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Review Article

Staphylococcus aureus cell wall maintenance – the multifaceted roles of peptidoglycan hydrolases in bacterial growth, fitness, and virulence

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Abstract

Staphylococcus aureus is an important human and livestock pathogen that is well-protected against environmental insults by a thick cell wall. Accordingly, the wall is a major target of present-day antimicrobial therapy. Unfortunately, *S. aureus* has mastered the art of antimicrobial resistance, as underscored by the global spread of methicillin-resistant *S. aureus* (MRSA). The major cell wall component is peptidoglycan. Importantly, the peptidoglycan network is not only vital for cell wall function, but it also represents a bacterial Achilles' heel. In particular, this network is continuously opened by no less than 18 different peptidoglycan hydrolases (PGHs) encoded by the *S. aureus* core genome, which facilitate bacterial growth and division. This focuses attention on the specific functions executed by these enzymes, their subcellular localization, their control at the transcriptional and post-transcriptional levels, their contributions to staphylococcal virulence and their overall importance in bacterial homeostasis. As highlighted in the present review, our understanding of the different aspects of PGH function in *S. aureus* has been substantially increased over recent years. This is important because it opens up new possibilities to exploit PGHs as innovative targets for next-generation antimicrobials, passive or active immunization strategies, or even to engineer them into effective antimicrobial agents.

Keywords: *Staphylococcus aureus*, peptidoglycan hydrolase, cell wall, subcellular protein localization, pathogenesis, antimicrobial susceptibility

Introduction

Staphylococcus aureus is a small and spherical Gram-positive bacterium (Fig. 1), belonging to the phylum of the Firmicutes. As a commensal, *S. aureus* frequently colonizes asymptomatically the nares, skin and gut of humans (Mainous *et al.* 2006, Raineri, Altulea and van Dijl 2022). Nonetheless, *S. aureus* is also one of the most renowned opportunistic pathogens, causing many different diseases that range from relatively mild skin and soft tissue infections to life-threatening diseases, such as bacteremia, pneumonia and endocarditis. The invasive behavior of *S. aureus* is often triggered through injury or medical interventions, where the barrier function of the host cells or tissues is compromised. However, *S. aureus* also produces a variety of virulence factors that allow this bacterium to invade the human body. Beyond the epithelial or endothelial barriers, *S. aureus* is able to bind to the extracellular matrix of host cells and tissues, to invade host cells, or to form biofilms on tissues and medical implants (Raineri, Altulea and van Dijl 2022). At the same time, it is able to effectively evade innate and adaptive immune defenses of the host, either through the secretion of particular virulence factors or the formation of biofilms (Thammavongsa *et al.* 2015, Goldmann and Medina 2018). Ultimately, this may result in persistent or chronic infections (Josse *et al.* 2017). In recent years, the treatment of *S. aureus* infections has become increasingly difficult due to the fact that this pathogen has acquired many different antibiotic resistances, as critically exemplified by the emergence of methicillin-resistant *S. aureus* (MRSA) lineages in hospitals and the community (Turner *et al.* 2019).

Gram-positive bacteria, like *S. aureus*, are surrounded by a thick cell wall, which plays vital roles in maintaining cell shape, cell integrity, cell viability and the protection against osmotic stress under different environmental conditions (Vollmer *et al.* 2008, Silhavy *et al.* 2010). The structure and composition of the cell wall varies between different Gram-positive bacterial species (Do *et al.* 2020). However, peptidoglycan is invariably the major cell wall component that surrounds the bacterial cytoplasmic membrane (Turner *et al.* 2014). *S. aureus* peptidoglycan contains relatively short glycan strands that are highly cross-linked through species-specific peptide bridges; 80%–90% of the so-called stem peptides are cross-linked via a pentaglycine bridge (Snowden and Perkins 1990, Monteiro *et al.* 2019, Sobral and Tomasz 2019). These cross-links are important for the integrity and physical strength of the peptidoglycan. Importantly, the peptidoglycan network surrounding the cell represents a highly dynamic structure that is maintained not only through the synthesis and modification of novel strands but also through the cleavage and degradation of existing

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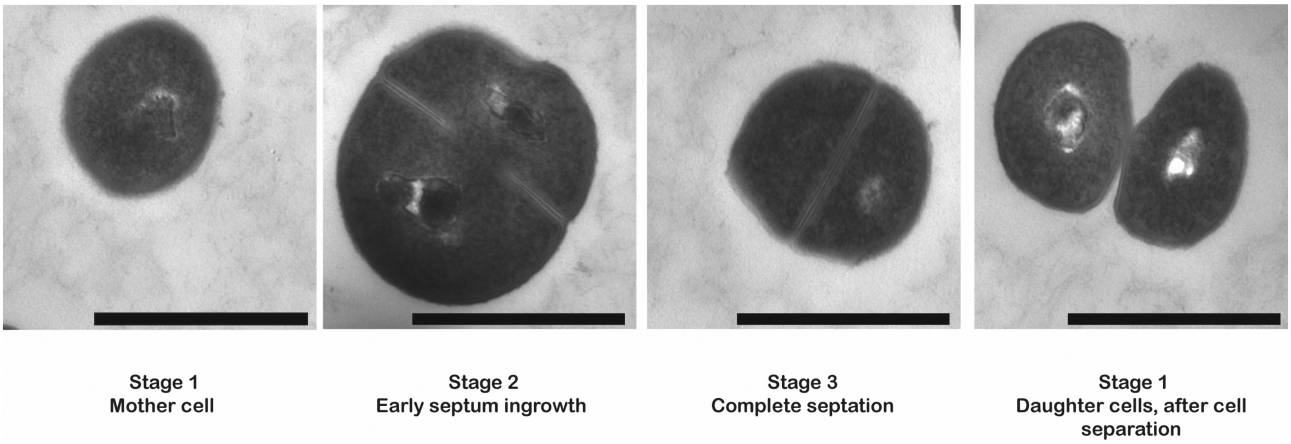

Figure 1. Different stages in the cell cycle of *Staphylococcus aureus* as visualized by transmission electron microscopy. During the initial phase of the cell cycle (stage 1), a spherical *S. aureus* mother cell will become slightly enlarged and forms a septum at the mid-cell position (stage 2). Once the synthesis of the septum is complete and the cell is further enlarged (stage3), bacterial autolytic enzymes will promote cell division, resulting in two daughter cells (stage 1). After cell separation, the septum of the daughter cells is reshaped from a flat surface into a hemisphere. Images were recorded by transmission electron microscopy. The magnification is indicated by scale bars (1 μm).

strands. These different activities are indispensable for bacterial growth and cell division, and they involve the concerted action of peptidoglycan synthases and hydrolases (Monteiro *et al.* 2015). Notably, an appropriate balance between bacterial peptidoglycan synthesis and hydrolysis is crucial for the bacterial cell viability. In particular, when peptidoglycan synthesis is slowed down while peptidoglycan hydrolysis proceeds, autolysis of the staphylococcal cell will occur (Ghuysen *et al.* 1966).

The mechanism of peptidoglycan synthesis has been investigated for a long time. The process starts with the synthesis of Lipid II precursors carrying disaccharide-peptide units inside the cell (Ruiz 2008, Sham *et al.* 2014), and their subsequent 'flipping' across the cytoplasmic membrane by specific transporters known as 'flippases' (Fig. 2) (Ruiz 2008). Afterwards, glycosyltransferases, such as the penicillin-binding protein 2 (PBP2), MGT and SgtA of *S. aureus* will polymerize the translocated Lipid II precursors into nascent glycan strands (Lovering *et al.* 2007, Reed *et al.* 2011, Lovering *et al.* 2012). These nascent strands will be cross-linked to stem peptides in the existing peptidoglycan matrix through the action of transpeptidases, such as PBP1, PBP3 and PBP4 (Sauvage *et al.* 2008). Because of its vital role in bacterial growth and survival, the process of peptidoglycan synthesis has been a preferred target for antibiotic development. This is underscored by important antibiotics like β-lactams (e.g. penicillin or next-generation cephalosporins) and vancomycin which, respectively, target penicillin-binding proteins and acyl-D-alanyl-D-alanine in the *S. aureus* cell wall (Nagarajan 1991, El-Shaboury *et al.* 2007, Rajagopal and Walker 2017). On the other hand, the equally important process of cell wall hydrolysis has, so far, received relatively little attention as a possible therapeutic target for antibiotics (Do *et al.* 2020).

The maturation of peptidoglycan requires not only the action of peptidoglycan synthases but also that of peptidoglycan hydrolases (PGHs) (Shockman and Höltje 1994, Shockman *et al.* 1996, Vollmer *et al.* 2008, Do *et al.* 2020). In general, Gram-positive bacteria contain multiple PGHs, which cleave specific sites in the peptidoglycan and have specific roles in cell wall biogenesis (Smith *et al.* 2000). Depending on the specific cleavage sites, three main types of PGHs can be distinguished, namely glycosidases, amidases and endopeptidases (Fig. 3) (Rigden *et al.* 2003, Fenton *et al.* 2010, Szweda *et al.* 2012). The physiological roles of PGHs are highly diverse, since they include the regulation of glycan strand length, control over the level of peptidoglycan cross-linking, the remodelling of peptidoglycan after cell separation, and the recycling of peptidoglycan fragments (Vollmer *et al.* 2008, Rajagopal and Walker 2017). The precise numbers and essential features of PGHs differ widely between bacteria. In particular, *S. aureus* genomes encode at least 18 PGHs with known or putative hydrolytic activities to support fundamental cellular processes during the cell cycle. Interestingly, none of these enzymes is essential for *S. aureus* survival with the exception of SsaA (SA2093), which is essential for strain NTCT8325, but not for strain N315 (Chaudhuri *et al.* 2009). The latter strain dependency with respect to the possible essentiality of PGHs implies that a precise distinction of the individual and concerted (inter)actions of PGHs in *S. aureus* is rather challenging. Accordingly, the fundamental functions of individual PGHs and their coordination with peptidoglycan synthesis are still poorly understood. However, disentangling the different roles of PGHs will be important, since they are potentially druggable targets for novel antibiotics, or passive or active anti-staphylococcal immunization approaches (Wang *et al.* 2021). PGHs are particularly attractive targets for drugs or immunization not only because they are exposed on the bacterial cell surface and have shown high immunogenicity (Pastrana *et al.* 2018, Dreisbach *et al.* 2020, Wang *et al.* 2021), but also because they play vital roles in cell growth and division. In view of this potential opportunity to develop novel preventive or therapeutic approaches to fight staphylococcal infections, the present review is aimed at providing a detailed overview of what is currently known about the physiological roles and specificity of the different PGHs of *S. aureus* and how they are expressed and function during different stages in the bacterial cell cycle and during infection. Of note, an important requirement for novel drug targets is that they are expressed by all the different lineages of a particular pathogen. Therefore, this review will be focused on the 18 PGHs encoded by the *S. aureus* core genome (Table 1). In contrast, PGHs encoded by plasmids and bacteriophages, such as LytP2 and LytP4 from the *S. aureus* Newman prophages ϕNM2 and ϕNM4 (Bae *et al.* 2006), respectively, will not be addressed as this topic has recently been reviewed (Fabijan *et al.* 2020, Rai and Khairnar 2021, Walsh *et al.* 2021).

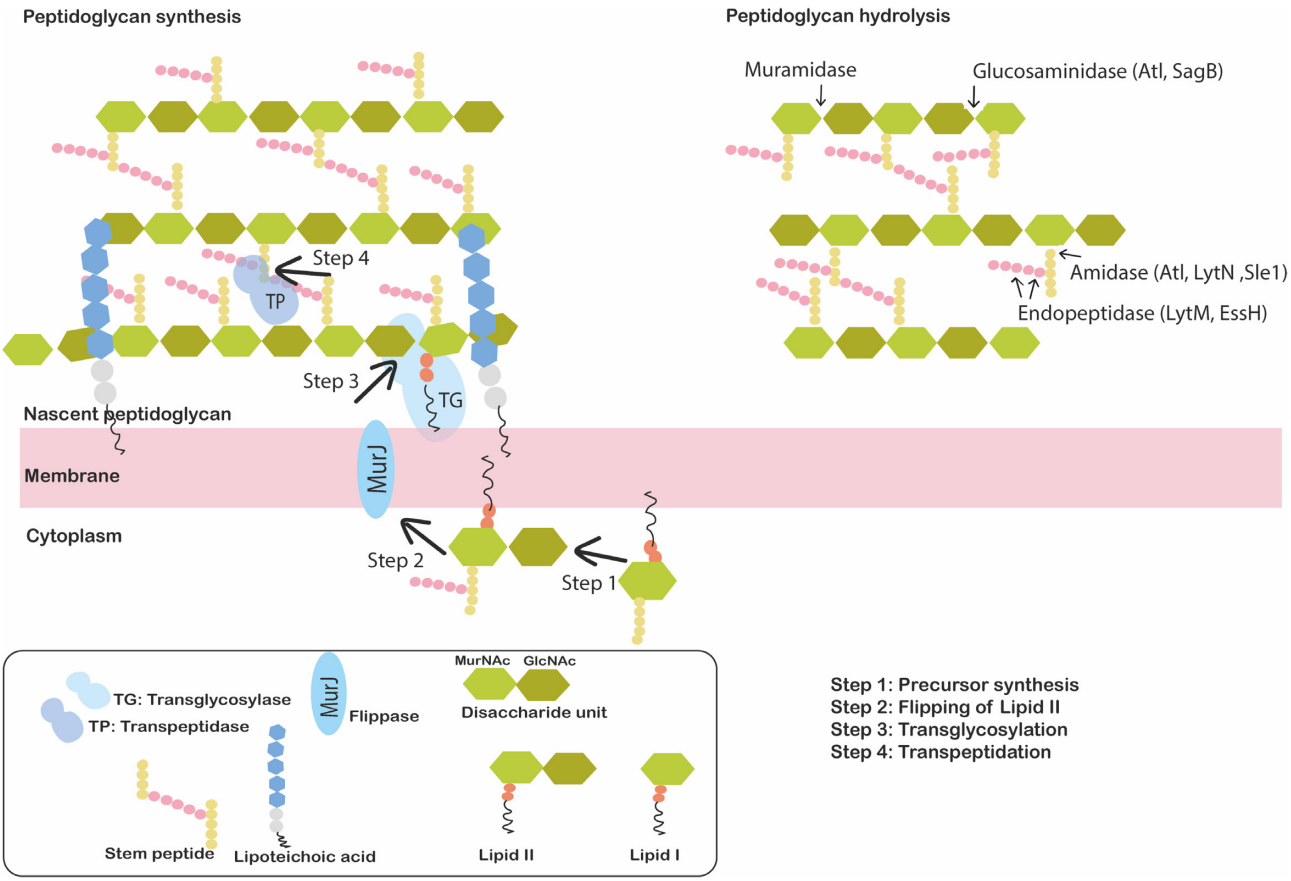

Figure 2. Schematic representation of the *S. aureus* cell envelope structure. The *S. aureus* cell envelope is composed of a cytoplasmic membrane that is surrounded by a thick layer of peptidoglycan. For peptidoglycan synthesis (left), Lipid II units carrying disaccharides are synthesized in the cytoplasm (Step 1) and exported across the cytoplasmic membrane by a flippase (MurJ) (Step 2). Subsequently, transglycosylases (TG) insert the disaccharides into a new glycan strand (Step 3). The stem peptides of the glycan strands are then cross-linked by transpeptidases (TP) to the existing peptidoglycan matrix (Step 4). Peptidoglycan hydrolysis (right) occurs due to the action of peptidoglycan hydrolases which are classified as glycosidases (glucosaminidase and muramidase), amidases or endopeptidases. The specificity of the cleavage sites of these PGH's of *S. aureus* are indicated by arrows.

Table 1. Overview of *S. aureus* peptidoglycan hydrolases.

Protein	Mw (kDa)	AA	N315	USA300	NCTC8325
SagA	29.6	258	SA2100	SAUSA300_2256	SAOUHSC_02580
SagB	32.5	284	SA1593	SAUSA300_1720	SAOUHSC_01895
Atl	136.7	1248	SA0905	SAUSA300_0955	SAOUHSC_00994
Aly/ScaH	69.2	619	SA2437	SAUSA300_2579	SAOUHSC_02979
LytN	43.1	383	SA1090	SAUSA300_1140	SAOUHSC_01219
LytH	32.7	291	SA1458	SAUSA300_1588	SAOUHSC_01739
SsaA	29.3	267	SA2093	SAUSA300_2249	SAOUHSC_02571
EssH	33.1	297	SA0270	SAUSA300_0277	SAOUHSC_00256
SA2353*	27.6	255	SA2353	SAUSA300_2503	SAOUHSC_02883
Sle1/Aaa	35.8	334	SA0423	SAUSA300_0438	SAOUHSC_00427
SA0710*	30.2	279	SA0710	SAUSA300_0739	SAOUHSC_00773
SA0620*	28.1	265	SA0620	SAUSA300_0651	SAOUHSC_00671
SA2097*	17.4	166	SA2097	SAUSA300_2253	SAOUHSC_02576
SA2332*	16.8	143	SA2332	SAUSA300_2482	SAOUHSC_02855
LytM	34.3	316	SA0265	SAUSA300_0270	SAOUHSC_00248
LytU	22	192	SA0205	SAUSA300_0207	SAOUHSC_00174
IsaA	24.2	233	SA2356	SAUSA300_2506	SAOUHSC_02887
SceD	24.1	231	SA1898	SAUSA300_2051	SAOUHSC_02333

Protein names and the respective accession codes for the *S. aureus* strains N315, USA300 and NCTC8325 are presented. *Functionally uncharacterized PGHs are named by their *S. aureus* N315 accession codes. Mw, molecular weight; AA, total numbers of amino acids including the signal peptide according to the annotation for *S. aureus* N315.

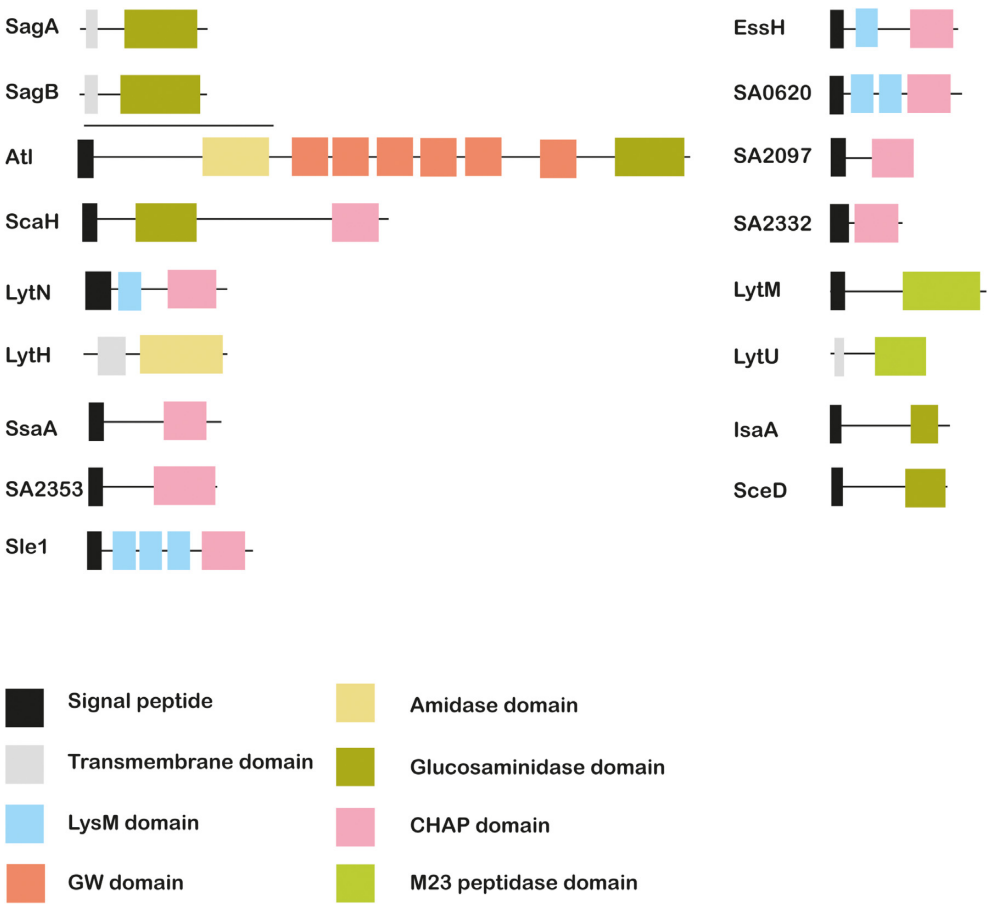

Figure 3. Modular structure of *S. aureus* peptidoglycan hydrolases. *S. aureus* peptidoglycan hydrolases have a modular structure. The different domains that make up these enzymes include catalytic domains, such as the amidase, glucosaminidase, CHAP, M23 peptidase and/or lytic transglycosylase domains. In addition, peptidoglycan hydrolases contain signal peptides, transmembrane domains and/or cell wall binding domains (e.g. LysM, GW) for subcellular localization.

Staphylococcal cell wall composition

The cell wall of Gram-positive bacteria contains not only multiple layers of peptidoglycan, but also other polymers like wall teichoic acids (WTAs), lipoteichoic acids (LTAs) and attached proteins (Strominger *et al.* 1967, Kojima *et al.* 1985, Schneewind *et al.* 1995). The wall may be coated with capsular polysaccharides (Keinhoerster *et al.* 2019). Typically, the thickness of the cell wall is 20 to 40 nm (Giesbrecht *et al.* 1998, Matias and Beveridge 2006). The peptidoglycan is composed of multiple long and stiff cross-linked glycan strands, with each strand consisting of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) units and attached stem peptides that are relatively short and flexible (de Pedro and Cava 2015). Different Gram-positive bacteria vary in the composition of the stem peptides and the respective cross-linked peptide bridges. The stem peptides of *S. aureus* peptidoglycan consist of L-alanine, D-isoglutamine, L-lysine, D-alanine and a terminal D-alanine (Vollmer *et al.* 2008, Silhavy *et al.* 2010). Further, the stem peptides are covalently cross-linked by cleavage of the amide bond of D-Ala-D-Ala and the generation of a new amide bond between the fourth D-Ala of a stem peptide and the first glycine of a pentaglycine bridge on a neighbouring glycan strand (de Pedro and Cava 2015). Notably, the pentaglycine interpeptide bridge is a unique and essential component of the *S. aureus* peptidoglycan, which permits the generation of a three-dimensional mesh that allows for cell plasticity. However, it also provides the cell wall with sufficient strength and rigidity needed under different environmental and infectious conditions (Delaune *et al.* 2011). The glycan strands of *S. aureus* are relatively short with, on average, 6 to 10 disaccharides (Boneca *et al.* 2000, Wheeler *et al.* 2015). This is apparently important for cell wall flexibility, as longer glycan strands resulting from inactivation of the *S. aureus* glucosaminidase SagB were shown to cause an increased cell wall stiffness (Wheeler *et al.* 2015). The level of glycan strand cross-linking also affects the cell wall stiffness. This view is underscored by experiments where *S. aureus* was treated with lysostaphin, an enzyme from *Staphylococcus simulans* that targets the pentaglycine bridge of the *S. aureus* cell wall, which resulted in decreased cell wall stiffness due to a reduced level of peptidoglycan cross-linking (Chan *et al.* 2016). Importantly, prolonged treatment with lysostaphin was shown to result in the formation of osmotically fragile cells (Schindler and Schuhardt 1964, Francius *et al.* 2008). Apart from variations in the peptidoglycan strand length, the peptidoglycan can also be chemically modified. Such peptidoglycan modifications play crucial roles in protecting *S. aureus* against innate host defenses. For instance, *O*-acetylation of the MurNAc C6 hydroxyl and *N*-deacetylation of the GlcNAc C2 acetyl will protect the bacteria against lysozyme produced by the host (Bera *et al.* 2006, Moynihan *et al.* 2014, Rajagopal and Walker 2017).

As indicated above, *S. aureus* incorporates complex teichoic acids (TAs) into its cell wall (Weidenmaier and Peschel 2008). These TAs serve several important roles related to cell wall main-

tenance and bacterial fitness under different environmental challenges. The TAs are either covalently bound to glycan strands in the case of WTAs, or anchored to the cytoplasmic membrane in the case of LTAs. The WTAs are glycopolymers consisting of repeating ribitol-5-phosphate units that are covalently linked to peptidoglycan via phosphodiester bonds between the GlcNAc-1-phosphate and the C6 position of the MurNAc of the peptidoglycan, which may be tailored by different amino acids and sugars (Brown *et al.* 2013). In the context of cell wall biogenesis, WTAs play important roles in peptidoglycan metabolism by regulating the localization of PBPs which, in turn, facilitates the cross-linking of peptidoglycan (Atilano *et al.* 2010, Sewell and Brown 2014, Rajagopal and Walker 2017). The WTA polymers have a negatively charged backbone, which provides proton-binding sites, thereby contributing to the regulation of autolytic activity and the localization of wall-associated PGHs (Jolliffe *et al.* 1981, Penyige *et al.* 2002). However, WTAs are involved in many more processes. For instance, WTAs impact directly or indirectly on protein secretion, and they provide a reservoir of metal ions that are needed in the bacterial physiology and the post-translocational folding of extracellular proteins (Sarvas *et al.* 2004). WTAs also serve roles in bacterial pathogenesis, including adhesion to host cells and tissues, and biofilm formation (Gross *et al.* 2001, Sieradzki and Tomasz 2003, Frankel and Schneewind 2012, Brown *et al.* 2013). In addition, WTAs regulate the localization of the transpeptidase PBP4 to control the crosslinking level of peptidoglycan (Atilano *et al.* 2010), and WTAs protect the bacteria against heat, low osmolarity, cationic antimicrobial peptides and antimicrobial fatty acids (Brown *et al.* 2013). Lastly, a connection between WTAs, autolysis, and vancomycin susceptibility of *S. aureus* was recently reported (Hort *et al.* 2021). An important conclusion from this study was that β-glycosylated WTAs result in decreased affinity of the major autolysin AtlA for binding to the cell wall, thereby reducing bacterial cell lysis.

On the other hand, LTAs are zwitterionic polymers composed of repeating glycerol-phosphate units that are anchored to the cytoplasmic membrane through fatty acids (Xia *et al.* 2010). Like the WTAs, the LTAs have been implicated in the control of cell morphology, growth, division and pathogenesis (Oku *et al.* 2009, Campbell *et al.* 2011).

The Gram-positive bacterial cell wall contains many proteins with roles in host adhesion, colonization and infection. These surface proteins are translocated across the cytoplasmic membrane via specific secretion pathways and they are subsequently either covalently or non-covalently attached to the cell wall (Schneewind *et al.* 1995, Navarre and Schneewind 1999). In particular, peptidoglycan provides the attachment sites for the covalent anchoring of surface proteins, which is mediated by transpeptidases that are better known as sortases (Navarre and Schneewind 1999, Tsompanidou *et al.* 2012). These sortases recognize a C-terminal LPXTG motif in nascent wall-associated proteins, cleave between the threonine and glycine residues of this motif, and couple the resulting terminal threonine residue to the terminal glycine of a pentapeptide bridge (Navarre and Schneewind 1999). Non-covalently cell wall-bound proteins interact with cell wall polymers, especially peptidoglycan, with the help of specific cell wall-binding domains. Alternatively, such proteins associate with the cell wall through specific physico-chemical features, including electrostatic and hydrophobic interactions (Chhatwal 2002, Dreisbach *et al.* 2011). Many of the covalently and non-covalently cell wall-bound proteins have roles in peptidoglycan metabolism and pathogenesis (Schneewind *et al.* 1995, Wardenburg *et al.* 2007).

Peptidoglycan hydrolases

PGHs are enzymes capable of cleaving the covalent bonds of glycan strands or stem peptides. These enzymes are widely conserved in bacteria where they are required for accurate cell growth, division and separation. However, genes encoding PGHs are highly redundant in most bacterial genomes (Vollmer *et al.* 2008). Accordingly, they are by themselves not essential for *S. aureus* growth and survival, at least under laboratory conditions, with exception of the aforementioned SsaA (SA2093) of *S. aureus* NTCT8325 (Antignac *et al.* 2007).

Depending on their specific cleavage sites in peptidoglycan, the PGHs are grouped into three classes. First, the glycosidases cleave the glycan strand, and here two different types of enzymes can be distinguished; the glucosaminidases cleave the β-1,4 glycosidic bond between GlcNAc and MurNAc whereas the lytic transglycosylases (muramidases) cleave the β-1,4 glycosidic bond between MurNAc and GlcNAc resulting in the formation of MurNAc with 1,6-anhydromuramic acid residues (Höltje *et al.* 1975). Second, the amidases cleave the amide bond between MurNAc and the first L-Ala residues of the stem peptide. Thirdly, the endopeptidases cleave the bond between two amino acid residues within a stem peptide or the pentaglycine bridge.

Notably, *S. aureus* uses *N*- acetylglucosaminidases instead of *N*-acetylmuramidases to cleave its own glycan strands. This may relate to the aforementioned *O*-acetylation of the MurNac residues, which *S. aureus* employs to protect itself from peptidoglycan cleavage by the host-derived *N*-acetylmuramidase lysozyme, which cleaves the MurNAc-β-1,4-GlcNAc bond of the glycan strand (Bera *et al.* 2005). Previous studies characterized various PGHs of *S. aureus*, including four glucosaminidases (Atl, SagA, SagB, ScaH), two lytic transglycosylases (IsaA, SceD), four putative amidases (Atl, Sle1, LytN, EssH) and five putative endopeptidases (LytN, LytH, LytM, LytU, EssH). Here it should be noted that this listing refers twice to some particular proteins because these proteins are associated with two distinct PGH activities. For instance, Atl has both a glucosaminidase domain and an amidase domain, which become physically separated by proteases produced by *S. aureus* during growth (Komatsuzawa *et al.* 1997). The amidase domain of Atl specifically targets the muramyl-tetrapeptide MurNAc-L-Ala-D-iGln-L-Lys-NHA-D-Ala-NH2 (MtetP) of the peptidoglycan of *S. aureus* (Büttner *et al.* 2014). Interestingly, another study showed that binding of the stem peptides of peptidoglycan is important for the specific association of the Atl homolog of *S. epidermidis* AmiE with its substrates (Lützner *et al.* 2009). The features of the different PGHs from *S. aureus* are summarized in Supplementary Table 1. The specific peptidoglycan cleavage sites of some of these PGHs, like those that contain cysteine, histidine-dependent amidohydrolase/peptidase (CHAP) domains have not yet been characterized. CHAP domain-containing PGHs are commonly present in many different bacteria, where they can act as amidases and/or endopeptidases (Bateman and Rawlings 2003). For example, the CHAP domain of the *S. aureus* PGH Sle1 only has amidase activity, while the CHAP domains of the *S. aureus* PGHs LytN and EssH have both amidase and endopeptidase activities (Bobrovskyy *et al.* 2018).

Frequently, PGHs show a modular architecture that combines different catalytic domains and/or cell wall-binding domains, and these can be located in the N-terminus or the C-terminus of a PGH (Fig. 3). The wall-binding domains allow the binding of PGHs to the peptidoglycan at an adequate concentration. They also properly position the respective PGH active sites towards the PG substrate cleavage site for efficient formation of enzyme-substrate

complexes (Vermassen *et al.* 2019). The type and number of cell wall-binding domains differ among PGHs (Steen *et al.* 2005). This is exemplified by the cell wall-binding Lysin Motif (LysM), which is widely distributed in both prokaryotes and eukaryotes (Buist *et al.* 2008). Bacterial LysM domains recognize and non-covalently bind to the *N*-acetylglucosamine moiety of peptidoglycan (Frankel and Schneewind 2012). LysM domains also direct the murein hydrolases Sle1 and LytN to the cross wall of *S. aureus*, where they assume different positions (Frankel *et al.* 2011, Frankel and Schneewind 2012, Pastrana *et al.* 2017). LytN contains a YSIRK/GS signal which also directs this protein to the septum of dividing cells (Frankel *et al.* 2011). SH3b, another cell wall-binding domain, targets proteins to the pentaglycine bridge with high affinity and specificity, and SH3b-mediated peptidoglycan binding is actually a key step in peptidoglycan hydrolysis (Visweswaran *et al.* 2014, Gonzalez-Delgado *et al.* 2020). The GW domain of Atl is a repeating cell wall-binding domain that binds to LTA (Sugai *et al.* 1995, Baba and Schneewind 1998, Zoll *et al.* 2012), which is responsible for the correct protein docking (Komatsuzawa *et al.* 1997, Frankel and Schneewind 2012, Zoll *et al.* 2012). Some PGHs are capable of cleaving cross-links of soluble muropeptides and intact peptidoglycan sacculi. For example, mutanolysin from *Streptomyces globisporus* cleaves the MurNAc-GlcNAc that carries a stem peptide (Kawata *et al.* 1983). In contrast, the glucosaminidase domain of Atl specifically cleaves the glycan strand without attached stem peptides (Nega *et al.* 2020). Compared to Atl, the glucosaminidase SagB prefers longer glycan strands as substrates which, in fact, implies an important role for SagB in controlling the glycan strand length (Wheeler *et al.* 2015, Chan *et al.* 2016). The amidase LytH exclusively cleaves peptides from non-cross-linked nascent peptidoglycan (Do *et al.* 2020).

Altogether, studies on the PGHs of *S. aureus* have shown that these enzymes are exported proteins that localize either to the bacterial septum (Sle1 [Frankel and Schneewind 2012], Atl [Schlag *et al.* 2010], LytN [Frankel *et al.* 2011], IsaA [Sakata *et al.* 2005]) or the peripheral wall region (LytM [Ramadurai *et al.* 1999] and LytU [Raulinaitis *et al.* 2017]) during cell growth and division. Some PGHs, like Atl and Sle1, are also secreted into the extracellular milieu and, subsequently, these PGHs cleave the septum at the cross wall (Komatsuzawa *et al.* 1997, Frankel and Schneewind 2012). Moreover, PGHs may even contain both a membrane anchor and an extracytoplasmic catalytic domain, as exemplified by LytU, LytH, SagA, and SagB. Their positioning close to the extracytoplasmic side of the membrane suggests that the latter four enzymes preferentially target nascent peptidoglycan.

Physiological roles of PGHs

PGHs have diverse functions, including the regulation of cell growth, cell enlargement, remodelling of peptidoglycan, peptidoglycan turnover and recycling, assembly of secretion systems, widening of pores in the wall to allow secretory protein passage and release of surface proteins, cell separation during cell division, and autolysis (Fig. 4) (Scheurwater *et al.* 2008, Vollmer *et al.* 2008). Some of the *S. aureus* PGHs play multiple functions in these processes (Vollmer *et al.* 2008, Frirdich and Gaynor 2013), and this probably explains why the loss of individual PGHs in Gram-positive bacteria is in most cases neither lethal, nor associated with major morphological phenotypes (Vollmer *et al.* 2008, Frirdich and Gaynor 2013).

PGH contributions to cell separation

One of the main functions of PGHs is to separate the daughter cells at the final stage in the cell division process. In the division phase, cells form a dynamic Z-ring through polymerization of the tubulin homologue FtsZ, which is an essential cytoplasmic cell division protein that recruits other proteins of the cell division machinery to synthesize the division septum (Ortiz *et al.* 2016, den Blaauwen *et al.* 2017). The two resulting hemispherical daughter cells are interlinked through a narrow peripheral ring, have their own cytoplasmic membranes, but share the septum, which is a layer of peptidoglycan that needs to be separated after cell division through the hydrolysis of crosslinked peptidoglycan by PGHs (Matias and Beveridge 2007, Vollmer *et al.* 2008). To this end, PGHs first create perforation holes in the peripheral ring that links the daughter cells. The resolution of the peripheral ring ('popping') occurs within milliseconds, which most likely depends on alterations in the cell surface stress (Zhou *et al.* 2015). Nonetheless, upon separation of the daughter cells there are no apparent changes in the volume of each daughter cell. Prior to cell separation, a large belt of peptidoglycan is present at the division sites, which may serve to protect the peripheral wall from hydrolysis by PGHs that participate in the 'pre-splitting' of the septal cell wall (Matias and Beveridge 2007, Turner *et al.* 2010). The concerted and precise action of PGHs in cell separation is thus very important because incorrect cell separation could affect proper division or delay the separation of daughter cells.

Amidases such as Atl, Sle1 and LytN have been implicated more prominently in cell separation by contributing to peptidoglycan cleavage at the septum. In particular, Atl localizes at the peripheral ring region of the surface of the septum prior cell separation (Yamada *et al.* 1996). In *atl* deletion mutants and mutants in which the amidase or glucosaminidase domains were inactivated, the cell wall of the 'cracked' daughter cell was shown to be smooth and flat, while the outer wall was rough (Nega *et al.* 2020). The rough surface was mostly detectable in the *atl* deletion mutant and the mutant with an inactivated glucosaminidase domain. Cluster formation was observed for all three mutants. Nonetheless, cell division was not principally affected in *atl* mutants with inactivated amidase or glucosaminidase domains (Nega *et al.* 2020). This may relate to the fact that in an *atl* mutant, the expression of Sle1 and other PGHs (i.e. SagA, SsaA-SA2093, ScaH, LytH, IsaA, LytM, SceD, EssH, SA2097, SA0620, SA2353, SA2332) was increased, likely compensating for a possible defect in cell separation due to the absence of Atl (Heilmann *et al.* 2005, Pasztor *et al.* 2010, Hirschhausen *et al.* 2012). Nevertheless, loss of Atl or Sle1 increased the duration of the cell cycle from 66 ± 9 min for the parental wild-type strain, to 82 ± 8 min or 86 ± 15 min, respectively (Monteiro *et al.* 2015). Although most upregulated genes in the *atl* mutant were shown to be controlled by the two-component system WalKR, no difference in expression of this regulatory system was detected in the *atl* mutant compared to the wild-type strain (Pasztor *et al.* 2010).

The amidase Sle1 plays important roles in cell separation by degrading the outer edge of the septum during cell division. Sle1 is not required for the resolution of the outer septum, but inactivation of Sle1 delays the onset of daughter cell separation, while elevated levels of Sle1 accelerate the onset of daughter cell separation and lead to a reduced cell size (Thalsø-Madsen *et al.* 2019). Of note, the aforementioned perforations in the peripheral ring at mid-cell, which are introduced prior to cell separation, are closely correlated to the Sle1 levels (Thalsø-Madsen *et al.* 2019). Furthermore, the lack of some amidases results in a partial dysregulation

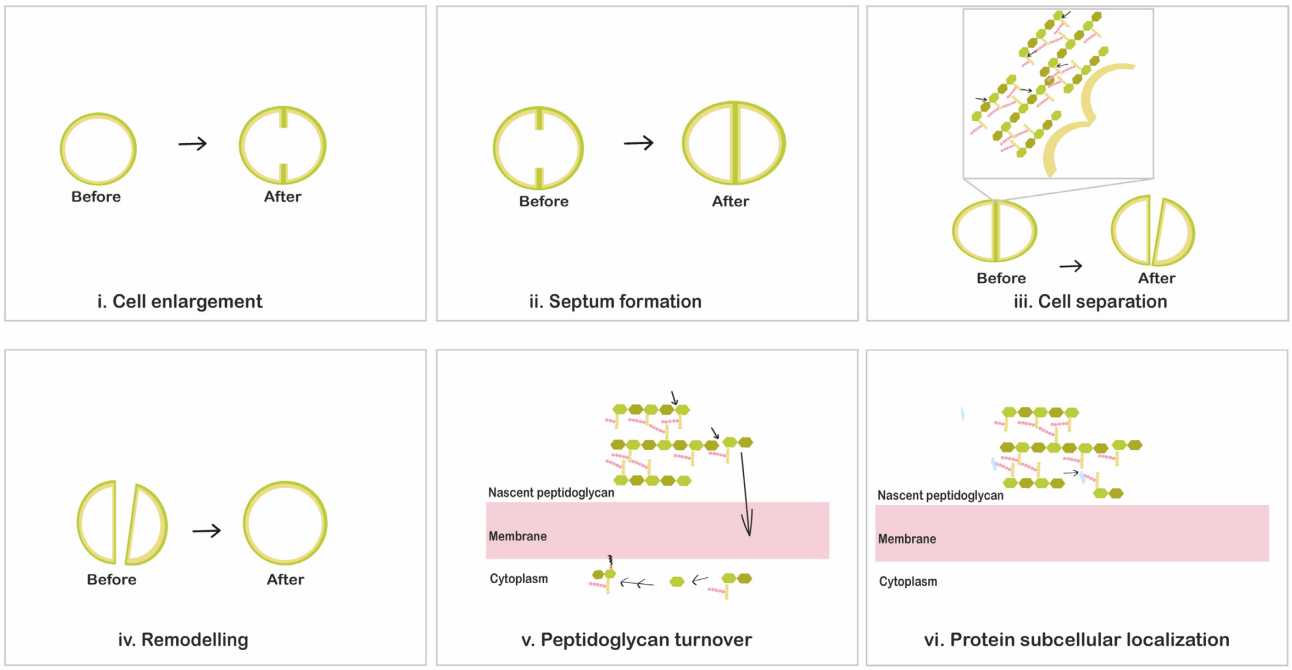

Figure 4. Physiological roles of *S. aureus* peptidoglycan hydrolases. Peptidoglycan hydrolases play different essential roles during bacterial growth and division. PGHs are involved in the entire cell cycle including cell enlargement (i), cell enlargement during septum formation (ii), daughter cell separation upon cell division (iii), and remodelling of the peptidoglycan network after daughter cell separation (iv). In addition, peptidoglycan hydrolases serve in peptidoglycan turnover (v) and they modulate cell wall passage of cell surface-located proteins (vi).

of peptidoglycan synthesis, and the respective mutant bacterial cells increase in size and show cell division defects. The loss of glucosaminidases, such as Atl and SagB, impact cell separation (Wheeler *et al.* 2015, Chan *et al.* 2016, Sutton *et al.* 2021). Bacteria lacking SceD and IsaA displayed impaired cell separation and clumping (Sakata *et al.* 2005, Stapleton *et al.* 2007).

PGH contributions to cell enlargement

During the cell cycle, the expansion of cells is important for maintaining the bacterial shape. This expansion probably starts at the cell periphery and also occurs at the division sites (Pinho *et al.* 2013, Lund *et al.* 2018). The proper subcellular location of FtsZ determines the final cell size (Jorge *et al.* 2011, Veiga *et al.* 2011). After cell division, *S. aureus* undergoes slight elongation at mid-cell along the lateral wall. This is mediated by PBP3 and RodA, which promote the sidewall insertion of peptidoglycan (Reichmann *et al.* 2019). *S. aureus* reshapes the flat septum into the curved hemispheres of two daughter cells once the cell separation has been completed (Pinho *et al.* 2013). PGHs drive the peptidoglycan remodelling after splitting by breaking existing bonds in the peptidoglycan, thereby increasing the peptidoglycan surface area and allowing the peptidoglycan to stretch. In turn, this allows the incorporation of new peptidoglycan units into the nascent glycan strands. A group of PGHs with glucosaminidase activity (i.e. Atl, SagA, ScaH and SagB) is required for cell enlargement after cell separation, and for reaching the mature cell shape (Wheeler *et al.* 2015). As mentioned above, physical properties, such as the cell wall stiffness and glycan strand length, may affect the cell wall elasticity, thereby impacting cell enlargement. Peptidoglycan strand length and cross-linking levels also affect the cell wall stiffness (Loskill *et al.* 2014, Wheeler *et al.* 2015). Accordingly, cell enlargement may depend on the reduction of cell wall stiffness. One model proposes that initially a dense and stiff peptidoglycan is formed. However, hydrolysis by PGHs makes it less dense, less cross-linked and less stiff, thereby allowing the insertion of new peptidoglycan and enabling the enlargement of cells into their mature shape (Loskill *et al.* 2014, Wheeler *et al.* 2015). SagB is dominantly responsible for determining the length of glycan strands, producing a GlcNAc at the end of one resulting strand and a MurNAc at the start of the other strand (Schaefer et al 2021, Willing *et al.* 2021). Accordingly, the inactivation of SagB results in long unprocessed glycan strands, which cause increased cell wall stiffness and decreased cell wall elasticity. Other glucosaminidases, like Atl, SagA and ScaH have an additional, though modest, impact on glycan strand length and cell enlargement, which may be due to reduced peptidoglycan cross-linking, especially by SagA (Wheeler *et al.* 2015, Chan *et al.* 2016, Pasquina-Lemonche *et al.* 2020).

PGH contributions to cell wall turnover

The turnover or recycling of bacterial peptidoglycan is constantly progressing during cell growth. Different bacteria have distinct strategies to breakdown the peptidoglycan and reutilize the liberated cell wall fragments for peptidoglycan recycling (Mayer *et al.* 2019). In fact, it has been reported that around 45% of the peptidoglycan is turned over in one generation in bacteria (Goodell 1985). In *S. aureus*, Atl plays an important role in cell wall turnover. In particular, Atl hydrolyzes peptidoglycan, thereby producing peptides and the disaccharide MurNAc-GlcNAc (Kluj *et al.* 2018). This unique disaccharide unit is transported into the bacterial cell and phosphorylated by the phosphotransferase system transporter MurP. The intracellular 6-phospho-N-acetylmuramidase MupG cleaves the MurNAc 6-phosphate-GlcNAc and produces MurNAc 6-phosphate and GlcNAc (Kluj *et al.* 2018). The MurQ esterase then converts MurNAc 6-phosphate into GlcNAc 6-phosphate (Borisova *et al.* 2016). Such cell wall turnover products may subsequently be perceived as signalling molecules for bacterial recognition by other (micro)organisms or mammalian hosts. In some bacteria this will lead to the induction of β-lactamase production (Jacobs

et al. 1997). Endopeptidases could also mediate the turnover of peptidoglycan. Importantly, the peptidoglycan turnover caused by PGHs regulates the release of some surface proteins that may be involved in host cell adhesion and invasion (Boneca 2005). The immunodominant staphylococcal antigen A (IsaA) and its paralogue SceD with putative soluble lytic transglycosylase domains may participate in peptidoglycan turnover and cell wall hydrolysis (Stapleton *et al.* 2007).

Role of PGHs in extracellular protein localization

The Sec pathway is the primary secretion pathway of *S. aureus*, which directs signal peptide-dependent translocation of proteins across the cytoplasmic membrane into the cell wall and extracellular milieu (Sibbald *et al.* 2006, Dreisbach *et al.* 2011, Schneewind and Missiakas 2014). However, among the surface-associated and extracellular proteins of *S. aureus*, many typically cytoplasmic proteins can be encountered, which are nowadays referred to as extracellular cytoplasmic proteins (ECPs) (Sibbald *et al.* 2006). PGHs have been implicated in the extracellular localization of these ECPs by modulating the peptidoglycan complexity and cross-linking (Ebner *et al.* 2015, 2017, Ebner *et al.* 2016, Ebner and Götz 2019, Zhao *et al.* 2020). For instance, this applies to SagB, which is evidenced by the fact that *sagB* mutant bacteria display defects in cell wall anchoring and protein secretion, whereas the liberation of ECPs is enhanced (Chan *et al.* 2016). Nonetheless, the release of ECPs by *sagB* mutant bacteria does not seem to be due to autolysis or membrane leakage (Chan *et al.* 2016). On the other hand, *atl* mutant bacteria display a significant decrease in the release of ECPs to the bacterial cell surface and into the extracellular milieu. This suggests a role of Atl-mediated cell wall weakening in the release of ECPs (Pasztor *et al.* 2010). In this context it is noteworthy that various ECPs have known roles in staphylococcal virulence, such as the adhesion to host cells. Importantly, there are also PGHs known to operate in close concert with dedicated protein secretion systems. This is exemplified by the EssH protein, which is a CHAP domain-containing PGH encoded by the *ess* locus. The activity of EssH is associated with the ESAT-6-like secretion system, also referred to as the type 7 secretion system (T7SS). In particular, EssH is required for assembly of the T7SS secretion system and secretion of the EsxC and EssD proteins by the T7SS-dependent pathway (Bobrovskyy *et al.* 2018). Through its amidase and endopeptidase activities EssH cleaves the stem peptides and pentaglycine bridges of the peptidoglycan to create space for protein passage across the bacterial cell wall. Likewise, PGHs involved in the assembly of secretion systems have been reported in other bacteria, such as *Escherichia coli* (Koraimann 2003).

Possible cross-talk of PGHs and peptidoglycan synthases

The peptidoglycan synthases and PGHs of Gram-positive bacteria are located at distinct subcellular positions, which sets limits to direct interactions between these enzymes (Vollmer *et al.* 2008). In particular, peptidoglycan synthases are positioned in the inner peptidoglycan layer, while PGHs are mostly positioned in the outer layers. However, there are examples of indirect cross-talk between peptidoglycan synthases and PGHs. For instance, the amidase LytH is a determinant for the subcellular localization of peptidoglycan synthases by controlling the density of peptidoglycan assembly sites. Consequently, the inactivation of LytH results in a displacement of division sites (Do *et al.* 2020). This can be compensated by a reduced activity of the PBP2 polymerase, which implies that LytH controls cell growth by regulating the PBP2-mediated synthesis of new peptidoglycan (Do *et al.* 2020). FtsZ is frequently mislocalized in *lytH* mutant cells, which implies that LytH is also important for FtsZ assembly (Do *et al.* 2020). Similarly, deletion of the glucosaminidase domain of Atl was also shown to result in a defective localization of the division septum, since in 25%–35% of glucosaminidase-mutated bacteria an asymmetric cell division was detected (Nega *et al.* 2020).

Regulation of PGHs

Control of PGH expression by gene regulatory systems

To maintain the rigid shape and structure of the peptidoglycan network, the expression and activity of PGHs needs to be spatially and temporally controlled (Egan *et al.* 2017). Several direct and indirect mechanisms involved in the regulation of PGHs are known to exist (Typas *et al.* 2012, Egan *et al.* 2017). These involve transcriptional control by gene regulatory systems of *S. aureus* (*e.g.* sigma factors, RNA binding proteins and small regulatory RNAs), altered biological activity of PGHs and their substrate binding by the presence of LTA and WTA or peptidoglycan modification, proteolytic processing by proteases, protein-protein interactions that activate or block the activity of PGHs, as well as environmental factors.

At the transcriptional level, two-component signal transduction systems and global regulators are involved in regulating the product of PGHs (Table 2 and Fig. 5). *S. aureus* is known to possess 16 so-called two-component regulatory systems (TCSs; *e.g.* WalKR, AgrAC, and ArlRS), which are composed of a sensor module with histidine kinase activity and a cognate DNA-binding response regulator that modulates transcription. Such TCSs monitor particular stresses or extracellular stimuli (Szurmant *et al.* 2007) and regulate the appropriate physiological responses through autophosphorylation of the sensor module and subsequent transfer of the phosphoryl group to the cognate response regulator, which leads to activation of the regulator (Kleerebezem *et al.* 1997). Particular TCSs may control the expression of multiple PGH-encoding genes. For instance, the WalR response regulator of the essential WalKR (YycGF) TCS specifically binds to the promoter regions of genes encoding the *S. aureus* PGHs Atl, LytM, SsaA (SA2093), IsaA, SceD, EssH, the three CHAP domain-containing proteins SA0620, SA2097, and SA2353) (Table 2) (Dubrac and Msadek 2004, Dubrac *et al.* 2007), Sle1 and SA2332 (Delauné *et al.* 2012), thereby positively controlling their expression. In addition, WalKR also positively regulates peptidoglycan biosynthesis and turnover (Dubrac *et al.* 2007). Importantly, WalKR can both regulate and be regulated by other regulatory components. For example, the YycH and YycI proteins stimulate WalK activity through WalKR phosphorylation (Gajdiss *et al.* 2020). Also, the WalR regulator stimulates the SaeSR TCS (Delauné *et al.* 2012), whereas SaeSR negatively regulates the *ssaA* gene (SA2093) (Rogasch *et al.* 2006). The expression of *ssaA* is positively regulated by GraSR (Falord *et al.* 2011), a TCS that has an important function in the regulation of the bacterial metabolism under oxidative stress conditions, at low pH and at higher temperature. Nine genes encoding PGHs are regulated by both GraSR and WalKR (Dubrac *et al.* 2007, Falord *et al.* 2011). Thus, the TCSs play multiple roles in the regulation of *S. aureus* cell wall metabolism (Dubrac *et al.* 2007, Delaune *et al.* 2011).

One of the key TCSs of *S. aureus*, the accessory gene regulation (*agr*) quorum-sensing system that modulates the expression of over 100 genes, is also involved in the regulation of cell wall metabolism (Dunman *et al.* 2001). Various genes for cell wall-associated proteins are repressed in the late exponential growth

Table 2. Peptidoglycan hydrolase regulation at the transcriptional level.

Gene encoding PGHs	Main transcriptional regulators
sagA	-
sagB	-
atl	**WalKR (↑)** (Dubrac *et al.* 2007), **SigB (↑)** (Houston *et al.* 2011), **AtlR (↓)** (Houston *et al.* 2011), **SarA (↓)** (Trotonda *et al.* 2009), **GraSR (↑)** (Herbert *et al.* 2007, Falord *et al.* 2011)
aly/scaH	-
lytN	**ArlRS (↓)** (Memmi *et al.* 2012), **MgrA (↓)** (Luong *et al.* 2006), **Rot (↓)** (Chu *et al.* 2013), **Rat (↓)** (Ingavale *et al.* 2003)
lytH	-
ssaA	**WalKR (↑)** (Dubrac *et al.* 2007), **GraSR (↑)** (Falord *et al.* 2011), **SaeSR (↓)** (Rogasch *et al.* 2006), **Agr (↓)** (Dunman *et al.* 2001), **putative RNAIII (↓)** (Lioliou *et al.* 2016)
essH	**GraSR (↑)** (Falord *et al.* 2011)
sa2353	**WalKR (↑)** (Dubrac *et al.* 2007), **RNAIII (↓)** (Boisset *et al.* 2007), **GraSR (↑)** (Falord *et al.* 2011)
sle1/aaa	**WalKR (↑)** (Delauné *et al.* 2012), **Agr (↓)** (Hirschhausen *et al.* 2012), **SarA (↓)** (Hirschhausen *et al.* 2012), **GraSR (↑)** (Herbert *et al.* 2007, Falord *et al.* 2011)
sa0710	**WalKR (↑)** (Dubrac *et al.* 2007), **ArlRS (↓)** (Crosby *et al.* 2020), **MgrA (↓)** (Luong *et al.* 2006)
sa0620	**WalKR (↑)** (Dubrac *et al.* 2007), **GraSR (↑)** (Falord *et al.* 2011)
sa2097	**WalKR (↑)** (Dubrac *et al.* 2007), **GraSR (↑)** (Falord *et al.* 2011)
sa2332	**WalKR (↑)** (Delauné *et al.* 2012), **GraSR (↑)** (Falord *et al.* 2011)
lytM	**WalKR (↑)** (Dubrac *et al.* 2007), **Rot (↓)** (Chu *et al.* 2013), **Agr (↓)** (Ramadurai *et al.* 1999), **SarA (↓)** (Ramadurai *et al.* 1999), **RNA III (↓)** (Chunhua *et al.* 2012), **Rat (↓)** (Ingavale *et al.* 2003)
lytU	**VraSR (↑)** (Pietiäinen *et al.* 2009)
isaA	**WalKR (↑)** (Dubrac *et al.* 2007, Stapleton *et al.* 2007), **SarA (↑)** (Stapleton *et al.* 2007), **SrrAB (↑)** (Stapleton *et al.* 2007), **putative RNAIII (↓)** (Lioliou *et al.* 2016), **GraSR (↑)** (Falord *et al.* 2011)
sceD	**WalKR (↑)** (Dubrac *et al.* 2007, Stapleton *et al.* 2007), **SigB (↑)** (Ziebandt *et al.* 2001, Stapleton *et al.* 2007), **Agr (↑)** (Stapleton *et al.* 2007), **putative RNAIII (↓)** (Lioliou *et al.* 2016), **LytSR (↓)** (Stapleton *et al.* 2007), **SaeSR (↓)** (Stapleton *et al.* 2007), **GraSR (↑)** (Falord *et al.* 2011), **SarA (↓)** (Stapleton *et al.* 2007)

*Genes for functionally uncharacterized PGHs are named by their *S. aureus* N315 accession codes; (↑) and (↓), up- or down-regulation of the respective PGH-encoding gene; -, unknown regulation mechanism.

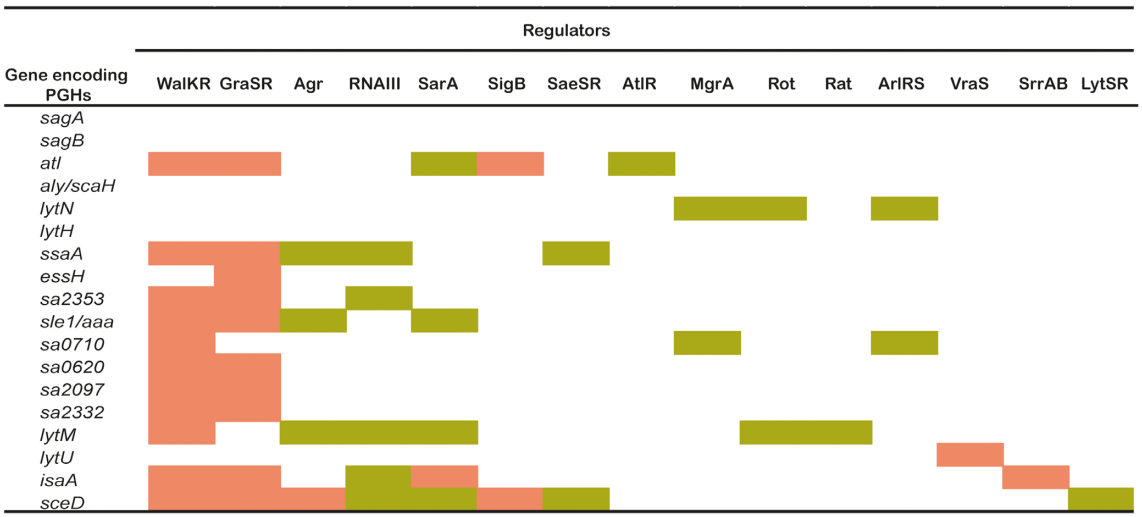

Figure 5. Peptidoglycan hydrolase gene regulation by different transcriptional regulators. The Figure provides an overview of PGH-encoding genes of *S. aureus* and translational regulators that are known to modulate the PGH gene expression. Up-regulation or down-regulation by particular gene regulators are indicated in pink or olive green, respectively.

phase by the *agr* system (Benito *et al.* 1998), or indirectly regulated via the small RNA III which is a key effector of the *agr* system (Abdelnour *et al.* 1993, Benito *et al.* 1998, Huntzinger *et al.* 2005). RNA III also targets other regulatory proteins and indirectly regulates the expression of several genes, including PGH-encoding genes. Thus, it was reported that *lytM* is negatively regulated by *agr* (Ramadurai *et al.* 1999). In particular, it was shown that the expression of *lytM* is negatively regulated by RNA III through interaction with the 5'UTR of the *lytM* mRNA and by blocking the ribosome-binding site of *lytM* at the post-transcriptional stage (Chunhua *et al.* 2012, Lioliou *et al.* 2016). Furthermore, RNA III was shown to negatively regulate *SA2353* (Boisset *et al.* 2007), and this small RNA was predicted to bind to the promoter regions of *sceD*, *isaA* and *ssaA* (*SA2093*) (Lioliou *et al.* 2016). Lastly, the *agr* system regulates the expression of protease genes, which negatively affect the activity of Atl and other PGHs at the post-transcriptional level.

The expression of various PGH-encoding genes of *S. aureus* is repressed by the TCSs LytSR (Brunskill and Bayles 1996a) and ArlRS (Fournier and Hooper 2000, Crosby *et al.* 2020), and by the transcriptional regulators MgrA (Rat) (Ingavale *et al.* 2003, Ingavale *et al.* 2005), Rot (Chu *et al.* 2013), SarA (Cheung *et al.* 2004, Trotonda *et al.* 2009) and AtlR (Houston *et al.* 2011). LytSR negatively regu-

lates PGH activity by regulating the expression of the *lytSR* operon and its downstream *lrgAB* operon (Brunskill and Bayles 1996b). In turn, the *lrgAB* operon, and also the paralogous *cidABC* operon apparently control the transport of PGHs across the membrane, thereby facilitating PGH regulation at the post-transcriptional level (Groicher *et al.* 2000, Rice *et al.* 2003, Rice and Bayles 2008, Bayles 2014). Here it should be noted that *cidA* encodes a putative holin that may disrupt the membrane integrity thereby promoting bacterial cell death and lysis, while *lrgA* encodes an anti-holin that counteracts cell death and lysis (Groicher *et al.* 2000, Rice *et al.* 2003, Rice and Bayles 2008). ArlRS and MgrA constitute a regulatory cascade that controls expression of a large number of genes (Crosby *et al.* 2016). ArlRS regulates autolysis by activating the expression of *mgrA* (Crosby *et al.* 2016, Crosby *et al.* 2020), where ArlRS and MgrA jointly mediate the repression of *lytN* (Luong and Lee 2006, Memmi *et al.* 2012). Furthermore, ArlRS, is a negative regulator of the aforementioned *essH* gene, but this does not involve MgrA (Crosby *et al.* 2020).

The regulator Rot, a DNA-binding protein from the SarA family of *S. aureus* regulators, can bind to the promoter regions of *lytM* and *lytN* to directly regulate the expression of these PGH-encoding genes (Chu *et al.* 2013). Rot also regulates the transcription of *lryAB*, which leads to an indirect regulation of PGH production (Chu *et al.* 2013). The negative regulation of autolysis by MgrA and SarA may be mediated indirectly by downregulation of *sarV*, which encodes a positive regulator of several PGHs (Manna *et al.* 2004, Trotonda *et al.* 2009). MgrA represses the expression of at least two transcriptional regulators, SarV and AtlR (Crosby *et al.* 2016). The repressor AtlR downregulates the transcription of *atl* (Houston *et al.* 2011), whereas the sigma factor SigB negatively regulates the *agr* locus, thereby positively influencing *atl* transcription (Houston *et al.* 2011). Lastly, the TCS VraSR is able to sense perturbations in cell wall synthesis and negatively regulates autolytic activity (Kuroda *et al.* 2003, Antignac *et al.* 2007).

Several factors have been reported to positively regulate the expression of PGH-encoding genes. For instance, the TCS SrrAB regulates gene expression in *S. aureus* in response to oxygen availability (Yarwood *et al.* 2001) and it positively regulates the transcription of *isaA* (Stapleton *et al.* 2007). Like the *agr* system, the regulators SarA, Rot, MgrA and SaeR also regulate the expression of extracytoplasmic proteases that can modulate PGH activity through proteolysis of, for instance, Atl (Gimza *et al.* 2019).

Lastly, some regulators may exert both positive and negative regulation of the expression of PGH-encoding genes. This is exemplified by the small RNA SprX of *S. aureus*, which exerts its regulatory function by base-pairing with *cis*- or *trans*-encoded target mRNAs, thereby influencing the mRNA stability and translation (Caldelari *et al.* 2013). Thus, SprX can directly interact with the WalR mRNA to positively regulate the expression of *lytM* and *atl*. On the other hand, SprX also binds to the *isaA* mRNA, thereby negatively influencing its stability (Buchad and Nair 2021). Another target of SprX is the *spoVG* gene, which encodes a site-specific DNA-binding protein responsible for the inhibition of cell wall degradation (Eyraud *et al.* 2014). In particular, it was shown that SpoVG can directly bind to the putative promoter region of *lytN*, *femA* and *lytSR* to repress their transcription (Liu *et al.* 2016).

Regulation of PGH activity by cell wall components

WTA and LTA are known to influence the activity of PGHs and their substrate binding capability. In particular, WTA was shown to act as a temporal and spatial regulator of peptidoglycan metabolism (Atilano *et al.* 2010, Schlag *et al.* 2010), being a key determinant for the localization and activity of Atl (Weidenmaier *et al.* 2004). WTA also prevents the association of the GW cell wall-binding domain of Atl with the side wall (also referred to as the lateral wall), but not with the septum where WTA is less abundant or altered in structure, which results in a more acidic milieu that reduces the activity of Atl (Schlag *et al.* 2010, Biswas *et al.* 2012, Tiwari *et al.* 2018). TagO is an *N*-acetylglucosamine-phosphate transferase, which catalyzes the first step of WTA biosynthesis. Accordingly, a *tagO* mutant was shown to be completely devoid of WTA. The absence of WTA resulted in an even distribution of Atl on the surface of a *tagO* mutant leading to increased autolytic activity (Schlag *et al.* 2010). WTA also mediates the specific binding of Sle1 and LytN to the cross-wall of the septum (Schlag *et al.* 2010) and, accordingly, a lack of WTA results in a disordered localization of Sle1 and LytN (Chan *et al.* 2013). Consistent with this finding, it was shown that Sle1 lacking the LysM domain is unable to fulfill its function. In contrast, the function of LytN was not affected by absence of the LysM domain (Frankel and Schneewind 2012). The latter finding can be explained by the presence of the YSIRK/GS motif in the signal peptide of LytN, which contributes to the localization of LytN to the cross-wall (DeDent *et al.* 2008). Of note, the GW cell wall-binding domain of Atl can also bind to LTA, which is highly abundant at the septum, resulting in Atl targeting to the septum (Biswas *et al.* 2012, Zoll *et al.* 2012). Similar to WTA, LTA has been implicated in controlling the activity of various PGHs (Fedtke *et al.* 2007, Tiwari *et al.* 2018).

Regulation of PGH activity by protein–protein interactions

Protein-protein interactions can affect the activity and localization of PGHs. For instance, PGHs can form complexes with other proteins, such as peptidoglycan synthases, to execute their biological functions (Ho¨ltje 1998, Typas *et al.* 2012). Thus, SagB and the cytoplasmic membrane protein SpdC (LyrA) were shown to form a complex and cleave nascent glycan strands to release newly synthesized glycan strands from the cell membrane and to produce lipid II-attached oligomers for further peptidoglycan elongation (Schaefer *et al.* 2021). The SagB partner protein SpdC orients the active site of SagB for cleaving glycan strands (Schaefer *et al.* 2021). SpdC also antagonizes the WalR-dependent expression of SceD, LytM, Atl, and SA2097 (Poupel *et al.* 2018). Another example of protein-protein interactions relevant for PGH activity concerns the membrane-localized amidase LytH, which requires catalytic activation by the polytopic membrane protein ActH (Schaefer *et al.* 2021). The LytH-ActH amidase complex can also regulate the activity of peptidoglycan synthases. In particular, this complex removes the stem peptides from a nascent glycan strand at the cell periphery and promotes the relocation of peptidoglycan synthases to the septum during cell division (Do *et al.* 2020). Intramolecular protein-protein interactions also appear to determine PGH activity. This is exemplified by the LytM protein that shows weak PGH activity (Osipovitch *et al.* 2015). In contrast, the PGH activity is strongly increased upon truncation of LytM to the so-called M23 endopeptidase domain (Fig. 3). This suggests that the activity of LytM's M23 domain is suppressed by the so-called occluding domain of LytM (Odintsov *et al.* 2004). Until now, it is not known how the intact LytM becomes activated, but this most likely occurs through the interaction with partner proteins rather than proteolytic processing since the full-size LytM is readily detectable in the growth medium of *S. aureus* (Wang *et al.* 2021).

Regulation of PGHs by proteolytic activity

Staphylococcus aureus possesses several cytoplasmic and secreted proteolytic enzymes that play important functions in regulating the expression and activities of PGHs (Shaw *et al.* 2004, Kolar *et al.* 2013). For example, Sle1 is a substrate of the highly conserved protease ClpXP, which is composed of the peptidase subunit ClpP and the chaperone subunit ClpX. Inactivation of ClpXP was shown to result in increased Sle1 activity (Feng *et al.* 2013, Thalsø-Madsen *et al.* 2019), which seems to be due to ClpXP mediated processing of the signal peptide-bearing Sle1 precursor protein (Feng *et al.* 2013, Liu *et al.* 2017). Likewise, enhanced levels of IsaA, SceD, LytM, LytN, SsaA (SA2093) were detected in both *clpP* and *clpX* mutant strains (Kirsch *et al.* 2020). Cytoplasmic proteases, such as ClpXP, have been implicated in the activation of some regulatory factors, such as Rot, thereby indirectly regulating the expression of PGHs (Frees *et al.* 2005). Importantly, also extracellular proteases seem to modulate the PGH levels by proteolytic processing, in order to dispose of damaged or no longer needed proteins, or in response to external or internal signals. These extracellular proteases include the metalloprotease Aur, the serine proteases SspA, SplA, and SplC, the serine protease-like proteins SplB, SplD, SplE, and SplF, as well as the cysteine proteases ScpA and SspB (Dubin 2002, Kolar *et al.* 2013). In particular, the SspA protease was shown to be involved in the proteolytic processing of Atl, thereby regulating Atl activity and autolysis (Chen *et al.* 2013, Sahukhal *et al.* 2015).

Regulation of PGHs by environmental factors

Lastly, the activity of PGHs can be modulated by environmental factors, such as the salt and metal concentrations, or the pH (Gilpin *et al.* 1972, Foster 1995, Calamita and Doyle 2002). The impact of metals is exemplified by the M23 domain of LytM, LytU and the amidase domain of Atl, which all display zinc-dependent enzymatic activity (Büttner *et al.* 2014, Raulinaitis *et al.* 2017). The PGH activity of LytM is strongly inhibited in the presence of Fe^{2+}, Cu^{2+}, and Mg^{2+} ions and low concentrations of NaCl or KCL (Ramadurai *et al.* 1999). In contrast, the presence of Cu^{2+} or Co^{2+} was shown to increase the activity of LytU (Raulinaitis *et al.* 2017). A clear pH dependency was reported for SagB, which is more active at pH 5 than at pH 7.5 (Wheeler *et al.* 2015). LytM shows optimal activity at pH values between 5 and 8 (Ramadurai *et al.* 1999), whereas Atl has optimal activity above pH 6.5. Of note, Atl activity seems to be enhanced at low temperature and high NaCl concentrations (Foster 1995). Accordingly, it has been proposed that changes in the ionic composition of the cell wall affect PGH activity (Cheung and Freese 1985). In particular, it was shown that WTA affects the concentration of proton-binding sites in the cell wall, thereby modulating the activity of Atl (Biswas *et al.* 2012). The optimal pH for activity may relate to the specific localization of a PGH within the cell wall, where a high concentration of protons is encountered at the membrane-cell wall interface due to the proton-motive force, while the proton concentration can be lower towards the cell surface (Calamita *et al.* 2001).

Impact of antibiotics on PGH activity

Inhibition of peptidoglycan synthesis by antibiotics can lead to bacterial autolysis. This was exemplified by perturbations of the peptidoglycan synthesis in *S. aureus*, either by growing the bacteria in the presence of sub-inhibitory concentrations of β-lactam antibiotics, or by downregulating the expression of the peptidoglycan synthase PBP2 (Antignac *et al.* 2007). However, in response to the respective cell wall stress, the synthesis of PGHs is repressed at the transcriptional level, leading to a decreased susceptibility to autolysis (Antignac *et al.* 2007). In turn, this may result in a reduced susceptibility for β-lactams, as evidenced by the observation that PGH inactivation typically enhances the resistance to β-lactam antibiotics (Tomasz *et al.* 1970). In different staphylococcal lineages, the antibiotic resistance profiles can be very different, which will impact on the correlation of antibiotic activity and PGHs to different extents. Accordingly, some PGH mutants show actually an increased susceptibility to antibiotics. For example, a *sagB* mutant of the MRSA USA300 LAC strain was shown to be more susceptible to the β-lactam antibiotic oxacillin (Chan *et al.* 2016). This may be due to the increased length of glycan strands as a consequence of the SagB-deficiency. The increased glycan strand length may, in fact, cause a perturbed recognition and transpeptidation by the PBP2a that is responsible for the methicillin resistance of the USA300 LAC strain (Chan *et al.* 2016). Furthermore, β-lactam resistance can be correlated to the level of Sle1 in MRSA isolates of the USA300 lineage (Thalsø-Madsen *et al.* 2019). In particular, in oxacillin-treated cells the expression of *sle1* is decreased, which leads to a cell separation defect. Conversely, high Sle1 levels accelerate the splitting of daughter cells and decrease the cell size under oxacillin stress conditions. This implies that the Sle1 level may be positively coupled to the transpeptidase activity of PBPs, and that an increased Sle1 level contributes to oxacillin resistance by promoting cell separation. In contrast, *sle1* mutant cells are more susceptible to oxacillin, which may be linked to a synthetically lethal effect on septum synthesis (Thalsø-Madsen *et al.* 2019). Another example where an antibiotic impacts on PGH activity concerns targocil, which targets the TarG subunit of the WTA translocase. Targocil treatment of methicillin-susceptible *S. aureus* was shown to decrease the cell lysis by sequestering Atl at the membrane instead of the septal surface, which was a consequence of the accumulation of untranslocated WTA molecules (Tiwari *et al.* 2018).

Role of PGHs in Pathogenesis

S. aureus can thrive and survive inside and outside a wide variety of eukaryotic cells. Accordingly, the peptidoglycan structure and dynamics can be altered in different niches provided by the host, which seems to be very important for bacterial survival and pathogenesis (Chamaillard *et al.* 2003, Boneca 2005). For instance, during biofilm formation, which frequently occurs on tissues and implanted biomaterials, the cross-linking of the peptidoglycan was found to be reduced (Kim *et al.* 2018). Also here the non-crosslinked pentaglycine bridges were shown to provide a support for surface proteins that promote the biofilm formation (Kim *et al.* 2018). During a *S. aureus* kidney infection, smaller bacterial cells with thicker cell walls and less crosslinking of the peptidoglycan were observed compared to *in vitro* cultured bacteria. It was suggested that the non-crosslinked pentaglycine bridges could be used as attachment sites for surface proteins that are needed in abscess formation (Sutton *et al.* 2021). In addition, during infection, the structure of peptidoglycan showed less complexity, which may relate to an adjustment of the peptidoglycan metabolism. This implies that the energy gained by the adjusted peptidoglycan metabolism could be used for other processes in order to allow the bacterial proliferation in a limited environment (Sutton *et al.* 2021). In extreme cases, *S. aureus* may even partially or completely lose its cell wall, thereby converting to the L-form as has been reported for recurrent and persistent infections. However, the L-form bacteria are unstable under normal growth conditions, so when the environment changes or the inducer (*e.g.* peni-

cillin or oxacillin) is removed, these bacteria will convert again to the normal state with a protective cell wall (Xu *et al.* 2020).

Importantly, peptidoglycan fragments can be perceived by the host as bacterial virulence factors. For instance, peptidoglycan turnover mediated by PGHs was shown to lead to the shedding of peptidoglycan fragments into the host environment, thereby eliciting a proinflammatory response in the host (Girardin *et al.* 2003). In particular, it is known that the products of peptidoglycan turnover, muropeptides, can target the human intracellular immune receptors Nod1 and Nod2 which, in turn, leads to NF-κB activation and the release of proinflammatory factors, such as cytokines, chemokines and various antimicrobial compounds (Davis and Weiser 2011).

The pathogenicity of *S. aureus* largely relies on secreted and cell wall-localized proteinaceous virulence factors that promote the interaction between bacteria and the host. Accordingly, various PGHs exposed on the bacterial cell surface also play important roles in bacterial pathogenesis, including host cell adhesion, host cell internalization, inflammatory responses, immune activation and the aforementioned biofilm formation (McCarthy *et al.* 2016, Schlesier *et al.* 2020, Sutton *et al.* 2021). PGH activities may alter the overall charge of the bacterial surface, or they may modify the exposure of adhesins which, in turn, leads to altered bacterial aggregation, adhesive properties or altered biofilm formation. For example, the major autolysin Atl also acts as a host adhesin and interacts with the extracellular matrix of host cells during attachment, colonization and infection. Atl has been shown to bind to fibronectin and vitronectin mainly via its GW-peptidoglycan binding domain (Kohler *et al.* 2014, Porayath *et al.* 2018), and it also binds to the human heat shock cognate protein (Hsc70) with high affinity (Schlesier *et al.* 2020), thereby promoting the efficient internalization into host cells, including non-professional phagocytes (Hirschhausen *et al.* 2010), such as endothelial cells (Schlesier *et al.* 2020) and human epithelial cells (Dziewanowska *et al.* 2000). Atl also binds to the extracellular matrix components heparin and gelatine, thereby promoting bacterial binding to multiple different host cells during an invasive infection (Porayath *et al.* 2018). An *atl* mutant was shown to be attenuated in nanopore propagation in implant materials *in vitro* (which mimics infection of bone canaliculi) and in abscess formation *in vivo*, which may be attributed to an altered promotion of cell clustering and rough cell surface formation (Masters *et al.* 2021). Intriguingly, *atl* mutant bacteria excreted almost no ECPs, such as aldolase (FbaA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). This could also explain the attenuation of *atl* mutants at least in part, because FbaA and GAPDH are known to promote the adherence of *S. aureus* to host cells (Pasztor *et al.* 2010, Ebner *et al.* 2016). The PGH Sle1 can act as an adhesin by binding to human fibrinogen, fibronectin and vitronectin (Heilmann *et al.* 2005). Both the LysM domain and CHAP domain of Sle1 have been shown to play roles in host adhesion (Scheurwater *et al.* 2008, Kim *et al.* 2018). Furthermore, both the *sle1* and the *atl* mutant bacteria showed a significant decrease in pathogenesis in acute murine infection models (Kajimura *et al.* 2005). In addition, loss of the glucosaminidase domain of Atl leads to cell clustering and attenuation in a murine model of osteomyelitis (Varrone *et al.* 2014). Nonetheless, another study obtained no evidence for a role of Atl in murine infection (McCarthy *et al.* 2016), and the lack of Atl by itself was not sufficient to cause attenuation in a Zebrafish infection model (Sutton *et al.* 2021). On the other hand, inactivation of SagA and/or ScaH did lead to attenuation in a Zebrafish infection model (Sutton *et al.* 2021). A *sagB* mutant also displayed significant attenuation in a murein sepsis model, which implies that the absence of SagB significantly reduces the fitness of *S. aureus* (Sutton *et al.* 2021). Altogether, the reason(s) why PGH mutations cause attenuation of *S. aureus* appear to be rather complex. For instance, inactivation of SagB results initially in thinner cell walls that take longer transition time to reach maturity which, in turn, may lead to altered, less effective interactions with the host (Balraadjsing *et al.* 2019, Pasquina-Lemonche *et al.* 2020, Sutton *et al.* 2021). However, *sagB* mutants also display aberrant excretion of cytoplasmic proteins and reduced release of membrane and secreted proteins (Chan *et al.* 2016), which may also be a cause of attenuation. Bacteria with *isaA* or *sceD* mutations did not show significant attenuation in a murine infection model, whereas an *isaA sceD* double mutant displayed clear attenuation (Stapleton *et al.* 2007). On the other hand, *isaA* single mutant bacteria were attenuated in a *Galleria mellonella* larval infection model (Zhao *et al.* 2019), whereas SceD was shown to be important for nasal colonization and dissemination of *S. aureus* in host tissues (Stapleton *et al.* 2007).

PGHs are frequently involved in the aforementioned formation of biofilms that, in general, consist of proteins, polysaccharides and extracellular DNA (i.e. eDNA) (Schilcher and Horswill 2020) and that also contribute to the bacterial pathogenesis (Archer *et al.* 2011). Importantly, PGHs can mediate the release of eDNA which is a key factor in biofilm formation. Both through its amidase and glucosaminidase domains, Atl is involved in the primary attachment of the bacteria to surfaces and, consequently, this PGH serves an essential function in biofilm formation (Biswas *et al.* 2006, Bose *et al.* 2012). Specifically, unprocessed Atl promotes the primary attachment of *S. aureus* to surfaces, while the proteolytically activated Atl promotes cell lysis, the release of eDNA and cell accumulation for early biofilm development (Biswas *et al.* 2006). Atl plays an essential role in the development of so-called *ica*-independent biofilms. The formation of such biolfilms is brought about by a diverse array of surface proteins, such as Aap and Bap. These proteins serve as adhesins that bind to the host's extracellular matrix, instead of extracellular polysaccharide adhesins, such as the polysaccharide intracellular adhesin or polymeric N-acetylglucosamine that are synthesized by enzymes encoded in the *ica* operon (O'Gara 2007, Houston *et al.* 2011). Next to Atl, previous research has also shown that Sle1 may have a role in the formation of bacterial biofilms (Liu *et al.* 2017). Lastly, an elevated expression of *isaA* as induced by the anti-biofilm reagent tannic acid or by protein overexpression may also lead to biofilm formation (Payne *et al.* 2013).

PGHs as potential targets for active or passive immunization

PGHs are commonly considered as promising targets for active and passive immunization against *S. aureus* infections, because most of these proteins are exposed on the bacterial cell surface, where they play important roles in cell wall metabolism. Proteomic analyses of the cell surface of *S. aureus* have shown that ScaH (Aly), LytM, IsaA, SA0620, SA2097 are nearly always present at this subcellular location of clinical *S. aureus* isolates (Ziebandt *et al.* 2010). For active immunization, PGHs could thus potentially be used to elicit protective host immune responses either through the generation of specific antibodies that opsonize *S. aureus* and lead to its elimination by complement and/or professional phagocytes, or by inducing protective cellular immune responses (Bredius *et al.* 1993). Importantly, there is an increasing need for vaccines against *S. aureus*, due to the wide-spread antibiotic resistance displayed by this pathogen. The idea that PGHs are suitable targets for vaccination is corroborated by the observation

that the amidase domain of the major PGH Atl can elicit protective immunity against *S. aureus* in a murine infection model (Nair *et al.* 2015). It is, however, presently unknown whether this protection takes place through the staphylococcal opsonization by anti-Atl antibodies and subsequent immune clearance, or through the inactivation of Atl by such antibodies. Another study showed that the amidase domain of Atl in combination with an Alum adjuvant efficiently protects mice against an *S. aureus* infection (Suresh *et al.* 2021). An octa-valent *S. aureus* antigen mixture was shown to induce significant IgG responses against LytM, IsaA and the propeptide of Atl in immunized mice, although this did not result in a protection against death upon *S. aureus* infection (van den Berg *et al.* 2015b). However, patients with the genetic blistering disease epidermolysis bullosa, whose wounds are highly colonized with multiple *S. aureus* lineages and who rarely develop serious invasive staphylococcal infections, do show high IgG levels against these antigens (van der Kooi-Pol *et al.* 2012,van der Kooi-Pol *et al.* 2013, van der Kooi-pol *et al.* 2013, van der Kooi-pol *et al.* 2014, van den Berg *et al.* 2015b, Pastrana *et al.* 2018). This seems to suggest that IgGs against LytM, IsaA and Atl may also confer some degree of protection against *S. aureus* infections in humans. Likewise, in a screen for immunogenic non-covalently cell wall-bound proteins, it was observed that Atl, IsaA, EssH, Sle1 and SsaA (SA2093) elicit high IgG levels in sera of patients with EB (Pastrana *et al.* 2018). These findings imply that surface-exposed PGHs could be potential targets for anti-staphylococcal vaccines. This idea is also supported by passive immunization experiments. For instance, the human monoclonal antibody 1D9, which binds to the N-terminal region of IsaA, can protect mice at least partially against *S. aureus* bacteraemia and skin infection (van den Berg *et al.* 2015a). Likewise the humanized monoclonal antibody hUK-66 that specifically binds IsaA was shown to protect against killing by *S. aureus* in murine infection models, and this monoclonal antibody also effectively enhanced *S. aureus* killing in whole blood (Lorenz *et al.* 2011, Oesterreich *et al.* 2014).

Interestingly, so-called lysibodies that were engineered by fusing the cell wall-binding domain of Atl and the Fc region of human IgG were reported to efficiently bind the *S. aureus* cell wall. In turn, this was shown to lead to the fixation of complement on the bacterial surface, the promotion of phagocytosis by neutrophils and macrophages, and the protection of mice from an MRSA infection (Raz *et al.* 2017). Another lysibody composed of the cell wall-binding domain of lysostaphin and the Fc region of human IgG was shown to promote phagocytosis by neutrophils and macrophages even more efficiently, as well as the protection of mice against MRSA infection (Raz *et al.* 2018). These observations indicate that PGHs can be effectively applied as targets for active or passive immunization and also for the development of completely new generations of engineered antimicrobials.

Conclusion

The Gram-positive bacterial cell wall is essentially one molecule of cross-linked peptidoglycan that shelters a protoplast within its boundaries, thereby providing solid support against high turgor pressures. On the other hand, the cell wall is highly dynamic, allowing the cell to grow and divide and, at the same time, it serves as a semi-porous filter that allows the uptake of essential nutrients, the exclusion of toxic compounds from the bacterial environment, and the excretion of metabolic wastes. Yet another function of the cell wall is to serve as a matrix that binds and retains proteins with key functions in nutrient acquisition, cell adhesion to surfaces and bacterial virulence (van Dijl and du Teil Espina 2021). To make all these vital cell wall functions happen, PGHs play central roles as highlighted in our present review. The PGHs are key in peptidoglycan modulation and, therefore, essential for the bacterial survival in widely differing environments where staphylococci can thrive, ranging from fomites to contaminated food products and different niches in the bodies of livestock and humans. To avoid the destruction of their essential cell wall structures and functions, bacterial cells need to carefully regulate the expression of PGHs both spatially and temporally. This is not a trivial challenge, as underscored by *S. aureus*, which needs to carefully control the activities of its 18 core PGHs. Our present review summarizes the current understanding of how *S. aureus* manages to control PGH activity, how the bacterium takes advantage of the different properties and localizations of its PGHs, and how it suppresses their potentially cytotoxic effects. Importantly, PGHs are much more than enzymes that degrade cell walls, since they serve multiple biological functions in bacterial growth and survival in widely varying environments. Moreover, they participate in the release of ECPs needed for the bacterial adhesion to surfaces of medical implants or host tissues, or in the regulated bacterial cell death to generate eDNA needed for biofilm formation. PGHs are also critical for the cell cycle and allow *S. aureus* to execute various functions in human pathogenesis. Altogether, these vital functions of PGHs imply that they can potentially be used as targets for the development of anti-staphylococcal therapies. This is exemplified by the possible application of PGHs to lyse bacteria, to target PGHs through passive or active anti-staphylococcal immunization approaches, or even to use parts of the staphylococcal PGHs in the engineering of novel antimicrobial drugs. Yet, in spite of the fact that our understanding of PGH functions has substantially increased over recent years, there are still many open questions that need to be answered before we can fully appreciate their possible application for novel antimicrobial therapies. For instance, when and where exactly in the bacterial cell do the different PGHs play their physiological roles, or how exactly do they facilitate the sorting and secretion of virulence factors (van Dijl and du Teil Espina 2021)? Also, we need to know more about the plethora of either cooperative or antagonistic interactions between these enzymes that are responsible for cell wall homeostasis, and about how PGH function is regulated by the levels of peptidoglycan crosslinking and other major cell wall components like WTA and LTA. A better understanding of this complex interplay between cell wall synthesis, the synthesized polymers and PGHs may ultimately allow us to define new targets for new-generation antibiotics, vaccines and therapeutic antibodies that can help us to disrupt the essential processes of cell growth and division in important multi-drug resistant pathogens like *S. aureus*.

Availability of data and Materials

Not applicable

Authors' contributions

M.W. drafted the manuscript. MW, GB, and JMvD collected literature and contributed manuscript sections. MW prepared the Figures. GB and JMvD supervised the project. All authors, critically revised the manuscript, gave final approval and agree to be accountable for all aspects of the review.

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References

Abdelnour A, Arvidson S, Bremell T *et al.* The accessory gene regulator (*agr*) controls *staphylococcus aureus* virulence in a murine arthritis model. *Infect Immun* 1993;**61**:3879–85.

Antignac A, Sieradzki K, Tomasz A. Perturbation of cell wall synthesis suppresses autolysis in *staphylococcus aureus*: evidence for coregulation of cell wall synthetic and hydrolytic enzymes. *J Bacteriol* 2007;**189**:7573–80.

Archer NK, Mazaitis MJ, Costerton JW *et al. Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. *Virulence* 2011;**2**:445–59.

Atilano ML, Pereira PM, Yates J *et al.* Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *staphylococcus aureus*. *Proc Natl Acad Sci* 2010;**107**:18991–6.

Baba T, Schneewind O. Targeting of muralytic enzymes to the cell division site of Gram-positive bacteria: repeat domains direct autolysin to the equatorial surface ring of *staphylococcus aureus*. *EMBO J* 1998;**17**:4639–46.

Bae T, Baba T, Hiramatsu K *et al.* Prophages of *staphylococcus aureus* newman and their contribution to virulence. *Mol Microbiol* 2006;**62**:1035–47.

Balraadjsing PP, Lund LD, Souwer Y *et al.* The nature of antibacterial adaptive immune responses against *staphylococcus aureus* is dependent on the growth phase and extracellular peptidoglycan. *Infect Immun* 2019;**88**:e00733–19.

Bateman A, Rawlings ND. The CHAP domain: a large family of amidases including GSP amidase and peptidoglycan hydrolases. *Trends Biochem Sci* 2003;**28**:234–7.

Bayles KW. Bacterial programmed cell death: making sense of a paradox. *Nat Rev Microbiol* 2014;**12**:63–9.

Benito Y, Lina G, Greenland T *et al.* Trans-complementation of a *staphylococcus aureus agr* mutant by *staphylococcus lugdunensis agr* RNAIII. *J Bacteriol* 1998;**180**:5780–3.

Bera A, Biswas R, Herbert S *et al.* The presence of peptidoglycan O-acetyltransferase in various staphylococcal species correlates with lysozyme resistance and pathogenicity. *Infect Immun* 2006;**74**:4598–604.

Bera A, Herbert S, Jakob A *et al.* Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of *staphylococcus aureus*. *Mol Microbiol* 2005;**55**:778–87.

van den Berg S, Bonarius HPJ, van Kessel KPM *et al.* A human monoclonal antibody targeting the conserved staphylococcal antigen IsaA protects mice against *staphylococcus aureus* bacteremia. *Int J Med Microbiol* 2015a;**305**:55–64.

van den Berg S, Koedijk DGAM, Back JW *et al.* Active immunization with an octa-valent *staphylococcus aureus* antigen mixture in models of *s . aureus* bacteremia and skin infection in mice. *PLoS One* 2015b;**10**:e0116847.

Biswas R, Martinez RE, Göhring N *et al.* Proton-binding capacity of *staphylococcus aureus* wall teichoic acid and its role in controlling autolysin activity. *PLoS One* 2012;**7**:e41415.

Biswas R, Voggu L, Simon UK *et al.* Activity of the major staphylococcal autolysin atl. *FEMS Microbiol Lett* 2006;**259**:260–8.

den Blaauwen T, Hamoen LW, Levin PA. The divisome at 25: the road ahead. *Curr Opin Microbiol* 2017;**36**:85–94.

Bobrovskyy M, Willing SE, Schneewind O *et al.* EssH peptidoglycan hydrolase enables *staphylococcus aureus* type VII secretion across the bacterial cell wall envelope. *J Bacteriol* 2018;**200**:e00268–18.

Boisset S, Geissmann T, Huntzinger E *et al. Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator rot by an antisense mechanism. *Genes Dev* 2007;**21**:1353–66.

Boneca IG. The role of peptidoglycan in pathogenesis. *Curr Opin Microbiol* 2005;**8**:46–53.

Boneca IG, Huang Z-H, Gage DA *et al.* Characterization of *staphylococcus aureus* cell wall glycan strands, evidence for a new β-N-acetylglucosaminidase activity. *J Biol Chem* 2000;**275**:9910–8.

Borisova M, Gaupp R, Duckworth A *et al.* Peptidoglycan recycling in Gram-positive bacteria is crucial for survival in stationary phase. *MBio* 2016;**7**:e00923–16.

Bose JL, Lehman MK, Fey PD *et al.* Contribution of the *staphylococcus aureus* atl AM and GL murein hydrolase activities in cell division, autolysis, and biofilm formation. *PLoS One* 2012;**7**:e42244.

Bredius RG, De Vries CE, Troelstra A *et al.* Phagocytosis of *staphylococcus aureus* and *haemophilus influenzae* type b opsonized with polyclonal human igg1 and igg2 antibodies. Functional hFc gamma RIIa polymorphism to IgG2. *J Immunol* 1993;**151**:1463–72.

Brown S, Santa Maria JP, Walker S. Wall teichoic acids of Gram-positive bacteria. *Annu Rev Microbiol* 2013;**67**:313–36.

Brunskill EW, Bayles KW. Identification and molecular characterization of a putative regulatory locus that affects autolysis in *staphylococcus aureus*. *J Bacteriol* 1996a;**178**:611–8.

Brunskill EW, Bayles KW. Identification of lytsr-regulated genes from *staphylococcus aureus*. *J Bacteriol* 1996b;**178**:5810–2.

Buchad H, Nair M. The small RNA SprX regulates the autolysin regulator WalR in *staphylococcus aureus*. *Microbiol Res* 2021;**250**:126785.

Buist G, Steen A, Kok J *et al.* LysM, a widely distributed protein motif for binding to (peptido) glycans. *Mol Microbiol* 2008;**68**:838–47.

Büttner FM, Zoll S, Nega M *et al.* Structure-function analysis of *staphylococcus aureus* amidase reveals the determinants of peptidoglycan recognition and cleavage. *J Biol Chem* 2014;**289**:11083–94.

Calamita HG, Doyle RJ. Regulation of autolysins in teichuronic acid-containing *bacillus subtilis* cells. *Mol Microbiol* 2002;**44**:601–6.

Calamita HG, Ehringer WD, Koch AL *et al.* Evidence that the cell wall of *bacillus subtilis* is protonated during respiration. *Proc Natl Acad Sci* 2001;**98**:15260–3.

Caldelari I, Chao Y, Romby P *et al.* RNA-mediated regulation in pathogenic bacteria. *Cold Spring Harb Perspect Med* 2013;**3**:a010298.

Campbell J, Singh AK, Santa Maria JP *et al.* Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in *staphylococcus aureus*. *ACS Chem Biol* 2011;**6**:106–16.

Chamaillard M, Hashimoto M, Horie Y *et al.* An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat Immunol* 2003;**4**:702–7.

Chan YGY, Frankel MB, Dengler V *et al. Staphylococcus aureus* mutants lacking the lytr-cpsa-Psr family of enzymes release cell wall teichoic acids into the extracellular medium. *J Bacteriol* 2013;**195**:4650–9.

Chan YGY, Frankel MB, Missiakas D *et al.* SagB glucosaminidase is a determinant of *staphylococcus aureus* glycan chain length, antibi-

otic susceptibility, and protein secretion. *J Bacteriol* 2016;**198**:1123–36.

Chaudhuri RR, Allen AG, Owen PJ *et al.* Comprehensive identification of essential *staphylococcus aureus* genes using transposon-mediated differential hybridisation (TMDH). *BMC Genomics* 2009;**10**:1–18.

Chen C, Krishnan V, Macon K *et al.* Secreted proteases control autolysin-mediated biofilm growth of *staphylococcus aureus*. *J Biol Chem* 2013;**288**:29440–52.

Cheung AL, Bayer AS, Zhang G *et al.* Regulation of virulence determinants *in vitro* and *in vivo* in *staphylococcus aureus*. *FEMS Immunol Med Microbiol* 2004;**40**:1–9.

Cheung HY, Freese E. Monovalent cations enable cell wall turnover of the turnover-deficient *lyt*-15 mutant of *bacillus subtilis*. *J Bacteriol* 1985;**161**:1222–5.

Chhatwal GS. Anchorless adhesins and invasins of Gram-positive bacteria: a new class of virulence factors. *Trends Microbiol* 2002;**10**:205–8.

Chu X, Xia R, He N *et al.* Role of rot in bacterial autolysis regulation of *staphylococcus aureus* NCTC8325. *Res Microbiol* 2013;**164**:695–700.

Chunhua M, Yu L, Yaping G *et al.* The expression of LytM is down-regulated by RNAIII in *staphylococcus aureus*. *J Basic Microbiol* 2012;**52**:636–41.

Crosby HA, Schlievert PM, Merriman JA *et al.* The *staphylococcus aureus* global regulator MgrA modulates clumping and virulence by controlling surface protein expression. *PLoS Pathog* 2016;**12**:e1005604.

Crosby HA, Tiwari N, Kwiecinski JM *et al.* The *staphylococcus aureus* ArlRS two-component system regulates virulence factor expression through mgrA. *Mol Microbiol* 2020;**113**:103–22.

Davis KM, Weiser JN. Modifications to the peptidoglycan backbone help bacteria to establish infection. *Infect Immun* 2011;**79**:562–70.

DeDent A, Bae T, Missiakas DM *et al.* Signal peptides direct surface proteins to two distinct envelope locations of *staphylococcus aureus*. *EMBO J* 2008;**27**:2656–68.

Delauné A, Dubrac S, Blanchet C *et al.* The WalKR system controls major staphylococcal virulence genes and is involved in triggering the host inflammatory response. *Infect Immun* 2012;**80**:3438–53.

Delaune A, Poupel O, Mallet A *et al.* Peptidoglycan crosslinking relaxation plays an important role in *staphylococcus aureus* walkr-dependent cell viability. *PLoS One* 2011;**6**:e17054.

van Dijl JM, du Teil Espina M. Proteins sorted by 'chaos and disorder.' *Nature Microbiology* 2021;**6**:973–4.

Do T, Page JE, Walker S. Uncovering the activities, biological roles, and regulation of bacterial cell wall hydrolases and tailoring enzymes. *J Biol Chem* 2020;**295**:3347–61.

Do T, Schaefer K, Santiago AG *et al.* *Staphylococcus aureus* cell growth and division are regulated by an amidase that trims peptides from uncrosslinked peptidoglycan. *Nat Microbiol* 2020;**5**:291–303.

Dreisbach A, van Dijl JM, Buist G. The cell surface proteome of *staphylococcus aureus*. *Proteomics* 2011;**11**:3154–68.

Dreisbach A, Wang M, van der Kooi-Pol MM *et al.* Tryptic shaving of *staphylococcus aureus* unveils immunodominant epitopes on the bacterial cell surface. *J Proteome Res* 2020;**19**:2997–3010.

Dubin G. Extracellular proteases of *staphylococcus* spp. *Biol Chem* 2002;**383**:1057–86.

Dubrac S, Boneca IG, Poupel O *et al.* New insights into the WalK/WalR (YycG/YycF) essential signal transduction pathway reveal a major role in controlling cell wall metabolism and biofilm formation in *staphylococcus aureus*. *J Bacteriol* 2007;**189**:8257–69.

Dubrac S, Msadek T. Identification of genes controlled by the essential YycG/YycF two-component system of *staphylococcus aureus*. *J Bacteriol* 2004;**186**:1175–81.

Dunman P áM, Murphy E, Haney S *et al.* Transcription profiling-based identification of *staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci. *J Bacteriol* 2001;**183**:17341–53.

Dziewanowska K, Carson AR, Patti JM *et al.* Staphylococcal fibronectin binding protein interacts with heat shock protein 60 and integrins: role in internalization by epithelial cells. *Infect Immun* 2000;**68**:6321–8.

Ebner P, Götz F. Bacterial excretion of cytoplasmic proteins (ECP): occurrence, mechanism, and function. *Trends Microbiol* 2019;**27**:176–87.

Ebner P, Luqman A, Reichert S *et al.* Non-classical protein excretion is boosted by PSMα-induced cell leakage. *Cell Rep* 2017;**20**:1278–86.

Ebner P, Prax M, Nega M *et al.* Excretion of cytoplasmic proteins (ECP) in *staphylococcus aureus*. *Mol Microbiol* 2015;**97**:775–89.

Ebner P, Rinker J, Götz F. Excretion of cytoplasmic proteins in *staphylococcus* is most likely not due to cell lysis. *Curr Genet* 2016;**62**:19–23.

Ebner P, Rinker J, Nguyen MT *et al.* Excreted cytoplasmic proteins contribute to pathogenicity in *staphylococcus aureus*. *Infect Immun* 2016;**84**:1672–81.

Egan AJF, Cleverley RM, Peters K *et al.* Regulation of bacterial cell wall growth. *FEBS J* 2017;**284**:851–67.

El-Shaboury SR, Saleh GA, Mohamed FA *et al.* Analysis of cephalosporin antibiotics. *J Pharm Biomed Anal* 2007;**45**:1–19.

Eyraud A, Tattevin P, Chabelskaya S *et al.* A small RNA controls a protein regulator involved in antibiotic resistance in *staphylococcus aureus*. *Nucleic Acids Res* 2014;**42**:4892–905.

Fabijan AP, Lin RCY, Ho J *et al.* Safety of bacteriophage therapy in severe *staphylococcus aureus* infection. *Nat Microbiol* 2020;**5**:465–72.

Falord M, Mäder U, Hiron A *et al.* Investigation of the *staphylococcus aureus* GraSR regulon reveals novel links to virulence, stress response and cell wall signal transduction pathways. *PLoS One* 2011;**6**:e21323.

Fedtke I, Mader D, Kohler T *et al.* A *staphylococcus aureus* ypfP mutant with strongly reduced lipoteichoic acid (LTA) content: LTA governs bacterial surface properties and autolysin activity. *Mol Microbiol* 2007;**65**:1078–91.

Feng J, Michalik S, Varming AN *et al.* Trapping and proteomic identification of cellular substrates of the ClpP protease in *staphylococcus aureus*. *J Proteome Res* 2013;**12**:547–58.

Fenton M, McAuliffe O, O'Mahony J *et al.* Recombinant bacteriophage lysins as antibacterials. *Bioengineered Bugs* 2010;**1**:9–16.

Foster SJ. Molecular characterization and functional analysis of the major autolysin of *staphylococcus aureus* 8325/4. *J Bacteriol* 1995;**177**:5723–5.

Fournier B, Hooper DC. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *staphylococcus aureus*. *J Bacteriol* 2000;**182**:3955–64.

Francius G, Domenech O, Mingeot-Leclercq MP *et al.* Direct observation of *staphylococcus aureus* cell wall digestion by lysostaphin. *J Bacteriol* 2008;**190**:7904–9.

Frankel MB, Hendrickx APA, Missiakas DM *et al.* LytN, a murein hydrolase in the cross-wall compartment of *staphylococcus aureus*, is involved in proper bacterial growth and envelope assembly. *J Biol Chem* 2011;**286**:32593–605.

Frankel MB, Schneewind O. Determinants of murein hydrolase targeting to cross-wall of *staphylococcus aureus* peptidoglycan. *J Biol Chem* 2012;**287**:10460–71.

Frees D, Sørensen K, Ingmer H. Global virulence regulation in *staphylococcus aureus*: pinpointing the roles of ClpP and ClpX in the *sar/agr* regulatory network. *Infect Immun* 2005;**73**:8100–8.

Frirdich E, Gaynor EC. Peptidoglycan hydrolases, bacterial shape, and pathogenesis. *Curr Opin Microbiol* 2013;**16**:767–78.

Gajdiss M, Monk IR, Bertsche U *et al.* YycH and YycI regulate expression of *staphylococcus aureus* autolysins by activation of WalRK phosphorylation. *Microorganisms* 2020;**8**:870.

Ghuysen J-M, Tipper DJ, Strominger JL. *et al.* (eds.) *Methods in Enzymology*. New York & London: Academic Press, 1966,685–99.

Giesbrecht P, Kersten T, Maidhof H *et al.* Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. *Microbiol Mol Biol Rev* 1998;**62**:1371–414.

Gilpin RW, Chatterjee AN, Young FE. Autolysis of microbial cells: salt activation of autolytic enzymes in a mutant of *staphylococcus aureus*. *J Bacteriol* 1972;**111**:272–83.

Gimza BD, Larias MI, Budny BG *et al.* Mapping the global network of extracellular protease regulation in *staphylococcus aureus*. *Msphere* 2019;**4**:e00676–19.

Girardin SE, Boneca IG, Viala J *et al.* Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 2003;**278**:8869–72.

Goldmann O, Medina E. *Staphylococcus aureus* strategies to evade the host acquired immune response. *Int J Med Microbiol* 2018;**308**:625–30.

Gonzalez-Delgado LS, Walters-Morgan H, Salamaga B *et al.* Two-site recognition of *staphylococcus aureus* peptidoglycan by lysostaphin SH3b. *Nat Chem Biol* 2020;**16**:24–30.

Goodell E. Recycling of murein by *escherichia coli*. *J Bacteriol* 1985;**163**:305–10.

Groicher KH, Firek BA, Fujimoto DF *et al.* The *staphylococcus aureus lrgAB* operon modulates murein hydrolase activity and penicillin tolerance. *J Bacteriol* 2000;**182**:1794–801.

Gross M, Cramton SE, Go¨tz F *et al.* Key role of teichoic acid net charge in *staphylococcus aureus* colonization of artificial surfaces. *Infect Immun* 2001;**69**:3423–6.

Heilmann C, Hartleib J, Hussain MS *et al.* The multifunctional *staphylococcus aureus* autolysin aaa mediates adherence to immobilized fibrinogen and fibronectin. *Infect Immun* 2005;**73**:4793–802.

Herbert S, Bera A, Nerz C *et al.* Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. *PLoS Pathog* 2007;**3**:e102.

Hirschhausen N, Schlesier T, Peters G *et al.* Characterization of the modular design of the autolysin/adhesin aaa from *staphylococcus aureus*. *PLoS One* 2012;**7**:e40353.

Hirschhausen N, Schlesier T, Schmidt MA *et al.* A novel staphylococcal internalization mechanism involves the major autolysin atl and heat shock cognate protein hsc70 as host cell receptor. *Cell Microbiol* 2010;**12**:1746–64.

Ho¨ltje J-V. Growth of the stress-bearing and shape-maintaining murein sacculus of *escherichia coli*. *Microbiol Mol Biol Rev* 1998;**62**:181–203.

Höltje J V, Mirelman D, Sharon N *et al.* Novel type of murein transglycosylase in *escherichia coli*. *J Bacteriol* 1975;**124**:1067–76.

Hort M, Bertsche U, Nozinovic S *et al.* The role of β-glycosylated wall teichoic acids in the reduction of vancomycin susceptibility in vancomycin-intermediate *staphylococcus aureus*. *Microbiol Spect* 2021;**9**:e0052821.

Houston P, Rowe SE, Pozzi C *et al.* Essential role for the major autolysin in the fibronectin-binding protein-mediated *staphylococcus aureus* biofilm phenotype. *Infect Immun* 2011;**79**:1153–65.

Huntzinger E, Boisset S, Saveanu C *et al. Staphylococcus aureus* RNAIII and the endoribonuclease III coordinately regulate *spa* gene expression. *EMBO J* 2005;**24**:824–35.

Ingavale S, van Wamel W, Luong TT *et al.* Rat/MgrA, a regulator of autolysis, is a regulator of virulence genes in *staphylococcus aureus*. *Infect Immun* 2005;**73**:1423–31.

Ingavale SS, Van Wamel W, Cheung AL. Characterization of RAT, an autolysis regulator in *staphylococcus aureus*. *Mol Microbiol* 2003;**48**:1451–66.

Jacobs C, Frère J-M, Normark S. Cytosolic intermediates for cell wall biosynthesis and degradation control inducible β-lactam resistance in Gram-negative bacteria. *Cell* 1997;**88**:823–32.

Jolliffe LK, Doyle RJ, Streips UN. The energized membrane and cellular autolysis in *bacillus subtilis*. *Cell* 1981;**25**:753–63.

Jorge AM, Hoiczyk E, Gomes JP *et al.* EzrA contributes to the regulation of cell size in *staphylococcus aureus*. *PLoS One* 2011;**6**:e27542.

Josse J, Laurent F, Diot A. Staphylococcal adhesion and host cell invasion: fibronectin-binding and other mechanisms. *Frontiers in Microbiology* 2017;**8**:2433.

Kajimura J, Fujiwara T, Yamada S *et al.* Identification and molecular characterization of an N-acetylmuramyl-l-alanine amidase sle1 involved in cell separation of *staphylococcus aureus*. *Mol Microbiol* 2005;**58**:1087–101.

Kawata S, Takemura T, Yokogawa K. Characterization of two N-acetylmuramidases from *streptomyces globisporus* 1829. *Agric Biol Chem* 1983;**47**:1501–8.

Keinhoerster D, George SE, Weidenmaier C *et al.* Function and regulation of *staphylococcus aureus* wall teichoic acids and capsular polysaccharides. *Int J Med Microbiol* 2019;**309**:151333.

Kim SJ, Chang J, Rimal B *et al.* Surface proteins and the formation of biofilms by *staphylococcus aureus*. *Biochimica Et Biophysica Acta (BBA) - Biomembranes* 2018;**1860**:749–56.

Kirsch VC, Fetzer C, Sieber SA. Global inventory of clpp-and clpx-Regulated proteins in *staphylococcus aureus*. *J Proteome Res* 2020;**20**:867–79.

Kleerebezem M, Quadri LEN, Kuipers OP *et al.* Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol Microbiol* 1997;**24**:895–904.

Kluj RM, Ebner P, Adamek M *et al.* Recovery of the peptidoglycan turnover product released by the autolysin atl in *staphylococcus aureus* involves the phosphotransferase system transporter MurP and the novel 6-phospho-N-acetylmuramidase mupG. *Frontiers in Microbiology* 2018;**9**:2725.

Kohler TP, Gisch N, Binsker U *et al.* Repeating structures of the major staphylococcal autolysin are essential for the interaction with human thrombospondin 1 and vitronectin. *J Biol Chem* 2014;**289**:4070–82.

Kojima N, Araki Y, Ito E. Structure of the linkage units between ribitol teichoic acids and peptidoglycan. *J Bacteriol* 1985;**161**:299–306.

Kolar SL, Antonio Ibarra J, Rivera FE *et al.* Extracellular proteases are key mediators of *s taphylococcus aureus* virulence via the global modulation of virulence-determinant stability. *Microbiologyopen* 2013;**2**:18–34.

Komatsuzawa H, Sugai M, Nakashima S *et al.* Subcellular localization of the major autolysin, ATL and its processed proteins in *staphylococcus aureus*. *Microbiol Immunol* 1997;**41**:469–79.

van der Kooi-Pol MM, Duipmans JC, Jonkman MF *et al.* Host–pathogen interactions in epidermolysis bullosa patients colonized with *staphylococcus aureus*. *Int J Med Microbiol* 2014;**304**:195–203.

van der Kooi-Pol MM, Sadaghian Sadabad M, Duipmans JC *et al.* Topography of distinct *staphylococcus aureus* types in chronic wounds of patients with epidermolysis bullosa. *PLoS One* 2013;**8**:e67272.

van der Kooi-Pol MM, De Vogel CP, Westerhout-Pluister GN *et al.* High anti-staphylococcal antibody titers in patients with epidermolysis bullosa relate to long-term colonization with alternating types of *staphylococcus aureus*. *J Invest Dermatol* 2013;**133**:847–50.

van der Kooi-Pol MM, Veenstra-Kyuchukova YK, Duipmans JC *et al.* High genetic diversity of *staphylococcus aureus* strains colonizing patients with epidermolysis bullosa. *Exp Dermatol* 2012;**21**:463–6.

Koraimann G. Lytic transglycosylases in macromolecular transport systems of Gram-negative bacteria. *Cell Mol Life Sci (CMLS)* 2003;**60**:2371–88.

Kuroda M, Kuroda H, Oshima T *et al.* Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *staphylococcus aureus*. *Mol Microbiol* 2003;**49**:807–21.

Lioliou E, Fechter P, Caldelari I *et al.* Various checkpoints prevent the synthesis of *staphylococcus aureus* peptidoglycan hydrolase LytM in the stationary growth phase. *RNA Biology* 2016;**13**:427–40.

Liu Q, Wang X, Qin J *et al.* The ATP-dependent protease ClpP inhibits biofilm formation by regulating agr and cell wall hydrolase sle1 in *staphylococcus aureus*. *Front Cell Infect Microbiol* 2017;**7**:181.

Liu X, Zhang S, Sun B. SpoVG regulates cell wall metabolism and oxacillin resistance in methicillin-resistant *staphylococcus aureus* strain N315. *Antimicrob Agents Chemother* 2016;**60**:3455–61.

Lorenz U, Lorenz B, Schmitter T *et al.* Functional antibodies targeting IsaA of *staphylococcus aureus* augment host immune response and open new perspectives for antibacterial therapy. *Antimicrob Agents Chemother* 2011;**55**:165–73.

Loskill P, Pereira PM, Jung P *et al.* Reduction of the peptidoglycan crosslinking causes a decrease in stiffness of the *staphylococcus aureus* cell envelope. *Biophys J* 2014;**107**:1082–9.

Lovering AL, De Castro LH, Lim D *et al.* Structural insight into the transglycosylation step of bacterial cell-wall biosynthesis. *Science* 2007;**315**:1402–5.

Lovering AL, Safadi SS, Strynadka NCJ. Structural perspective of peptidoglycan biosynthesis and assembly. *Annu Rev Biochem* 2012;**81**:451–78.

Lund VA, Wacnik K, Turner RD *et al.* Molecular coordination of *staphylococcus aureus* cell division. *Elife* 2018;**7**:e32057.

Luong TT, Lee CY. The *arl* locus positively regulates *staphylococcus aureus* type 5 capsule via an *mgrA*-dependent pathway. *Microbiology* 2006;**152**:3123–31.

Luong TT, Dunman PM, Murphy E *et al.* Transcription profiling of the MgrA regulon in *staphylococcus aureus*. *J Bacteriol* 2006;**188**:1899–910.

Lützner N, Pätzold B, Zoll S *et al.* Development of a novel fluorescent substrate for autolysin e, a bacterial type II amidase. *Biochem Biophys Res Commun* 2009;**380**:554–8.

Mainous AG, Hueston WJ, Everett CJ *et al.* Nasal carriage of *staphylococcus aureus* and methicillin-resistant *s aureus* in the united states, 2001–2002. *Ann Family Med* 2006;**4**:132–7.

Manna AC, Ingavale SS, Maloney M *et al.* Identification of *sarV* (SA2062), a new transcriptional regulator, is repressed by SarA and MgrA (SA0641) and involved in the regulation of autolysis in *staphylococcus aureus*. *J Bacteriol* 2004;**186**:5267–80.

Masters EA, Muthukrishnan G, Ho L *et al.* *Staphylococcus aureus* cell wall biosynthesis modulates bone invasion and osteomyelitis pathogenesis. *Front Microbiol* 2021;**12**:723498.

Matias VRF, Beveridge TJ. Native cell wall organization shown by cryo-electron microscopy confirms the existence of a periplasmic space in *staphylococcus aureus*. *J Bacteriol* 2006;**188**:1011–21.

Matias VRF, Beveridge TJ. Cryo-electron microscopy of cell division in *staphylococcus aureus* reveals a mid-zone between nascent cross walls. *Mol Microbiol* 2007;**64**:195–206.

Mayer C, Kluj RM, Muehleck M *et al.* Bacteria's different ways to recycle their own cell wall. *Int J Med Microbiol* 2019;**309**:151326.

McCarthy H, Waters EM, Bose JL *et al.* The major autolysin is redundant for *staphylococcus aureus* USA300 LAC JE2 virulence in a murine device-related infection model. *FEMS Microbiol Lett* 2016;**363**:fnw087.

Memmi G, Nair DR, Cheung A. Role of ArlRS in autolysis in methicillin-sensitive and methicillin-resistant *staphylo coccus aureus* strains. *J Bacteriol* 2012;**194**:759–67.

Monteiro JM, Covas G, Rausch D *et al.* The pentaglycine bridges of *staphylococcus aureus* peptidoglycan are essential for cell integrity. *Sci Rep* 2019;**9**:5010.

Monteiro JM, Fernandes PB, Vaz F *et al.* Cell shape dynamics during the staphylococcal cell cycle. *Nat Commun* 2015;**6**:1–12.

Moynihan PJ, Sychantha D, Clarke AJ. Chemical biology of peptidoglycan acetylation and deacetylation. *Bioorg Chem* 2014;**54**:44–50.

Nagarajan R. Antibacterial activities and modes of action of vancomycin and related glycopeptides. *Antimicrob Agents Chemother* 1991;**35**:605–9.

Nair N, Vinod V, Suresh MK *et al.* Amidase, a cell wall hydrolase, elicits protective immunity against *staphylococcus aureus* and *s . epidermidis*. *Int J Biol Macromol* 2015;**77**:314–21.

Navarre WW, Schneewind O. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol Mol Biol Rev* 1999;**63**:174–229.

Nega M, Tribelli PM, Hipp K *et al.* New insights in the coordinated amidase and glucosaminidase activity of the major autolysin (Atl) in *staphylococcus aureus*. *Communications Biology* 2020;**3**:1–10.

O'Gara JP. *ica* and beyond: biofilm mechanisms and regulation in *staphylococcus epidermidis* and *staphylococcus aureus*. *FEMS Microbiol Lett* 2007;**270**:179–88.

Odintsov SG, Sabala I, Marcyjaniak M *et al.* Latent LytM at 1.3 Å resolution. *J Mol Biol* 2004;**335**:775–85.

Oesterreich B, Lorenz B, Schmitter T *et al.* Characterization of the biological anti-staphylococcal functionality of hUK-66 igg1, a humanized monoclonal antibody as substantial component for an immunotherapeutic approach. *Hum Vaccin Immunother* 2014;**10**:926–37.

Oku Y, Kurokawa K, Matsuo M *et al.* Pleiotropic roles of polyglycerolphosphate synthase of lipoteichoic acid in growth of *staphylococcus aureus* cells. *J Bacteriol* 2009;**191**:141–51.

Ortiz C, Natale P, Cueto L *et al.* The keepers of the ring: regulators of FtsZ assembly. *FEMS Microbiol Rev* 2016;**40**:57–67.

Osipovitch DC, Therrien S, Griswold KE. Discovery of novel *s . aureus* autolysins and molecular engineering to enhance bacteriolytic activity. *Appl Microbiol Biotechnol* 2015;**99**:6315–26.

Pasquina-Lemonche L, Burns J, Turner RD *et al.* The architecture of the Gram-positive bacterial cell wall. *Nature* 2020;**582**:294–7.

Pastrana FR, Neef J, van Dijl JM *et al.* A *lactococcus lactis* expression vector set with multiple affinity tags to facilitate isolation and direct labeling of heterologous secreted proteins. *Appl Microbiol Biotechnol* 2017;**101**:8139–49.

Pastrana FR, Neef J, Koedijk DGAM *et al.* Human antibody responses against non-covalently cell wall-bound *staphylococcus aureus* proteins. *Sci Rep* 2018;**8**:1–11.

Pasztor L, Ziebandt A-K, Nega M *et al.* Staphylococcal major autolysin (Atl) is involved in excretion of cytoplasmic proteins. *J Biol Chem* 2010;**285**:36794–803.

Payne DE, Martin NR, Parzych KR *et al.* Tannic acid inhibits *staphylococcus aureus* surface colonization in an isaa-dependent manner. *Infect Immun* 2013;**81**:496–504.

de Pedro MA, Cava F. Structural constraints and dynamics of bacterial cell wall architecture. *Front Microbiol* 2015;**6**:449.

Penyige A, Matkó J, Deák E *et al.* Depolarization of the membrane potential by β-lactams as a signal to induce autolysis. *Biochem Biophys Res Commun* 2002;**290**:1169–75.

Pinho MG, Kjos M, Veening J-W. How to get (a) round: mechanisms controlling growth and division of coccoid bacteria. *Nat Rev Microbiol* 2013;**11**:601–14.

Porayath C, Suresh MK, Biswas R *et al.* Autolysin mediated adherence of *staphylococcus aureus* with fibronectin, gelatin and heparin. *Int J Biol Macromol* 2018;**110**:179–84.

Poupel O, Proux C, Jagla B *et al.* SpdC, a novel virulence factor, controls histidine kinase activity in *staphylococcus aureus*. *PLoS Pathog* 2018;**14**:e1006917.

Rai A, Khairnar K. Overview of the risks of *staphylococcus aureus* infections and their control by bacteriophages and bacteriophage-encoded products. *Brazilian J Microbiol* 2021;**52**:2031–42.

Raineri EJM, Altulea D, van Dijl JM. Staphylococcal trafficking and infection—from 'nose to gut' and back. *FEMS Microbiol Rev* 2022;**46**:fuab041.

Rajagopal M, Walker S. Envelope structures of Gram-positive bacteria. *Curr Top Microbiol Immunol* 2017;**404**:1–44.

Ramadurai L, Lockwood KJ, Lockwood J *et al.* Characterization of a chromosomally encoded glycylglycine endopeptidase of *staphylococcus aureus*. *Microbiology* 1999;**145**:801–8.

Raulinaitis V, Tossavainen H, Aitio O *et al.* Identification and structural characterization of LytU, a unique peptidoglycan endopeptidase from the lysostaphin family. *Sci Rep* 2017;**7**:1–14.

Raz A, Serrano A, Lawson C *et al.* Lysibodies are IgG fc fusions with lysin binding domains targeting *staphylococcus aureus* wall carbohydrates for effective phagocytosis. *Proc Natl Acad Sci* 2017;**114**:4781–6.

Raz A, Serrano A, Thaker M *et al.* Lysostaphin lysibody leads to effective opsonization and killing of methicillin-resistant *staphylococcus aureus* in a murine model. *Antimicrob Agents Chemother* 2018;**62**:e01056–18.

Reed P, Veiga H, Jorge AM *et al.* Monofunctional transglycosylases are not essential for *staphylococcus aureus* cell wall synthesis. *J Bacteriol* 2011;**193**:2549–56.

Reichmann NT, Tavares AC, Saraiva BM *et al.* SEDS–bPBP pairs direct lateral and septal peptidoglycan synthesis in *staphylococcus aureus*. *Nature Microbiology* 2019;**4**:1368–77.

Rice KC, Bayles KW. Molecular control of bacterial death and lysis. *Microbiol Mol Biol Rev* 2008;**72**:85–109.

Rice KC, Firek BA, Nelson JB *et al.* The *staphylococcus aureus cidAB* operon: evaluation of its role in regulation of murein hydrolase activity and penicillin tolerance. *J Bacteriol* 2003;**185**:2635–43.

Rigden DJ, Jedrzejas MJ, Galperin MY. Amidase domains from bacterial and phage autolysins define a family of γ-d, l-glutamate-specific amidohydrolases. *Trends Biochem Sci* 2003;**28**:230–4.

Rogasch K, Ru¨hmling V, Pané-Farré J *et al.* Influence of the two-component system SaeRS on global gene expression in two different *staphylococcus aureus* strains. *J Bacteriol* 2006;**188**:7742–58.

Ruiz N. Bioinformatics identification of MurJ (MviN) as the peptidoglycan lipid II flippase in *escherichia coli*. *Proc Natl Acad Sci* 2008;**105**:15553–7.

Sahukhal GS, Batte JL, Elasri MO. msaABCR operon positively regulates biofilm development by repressing proteases and autolysis in *staphylococcus aureus*. *FEMS Microbiol Lett* 2015;**362**:1.

Sakata N, Terakubo S, Mukai T. Subcellular location of the soluble lytic transglycosylase homologue in *staphylococcus aureus*. *Curr Microbiol* 2005;**50**:47–51.

Sarvas M, Harwood CR, Bron S *et al.* Post-translocational folding of secretory proteins in Gram-positive bacteria. *Biochim Biophys Acta* 2004;**1694**:311–27.

Sauvage E, Kerff F, Terrak M *et al.* The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* 2008;**32**:234–58.

Schaefer K, Owens TW, Page JE *et al.* Structure and reconstitution of a hydrolase complex that may release peptidoglycan from the membrane after polymerization. *Nat Microbiol* 2021;**6**:34–43.

Scheurwater E, Reid CW, Clarke AJ. Lytic transglycosylases: bacterial space-making autolysins. *Int J Biochem Cell Biol* 2008;**40**:586–91.

Schilcher K, Horswill AR. Staphylococcal biofilm development: structure, regulation, and treatment strategies. *Microbiol Mol Biol Rev* 2020;**84**:e00026–19.

Schindler CA, Schuhardt VT. Lysostaphin: a new bacteriolytic agent for the *staphylococcu s*. *Proc Natl Acad Sci* 1964;**51**:414.

Schlag M, Biswas R, Krismer B *et al.* Role of staphylococcal wall teichoic acid in targeting the major autolysin atl. *Mol Microbiol* 2010;**75**:864–73.

Schlesier T, Siegmund A, Rescher U *et al.* Characterization of the Atl-mediated staphylococcal internalization mechanism. *Int J Med Microbiol* 2020;**310**:151463.

Schneewind O, Fowler A, Faull KF. Structure of the cell wall anchor of surface proteins in *staphylococcus aureus*. *Science* 1995;**268**:103–6.

Schneewind O, Missiakas D. Sec-secretion and sortase-mediated anchoring of proteins in Gram-positive bacteria. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research* 2014;**1843**:1687–97.

Sewell EWC, Brown ED. Taking aim at wall teichoic acid synthesis: new biology and new leads for antibiotics. *J Antibiot (Tokyo)* 2014;**67**:43–51.

Sham L-T, Butler EK, Lebar MD *et al.* MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. *Science* 2014;**345**:220–2.

Shaw L, Golonka E, Potempa J *et al.* The role and regulation of the extracellular proteases of *staphylococcus aureus*. *Microbiology* 2004;**150**:217–28.

Shockman GD, Daneo-Moore L, Kariyama R *et al.* Bacterial walls, peptidoglycan hydrolases, autolysins, and autolysis. *Microb Drug Resist* 1996;**2**:95–8.

Shockman GD, Höltje J-V. Microbial peptidoglycan (murein) hydrolases. In: Ghuysen J.-M. *et al.* (eds) *New Comprehensive Biochemistry*. Amsterdam: Elsevier Science B.V., 1994,131–66.

Sibbald M, Ziebandt AK, Engelmann S *et al.* Mapping the pathways to staphylococcal pathogenesis by comparative secretomics. *Microbiol Mol Biol Rev* 2006;**70**:755–88.

Sieradzki K, Tomasz A. Alterations of cell wall structure and metabolism accompany reduced susceptibility to vancomycin in an isogenic series of clinical isolates of *staphylococcus aureus*. *J Bacteriol* 2003;**185**:7103–10.

Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2010;**2**:a000414.

Smith TJ, Blackman SA, Foster SJ. Autolysins of *bacillus subtilis*: multiple enzymes with multiple functions. *Microbiology* 2000;**146**:249–62.

Snowden MA, Perkins HR. Peptidoglycan cross-linking in *staphylococcus aureus*: an apparent random polymerisation process. *Eur J Biochem* 1990;**191**:373–7.

Sobral R, Tomasz A. The staphylococcal cell wall. *Microbiology Spectrum* 2019;**7**:4–7.

Stapleton MR, Horsburgh MJ, Hayhurst EJ *et al.* Characterization of IsaA and SceD, two putative lytic transglycosylases of *staphylococcus aureus*. *J Bacteriol* 2007;**189**:7316–25.

Steen A, Buist G, Horsburgh GJ *et al.* AcmA of *lactococcus lactis* is an N-acetylglucosaminidase with an optimal number of LysM domains for proper functioning. *FEBS J* 2005;**272**:2854–68.

Strominger JL, Izaki K, Matsuhashi M *et al.* Peptidoglycan transpeptidase and D-alanine carboxypeptidase: penicillin-sensitive enzymatic reactions. *Fed Proc* 1967;**26**:9–22.

Sugai M, Komatsuzawa H, Akiyama T *et al.* Identification of endo-beta-N-acetylglucosaminidase and N-acetylmuramyl-L-alanine amidase as cluster-dispersing enzymes in *staphylococcus aureus*. *J Bacteriol* 1995;**177**:1491–6.

Suresh MK, Kumar VA, Biswas L *et al.* Protective efficacy of alum adjuvanted amidase protein vaccine against *staphylococcus aureus* infection in multiple mouse models. *J Appl Microbiol* 2021.

Sutton JAF, Carnell OT, Lafage L *et al. Staphylococcus aureus* cell wall structure and dynamics during host-pathogen interaction. *PLoS Pathog* 2021;**17**:e1009468.

Szurmant H, White RA, Hoch JA. Sensor complexes regulating two-component signal transduction. *Curr Opin Struct Biol* 2007;**17**:706–15.

Szweda P, Schielmann M, Kotlowski R *et al.* Peptidoglycan hydrolases-potential weapons against *staphylococcus aureus*. *Appl Microbiol Biotechnol* 2012;**96**:1157–74.

Takahashi J, Komatsuzawa H, Yamada S *et al.* Molecular characterization of an *atl* null mutant of *staphylococcus aureus*. *Microbiol Immunol* 2002;**46**:601–12.

Thalsø-Madsen I, Torrubia FR, Xu L *et al.* The sle1 cell wall amidase is essential for β-lactam resistance in community-acquired methicillin-resistant *staphylococcus aureus* USA300. *Antimicrob Agents Chemother* 2019;**64**:e01931–19.

Thammavongsa V, Kim HK, Missiakas D *et al.* Staphylococcal manipulation of host immune responses. *Nat Rev Microbiol* 2015;**13**:529–43.

Tiwari KB, Gatto C, Walker S *et al.* Exposure of *staphylococcus aureus* to targocil blocks translocation of the major autolysin atl across the membrane, resulting in a significant decrease in autolysis. *Antimicrob Agents Chemother* 2018;**62**:e00323–18.

Tomasz A, Albino A, Zanati EVE. Multiple antibiotic resistance in a bacterium with suppressed autolytic system. *Nature* 1970;**227**:138–40.

Trotonda MP, Xiong YQ, Memmi G *et al.* Role of *mgrA* and *sarA* in methicillin-resistant *staphylococcus aureus* autolysis and resistance to cell wall-active antibiotics. *J Infect Dis* 2009;**199**:209–18.

Tsompanidou E, Denham EL, Sibbald MJJB *et al.* The sortase a substrates FnbpA, FnbpB, ClfA and ClfB antagonize colony spreading of *staphylococcus aureus*. *PLoS One* 2012;**7**:e44646.

Turner NA, Sharma-Kuinkel BK, Maskarinec SA *et al.* Methicillin-resistant *staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol* 2019;**17**:203–18.

Turner RD, Ratcliffe EC, Wheeler R *et al.* Peptidoglycan architecture can specify division planes in *staphylococcus aureus*. *Nat Commun* 2010;**1**:1–9.

Turner RD, Vollmer W, Foster SJ. Different walls for rods and balls: the diversity of peptidoglycan. *Mol Microbiol* 2014;**91**:862–74.

Typas A, Banzhaf M, Gross CA *et al.* From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat Rev Microbiol* 2012;**10**:123–36.

Varrone JJ, de Mesy Bentley KL, Bello-Irizarry SN *et al.* Passive immunization with anti-glucosaminidase monoclonal antibodies protects mice from implant-associated osteomyelitis by mediating opsonophagocytosis of *staphylococcus aureus* megaclusters. *J Orthop Res* 2014;**32**:1389–96.

Veiga H, Jorge AM, Pinho MG. Absence of nucleoid occlusion effector noc impairs formation of orthogonal FtsZ rings during *staphylococcus aureus* cell division. *Mol Microbiol* 2011;**80**:1366–80.

Vermassen A, Leroy S, Talon R *et al.* Cell wall hydrolases in bacteria: insight on the diversity of cell wall amidases, glycosidases and peptidases toward peptidoglycan. *Front Microbiol* 2019;**10**:331.

Visweswaran GRR, Leenhouts K, van Roosmalen M *et al.* Exploiting the peptidoglycan-binding motif, LysM, for medical and industrial applications. *Appl Microbiol Biotechnol* 2014;**98**:4331–45.

Vollmer W, Blanot D, De Pedro MA. Peptidoglycan structure and architecture. *FEMS Microbiol Rev* 2008;**32**:149–67.

Vollmer W, Joris B, Charlier P *et al.* Bacterial peptidoglycan (murein) hydrolases. *FEMS Microbiol Rev* 2008;**32**:259–86.

Walsh L, Johnson CN, Hill C *et al.* Efficacy of phage-and bacteriocin-based therapies in combatting nosocomial MRSA infections. *Front Mol Biosci* 2021;**8**:295.

Wang M, van den Berg S, Hernández YM *et al.* Differential binding of human and murine iggs to catalytic and cell wall binding domains of *staphylococcus aureus* peptidoglycan hydrolases. *Sci Rep* 2021;**11**:1–16.

Wardenburg JB, Patel RJ, Schneewind O. Surface proteins and exotoxins are required for the pathogenesis of *staphylococcus aureus* pneumonia. *Infect Immun* 2007;**75**:1040–4.

Weidenmaier C, Kokai-Kun JF, Kristian SA *et al.* Role of teichoic acids in *staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nat Med* 2004;**10**:243–5.

Weidenmaier C, Peschel A. Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nat Rev Microbiol* 2008;**6**:276–87.

Wheeler R, Turner RD, Bailey RG *et al.* Bacterial cell enlargement requires control of cell wall stiffness mediated by peptidoglycan hydrolases. *MBio* 2015;**6**:e00660–15.

Willing S, Schneewind O, Missiakas D. Regulated cleavage of glycan strands by the murein hydrolase SagB in *staph ylococcus aureus* involves a direct interaction with LyrA (SpdC). *J Bacteriol* 2021;**203**:e00014–21.

Xia G, Kohler T, Peschel A. The wall teichoic acid and lipoteichoic acid polymers of *staphylococcus aureus*. *Int J Med Microbiol* 2010;**300**:148–54.

Xu Y, Zhang B, Wang L *et al.* Unusual features and molecular pathways of *staphylococcus aureus* L-form bacteria. *Microb Pathog* 2020;**140**:103970.

Yamada S, Sugai M, Komatsuzawa H *et al.* An autolysin ring associated with cell separation of *staphylococcus aureus*. *J Bacteriol* 1996;**178**:1565–71.

Yarwood JM, McCormick JK, Schlievert PM. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *staphylococcus aureus*. *J Bacteriol* 2001;**183**:1113–23.

Zhao X, Chlebowicz-Flissikowska MA, Wang M *et al.* Exoproteomic profiling uncovers critical determinants for virulence of livestock-associated and human-originated *staphylococcus aureus* ST398 strains. *Virulence* 2020;**11**:947–63.

Zhao X, Palma Medina LM, Stobernack T *et al.* Exoproteome heterogeneity among closely related *staphylococcus aureus* t437 isolates and possible implications for virulence. *J Proteome Res* 2019;**18**:2859–74.

Zhou X, Halladin DK, Rojas ER *et al.* Mechanical crack propagation drives millisecond daughter cell separation in *staphylococcus aureus*. *Science* 2015;**348**:574–8.

Ziebandt A, Weber H, Rudolph J *et al.* Extracellular proteins of *staphylococcus aureus* and the role of SarA and σB. *Protomecis* 2001;**1**:480–93.

Ziebandt A, Kusch H, Degner M *et al.* Proteomics uncovers extreme heterogeneity in the *staphylococcus aureus* exoproteome due to genomic plasticity and variant gene regulation. *Proteomics* 2010;**10**:1634–44.

Zoll S, Schlag M, Shkumatov AV *et al.* Ligand-binding properties and conformational dynamics of autolysin repeat domains in staphylococcal cell wall recognition. *J Bacteriol* 2012;**194**:3789–802.