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Recent Advances in Pneumococcal Peptidoglycan Biosynthesis Suggest New Vaccine and Antimicrobial Targets

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Abstract

Streptococcus pneumoniae is a serious human respiratory pathogen that has the capacity to evade capsule-based vaccines and to develop multidrug antibiotic resistance. This review summarizes recent advances in understanding the mechanisms and regulation of peptidoglycan (PG) biosynthesis that result in ellipsoid-shaped, ovococcus *Streptococcus* cells. New results support a two-state model for septal and peripheral PG synthesis at mid-cell, involvement of essential cell division proteins in PG remodeling, and mid-cell localization of proteins that organize PG biosynthesis and that form the protein translocation apparatus. PG biosynthesis proteins have already turned up as promising vaccine candidates and targets of antibiotics. Properties of several recently characterized proteins that mediate or regulate PG biosynthesis suggest a source of additional targets for therapies against pneumococcus.

Introduction

Streptococcus pneumoniae (pneumococcus) is an extremely serious, opportunistic pathogen that annually kills millions of people worldwide [1,2]. *S. pneumoniae* colonizes the nasopharynx of children and adults, and carriage is thought to be required for its spread among its human hosts [1,3]. *S. pneumoniae* can also cause a variety of invasive diseases that range from middle ear infections (otitis media) to pneumonia, bacteremia, and meningitis [1,2,4]. Pneumococcal invasive diseases are particularly prevalent among individuals with underdeveloped or compromised immune systems, such as infants, the elderly, and HIV patients [5,6], and following certain respiratory infections, such as influenza [6]. Because of the severe toll of *S. pneumoniae* on human health, vaccine development against pneumococcal infection continues to be a major area of research activity (reviewed in [7]). The goal of this review is to summarize some recent developments from this and other laboratories in understanding PG biosynthesis and cell division in *S. pneumoniae* and other ovococcus species. Essential proteins that have emerged from these studies could provide new vaccine candidates and targets for antibiotic and passive-immunity therapies.

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Recent trends in vaccine development against pneumococcal protein antigens

All vaccines currently in use target the pneumococcal exopolysaccharide capsule, of which there are greater than 90 different serotype varieties [7-9]. The capsule is a major virulence factor that prevents phagocytosis by the immune system [3]. Capsule-based (23-valent) vaccines have been relatively successful in providing protection to adults, and protein-conjugated-carbohydrate-based (7-valent) vaccines have recently provided considerable protection to infants in developed countries [6,7]. Nevertheless, serotypes not included in the 7-valent vaccine appeared rapidly in clinical isolates [10], prompting the development of new 10- and 13-valent conjugated vaccines tailored to the prevalent clinical isolates in different regions [6,7]. Ominously, in parallel to vaccine evasion, resistance of *S. pneumoniae* clinical isolates to multiple antibiotics has started to increase at alarming rates [11-13].

The expense of developing conjugated vaccines and the difficulty of outpacing the genetic plasticity of an organism with natural transformation [14] has prompted the consideration of conserved proteins as possible vaccine targets [7,9,15]. Two types of potential protein targets have emerged from recent screens based on proteomic and surface-protein display approaches [8,16,17], as well as the more traditional approach of testing surface-exposed protein virulence factors [18,19]. One set of targets induces humoral immunity leading to protective antibody production against invasive infection [7,8]. The other set stimulates protection against colonization by a mechanism that depends on CD4⁺ T helper cells and IL-17A cytokine production [16,19]. Several ABC transporter substrate binding proteins (e.g., PsaA involved in Mn²⁺ uptake) act as protective antigens against invasive pneumococcal diseases (see [3,7,9]). PsaA is a lipoprotein that is tethered close to the extracellular surface and bound to transmembrane ABC channel proteins (see [3]). Nevertheless, PsaA and other lipoproteins are accessible to antibodies at some level, despite the presence of the capsule and PG. As expected, antibody accessibility to these surface proteins increases in unencapsulated mutants, as determined by flow cytometry (see [20]).

Several pneumococcal surface proteins that elicit an antibody response mediate peptidoglycan (PG) biosynthesis (PcsB, PBP1b, and LytB), cell division (FtsL and DivIC), autolysis (LytA, LytC, and CbpD), cell wall stress response (StkP), and proteolysis (ZmpB and PrtA) [7-9,16,19]. Of these, PcsB and StkP, along with the PsaA Mn²⁺ receptor protein, have proven to be particularly promising as vaccine candidates [8,9,19]. The ability of these surface proteins located close to the pneumococcal cell membrane to elicit immune responses raises the possibility that other PG biosynthetic and cell division proteins could serve as vaccine targets. In addition, the essentiality, “druggable” location on the cell surface, relatively high abundance, and strict conservation across serotypes suggest that some of the pneumococcal proteins involved in these processes may be possible targets for antibiotic and passive-immunity therapies.

The two-state model of PG synthesis involves essential proteins with large extracellular domains

The early work of Higgins and Shockman suggested that ellipsoid (American-football)-shaped bacteria, such as *Streptococcus*, *Enterococcus*, and *Lactococcus* species, synthesize their PGs by a combination of peripheral (sidewall-like) and septal synthesis that occurs in the mid-cell regions of dividing cells (Fig. 1) [21,22]. These two modes are analogous to the lateral sidewall and septal PG synthesis that occur at different locations in rod-shaped bacteria [23-25]. However, unlike rod-shaped bacteria, ovococcus species lack an MreB

homologue, and peripheral and septal PG synthesis are likely coordinated with and organized by FtsZ ring formation [25,26]. Because two modes of PG biosynthesis are required to form ellipsoid-shaped bacteria, models have been proposed for two different PG synthesis machineries (Fig. 1) (see [23-25]). Based on the composition of the complexes in rod-shaped cells, it has been hypothesized that specific sets of homologous cell division proteins mediate peripheral (MreC, MreD, RodA, GpsB, and PBP2b) and septal (FtsZ, EzrA, GpsB, PBP2x, FtsW, and DivIB/FtsL/DivIC) PG synthesis in ovococcus bacteria (Fig. 2) [24,25]. Many aspects of this model are largely untested, and it remains to be determined whether the two ovococcus PG synthesis machineries exist as two distinct complexes (Fig. 1) or form one large complex at mid-cell [25]. In addition, the roles of Class A (dual-function transglycosylase-transpeptidase) PBP1a, PBP1b, and PBP2a are only beginning to emerge [13,23,25]. Although useful, this two-state model is obviously an oversimplification, even in rod-shaped bacteria (see [27]), where some penicillin-binding proteins (PBPs) and division regulators, such as PBP1 and GpsB of *B. subtilis*, shuttle between the lateral and septal PG synthesis machineries [28].

There are several noteworthy properties of these PG synthesis proteins relevant to their possible use as antimicrobial targets. Some membrane-bound PG synthesis proteins (e.g., PBPs and MreC) contain large extracellular domains or extracellular loops (Fig. 2) [24,25,28]. In addition, many of these surface PG synthesis proteins are relatively abundant. For example, *S. pneumoniae* MreC is present at about $\approx 8,500$ dimers per exponentially growing cell [23]. Finally, some of these PG synthesis proteins that are essential in *S. pneumoniae* [13,23,29] are not essential in the model Gram-positive bacterium, *Bacillus subtilis* (e.g., PBP2a_{Bsu} (homologue of PBP2b_{Spn}), GpsB, EzrA, and MreD) [28,30], or in the coccus, *Staphylococcus aureus* (e.g., MreCD, RodA) [31]. Reasons for these differences could be functional redundancy in *B. subtilis* [28,30] or lack of peripheral PG synthesis in spherical *S. aureus* [24,25]. Consistent with this interpretation, proteins required for PG synthesis, like EzrA, are essential in *S. pneumoniae* and some strains of *S. aureus* [29,32,33]. However, even among *Streptococcus* species there is diversity in the composition of the PG synthesis machines, as exemplified by the fact that MreC is essential in *S. pneumoniae*, but not even present in *Streptococcus pyogenes* (see [27]). This diversity will likely affect the mechanisms and relative timing of peripheral and septal PG synthesis in different ovococcus species.

Recent evidence supporting the two-state model of ovococcus PG synthesis

A useful observation from studies of rod-shaped bacteria is that mutational or antibiotic impairment of lateral sidewall or septal PG synthesis leads to the formation of spherical or elongated, cylindrical cells, respectively (see [28]). Three recent studies have used this simplifying principle to provide support for the two-state model of PG synthesis in ovococcus bacteria [23,34,35]. Unexpectedly, the ovococcus, *Lactococcus lactis*, is pleomorphic and forms elongated rod-shaped cells in biofilms and during planktonic growth in some media [35]. Filament formation was ascribed to transient arrest of cell division resulting in multiple sites of PG synthesis along the lengths of the filamented cells [35]. Filamentation of *L. lactis* cells also occurs upon treatment with the β -lactam antibiotic, methicillin, which specifically inhibits PBP2x, a Class B (single-function transpeptidase) PBP essential in *L. lactis* and *S. pneumoniae* [34,35]. Remarkably, methicillin-resistant mutants of *L. lactis* contain amino acid changes in PBP2x that prevent filament formation in response to the antibiotic or during growth. Together, these results directly support the model that PBP2x is involved in septal PG synthesis (Fig. 2) and that inhibition of PBP2x function contributes to filament formation in wild-type cells under some growth conditions [35]. Another surprise from this work is that the Class B PBP2b is not essential in *L. lactis*

[35], whereas its homologue is essential in *S. pneumoniae* [13,29]. *L. lactis pbp2b* mutants formed spherically shaped cells, even upon treatment with methicillin [35], consistent with a role of PBP2b in peripheral PG synthesis (Fig. 2).

A third recent paper supports a role for MreCD in peripheral PG biosynthesis in *S. pneumoniae* (Fig. 2) [23]. MreC and MreD are required to maintain the shape of rod-shaped bacteria, along with the actin homologue, MreB [24,27]. Depletion of MreCD in rod-shaped bacteria leads to the formation of spherical cells and the accumulation of suppressor mutations [25,36,37]. *Streptococcus pneumoniae* and other ovococci lack MreB homologues, and the functions of the MreCD proteins were unknown. Similar to rod-shaped bacteria, *mreCD* are essential in a virulent serotype 2 strain of *S. pneumoniae*, and depletion of MreCD results in cell rounding and lysis, consistent with a role in peripheral PG biosynthesis. Similar to rod-shaped bacteria, $\Delta mreCD$ mutants accumulate bypass suppressors that allow growth, but do not restore normal cell morphology [23]. One class of suppressors eliminates function of Class A PBP1a, which plays a major role in the development of pneumococcal β -lactam resistance [23]. PBP1a is not essential in *S. pneumoniae*, but the absence of PBP1a causes cells to have smaller diameters than their PBP1a⁺ parents [23]. This morphology phenotype is especially visible in unencapsulated cells [23,38]. In contrast, the absence of the other Class A PBPs, PBP2a or PBP1b, does not appreciably affect cell size or allow growth of $\Delta mreCD$ mutants. In *S. pneumoniae*, deletion mutations that knock out PBP1a and PBP2a are synthetically lethal and prevent growth. However, the different effects of $\Delta pbp1a$ and $\Delta pbp2a$ mutations on cell shape and $\Delta mreCD$ mutation suppression indicate that PBP1a and PBP2a are not simply redundant or equivalent [23]. Finally, immunofluorescent microscopy located MreCD to the equators and septa of dividing pneumococcal cells, similar to the PBPs and PG pentapeptides indicative of PG synthesis [23]. These combined results are consistent with a model in which MreCD direct peripheral PG synthesis and control PBP1a localization or activity in *S. pneumoniae* [23]. This work also points up the general caution that mutations that impair the growth of *S. pneumoniae* often readily accumulate bypass suppressor mutations that mask and alter primary phenotypes (see [38,39]).

Pneumococcal StkP serine/threonine kinase locates to regions of PG biosynthesis and cell division and phosphorylates DivIVA

As noted above, the StkP regulatory protein has emerged as a major vaccine candidate for antibody and T-cell-mediated immunity [16,19]. A review by Burnside and Rajaopalan in this issue summarizes the current understanding of targets of serine/threonine kinases and phosphatases that mediate gene expression in prokaryotes [40]. In *S. pneumoniae*, StkP encodes the sole serine/threonine kinase and consists of an amino-terminal kinase domain, a transmembrane domain, and an extracellular domain containing 4 tandem PASTA motifs [41,42]. The PASTA motifs are exposed sufficiently on the cell surface to be detected by flow cytometry, and the 3 outermost PASTA domains act as antigens [42]. Each PASTA motif in StkP contains only about 70 amino acids [41,42]. The immunogenicity of the second PASTA motif supports the idea that some proteins close to the pneumococcal cell surface, including those located at the division septum like StkP (see below), can act as antigens, despite the presence of capsule.

Accumulating data suggests that StkP plays an important regulatory role in pneumococcal cell division, even during exponential growth in the absence of overt stress conditions [42,43]. Notably, StkP localizes predominantly to division septa and equators [42], similar to other cell division proteins, including FtsZ [44-46], PBP2b and PBP2x [13], and MreCD [23]. StkP disperses around the periphery of cells as cultures enter stationary phase [42]. Consistent with a role in division, StkP phosphorylates six protein targets continuously

during different stages of growth, and the PASTA domain is necessary for this substrate phosphorylation [43]. *stkP* mutants grow slower than their parent strains and show an elongated cell morphology in which septation seems to be interrupted [8,43]. One of the major substrates of StkP phosphorylation *in vitro* and *in vivo* is the DivIVA division protein [43,47]. Like its homologue in *B. subtilis* [48,49], DivIVA binds to negatively curved membrane surfaces at division septa and cell poles of *S. pneumoniae* cells [50]. However, *S. pneumoniae* lacks the Min system that interacts with DivIVA in *B. subtilis* [50-52]. Based on the morphology of pneumococcal *divIVA* mutants, it has been postulated that DivIVA may play multifaceted roles in pneumococcal cell division, separation, and maturation of cell poles [50]. Phosphorylation of DivIVA by StkP may partly regulate its activity, and it is noteworthy that *divIVA* and *stkP* mutants show elongated cell morphologies with certain resemblances [8,43,50]. However, it has not yet been determined whether DivIVA regulates PG synthesis, PG remodeling by hydrolases (see below), or both processes in PG biosynthesis. In addition, the StkP PASTA domain binds β -lactam antibiotics and synthetic PG mucopeptides directly [41], indicating that phosphorylation by StkP likely plays a role in cell wall stress responses.

PG remodeling by PG hydrolases is linked to cell division by interactions with signal transduction complexes on the cell surface

The process of PG biosynthesis involves not only PG synthesis, but PG remodeling by hydrolase enzymes (Fig. 1) [53]. The connection between PG remodeling and cell division is poorly understood in ovococcus bacteria. In *S. pneumoniae* D39, 13 genes encode proteins that are known or putative PG hydrolases or that are likely associated with PG hydrolysis [45,53,54]. Single knockout mutations in 5 (*pcsB*, *pmp23*, *dacA*, *dacB*, and *spd_0703*) of these 13 genes result in overt effect on pneumococcal cell shape or division, possibly implicating them in PG remodeling [54]. Of these 5 genes, only *pcsB* is essential in serotype 2 strains [38,54,55]. Deletion of *pcsB* has been reported in some other capsule serotype strains, but the resulting $\Delta pcsB$ mutants are severely impaired for growth [56], and it has not been completely resolved whether bypass suppressor mutations accumulate in $\Delta pcsB$ mutants. The *pcsB* gene is a member of the regulon controlled by the WalRK_{Spn} (VicRK) two-component regulatory system [55,57]. Besides *pcsB*, WalRK_{Spn} controls non-essential PG hydrolase genes in *S. pneumoniae* [57], and homologues of WalRK generally control division PG hydrolases in other low-GC Gram-positive bacteria [58,59]. PcsB is secreted to the cell surface by the Sec translocase [38] and consists of two conserved domains: an amino-terminal coiled-coil domain and a carboxyl terminal CHAP domain (Fig. 3) [38,45,56]. CHAP-domain proteins usually act as PG hydrolases, but purified PcsB lacks detectable enzymatic activity [38,56].

The paradox of PcsB essentiality, but lack of enzymatic activity, suggested that PcsB binds to membrane proteins that may regulate a PG hydrolytic or other activity of PcsB. This hypothesis was recently confirmed in *S. pneumoniae* [45]. To explore the functions of PcsB, its subcellular localization was determined. Fractionation experiments showed that exported PcsB lacking its signal peptide was bound by hydrophobic interactions on the external membrane surface of pneumococcal cells [20,45]. Immunofluorescent microscopy localized PcsB mainly to the septa and equators of dividing cells [45], but not at cell poles as claimed in a previous study [45]. Chemical crosslinking combined with immunoprecipitation showed that PcsB interacts with the cell division complex formed by membrane-bound FtsX and cytoplasmic FtsE (Fig. 3) [45], which structurally resemble the channel and ATPase subunits of an ABC transporter, respectively [60]. Far-Western blotting showed that this interaction was partly through the large extracellular loop of FtsX and the amino-terminal coiled-coil domain of PcsB [45]. Unlike in *Bacillus subtilis* and *Escherichia coli*, FtsX and FtsE are essential in *S. pneumoniae*, confirming the corollary that essential PcsB would

interact with an essential membrane protein. Consistent with an interaction between PcsB and FtsX, pneumococcal cells depleted of PcsB or FtsX had strikingly similar defects in cell division, and depletion of FtsX caused mislocalization of PcsB, but not the FtsZ early division protein [45].

These results support a model in which the interaction of the FtsEX complex with PcsB activates its PG hydrolysis activity and couples peptidoglycan remodeling to pneumococcal cell division (Fig. 3). This model fulfills the expectation that PG remodeling, like PG synthesis, will be tightly regulated and coupled to FtsZ-mediated cell division. This model is likely to be general, because an analogous interaction was found concurrently in *E. coli* between FtsX and a PG hydrolysis system [61]. Indirect support for this model is also provided by the recent report that disruption of *ftsX* or the *lytE* PG autolysin gene imparts chemokine resistance to *Bacillus anthracis* [62]. Despite being located close to the cell surface of *S. pneumoniae*, PcsB is accessible to antibodies [20] and has emerged as a leading vaccine candidate that is already in clinical trials [7,8,19]. Moreover, the interaction between PcsB and the extracellular loop of the essential FtsX protein suggests that large loop domains of integral membrane proteins may be candidates for vaccines or targets for passive immunity. Finally, if the CHAP domain of PcsB turns out to have a regulated PG hydrolase activity, then antibiotics directed to conserved papain-family CHAP domains might be sought.

SecA, SecY, and HtrA localize to regions of PG biosynthesis and cell division in growing *S. pneumoniae*, whereas SrtA sortase is randomly distributed on cell surfaces

The Sec translocase pathway is the major route for protein transport across and into the cytoplasmic membrane of bacteria. Previous studies reported that the SecA translocase ATP-binding subunit and the cell-surface HtrA protease/chaperon formed a single microdomain, termed ExPortal, in certain ovococcus species like *S. pyogenes* [63,64]. However, new results do not support the existence of an ExPortal microdomain in *S. pneumoniae* [46], which is evolutionarily distant from *S. pyogenes* (reviewed in [65]). During exponential growth of *S. pneumoniae*, the SecA ATPase motor and SecY channel protein locate dynamically in cells at different stages of division [46]. In early divisional cells, both Sec subunits concentrate at equators, which are future sites of constriction. Further along in division, SecA and SecY remain localized at mid-cell septa, whereas in late divisional cells, both Sec subunits are hemispherically distributed in the regions between septa and the future equators of dividing cells (Fig. 4). In contrast, the pneumococcal HtrA protease/chaperon localizes to the equators and septa of most dividing cells (Fig. 4), whereas the SrtA sortase locates over the surface of cells in no discernible pattern. The dynamic pattern of Sec distribution is largely absent in most cells in early stationary phase and in ΔcIs mutants lacking cardiolipin synthase [46]. In addition, Sec distribution is not perturbed by the absence of Flotillin-family proteins, which are components of membrane microdomain rafts that also contain signaling and transport proteins in eukaryotic and bacterial cells (see [66]). The coincident localization of SecA, SecY, and HtrA to regions of PG biosynthesis in unstressed, growing cells suggests that the pneumococcal Sec translocase directs assembly of the PG biosynthesis apparatus to regions where it is needed during division and that HtrA may play a general role in quality control of proteins exported by the Sec translocase.

Conclusions

New approaches and refinements of older approaches are elucidating the machineries and mechanisms that synthesize and remodel PG and coordinate PG biosynthesis with cell

division in *S. pneumoniae* and other Gram-positive pathogens. Several themes are emerging from this research. Pathogens, such as *S. pneumoniae*, have substantially smaller genomes than some model bacteria, such as *B. subtilis* ($\approx 2,000$ versus $>4,000$ encoded proteins), and this reduction is paralleled in some cases by less functional redundancy in *S. pneumoniae*. Consequently, several PG biosynthesis or cell division proteins that are not essential individually in *B. subtilis* are essential in *S. pneumoniae* (e.g., EzrA, GpsB, and FtsEX) [28-30,45]. In addition, there are both similarities and some remarkable differences in the functions, interactions, and localization of some PG biosynthesis and cell division proteins between *B. subtilis* and *S. pneumoniae* (e.g., MreCD and DivIVA) [23,50]. It seems likely that subsets of these proteins will function in the same overall processes of peripheral, septal, or both forms of PG biosynthesis, but in different ways.

Another level of complexity is that the PG acts as the scaffolding for the covalent attachment of teichoic acids, proteins by sortase, and the capsule of *S. pneumoniae*. Hanson and Neely discuss the coordinate regulation of these surface components in Gram-positive bacteria in this issue [67] (also see [25,68]). Therefore, the PG itself is a fundamental determinant of bacterial virulence and pathogenicity, especially in gram-positive species. Moreover, fragments of the PG stimulate the TLR- and NOD-mediated innate immune responses (see [69,70]), and PG is the major target for clinically important classes of antibiotics, including β -lactams and glycopeptides (see [13]). As discussed above, several pneumococcal PG biosynthesis proteins have emerged as antigens for possible vaccine development (see [7]). Another theme from recent publications is the concentration of the apparatuses for PG synthesis, PG hydrolysis, cell division, protein secretion, and control of PG biosynthesis and protein quality at the division septa and equators of dividing pneumococcal cells (e.g., [23,25,45,46,54,56]). The division septa and equators of pneumococcal cells are astonishingly busy and dynamic places. Several surface proteins in these division-site complexes are accessible to antibodies, despite the fact that the proteins are not extended and are located close to the extracellular surface (e.g., PcsB, StkP) [20,42].

Together, the essentiality, extracellular locations, antibody accessibility, relative abundance, and amino acid conservation in different pneumococcal serotypes suggest that the PG biosynthetic apparatus will provide additional vaccine candidates and targets for passive immunity therapies. The enzymatic activities of some of these druggable PG biosynthetic proteins could also become targets for new classes of antibiotics. For example, the essentiality or requirement for normal division of CHAP-domain PG hydrolases makes them an attractive potential antibiotic target. CHAP domain hydrolases are a kind of cysteine protease [71], and large numbers of cysteine protease and peptidase inhibitors have been reported for use in treatment of intrinsic diseases, including cancer, bone disease, and Alzheimer, and as possible therapies against viruses and parasites (see [72]). As evidenced by these examples, it has been possible to modify the side chains of starting inhibitors to achieve highly selective inhibition of specific kinds of cysteine proteases involved in different cellular functions. Achieving a similar high level of specificity against bacterial CHAP-domain hydrolases seems challenging, but possible, given the extensive chemical libraries of protease inhibitors that already exist [72].

Finally, due to space limitations, this review has focused on PG biosynthesis proteins that are essential, highly conserved, and cause pronounced defects in cell division when absent during exponential growth. Other pneumococcal PG hydrolases have been implicated in cell separation at poles (e.g., LytB [73]), in competence-dependent fratricide (e.g., CbpD and LytC [74]), or stationary-phase autolysis (e.g., LytA [75]) (see also [54]). Mutants lacking LytB and LytC were recently shown to be attenuated for colonization and avoiding host immunity during invasive disease [73], and LytA, LytB, and LytC have already been shown to be protective antigens against pneumococcal invasive disease in murine models (see [7]).

These and other PG metabolism enzymes may also emerge as new vaccine candidates and antimicrobial targets.

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Highlights

- > Recent trends in vaccine development against pneumococcal surface protein antigens
- > Relates precedents to newly characterized peptidoglycan (PG) biosynthesis proteins
- > Recent evidence for the two-state model of PG synthesis in *S. pneumoniae*
- > Involvement of pneumococcal StkP serine/threonine kinase in PG biosynthesis
- > FtsEX cell division protein complex interacts with PcsB PG hydrolase in PG remodeling
- > Pneumococcal SecA/HtrA protein translocase localizes to regions of PG biosynthesis
- > New PG biosynthesis proteins as targets for antibiotic and passive-immunity therapies

FIG. 1.

Two-state model of PG biosynthesis in ovococcus bacteria, such as *S. pneumoniae*. To attain an ellipsoid shape, two machineries have been proposed that carry out peripheral and septal PG synthesis (see [23-25]). At the start of a division cycle, components of both machines locate to the equators of cells (top in figure). One machine (orange dots) carries out peripheral PG synthesis (light blue) between the future equator and septum of dividing cells. Peripheral PG synthesis corresponds to a form of lateral (sidewall) PG synthesis in rod-shaped bacteria. At some point, septal PG synthesis (medium blue; green dots) commences to divide the cell in two. For clarity, the peripheral and septal machines are shown at separate locations at the middle of dividing cells, although the spatial location and compositions of the two machines have not been established (see [25]). The red dot (Pac-Man) corresponds to PG hydrolases that remodel the PG and allow septal separation. The figure was modified based on references [23,25].

FIG. 2.

Model for the composition of the septal (left) and peripheral (right) PG synthesis machines in *S. pneumoniae*. Based on the compositions of PG machines in rod-shaped bacteria, it is postulated that the septal or peripheral PG synthesis complex in ovococcus species contains EzrA, FtsW and PBP2x (left), or MreC, MreD, PBP2b, and RodA (right), respectively (see [24,25,28]). GpsB and PBP1a are postulated to be present in both complexes (see [23,28]), whereas the location and functions of the remaining pneumococcal Class A PBPs (PBP2a and PBP2b) are unknown [23,25]. EzrA interactions with FtsZ (dashed lines) couple cell division to septal PG synthesis (see [28,32,33]), whereas interactions between the peripheral machine and the FtsZ divisome are unknown. This model is supported in ovococcus species by recent papers described in the text [23,25,34,35]. Possible roles of the low-molecular weight PBP DacA D,D carboxypeptidase or the DacB L,D carboxypeptidase in limiting PG pentapeptide substrate from other PBPs is not considered in this review (see [25,38,54]).

Fig. 3.

Models for PcsB and FtsEX_{Spn} interactions and functions in *S. pneumoniae*. In the direct interaction model (left), a PG hydrolytic activity of PcsB is activated by interactions that include binding of the coiled-coil domain of PcsB and the ECL1 (extracellular loop 1) of FtsX_{Spn}. Membrane-bound FtsX_{Spn} interacts with the cytoplasmic FtsE_{Spn} ATPase subunit. This model postulates that PG remodeling by a PcsB hydrolytic activity is coordinated with cell division through its interaction with the FtsEX_{Spn} complex, which interacts with FtsZ_{Spn} and other division proteins (see [45]). In the indirect model, PG remodeling is catalyzed by activation of an unknown PG hydrolase that interacts with PcsB bound to the FtsEX_{Spn} complex. In the indirect model, PcsB acts as a scaffolding protein between this hypothetical PG hydrolase and the FtsEX_{Spn} complex. The two models are not mutually exclusive.

Fig. 4.

Dynamic distribution of the SecA translocase subunits (top panel) and septal localization of the HtrA surface chaperon/protease (bottom panel) during cell division of *S. pneumoniae*. Immunolabeled FLAG-tagged SecA and HtrA are pseudo-colored green, while DAPI stained nucleoids are pseudo-colored red. During early division (top row of top panel), SecA is localized at mid-cell septa, and becomes hemispherically distributed at late division stages (bottom row of top panel). HtrA localizes to the equators and septa of dividing cells at all stages of division. Scale bar = 1 μ m. See text and [46] for additional details.