Postprint: Advances in the Regulatory Mechanisms of Bacterial Cell Wall Growth

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Abstract

During bacterial growth, the cell wall plays a critical role in maintaining cell shape and integrity, as well as resisting internal turgor pressure. The synthesis, division, regeneration, and recycling of the cell wall are closely associated with bacterial growth, reproduction, and adaptation to environmental stress. At present, the mechanisms of cell wall growth, how bacteria regulate this process, and how it coordinates with other cellular processes remain poorly understood. Deciphering cell wall regulatory mechanisms is essential for understanding bacterial cell wall function, elucidating drug modes of action, and developing novel therapeutic strategies. This review summarizes international research progress on the mechanisms of bacterial cell wall growth regulation, focusing on the roles of scaffold proteins, transcription factors, non-coding small RNAs, and protein-protein interactions in modulating cell wall synthesis, cell division, and stress responses. Furthermore, it discusses the application of cell wall regulatory mechanisms in antimicrobial drug development and provides perspectives on future research directions.

Full Text

Research Progress on the Regulatory Mechanisms of Bacterial Cell Wall Growth

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Abstract

During bacterial growth, the cell wall maintains cell shape and integrity while resisting internal turgor pressure. The synthesis, division, regeneration, and recycling of the cell wall are intimately linked to bacterial proliferation and environmental stress responses. Currently, the mechanisms underlying cell wall growth, its regulation, and its coordination with other cellular processes remain poorly understood. Elucidating these regulatory mechanisms is crucial for understanding bacterial cell wall function, determining drug action modes, and developing next-generation therapeutics. This review summarizes recent international research progress on bacterial cell wall growth regulation, focusing on mechanisms by which scaffold proteins, transcription factors, non-coding small RNAs, and protein-protein interactions control cell wall synthesis, cell division, and stress responses. We also summarize applications of these regulatory mechanisms in antimicrobial drug development and propose future research directions.

Keywords: cell wall; scaffolding proteins; transcriptional regulators; sRNA; protein-protein interaction

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Bacteria possess strong environmental adaptability, adjusting their nutrient uptake, metabolic pathways, and morphological structures to cope with various environmental stresses such as temperature, oxidative pressure, and antibiotics [1-3]. The cell wall not only shapes bacterial morphology but also serves as the primary protective barrier [4].

Both Gram-negative and Gram-positive bacterial cell walls consist primarily of a peptidoglycan meshwork—a robust scaffold structure formed by alternating N-acetylmuramic acid and N-acetylglucosamine linked via -1,4 glycosidic bonds [5]. The rates of peptidoglycan synthesis and degradation determine bacterial cell morphology [6], making the peptidoglycan scaffold the most critical component of the cell wall. The "maintenance"and "upkeep"of peptidoglycan structure represent metabolically expensive processes requiring multiple enzymes, scaffold proteins, or multi-enzyme complexes [7]. Additionally, transcription factors and non-coding small RNAs regulate cell wall metabolism through transcriptional, translational, and protein-protein interaction mechanisms [Figure 1: see original paper]. Studies have identified proteins involved in cell wall growth and regulation as key or potential targets for antibiotic drugs [8,9], making the elucidation of cell wall regulatory mechanisms crucial for understanding bacterial drug resistance and developing novel antimicrobial agents.

2. Peptidoglycan Synthesis

Peptidoglycan sacculus growth is a dynamic process of synthesis and hydrolysis, requiring synthetases to produce peptidoglycan and attach it to the existing sacculus, while hydrolases cleave the sacculus to insert newly synthesized material. Peptidoglycan synthesis occurs in three stages: (1) intracellu-

lar synthesis of nucleotide precursors (UDP-N-acetylglucosamine and UDP-N-acetylmuramyl pentapeptide); (2) formation of undecaprenyl-pyrophosphoryl-disaccharide-pentapeptide (Lipid II) anchored in the cell membrane, followed by flipping to the outer leaflet by flippases [10-12]; and (3) insertion of Lipid II into the peptidoglycan sacculus via penicillin-binding proteins (PBPs). This process requires glycosyltransferases (GTases) to polymerize glycan chains and transpeptidases (TPases) to crosslink peptide chains. TPases, also known as PBPs, are targets of penicillin and -lactam antibiotics. Peptidoglycan synthetases are classified into three categories [13,14]: Class A PBPs contain both GTase and TPase catalytic domains, connecting Lipid II precursors to the cell wall meshwork through sequential glycosyltransfer and transpeptidation reactions; Class B PBPs possess only TPase activity and play more dominant roles in cell division and stress response; and monofunctional glycosyltransferases (such as MraY and MurG) have only GTase structure and activity.

3. The Regulatory Role of Scaffold Proteins in Cell Wall Dynamics

Scaffold proteins, present in most bacteria, provide a stable structure that binds multiple proteins or enzymes, ensuring their stable function [15]. During cell growth and division, scaffold proteins regulate the spatial arrangement of peptidoglycan synthetases and hydrolases, maintaining peptidoglycan meshwork integrity. The most extensively studied scaffold proteins include: (1) MreB—associated with elongation in rod-shaped bacteria; (2) FtsZ—forms the Z-ring to regulate cell division; (3) GpsB—regulates cell division; (4) DivIVA—regulates cell division and sporulation; and (5) EzrA—cooperates with GpsB to regulate the cell wall.

3.1 MreB Protein Regulates Rod-Shaped Cell Wall Formation MreB is a key protein determining cell morphology and size, sensing and altering cell shape to promote rod formation and maintain morphological stability through a self-organizing feedback system [16]. In many rod-shaped bacteria such as *Escherichia coli* and *Bacillus subtilis*, MreB forms antiparallel double filaments that bind the inner membrane surface and move in an approximately circular pattern [17]. Some bacteria like *B. subtilis* possess multiple MreB homologs that partially redundantly regulate cell morphology [18], whereas *E. coli* has only one MreB protein, whose deletion causes loss of rod shape [19].

In *E. coli*, since the cell wall resides in the periplasmic space, intracellular MreB requires transmembrane linker proteins to regulate the cell wall. As shown in [Figure 2: see original paper]A, MreC, MreD, and RodZ are linker proteins whose cytoplasmic domains connect to MreB and periplasmic domains bind cell wall synthetases [20], thereby influencing MreB rotation and regulating cell morphology [21]. Increasing evidence indicates that local geometric features such as cell surface curvature affect MreB localization, thereby determining insertion sites for new cell wall material [22].

3.2 FtsZ Protein Influences Cell Division FtsZ, a tubulin homolog, regulates cell division by forming a ring-like structure called the Z-ring at midcell. Over ten cell division proteins localize to this scaffold, coordinating to form the divisome [Figure 2: see original paper]B. In *E. coli* during early division stages, FtsZ at the division site serves as a scaffold that binds division proteins FtsA and ZipA [23], forming a dynamic Z-ring at midcell. FtsA and ZipA also bind each other to form a complex that stabilizes the Z-ring, which can then recruit Lipid II synthetase MurG and other peptidoglycan synthetases to enhance cell wall synthesis in preparation for division [24].

3.3 GpsB and EzrA Influence Cell Division Two additional conserved proteins in Gram-positive bacteria, GpsB and EzrA, regulate cell wall synthesis through scaffolding functions. GpsB is a hexameric protein with N-terminal dimeric and C-terminal trimeric structures forming an asymmetric tripod-like arrangement [25,26]. This closed tripod configuration enables GpsB hexamers to simultaneously interact with multiple membrane-embedded PBP1 proteins, influencing PBP1 arrangement during division and thereby regulating cell wall synthesis.

EzrA interacts with PBPs, FtsA, FtsZ, and GpsB, participating in Z-ring assembly and cell wall synthesis regulation [27]. In *B. subtilis*, deletion of *ezrA* produces the same phenotype as *ponA* (encoding PBP1) deletion strains—cells become elongated with thinner cell walls [28]. FtsZ connects with EzrA dimers while binding FtsA to form a complex that further associates with GpsB in the cytoplasm to create a stable structure [25,27].

3.4 DivIVA Protein Regulates Cell Division and Sporulation The DivIVA scaffold protein shares high N-terminal homology with GpsB [29], and its N-terminal function is similar to GpsB. In both *B. subtilis* and *Listeria monocytogenes*, DivIVA can bind bifunctional peptidoglycan synthetases PBPs [27]. Additionally, in *B. subtilis*, its N-terminus interacts with MinJ while its C-terminal binding domain associates with the DNA-binding protein RacA during chromosome segregation [30]. In *Streptococcus pneumoniae*, DivIVA also binds cell division proteins FtsZ, FtsA, ZapA, FtsK, and peptidoglycan hydrolase PcsB, forming a complex that influences cell division and peptidoglycan hydrolysis [31]. Studies show that dumbbell-shaped DivIVA tetramers have membrane-binding sites at both ends that can bind to negatively curved membranes [32], and proteins associated with DivIVA promote peptidoglycan synthesis or hydrolysis, thereby affecting cell division and sporulation [33,34].

4. Transcriptional Regulators Control Cell Wall Synthesis Genes at the Transcriptional Level

During stress responses, bacteria utilize factors, two-component systems (TCS), and transcriptional regulators to control gene expression. TCS consist of histidine kinase/response regulator pairs (HK/RR) that phosphorylate cytoplasmic

response regulators via membrane-anchored histidine kinases, thereby regulating stress response genes. In *Vibrio cholerae*, environmental -lactams activate the WigKR TCS signaling pathway. RNA-seq analysis of *wigR* overexpression strains revealed that WigR regulates the complete cell wall synthesis pathway (including peptidoglycan precursor synthesis, precursor translocation, cell wall assembly, remodeling, and degradation), enhancing -lactam resistance [35].

In *Staphylococcus aureus* NCTC8325, the AirSR TCS positively regulates expression of over 20 genes involved in cell wall metabolism. Haipeng Sun et al. [36] found that *airSR* deletion caused autolysis and confirmed that AirR directly binds to cell wall metabolism genes (*cap*, *pbp1*, *ddl*, etc.) to regulate cell wall metabolism. *Bacillus subtilis* expresses over 30 TCS, among which WalRK (YycFG) plays a key role in regulating cell wall metabolism, with WalR directly controlling cell wall homeostasis, membrane integrity, and cell division [37]. Additionally, under phosphate limitation, the PhoPR and WalRK systems co-regulate cell wall metabolism: PhoPR represses wall teichoic acid genes *tagAB* while activating teichuronic acid genes *tuaA-H* [38], reducing anionic polymer composition in the cell wall from 100% wall teichoic acid to 30% [39].

Furthermore, factors and transcriptional regulators control cell wall synthesis. In *E. coli*, the S factor senses environmental conditions to influence BolA transcription factor expression, which binds the *mreB* transcription start site to inhibit *mreB* transcription, causing round cell morphology [40,41]. The highly conserved transcription factor MraZ, located in the division and cell wall gene cluster (*dcw* cluster), represses its promoter Pmra, affecting expression of 14 other division and peptidoglycan synthesis genes including *ftsL*, *ftsI*, *murC*, *murD*, *murE*, *murF*, *ddl*, and *mraY* [42].

5. sRNA Regulation of Cell Wall at the Post-Transcriptional Level

Non-coding sRNAs are 50-500 nt RNAs that regulate target gene expression in response to environmental changes, affecting protein activity, mRNA stability, and translation. In *Listeria*, the cell wall synthesis-related protein Lmo0514 contains an LPXTG motif recognized by sortases, enabling covalent attachment to the cell wall [43,44]. The sRNA Rli27 binds the 5' -UTR of *lmo0514* mRNA, stabilizing its secondary structure and increasing Lmo0514 expression to enhance cell wall tolerance [44]. Bastien Cayrol et al. [45] found that sRNA DsrA directly binds the 5' -UTR of *mreB* mRNA, affecting its transcription and structural stability, reducing intracellular MreB protein and altering cell morphology. The Hfq-dependent sRNA DicF inhibits FtsZ translation by complementary binding to the ribosome binding site of *ftsZ*, affecting Z-ring formation during division [46]. Additionally, in *E. coli*, UDP-N-acetylglucosamine is synthesized by glucosamine-6-phosphate synthase GlmS from L-glutamine and fructose-6-phosphate, with its expression directly regulated by the conserved sRNA GlmZ, which activates *glmS* expression through base pairing [47]. Currently, reports on sRNA regulation of cell wall remain limited and require further investigation.

6. Protein-Protein Interactions Influence Cell Wall Synthesis and Hydrolysis

Peptidoglycan synthesis requires participation of multiple proteins/enzymes that cannot function independently. Since Höltje et al. [48] proposed that peptidoglycan synthesis and degradation occur through multi-enzyme complexes, many studies have reported enzymes involved in peptidoglycan synthesis and their interactions. Protein-protein interactions are common, altering the structure and conformation of themselves or linker proteins to increase or decrease activity, thereby regulating cell wall growth.

The dynamic flux of peptidoglycan synthesis and degradation is the main determinant of bacterial cell morphology. Peptidoglycan synthesis-related proteins MreC, MreD, and bifunctional penicillin-binding proteins (PBPs) are collectively termed peptidoglycan synthetases [49]. PBPs are major components of the cell wall peptidoglycan synthesis system and antibiotic targets; penicillin inactivates them, disrupting peptidoglycan metabolic flux and causing bacterial lysis. *E. coli* has three bifunctional PBPs (PBP1A, PBP1B, and PBP1C). Outer membrane-anchored lipoproteins (Lop) can traverse peptidoglycan pores to regulate PBP activity [50]. LopA and LopB directly bind to the non-catalytic UB2H domain of PBP1A and PBP1B, respectively, with the resulting dimer inducing conformational changes that activate transpeptidase activity, increasing crosslinking of branched pentapeptides and cell wall mechanical strength [51-54]. Another membrane protein, FtsN, binds near the membrane-proximal region of PBP1B and cooperates with LopB to increase glycan chain synthesis rates. During cell division, the Tol-Pal system, which ensures proper outer membrane constriction, directly binds and regulates the PBP1B-LopB dimer [55]. However, some Gram-negative bacteria lack LopB homologs, such as *Pseudomonas aeruginosa*, where Neil G. Greene et al. identified an alternative protein, LpoP, with similar regulatory mechanisms [56]. The maximum molecular length of Lpo activators may serve as a molecular ruler limiting peptidoglycan thickness, while thick, multi-layered peptidoglycan can prevent outer membrane regulators from accessing synthetases. This pore-mediated activation mechanism represents a powerful autoregulatory system that adjusts peptidoglycan growth rate to match overall cell growth. When cell growth rate is slower than peptidoglycan synthesis rate, increased peptidoglycan density reduces pore size, weakening Lop activation of PBPs and decreasing synthesis rate to match cell growth, and vice versa [7].

FtsW, a transmembrane Lipid II flippase, interacts with peptidoglycan synthetases PBP3 and PBP1B and recruits PBP3 to the division site [57,58]. Additionally, FtsN is an essential division protein that interacts with FtsA, FtsBLQ, ZapA, PBP3, FtsW, PBP1B, and FtsQ [59-61]. It induces conformational changes in FtsA and FtsBLQ subcomplexes on both membrane sides to inhibit septum formation and membrane invagination [59], and promotes PBP1B activity by stabilizing its dimeric structure [62].

Beyond synthetases, hydrolase activity regulation is crucial for peptidoglycan growth, cell division, and morphological changes. Hydrolases include DD-carboxypeptidases, amidases, endopeptidases, and lytic transglycosylases [63]. Proteins containing LytM domains (EnvC, NlpD, YebA, and YgeR) regulate amidase activity, with NlpD activating amidases AmiA and AmiB, and EnvC activating AmiC [64,65]. In *P. aeruginosa*, Ivyp1 and Ivyp2 proteins inhibit lytic transglycosylase MltB activity [66].

7. Applications in Antimicrobial Drug Development

Since intact peptidoglycan is essential for bacterial survival, all cell wall synthesis and regulatory proteins are considered important targets for novel antimicrobial agents [67]. Currently widely used antibiotics that inhibit cell wall synthesis include: (1) fosfomycin, which inhibits cytoplasmic disaccharide-peptide precursor formation [68]; and (2) -lactams such as penicillin, which inhibit PBPs during cell wall assembly [69]. The emergence and increasing prevalence of multidrug-resistant bacteria such as methicillin-resistant *S. aureus* and vancomycin-resistant enterococci have spurred intense interest in developing drugs with novel mechanisms of action. Over the past five years, the most extensively studied drug target has been the cell division scaffold protein FtsZ [70-72].

FtsZ inhibition prevents divisome formation, leading to filamentation and eventual cell death. No drugs currently target this protein, but many laboratories have made significant progress in FtsZ inhibitor research, demonstrating that FtsZ inhibition causes bacterial cell death.

Current FtsZ inhibitors include four major categories: natural products, small molecules, peptides, and nucleic acids. Natural products include curcumin, coumarins, plumbagin, resveratrol, berberine, phenylpropanoids, and cinnamaldehyde. Small molecules primarily include benzamides, 2-nitro-vanillin-aniline Schiff bases, arene-diol digallates, rhodanine derivatives, pyrimidine-quinuclidine derivatives, taxanes, and benzimidazoles. Additionally, peptides such as cathelin-related antimicrobial peptide (CRAMP) and edeine, and nucleic acids such as peptide nucleic acid (PNA) and locked nucleic acid (LNA) have been developed [70-72]. These natural and synthetic inhibitors provide a foundation for novel antimicrobial drug development and clinical applications.

Bacterial cell wall research has attracted considerable attention, particularly for pathogenic bacteria, with substantial progress over the past decade. However, many cell wall growth and regulatory mechanisms remain unresolved. Advances in cryo-electron microscopy and emerging interdisciplinary fields have established a solid foundation for in-depth mechanistic studies. Peptidoglycan synthesis is closely related to overall cell growth, yet how cells change size while maintaining integrity and shape remains unclear. The complex interaction networks among scaffold proteins, peptidoglycan synthetases, hydrolases, and their

transcriptional regulators require further investigation. Advances in biochemical techniques such as high-throughput mutagenesis, genetic screening, in vitro activity analysis, and structural determination will greatly promote related research. Elucidating cell wall growth and regulatory mechanisms provides strong support for developing novel drugs and next-generation therapeutics.

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