Pazos M, Peters K, Vollmer W. [Robust peptidoglycan growth by dynamic and variable multi-protein complexes](#). *Current Opinion in Microbiology* 2017, 36, 55–61.

Copyright:

© 2017. This manuscript version is made available under the [CC-BY-NC-ND 4.0 license](#)

DOI link to article:

<http://dx.doi.org/10.1016/j.mib.2017.01.006>

Date deposited:

28/02/2017

Embargo release date:

15 February 2018

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](#)

1 **Robust peptidoglycan growth by dynamic and variable multi-protein complexes**

2

3 Manuel Pazos, Katharina Peters and Waldemar Vollmer*

4

5 Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, University of
6 Newcastle, Newcastle upon Tyne, United Kingdom, NE2 4HH

7

8 *Correspondence to: Waldemar Vollmer, Institute for Cell and Molecular Biosciences, University of
9 Newcastle, Newcastle upon Tyne, United Kingdom, NE2 4HH; Email: w.vollmer@ncl.ac.uk; Tel:
10 +44 (0)191 208 3216.

11

12 **Abstract**

13 In Gram-negative bacteria such as *Escherichia coli* the peptidoglycan sacculus resides in the
14 periplasm, a compartment that experiences changes in pH value, osmolality, ion strength and other
15 parameters depending on the cell's environment. Hence, the cell needs robust peptidoglycan growth
16 mechanisms to grow and divide under different conditions. Here we propose a model according to
17 which the cell achieves robust peptidoglycan growth by employing dynamic multi-protein complexes,
18 which assemble with variable composition from freely diffusing sets of peptidoglycan synthases,
19 hydrolases and their regulators, whereby the composition of the active complexes depends on the cell
20 cycle state - cell elongation or division - and the periplasmic growth conditions.

21

22 **Introduction**

23 Bacteria surround their cytoplasmic membrane with a net-like layer made of peptidoglycan (PG),
24 which consists of glycan chains connected by short peptides, to protect the cell from bursting while
25 maintaining cell shape [1]. Gram-negative bacteria such as *Escherichia coli* have a mainly single
26 layer of PG residing in the periplasm. The PG layer is elastic, expanding and shrinking in response
27 to changes in the cell's turgor. It is fascinating that this thin PG layer with its essential, stabilizing role
28 is constantly remodelled: an *E. coli* cell releases almost half of the material from the PG layer per
29 generation in a process called PG turnover [2]. According to a model proposed by Høltje more than
30 two decades ago [3], the high turnover is the consequence of a 3-for-1 PG growth mechanism
31 involving the coordinated activities of synthetic and hydrolytic enzymes. Indeed, *E. coli* requires not
32 only PG synthesis activities to expand its PG layer during growth and cell division, but also needs the
33 activities of PG hydrolases for length growth and cell separation [4,5].

34 PG synthases are either glycosyltransferases (GTases), which polymerize the glycan strands,
35 transpeptidases (TPases), which form peptide cross-links, or bi-functional enzymes which catalyze
36 both reactions [6]. *E. coli* has three non-essential bi-functional GTase-TPases (PBP1A, PBP1B and
37 PBP1C) but requires one of the major enzymes, PBP1A and PBP1B, for growth, while PBP1C is
38 dispensable. PBP1A and PBP1B require the interaction with a cognate outer-membrane lipoprotein,
39 LpoA and LpoB, respectively, for stimulation and functioning in the cell [7-10]. There is also a non-
40 essential GTase (MtgA) and two TPases with specific and essential roles in cell elongation (PBP2)
41 or division (PBP3). PBP2 and PBP3 are genetically and functionally linked to a member of the SEDS
42 (shape, elongation, division and sporulation) family of integral membrane proteins (RodA and FtsW,
43 respectively), for which different functions have been proposed. While biochemical evidence showed
44 that FtsW and other SEDS proteins are capable of flipping the PG precursor lipid II and phospholipids
45 across the membrane of proteoliposomes [11,12], whole cell assays and genetic data were used to
46 conclude that MurJ, and not RodA/FtsW, is a lipid II flippase [13]. The new crystal structure of MurJ
47 supports a transport function but does not identify the ligand [14]. Based on genetic data and assays

with a partially purified *B. subtilis* RodA fraction it was also suggested that SEDS proteins function as GTases and not as lipid II flippases [15]. However, *E. coli* FtsW did not show GTase activity but formed a trimeric complex with PBP3 and PBP1B and controlled lipid II polymerization of PBP1B [16]. Due to these conflicting conclusions made from biochemical assays and *in vivo* genetic data further experiments are needed to clarify the roles of MurJ and SEDS proteins.

E. coli has more than 20 PG hydrolases [lytic transglycosylases (LTs); *N*-acetylmuramyl-L-Ala amidases (amidases); endopeptidases (EPases); carboxypeptidases (CPases)] in its periplasm cleaving covalent bonds in the PG layer to release PG fragments (see Table 1 for enzyme names). It is not known how most of these hydrolases are regulated, but the uncontrolled activities of the autolysins (LTs, amidases and EPases) cause cell lysis under certain conditions, for example when PG synthesis is blocked by a β -lactam. Elegant work from the Bernhardt laboratory showed that EnvC and NlpD, which were originally thought to have PG hydrolase activity [17,18], are not enzymes but are needed to stimulate the activities of the PG amidases AmiA, AmiB and AmiC for septum PG cleavage and daughter cell separation [19].

In this opinion article, we present our current view on the mechanisms of PG synthesis in *E. coli*, with the particular aim to emphasize the dynamics of the process with respect to the assembly and disassembly of PG synthesis complexes that move along the PG layer to incorporate new PG and cleave the old material. We present a model according to which the conditions in the periplasm (such as pH value, osmolality, etc.), which are affected by the growth medium, dictate the composition of dynamically assembled PG synthesis complexes, ensuring that PG synthases and hydrolases are optimally active and regulated at any growth condition. This model explains why *E. coli* maintains large sets of seemingly redundant PG enzymes.

Assembly and disassembly of dynamic PG synthesis complexes

Theoretically, PG growth could be carried out from lipid II precursor by a minimal system of only three or four active domains (Figure 1) [20]. A GTase produces a glycan chain, which is attached to

74 the existing PG layer by one or two TPases, while an endopeptidase (EPase) cleaves in the existing
75 layer to allow the insertion of the new strand for surface expansion of the PG layer. A more
76 complicated model, which explains PG turnover, assumes the incorporation of three glycan chains
77 and the concomitant removal of one old strand (Höltje's 3-for-1 model) [3]. While the precise
78 mechanism of PG growth remains unknown, these models are consistent with the observed large
79 number of interactions detected for PG enzymes (reviewed in [6,21]), suggesting the existence of PG
80 synthesizing multi-enzyme complexes. However, it became clear that several structural and
81 regulatory proteins are involved to control the assembly and disassembly of these complexes and/or
82 to position them in the cell envelope and control their activity [7].

83 The dynamic bacterial cytoskeleton regulates PG synthesis from inside the cell, presumably
84 by facilitating the assembly, positioning and/or activation of the PG synthesis complexes [22-24].
85 During cell elongation the bacterial actin-like MreB and the associated proteins MreC, MreD and
86 RodZ, which are essential to maintain the rod-shape of the cell, regulate PG synthesis complexes with
87 PBP2 and PBP1A. MreB directly interacts with the main cell division protein, the tubulin-like FtsZ,
88 and this interaction is important to initiate division [25]. During cell division, FtsZ and more than 9
89 associated essential cell division proteins, facilitate the formation of the ring-like division septum at
90 mid-cell [26], including the synthesis of the septal PG catalyzed mainly by PBP3 and PBP1B [27,28].
91 Interestingly, recent work shows that in *Bacillus subtilis* and *E. coli* FtsZ moves in patches around
92 the closing division ring by a GTP-dependent treadmilling mechanisms [22,29]. FtsZ movement
93 drives a similar movement of the PG synthase PBP2B, the class B PBP essential for cell division in
94 *B. subtilis* [22]. Hence, during cell division, the septal PG grows inwards by circular attachment of
95 new material until the septum is closed.

96 During cell elongation MreB forms dynamic filamentous structures or foci that rotate around
97 the short axis of the cell in the direction of the glycan chains [24]. The movement of these MreB foci
98 depends on ongoing PG synthesis, which is in sharp contrast to the movement of FtsZ patches during
99 cell division, which does not require PG synthesis [22]. Remarkably, MreB does not rotate in certain

100 rod-shaped mutants of *E. coli* carrying an altered MreB and lacking RodZ, which is an cell elongation
101 protein that couples MreB rotation with cell wall synthesis [23]. The rotation of MreB is not required
102 to maintain rod-shape under standard laboratory condition, but is important for the robustness of
103 shape-maintenance under conditions of cell wall stress [23]. Although the specific inactivation of
104 PBP2 halts the rotation of the MreB foci [24], PBP2 molecules were reported to move much faster
105 and erratic in a diffusive manner arguing against long-lived complexes containing MreB and PBP2
106 [30]. However, a more recent study identified sub-populations of PBP2 and the SEDS protein RodA
107 that rotate with similar, slow velocity as MreB foci around the short axis of the cell, suggesting that
108 these slower molecules of PBP2 and RodA move together in a complex with MreB (although co-
109 localization has not been demonstrated) [31]. The rotation of MreB was independent of the activity
110 of class A PBPs and, under these conditions, only fast moving, diffusive PBP1B molecules could be
111 observed [31]. This diffusive, unidirectional motion of PBP1A and PBP1B was confirmed in another
112 study [32] which also identified sub-populations of fast and slow molecules of PBP1A and showed
113 that the motion of PBP1A is modulated by an inhibitor (cefsulodin) and an activator (LpoA) [32].
114 However, it remains to be seen which of the sub-populations of PBP molecules are engaged in PG
115 synthesis. PBP1A has been functionally linked to cell elongation by its cooperative interaction with
116 PBP2 and side-wall localization [33]; hence, one would predict a yet unobserved sub-population of
117 PBP1A that shows a similar dynamical behaviour as PBP2 during rounds of PG synthesis.
118 Alternatively, class A and B PBPs act mostly independent of each other, interacting only sporadically
119 with each other [31]. Overall, the available single molecule tracking data suggest that PG synthesis
120 complexes are not static entities. Rather, functional complexes assemble from their components when
121 PG synthesis is required, regulated by cytoskeletal elements, and they become fully activated by outer
122 membrane anchored lipoproteins. After some time of active PG synthesis the complexes disassemble
123 leaving the individual components to freely diffuse in the cell membrane or periplasm until the next
124 round of PG synthesis is initiated. Rounds of dynamic assembly, synthesis activity and disassembly

125 are also known from other complexes synthesizing macromolecules, such as the ribosome or the RNA
126 polymerase, which have well characterized initiation, elongation and termination steps.

127

128 **Redundancy in PG synthesis and hydrolysis**

129 *E. coli* has more than 50 enzymes for PG synthesis and hydrolysis, and the enzymatic reactions take
130 place in the cytoplasm, at the cytoplasmic membrane and in the periplasm [6]. Ten out of the twelve
131 cytoplasmic and membrane-bound reactions leading to lipid II precursor are catalysed by single,
132 essential enzymes (Mur enzymes and MraY), and only the D-alanine racemase and D-Ala-D-Ala
133 ligase steps are catalyzed by two redundant enzymes. In sharp contrast, there is significantly more
134 redundancy associated with the enzymatic steps that take place at the periplasmic side of the
135 cytoplasmic membrane and in the periplasm itself. In fact, with the notable exception of the class B
136 PBP - flippase (and/or GTase) pairs PBP2 - RodA and PBP3 - FtsW, which have specific roles in cell
137 elongation and division, respectively, all other reactions are catalysed by (semi)-redundant enzymes
138 (Table 1). This includes essential functions such as the GTase/TPase activities of the class A PBPs
139 and their regulators, PBP1A - LpoA and PBP1B - LpoB, respectively. A double mutant lacking both
140 class A PBPs (or both regulators) is not viable, but each of the single mutants is [34]. The single
141 mutants have mild phenotypes under standard laboratory conditions, but severe ones under more
142 stringent conditions (for example, [33,35]) suggesting that PBP1B - LpoB and PBP1A - LpoA are
143 not completely interchangeable. This semi-redundancy is consistent with the greater enrichment of
144 PBP1B at the cell division site and its interactions with the cell division proteins PBP3 and FtsN
145 [27,36], and with members of the Tol-system, CpoB and TolA [37], suggesting a role in cell division.
146 By contrast, PBP1A is more specialised in cell elongation based on its side-wall localization and
147 interaction with PBP2 [33]. It is feasible to speculate that the semi-redundant PBP1A and PBP1B
148 share some common interaction partners in the elongation and division complexes and with the Tol-
149 system. *E. coli* has two further, non-essential PG synthases, PBP1C and MtgA, whose functions are

150 not well understood, although MgtA localizes to the division site and interacts with PBP3, FtsW and
151 the cell division protein FtsN [38].

152 There are two other periplasmic PG synthesis reactions that both involve redundant, non-
153 essential LD-TPase enzymes. YnhG and YcbB synthesize the rare LD-cross-links [39], and ErfK,
154 YbiS and YcfS attach Braun's lipoprotein Lpp to the PG contributing to the tight linkage between the
155 sacculus and the outer membrane to stabilize the cell envelope [40].

156 The enzyme redundancy is greatest in the case of the PG hydrolases that act on the PG
157 sacculus. None of the 8 LTs, 6 EPases, 4 amidases or 7 CPases is essential, and many multiple mutant
158 strains have no or mild phenotypes, making it difficult to define a clear role for each of these enzymes.
159 Nonetheless, some of the hydrolases appear to have specialized functions. Three amidases (AmiA,
160 AmiB and AmiC) and their regulators (EnvC and NlpD) have redundant and significant roles in
161 septum cleavage during cell division, and a mutant lacking all three enzymes grows in chains of
162 unseparated cells [4]. Additional deletions of LTs and EPase encoding genes exacerbate the chaining
163 phenotype, indicating that other hydrolases can participate in septum cleavage to partially compensate
164 for the loss of amidases [41]. During cell elongation three EPases (MepS, MepM and MepH) have a
165 redundant and essential role in cleaving cross-links in the sacculus for PG growth, such that
166 inactivation of all three enzymes inhibits PG growth during cell elongation leading to cell lysis [5,42].
167 DD-CPase activity is not essential, but the collective pentapeptide-trimming activity of the 7 CPases
168 is required to maintain normal rod-shape, presumably by regulating the amount and/or position of
169 available pentapeptides for PBP-mediated cross-linking activity [43,44]. While single deletion of
170 DD-CPase genes has no or only minor consequences, certain multiple mutants produce misshapen
171 cells with enlarged diameter and even branches [43,44]. Besides their PG synthase activity, PBP1A
172 and PBP1B also exhibit CPase activity at lower pH values and in the presence of their cognate Lpo
173 activators [7]. Hence, the DD-CPases represent another example of a set of seemingly redundant PG
174 enzymes.

175

176 **Multiple periplasmic enzymes for robust PG growth**

177 Why does *E. coli* maintain multiple, seemingly redundant PG enzymes for each reaction in the
178 periplasm, while most cytoplasmic steps are catalysed by a single, essential enzyme? We hypothesize
179 that the reason for the higher redundancy is that the properties and composition of the periplasm vary
180 with changes in the cell's environment, while the cytoplasm is a less-changing, well-buffered
181 compartment. Hence, the cytoplasmic enzymes are optimized to function under stable condition
182 without the need of having multiple enzymes for the same biosynthetic step. However, the periplasm
183 is less buffered and significantly affected by the pH value, ion strength and osmolality of the growth
184 medium, because the outer-membrane allows the entry of molecules with less than ~600 Da [45]. *E.*
185 *coli* maintains robust growth at a range of conditions, for example at acidic pH or in the presence of
186 high concentration of NaCl, which can affect periplasmic processes such as the essential PG
187 synthesis. Hence, we suggest that the redundancy of periplasmic PG enzymes has evolved to allow
188 *E. coli* to grow at a wide range of growth conditions. The various PG enzymes have different
189 properties and optimal conditions for activity, but the combined activities of the enzymes of each set
190 (PBPs, TPases, LTs, EPases, amidases, CPases) may cover the whole range of conditions at which *E.*
191 *coli* grows (Figure 2). In support of this view, we have recently found that one of the so-called 'minor'
192 CPases, PBP6b, which is poorly expressed and has no known role at standard growth conditions,
193 becomes important in *E. coli* cells growing at pH 5 [46]. Under this condition, the PBP6b gene is
194 more expressed, the produced protein is more stable and more active, and cells require PBP6b to
195 maintain normal cell shape [46]. By contrast, when cells grow at near neutral pH the major DD-
196 CPase, PBP5 is more important. Another hydrolase with an activity optimum at non-standard
197 conditions is the LT MltA, which is ~10 times more active at 30°C than at 37°C [47]. Although more
198 work is needed to characterize the different PG enzymes at various conditions, these examples support
199 the notion that *E. coli* achieves robust PG synthesis using sets of (seemingly redundant) enzymes that
200 differ in their activity ranges.

202 **Model for condition-dependent complex composition**

203 Taking into account the interactions of PG enzymes and their regulators, their cellular localization
204 and dynamics, and the multiplicity of PG enzymes in the periplasm, we propose a model according
205 to which PG synthesis is facilitated by dynamic multi-enzyme complexes, which assemble for rounds
206 of PG synthesis for a certain period of time and then disassemble. Importantly, the composition of
207 these complexes, which contain synthases, hydrolases and their regulators, depends not only on the
208 state in the cell cycle (cell elongation or division) but on the conditions in the periplasm, which are
209 significantly affected by the cell's environment (Figure 2). Given the high level of redundancies
210 observed, it is possible that different complexes are active even at a given growth condition. This
211 model assumes that always optimally active complexes assemble in response to the growth
212 conditions, providing a mechanism that ensures robust PG synthesis for cell growth in a changing
213 environment.

214

215 **Concluding remarks**

216 So far PG synthesis has been studied in the cell and with purified enzymes mainly at standard growth
217 or reaction conditions, and the seeming redundancy in the periplasmic enzymes and their regulators
218 has not been well understood. We propose here a model of dynamic and variable multi-protein
219 complexes for PG growth that will hopefully prompt PG studies at other than standard conditions.
220 There are many questions to answer, for example: How do protein-protein interactions change with
221 periplasmic conditions? Do complexes with different composition employ the same molecular
222 mechanisms of PG growth? How is the regulation of PG synthesis and hydrolysis affected by the
223 properties of the periplasm? How could robustness in cell envelope biogenesis evolve in *E. coli* and
224 other species that are capable of growing in a range of environments? Trying to answer these and
225 other questions will surely keep us busy in the years to come.

226

227 **Acknowledgements**

228 This work was supported by the Wellcome Trust (101824/Z/13/Z), the MRC (MR/N002679/1) and
229 European Commission (FP7-PEOPLE-2013-IEF-623868). We thank Alexander Egan for critical
230 reading of the manuscript.

231

232 **References**

- 233 1. Vollmer W, Blanot D, de Pedro MA: **Peptidoglycan structure and architecture.** *FEMS Microbiol*
234 *Rev* 2008, **32**:149-167.
- 235 2. Goodell EW, Schwarz U: **Release of cell-wall peptides into culture-medium by exponentially**
236 **growing *Escherichia coli*.** *J Bacteriol* 1985, **162**:391-397.
- 237 3. Höltje JV: **Growth of the stress-bearing and shape-maintaining murein sacculus of**
238 ***Escherichia coli*.** *Microbiol Mol Biol Rev* 1998, **62**:181-203.
- 239 4. Heidrich C, Templin MF, Ursinus A, Merdanovic M, Berger J, Schwarz H, de Pedro MA, Höltje
240 JV: **Involvement of *N*-acetylmuramyl-L-alanine amidases in cell separation and**
241 **antibiotic-induced autolysis of *Escherichia coli*.** *Mol Microbiol* 2001, **41**:167-178.
- 242 5. Singh SK, SaiSree L, Amrutha RN, Reddy M: **Three redundant murein endopeptidases catalyse**
243 **an essential cleavage step in peptidoglycan synthesis of *Escherichia coli* K12.** *Mol*
244 *Microbiol* 2012, **86**:1036-1051.
- 245 6. Typas A, Banzhaf M, Gross CA, Vollmer W: **From the regulation of peptidoglycan synthesis to**
246 **bacterial growth and morphology.** *Nat Rev Microbiol* 2012, **10**:123-136.
- 247 * 7. Egan AJ, Biboy J, van't Veer I, Breukink E, Vollmer W: **Activities and regulation of**
248 **peptidoglycan synthases.** *Philos Trans R Soc Lond B Biol Sci* 2015, **370**.
- 249 [Comprehensive summary about the current knowledge on the interactions and regulation of](#)
250 [PG synthases from *E. coli*. Shows for the first time the DD-CPase activity of stimulated class](#)
251 [A PBPs and their activities in proteoliposomes.](#)

- 252 8. Markovski M, Bohrhunter JL, Lupoli TJ, Uehara T, Walker S, Kahne DE, Bernhardt TG: **Cofactor**
253 **bypass variants reveal a conformational control mechanism governing cell wall**
254 **polymerase activity**. *Proc Natl Acad Sci U S A* 2016, **113**:4788-4793.
- 255 9. Paradis-Bleau C, Markovski M, Uehara T, Lupoli TJ, Walker S, Kahne DE, Bernhardt TG:
256 **Lipoprotein cofactors located in the outer membrane activate bacterial cell wall**
257 **polymerases**. *Cell* 2010, **143**:1110-1120.
- 258 10. Typas A, Banzhaf M, van den Berg van Saparoea B, Verheul J, Biboy J, Nichols RJ, Zietek M,
259 Beilharz K, Kannenberg K, von Rechenberg M, *et al.*: **Regulation of peptidoglycan**
260 **synthesis by outer-membrane proteins**. *Cell* 2010, **143**:1097-1109.
- 261 * 11. Mohammadi T, Sijbrandi R, Lutters M, Verheul J, Martin NI, den Blaauwen T, de Kruijff B,
262 Breukink E: **Specificity of the transport of lipid II by FtsW in *Escherichia coli***. *J Biol*
263 *Chem* 2014, **289**:14707-14718.
- 264 Provides biochemical evidence for the lipid II flippase activity of FtsW from *E. coli*
265 reconstituted in proteoliposomes, whereas Reference 13 concludes from genetic data that
266 MurJ is the lipid II flippase. Reference No. 15 provides genetic evidence and biochemical data
267 with a partially purified RodA from *B. subtilis* and these authors suggest that SEDS proteins
268 have PG glycosyltransferase activity.
- 269 12. Mohammadi T, van Dam V, Sijbrandi R, Vernet T, Zapun A, Bouhss A, Diepeveen-de Bruin M,
270 Nguyen-Disteche M, de Kruijff B, Breukink E: **Identification of FtsW as a transporter of**
271 **lipid-linked cell wall precursors across the membrane**. *EMBO J* 2011, **30**:1425-1432.
- 272 * 13. Sham LT, Butler EK, Lebar MD, Kahne D, Bernhardt TG, Ruiz N: **Bacterial cell wall. MurJ**
273 **is the flippase of lipid-linked precursors for peptidoglycan biogenesis**. *Science* 2014,
274 **345**:220-222.
- 275 See comment at Reference No. 11.
- 276 14. Kuk AC, Mashalidis EH, Lee SY: **Crystal structure of the MOP flippase MurJ in an inward-**
277 **facing conformation**. *Nat Struct Mol Biol* 2016, doi: 10.1038/nsmb.3346.

- 278 * 15. Meeske AJ, Riley EP, Robins WP, Uehara T, Mekalanos JJ, Kahne D, Walker S, Kruse AC,
279 Bernhardt TG, Rudner DZ: **SEDS proteins are a widespread family of bacterial cell wall**
280 **polymerases**. *Nature* 2016, **537**:634-638.
- 281 [See comment at Reference No. 11.](#)
- 282 16. Leclercq S, Derouaux A, Olatunji S, Fraipont F, Egan AJF, Vollmer W, Breukink E, Terrak M:
283 **Interplay between Penicillin-binding proteins and SEDS proteins promotes bacterial cell**
284 **wall synthesis**. *Scientific Report, accepted*.
- 285 17. Bernhardt TG, de Boer PA: **Screening for synthetic lethal mutants in *Escherichia coli* and**
286 **identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase**
287 **activity**. *Mol Microbiol* 2004, **52**:1255-1269.
- 288 18. Uehara T, Dinh T, Bernhardt TG: **LytM-Domain Factors Are Required for Daughter Cell**
289 **Separation and Rapid Ampicillin-Induced Lysis in *Escherichia coli***. *J Bacteriol* 2009,
290 **191**:5094-5107.
- 291 19. Uehara T, Parzych KR, Dinh T, Bernhardt TG: **Daughter cell separation is controlled by**
292 **cytokinetic ring-activated cell wall hydrolysis**. *EMBO J* 2010, **29**:1412-1422.
- 293 20. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P: **The penicillin-binding proteins: structure**
294 **and role in peptidoglycan biosynthesis**. *FEMS Microbiol Rev* 2008, **32**:234-258.
- 295 21. Egan AJ, Vollmer W: **The physiology of bacterial cell division**. *Ann N Y Acad Sci* 2013, **1277**:8-
296 28.
- 297 ** 22. Bisson-Filho AW, Hsu YP, Squyres G, Kuru E, Wu F, Jukes C, Dekker C, Holden S,
298 VanNieuwenhze M, Brun Y, *et al.*: **Treadmilling by FtsZ filaments drives peptidoglycan**
299 **synthesis and bacterial cell division**. *bioRxiv* 2016. doi: <http://dx.doi.org/10.1101/077560>.
- 300 [Shows together with Reference 29 the treadmilling of FtsZ at the division site. Provides also](#)
301 [evidence that the rotation of FtsZ is required for the movement of an essential PG synthase](#)
302 [and that septal PG synthesis processive.](#)

- 303 * 23. Morgenstein RM, Bratton BP, Nguyen JP, Ouzounov N, Shaevitz JW, Gitai Z: **RodZ links**
304 **MreB to cell wall synthesis to mediate MreB rotation and robust morphogenesis.** *Proc*
305 *Natl Acad Sci U S A* 2015, **112**:12510-12515.
306 Shows together with Reference 30 that MreB (dynamics) is required for robust maintainance
307 of rod-shape.
- 308 24. van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, Gitai Z: **The**
309 **bacterial actin MreB rotates, and rotation depends on cell-wall assembly.** *Proc Natl Acad*
310 *Sci U S A* 2011, **108**:15822-15827.
- 311 25. Fenton AK, Gerdes K: **Direct interaction of FtsZ and MreB is required for septum synthesis**
312 **and cell division in *Escherichia coli*.** *EMBO J* 2013, **32**:1953-1965.
- 313 26. Natale P, Pazos M, Vicente M: **The *Escherichia coli* divisome: born to divide.** *Environ*
314 *Microbiol* 2013, **15**:3169-3182.
- 315 27. Bertsche U, Kast T, Wolf B, Fraipont C, Aarsman ME, Kannenberg K, von Rechenberg M,
316 Nguyen-Disteche M, den Blaauwen T, Höltje JV, *et al.*: **Interaction between two murein**
317 **(peptidoglycan) synthases, PBP3 and PBP1B, in *Escherichia coli*.** *Mol Microbiol* 2006,
318 **61**:675-690.
- 319 28. de Pedro MA, Quintela JC, Höltje JV, Schwarz H: **Murein segregation in *Escherichia coli*.** *J*
320 *Bacteriol* 1997, **179**:2823-2834.
- 321 ** 29. Yang X, Lyu Z, Miguel A, McQuillen R, Huang KC, Xiao J: **GTPase activity-coupled**
322 **treadmilling of the bacterial tubulin FtsZ organizes septal cell-wall synthesis.** *bioRxiv*
323 2016. doi: <http://dx.doi.org/10.1101/077610>.
324 See comment at Reference No. 22.
- 325 * 30. Lee TK, Tropini C, Hsin J, Desmarais SM, Ursell TS, Gong EH, Gitai Z, Monds RD, Huang
326 KC: **A dynamically assembled cell wall synthesis machinery buffers cell growth.** *Proc*
327 *Natl Acad Sci U S A* 2014, **111**:4554-4559.
328 See comment at Reference No. 23.

- 329 31. Cho H, Wivagg CN, Kapoor M, Barry Z, Rohs PD, Suh H, Marto JA, Garner EC, Bernhardt TG:
330 **Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families**
331 **functioning semi-autonomously.** *Nat Microbiol* 2016, **1**:16172.
- 332 32. Lee TK, Meng K, Shi H, Huang KC: **Single-molecule imaging reveals modulation of cell wall**
333 **synthesis dynamics in live bacterial cells.** *Nat Commun* 2016, **7**:13170.
- 334 33. Banzhaf M, van den Berg van Saparoea B, Terrak M, Fraipont C, Egan A, Philippe J, Zapun A,
335 Breukink E, Nguyen-Disteche M, den Blaauwen T, *et al.*: **Cooperativity of peptidoglycan**
336 **synthases active in bacterial cell elongation.** *Mol Microbiol* 2012, **85**:179-194.
- 337 34. Yousif SY, Broome-Smith JK, Spratt BG: **Lysis of *Escherichia coli* by beta-lactam antibiotics:**
338 **deletion analysis of the role of penicillin-binding proteins 1A and 1B.** *J Gen Microbiol*
339 1985, **131**:2839-2845.
- 340 35. Garcia del Portillo F, de Pedro MA: **Differential effect of mutational impairment of penicillin-**
341 **binding proteins 1A and 1B on *Escherichia coli* strains harboring thermosensitive**
342 **mutations in the cell division genes *ftsA*, *ftsQ*, *ftsZ*, and *pbpB*.** *J Bacteriol* 1990, **172**:5863-
343 5870.
- 344 36. Müller P, Ewers C, Bertsche U, Anstett M, Kallis T, Breukink E, Fraipont C, Terrak M, Nguyen-
345 Distèche M, Vollmer W: **The essential cell division protein FtsN interacts with the murein**
346 **(peptidoglycan) synthase PBP1B in *Escherichia coli*.** *J Biol Chem* 2007, **282**:36394-36402.
- 347 * 37. Gray AN, Egan AJ, Van't Veer IL, Verheul J, Colavin A, Koumoutsis A, Biboy J, Altelaar AF,
348 Damen MJ, Huang KC, *et al.*: **Coordination of peptidoglycan synthesis and outer**
349 **membrane constriction during *Escherichia coli* cell division.** *eLife* 2015, **4**.
350 [Identified functional interactions between the septal PG synthase PBP1B and two members](#)
351 [of the Tol complex to coordinate PG synthesis with outer membrane constriction.](#)
- 352 38. Derouaux A, Wolf B, Fraipont C, Breukink E, Nguyen-Distèche M, Terrak M: **The**
353 **monofunctional glycosyltransferase of *Escherichia coli* localizes to the cell division site**

354 **and interacts with penicillin-binding protein 3, FtsW, and FtsN. *J Bacteriol* 2008,**
355 **190:1831-1834.**

356 39. Magnet S, Dubost L, Marie A, Arthur M, Gutmann L: **Identification of the L,D-transpeptidases**
357 **for peptidoglycan cross-linking in *Escherichia coli*. *J Bacteriol* 2008, 190:4782-4785.**

358 40. Magnet S, Bellais S, Dubost L, Fourgeaud M, Mainardi JL, Petit-Frere S, Marie A, Mengin-
359 Lecreulx D, Arthur M, Gutmann L: **Identification of the L,D-transpeptidases responsible**
360 **for attachment of the Braun lipoprotein to *Escherichia coli* peptidoglycan. *J Bacteriol***
361 **2007, 189:3927-3931.**

362 41. Heidrich C, Ursinus A, Berger J, Schwarz H, Höltje JV: **Effects of multiple deletions of murein**
363 **hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in**
364 ***Escherichia coli*. *J Bacteriol* 2002, 184:6093-6099.**

365 42. Singh SK, Parveen S, SaiSree L, Reddy M: **Regulated proteolysis of a cross-link-specific**
366 **peptidoglycan hydrolase contributes to bacterial morphogenesis. *Proc Natl Acad Sci U S***
367 **A 2015, 112:10956-10961.**

368 43. Nelson DE, Young KD: **Penicillin-binding protein 5 affects cell diameter, contour, and**
369 **morphology of *Escherichia coli*. *J Bacteriol* 2000, 182:1714-1721.**

370 44. Nelson DE, Young KD: **Contributions of PBP 5 and DD-carboxypeptidase penicillin binding**
371 **proteins to maintenance of cell shape in *Escherichia coli*. *J Bacteriol* 2001, 183:3055-3064.**

372 45. Rosenbusch JP: **Structural and functional properties of porin channels in *E. coli* outer**
373 **membranes. *Experientia* 1990, 46:167-173.**

374 ** 46. Peters K, Kannan S, Rao VA, Biboy J, Vollmer D, Erickson SW, Lewis RJ, Young KD,
375 Vollmer W: **The Redundancy of Peptidoglycan Carboxypeptidases Ensures Robust Cell**
376 **Shape Maintenance in *Escherichia coli*. *MBio* 2016, 7.**

377 This work revealed the important function of a previously considered minor DD-CPase,
378 PBP6b in *E. coli* growing at low pH and, hence, this paper shows that seemingly redundant
379 PG hydrolases can have important roles at certain growth conditions.

380 47. Lommatzsch J, Templin MF, Kraft AR, Vollmer W, Höltje JV: **Outer membrane localization**
381 **of murein hydrolases: MltA, a third lipoprotein lytic transglycosylase in *Escherichia coli*.**
382 *J Bacteriol* 1997, **179**:5465-5470.

383

384

385 **Table 1:** Essential enzymes and redundancies in the PG synthesis pathway of *E. coli*. See main text
 386 for discussion and references.

Function	Proteins
Intracellular lipid II synthesis	<ul style="list-style-type: none"> ▪ Essential enzymes: MurA; MurB; MurC; MurC; MurD; MurE; MurF; MurI; MraY; MurG ▪ Redundancy in essential steps: Alr, DadX (alanine racemases); D-DdlA, DdlB (alanyl-D-alanine ligases)
PG synthesis	<ul style="list-style-type: none"> ▪ Essential class B PBP - SEDS pairs for different processes: PBP2-RodA (cell elongation); PBP3-FtsW (cell division) ▪ Semi-redundancy in class A PBP PG synthases - activator function: PBP1A - LpoA; PBP1B - LpoB ▪ Non-essential components: PBP1C, MtgA
LD-trans-peptidases	<ul style="list-style-type: none"> ▪ Multiple enzymes for LD-crosslink formation: YnhG, YcbB ▪ Multiple enzymes for lipoprotein attachment: ErfK, YbiS, YcfS
PG hydrolysis	<ul style="list-style-type: none"> ▪ Multiple non-essential lytic transglycosylases: Slt, MltA, MltB, MltC, MltD, MltE, MltF, MltG ▪ Multiple non-essential DD-endopeptidases: PBP4, PBP7, MepA, MepS, MepM, MepH ▪ Multiple non-essential DD-carboxypeptidases: PBP4, PBP4b, PBP5, PBP6a, PBP6b, PBP7, AmpH ▪ DD-endopeptidases that are redundantly essential for cell elongation: MepS, MepM, MepH ▪ Redundant MurNAc-L-Ala amidases - activators for cell separation: AmiA - EnvC; AmiB - EnvC; AmiC - NlpD ▪ Semi-redundant DD-carboxypeptidases to maintain cell shape at different pH values: PBP5; PBP6b

388 **Figure legends**

389

390 **Figure 1. Theoretical minimal system of PG growth [20].** The GTase domain of a class A PBPs
391 (yellow) utilizes lipid II (red) to synthesize a glycan chain, which is incorporated into the existing PG
392 layer by the TPase domains of class A and B PBPs (grey and purple), while an EPase (green) cleaves
393 in a cross-link in the PG to allow the insertion of the new glycan chain. Alternatively, the system
394 could work with only one TPase that is flexible enough to utilize peptides from different glycan
395 chains.

396

397 **Figure 2. Model for growth condition-dependent composition of dynamic complexes.** In non-
398 growing cells and in the absence of PG synthesis single molecules or partial complexes of PG
399 enzymes, their regulators and cytoskeletal elements (represented as colored circles) diffuse in the cell
400 membrane or periplasm as indicated by the small arrows (upper part). Protein redundancy is indicated
401 by different colors but for simplicity only two colors were used for each category of PG enzymes and
402 their regulators, although in the cell the level of redundancy is higher. Dynamic PG-synthesizing
403 complexes (enclosed by dashed circles) assemble for rounds of PG synthesis at the side wall (for cell
404 elongation) or mid-cell (for cell division) (middle and bottom part), and active complexes move
405 processively in the direction of glycan chains (large arrows). The composition of the active complexes
406 depends on the growth condition, which affects the properties of the periplasm (left side). For
407 example, growth condition A (blue color) could stand for growth at pH 7.5 and growth condition B
408 for growth at pH 5. Other growth variables such as osmolality, ion strength and temperature can also
409 affect the composition of the PG synthesis complexes. OM, outer membrane; PG, peptidoglycan
410 layer; IM, inner membrane.

411

Non-growing cell

Elongation

Septation

Growth
condition A

Growth
condition B

- PG synthases
- Regulators of synthases
- MreB and regulators

- PG hydrolases
- Regulators of hydrolases
- FtsZ and regulators

- Active elongation complex
- Active septation complex