Structural Insights into the Lipoprotein Outer Membrane Regulator of Penicillin-binding Protein 1B*

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In bacteria, the synthesis of the protective peptidoglycan sacculus is a dynamic process that is tightly regulated at multiple levels. Recently, the lipoprotein co-factor LpoB has been found essential for the in vivo function of the major peptidoglycan synthase PBP1b in Enterobacteriaceae. Here, we reveal the crystal structures of Salmonella enterica and Escherichia coli LpoB. The LpoB protein can be modeled as a ball and tether, consisting of a disordered N-terminal region followed by a compact globular C-terminal domain. Taken together, our structural data allow us to propose new insights into LpoB-mediated regulation of peptidoglycan synthesis.

Most bacteria enclose themselves within a peptidoglycan sacculus. In Gram-negative bacteria, the PG layer is an essential cell surface feature that sits between the inner and outer membranes and helps to protect the bacterium from osmotic rupture due to membrane turgor pressure and is the defining layer that governs cell shape and morphogenesis (1). Due to its essential role, periplasmic location, and lack of human orthologs, the majority of human antibacterials target PG synthesis (2). However, the rapid emergence of bacterial resistance mechanisms to these agents demands that we better understand additional regulatory factors required for PG synthesis to identify novel drug targets.

The final stages of PG synthesis involve attaching new lipid II precursor molecules onto the pre-existing sacculus. To accomplish this task, two enzymatic activities are required: (i) glycosyltransferases (GTases) covalently link the GlcNac-MurNac pentapeptide unit from lipid II onto the GlcNac-MurNac backbone of a nascent PG chain, and (ii) transpeptidases (TPases) cross-link newly incorporated donor pentapeptides to pre-existing acceptor peptides in the PG layer (3). Two main bifunctional molecular class A enzymes having both GTase and TPase activity (PBP1a and PBP1b) are anchored within the cytoplasmic membrane and are responsible for the incorporation of nascent PG monomers onto the growing sacculus in Escherichia coli. PBP1a and PBP1b appear to have partially redundant roles in that the presence of only one or the other is sufficient for cell viability (1). In E. coli, PBP1a is commonly associated with cell wall elongation whereas PBP1b is involved predominantly in septal PG synthesis under normal cell growth conditions (1). E. coli PBP1b consists of five domains: (i) an N-terminal trans-membrane (TM) α-helix (residues 64–87); (ii) a UvrB domain 2 homolog (UB2H) domain, which is proposed to interact with periplasmic binding partners (residues 109–200) (4); (iii) a membrane-associated GTase domain (residues 203–367); (iv) a linker region connecting the GTase and TPase domains (residues 391–443); and (v) a C-terminal TPase domain (residues 444–736).

PBP1a and PBP1b do not function in isolation, but rather are part of a multicomponent synthase complex that contains a myriad of other biosynthetic enzymes, catalytic enzymes, and regulatory factors (1). The rate and localization of PG deposition by these complexes must be finely tuned to recognize the metabolic state of the cell and facilitate morphological changes. We are just beginning to understand the molecular mechanisms governing regulation of PG synthase activity and the overall architecture of the synthetic machinery. For example, the bacterial MreB cytoskeletal elements have been found to direct the placement of PG synthases passively from inside the cell and thereby regulate circumferential PG deposition during the elongative phase of cell growth (5, 6).

Recently, two unrelated outer membrane lipoproteins (LpoA and LpoB, also known as YraM and YefM) have been found essential for the in vivo function of PBP1a and PBP1b in Entero-
Each Lpo protein interacts directly with its cognate partner in vitro and was found to stimulate its enzymatic activity (8, 7). Recently, it has been demonstrated that LpoA and LpoB stimulate the activity of their PBP-binding partner by affecting different domains. LpoA directly activates the PBP1a transpeptidase domain, whereas LpoB activates PBP1b-catalyzed glycosyltransfer. Interestingly, in both cases, activation of one domain leads to enhanced activity of the other enzymatic domain, suggesting that the GTase and TPase domains function in a cooperative manner (9). A PBP1bΔUB2H deletion mutant was unable to cross-link with LpoB, and overexpression of this construct did not rescue a PBP1a knock-out phenotype, suggesting that this domain is likely involved in interaction with LpoB (8). The LpoB-PBP1b complex localizes to the division site during septation and can presumably complement the role of the Tol-Pal complex, which creates a continuous connection between the inner and outer membrane and is implicated in outer membrane invagination during cell division (10). LpoB has also been shown to interact directly with isolated PG sacculi using sedimentation assays (8). A recent report has indicated that both PBP1b and LpoB are required for PG recovery following lysozyme-induced spheroplast formation in *E. coli* (11). These reports provide the first evidence that PG synthesis is in part regulated by the bacterial outer membrane in a species-specific manner.

To date, very little is known about the direct role of LpoB in peptidoglycan synthesis and the molecular details of its interaction with PBP1b, in large part due to a lack of structural information. Here, we disclose the 2.8, 2.0, and 2.2 Å resolution crystal structures of *E. coli* LpoB and *Salmonella enterica* LpoB in the Sel-Met derivative and native forms. The structures allow us to propose a revised model for LpoB-mediated regulation of PBP1b activity.

**EXPERIMENTAL PROCEDURES**

**LpoB: Cloning, Expression, and Purification**—The *S. enterica* and *E. coli* LpoB DNA sequences (NCBI reference sequence: NC_022525.1 and NC_002695.1) corresponding to amino acids (Tyr-77 to Gln-212, Val-21 to Gln-213, and Tyr-78 to Gln-213) were synthesized and cloned into the pUC57 vector by BioBasic. The genes were then subcloned into pET-41b-GST expression vectors with C-terminal thrombin-cleavable His8 tags using restriction free cloning as described previously (12). The expression vectors were then transformed into *E. coli* BL21 DE3 cells. Cells were grown in LB broth at 37 °C until an *A*$_{600}$ of 0.7 was reached, and the culture was cooled to room temperature. Sel-Met-derived *S. enterica* LpoB-containing cells were grown in minimal medium as described previously (13). Expression was then induced by addition of 1 mM isopropyl-β-D-1-thiogalactopyranoside. Following induction, the cultures were grown at 25 °C for 14 h, and cells (~20 g) were harvested and resuspended in lysis buffer (300 mM NaCl, 50 mM Tris-Cl, pH 8, one EDTA-free protease inhibitor tablet from Roche Applied Science). Cell lysis was induced by passing the sample through a French press at 12,000 p.s.i., and the lysate was centrifuged (45,000 rpm in a Beckman 70Ti rotor) for 30 min. The supernatant was then filtered using a 0.22-μm syringe filter. The filtered supernatant was passed through a 1-ml HiTrap HP His column that was pre-equilibrated in lysis buffer. The His$_8$-tagged LpoB proteins were eluted using elution buffer (50 mM Tris, pH 8, 300 mM NaCl, 1 mM imidazole) with a gradient of imidazole from 0 to 500 mM in 40 min. A total of 1 unit of
bovine α-thrombin was added per mg of pooled LpoB-enriched fractions and the sample was dialyzed overnight into size exclusion buffer (20 mM Tris, pH 7.0, 150 mM NaCl). The LpoB fractions were then further purified using a Superdex 75 26/60 (GE Healthcare), with size exclusion buffer as running buffer. The final protein yield was ~30 mg/liter of bacterial culture. The purified protein was concentrated to 60 mg/ml for crystallization trials.

**Circular Dichroism Spectroscopy**—Far-UV CD experiments were performed using a Jasco J810 CD spectrophotometer using quartz cuvettes with a 1-mm path length. Data were collected at 20 °C, with a wavelength interval of 1.0 nm. Wavelength scans were taken from 190 to 300 nm on *E. coli* LpoB (Val-21 to Gln-213) and *E. coli* LpoB (Tyr-78 to Gln-213) in CD buffer (10 mM phosphate buffer, pH 7.0, 150 mM NaF). The millidegree machine output units were converted to mean residue ellipticity (ε\[^{MR}\]) using the following equation: ε\[^{MR}\] = ΔA/(10 × Nr × l × C), where ΔA is the machine output in millidegrees, Nr is the number of protein residues, l is the path length in centimeters, and C is the molar protein concentration. Finally, ε\[^{MR}\] was plotted as a function of wavelength using Microsoft Excel.

**FIGURE 2.** LpoB has a disordered N-terminal region. A, prediction of *E. coli* LpoB disordered regions. Programs used were DisEMBL, PONDR-FIT, and DISOPRED (26–28). B, representative far-UV circular dichroism spectra for LpoB. The vertical axis shows CD intensity in units of mean residue ellipticity as a function of wavelength. Spectra corresponding to (i) *E. coli* LpoB (Tyr-78 to Gln-213), (ii) *E. coli* LpoB (Val-21 to Gln-213), and (iii) ii–i are shown in blue, red, and cyan. Error bars for i and ii correspond to the S.D. from duplicate measurements, whereas the error bars for iii represent uncertainty values based on propagation of error from subtracting i from ii.
Crystallization, Data Collection, and Structure Determination—Crystals were grown using the sitting drop vapor diffusion method at 25 °C with 1 μl of protein solution, mixed with an equal volume of precipitant. The *S. enterica* LpoB native and Sel-Met derivative protein crystallization precipitant was (0.1 M NaAc, pH 4.6, 2 M ammonium sulfate). Initial crystal hits were used as a seed stock to grow larger crystals in the same conditions and 199–212) were utilized. Waters were added manually in translation-liberation-screw (TLS) groups (residues 80–95, 96–119, 120–133, 134–151, 152–162, 163–185, 186–198, and 199–212) were utilized. Waters were added manually in Coot by examining both the F_o − F_c and 2F_o − F_c electron density maps, where F_o and F_c are observed and calculated structure factors, respectively. The final models are of excellent stereochemical quality and display no Ramachandran outlier residues as observed using the Dynarama statistics in Coot (20). Coordinates and structure factors were deposited in the Protein Data Bank with accession codes (4Q6V, 4Q6L, and 4Q6Z) for *S. enterica* Sel-Met LpoB, *S. enterica* LpoB, and *E. coli* LpoB, respectively. Figures representing the LpoB crystal structures were generated using the program PyMOL (21).

Size Exclusion Chromatography-Multilangle Light Scattering (SEC-MALS)—Purified *S. enterica* (Tyr-77 to Gln-212) LpoB and *E. coli* (Val-21 to Gln-213) LpoB were independently applied to a Superdex 75 HR 10/30 column (GE Healthcare) using an Agilent 1100 series HPLC that was coupled in-line to a Dawn® Helios™ II 18-angle MALS light scattering detector and Optilab® T-Rex™ differential refractometer detector (both from Wyatt Technology) as a protein detector. The light scattering detectors were normalized using monomeric bovine serum albumin (Sigma-Aldrich). A total of 100 μg of protein sample was injected on the column, previously equilibrated in running buffer (30 mM Tris, pH 7.0, 150 mM NaCl), at a 0.2 ml/min flow rate. Data were collected and analyzed using the Astra 6 software platform provided by the manufacturer, Wyatt Technology. The protein absolute molecular mass was calculated assuming a dn/dc value of 0.185 ml/g and a theoretical extinction coefficient of 1.0779 ml/(mg × cm). All SEC-MALS experiments and analysis were repeated in duplicate.

RESULTS AND DISCUSSION

Construct Design and Structure Solution—The *S. enterica* and *E. coli* LpoB proteins (NCBI accession numbers: Q8ZQ08.1 and AAC74189.1) have 90% sequence identity and consist of a single chain polypeptide, with mature sequences from Val-21 to Gln-212

### TABLE 1

| LpoB construct | Se-Met® *S. enterica* LpoB | Native *S. enterica* LpoB | Native *E. coli* LpoB |
|----------------|---------------------------|--------------------------|----------------------|
| **Data collection** |                           |                          |                      |
| Wavelength (Å)    | 0.9795                    | 1.000                    | 1.000                |
| Space group      | P2,1,2                    | P2,1,2                   | P2,3                 |
| Cell dimensions  |                           |                          |                      |
| a, b, c (Å)      | 29.35, 37.92, 98.59       | 28.98, 37.76, 98.40      | 96.93, 96.93, 96.93   |
| a, b, c (°)      | 90.00, 90.00              | 90.00, 90.00             | 90.00, 90.00         |
| Resolution (Å)^a | 49.30–1.97 (2.04–1.97)    | 49.20–2.20 (2.28–2.20)   | 68.54–2.8 (2.95–2.8)  |
| Completeness (%) | 99.7 (98.2)               | 99.5 (98.7)              | 99.6 (99.3)          |
| Unique reflections | 8,206 (774)              | 5,888 (813)             | 7,727 (1103)         |
| Redundancy^a     | 2.0 (1.9)                 | 12.1 (11.9)             | 5.4 (5.5)            |
| I/σ(I)^b         | 10.0 (3.6)                | 18.7 (9.5)              | 9.4 (2.4)            |
| R_mold (%)       | 6.8 (18.2)                | 2.7 (7.2)               | 5.60 (34.0)          |
| SAD phasing      |                           |                          |                      |
| No. of heavy atom sites | 5                       | NA^c                    | NA                   |
| Mean figure of merit^d | 0.4                    | NA                      | NA                   |
| **Refinement statistics** |             |                          |                      |
| R_work/R_free  | 22.0/29.0                 | 24.9/29.7                | 22.5/28.9            |
| No. of atoms    | 995                       | 1,014                    | 1,970                |
| Protein         | 995                       | 1,014                    | 1,970                |
| Water           | 27                        | 12                       | 24                   |
| r.m.s.d. bonds (Å) | 0.012                 | 0.010                    | 0.010                |
| r.m.s.d. angles (°) | 1.42                 | 1.46                     | 1.37                 |
| Average B-factors (Å^2) | 29.4            | 33.70                    | 63.0                 |
| Protein         | 29.4                      | 33.70                    | 63.0                 |
| Water           | 30.9                      | 45.90                    | 41.4                 |

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^a SeMet, selenomethionine.

^b Values in parentheses represent the highest resolution shell.

^c NA, non applicable.

^d Figure of merit from Phenix AutoSol after density modification.
and Met-21 to Gln-213, respectively. Previously, LpoB was found to localize to the bacterial outer membrane (7, 8). Furthermore, the LipoP server predicts a high confidence score (20.7) (22) for a lipidation signal peptide. A multiple sequence alignment of LpoB from multiple different species shows that the proposed cleavage site is universally conserved in LpoB variants throughout Enterobacteriaceae (Fig. 1, alignment generated using ClustalW and depicted using the Chimera software) (23, 24). LpoB has an amino acid other than aspartic acid at position +2 from the lipidation signal cleavage site, indicating localization to the outer rather than cytoplasmic membrane by the lipoprotein outer-membrane localization (Lol) pathway (25). Surprisingly, mutation of the LpoB sorting signal to D\textsuperscript{+2}E\textsuperscript{+3} or incorporation of the signal sequence of a soluble periplasmic protein (DsbA) in a LpoA\textsuperscript{−}PBP1a\textsuperscript{−} knock-out mutant resulted in viable cells, suggesting that LpoB does not require outer membrane localization or lipidation to stimulate PBP1b function \textit{in vivo} (7).

Immediately following the lipidation signal peptide, there is a predicted disordered region constituting the first 58 amino acids in the N-terminal portion of the mature (membrane-tethered) protein (disorder probability \textit{70\%} using DisEMBL, PONDR-FIT, and DISOPRED2; Fig. 2A) (26–28). This predicted disorder is in
Crystal Structure of LpoB

large part due to the presence of 16 proline residues distributed randomly throughout this stretch of amino acids, comprising roughly 30% of the sequence in E. coli. In addition, this region is not predicted to form a polyproline helix (DSP server, 29). To validate this potential disordered region experimentally we utilized far-UV circular dichroism spectroscopy with the E. coli LpoB construct (Val-21 to Gln-213) and the N-terminally truncated construct (Tyr-78 to Gln-212). Both constructs provided spectra indicative of a mixed α/β protein (with the typical negative ellipticity minima at 222 and 208 nm and maxima at 193 nm; Fig. 2B). To infer the secondary structure characteristics of the first 58 residues, a difference spectrum was computed by subtracting the ensemble spectrum from the original spectrum for both constructs. The difference spectrum displays a characteristically low ellipticity above 210 nm and a negative ellipticity minimum in the 195–200 nm range, features that are indicative of an intrinsically disordered peptide (Fig. 2B) (30, 31).

Presumably, LpoB must stretch from the outer membrane to contact the UB2H domain of PBP1b, a total of ~100 Å. Given that the entire protein sequence is 213 amino acids, and, from the crystal structure, the globular domain is very small (~40 Å in diameter at the widest point), we propose a ball and tether model in which the disordered proline-rich N-terminal region of LpoB facilitates the stretching required to contact the PBP1b UB2H domain. This type of mechanism is utilized in other systems including the CheR receptor involved in bacterial chemotaxis and the inner membrane division specific lipoprotein FtsN, which also binds and regulates PBP1b activity (32, 33). The ball and tether system has been studied in detail, and a stochastic model for chain elasticity has been developed using Brownian dynamics and experimentally validated using high force atomic force microscopy for the ubiquitin model system (under conditions where no secondary structure elements remain) (34, 35). These reports suggest that the globular domain (the “ball”) sterically prohibits conformations in close proximity to the membrane attachment point, thus necessarily promoting elongated tether (the “chain”) conformations. Therefore, we propose that the role of the LpoB tether may in part be to increase its frequency of interaction with PBP1b by imposing such diffusional restrictions.

For crystallographic purposes, the S. enterica and E. coli LpoB constructs (Tyr-77 to Gln-212 and Tyr-78 to Gln-213), lacking the N-terminal signal peptide and disordered region, were utilized. The crystal structure of Sel-Met S. enterica LpoB was solved by Se-SAD phasing in space group P212121 and refined to a resolution of 2.0 Å (Table 1). Subsequently, the crystal structures of native S. enterica and E. coli LpoB were solved by molecular replacement to 2.2 Å and 2.8 Å resolution in space groups P21, 2121, and P23, respectively (Table 1). The S. enterica and E. coli LpoB structures contain one and two protein monomers within the asymmetric unit. Because of missing density at the termini of various chains, the following amino acids could be modeled for S. enterica LpoB chain A (Tyr-77 to Gln-211) and for E. coli LpoB (chain A and B = Tyr-78 to Gln-212). The final Sel-Met S. enterica LpoB, native S. enterica LpoB, and E. coli LpoB models have R and R_free values of (22 and 29, 24.9 and 29.7, 22.5 and 28.9), respectively, and display excellent stereochemistry with no Ramachandran outliers as defined by Dynaroma (Table 1) (20). The Sel-Met LpoB structures display high overall structural similarity (r.m.s.d. for overlay of chain A for both structures on 128 common CA atoms: 1.7 Å). Furthermore, the native E. coli and S. enterica LpoB structures overlay remarkably well (r.m.s.d for all 136 common CA atoms: 0.30 Å), therefore we have used the higher resolution Sel-Met derivative data in our analysis of the S. enterica LpoB model.

Overall Structure of LpoB—The LpoB fold is characterized by an internal four-stranded mixed β-sheet that is flanked by N- and C-terminal helix pairs. The N and C termini project into roughly the same three-dimensional space and are separated by a mere 4 Å (measured from Ca-Ca) (Fig. 3, A and B). The N-terminal (α1/α2) helix pair packs against one face of the β-sheet, constituting the hydrophobic core of the protein. One face of the β-sheet is solvent-exposed; therefore, the core region of the sheet alternates highly conserved hydrophobic and hydrophilic residues (Figs. 1 and 3C). The N- and C-terminal helix pairs are oriented at roughly 90° relative to each other and in conjunction with the strand β2, as well as loops L1 and...
L4, define a single large cleft \( V = 2044 \, \text{Å}^3 \), and average depth = 12.1 Å, generated using PDBsum (36), which consists largely of neutral and aliphatic residues. A multiple sequence alignment of the LpoB protein from several available Enterobacteriaceae species reveals that many residues in and around this cleft are highly conserved; however, their exact functional role is yet to be elucidated (Fig. 3C). The electrostatic surface of LpoB was computed using the APBS software (37) and displays a single positively charged patch that runs roughly parallel to the solvent-exposed face of the ß-sheet (Fig. 3D).

**Oligomeric State**—The oligomeric state of (Tyr-77 to Gln-212) S. enterica LpoB and (Val-21 to Gln-213) E. coli LpoB in solution was determined using high performance size exclusion chromatography coupled in-line to a SEC-MALS. The differential refractive index and UV traces clearly show that LpoB exists solely as a monomer in solution (experimental molecular mass for (Tyr-77 to

![Structural comparison of LpoB with related proteins.](image)
Crystal Structure of LpoB

Gln-212) *S. enterica* LpoB and (Val-21 to Gln-213) *E. coli* LpoB: 14.47 ± 0.20 kDa and 19.27 ± 0.079 versus actual molecular mass: 14.35 and 20.34 kDa (Fig. 4, A and B). These findings are further corroborated by the fact that there are no predicted dimer interfaces within either the *S. enterica* or *E. coli* LpoB crystal lattices as defined by the program PISA (38). LpoB is thus a monomeric protein of dimensions 22 Å × 35 Å × 40 Å.

**Structural Comparisons**—A search for structural homologs of LpoB using the Dali server (39) identified several possible candidates. The top hits correspond to (i) the *Neisseria meningitidis* lipoprotein GNA1162, a protein of unknown function of dimensions 22 Å (r.m.s.d. on 112 common Ca atoms: 2.9 Å, PDB ID 4HRV, Fig. 5A) (40), (ii) the N-terminal domain of TolB, a soluble periplasmic protein that is part of the Tol-Pal system responsible for colicin import through OmfF and for outer membrane constriction during cell division (r.m.s.d. = 3.2 Å for 96 common Ca atoms, PDB ID 3IAX, Fig. 5B) (41), and (iii) the *Vibrio* species-specific flagellar ring stabilizing protein FlgT, which forms part of the flagellar H-ring and is thought to have a role in stabilizing the T-ring, which is essential for formation of the torque-generating stator in *Vibrio cholerae* (r.m.s.d. on 104 common Ca atoms, 4.2 Å, PDB ID 3W1E, Fig. 5C) (42). Taken together, the above-mentioned structural homologies suggest an evolutionary relationship among the flagellar motor system, Tol-Pal, and the LpoB-PBP1b system. The proteins identified are all involved in protein-protein interactions. Therefore, we suggest that the LpoB C-terminal domain is a protein–protein interaction domain. Interestingly, LpoB is the first of these proteins to be membrane-tethered by a flexible linker.

**Genetic Context**—*E. coli* LpoB exists within an operon harboring six genes; hinT, a histidine triad nucleotide-binding protein involved in d-alanine catabolism; Ycfl, a putative lipoprotein potentially involved in cell adhesion; thiK, a thiamine kinase; NagZ, a β-hexosaminidase involved in muropeptide recycling; and Ycfl, a predicted esterase (Fig. 5D). LpoB and Ycfl have been identified as immunogenic bacterial proteins that are induced during human infection by *S. enterica*, suggesting a potential role in pathogenicity (43). Both hinT and NagZ are involved in peptidoglycan recycling, a process that is intimately linked to cell wall growth and hence LpoB activity due to the necessary removal and recycling of old PG fragments during sacculus synthesis (44–46).

**CONCLUSION**

There are likely several cellular factors that affect LpoB-PBP1b complex formation and consequently peptidoglycan synthesis. Both binding partners are anchored to distinct biological membranes and thus have restricted diffusion, a factor that has been proposed in other systems to directly modulate complex formation (47). Additional interactions by PBP1b, both to self in the observed dimeric oligomerization state of the enzyme as well as to a number of proposed cellular partners, including the cell-division-specific lipoprotein FtsN, the septal transpeptidase PBP3, and the lytic transglycosylase MipA (48–50), are also important factors to consider in potential modulation of LpoB-PBP1b complex formation.

We propose that LpoB can be modeled as a ball and tether protein, which has a flexible N-terminal linker region that facilitates the stretching required to contact the PBP1b UB2H domain located near the bacterial inner membrane. Our observation of the LpoB monomeric nature and overall molecular dimensions supports a potential model in which LpoB accessibility/action on its target PBP1b is modulated in sparse areas of PG matrix by appropriately sized pores allowing LpoB to more freely traverse the periplasm and stimulate the activity of PBP1b at the inner membrane (8, 51). Whether the observation that LpoB binds peptidoglycan directly (8) may also imply an additional modulatory role in its accessibility to or action on PBP1b remains to be elucidated. Previously, it was suggested that the trans-envelope nature of the PBP1b-LpoB complex may serve to guide the synthase machinery along peripheral tracks oriented in the direction of pre-existing cables of peptidoglycan (filigree) that constitute the sacculus (7). Future experiments directed at studying PBP1b PG synthase patch motility in an LpoB knock-out would be informative in deciphering whether LpoB plays a defining role in directing patch motility. Taken together, this structural study serves as a starting point to better understand the LpoB-PBP1b interaction and its role in peptidoglycan growth and morphogenesis.

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**REFERENCES**

1. Typas, A., Banzhaf, M., Gross, C. A., and Vollmer, W. (2012) From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat. Rev. Microbiol.* 10, 123–136.

2. Page, M. P. (2012) in *Antibiotic Discovery and Development* (Dougherty, T. J., and Pucci, M. J., eds) pp. 79–117, Springer Science + Business Media, Basel.

3. Sobhani, S., King, D. T., and Strynadka, N. C. (2013) Fortifying the wall: synthesis, regulation and degradation of bacterial peptidoglycans. *Curr. Opin. Struct. Biol.* 23, 695–703.

4. Sung, M. T., Lai, Y. T., Huang, C. Y., Chou, L. Y., Shih, H. W., Cheng, W. C., Wong, C. H., and Ma, C. (2009) Crystal structure of the membrane-bound bifunctional transglycosylase PBP1b from *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8824–8829.

5. Domínguez-Escobar, J., Chastanet, A., Crevenna, A. H., Fromion, V., Wedlich-Söldner, R., and Carballido-López, R. (2011) Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science* 333, 225–228.

6. Garner, E. C., Bernard, R., Wang, W., Zhuang, X., Rudner, D. Z., and Mitchison, T. (2011) Coupled, circumferential motions of the cell Wall synthesis machinery and MreB filaments in *B. subtilis*. *Science* 333, 222–225.

7. Paradis-Bleau, C., Markowski, M., Uehara, T., Lupoli, T. J., Walker, S., Kähne, D. E., and Bernhardt, T. G. (2010) Lipoprotein cofactors located in the outer membrane activate bacterial cell wall polymerases. *Cell* 143, 1110–1120.

8. Typas, A., Banzhaf, M., Saparoea, B., Verheul, J., Biboy, J., Nichols, R. J., Zietek, M., Beilharz, K., Kannenberg, K., von Rechenberg, M., Breukink, E., den Blaauwen, T., Gross, C. A., and Vollmer, W. (2010) Regulation of peptidoglycan synthesis by outer-membrane proteins. *Cell* 143, 1097–1109.

9. Lupoli, T. J., Lebar, M. D., Markowski, M., Bernhardt, T., Kähne, D., and Walker, S. (2014) Lipoprotein activators stimulate *Escherichia coli* penicillin-binding proteins by different mechanisms. *J. Am. Chem. Soc.* 136, 52–55.
