bacteria. *Microbiol Mol Biol Rev* 67:686–723 10.1128/MMBR.67.4.686-723.2003. [PubMed: 14665680]

30. Heaton MP, Neuhaus FC. 1994 Role of the D-alanyl carrier protein in the biosynthesis of D-alanyl-lipoteichoic acid. *J Bacteriol* 176:681–690 10.1128/jb.176.3.681-690.1994. [PubMed: 8300523]
31. Atilano ML, Pereira PM, Yates J, Reed P, Veiga H, Pinho MG, Filipe SR. 2010 Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *Staphylococcus aureus*. *Proc Natl Acad Sci USA* 107:18991–18996 10.1073/pnas.1004304107. [PubMed: 20944066]
32. Gründling A, Schneewind O. 2007 Genes required for glycolipid synthesis and lipoteichoic acid anchoring in *Staphylococcus aureus*. *J Bacteriol* 189:2521–2530 10.1128/JB.01683-06. [PubMed: 17209021]
33. Gründling A, Schneewind O. 2007 Synthesis of glycerol phosphate lipoteichoic acid in *Staphylococcus aureus*. *Proc Natl Acad Sci USA* 104:8478–8483 10.1073/pnas.0701821104. [PubMed: 17483484]
34. Kiriukhin MY, Debatov DV, Shinabarger DL, Neuhaus FC. 2001 Biosynthesis of the glycolipid anchor in lipoteichoic acid of *Staphylococcus aureus* RN4220: role of Ypfp, the diglucosyldiacylglycerol synthase. *J Bacteriol* 183:3506–3514 10.1128/JB.183.11.3506-3514.2001. [PubMed: 11344159]
35. Reichmann NT, Cassona CP, Gründling A. 2013 Revised mechanism of D-alanine incorporation into cell wall polymers in Gram-positive bacteria. *Microbiology* 159:1868–1877 10.1099/mic.0.069898-0. [PubMed: 23858088]
36. Reichmann NT, Piçarra Cassona C, Monteiro JM, Bottomley AL, Corrigan RM, Foster SJ, Pinho MG, Gründling A. 2014 Differential localization of LTA synthesis proteins and their interaction with the cell division machinery in *Staphylococcus aureus*. *Mol Microbiol* 92:273–286 10.1111/mmi.12551. [PubMed: 24533796]
37. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K. 2001 Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 357:1225–1240 10.1016/S0140-6736(00)044032. [PubMed: 11418146]
38. Kusch H, Engelmann S. 2014 Secrets of the secretome in *Staphylococcus aureus*. *Int J Med Microbiol* 304:133–141 10.1016/j.ijmm.2013.11.005. [PubMed: 24424242]
39. Benson SA, Hall MN, Silhavy TJ. 1985 Genetic analysis of protein export in *Escherichia coli* K12. *Annu Rev Biochem* 54:101–134 10.1146/annurev.bi.54.070185.000533. [PubMed: 3896116]
40. Schatz PJ, Beckwith J. 1990 Genetic analysis of protein export in *Escherichia coli*. *Annu Rev Genet* 24:215–248 10.1146/annurev.ge.24.120190.001243. [PubMed: 2088168]
41. Tsirigotaki A, De Geyter J, Šoštarić N, Economou A, Karamanou S. 2017 Protein export through the bacterial Sec pathway. *Nat Rev Microbiol* 15:21–36 10.1038/nrmicro.2016.161. [PubMed: 27890920]
42. Sibbald MJ, Ziebandt AK, Engelmann S, Hecker M, de Jong A, Harmsen HJ, Raangs GC, Stokroos I, Arends JP, Dubois JY, van Dijk JM. 2006 Mapping the pathways to staphylococcal pathogenesis by comparative secretomics. *Microbiol Mol Biol Rev* 70:755–788 10.1128/MMBR.00008-06. [PubMed: 16959968]
43. Economou A, Pogliano JA, Beckwith J, Oliver DB, Wickner W. 1995 SecA membrane cycling at SecYEG is driven by distinct ATP binding and hydrolysis events and is regulated by SecD and SecE. *Cell* 83:1171–1181 10.1016/0092-8674(95)90143-4. [PubMed: 8548804]
44. Economou A, Wickner W. 1994 SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell* 78:835–843 10.1016/S0092-8674(94)90582-7. [PubMed: 8087850]
45. Oliver DB, Beckwith J. 1981 *E. coli* mutant pleiotropically defective in the export of secreted proteins. *Cell* 25:765–772 10.1016/0092-8674(81)90184-7. [PubMed: 7026050]
46. Tsukazaki T, Mori H, Echizen Y, Ishitani R, Fukai S, Tanaka T, Perederina A, Vassilyev DG, Kohno T, Maturana AD, Ito K, Nureki O. 2011 Structure and function of a membrane component SecDF that enhances protein export. *Nature* 474:235–238 10.1038/nature09980. [PubMed: 21562494]

47. Pogliano JA, Beckwith J. 1994 SecD and SecF facilitate protein export in *Escherichia coli*. EMBO J 13:554–561 10.1002/j.1460-2075.1994.tb06293.x. [PubMed: 8313900]
48. Quiblier C, Zinkernagel AS, Schuepbach RA, Berger-Bächi B, Senn MM. 2011 Contribution of SecDF to *Staphylococcus aureus* resistance and expression of virulence factors. BMC Microbiol 11:72 10.1186/1471-2180-11-72. [PubMed: 21486434]
49. Quiblier C, Seidl K, Roschitzki B, Zinkernagel AS, Berger-Bächi B, Senn MM. 2013 Secretome analysis defines the major role of SecDF in *Staphylococcus aureus* virulence. PLoS One 8:e63513 10.1371/journal.pone.0063513. [PubMed: 23658837]
50. Yu W, Missiakas D, Schneewind O. 2018 Septal secretion of protein A in *Staphylococcus aureus* requires SecA and lipoteichoic acid synthesis. eLife 7:e34092 10.7554/eLife.34092. [PubMed: 29757141]
51. Date T, Wickner W. 1981 Isolation of the *Escherichia coli* leader peptidase gene and effects of leader peptidase overproduction in vivo. Proc Natl Acad Sci USA 78:6106–6110 10.1073/pnas.78.10.6106. [PubMed: 6273848]
52. Cregg KM, Wilding I, Black MT. 1996 Molecular cloning and expression of the *spsB* gene encoding an essential type I signal peptidase from *Staphylococcus aureus*. J Bacteriol 178:5712–5718 10.1128/jb.178.19.5712-5718.1996. [PubMed: 8824617]
53. Morisaki JH, Smith PA, Date SV, Kajihara KK, Truong CL, Modrusan Z, Yan D, Kang J, Xu M, Shah IM, Mintzer R, Kofoed EM, Cheung TK, Arnott D, Koehler MF, Heise CE, Brown EJ, Tan MW, Hazenbos WL. 2016 A putative bacterial ABC transporter circumvents the essentiality of signal peptidase. MBio 7:e00412–16 10.1128/mBio.00412-16. [PubMed: 27601569]
54. Craney A, Romesberg FE. 2015 A putative cro-like repressor contributes to arylomycin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 59:3066–3074 10.1128/AAC.04597-14. [PubMed: 25753642]
55. Frankel MB, Hendrickx AP, Missiakas DM, Schneewind O. 2011 LytN, a murein hydrolase in the cross-wall compartment of *Staphylococcus aureus*, is involved in proper bacterial growth and envelope assembly. J Biol Chem 286:32593–32605 10.1074/jbc.M111.258863. [PubMed: 21784864]
56. Sugai M, Yamada S, Nakashima S, Komatsuzawa H, Matsumoto A, Oshida T, Suganaka H. 1997 Localized perforation of the cell wall by a major autolysin: *atl* gene products and the onset of penicillin-induced lysis of *Staphylococcus aureus*. J Bacteriol 179:2958–2962 10.1128/jb.179.9.2958-2962.1997. [PubMed: 9139914]
57. Takahashi J, Komatsuzawa H, Yamada S, Nishida T, Labischinski H, Fujiwara T, Ohara M, Yamagishi J, Sugai M. 2002 Molecular characterization of an *atl* null mutant of *Staphylococcus aureus*. Microbiol Immunol 46:601–612 10.1111/j.1348-0421.2002.tb02741.x. [PubMed: 12437027]
58. Kajimura J, Fujiwara T, Yamada S, Suzawa Y, Nishida T, Oyamada Y, Hayashi I, Yamagishi J, Komatsuzawa H, Sugai M. 2005 Identification and molecular characterization of an N-acetylmuramyl-L-alanine amidase SleI involved in cell separation of *Staphylococcus aureus*. Mol Microbiol 58:1087–1101 10.1111/j.1365-2958.2005.04881.x. [PubMed: 16262792]
59. Oshida T, Sugai M, Komatsuzawa H, Hong Y-M, Suganaka H, Tomasz A. 1995 A *Staphylococcus aureus* autolysin that has an N-acetylmuramoyl-L-alanine amidase domain and an endo-beta-N-acetylglucosaminidase domain: cloning, sequence analysis, and characterization. Proc Natl Acad Sci USA 92:285–289 10.1073/pnas.92.1.285. [PubMed: 7816834]
60. Schneewind O, Fowler A, Faull KF. 1995 Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. Science 268:103–106 10.1126/science.7701329. [PubMed: 7701329]
61. Schneewind O, Model P, Fischetti VA. 1992 Sorting of protein A to the staphylococcal cell wall. Cell 70:267–281 10.1016/0092-8674(92)90101-H. [PubMed: 1638631]
62. Navarre WW, Schneewind O. 1994 Proteolytic cleavage and cell wall anchoring at the LPXTG motif of surface proteins in Gram-positive bacteria. Mol Microbiol 14:115–121 10.1111/j.1365-2958.1994.tb01271.x. [PubMed: 7830549]
63. Mazmanian SK, Liu G, Ton-That H, Schneewind O. 1999 *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. Science 285:760–763 10.1126/science.285.5428.760. [PubMed: 10427003]

64. Ton-That H, Liu G, Mazmanian SK, Faull KF, Schneewind O. 1999 Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of *Staphylococcus aureus* at the LPXTG motif. *Proc Natl Acad Sci USA* 96:12424–12429 10.1073/pnas.96.22.12424. [PubMed: 10535938]
65. Ton-That H, Mazmanian SK, Faull KF, Schneewind O. 2000 Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. Sortase-catalyzed in vitro transpeptidation reaction using LPXTG peptide and NH(2)-Gly(3) substrates. *J Biol Chem* 275:9876–9881 10.1074/jbc.275.13.9876. [PubMed: 10734144]
66. Perry AM, Ton-That H, Mazmanian SK, Schneewind O. 2002 Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. III. Lipid II is an in vivo peptidoglycan substrate for sortase-catalyzed surface protein anchoring. *J Biol Chem* 277:16241–16248 10.1074/jbc.M109194200. [PubMed: 11856734]
67. Ton-That H, Schneewind O. 1999 Anchor structure of staphylococcal surface proteins. IV. Inhibitors of the cell wall sorting reaction. *J Biol Chem* 274:24316–24320 10.1074/jbc.274.34.24316. [PubMed: 10446208]
68. DeDent AC, McAdow M, Schneewind O. 2007 Distribution of protein A on the surface of *Staphylococcus aureus*. *J Bacteriol* 189:4473–4484 10.1128/JB.00227-07. [PubMed: 17416657]
69. DeDent A, Bae T, Missiakas DM, Schneewind O. 2008 Signal peptides direct surface proteins to two distinct envelope locations of *Staphylococcus aureus*. *EMBO J* 27:2656–2668 10.1038/emboj.2008.185. [PubMed: 18800056]
70. Mazmanian SK, Ton-That H, Schneewind O. 2001 Sortase-catalysed anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. *Mol Microbiol* 40:1049–1057 10.1046/j.1365-2958.2001.02411.x. [PubMed: 11401711]
71. Bae T, Schneewind O. 2003 The YSIRK-G/S motif of staphylococcal protein A and its role in efficiency of signal peptide processing. *J Bacteriol* 185:2910–2919 10.1128/JB.185.9.2910-2919.2003. [PubMed: 12700270]
72. Cheng AG, Missiakas D, Schneewind O. 2014 The giant protein Ehb is a determinant of *Staphylococcus aureus* cell size and complement resistance. *J Bacteriol* 196:971–981 10.1128/JB.01366-13. [PubMed: 24363342]
73. Yu W, Götz F. 2012 Cell wall antibiotics provoke accumulation of anchored mCherry in the cross wall of *Staphylococcus aureus*. *PLoS One* 7:e30076 10.1371/journal.pone.0030076. [PubMed: 22253886]
74. Rosenstein R, Götz F. 2000 Staphylococcal lipases: biochemical and molecular characterization. *Biochimie* 82:1005–1014 10.1016/S0300-9084(00)01180-9. [PubMed: 11099797]
75. Cadieux B, Vijayakumaran V, Bernards MA, McGavin MJ, Heinrichs DE. 2014 Role of lipase from community-associated methicillin-resistant *Staphylococcus aureus* strain USA300 in hydrolyzing triglycerides into growth-inhibitory free fatty acids. *J Bacteriol* 196:4044–4056 10.1128/JB.02044-14. [PubMed: 25225262]
76. Nikoleit K, Rosenstein R, Verheij HM, Götz F. 1995 Comparative biochemical and molecular analysis of the *Staphylococcus hyicus*, *Staphylococcus aureus* and a hybrid lipase. Indication for a C-terminal phospholipase domain. *Eur J Biochem* 228:732–738 10.1111/j.1432-1033.1995.tb0317.x. [PubMed: 7737171]
77. Simons JW, Adams H, Cox RC, Dekker N, Götz F, Slotboom AJ, Verheij HM. 1996 The lipase from *Staphylococcus aureus*. Expression in *Escherichia coli*, large-scale purification and comparison of substrate specificity to *Staphylococcus hyicus* lipase. *Eur J Biochem* 242:760–769 10.1111/j.1432-1033.1996.0760r.x. [PubMed: 9022707]
78. Abrahmsén L, Moks T, Nilsson B, Hellman U, Uhlén M. 1985 Analysis of signals for secretion in the staphylococcal protein A gene. *EMBO J* 4(13B):3901–3906 10.1002/j.1460-2075.1985.tb04164.x. [PubMed: 3912176]
79. Schneewind O, Mihaylova-Petkov D, Model P. 1993 Cell wall sorting signals in surface proteins of Gram-positive bacteria. *EMBO J* 12:4803–4811 10.1002/j.1460-2075.1993.tb06169.x. [PubMed: 8223489]
80. Sjöquist J, Movitz J, Johansson I-B, Hjelm H. 1972 Localization of protein A in the bacteria. *Eur J Biochem* 30:190–194 10.1111/j.1432-1033.1972.tb02086.x. [PubMed: 5086604]

81. Becker S, Frankel MB, Schneewind O, Missiakas D. 2014 Release of protein A from the cell wall of *Staphylococcus aureus*. *Proc Natl Acad Sci USA* 111:1574–1579 10.1073/pnas.1317181111. [PubMed: 24434550]
82. Foster TJ, Geoghegan JA, Ganesh VK, Höök M. 2014 Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat Rev Microbiol* 12:49–62 10.1038/nrmicro3161. [PubMed: 24336184]
83. Forsgren A. 1970 Significance of protein A production by staphylococci. *Infect Immun* 2:672–673. [PubMed: 16557894]
84. Votintseva AA, Fung R, Miller RR, Knox K, Godwin H, Wyllie DH, Bowden R, Crook DW, Walker AS. 2014 Prevalence of *Staphylococcus aureus* protein A (spa) mutants in the community and hospitals in Oxfordshire. *BMC Microbiol* 14:63 10.1186/1471-2180-14-63. [PubMed: 24621342]
85. Falugi F, Kim HK, Missiakas DM, Schneewind O. 2013 Role of protein A in the evasion of host adaptive immune responses by *Staphylococcus aureus*. *MBio* 4:e00575–e13 10.1128/mBio.00575-13. [PubMed: 23982075]
86. Forsgren A, Sjöquist J. 1966 “Protein A” from *S. aureus*. I. Pseudoimmune reaction with human gamma-globulin. *J Immunol* 97:822–827. [PubMed: 4163007]
87. Forsgren A, Quie PG. 1974 Effects of staphylococcal protein A on heat labile opsonins. *J Immunol* 112:1177–1180. [PubMed: 4590699]
88. Pauli NT, Kim HK, Falugi F, Huang M, Dulac J, Henry Dunand C, Zheng NY, Kaur K, Andrews SF, Huang Y, DeDent A, Frank KM, Charnot-Katsikas A, Schneewind O, Wilson PC. 2014 *Staphylococcus aureus* infection induces protein A-mediated immune evasion in humans. *J Exp Med* 211:2331–2339 10.1084/jem.20141404. [PubMed: 25348152]
89. Goodyear CS, Silverman GJ. 2004 Staphylococcal toxin induced preferential and prolonged in vivo deletion of innate-like B lymphocytes. *Proc Natl Acad Sci USA* 101:11392–11397 10.1073/pnas.0404382101. [PubMed: 15273292]
90. Kim HK, Falugi F, Missiakas DM, Schneewind O. 2016 Peptidoglycan-linked protein A promotes T cell-dependent antibody expansion during *Staphylococcus aureus* infection. *Proc Natl Acad Sci USA* 113:5718–5723 10.1073/pnas.1524267113. [PubMed: 27140614]
91. Mazmanian SK, Ton-That H, Su K, Schneewind O. 2002 An iron-regulated sortase anchors a class of surface protein during *Staphylococcus aureus* pathogenesis. *Proc Natl Acad Sci USA* 99:2293–2298 10.1073/pnas.032523999. [PubMed: 11830639]
92. Marraffini LA, Schneewind O. 2005 Anchor structure of staphylococcal surface proteins. V. Anchor structure of the sortase B substrate IsdC. *J Biol Chem* 280:16263–16271 10.1074/jbc.M500071200. [PubMed: 15718231]
93. Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, Joachmiak A, Missiakas DM, Schneewind O. 2003 Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* 299:906–909 10.1126/science.1081147. [PubMed: 12574635]
94. Skaar EP, Humayun M, Bae T, DeBord KL, Schneewind O. 2004 Iron-source preference of *Staphylococcus aureus* infections. *Science* 305:1626–1628 10.1126/science.1099930. [PubMed: 15361626]
95. Torres VJ, Pishchany G, Humayun M, Schneewind O, Skaar EP. 2006 *Staphylococcus aureus* IsdB is a hemoglobin receptor required for heme iron utilization. *J Bacteriol* 188:8421–8429 10.1128/JB.01335-06. [PubMed: 17041042]
96. Pilpa RM, Robson SA, Villareal VA, Wong ML, Phillips M, Clubb RT. 2009 Functionally distinct NEAT (NEAr transporter) domains within the *Staphylococcus aureus* IsdH/HarA protein extract heme from methemoglobin. *J Biol Chem* 284:1166–1176 10.1074/jbc.M806007200. [PubMed: 18984582]
97. Muryoi N, Tiedemann MT, Pluym M, Cheung J, Heinrichs DE, Stillman MJ. 2008 Demonstration of the iron-regulated surface determinant (Isd) heme transfer pathway in *Staphylococcus aureus*. *J Biol Chem* 283:28125–28136 10.1074/jbc.M802171200. [PubMed: 18676371]
98. Zhu H, Xie G, Liu M, Olson JS, Fabian M, Dooley DM, Lei B. 2008 Pathway for heme uptake from human methemoglobin by the iron-regulated surface determinants system of *Staphylococcus aureus*. *J Biol Chem* 283:18450–18460 10.1074/jbc.M801466200. [PubMed: 18467329]

99. Wu R, Skaar EP, Zhang R, Joachimiak G, Gornicki P, Schneewind O, Joachimiak A. 2005 *Staphylococcus aureus* IsdG and IsdI, heme-degrading enzymes with structural similarity to monooxygenases. *J Biol Chem* 280:2840–2846 10.1074/jbc.M409526200. [PubMed: 15520015]
100. Reniere ML, Ukpabi GN, Harry SR, Stec DF, Krull R, Wright DW, Bachmann BO, Murphy ME, Skaar EP. 2010 The IsdG-family of haem oxygenases degrades haem to a novel chromophore. *Mol Microbiol* 75:1529–1538 10.1111/j.1365-2958.2010.07076.x. [PubMed: 20180905]
101. Skaar EP, Schneewind O. 2004 Iron-regulated surface determinants (Isd) of *Staphylococcus aureus*: stealing iron from heme. *Microbes Infect* 6:390–397 10.1016/j.micinf.2003.12.008. [PubMed: 15101396]
102. Pilpa RM, Fadeev EA, Villareal VA, Wong ML, Phillips M, Clubb RT. 2006 Solution structure of the NEAT (NEAr transporter) domain from IsdH/HarA: the human hemoglobin receptor in *Staphylococcus aureus*. *J Mol Biol* 360:435–447 10.1016/j.jmb.2006.05.019. [PubMed: 16762363]

FIGURE 1.

Schematic illustrating *S. aureus* cell division, PBP2-mediated synthesis of cross-wall peptidoglycan, LtaS-mediated synthesis of lipoteichoic acid (LTA) in septal membranes, and the trafficking of surface protein precursors with YSIRK-GXXS motif signal peptides (blue circles) to septal membranes. Following translocation, sortase A-mediated cell wall sorting, and incorporation into the cross-wall, the peptidoglycan layer is split and divided cells are separated, exposing cross-wall-incorporated proteins on the bacterial surface. Surface proteins with canonical signal peptides (red circles) are deposited into polar segments of the peptidoglycan layer.

FIGURE 2.

Surface protein anchoring to the cell wall of *S. aureus*. (A) Primary structure of the precursor for staphylococcal protein A (SpA) with an N-terminal signal peptide, signal peptidase (SpsB) cleavage site, five immunoglobulin binding domains (IgBDs), region X (Xr), LysM domain, and C-terminal LPXTG-motif sorting signal. Cell wall-anchored SpA is tethered to the envelope via an amide bond between the carboxyl-group of its C-terminal threonyl (T in the LPXTG motif) and the amino group of the pentaglycine cross-bridge within peptidoglycan. Released nSpA encompasses murein tetrapeptide-tetraglycyl [L-Ala-D-iGln-(SpA-Gly₅)-L-Lys-D-Ala-Gly₄] linked to the C-terminal threonyl of the surface protein. (B) Cell wall sorting pathway of surface proteins in *S. aureus*. Following Sec-mediated translocation of surface protein precursors across the membrane, sortase A cleaves the LPXTG-motif of the C-terminal sorting signal between the threonyl (T) and the glycyl (G) residues and forms a thioester bond between its active site cysteinyl and the carboxyl-group of the C-terminal threonyl. The sortase acyl-enzyme intermediate is relieved by the nucleophilic attack of lipid II, thereby generating surface protein linked to peptidoglycan precursor, which is subsequently incorporated by penicillin binding protein 2 into the cell wall peptidoglycan. Through the action of murein hydrolases, surface protein is released from the cell wall into the extracellular milieu.