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Pneumococcal cell biology in a new light

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References

1. Avery, O. T., Macleod, C. M. & McCarty, M. Studies on the chemical nature of the sustance inducing transformation of pneumococcal types: Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. *J. Exp. Med.* **79,** 137–158 (1944).
2. Bogaert, D., De Groot, R. & Hermans, P. W. M. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect. Dis.* **4,** 144–154 (2004).
3. Kadioglu, A., Weiser, J. N., Paton, J. C. & Andrew, P. W. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. *Nat. Rev. Microbiol.* **6,** 288–301 (2008).
4. Rotimi, V. O. & Duerden, B. I. The development of the bacterial flora in normal neonates. *J. Med. Microbiol.* **14,** 51–62 (1981).
5. Faden, H., Stanievich, J., Brodsky, L., Bernstein, J. & Ogra, P. L. Changes in nasopharyngeal flora during otitis media of childhood. *Pediatr. Infect. Dis. J.* **9,** 623–626 (1990).
6. García-Rodríguez, J. Á. & Martínez, M. J. F. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J. Antimicrob. Chemother.* **50,** 59–74 (2002).
7. Walker, C. L. F. *et al.* Global burden of childhood pneumonia and diarrhoea. *Lancet* **381,** 1405–1416 (2013).
8. Erdmann, J. Keeping pneumonia's vaccines effective. *Chem. Biol.* **17,** 1043–1044 (2010).
9. Calix, J. J. & Nahm, M. H. A new pneumococcal serotype, 11E, has a variably inactivated *wcjE* gene. *J. Infect. Dis.* **202,** 29–38 (2010).
10. Calix, J. J. *et al.* Biochemical, genetic, and serological characterization of two capsule subtypes among *Streptococcus pneumoniae* Serotype 20 strains: discovery of a new pneumococcal serotype. *J. Biol. Chem.* **287,** 27885–27894 (2012).
11. Jin, P. *et al.* First report of putative *Streptococcus pneumoniae* serotype 6D among nasopharyngeal isolates from Fijian children. *J. Infect. Dis.* **200,** 1375–1380 (2009).
12. Griffith, F. The Significance of Pneumococcal Types. *J. Hyg. (Lond.)* **27,** 113–159 (1928).
13. Marks, L. R., Parameswaran, G. I. & Hakansson, A. P. Pneumococcal interactions with epithelial cells are crucial for optimal biofilm formation and colonization in vitro and in vivo. *Infect. Immun.* **80,** 2744–2760 (2012).
14. Shimomura, O., Johnson, F. H. & Saiga, Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J. Cell. Comp. Physiol.* **59,** 223–239 (1962).
15. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. & Prasher, D. C. Green fluorescent protein as a marker for gene expression. *Science* **263,** 802–805 (1994).
16. Tsien, R. Y. The green fluorescent protein. *Annu. Rev. Biochem.* **67,** 509–544 (1998).
17. Acebo, P., Nieto, C., Corrales, M. A., Espinosa, M. & López, P. Quantitative detection of *Streptococcus pneumoniae* cells harbouring single or multiple copies of the gene encoding the green fluorescent protein. *Microbiol. Read. Engl.* **146 (Pt 6),** 1267–1273 (2000).
18. Morlot, C., Zapun, A., Dideberg, O. & Vernet, T. Growth and division of *Streptococcus pneumoniae*: localization of the high molecular weight penicillin-binding proteins during the cell cycle. *Mol. Microbiol.* **50,** 845–855 (2003).

References

19. Zapun, A., Vernet, T. & Pinho, M. G. The different shapes of cocci. *FEMS Microbiol. Rev.* **32,** 345–360 (2008).
20. Schnell, U., Dijk, F., Sjollema, K. A. & Giepmans, B. N. G. Immunolabeling artifacts and the need for live-cell imaging. *Nat. Methods* **9,** 152–158 (2012).
21. Eberhardt, A., Wu, L. J., Errington, J., Vollmer, W. & Veening, J.-W. Cellular localization of choline-utilization proteins in *Streptococcus pneumoniae* using novel fluorescent reporter systems. *Mol. Microbiol.* **74,** 395–408 (2009).
22. Henriques, M. X., Catalão, M. J., Figueiredo, J., Gomes, J. P. & Filipe, S. R. Construction of improved tools for protein localization studies in *Streptococcus pneumoniae*. *PloS One* **8,** e55049 (2013).
23. Pinho, M. G., Kjos, M. & Veening, J.-W. How to get (a)round: mechanisms controlling growth and division of coccoid bacteria. *Nat. Rev. Microbiol.* **11,** 601–614 (2013).
24. Siefert, J. L. & Fox, G. E. Phylogenetic mapping of bacterial morphology. *Microbiol. Read. Engl.* **144 (Pt 10),** 2803–2808 (1998).
25. Schleifer, K. H. & Kandler, O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36,** 407–477 (1972).
26. Mohammadi, T. *et al.* Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. *EMBO J.* **30,** 1425–1432 (2011).
27. Brown, S., Santa Maria, J. P. & Walker, S. Wall Teichoic Acids of Gram-Positive Bacteria. *Annu. Rev. Microbiol.* **67,** 313–336 (2013).
28. Lovering, A. L., Safadi, S. S. & Strynadka, N. C. J. Structural perspective of peptidoglycan biosynthesis and assembly. *Annu. Rev. Biochem.* **81,** 451–478 (2012).
29. Severin, A. & Tomasz, A. Naturally occurring peptidoglycan variants of *Streptococcus pneumoniae*. *J. Bacteriol.* **178,** 168–174 (1996).
30. Bui, N. K. *et al.* Isolation and analysis of cell wall components from *Streptococcus pneumoniae*. *Anal. Biochem.* **421,** 657–666 (2012).
31. Filipe, S. R., Pinho, M. G. & Tomasz, A. Characterization of the murMN operon involved in the synthesis of branched peptidoglycan peptides in *Streptococcus pneumoniae*. *J. Biol. Chem.* **275,** 27768–27774 (2000).
32. Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A. & Charlier, P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* **32,** 234–258 (2008).
33. Vollmer, W., Blanot, D. & De Pedro, M. A. Peptidoglycan structure and architecture. *FEMS Microbiol. Rev.* **32,** 149–167 (2008).
34. Barreteau, H. *et al.* Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* **32,** 168–207 (2008).
35. Van den Ent, F., Amos, L. A. & Löwe, J. Prokaryotic origin of the actin cytoskeleton. *Nature* **413,** 39–44 (2001).
36. Jones, L. J., Carballido-López, R. & Errington, J. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* **104,** 913–922 (2001).
37. Carballido-López, R. Orchestrating bacterial cell morphogenesis. *Mol. Microbiol.* **60,** 815–819 (2006).
38. Pinho, M. G. & Errington, J. Recruitment of penicillin-binding protein PBP2 to the division site of *Staphylococcus aureus* is dependent on its transpeptidation substrates. *Mol. Microbiol.* **55,** 799–807 (2005).
39. Tomasz, A., Jamieson, J. D. & Ottolenghi, E. The fine structure of Diplococcus Pneumoniae. *J. Cell Biol.* **22,** 453–467 (1964).
40. Daniel, R. A. & Errington, J. Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell* **113,** 767–776 (2003).

41. Ng, W.-L., Kazmierczak, K. M. & Winkler, M. E. Defective cell wall synthesis in *Streptococcus pneumoniae* R6 depleted for the essential PcsB putative murein hydrolase or the VicR (YycF) response regulator. *Mol. Microbiol.* **53,** 1161–1175 (2004).
42. Higgins, M. L. & Shockman, G. D. Procaryotic cell division with respect to wall and membranes. *CRC Crit. Rev. Microbiol.* **1,** 29–72 (1971).
43. Beilharz, K. *et al.* Control of cell division in *Streptococcus pneumoniae* by the conserved Ser/Thr protein kinase StkP. *Proc. Natl. Acad. Sci. U. S. A.* **109,** E905–913 (2012).
44. Berg, K. H., Stamsås, G. A., Straume, D. & Håvarstein, L. S. The effect of low Pbp2b levels on cell morphology and peptidoglycan composition in *Streptococcus pneumoniae* R6. *J. Bacteriol.* (2013). doi:10.1128/JB.00184-13
45. Le Gouëllec, A. *et al.* Roles of pneumococcal DivIB in cell division. *J. Bacteriol.* **190,** 4501–4511 (2008).
46. Lleo, M. M., Canepari, P. & Satta, G. Bacterial cell shape regulation: testing of additional predictions unique to the two-competing-sites model for peptidoglycan assembly and isolation of conditional rod-shaped mutants from some wild-type cocci. *J. Bacteriol.* **172,** 3758–3771 (1990).
47. Morlot, C. *et al.* Interaction of Penicillin-Binding Protein 2x and Ser/Thr protein kinase StkP, two key players in *Streptococcus pneumoniae* R6 morphogenesis. *Mol. Microbiol.* (2013). doi:10.1111/mmi.12348
48. Noirclerc-Savoye, M., Morlot, C., Gérard, P., Vernet, T. & Zapun, A. Expression and purification of FtsW and RodA from *Streptococcus pneumoniae*, two membrane proteins involved in cell division and cell growth, respectively. *Protein Expr. Purif.* **30,** 18–25 (2003).
49. Peters, K. *et al. Streptococcus pneumoniae* PBP2x mid-cell localization requires the C-terminal PASTA domains and is essential for cell shape maintenance. *Mol. Microbiol.* n/a–n/a (2014). doi:10.1111/mmi.12588
50. Wheeler, R., Mesnage, S., Boneca, I. G., Hobbs, J. K. & Foster, S. J. Super-resolution microscopy reveals cell wall dynamics and peptidoglycan architecture in ovococcal bacteria. *Mol. Microbiol.* **82,** 1096–1109 (2011).
51. Sham, L.-T., Tsui, H.-C. T., Land, A. D., Barendt, S. M. & Winkler, M. E. Recent Advances in Pneumococcal Peptidoglycan Biosynthesis Suggest New Vaccine and Antimicrobial Targets. *Curr. Opin. Microbiol.* **15,** 194–203 (2012).
52. Higgins, M. L. & Shockman, G. D. Model for cell wall growth of Streptococcus faecalis. *J. Bacteriol.* **101,** 643–648 (1970).
53. Higgins, M. L. & Shockman, G. D. Study of cycle of cell wall assembly in Streptococcus faecalis by three-dimensional reconstructions of thin sections of cells. *J. Bacteriol.* **127,** 1346–1358 (1976).
54. Sham, L.-T., Barendt, S. M., Kopecky, K. E. & Winkler, M. E. Essential PcsB putative peptidoglycan hydrolase interacts with the essential FtsXSpn cell division protein in *Streptococcus pneumoniae* D39. *Proc. Natl. Acad. Sci. U. S. A.* **108,** E1061–1069 (2011).
55. Vollmer, W., Joris, B., Charlier, P. & Foster, S. Bacterial peptidoglycan (murein) hydrolases. *FEMS Microbiol. Rev.* **32,** 259–286 (2008).
56. García, P., González, M. P., García, E., López, R. & García, J. L. LytB, a novel pneumococcal murein hydrolase essential for cell separation. *Mol. Microbiol.* **31,** 1275–1281 (1999).
57. De Las Rivas, B., García, J. L., López, R. & García, P. Purification and polar localization of pneumococcal LytB, a putative endo-beta-N-acetylglucosaminidase: the chain-dispersing murein hydrolase. *J. Bacteriol.* **184,** 4988–5000 (2002).
58. Arends, S. J. R., Kustusch, R. J. & Weiss, D. S. ATP-binding site lesions in FtsE impair cell division. *J. Bacteriol.* **191,** 3772–3784 (2009).

59. Sham, L.-T., Jensen, K. R., Bruce, K. E. & Winkler, M. E. Involvement of FtsE ATPase and FtsX Extracellular Loops 1 and 2 in FtsEX-PcsB Complex Function in Cell Division of *Streptococcus pneumoniae* D39. *mBio* **4,** (2013).
60. Ng, W.-L., Tsui, H.-C. T. & Winkler, M. E. Regulation of the pspA virulence factor and essential pcsB murein biosynthetic genes by the phosphorylated VicR (YycF) response regulator in *Streptococcus pneumoniae*. *J. Bacteriol.* **187,** 7444–7459 (2005).
61. Bisicchia, P. *et al.* Peptidoglycan metabolism is controlled by the WalRK (YycFG) and PhoPR two-component systems in phosphate-limited *Bacillus subtilis* cells. *Mol. Microbiol.* **75,** 972–989 (2010).
62. Dubrac, S., Bisicchia, P., Devine, K. M. & Msadek, T. A matter of life and death: cell wall homeostasis and the WalKR (YycGF) essential signal transduction pathway. *Mol. Microbiol.* **70,** 1307–1322 (2008).
63. Giefing-Kröll, C., Jelencsics, K. E., Reipert, S. & Nagy, E. Absence of pneumococcal PcsB is associated with overexpression of LysM domain-containing proteins. *Microbiol. Read. Engl.* **157,** 1897–1909 (2011).
64. Lewis, P. J. Bacterial subcellular architecture: recent advances and future prospects. *Mol. Microbiol.* **54,** 1135–1150 (2004).
65. Tipper, D. J. & Strominger, J. L. Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. *Proc. Natl. Acad. Sci. U. S. A.* **54,** 1133–1141 (1965).
66. Goffin, C. & Ghuysen, J. M. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. *Microbiol. Mol. Biol. Rev. MMBR* **62,** 1079–1093 (1998).
67. Lovering, A. L., de Castro, L. H., Lim, D. & Strynadka, N. C. J. Structural insight into the transglycosylation step of bacterial cell-wall biosynthesis. *Science* **315,** 1402–1405 (2007).
68. Macheboeuf, P., Contreras-Martel, C., Job, V., Dideberg, O. & Dessen, A. Penicillin binding proteins: key players in bacterial cell cycle and drug resistance processes. *FEMS Microbiol. Rev.* **30,** 673–691 (2006).
69. Den Blaauwen, T., de Pedro, M. A., Nguyen-Distèche, M. & Ayala, J. A. Morphogenesis of rod-shaped sacculi. *FEMS Microbiol. Rev.* **32,** 321–344 (2008).
70. Hakenbeck, R. *et al.* Penicillin-binding proteins in beta-lactam-resistant streptococcus pneumoniae. *Microb. Drug Resist. Larchmt. N* **5,** 91–99 (1999).
71. Schuster, C., Dobrinski, B. & Hakenbeck, R. Unusual septum formation in *Streptococcus pneumoniae* mutants with an alteration in the D,D-carboxypeptidase penicillin-binding protein 3. *J. Bacteriol.* **172,** 6499–6505 (1990).
72. Song, J.-H. *et al.* Identification of essential genes in *Streptococcus pneumoniae* by allelic replacement mutagenesis. *Mol. Cells* **19,** 365–374 (2005).
73. Land, A. D. & Winkler, M. E. The Requirement for Pneumococcal MreC and MreD Is Relieved by Inactivation of the Gene Encoding PBP1a. *J. Bacteriol.* **193,** 4166–4179 (2011).
74. Pérez-Núñez, D. *et al.* A new morphogenesis pathway in bacteria: unbalanced activity of cell wall synthesis machineries leads to coccus-to-rod transition and filamentation in ovococci. *Mol. Microbiol.* **79,** 759–771 (2011).
75. Mileykovskaya, E., Sun, Q., Margolin, W. & Dowhan, W. Localization and function of early cell division proteins in filamentous Escherichia coli cells lacking phosphatidylethanolamine. *J. Bacteriol.* **180,** 4252–4257 (1998).
76. Morlot, C., Noirclerc-Savoye, M., Zapun, A., Dideberg, O. & Vernet, T. The D,D-carboxypeptidase PBP3 organizes the division process of *Streptococcus pneumoniae*. *Mol. Microbiol.* **51,** 1641–1648 (2004).

77. Barendt, S. M. *et al.* Influences of Capsule on Cell Shape and Chain Formation of Wild-Type and pcsB Mutants of Serotype 2 *Streptococcus pneumoniae*. *J. Bacteriol.* **191,** 3024–3040 (2009).
78. Typas, A., Banzhaf, M., Gross, C. A. & Vollmer, W. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat. Rev. Microbiol.* **10,** 123–136 (2012).
79. Domínguez-Escobar, J. *et al.* Processive Movement of MreB-Associated Cell Wall Biosynthetic Complexes in Bacteria. *Science* **333,** 225–228 (2011).
80. Garner, E. C. *et al.* Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in B. subtilis. *Science* **333,** 222–225 (2011).
81. Morlot, C. *et al.* Interaction of Penicillin-Binding Protein 2x and Ser/Thr protein kinase StkP, two key players in *Streptococcus pneumoniae* R6 morphogenesis. *Mol. Microbiol.* **90,** 88–102 (2013).
82. Lages, M. C. A., Beilharz, K., Morales Angeles, D., Veening, J.-W. & Scheffers, D.-J. The localization of key *Bacillus subtilis* penicillin binding proteins during cell growth is determined by substrate availability. *Environ. Microbiol.* (2013). doi:10.1111/1462-2920.12206
83. Helassa, N., Vollmer, W., Breukink, E., Vernet, T. & Zapun, A. The membrane anchor of penicillin-binding protein PBP2a from *Streptococcus pneumoniae* influences peptidoglycan chain length. *FEBS J.* **279,** 2071–2081 (2012).
84. Lovering, A. L., De Castro, L., Lim, D. & Strynadka, N. C. J. Structural analysis of an 'open' form of PBP1B from *Streptococcus pneumoniae*. *Protein Sci. Publ. Protein Soc.* **15,** 1701–1709 (2006).
85. Maggi, S. *et al.* Division protein interaction web: identification of a phylogenetically conserved common interactome between *Streptococcus pneumoniae* and Escherichia coli. *Microbiol. Read. Engl.* **154,** 3042–3052 (2008).
86. Barendt, S. M., Sham, L.-T. & Winkler, M. E. Characterization of mutants deficient in the L,D-carboxypeptidase (DacB) and WalRK (VicRK) regulon, involved in peptidoglycan maturation of *Streptococcus pneumoniae* serotype 2 strain D39. *J. Bacteriol.* **193,** 2290–2300 (2011).
87. Stock, A. M., Robinson, V. L. & Goudreau, P. N. Two-component signal transduction. *Annu. Rev. Biochem.* **69,** 183–215 (2000).
88. Bakal, C. J. & Davies, J. E. No longer an exclusive club: eukaryotic signalling domains in bacteria. *Trends Cell Biol.* **10,** 32–38 (2000).
89. Muñoz-Dorado, J., Inouye, S. & Inouye, M. A gene encoding a protein serine/threonine kinase is required for normal development of M. xanthus, a gram-negative bacterium. *Cell* **67,** 995–1006 (1991).
90. Galperin, M. Y., Higdon, R. & Kolker, E. Interplay of heritage and habitat in the distribution of bacterial signal transduction systems. *Mol. Biosyst.* **6,** 721–728 (2010).
91. Cozzone, A. J. Role of protein phosphorylation on serine/threonine and tyrosine in the virulence of bacterial pathogens. *J. Mol. Microbiol. Biotechnol.* **9,** 198–213 (2005).
92. Kang, C.-M. *et al.* The Mycobacterium tuberculosis serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. *Genes Dev.* **19,** 1692–1704 (2005).
93. Molle, V. & Kremer, L. Division and cell envelope regulation by Ser/Thr phosphorylation: Mycobacterium shows the way. *Mol. Microbiol.* **75,** 1064–1077 (2010).
94. Echenique, J., Kadioglu, A., Romao, S., Andrew, P. W. & Trombe, M.-C. Protein Serine/Threonine Kinase StkP Positively Controls Virulence and Competence in *Streptococcus pneumoniae*. *Infect. Immun.* **72,** 2434–2437 (2004).

95. Nováková, L. *et al.* Characterization of a eukaryotic type serine/threonine protein kinase and protein phosphatase of *Streptococcus pneumoniae* and identification of kinase substrates. *FEBS J.* **272,** 1243–1254 (2005).
96. Yeats, C., Finn, R. D. & Bateman, A. The PASTA domain: a beta-lactam-binding domain. *Trends Biochem. Sci.* **27,** 438 (2002).
97. Dessen, A., Mouz, N., Gordon, E., Hopkins, J. & Dideberg, O. Crystal structure of PBP2x from a highly penicillin-resistant *Streptococcus pneumoniae* clinical isolate: a mosaic framework containing 83 mutations. *J. Biol. Chem.* **276,** 45106–45112 (2001).
98. Pares, S., Mouz, N., Pétillot, Y., Hakenbeck, R. & Dideberg, O. X-ray structure of *Streptococcus pneumoniae* PBP2x, a primary penicillin target enzyme. *Nat. Struct. Biol.* **3,** 284–289 (1996).
99. Gordon, E., Mouz, N., Duée, E. & Dideberg, O. The crystal structure of the penicillin-binding protein 2x from *Streptococcus pneumoniae* and its acyl-enzyme form: implication in drug resistance. *J. Mol. Biol.* **299,** 477–485 (2000).
100. Shah, I. M., Laaberki, M.-H., Popham, D. L. & Dworkin, J. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135,** 486–496 (2008).
101. Maestro, B. *et al.* Recognition of peptidoglycan and [beta]-lactam antibiotics by the extracellular domain of the Ser/Thr protein kinase StkP from *Streptococcus pneumoniae*. *FEBS Lett.* **585,** 357–363 (2011).
102. Giefing, C., Jelencsics, K. E., Gelbmann, D., Senn, B. M. & Nagy, E. The pneumococcal eukaryotic-type serine/threonine protein kinase StkP co-localizes with the cell division apparatus and interacts with FtsZ in vitro. *Microbiol. Read. Engl.* **156,** 1697–1707 (2010).
103. Sun, X. *et al.* Phosphoproteomic analysis reveals the multiple roles of phosphorylation in pathogenic bacterium *Streptococcus pneumoniae*. *J. Proteome Res.* **9,** 275–282 (2010).
104. Nováková, L. *et al.* Identification of multiple substrates of the StkP Ser/Thr protein kinase in *Streptococcus pneumoniae*. *J. Bacteriol.* **192,** 3629–3638 (2010).
105. Ulijasz, A. T., Falk, S. P. & Weisblum, B. Phosphorylation of the RitR DNA-binding domain by a Ser-Thr phosphokinase: implications for global gene regulation in the streptococci. *Mol. Microbiol.* **71,** 382–390 (2009).
106. Falk, S. P. & Weisblum, B. Phosphorylation of the *Streptococcus pneumoniae* cell wall biosynthesis enzyme MurC by a eukaryotic-like Ser/Thr kinase. *FEMS Microbiol. Lett.* **340,** 19–23 (2013).
107. Sasková, L., Nováková, L., Basler, M. & Branny, P. Eukaryotic-Type Serine/Threonine Protein Kinase StkP Is a Global Regulator of Gene Expression in *Streptococcus pneumoniae*. *J. Bacteriol.* **189,** 4168–4179 (2007).
108. Massagué, J. TGF-beta signal transduction. *Annu. Rev. Biochem.* **67,** 753–791 (1998).
109. Mieczkowski, C., Iavarone, A. T. & Alber, T. Auto-activation mechanism of the Mycobacterium tuberculosis PknB receptor Ser/Thr kinase. *EMBO J.* **27,** 3186–3197 (2008).
110. Pereira, S. F. F., Goss, L. & Dworkin, J. Eukaryote-Like Serine/Threonine Kinases and Phosphatases in Bacteria. *Microbiol. Mol. Biol. Rev.* **75,** 192–212 (2011).
111. Pallová, P., Hercík, K., Sasková, L., Nováková, L. & Branny, P. A eukaryotic-type serine/threonine protein kinase StkP of *Streptococcus pneumoniae* acts as a dimer in vivo. *Biochem. Biophys. Res. Commun.* **355,** 526–530 (2007).
112. Osaki, M. *et al.* The StkP/PhpP signaling couple in *Streptococcus pneumoniae*: cellular organization and physiological characterization. *J. Bacteriol.* **191,** 4943–4950 (2009).

113. Aarsman, M. E. G. *et al.* Maturation of the Escherichia coli divisome occurs in two steps. *Mol. Microbiol.* **55,** 1631–1645 (2005).
114. Gamba, P., Veening, J.-W., Saunders, N. J., Hamoen, L. W. & Daniel, R. A. Two-step assembly dynamics of the *Bacillus subtilis* divisome. *J. Bacteriol.* **191,** 4186–4194 (2009).
115. Fleurie, A. *et al.* Mutational dissection of the S/T-kinase StkP reveals crucial roles in cell division of *Streptococcus pneumoniae*. *Mol. Microbiol.* **83,** 746–758 (2012).
116. Beier, D. & Gross, R. Regulation of bacterial virulence by two-component systems. *Curr. Opin. Microbiol.* **9,** 143–152 (2006).
117. Guenzi, E., Gasc, A. M., Sicard, M. A. & Hakenbeck, R. A two-component signal-transducing system is involved in competence and penicillin susceptibility in laboratory mutants of *Streptococcus pneumoniae*. *Mol. Microbiol.* **12,** 505–515 (1994).
118. Zähner, D. *et al.* The ciaR/ciaH regulatory network of *Streptococcus pneumoniae*. *J. Mol. Microbiol. Biotechnol.* **4,** 211–216 (2002).
119. Dagkessamanskaia, A. *et al.* Interconnection of competence, stress and CiaR regulons in *Streptococcus pneumoniae*: competence triggers stationary phase autolysis of ciaR mutant cells. *Mol. Microbiol.* **51,** 1071–1086 (2004).
120. Mascher, T., Heintz, M., Zähner, D., Merai, M. & Hakenbeck, R. The CiaRH system of *Streptococcus pneumoniae* prevents lysis during stress induced by treatment with cell wall inhibitors and by mutations in pbp2x involved in beta-lactam resistance. *J. Bacteriol.* **188,** 1959–1968 (2006).
121. Dawid, S., Sebert, M. E. & Weiser, J. N. Bacteriocin activity of *Streptococcus pneumoniae* is controlled by the serine protease HtrA via posttranscriptional regulation. *J. Bacteriol.* **191,** 1509–1518 (2009).
122. Ibrahim, Y. M., Kerr, A. R., McCluskey, J. & Mitchell, T. J. Role of HtrA in the virulence and competence of *Streptococcus pneumoniae*. *Infect. Immun.* **72,** 3584–3591 (2004).
123. Sebert, M. E., Palmer, L. M., Rosenberg, M. & Weiser, J. N. Microarray-based identification of htrA, a *Streptococcus pneumoniae* gene that is regulated by the CiaRH two-component system and contributes to nasopharyngeal colonization. *Infect. Immun.* **70,** 4059–4067 (2002).
124. Halfmann, A., Hakenbeck, R. & Brückner, R. A new integrative reporter plasmid for *Streptococcus pneumoniae*. *FEMS Microbiol. Lett.* **268,** 217–224 (2007).
125. Cao, M., Wang, T., Ye, R. & Helmann, J. D. Antibiotics that inhibit cell wall biosynthesis induce expression of the *Bacillus subtilis* sigma(W) and sigma(M) regulons. *Mol. Microbiol.* **45,** 1267–1276 (2002).
126. Haas, W., Kaushal, D., Sublett, J., Obert, C. & Tuomanen, E. I. Vancomycin stress response in a sensitive and a tolerant strain of *Streptococcus pneumoniae*. *J. Bacteriol.* **187,** 8205–8210 (2005).
127. Kuroda, M. *et al.* Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. *Mol. Microbiol.* **49,** 807–821 (2003).
128. Rogers, P. D. *et al.* Gene expression profiling of the response of *Streptococcus pneumoniae* to penicillin. *J. Antimicrob. Chemother.* **59,** 616–626 (2007).
129. Cassone, M., Gagne, A. L., Spruce, L. A., Seeholzer, S. H. & Sebert, M. E. The HtrA protease from *Streptococcus pneumoniae* digests both denatured proteins and the competence-stimulating peptide. *J. Biol. Chem.* (2012). doi:10.1074/jbc.M112.391482
130. Peterson, S. N. *et al.* Identification of competence pheromone responsive genes in *Streptococcus pneumoniae* by use of DNA microarrays. *Mol. Microbiol.* **51,** 1051–1070 (2004).

131. Håvarstein, L. S., Coomaraswamy, G. & Morrison, D. A. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. *Proc. Natl. Acad. Sci. U. S. A.* **92,** 11140–11144 (1995).
132. Stevens, K. E., Chang, D., Zwack, E. E. & Sebert, M. E. Competence in *Streptococcus pneumoniae* is regulated by the rate of ribosomal decoding errors. *mBio* **2,** (2011).
133. Tsui, H.-C. T., Keen, S. K., Sham, L.-T., Wayne, K. J. & Winkler, M. E. Dynamic distribution of the SecA and SecY translocase subunits and septal localization of the HtrA surface chaperone/protease during *Streptococcus pneumoniae* D39 cell division. *mBio* **2,** (2011).
134. Jones, G. & Dyson, P. Evolution of transmembrane protein kinases implicated in coordinating remodeling of gram-positive peptidoglycan: inside versus outside. *J. Bacteriol.* **188,** 7470–7476 (2006).
135. Southward, C. M. & Surette, M. G. The dynamic microbe: green fluorescent protein brings bacteria to light. *Mol. Microbiol.* **45,** 1191–1196 (2002).
136. Gustafsson, C., Govindarajan, S. & Minshull, J. Codon bias and heterologous protein expression. *Trends Biotechnol.* **22,** 346–353 (2004).
137. Veening, J.-W. *et al.* Bet-hedging and epigenetic inheritance in bacterial cell development. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 4393–4398 (2008).
138. Cormack, B. P., Valdivia, R. H. & Falkow, S. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173,** 33–38 (1996).
139. Scholz, O., Thiel, A., Hillen, W. & Niederweis, M. Quantitative analysis of gene expression with an improved green fluorescent protein. *Eur. J. Biochem.* **267,** 1565–1570 (2000).
140. Lewis, P. J. & Marston, A. L. GFP vectors for controlled expression and dual labelling of protein fusions in *Bacillus subtilis*. *Gene* **227,** 101–110 (1999).
141. Pédelacq, J.-D., Cabantous, S., Tran, T., Terwilliger, T. C. & Waldo, G. S. Engineering and characterization of a superfolder green fluorescent protein. *Nat. Biotechnol.* **24,** 79–88 (2006).
142. Puigbò, P., Guzmán, E., Romeu, A. & Garcia-Vallvé, S. OPTIMIZER: a web server for optimizing the codon usage of DNA sequences. *Nucleic Acids Res.* **35,** W126–131 (2007).
143. Zacharias, D. A., Violin, J. D., Newton, A. C. & Tsien, R. Y. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. *Science* **296,** 913–916 (2002).
144. Halfmann, A., Hakenbeck, R. & Brückner, R. A new integrative reporter plasmid for *Streptococcus pneumoniae*. *FEMS Microbiol. Lett.* **268,** 217–224 (2007).
145. Martin, B. *et al.* Expression and maintenance of ComD-ComE, the two-component signal-transduction system that controls competence of *Streptococcus pneumoniae*. *Mol. Microbiol.* **75,** 1513–1528 (2010).
146. parts.igem.org. Part:BBa I746909. at <http://parts.igem.org/Part:BBa_I746909>
147. Andersen, J. B. *et al.* New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl. Environ. Microbiol.* **64,** 2240–2246 (1998).
148. Sliusarenko, O., Heinritz, J., Emonet, T. & Jacobs-Wagner, C. High-throughput, subpixel precision analysis of bacterial morphogenesis and intracellular spatio-temporal dynamics. *Mol. Microbiol.* **80,** 612–627 (2011).
149. Kaern, M., Blake, W. J. & Collins, J. J. The engineering of gene regulatory networks. *Annu. Rev. Biomed. Eng.* **5,** 179–206 (2003).
150. Ozbudak, E. M., Thattai, M., Kurtser, I., Grossman, A. D. & van Oudenaarden, A. Regulation of noise in the expression of a single gene. *Nat. Genet.* **31,** 69–73 (2002).

151. Thattai, M. & van Oudenaarden, A. Intrinsic noise in gene regulatory networks. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 8614–8619 (2001).
152. Thattai, M. & van Oudenaarden, A. Intrinsic noise in gene regulatory networks. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 8614–8619 (2001).
153. Stewart, E. J., Madden, R., Paul, G. & Taddei, F. Aging and death in an organism that reproduces by morphologically symmetric division. *PLoS Biol.* **3,** e45 (2005).
154. Rosenfeld, N., Young, J. W., Alon, U., Swain, P. S. & Elowitz, M. B. Gene regulation at the single-cell level. *Science* **307,** 1962–1965 (2005).
155. Sambrook, J. & Russell, D. W. *Molecular Cloning: A Laboratory Manual*. (Cold Spring Harbor Laboratory Press, 2001).
156. Martin, B., Garcia, P., Castanié, M.-P. & Claverys, J.-P. The recA gene of *Streptococcus pneumoniae* is part of a competence-induced operon and controls lysogenic induction. *Mol. Microbiol.* **15,** 367–379 (1995).
157. Hoskins, J. *et al.* Genome of the bacterium *Streptococcus pneumoniae* strain R6. *J. Bacteriol.* **183,** 5709–5717 (2001).
158. O'Brien, K. L. *et al.* Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* **374,** 893–902 (2009).
159. Overkamp, W. *et al.* Benchmarking various GFP variants in *Bacillus subtilis*, *Streptococcus pneumoniae* and Lactococcus lactis for live cell imaging. *Appl. Environ. Microbiol.* (2013). doi:10.1128/AEM.02033-13
160. Prasher, D. C., Eckenrode, V. K., Ward, W. W., Prendergast, F. G. & Cormier, M. J. Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* **111,** 229–233 (1992).
161. Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22,** 1567–1572 (2004).
162. Shaner, N. C. *et al.* Improving the photostability of bright monomeric orange and red fluorescent proteins. *Nat. Methods* **5,** 545–551 (2008).
163. Shcherbo, D. *et al.* Far-red fluorescent tags for protein imaging in living tissues. *Biochem. J.* **418,** 567–574 (2009).
164. Merzlyak, E. M. *et al.* Bright monomeric red fluorescent protein with an extended fluorescence lifetime. *Nat. Methods* **4,** 555–557 (2007).
165. Shcherbo, D. *et al.* Bright far-red fluorescent protein for whole-body imaging. *Nat. Methods* **4,** 741–746 (2007).
166. Fleurie, A. *et al.* Interplay of the Serine/Threonine-Kinase StkP and the Paralogs DivIVA and GpsB in Pneumococcal Cell Elongation and Division. *PLoS Genet.* **10,** e1004275 (2014).
167. Lanie, J. A. *et al.* Genome sequence of Avery's virulent serotype 2 strain D39 of *Streptococcus pneumoniae* and comparison with that of unencapsulated laboratory strain R6. *J. Bacteriol.* **189,** 38–51 (2007).
168. Kjos, M. & Veening, J.-W. Tracking of chromosome dynamics in live *Streptococcus pneumoniae* reveals that transcription promotes chromosome segregation. *Mol. Microbiol.* **91,** 1088–1105 (2014).
169. Tomasz, A. *Streptococcus pneumoniae: molecular biology & mechanisms of disease*. (Mary Ann Liebert, Inc., 2000).
170. Av-Gay, Y. & Everett, M. The eukaryotic-like Ser/Thr protein kinases of Mycobacterium tuberculosis. *Trends Microbiol.* **8,** 238–244 (2000).
171. Inouye, S. *et al.* A large family of eukaryotic-like protein Ser/Thr kinases of Myxococcus xanthus, a developmental bacterium. *Microb. Comp. Genomics* **5,** 103–120 (2000).

172. Petrícková, K. & Petrícek, M. Eukaryotic-type protein kinases in Streptomyces coelicolor: variations on a common theme. *Microbiol. Read. Engl.* **149,** 1609–1621 (2003).
173. Wang, L., Sun, Y.-P., Chen, W.-L., Li, J.-H. & Zhang, C.-C. Genomic analysis of protein kinases, protein phosphatases and two-component regulatory systems of the cyanobacterium Anabaena sp. strain PCC 7120. *FEMS Microbiol. Lett.* **217,** 155–165 (2002).
174. Young, T. A., Delagoutte, B., Endrizzi, J. A., Falick, A. M. & Alber, T. Structure of Mycobacterium tuberculosis PknB supports a universal activation mechanism for Ser/Thr protein kinases. *Nat. Struct. Biol.* **10,** 168–174 (2003).
175. Banu, L. D. *et al.* The Streptococcus mutans serine/threonine kinase, PknB, regulates competence development, bacteriocin production, and cell wall metabolism. *Infect. Immun.* **78,** 2209–2220 (2010).
176. Beltramini, A. M., Mukhopadhyay, C. D. & Pancholi, V. Modulation of cell wall structure and antimicrobial susceptibility by a *Staphylococcus aureus* eukaryote-like serine/threonine kinase and phosphatase. *Infect. Immun.* **77,** 1406–1416 (2009).
177. Fiuza, M. *et al.* From the characterization of the four serine/threonine protein kinases (PknA/B/G/L) of Corynebacterium glutamicum toward the role of PknA and PknB in cell division. *J. Biol. Chem.* **283,** 18099–18112 (2008).
178. Jin, H. & Pancholi, V. Identification and biochemical characterization of a eukaryotic-type serine/threonine kinase and its cognate phosphatase in Streptococcus pyogenes: their biological functions and substrate identification. *J. Mol. Biol.* **357,** 1351–1372 (2006).
179. Rajagopal, L., Clancy, A. & Rubens, C. E. A Eukaryotic Type Serine/Threonine Kinase and Phosphatase inStreptococcus agalactiae Reversibly Phosphorylate an Inorganic Pyrophosphatase and Affect Growth, Cell Segregation, and Virulence. *J. Biol. Chem.* **278,** 14429–14441 (2003).
180. Silvestroni, A. *et al.* Identification of serine/threonine kinase substrates in the human pathogen group B streptococcus. *J. Proteome Res.* **8,** 2563–2574 (2009).
181. Lee, M. *et al.* Synthetic peptidoglycan motifs for germination of bacterial spores. *Chembiochem Eur. J. Chem. Biol.* **11,** 2525–2529 (2010).
182. Dias, R., Félix, D., Caniça, M. & Trombe, M.-C. The highly conserved serine threonine kinase StkP of *Streptococcus pneumoniae* contributes to penicillin susceptibility independently from genes encoding penicillin-binding proteins. *BMC Microbiol.* **9,** 121 (2009).
183. Giefing, C. *et al.* Discovery of a novel class of highly conserved vaccine antigens using genomic scale antigenic fingerprinting of pneumococcus with human antibodies. *J. Exp. Med.* **205,** 117–131 (2008).
184. Kahne, D., Leimkuhler, C., Lu, W. & Walsh, C. Glycopeptide and lipoglycopeptide antibiotics. *Chem. Rev.* **105,** 425–448 (2005).
185. Prudhomme, M., Attaiech, L., Sanchez, G., Martin, B. & Claverys, J.-P. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science* **313,** 89–92 (2006).
186. Drlica, K. Mechanism of fluoroquinolone action. *Curr. Opin. Microbiol.* **2,** 504–508 (1999).
187. Fadda, D. *et al. Streptococcus pneumoniae* DivIVA: localization and interactions in a MinCD-free context. *J. Bacteriol.* **189,** 1288–1298 (2007).
188. Lara, B. *et al.* Cell division in cocci: localization and properties of the *Streptococcus pneumoniae* FtsA protein. *Mol. Microbiol.* **55,** 699–711 (2005).
189. Zapun, A., Contreras-Martel, C. & Vernet, T. Penicillin-binding proteins and beta-lactam resistance. *FEMS Microbiol. Rev.* **32,** 361–385 (2008).

190. Fadda, D. *et al.* Characterization of divIVA and other genes located in the chromosomal region downstream of the dcw cluster in *Streptococcus pneumoniae*. *J. Bacteriol.* **185,** 6209–6214 (2003).
191. Manteca, A., Ye, J., Sánchez, J. & Jensen, O. N. Phosphoproteome analysis of Streptomyces development reveals extensive protein phosphorylation accompanying bacterial differentiation. *J. Proteome Res.* **10,** 5481–5492 (2011).
192. Schultz, C. *et al.* Genetic and biochemical analysis of the serine/threonine protein kinases PknA, PknB, PknG and PknL of Corynebacterium glutamicum: evidence for non-essentiality and for phosphorylation of OdhI and FtsZ by multiple kinases. *Mol. Microbiol.* **74,** 724–741 (2009).
193. Margolin, W. Sculpting the bacterial cell. *Curr. Biol. CB* **19,** R812–822 (2009).
194. Massidda, O., Anderluzzi, D., Friedli, L. & Feger, G. Unconventional organization of the division and cell wall gene cluster of *Streptococcus pneumoniae*. *Microbiol. Read. Engl.* **144 (Pt 11),** 3069–3078 (1998).
195. Albarracín Orio, A. G., Piñas, G. E., Cortes, P. R., Cian, M. B. & Echenique, J. Compensatory evolution of pbp mutations restores the fitness cost imposed by β-lactam resistance in *Streptococcus pneumoniae*. *PLoS Pathog.* **7,** e1002000 (2011).
196. Mir, M. *et al.* The extracytoplasmic domain of the Mycobacterium tuberculosis Ser/Thr kinase PknB binds specific muropeptides and is required for PknB localization. *PLoS Pathog.* **7,** e1002182 (2011).
197. Minnen, A., Attaiech, L., Thon, M., Gruber, S. & Veening, J.-W. SMC is recruited to oriC by ParB and promotes chromosome segregation in *Streptococcus pneumoniae*. *Mol. Microbiol.* **81,** 676–688 (2011).
198. De Jong, I. G., Beilharz, K., Kuipers, O. P. & Veening, J.-W. Live Cell Imaging of *Bacillus subtilis* and *Streptococcus pneumoniae* using Automated Time-lapse Microscopy. *J. Vis. Exp. JoVE* (2011). doi:10.3791/3145
199. Kloosterman, T. G., van der Kooi-Pol, M. M., Bijlsma, J. J. E. & Kuipers, O. P. The novel transcriptional regulator SczA mediates protection against Zn2+ stress by activation of the Zn2+-resistance gene czcD in *Streptococcus pneumoniae*. *Mol. Microbiol.* **65,** 1049–1063 (2007).
200. Canova, M. J., Kremer, L. & Molle, V. pETPhos: a customized expression vector designed for further characterization of Ser/Thr/Tyr protein kinases and their substrates. *Plasmid* **60,** 149–153 (2008).
201. Claverys, J. P., Dintilhac, A., Pestova, E. V., Martin, B. & Morrison, D. A. Construction and evaluation of new drug-resistance cassettes for gene disruption mutagenesis in *Streptococcus pneumoniae*, using an ami test platform. *Gene* **164,** 123–128 (1995).
202. Atilano, M. L. *et al.* Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 18991–18996 (2010).
203. Ravin, A. W. Reciprocal capsular transformations of pneumococci. *J. Bacteriol.* **77,** 296–309 (1959).
204. Leenhouts, K. *et al.* A general system for generating unlabelled gene replacements in bacterial chromosomes. *Mol. Gen. Genet. MGG* **253,** 217–224 (1996).
205. Sun, X. *et al.* Phosphoproteomic analysis reveals the multiple roles of phosphorylation in pathogenic bacterium *Streptococcus pneumoniae*. *J. Proteome Res.* **9,** 275–282 (2010).
206. Piñas, G. E., Cortes, P. R., Orio, A. G. A. & Echenique, J. Acidic stress induces autolysis by a CSP-independent ComE pathway in *Streptococcus pneumoniae*. *Microbiol. Read. Engl.* **154,** 1300–1308 (2008).

207. Cole, J. N. *et al.* Role of group A Streptococcus HtrA in the maturation of SpeB protease. *Proteomics* **7,** 4488–4498 (2007).
208. Rosch, J. W. & Caparon, M. G. The ExPortal: an organelle dedicated to the biogenesis of secreted proteins in Streptococcus pyogenes. *Mol. Microbiol.* **58,** 959–968 (2005).
209. Strauch, K. L., Johnson, K. & Beckwith, J. Characterization of degP, a gene required for proteolysis in the cell envelope and essential for growth of Escherichia coli at high temperature. *J. Bacteriol.* **171,** 2689–2696 (1989).
210. Giammarinaro, P., Sicard, M. & Gasc, A. M. Genetic and physiological studies of the CiaH-CiaR two-component signal-transducing system involved in cefotaxime resistance and competence of *Streptococcus pneumoniae*. *Microbiol. Read. Engl.* **145 (Pt 8),** 1859–1869 (1999).
211. Lindquist, S. & Craig, E. A. The heat-shock proteins. *Annu. Rev. Genet.* **22,** 631–677 (1988).
212. Ramos-Montañez, S., Kazmierczak, K. M., Hentchel, K. L. & Winkler, M. E. Instability of ackA (acetate kinase) mutations and their effects on acetyl phosphate and ATP amounts in *Streptococcus pneumoniae* D39. *J. Bacteriol.* **192,** 6390–6400 (2010).
213. Daniel, R. A., Harry, E. J. & Errington, J. Role of penicillin-binding protein PBP 2B in assembly and functioning of the division machinery of *Bacillus subtilis*. *Mol. Microbiol.* **35,** 299–311 (2000).
214. Wei, Y., Havasy, T., McPherson, D. C. & Popham, D. L. Rod shape determination by the *Bacillus subtilis* class B penicillin-binding proteins encoded by pbpA and pbpH. *J. Bacteriol.* **185,** 4717–4726 (2003).
215. Van Teeffelen, S. *et al.* The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proc. Natl. Acad. Sci. U. S. A.* **108,** 15822–15827 (2011).
216. Scheffers, D.-J. & Pinho, M. G. Bacterial cell wall synthesis: new insights from localization studies. *Microbiol. Mol. Biol. Rev. MMBR* **69,** 585–607 (2005).
217. Potluri, L. *et al.* Septal and lateral wall localization of PBP5, the major D,D-carboxypeptidase of Escherichia coli, requires substrate recognition and membrane attachment. *Mol. Microbiol.* **77,** 300–323 (2010).
218. Costa, T., Priyadarshini, R. & Jacobs-Wagner, C. Localization of PBP3 in Caulobacter crescentus is highly dynamic and largely relies on its functional transpeptidase domain. *Mol. Microbiol.* **70,** 634–651 (2008).
219. Breukink, E. & de Kruijff, B. Lipid II as a target for antibiotics. *Nat. Rev. Drug Discov.* **5,** 321–332 (2006).
220. Hasper, H. E. *et al.* An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. *Science* **313,** 1636–1637 (2006).
221. Atrih, A., Bacher, G., Allmaier, G., Williamson, M. P. & Foster, S. J. Analysis of peptidoglycan structure from vegetative cells of *Bacillus subtilis* 168 and role of PBP 5 in peptidoglycan maturation. *J. Bacteriol.* **181,** 3956–3966 (1999).
222. Daniel, R. A. & Errington, J. Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell* **113,** 767–776 (2003).
223. Tiyanont, K. *et al.* Imaging peptidoglycan biosynthesis in *Bacillus subtilis* with fluorescent antibiotics. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 11033–11038 (2006).
224. Wiedemann, I. *et al.* Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. *J. Biol. Chem.* **276,** 1772–1779 (2001).
225. Hasper, H. E., de Kruijff, B. & Breukink, E. Assembly and stability of nisin-lipid II pores. *Biochemistry (Mosc.)* **43,** 11567–11575 (2004).
226. Scheffers, D.-J. & Errington, J. PBP1 is a component of the *Bacillus subtilis* cell division machinery. *J. Bacteriol.* **186,** 5153–5156 (2004).

227. Scheffers, D.-J., Jones, L. J. F. & Errington, J. Several distinct localization patterns for penicillin-binding proteins in *Bacillus subtilis*. *Mol. Microbiol.* **51,** 749–764 (2004).
228. Kawai, Y., Daniel, R. A. & Errington, J. Regulation of cell wall morphogenesis in *Bacillus subtilis* by recruitment of PBP1 to the MreB helix. *Mol. Microbiol.* **71,** 1131–1144 (2009).
229. Strahl, H. & Hamoen, L. W. Membrane potential is important for bacterial cell division. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 12281–12286 (2010).
230. Johnson, A. S., van Horck, S. & Lewis, P. J. Dynamic localization of membrane proteins in *Bacillus subtilis*. *Microbiol. Read. Engl.* **150,** 2815–2824 (2004).
231. Kahan, F. M., Kahan, J. S., Cassidy, P. J. & Kropp, H. The mechanism of action of fosfomycin (phosphonomycin). *Ann. N. Y. Acad. Sci.* **235,** 364–386 (1974).
232. Wolf, D., Domínguez-Cuevas, P., Daniel, R. A. & Mascher, T. Cell envelope stress response in cell wall-deficient L-forms of *Bacillus subtilis*. *Antimicrob. Agents Chemother.* **56,** 5907–5915 (2012).
233. Bonev, B. B., Breukink, E., Swiezewska, E., De Kruijff, B. & Watts, A. Targeting extracellular pyrophosphates underpins the high selectivity of nisin. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **18,** 1862–1869 (2004).
234. Ouellette, S. P., Karimova, G., Subtil, A. & Ladant, D. Chlamydia co-opts the rod shape-determining proteins MreB and Pbp2 for cell division. *Mol. Microbiol.* **85,** 164–178 (2012).
235. Divakaruni, A. V., Baida, C., White, C. L. & Gober, J. W. The cell shape proteins MreB and MreC control cell morphogenesis by positioning cell wall synthetic complexes. *Mol. Microbiol.* **66,** 174–188 (2007).
236. Ganchev, D. N., Hasper, H. E., Breukink, E. & de Kruijff, B. Size and orientation of the lipid II headgroup as revealed by AFM imaging. *Biochemistry (Mosc.)* **45,** 6195–6202 (2006).
237. Chugunov, A. *et al.* Lipid-II forms potential 'landing terrain' for lantibiotics in simulated bacterial membrane. *Sci. Rep.* **3,** 1678 (2013).
238. Henrichfreise, B. *et al.* Functional conservation of the lipid II biosynthesis pathway in the cell wall-less bacteria Chlamydia and Wolbachia: why is lipid II needed? *Mol. Microbiol.* **73,** 913–923 (2009).
239. Spira, F., Dominguez-Escobar, J., Müller, N. & Wedlich-Söldner, R. Visualization of cortex organization and dynamics in microorganisms, using total internal reflection fluorescence microscopy. *J. Vis. Exp. JoVE* e3982 (2012). doi:10.3791/3982
240. Breeuwer, P. & Abee, T. Assessment of viability of microorganisms employing fluorescence techniques. *Int. J. Food Microbiol.* **55,** 193–200 (2000).
241. Scott, J. A. G., Brooks, W. A., Peiris, J. S. M., Holtzman, D. & Mulhollan, E. K. Pneumonia research to reduce childhood mortality in the developing world. *J. Clin. Invest.* **118,** 1291–1300 (2008).
242. Betzig, E. *et al.* Imaging intracellular fluorescent proteins at nanometer resolution. *Science* **313,** 1642–1645 (2006).
243. Shcherbakova, D. M., Sengupta, P., Lippincott-Schwartz, J. & Verkhusha, V. V. Photocontrollable Fluorescent Proteins for Superresolution Imaging. *Annu. Rev. Biophys.* **43,** 303–329 (2014).
244. Buss, J., Coltharp, C. & Xiao, J. Super-resolution Imaging of the Bacterial Division Machinery. *J. Vis. Exp.* (2013). doi:10.3791/50048
245. Land, A. D. *et al.* Requirement of essential Pbp2x and GpsB for septal ring closure in *Streptococcus pneumoniae* D39. *Mol. Microbiol.* **90,** 939–955 (2013).
246. Mohammadi, T. *et al.* Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. *EMBO J.* **30,** 1425–1432 (2011).

247. Berg, K. H., Stamsås, G. A., Straume, D. & Håvarstein, L. S. The effect of low Pbp2b levels on cell morphology and peptidoglycan composition in *Streptococcus pneumoniae* R6. *J. Bacteriol.* (2013). doi:10.1128/JB.00184-13
248. Macek, B. *et al.* The serine/threonine/tyrosine phosphoproteome of the model bacterium *Bacillus subtilis*. *Mol. Cell. Proteomics MCP* **6,** 697–707 (2007).
249. Burnside, K. & Rajagopal, L. Aspects of eukaryotic-like signaling in Gram-positive cocci: a focus on virulence. *Future Microbiol.* **6,** 747–761 (2011).
250. Hempel, A. M. *et al.* The Ser/Thr protein kinase AfsK regulates polar growth and hyphal branching in the filamentous bacteria Streptomyces. *Proc. Natl. Acad. Sci.* **109,** E2371–E2379 (2012).
251. Kang, C.-M. *et al.* The Mycobacterium tuberculosis serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. *Genes Dev.* **19,** 1692–1704 (2005).
252. Tavares, J. R., de Souza, R. F., Meira, G. L. S. & Gueiros-Filho, F. J. Cytological characterization of YpsB, a novel component of the *Bacillus subtilis* divisome. *J. Bacteriol.* **190,** 7096–7107 (2008).
253. Steele, V. R., Bottomley, A. L., Garcia-Lara, J., Kasturiarachchi, J. & Foster, S. J. Multiple essential roles for EzrA in cell division of *Staphylococcus aureus*. *Mol. Microbiol.* **80,** 542–555 (2011).
254. Merdanovic, M., Clausen, T., Kaiser, M., Huber, R. & Ehrmann, M. Protein quality control in the bacterial periplasm. *Annu. Rev. Microbiol.* **65,** 149–168 (2011).

Nederlandse Samenvatting

Streptococcus pneumoniae, ofwel de pneumokok, is een belangrijke menselijke pathogeen die ernstige aandoeningen kan veroorzaken, waaronder longontsteking, hersenvliesontsteking, middenoorontsteking en sepsis. In ontwikkelingslanden in het bijzonder is door *S. pneumoniae* veroorzaakte bloedvergiftiging verantwoordelijk voor 25% van alle voorkombare sterfgevallen van kinderen onder de 5 jaar. In de afgelopen decennia zijn verscheidene antibioticaresistente varianten van deze bacterie verschenen, wat het belang benadrukt van de zoektocht naar nieuwe, efficiënte strategieën om pneumokokkeninfecties te bestrijden. Daarom is het belangrijk om inzicht te krijgen in hoe deze bacteriën overleven en ziekte veroorzaken in het menselijk lichaam.

Ontwikkeling van moleculaire technieken

Er is nog relatief weinig bekend over de moleculaire mechanismen die de fundering vormen van processen als celdeling, chromosoomsegregatie, celgroei en pathogeniciteit in *S. pneumoniae*. Een van de redenen hiervoor is dat de moleculair-biologische technieken om dit soort processen te bestuderen beperkt zijn. De ontdekking van het groen-fluorescente proteïne (GFP) en later ook andere fluorescente eiwitten hebben het onderzoeksgebied veranderd en nieuwe mogelijkheden gebracht voor het bestuderen van individuele cellen. Het gebruik van GFP in het onderzoek naar pneumokokken is echter pas vrij recent ingevoerd. Deze verlate invoering van GFP in het veld kan te maken hebben met het feit dat oxidatie van het GFP-fluorofoor noodzakelijk is voor de maturatie van het eiwit, terwijl *S. pneumoniae* juist een micro-aerofiel organisme is.

In hoofdstuk 2 en 3 beschrijven we de eigenschappen van verschillende GFP- en RFP-varianten voor het gebruik in *S. pneumoniae*. Deze fluorescente eiwitten maken het niet alleen mogelijk om eiwitlokalisatie te bestuderen, maar ook om genexpressie in individuele levende cellen te visualiseren. Verder presenteren we een protocol voor time-lapse microscopie (hoofdstuk 2), waarmee eiwitdynamica op het niveau van individuele cellen kan worden bestudeerd. De tools die in dit proefschrift worden gepresenteerd, maken het dus mogelijk om zowel ruimtelijke als tijdsafhankelijke aspecten van eiwitlokalisatie te bekijken over de gehele celcyclus van *S. pneumoniae*.

Samenvatting

Bacteriële celdeling

Een van de meest fundamentele processen in de bacteriële levenscyclus is de celdeling. Hierbij treedt een goed gecoördineerde wisselwerking van complexe eiwitmachinerieën op. Vele aspecten van dit proces zijn uitgebreid bestudeerd, met name in staafvormige modelorganismen zoals de Gram-negatieve *Escherichia coli* en *Caulobacter crescentus* en de Gram-positieve *Bacillus subtilis*. Veel hoofdrolspelers in celdeling en de synthese van peptidoglycan (PG) zijn reeds geïdentificeerd en gekarakteriseerd. Veel van onze huidige kennis van celdeling is afkomstig uit deze studies en dit heeft tot een beter begrip van celdeling in het algemeen geleid en kan ook het inzicht in dit proces in andere organismen verbeteren. Desalniettemin is een deel van deze kennis beperkt, vooral wanneer we willen begrijpen hoe de celvorm in stand wordt gehouden, gelet bijvoorbeeld op de celdeling van ovococci als *Streptococcus pneumoniae*. Hoewel een aantal geconserveerde celdelingseiwitten reeds is bestudeerd in ovococci, worden de mechanismen die celdeling en PG-synthese aansturen nog slecht begrepen. Eén grote vraag die we gedeeltelijk in dit proefschrift behandelen, is hoe perifere en septale celwandsynthese met elkaar worden gecoördineerd in zulke ellipsvormige bacteriën.

Men heeft gesuggereerd dat er in ovococci twee vormen van PG-synthese, septaal en perifeer, plaatsvinden. Volgens het huidige model elongeert een cel als gevolg van perifere PG-synthese. Hoewel de elongatie van cellen van staafvormige bacteriesoorten afhankelijk is van MreB, zijn er interessant genoeg geen homologen van dit eiwit geïdentificeerd in *S. pneumoniae* of andere (ovo)cocci. MreB vormt lapjes langs de laterale celwand, waaraan de elongatiemachinerie zich hecht. Daarom werd voor ovococci aangenomen dat PG-synthese door FtsZ wordt gecoördineerd en dat de machinerieën langs de Z-ring assembleren. Er werd een model voorgesteld dat bestond uit twee toestanden. In dit model vinden we twee machinerieën die respectievelijk verantwoordelijk zijn voor perifere en septale celwandsynthese. In analogie met de compositie van de elongatie- en celdelingscomplexen in *E. coli* en *B. subtilis* zijn er twee complexen gesuggereerd die nodig zijn voor de synthese van perifere en septale peptidoglycan. Volgend uit deze analogie bestaat het voorgestelde complex voor septale synthese uit FtsZ, EzrA, de DivIVA-paraloog GpsB, de lipide-II-flippase FtsW, het subcomplex van DivIB/FtsL/DivIC en de transpeptidase PBP2x. Het voorgestelde complex voor perifere synthese bevat MreC/MreD, de lipide-II-flippase RodA, GpsB en de monofunctionele transpeptidase PBP2b. De regulatie en coördinatie van deze twee machinerieën blijft echter onduidelijk. Land en Winkler lieten zien dat PBP2x en PBP1a volgens een vergelijkbaar patroon lokaliseren, maar dat deze

patronen van elkaar verschillen op het moment dat het septum sluit. Soortgelijke waarnemingen werden gedaan door Peters et al., waaruit bleek dat PBP2x en PBP1a in de meeste cellen colokaliseren. Deze data hebben het huidige tweedelige model, waarbij PBP1a heen en weer wordt gestuurd tussen perifere en septale PG-synthese, versterkt.

Invloed van Ser/Thr-fosforylering door StkP op celdeling

De fosforylering van eiwitten door eiwitkinases en fosfatases is een veelgebruikte strategie om celcyclussignalen door te geven, opdat op de omgeving gereageerd kan worden. Tweecomponentensystemen (TCS) zijn de eerst beschreven en ook meest aanwezige signaleringssystemen voor prokaryoten. Meer recent werden ook eukaryoot-type serine-threonine-eiwitkinases (ESTKP) ontdekt. *S. pneumoniae* codeert slechts voor een enkele ESTKP, StkP, en zijn corresponderende fosfatase PhpP.

Het eerste bacteriële fosfoproteoom dat serine-, threonine- en tyrosinefosforyleringen beschreef werd in 2007 gepubliceerd, voor *B. subtilis*. Daarna is deze methode ook toegepast voor enkele andere organismen, waaronder *S. pneumoniae*. Met behulp van verschillende *in vivo* en/of *in vitro* technieken zijn targets van StkP die betrokken zijn bij celdeling en celwandsynthese geïdentificeerd, zoals DivIVA, FtsZ, FtsA, GlmM. De gevonden targets, die belangrijke celdelingseiwitten vertegenwoordigen, en het feit dat StkP-gedepleteerde cellen geëlongeerd lijken te zijn, geven een duidelijk verband aan tussen StkP en de regulatie van celdeling.

Zoals hierboven al werd genoemd, heeft *S. pneumoniae* een karakteristieke ovaalvorm. Deze wordt waarschijnlijk bepaald door een gecontroleerde wisselwerking tussen perifere en septale peptidoglycansynthese, maar hoe deze twee processen worden gecontroleerd en gecoördineerd is nog onduidelijk. In hoofdstuk 4 wordt beschreven dat StkP een belangrijke rol speelt in het coördineren van celwandsynthese tijdens celgroei en celdeling.

StkP is onderdeel van de familie van ultrageconserveerde Ser/Thr-kinases in Gram-positieve bacteriën, die allen bestaan uit intracellulaire kinasedomeinen en extracellulaire PASTA-domeinen die met een transmembraanhelix aan elkaar verbonden zijn. Men heeft laten zien dat PrkC, een ESTKP van *B. subtilis*, wordt geactiveerd bij binding van vrije muropeptiden, met germinatie als gevolg. Interessant genoeg kan het PASTA-domein van StkP PG-subeenheden en bèta-lactam-antibiotica binden. In hoofdstuk 4 beschrijven we dat StkP lokaliseert naar celdelingssites en dat de activering ervan afhankelijk is van de celcyclus en de beschikbaarheid van substraat (ongelinkt PG). Cellen die *stkP* missen, tonen een ernstig celdelingsdefect en hebben een geëlongeerd fenotype.

Tot slot laten we zien dat *stkP* essentieel is voor correcte septumvorming en septumsluiting. Daarom nemen we aan dat StkP optreedt als een moleculaire schakelaar tussen perifere en septale PG-synthese die celdeling stuurt (hoofdstuk 4). Dit kan worden gezien als een cumulatief effect, door het ontbreken van goed-getimede fosforylering van celdelingseiwitten. Voor het sterk gerelateerde ovaalvormige organisme *Lactococcus lactis* is getoond dat een ongebalanceerde PG-synthese resulteert in geëlongeerde, staafvormige cellen. Goed gecoördineerde constructie en deconstructie van divisie complex is belangrijk voor juiste voortgang van de celcyclus en wordt mogelijk gestuurd door fosforylering en defosforylering door StkP en zijn corresponderende fosfatase PhpP. Het exacte mechanisme waarmee StkP celdeling en PG-synthese reguleert blijft echter onduidelijk.

Overexpressie van gefosforyleerd StkP (StkP~P) resulteert in het tegenovergestelde fenotype van StkP-depletie; cellen zijn significant korter (hoofdstuk 5) en een hogere turn-over van StkP werd gedetecteerd, wat wijst op de activiteit van een protease dat StkP~P degradeert. Onder deze omstandigheden van StkP~P overexpressie zagen we dat het tweecomponentensysteem *ciaRH* was opgereguleerd. Het regulerende systeem van CiaRH is interessant genoeg betrokken bij de respons op verschillende vormen van stress, waaronder celwandstress, autolysis en de gevoeligheid voor bèta-lactam-antibiotica. HtrA is onderdeel van het CiaRH-regulon en is lid van de familie van serineproteases die een belangrijke rol spelen voor de controle van eiwitkwaliteit. Het is daarom mogelijk dat proteolyse van StkP~P door HtrA wordt gebruikt om een nauwkeurig gestuurd feedbacksysteem op te zetten voor het controleren van de voortgang van de celcyclus in *S. pneumoniae*. Maar hoewel *ciaRH* (coderend voor het TCS CiaRH) en daardoor ook *htrA* waren opgereguleerd, konden we niet bevestigen dat StkP~P een bona fide target is van HtrA. Het zou echter kunnen dat dit wel degelijk het geval is, maar dat we degradatie van StkP~P niet konden waarnemen door technische beperkingen (die we niet wisten te omzeilen). Het is interessant dat HtrA lokaliseert op de celdelingssites, de plekken waar PG-synthese plaatsvindt, en colokaliseert met StkP (hoofdstuk 5). Verder liet immunofluorescentiemicroscopie zien dat de subeenheden van de Sec-machinerie, SecA en SecY, en HtrA samenvallen in groeiende cellen. Daarom werd geopperd dat HtrA belangrijk is voor eiwitkwaliteitscontrole van door het Sec-systeem getransloceerde eiwitten. Peters et al. lieten zien dat derivaten van GFP-PBP2x door HtrA worden gedegradeerd. Niettemin werd geen directe degradatie van PBP2x of GFP-PBP2x waargenomen. Er wordt aangenomen dat de turnover van eiwit op celdelingssites hoog is en dat HtrA indirect een belangrijke rol speelt in de regulatie van de celcyclus, door het herkennen en degraderen

van misgevouwen eiwitten. Doordat het competentiestimulerende peptide CSP wordt gedegradeerd door HtrA, is het controleren van de ontwikkeling van competentie een andere rol van dit eiwit. Ook in de competitie tussen verschillende soorten heeft HtrA zijn rol door het controleren van de expressie van bacteriocines.

Positionering van penicillinebindende proteïnen

PG-synthese wordt gekatalyseerd door hoogmolecuulgewicht PBPs, hoewel de specifieke rol van de meeste individuele PBPs onduidelijk blijft. Tijdens PG-synthese wordt de PG-precursor lipide II geïncorporeerd in het groeiende peptidoglycannetwerk door middel van transpeptidatie- en transglycosylatiereacties. Deze reacties worden gestimuleerd door PBPs. Voor staafvormige organismen, zoals *B. subtilis*, werd getoond dat verschillende groepen PBPs betrokken zijn bij PG-elongatie en -deling. In *B. subtilis* is PBP2b betrokken bij en essentieel voor deling, terwijl PbpH en PBP2a nodig zijn voor PG-synthese tijdens elongatie. In *S. pneumoniae* en andere ovococci vindt PG-synthese met name plaats op celdelingssites en twee nieuwe hemisferen worden gesynthetiseerd tussen de twee splitsende oude hemisferen. Er blijven echter twee hoofdvragen open: hoe wordt de activiteit van PBPs gereguleerd en hoe worden ze gestuurd naar de plek waar ze hun werk moeten doen?

Er zijn twee verschillende modellen voorgesteld om deze vragen te beantwoorden, waarin PBP-lokalisatie ofwel wordt gedreven door de structuur van het cytoskelet ofwel door het substraat zelf. PBPs hebben een interactie met het cytoskelet en vormen daarbij dynamische structuren, waarvan werd aangenomen dat ze de drijvende kracht waren achter de lokalisatie van PBPs. Andere studies lieten zien dat actieve PG-synthese, met behulp van PbpH en PBP2a, nodig is voor de dynamica van MreB. Het model waarin de positionering van PBP afhangt van de beschikbaarheid van substraat werd al eerder voorgesteld. Om deze hypothese te testen (hoofdstuk 6) maakten we gebruik van het feit dat lipide II, het substraat van PBPs, wordt gebonden en gedelokaliseerd door nisine, een posttranslationeel gemodificeerd antimicrobieel peptide. Door de lokalisatie van PBPs van *B. subtilis* te volgen, waarvan bekend is dat ze de beweeglijkheid van MreB faciliteren, konden we laten zien dat de positionering van deze PBPs wordt bepaald door het substraat in plaats van door MreB. Ook verscheidene PBPs van *S. pneumoniae* raakten gedelokaliseerd door nisine (hoofdstuk 6). Samen met eerder werk aan *S. aureus* en *S. pneumoniae*, wekken de data in hoofdstuk 6 sterk de suggestie dat lipide II de lokalisatie van belangrijke PBPs aanstuurt. Substraatafhankelijke lokalisatie werd ook gerapporteerd voor StkP, waarschijnlijk dankzij zijn extracellulaire PASTA-domeinen, die voor het eerst

werden ontdekt in PBP2x. Morlot et al. lieten met immunofluorescentiemicroscopie zien dat de deletie van D,D-carboxypeptidase PBP3 leidde tot de delokalisatie van PBP2x. Daarom werd gepostuleerd dat de lokalisatie van PBP2x afhangt van de herkenning en binding door zijn PASTA-domeinen van niet-gecrosslinkt peptidoglycan. Wat de drijvende kracht achter de specifieke lokalisatie van lipide II naar nieuwe delingssites is, blijft een open vraag.

Dankwoord

At the end of my Diploma project in Newcastle, I got into contact with Jan-Willem and we talked about the option to start a PhD in his new group in Groningen. I got very excited about that idea. First of all due to the fact to work with such a great and inspiring scientist as Jan-Willem is. And second, that I would work with a human pathogen and establish new techniques to study its cell biology using microscopy. The only question that remained at the time: where the heck is this Groningen?

Soon, I found out and realized that life in the North-East of this flat and windy neighbor country *kon minder* (could be worse - to keep it in *Gronings* words) and the science is just great!

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List Of Publications

Beilharz K., Kjos M., Veening J.-W. Improved red fluorescent proteins for gene expression and protein localization studies in *Streptococcus pneumoniae. Submitted.* **(Chapter 3)**

Peters K., Schweizer I., **Beilharz K.**, Stahlmann C., Veening J.-W., Hakenbeck R. and Denapaite D. *Streptococcus pneumoniae* PBP2x mid-cell localization requires the C-terminal PASTA domains and is essential for cell shape maintenance. *Mol. Microbiol.* n/a–n/a (2014). doi:10.1111/mmi.12588

Burghout P., Quintero B., Bos L., **Beilharz K.**, Veening J.-W., de Jonge M. I., van der Linden M., van der Ende A. and Hermans P. W. M. A single amino acid substitution in the MurF UDP-MurNAc-pentapeptide synthetase renders *Streptococcus pneumoniae* dependent on CO_2 and temperature. *Mol. Microbiol.* **89,** 494–506 (2013).

Overkamp W., **Beilharz K.**, Weme R. D. O., Solopova A., Karsens H., Kovács Á. T., Kok J., Kuipers O. P. and Veening J.-W. Benchmarking various GFP variants in *Bacillus subtilis*, *Streptococcus pneumoniae* and *Lactococcus lactis* for live cell imaging. *Appl. Environ. Microbiol.* (2013). doi:10.1128/AEM.02033-13. **(Chapter 2)**

Beilharz K.*, Lages M. C. A*., Morales Angeles D., Veening J.-W. and Scheffers D.-J. The localization of key *Bacillus subtilis* penicillin binding proteins during cell growth is determined by substrate availability. *Environ. Microbiol.* (2013). doi:10.1111/1462-2920.12206. **(Chapter 6)**

Beilharz K.*, Nováková L.*, Fadda D., Branny P., Massidda O. and Veening J.-W. Control of cell division in *Streptococcus pneumoniae* by the conserved Ser/Thr protein kinase StkP. *Proc. Natl. Acad. Sci. U. S. A.* **109,** E905–913 (2012). **(Chapter 4)**

de Jong G., **Beilharz K.**, Kuipers O. P. and Veening J.-W. Live Cell Imaging of *Bacillus subtilis* and *Streptococcus pneumoniae* using Automated Time-lapse Microscopy. *J. Vis. Exp. JoVE* (2011). doi:10.3791/3145. **(Chapter 2)**

Typas A., Banzhaf M., van den Berg van 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. Regulation of peptidoglycan synthesis by outer-membrane proteins. *Cell* **143,** 1097–1109 (2010).

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