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How to get (a)round: mechanisms controlling growth and division of coccoid bacteria

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Abstract | Bacteria come in a range of shapes, including round, rod-shaped, curved and spiral cells. This morphological diversity implies that different mechanisms exist to guide proper cell growth, division and chromosome segregation. Although the majority of studies on cell division have focused on rod-shaped cells, the development of new genetic and cell biology tools has provided mechanistic insight into the cell cycles of bacteria with different shapes, allowing us to appreciate the underlying molecular basis for their morphological diversity. In this Review, we discuss recent progress that has advanced our knowledge of the complex mechanisms for chromosome segregation and cell division in bacteria which have, deceptively, the simplest possible shape: the cocci.

Bacteria come in a variety of shapes and sizes[1,2]. This diversity in cell shape, from round and rod-shaped cells to curved, spiral or even square bacteria, implies that there are different mechanisms guiding proper cell growth and division. In most bacteria, cell shape is maintained by the cell wall peptidoglycan sacculus, a sack-like macromolecule that encases the cytoplasmic membrane and is composed of glycan chains crosslinked by short peptides[3]. The cell wall prevents cell lysis by providing stability against the high intracellular–extracellular osmotic pressure differential (turgor). However, the cell wall also needs to be flexible to allow for changes in cell shape during growth and division. The structure of the cell wall is maintained by the combined activities of penicillin-binding proteins (PBPs), which synthesize peptidoglycan, and autolysins, which hydrolyse peptidoglycan; together, these proteins continuously remodel the sacculus during growth and division.

How rod-shaped bacteria maintain their characteristic shape, segregate their chromosomes after DNA replication and find the correct division site to generate equally sized daughter cells after division is reasonably well, but not completely, understood. By contrast, it has been only recently that researchers have been able to obtain the first glimpses of the mechanisms underlying these processes in cocci, despite the simpler shape of these cells. These advances have been facilitated by the availability of new tools, such as new gene expression systems, codon-optimized variants of fast-folding fluorescent proteins and super-resolution microscopy[4–14]. What has become strikingly clear is that important differences exist in the mechanisms used to coordinate morphogenesis and cell cycle events in bacteria with different shapes. For example, rods divide in one plane only, whereas cocci can divide in one plane (streptococci and enterococci), two planes (neisseriae and deinococci) or even three planes (staphylococci and micrococci) (TABLE 1).

Here, we discuss the recent developments that have enhanced our understanding of cell wall synthesis, chromosome segregation, division site selection and cytokinesis in cocci. The term cocci refers to round-shaped, spherical cells as well as to ovoid or rugby-ball-shaped cells, which are more commonly termed ovococci. Some of the most well-known coccoid bacteria are the ovoid *Streptococcus pneumoniae* and the round *Staphylococcus aureus*, two pathogens that are excellent 'non-traditional' model organisms which can be used to study fundamental biological questions. In fact, much of our current knowledge about cell cycle processes in cocci has come from the study of these two Gram-positive species, and therefore this Review has a strong focus on these bacteria.

Peptidoglycan synthesis and cell division

Most bacterial cells grow and divide by elongating the lateral cell wall and building a new cell wall disc, the septum, which divides the mother cell into two identical daughter cells. Therefore, both elongation and septation require synthesis of new peptidoglycan[3,15–17] (BOX 1). Here, we discuss only the later stages of peptidoglycan

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Table 1 | **Conservation of some bacterial proteins involved in cell wall synthesis, chromosome segregation and division site selection**

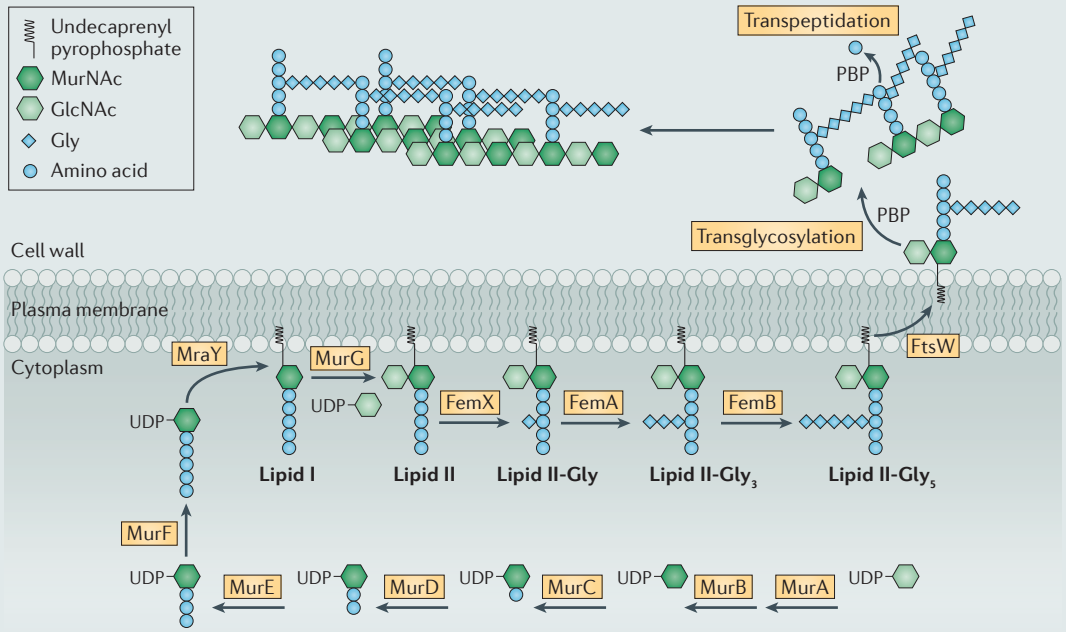
Species	Shape*	Cell wall synthesis and cytokinesis								Chromosome biology and division site selection										
		MreB	MreC	MreD	FtsZ	FtsA	StkP	EzrA	GpsB	DivIVA	MinC	MinD	MinE	MinJ	Noc‡	SlmA	ParA	ParB	Smc	FtsK
Firmicutes																				
Bacillus subtilis		+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+
Streptococcus pneumoniae		–	+	+§	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+
Streptococcus agalactiae‖		–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+
Lactococcus lactis		–	+	+§	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+
Enterococcus faecalis		–	+	+§	+	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+
Pediococcus pentosaceus		–	+	+§	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+
Leuconostoc mesenteroides		+	+	+§	+	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+
Staphylococcus aureus		–	+	+§	+	+	+	+	+	+	–	–	–	–	+	–	–	+	+	+
Veillonella parvula		+	+	–	+	–	+	–	–	–	–	+	–	–	–	–	+	+	+	+
Proteobacteria																				
Escherichia coli		+	+	+	+	+	–¶	–	–	–	+	+	+	–	–	+	–	–#	–**	+
Caulobacter crescentus		+	+	+‡‡	+	+	–	–	–	–	–	–	–	–	–	–	+	+	+	+
Actinobacteria																				
Micrococcus luteus		–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	+	+	–	+
Proteobacteria																				
Neisseria gonorrhoeae		–	–	–	+	+	–	–	–	–	+	+	+	–	–	–	+	+	+	+
Moraxella catarrhalis		–	–	–	+	+	–	–	–	–	–§§	+	+	–	–	–	+	+	+	+
Azotobacter vinelandii		+	+	+	+	+	–	–	–	–	+	+	+	–	–	–	+	+	+	+
Deinococcus–Thermus																				
Deinococcus radiodurans		–	–	–	+	+	–¶	–	–	+	+	+	+	–	–	–	+	+	+	+
Cyanobacteria																				
Synechocystis spp.‖‖		+	+	–	+	–	–¶	–	–	–	–§§	+	+	–	–	–	+	+	+	+

The presence or absence of proteins were determined by BLASTP searches against fully annotated genomes using the respective sequences from the *B. subtilis* and/or *E. coli* proteins as queries and by using Proteinortho BLAST searches to find orthologues[159]. Noc, nucleoid occlusion protein; *Ovococcal cells, with an ellipsoid shape, divide in one plane over successive division cycles, whereas spherical cells can divide in two or three alternating orthogonal planes over successive division cycles. The dashed lines represent division planes. Red and blue indicate Gram-negative and Gram-positive species, respectively. ‡Noc is a homologue of ParB. Proteins were assigned as Noc only when another protein was identified as a ParB homologue in the same species. §Proteins with a low degree of sequence similarity to *B. subtilis* MreD but described as MreD in the literature[39]. ‖*S. agalactiae* has been reported as being ovoid as well as spherical. ¶Proteins with a serine/threonine kinase domain, but without a PASTA (penicillin-binding protein and serine/threonine kinase-associated) domain. #Proteins with a low degree of similarity to *B. subtilis* ParB. **Instead of a structural maintenance of chromosomes (SMC) complex, *E. coli* contains the functional analogue, MukBEF. ‡‡*C. crescentus* MreD[160] does not have sequence similarity with *E. coli* and *B. subtilis* MreD. §§Proteins with a low degree of similarity to *E. coli* MinC. ‖‖*Synechocystis* spp. is described as dividing in two or three planes[161].

synthesis, which are catalysed by PBPs on the exterior of the cytoplasmic membrane. The substrate for PBPs is the dissacharide–pentapeptide lipid-linked precursor lipid II, which is incorporated into nascent peptidoglycan through PBP-mediated transglycosylation and transpeptidation reactions, leading to the synthesis of glycan strands and their crosslinking via flexible peptides, respectively. As mentioned above, coccoid bacteria come in two different shapes: spheres and elongated ellipsoids. Underlying this difference are two distinct modes of cell wall synthesis[15] (FIG. 1). Spherical cocci synthesize cell wall mainly, if not only, at the division septum, in a process catalysed by one type of cell wall synthesis machinery. This machinery consists of a

Box 1 | **Peptidoglycan synthesis**

Synthesis of peptidoglycan, the major constituent of the bacterial cell wall, takes place in three stages that occur at three different locations in the cell (see the figure). The process begins in the cytoplasm, where the nucleotide sugar-linked precursors UDP-*N*-acetylmuramyl (UDP-MurNAc)-pentapeptide and UDP-*N*-acetylglucosamine (UDP-GlcNAc) are synthesized. The second stage takes place at the cytoplasmic membrane, where the UDP-MurNAc-pentapeptide precursor is linked to the transport lipid (undecaprenyl pyrophosphate), resulting in the formation of lipid I. The subsequent addition of GlcNAc from UDP-GlcNAc produces lipid II. A peptide crossbridge (in the case of *Staphylcoccus aureus*, five glycine residues, as shown) is added at the third amino acid in species in which peptidoglycan is not directly crosslinked. Lipid II is then flipped to the external side of the cell membrane (most probably by FtsW proteins), where it is incorporated into nascent peptidoglycan by penicillin-binding proteins (PBPs). During the third stage, PBPs catalyse transglycosylation and transpeptidation reactions, resulting in the respective polymerization and crosslinking of the glycan strands via flexible peptides. PBPs are often divided into high-molecular-mass (HMM) and low-molecular-mass (LMM) PBPs[131]. HMM PBPs can be further classified as class A or class B PBPs according to their functional domains[131]. Class A PBPs are bifunctional, having both transglycosylase and transpeptidase activities, whereas class B PBPs have only transpeptidase activity. LMM PBPs have a penicillin-binding domain and are usually D,D-peptidases[132], although some, such as *S. aureus* PBP4, have transpeptidase activity[133].



(possibly transient) complex of proteins that catalyses the synthesis of peptidoglycan and might also include hydrolytic autolysins[18,19]. Ovococci, however, have two modes of cell wall synthesis, septal and peripheral, and it has been postulated that this might require two types of cell wall synthesis machineries, each containing specific PBPs dedicated to either cell elongation or cell division. However, whether two distinct types of machinery do indeed operate at these different sites has not been fully elucidated[17].

Peptidoglycan synthesis in spherical cocci. *S. aureus* can be considered a minimalist model for cell wall synthesis, as it contains only four native PBPs, in contrast to the best studied Gram-positive bacterium, *Bacillus subtilis*, which has 16 PBPs[20]. The high-molecular-mass (HMM) class A protein PBP2 of *S. aureus* is bifunctional, having both transglycosylase and transpeptidase activity, whereas the HMM class B proteins PBP1 and PBP3, as well as the low-molecular-mass (LMM) protein PBP4, possess transpeptidase activity only. PBP1, PBP2 and PBP4 localize at the septum[18,21,22] (the localization of PBP3 has not yet been determined) (FIG. 1a), consistent with the observation that peptidoglycan synthesis occurs only at this site[18,19]. Interestingly, different mechanisms are responsible for the recruitment of each staphylococcal PBP to the septum. PBP1 seems to be part of the divisome and is hypothesized to be recruited by an unidentified divisome protein in a manner that is independent of a functional PBP transpeptidase domain[21,23]. PBP2 migrates to the septum by recognizing its substrate, lipid II[24]. In rod-shaped *Escherichia coli*, lipid II is translocated from the inner side to the external side of the cytoplasmic membrane by the septally localized lipid II flippase, FtsW[25,26]. Because *S. aureus* encodes two homologues of FtsW, it is likely that lipid II is translocated at the septum by a similar mechanism in this species, thereby inducing the recruitment of PBP2 to the division site. PBP4, which generates highly crosslinked peptidoglycan, is recruited to the septum by an unidentified intermediate of wall teichoic acid synthesis[22]. This mechanism provides both spatial and temporal regulation of PBP4 localization. Wall teichoic acids are synthesized only at the division septum[22], in a process that is likely to occur

Divisome
A large complex of proteins that assembles at the division site and drives cytokinesis.

Wall teichoic acid
An anionic glycopolymer that is bound to the peptidoglycan of Gram-positive bacteria.

only after septal peptidoglycan synthesis has been initiated (presumably by PBP1 and PBP2), as wall teichoic acids are attached to peptidoglycan. As a result, PBP4 is recruited later than PBP1 and PBP2, and this delay might be required to allow the incorporation of polysaccharides and proteins into the cell wall, which might be hampered if peptidoglycan were to become highly crosslinked at an earlier stage.

When the septal peptidoglycan has been synthesized by the PBPs, autolysins are required to split the septum and generate two equally sized daughter cells (FIG. 1a). Little is known about cell wall remodelling in cocci, but cryo-electron microscopy of thin sections of *S. aureus* cells has shown that complete septa are composed of a low-density zone that separates two zones of high density which correspond to two adjacent cross walls[27] (FIG. 1a). The presence of the low-density region suggests that the cross walls have already formed two independent structures in the complete septum. The low-density zone in the septum does not extend into the surface cell wall, so it is possible that the autolysins (which degrade the cell wall and induce septum splitting) act only at the periphery of the septum and not along the entire length of the septum. After splitting, the septum is immediately exposed to the external milieu, and the high internal osmotic pressure pushes the flat septum outwards, forcing it to adopt a curved surface that corresponds to one hemisphere of the new daughter cell. This process is so fast that splitting intermediates are rarely observed by electron or fluorescence microscopy. This suggests that osmotic pressure alone (and not enzyme-mediated remodelling) is sufficient to induce this morphological transition, possibly by inducing changes in the topological arrangement of the glycan and peptide chains, which might provide an increased surface area without needing new cell wall synthesis[28]. However, this hypothesis has not yet been tested.

Peptidoglycan synthesis in ovococci. *S. pneumoniae* has 6 PBPs: the bifunctional HMM class A proteins PBP1a, PBP1b and PBP2a, the HMM class B transpeptidases PBP2b and PBP2x, and the LMM D,D-carboxypeptidase PBP3. In contrast to spherical cocci, ovococci display both septal and so-called peripheral growth. Peripheral peptidoglycan synthesis occurs at mid-cell, between the equatorial rings, and is responsible for the slight longitudinal elongation that results in the ovoid shape of these cells[15,29] (FIG. 1b). Thus, in terms of cell wall synthesis, ovococci more closely resemble rod-shaped bacteria, which have at least two cell wall synthesis machineries, one dedicated to cell elongation and another to synthesis of the division septum[3,30]. However, peripheral growth in ovococci is mechanistically different from elongation in rods, as the latter is dependent on the MreB-like cytoskeletal proteins (BOX 2), which are absent in most ovococci (TABLE 1). More recently, a third cell wall synthesis machinery has been suggested for *E. coli*. This machinery is dependent on FtsZ (BOX 2) and might be responsible for a stage of preseptal elongation that occurs before septum constriction[3,31]. Peripheral growth in ovococci might be similar to preseptal growth in rods. In agreement with this hypothesis, fluorescence microscopy of nascent peptidoglycan in ovococci has revealed that peptidoglycan synthesis occurs in a broad band at mid-cell in *S. pneumoniae* and *Lactococcus lactis* (another ovococcus), but not along the lateral wall[19,30,32] (FIG. 1c). This suggests that both septal and peripheral synthesis occur at the division site. Accordingly, *S. pneumoniae* PBP2x (which is involved in septal synthesis) and PBP2b (which is involved in peripheral synthesis) both localize to the division site at mid-cell[15], although this does not necessarily indicate that the two proteins are part of the same machinery. In fact, super-resolution microscopy (three-dimensional structured-illumination microscopy) of *S. pneumoniae* labelled with two different fluorescent probes, one which preferentially binds PBP1b and PBP3, and a second which labels all pneumococcal PBPs, has shown that there is surprisingly little overlap in the localization of the two stains[10].

Little is known about how pneumococcal PBPs are recruited to the division site. However, it seems that substrate recognition has a role in the localization of some HMM PBPs. In *S. pneumoniae*, PBP3 trims the last

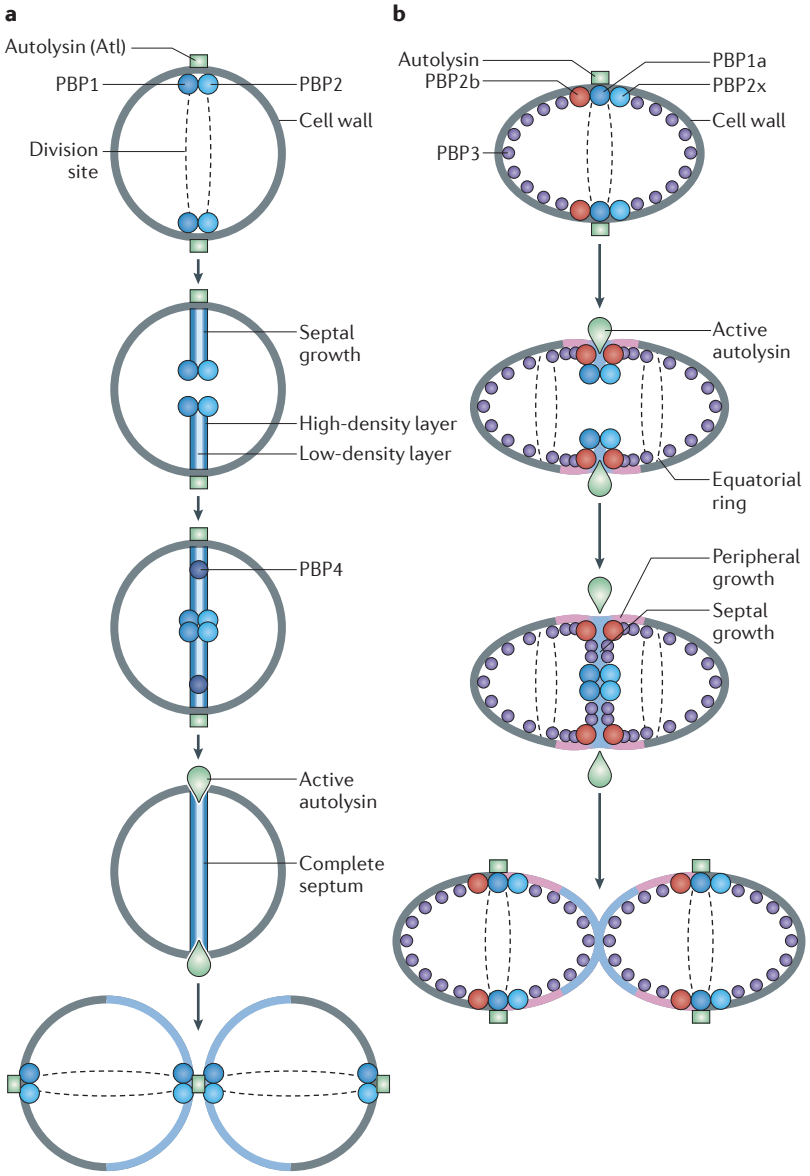
Figure 1 | **Cell wall synthesis in cocci and ovococci.** **a** | Spherical cocci (such as *Staphylococcus aureus*) synthesize peptidoglycan at the septum only, a process that involves at least penicillin-binding protein 1 (PBP1) and PBP2. At a later stage of the cell cycle, PBP4 is recruited to the septum and functions in increasing peptidoglycan crosslinking. The exact role of PBP3 is currently unknown. The complete septum is composed of a low-density central layer separating two high-density layers, corresponding to adjacent cross walls, which will each become one-half of the new cell wall in daughter cells. *S. aureus* encodes multiple autolysins, but thus far only Atl is known to have a role in septum splitting. **b** | In ovococci (such as *Streptococcus pneumoniae*), both septal and peripheral peptidoglycan synthesis occur during division. Septal peptidoglycan synthesis (catalysed by PBP1a and PBP2x) occurs at the division site, and peripheral peptidoglycan synthesis (catalysed by protein PBP2b) occurs in close proximity to the division site, leading to peptidoglycan insertion between the present and the future division sites (equatorial rings) and causing the cell to elongate. Other PBPs (for example, PBP1b, PBP2a and PBP3) are also involved in this process, but their exact roles are unknown. **c** | Peptidoglycan synthesis visualized through the incorporation of fluorescent D-amino acid derivatives. The cells were grown in the presence of TDL (a fluorescent carboxytetramethylrhodamine derivative of D-alanine; red) for 1–2 generations to label the cell contour, followed by a short pulse of HADA (a fluorescent hydroxy coumarin derivative of D-alanine; blue), to label nascent peptidoglycan. The image shows that both spherical cocci (*S. aureus*) and ovococci (*Lactococcus lactis* and *S. pneumoniae*) incorporate peptidoglycan at the septum. Therefore, the peripheral cell wall synthesis that leads to elongation of ovococci occurs in close proximity to the division site, in contrast to the elongation of rods, in which new peptidoglycan is inserted into the lateral wall. Peptidoglycan older than two generations does not appear as labelled (white arrows). Part **c** images courtesy of E. Kuru, M. S. Van Nieuwenhze and Y. Brun, Indiana Univeristy Bloomington, USA.

Equatorial rings
Annular rings of peptidoglycan that are present in the middle of the cell during cell division in some ovococci. These rings mark the future division sites in new daughter cells.

MreB
An actin-like cytoskeletal protein that assembles in short discrete patches which move processively along the cell periphery, perpendicular to the long axis of the cell, powered by peptidoglycan synthesis. MreB might spatially organize the proteins that are required for cell wall synthesis.

FtsZ
A tubulin-like protein with GTPase activity and the first protein found to be recruited to the future division site, where it polymerizes to form the Z ring.

Structured-illumination microscopy
A technique that uses spatially structured illumination and increases the spatial resolution of wide-field fluorescence microscopy to beyond the classical limit.



residue of the pentapeptide present in the substrate of the HMM PBPs, rendering this substrate unsuitable for transpeptidation. As PBP3 is evenly distributed in both hemispheres of wild-type pneumococcal cells and seems to be absent from the future division site at mid-cell during the initial stages of the cell cycle[33], it has been suggested that this PBP3 distribution restricts the substrate of HMM PBPs to the division site. Accordingly, in a mutant lacking PBP3, colocalization of the HMM PBP rings and the FtsZ ring at mid-cell is lost[33]. However, in another study, PBP3 was found to be evenly distributed along the periphery of pneumococcal cells, but it also localized at the division site[34]. Thus, further work is needed to resolve this discrepancy. More recently, *S. pneumoniae* PBP1a and PBP2x have been shown to delocalize from mid-cell on addition of the lipid II-sequestering lantibiotic nisin[35], suggesting that the localization of these two HMM PBPs is also guided by substrate availability (M. C. A. Lages, K. Beilharz, D. Morales-Angeles, J.-W.V. and D. J. Scheffers, unpublished observations).

The two-state model of peptidoglycan synthesis. On the basis of these observations, a two-state model for peptidoglycan biosynthesis has been proposed to account for the existence of two cell wall synthesis machineries at the division site of ovococci[15,17,32,36], although biochemical evidence to support this model is lacking. The model proposes that both the peripheral and the septal machineries localize to the division site at the beginning of a division cycle. The peripheral machinery remains at the edges of the septal disc, inserting material between the equatorial rings and the septum and resulting in elongation of the cell, whereas the septal machinery follows the leading edge of the constricting septum, synthesizing the cross wall. It is currently unknown whether the two machineries function simultaneously or successively during the cell cycle, and whether elongation is solely due to the insertion of new material by the peripheral machinery or whether, owing to concomitant splitting of the new material by autolysins, the septal machinery is also required to drive elongation.

The two-state model predicts that ovococci can be converted into elongated, rod-shaped cells if septal growth only is inhibited and, conversely, that ovococci can be converted into spherical cells if only peripheral growth is inhibited. This hypothesis has been elegantly tested in *L. lactis*, in which the activity of PBP2x (a PBP responsible for septal growth) was inhibited by methicillin, resulting in elongation of the ovococcal cells. By contrast, deletion of PBP2b (a PBP required for peripheral growth) caused cells to adopt a spherical morphology[32]. Similarly, ovococcus-to-rod transitions have been observed for other organisms exposed to methicillin, such as *Streptococcus agalactiae*, *Streptococcus bovis* and *Enterococcus hirae*[37].

Given that most ovococci lack an MreB homologue, FtsZ might coordinate and organize not only septal peptidoglycan synthesis but also peripheral peptidoglycan synthesis, similarly to the potential role of this protein in preseptal elongation in rods[3,31]. However, it is also possible that other cytoskeletal elements are involved in peripheral peptidoglycan synthesis in ovococci. In *B. subtilis*, MreC and MreD have been suggested to couple the MreB-like intracellular cytoskeletal proteins to the extracellular PBPs that are involved in cell elongation[38]. Indeed, depletion of MreCD in *S. pneumoniae* results in cell rounding, suggesting that these proteins are involved in synthesis of the peripheral cell wall[39].

Regulation of cell wall synthesis in ovococci. It was recently suggested that eukaryotic-type serine/threonine kinases (STKs) are involved in coordinating the activity

Septal disc
A structure that forms in the middle of the mother cell during cell division, by invagination of the cell membrane and ingrowth of the cell wall.

Box 2 | **The basics of bacterial cell division**

In most bacteria, cell division begins with the assembly of the highly conserved FtsZ tubulin-like protein at mid-cell. FtsZ is anchored to the membrane via the conserved protein FtsA. Similarly to eukaryotic tubulin, FtsZ is a self-activating GTPase, and GTP hydrolysis provides the energy required for FtsZ monomers to polymerize into a so-called Z ring in the largely nucleoid-free region at mid-cell[134]. Formation of the Z ring is tightly regulated by a number of proteins that either stimulate or inhibit FtsZ polymerization. The Z ring provides a scaffold for the binding of several highly conserved cell division proteins (including the penicillin-binding proteins (PBPs)) that together form the divisome. After the divisome has assembled, the ring constricts as the cell membrane invaginates, and peptidoglycan is synthesized, dividing the mother cell into two equally sized daughter cells. The force required for ring and membrane constriction might be generated in part by FtsZ itself[135]. Many of the divisome proteins are conserved (TABLE 1), and the general mechanisms underlying cytokinesis are similar in both rods and cocci (for reviews, see REFS 2,3,136,137).

In rods, the PBPs required for septal peptidoglycan synthesis are directed to the Z ring, whereas the PBPs required for peripheral peptidoglycan synthesis (which is required for cell elongation) colocalize with the MreB-containing actin-like cytoskeleton. MreB was originally thought to form long helical structures extending along the entire length of the cell[127], but recent data suggest that the protein forms patches which move processively along tracks perpendicular to the long axis of the cell, and that their movement is powered by peptidoglycan synthesis[138–140]. Because cocci lack MreB homologues (TABLE 1), it is still unclear how peripheral cell wall synthesis is controlled in ovococci. One possible scenario is that FtsZ acts as a topological scaffold for the PBPs required for both septal and peripheral cell wall synthesis in ovococci (see main text for details). Alternatively (or in addition), it was recently shown that the eukaryotic-like serine/threonine kinase StkP has a role in coordinating the activities of the peripheral and septal cell wall synthesis machineries in *Streptococcus pneumoniae*, indicating that this protein might functionally substitute for MreB in ovococci[50].

of the pneumococcal cell wall biosynthesis machineries. STKs are widespread in prokaryotic genomes and regulate diverse cellular processes such as hyphal branching in *Streptomyces coelicolor*[40], spore germination in *B. subtilis*[41], antimicrobial resistance in *Enterococcus faecalis*[42] and fruiting-body formation in *Myxococcus xanthus*[43] (for reviews, see REFS 44,45). The current paradigm for prokaryotic STKs is based in part on the structure of PknB, an STK from *Mycobacterium tuberculosis* that is structurally related to eukaryotic Hanks-type kinases[46]. On autophosphorylation, the phosphoryl group of the active kinase is transferred to the hydroxyl group of a serine or threonine residue in the target protein, thereby modulating the activity and/or localization of the target[47]. As rod-shaped bacteria that lack MreB (such as *M. tuberculosis* and *Corynebacterium glutamicum*) control cell elongation using STKs[48,49], it has been hypothesized that these proteins act as molecular switches to control the shift from peripheral to septal cell wall synthesis (and vice versa), thereby coordinating cell elongation in the absence of an actin-like cytoskeleton[50]. Consistent with this idea, most ovococci lack MreB but contain at least one STK, whereas STKs are less commonly found in the genomes of spherical bacteria (TABLE 1).

The *S. pneumoniae* genome encodes a single STK, StkP, which contains a signalling domain with four so-called PASTA (PBP and STK-associated) repeats[51,52]. The PASTA domains of StkP bind to uncrosslinked peptidoglycan[53], and it has been postulated that STKs with a PASTA signature are key regulators of cell wall biosynthesis[54]. StkP localizes to the division site in a cell cycle-dependent manner[50,55], and one of the main targets of this kinase is the cell division protein DivIVA, which is highly conserved in Gram-positive species[56] (TABLE 1). DivIVA is one of the last proteins to localize to the cell division site and, depending on the organism, is involved in several cell cycle-related processes, such as chromosome segregation and cell division (see below). In the absence of StkP, cells become elongated, as peripheral cell wall synthesis exceeds septal cell wall synthesis[50]. The mechanism by which StkP coordinates cell wall synthesis with cell division is still unclear, but an interesting hypothesis is that active phosphorylation and dephosphorylation of DivIVA and/or other unidentified cell division proteins somehow regulates the activity of FtsZ or the PBPs.

Cell wall remodelling by peptidoglycan autolysins is poorly understood in ovococci. The extracellular protein PcsB is the only essential peptidoglycan autolysin in *S. pneumoniae* and localizes to cell division sites[57]; however, it does not demonstrate hydrolytic activity when purified[58]. This paradox was partially solved recently, when it was shown that the conserved FtsEX complex is required for PcsB function in the cell[57]. The FtsEX complex, which probably localizes to the division site, structurally resembles an ABC transporter, suggesting that regulated ATP hydrolysis activates PcsB, thereby coupling cell wall remodelling with cell division[57]. In fact, a similar mechanism seems to control peptidoglycan hydrolysis in *E. coli*, in which the hydrolyase EnvC is controlled by FtsEX[59].

Chromosome organization and segregation

Synthesis of the division septum by the septal machineries cannot be completed until the dividing cell has segregated the newly replicated chromosomes towards opposite poles of the cell. Interestingly, chromosome segregation is one of the few processes that is better understood in eukaryotes than in prokaryotes. Although there has been no structure like the eukaryotic mitotic spindle identified in bacteria, a number of different mechanisms are used to ensure that the duplicated bacterial chromosome is correctly segregated. These mechanisms are thought to include active processes as well as general (that is, passive) cellular processes (for recent reviews, see REFS 60–64).

Active chromosome segregation processes. The structural maintenance of chromosomes complex (SMC complex) and the chromosome partitioning system ParABS, which together have an established role in actively segregating the chromosome in rod-shaped cells, have also been shown to function in cocci[61,62,65]. SMC complexes are found in eukaryotes and in most bacteria. In eukaryotes, several different SMC complexes function in diverse processes, such as sister chromatid cohesion, recombination, DNA repair and mitotic chromosome condensation[66]. Bacteria encode a single highly conserved Smc protein (TABLE 1) that forms complexes with ScpA and ScpB, generating asymmetrical tripartite rings, analogous to eukaryotic SMC complexes[67]. These complexes are suggested to have a crucial role in the organization and condensation of chromosomes[68,69]. The *E. coli* SMC complex analogue, MukBEF, seems to

Hanks-type kinases
Serine/threonine kinases with the so-called Hanks fold. The catalytic residues and overall structure of this fold are highly conserved, and it is found, for example, in the kinase domain of eukaryotic cyclic AMP-dependent protein kinase A and *Streptococcus pneumoniae* StkP.

Mitotic spindle
A microtubule-based eukaryotic subcellular structure that pulls sister chromatids apart during cell division.

Structural maintenance of chromosomes complex
A protein complex with a putative role in organizing the origin regions in bacteria during replication. The functional homologue of this complex in *Escherichia coli* and related alphaproteobacteria is called the MukBEF complex.

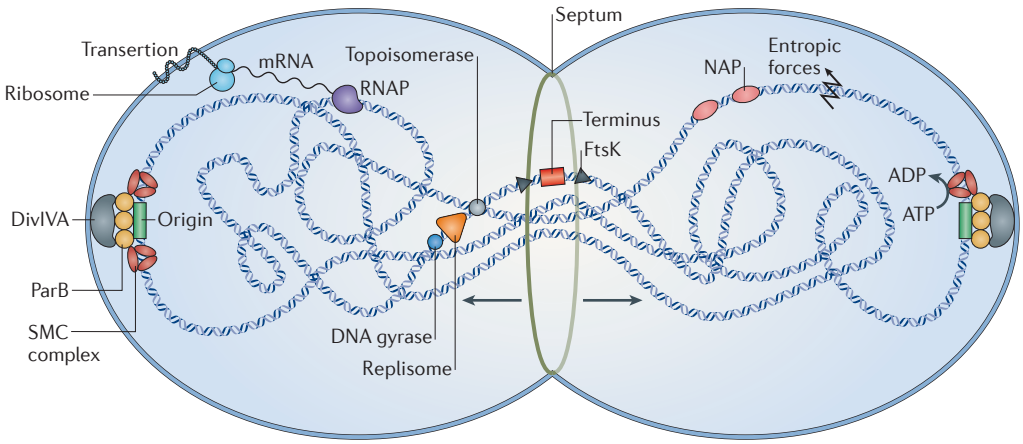


Figure 2 | **Chromosome segregation in cocci.** Both passive and active mechanisms are believed to be involved in chromosome segregation in cocci. *Streptococcus pneumoniae* is shown as a model for chromosome segregation in cocci, as the processes are best characterized in this organism. The active mechanisms probably include chromosome capture, which involves ParB binding to *parS* sites near the origin of replication. As ParB has been shown to interact with the protein DivIVA at the cell poles, this interaction could function as an origin-tethering mechanism and aid in chromosome segregation. ParB also interacts with structural maintenance of chromosomes (SMC) complexes, which travel along the DNA as molecular motors and promote DNA segregation by a capture-and-release mechanism. FtsK localizes to the division site and pumps DNA away from this site. In addition, the passive mechanisms include processes such as DNA replication and transcription; transertion; entropic forces generated by DNA supercoiling, through the action of the replisome, DNA gyrase and topoisomerase; and DNA compaction through the action of nucleoid-associated proteins (NAPs) such as histone-like proteins and SMC complexes. RNAP, RNA polymerase.

actively 'walk' along the chromosome by capturing and releasing DNA segments and is powered by ATP hydrolysis. Accordingly, it has been suggested that MukBEF organizes and segregates the chromosome in a manner comparable to a molecular motor[70]. Similarly to the SMC complex of rod-shaped *B. subtilis*, the *S. pneumoniae* SMC complex forms discrete foci that localize in the vicinity of the origin of replication (*ori* in this species), which depends on the binding of ParB to *parS* sites[68,69,71] (see below). The deletion of genes encoding components of the SMC complex in rod-shaped bacteria often results in lethality or poor growth under standard laboratory conditions[72,73]. By contrast, *smc* deletion in cocci (such as *S. pneumoniae*, *S. aureus* and *Deinococcus radiodurans*) results in only minor phenotypic aberrations, although a substantial fraction of cells are anucleate[71,74,75]. Furthermore, some cocci (such as *Micrococcus luteus*) do not encode an SMC complex (TABLE 1), suggesting that different cocci rely on different systems to organize their chromosomes.

The *parABS* locus is widely conserved among bacteria and was originally identified as a crucial factor for the partitioning of low-copy-number plasmids[65]. It was later demonstrated that chromosomal *parABS* loci are also involved in the segregation of sister chromosomes[76], although the control systems and mode of action are often different between plasmids and chromosomes[62,64]. ParB is a DNA-binding protein that binds specific *parS* sequences in the chromosome; ParA (a Walker-type ATPase) then attaches to ParB and polymerizes into long filaments. *In vitro* studies in crescent-shaped *Caulobacter crescentus* and spiral-shaped *Vibrio cholerae* have shown that ParA filaments extend from one cell pole to the vicinity of *ori* on the chromosome[77–79]. In *C. crescentus*, the ParB–*parS* nucleoprotein complexes located near *ori* are thought to induce ATP hydrolysis by ParA, thereby triggering shortening of the ParA filaments and pulling the newly replicated chromosome towards the new pole. This results in the movement of *ori*, given that *parABS* loci are generally found in close proximity to *ori*[80]. Interestingly, the genomes of some cocci, including *S. pneumoniae* and *S. aureus*, lack *parA* but contain *parB* (TABLE 1). Despite *S. pneumoniae* and *S. aureus* lacking ParA, the ParB homologues in these species (known as Spo0J) also localize to *oriC* (the origin of replication in these species), similarly to *B. subtilis* ParB[71,81] (FIG. 2). Furthermore, *S. pneumoniae parB* mutants generate anucleate cells[71], suggesting that the ParB–*parS* system also promotes chromosome segregation in cocci.

Passive chromosome segregation processes. Although active segregation systems (such as the ParABS and SMC complex systems) seem to be involved in chromosome segregation, they are non-essential, suggesting that passive or indirect processes are important drivers of chromosome segregation in cocci (FIG. 2). For example, DNA replication provides an extrusion force on the newly synthesized strands of DNA[82], and movement of the replisomes along the left and right replichores might further direct chromosome segregation[83,84]. Strikingly, chromosomal organization in rods and crescent-shaped cells is maintained during sequential segregation of the replicated DNA[85–87], probably because the leading and lagging strands occupy specific cellular locations after replication[88]. Localization of the replisome and of

different chromosomal loci has not yet been studied in cocci, but these factors might be particularly important for spherical bacteria such as *S. aureus*, for which the directionality of chromosome segregation seems to guide division site selection[89] (see below).

It has also been suggested that transcription functions as a locomotive force for chromosome segregation in both rod-shaped *B. subtilis*[90] and oval-shaped *S. pneumoniae* (M. Kjos and J.-W. Veening, unpublished observations), although this is not the case in rod-shaped *E. coli*[91]. Similarly to movement of the replisome, movement of RNA polymerase could function in extruding the DNA template, thereby contributing to directed chromosome segregation. In addition, transertion has been proposed to aid in chromosome segregation[92], but experimental evidence supporting this hypothesis is scarce. A recent study[93] provided direct evidence that membrane protein expression affects positioning of chromosomal loci in rod-shaped *E. coli*. However, as transcription seems to be of minor importance for *E. coli* chromosome segregation[91], the transertion mechanism is unlikely to have an important role in this organism. Future experiments need to address whether transertion is important for chromosome segregation in cocci.

DNA supercoiling, chromosome decatenation and DNA compaction are other mechanisms that facilitate chromosome segregation. In fact, physical models suggest that chromosome segregation can be completely driven by entropic forces. According to this model, the major role of the nucleoid-associated proteins (NAPs) that affect chromosome structure and segregation is to regulate the physical state of chromosomes in order to enhance entropy-driven segregation, leading to spontaneous demixing of DNA daughter strands[94,95].

Coordinating chromosome segregation with division. When the newly replicated chromosomes are positioned within the daughter cells by one (or a combination) of the above mechanisms, the final step of chromosome segregation is coordinated with cell division to ensure that the DNA is not guillotined by the cytokinetic machinery. This is achieved by specific selection of the division site (see below) as well as by highly conserved DNA pumps similar to FtsK and SpoIIIE, which are present in several cocci (TABLE 1). FtsK localizes to the cell division site in *L. lactis*[96,97] and ensures that unsegregated DNA is pumped into the daughter cells before the septum closes (FIG. 2). The directionality of DNA pumping is conferred by the γ-domain of FtsK, which binds to specific short DNA sequences called FtsK-orienting polar sequences (KOPS). These KOPS motifs act in chromosome segregation by directing the activity of the DNA translocase (FtsK) towards the terminus, so that newly replicated termini are brought together at the closing septum, thus facilitating the completion of chromosome segregation[98]. During sporulation in *B. subtilis*, SpoIIIE pumps one of the chromosomes from the large mother cell into the smaller forespore[99]. FtsK-like proteins might be more important for cocci than they are for rods because of the smaller volume into which the newly replicated chromosome has to be segregated, given that the chromosomes typically occupy most of the cytoplasm in cocci, whereas in rods the newly replicated chromosomes are usually well separated from each other following segregation[64,81].

Division site selection and cytokinesis

In most bacteria, mechanisms that determine selection of the division site are essential not only to coordinate cytokinesis and chromosome segregation but also to ensure that the two daughter cells have the same size. To achieve this, it is crucial that the septum is placed exactly in the middle of the mother cell. Accordingly, septum placement in rod-shaped bacteria occurs with a deviation of less than ~2.5% from the centre of the cell in *E. coli*[100] and *B. subtilis*[101]. To ensure this level of accuracy, bacteria use two main mechanisms: the Min system and nucleoid occlusion (BOX 3).

In contrast to rods, spherical bacteria can generate two identical daughter cells by dividing in any plane that crosses the centre of the sphere. But how is the division plane selected, considering that there are multiple options? Deletion of rod-shape-determining gene A (*rodA*) in *E. coli* or treatment of the cell with the β-lactam mecillinam induces cell rounding and prevents division from occurring in only one plane. In these cells, nucleoid occlusion determines localization of the division plane[102] and results in FtsZ polymerization in random perpendicular planes between the nucleoids[103]. The Min system also seems to have a role in *rodA*-depleted *E. coli* cells, as its absence results in cells with even more severe division defects than *rodA*-deficient cells[104]. *S. aureus* lacks homologues of MinC and MinD but encodes a protein similar to *B. subtilis* nucleoid occlusion protein (Noc)[89] (BOX 3; TABLE 1). This *B. subtilis* protein binds a large region of the chromosome close to *oriC* and is absent from the terminus-proximal region[105]. In *S. aureus*, the nucleoid occupies most of the volume of the cell, but when chromosome segregation is initiated, the central region of the cell becomes free of the Noc-bound DNA, and consequently only one possible division plane (which would not bisect the nucleoid) becomes available. Thus, the establishment of the chromosome segregation axis in *S. aureus* might be the only cue required to determine the placement of the division septum. Therefore, it is essential to understand how the directionality of chromosome segregation is established in three alternative orthogonal axes, as is needed to accomplish the characteristic cell division pattern of *S. aureus*.

Solving the directionality problem. When a spherical cell divides, it generates two temporarily asymmetrical daughter cells that have one longer axis (parallel to the division septum) and one shorter axis (perpendicular to the division septum) (FIG. 3a). As mentioned above, entropic forces are thought to have a major role in chromosome segregation[94,95]. These forces could explain division in two perpendicular planes: on division of spherical cells, the favoured axis of chromosome segregation in each daughter cell will necessarily be parallel to the complete septum, as segregation along an axis perpendicular to the division septum would

Transertion
The coupling of transcription–translation and protein insertion into the membrane. This results in localization of the DNA–RNA polymerase–RNA–ribosome–peptide complex at the membrane.

Decatenation
The resolution of interlinked circular chromosomes through the breaking and re-ligating of DNA bonds by topoisomerase.

Entropic forces
Conformational entropy generated by processes such as DNA supercoiling and compaction. Entropic forces are proposed to be major guiding forces for the segregation of bacterial chromosomes, leading to the spontaneous demixing of daughter strands.

Box 3 | **The Min and nucleoid occlusion systems**

Rod-shaped bacteria have two main systems to accurately direct localization of the division plane to mid-cell: the Min system prevents aberrant division at the cell poles (reviewed in REFS 141,142), and nucleoid occlusion prevents division from occurring over the nucleoids (reviewed in REF. 105).

In the Min system of *Escherichia coli* (see the figure), the ATPase MinD binds to the membrane in a complex with ATP and recruits MinC. MinC is an inhibitor of FtsZ polymerization, so a zone is formed in which the Z ring (consisting of polymerized FtsZ monomers) cannot assemble. MinE molecules form a ring-like structure (known as the E ring) that gradually releases MinD, and thus MinC, from the membrane by stimulating the ATPase activity of MinD. In the cytoplasm, the released MinD–ADP undergoes nucleotide exchange, re-generating MinD–ATP, which assembles at the membrane of the opposite cell pole (at the site where the concentration of MinE is lowest). Repetition of this process results in the oscillation of MinCD from pole to pole, establishing a gradient wherein the concentration of MinCD is highest at the poles and lowest at mid-cell. Thus, FtsZ polymerization is directed to mid-cell[143]. In *Bacillus subtilis*, MinCD does not oscillate but is sequestered at the cell pole by MinJ–DivIVA. Late during the cell cycle, DivIVA is recruited to mid-cell, where there is a strong negative membrane curvature owing to septum formation, and is retained at the cell poles after division[144–148].

The molecular mechanism of nucleoid occlusion[149,150] remained obscure until two proteins, *B. subtilis* nucleoid occlusion protein (Noc)[117] and *E. coli* SlmA[151], were identified as nucleoid occlusion effectors. These proteins bind specific DNA sequences that are scattered throughout the chromosome but are absent from the terminus region (*ter*)[152–154]. Before DNA replication is initiated, the mid-cell region is occupied by the origin of replication (*oriC*)-proximal region of the chromosome, which is protected by the nucleoid effector protein, and therefore the Z ring cannot assemble at this site. As replication proceeds, the *oriC*-proximal regions of the chromosome and the associated effector protein are moved towards the poles, away from mid-cell. When the protected chromosomal regions are completely segregated, a nucleoid effector-free zone is generated at mid-cell, allowing polymerization of FtsZ. Nucleoid occlusion thus has a role not only in preventing FtsZ ring assembly on top of the nucleoid but also in coordinating the correct timing of cell division with chromosome segregation[105].

Importantly, in the absence of both the Min system and nucleoid occlusion, rod-shaped cells still show a modest bias for FtsZ polymerization at mid-cell[117,155,156], suggesting that additional control mechanisms exist.

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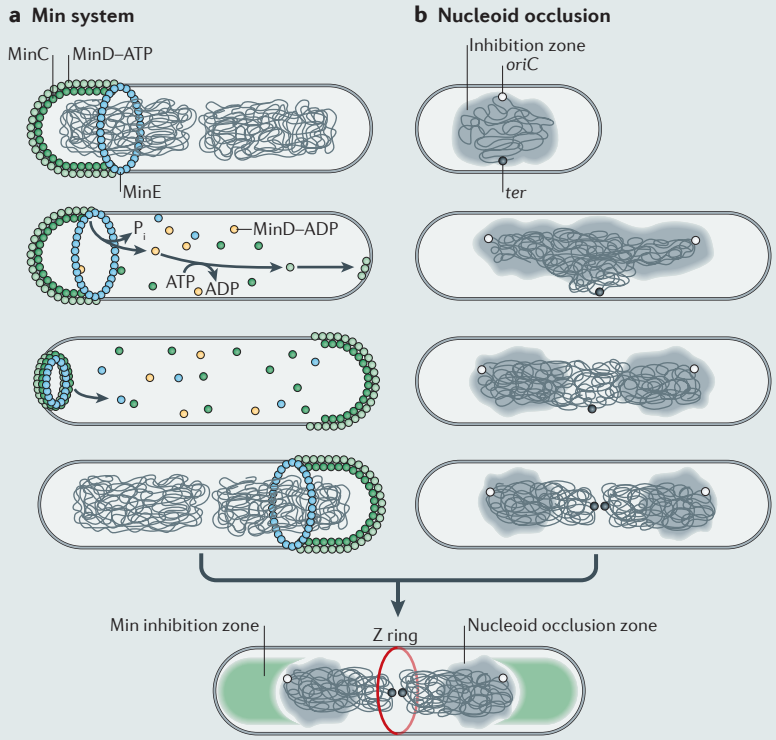

be more constrained in terms of space, and therefore entropically less favourable (FIG. 3a). When the chromosome segregates parallel to the equatorial division septum, it provides only one plane that is lacking Noc. This plane is inevitably perpendicular to the previous division plane, resulting in division in two orthogonal planes (FIG. 3a). To ensure division in three perpendicular planes, a second geometric cue must exist to define which of all the potential planes perpendicular to the previous septum will be chosen. A recent model proposes that an unidentified *oriC*-binding protein has its highest concentration at the cross-junctions of the two previous division planes, directing chromosome segregation towards these two points on opposite sides of the cell[89] (FIG. 3b). In this model, two principles are sufficient to explain cell division in three orthogonal planes during three consecutive division cycles: first, chromosome segregation is directed towards the cross-junctions of the previous division planes, and second, the division septum is placed in the Noc-free area that is generated on chromosome segregation. This model is in accordance with a theoretical model for division in three orthogonal planes, proposed more than a decade ago, which also postulated the existence of DNA-binding sites on the cell wall at 90° angles relative to the previous axis of chromosome segregation[106]. Interestingly, *S. aureus* cells show 'scars' of previous divisions, which can be seen as perfectly perpendicular rings by electron microscopy of immunogold-labelled cells using an antibody that recognizes the autolysin Atl[107]. More recently, atomic force microscopy was used to visualize ring-like structures in the peptidoglycan of *S. aureus* cells, which were also placed at right angles[108]. Thus, it is possible that peptidoglycan or other surface structures contain epigenetic information that is used by the cell as geometrical cues for division.

Division site selection in other cocci. Not all spherical bacteria lack the Min system (TABLE 1). For example, *Neisseria gonorrhoeae* lacks a known nucleoid occlusion effector but does encode MinC and MinD, which function to inhibit cell division. Overexpression of *minCD* causes enlargement of gonococcal cells, whereas mutations in *minCD* result in heterogeneously sized cells with multiple and sometimes incomplete septa[109,110], features that are compatible with cell division occurring along random planes instead of along the perpendicular planes observed for wild-type cells[109,110]. In contrast to *E. coli minCD* mutants, gonococcal *minCD* mutants have reduced viability, suggesting that the Min system (and its role in regulating cell division) is more important for maintaining fitness in *N. gonorrhoeae* than in *E. coli*[109,110]. The localization of Min proteins in *N. gonorrhoeae* is unknown; however, heterologous expression of GFP-tagged *N. gonorrhoeae* MinD in *E. coli*[111] has shown that the protein oscillates from pole to pole (similarly to the *E. coli* system). Moreover, when *N. gonorrhoeae* MinD is expressed in *rodA*-deficient (that is, round) *E. coli* cells, it oscillates in a plane that is parallel to the complete septum. This oscillation pattern is expected to generate a region in which the average concentration of

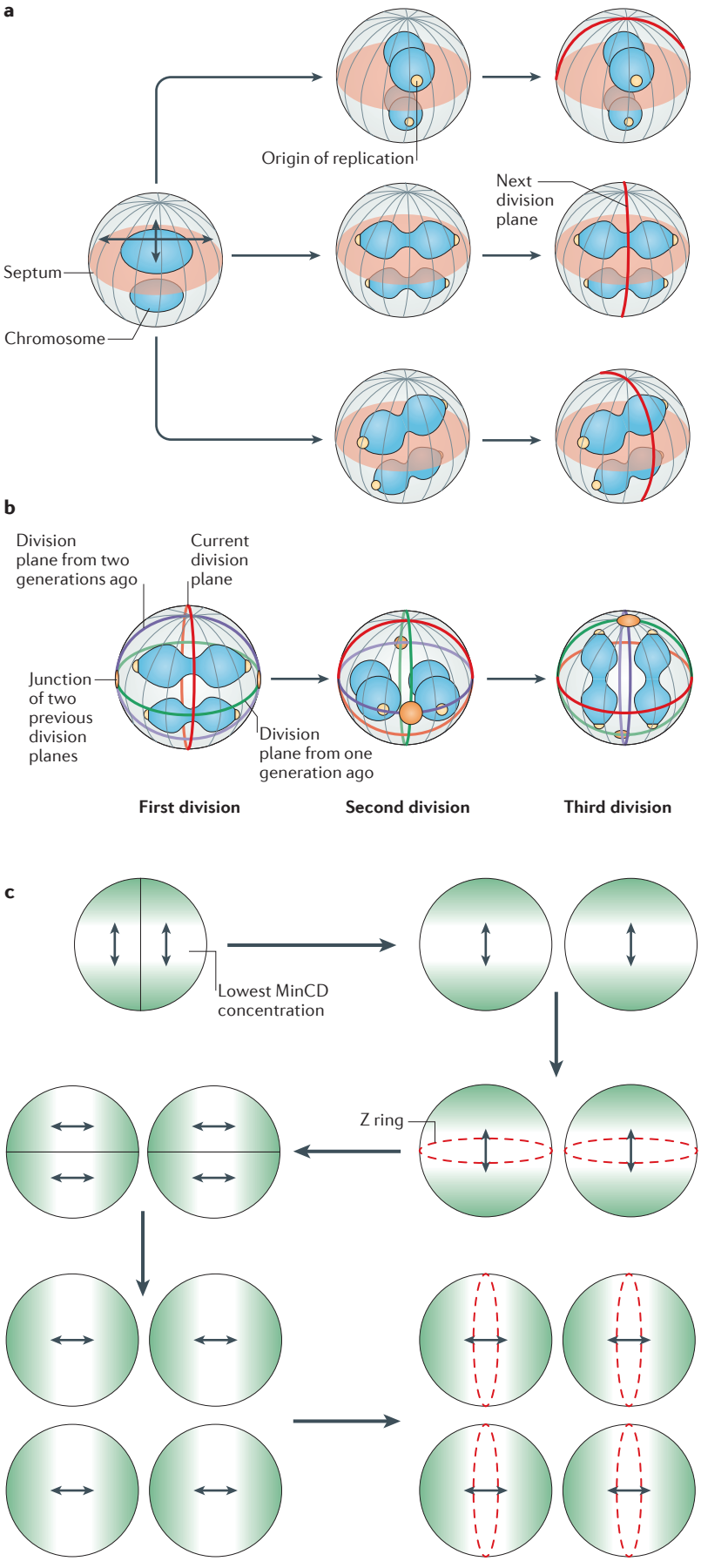

Figure 3 | **Models for the mechanism of division site selection in spherical cocci.** **a** | Division site selection in two perpendicular planes could rely on entropic forces and nucleoid occlusion. The schematic shows a cell that has formed the septum at the equatorial plane of division. The axis of chromosome segregation in each half of the original cell (that is, in each future daughter cell) is parallel to the septum and is shown as a long black arrow; this axis is less constrained than the others in terms of space and is therefore favoured by entropy. On chromosome segregation, one plane (shown as the red meridian) perpendicular to the previous division plane is free from DNA and also therefore free from nucleoid occlusion protein (Noc)-mediated inhibition of FtsZ polymerization. Without an additional topological cue, the chromosome has an equal probability of segregating along any axis parallel to the previous septum (that is, along any of the meridians shown in grey). Three of the possible orientations of the division site are shown. **b** | Division site selection in three perpendicular planes could rely on the directionality of chromosome segregation and nucleoid occlusion. The schematic shows a cell with the orientation of the current division plane and of the previous two division planes indicated, and it depicts how these planes determine the division plane for two further rounds of cell division. The chromosomal origins of replication segregate towards the junctions of the last two division planes. This defines the current division plane as the only plane that is not subjected to nucleoid occlusion. **c** | Division site selection in two perpendicular planes could rely on the Min system. When a spherical cell divides, it generates two temporarily asymmetrical daughter cells that have one longer axis (parallel to the division septum) and one shorter axis (perpendicular to the division septum). MinCD proteins are predicted to oscillate along the longer axis of the cell (BOX 3), even if the two axes differ by only 5% in length[104,158], generating a gradient that has the lowest concentration in a plane perpendicular to the previous division plane. As MinC inhibits FtsZ polymerization, Z ring assembly will occur only in this perpendicular plane of low MinCD concentration. Parts **a**,**b** images are reproduced, with permission, from REF. 89 © (2008) Wiley.

MinCD is minimal, in a plane orthogonal to the previous division septum, making this plane the most likely division site (FIG. 3c).

Interestingly, the genome of *S. pneumoniae* (similarly to those of most other ovococci and to curved *Caulobacter crescentus*) does not encode homologues of the Min system or any of the previously identified nucleoid occlusion effectors (TABLE 1). This suggests that *S. pneumoniae* uses a novel functional homologue of SlmA or Noc to determine septum localization. Alternatively, nucleoid occlusion might be absent in *S. pneumoniae*, and an unidentified mechanism might be used to position the septum. Cocci that lack a Min system typically encode DivIVA (TABLE 1), and the absence of DivIVA can lead to aberrant cell morphologies and severe defects in chromosome segregation[112,113]. For example, DivIVA is essential in *E. faecalis*[112] and the filamentous bacterium *S. coelicolor*[114], which also lack a Noc homologue and a Min system. It is tempting to speculate that DivIVA, perhaps in partnership with ParB, provides an anchor for the chromosome,

Epigenetic information
Cues or signals that result in changes in gene expression or phenotypes independently of changes in DNA sequence.

dcw cluster
A region in bacterial chromosomes that encodes various genes involved in cell division and cell wall synthesis

thus generating a polar gradient of DNA with the lowest concentration at mid-cell. Indeed, in *S. pneumoniae,* DivIVA has been shown to interact with ParB[113], which binds specifically to *parS* sites located near *oriC*[71]. Therefore, the DivIVA–ParB complex might anchor the origins of the newly segregated chromosomes at the old cell poles of the dividing cell and thereby provide positional information for the Z ring. Some evidence supporting this hypothesis comes from *C. glutamicum* (a rod-shaped bacterium that also lacks a Min system and a Noc protein), in which DivIVA interacts with ParB and functions as an origin-tethering factor[115].

Another protein possibly used for division site selection is the FtsZ inhibitor EzrA. EzrA is an early cell division protein that has a partially redundant role in *B. subtilis*. In this organism, EzrA depletion results in a strong phenotype only in combination with mutations in other cell division genes, such as *noc* or *gpsB* (a homologue of *divIVA* that is involved in controlling the cell-elongation division cycle)[116–118]. Interestingly, EzrA is essential in *S. pneumoniae* and in certain *S. aureus* strains[119–121], suggesting that it is not as functionally redundant in cocci as it is in *B. subtilis*. More recent work shows that EzrA is not essential for *S. aureus* viability, but is required for cell size homeostasis through the coordination of proper FtsZ dynamics at mid-cell[122]. EzrA might prevent Z ring formation near the cell poles of ovococci, as has been suggested for *B. subtilis*[116]. However, whether such mechanisms aid division site selection and cell division in cocci remains to be tested.

Summary and future perspectives

It has become increasingly clear that bacteria of different shapes have adopted distinct mechanisms to faithfully segregate their chromosomes and divide. Because cocci have the simplest possible shape, it is perhaps intuitive to propose that rods evolved from cocci through the acquisition of the cell elongation machinery and the loss of division site selection systems, as these systems can restrict the number of division planes to one. However, phylogeny studies indicate the opposite. In the late 1970s, Woese and colleagues showed that cocci are dispersed in various branches of the prokaryotic phylogenetic tree, and therefore suggested that spherical bacteria be considered degenerate forms of bacteria with more complex shapes[123,124]. More recently, phylogenetic mapping has shown that the deepest branches of the tree contain exclusively rod-shaped bacteria[125]. This finding would suggest that the first bacterium containing a peptidoglycan sacculus was rod shaped. Moreover, coccoid morphology seems to be an evolutionary dead-end, as lineages of spherical bacteria in the phylogenetic tree never revert to a rod-shaped morphology. Consistent with this idea, there are no reports of genetic alterations resulting in the transition of truly spherical cocci to rods. On the contrary, there are several examples of mutations that result in the rod-to-coccus transition, such as the loss of *rodA*[126] or *mreB*[127] in *B. subtilis*, and overexpression of *bolA* in *E. coli*[128]. Other phylogenetic studies that have been carried out have been based on the distribution of DNA insertions and deletions[129] or on the genetic organization of the *dcw* cluster[130], and these studies also suggest that rods evolved before cocci. Because *mreB*-like genes are absent in the genomes of most coccoid bacteria (TABLE 1), it is tempting to speculate that loss of the MreB cytoskeleton is the main factor that prevents cocci from elongating into rods. This inability to elongate results in a lower number of peptidoglycan synthesis proteins in cocci, which makes these organisms more amenable to studies aimed at unravelling the functional roles of different PBPs. One major goal of the bacterial cell division field is to reconstitute an active divisome and an active peptidoglycan synthesis machinery *in vitro*. We predict that this might be easier to achieve if cocci are used as models, given that fewer components are involved in these cells. Despite recent advances, elucidation of the mechanistic details of cell division in cocci still represents a major challenge for future studies in the field.

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Competing interests statement
The authors declare no competing financial interests.