SPATIAL CONTROL OF BACTERIAL DIVISION-SITE PLACEMENT

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Abstract | The site of cell division in bacterial cells is placed with high fidelity at a designated position, usually the midpoint of the cell. In normal cell division in *Escherichia coli* this is accomplished by the action of the Min proteins, which maintain a high concentration of a septation inhibitor near the ends of the cell, and a low concentration at midcell. This leaves the midcell site as the only available location for formation of the division septum. In other species, such as *Bacillus subtilis*, this general paradigm is maintained, although some of the proteins differ and the mechanisms used to localize the proteins vary. A second mechanism of negative regulation, the nucleoid-occlusion system, prevents septa forming over nucleoids. This system functions in Gram-negative and Gram-positive bacteria, and is especially important in cells that lack the Min system or in cells in which nucleoid replication or segregation are defective. Here, we review the latest findings on these two systems.

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In most prokaryotic and eukaryotic species, cell division occurs by the formation of a division septum and the subsequent formation of two equivalent daughter cells. After completion of chromosome replication and segregation of the daughter chromosomes to the two halves of the cell, the septum assembles at a predetermined site and two progeny cells are produced. The accurate placement of the division site, usually at midcell, is essential for the propagation of the species, which requires that each daughter cell in a vegetatively growing culture receives one copy of the cellular genome. In addition, some organisms undergo differentiation pathways that give rise to the production of two dissimilar cells, and this also requires that the division site is appropriately placed. In view of these crucially important roles for proper septal placement, it is not surprising that both eukaryotic and prokaryotic organisms have evolved highly reliable division-site-selection systems. The basic question to be answered is: what are the spatial recognition mechanisms that allow a cell to identify a specific location along the long axis, usually the midpoint, and mark it as the proper site for division-site differentiation?

In bacteria, most work on division-site selection has been carried out in the rod-shaped species *Escherichia coli* and *Bacillus subtilis*, but information is also emerging from other organisms. Identifying the correct site for placement of the bacterial septation machinery is accomplished by systems of negative control in which septation is prevented at unwanted sites, leaving the desired site, usually at midcell, as the default site for septal placement.

The Min site-selection system

The MinCDE system. The first evidence that genetics could be used to unravel the division-site selection process came four decades ago with the discovery of minicell mutants of *E. coli* by Adler and collaborators[1]. Septal placement is perturbed in these mutants, which are characterized by the frequent occurrence of septation near a cell pole instead of at midcell. Each polar septation event gives rise to the production of a small spherical cell (minicell) that lacks chromosomal DNA, and a residual mother cell that is longer than a normal cell and contains two nucleoids. Interestingly, plasmid DNA can freely partition into the minicells, where plasmid-encoded proteins

Box 1 | **FtsZ**

FtsZ is an essential protein that is required for cell division. FtsZ is present in virtually all Bacteria, in most Archaea, and in chloroplasts and mitochondria in Eukarya, and is the most conserved of the known bacterial cell-division proteins.

FtsZ is the earliest known protein to localize to the future division site, where the protein forms a ring-like structure (the Z-ring) that extends around the circumference of the cell. The Z-ring is anchored to the inner surface of the plasma membrane by the FtsA protein[93] and probably also by ZipA[94]. The Z-ring is believed to be composed of several extended FtsZ polymers organized into protofilaments. It is not known whether these are present as true rings or whether they might be part of a tightly compressed spiral structure.

The assembly of at least ten other division proteins into a multiprotein complex, the cytokinetic ring ('septasome'), is dependent on the presence of the Z-ring at the division site (reviewed in REF. 95). Because of this, the Z-ring is thought to act as the initial scaffold for the assembly of most or all of the essential components of the cell-division machinery. The Z-ring constricts together with the other septasomal components as the division septum is formed. The Z-ring determines the normal shape of the invaginating septum, as shown by the observation that, in an *ftsZ* mutant that forms a coiled instead of a ring-like FtsZ structure, the septum is twisted in a similar manner[96]. It is not known whether the role of FtsZ is limited to its scaffold function during divisome assembly, or whether it has other roles in regulating or actively participating in the ingrowth of the new septum.

Interestingly, FtsZ is a prokaryotic homologue of tubulin, the main protein component of microtubules in eukaryotic cells. This was initially inferred from the modest homology of their sequence and from the GTP-dependent ability of FtsZ to polymerize into filaments, a property it shares with tubulins. The close structural relationship between FtsZ and tubulin was firmly established when it was shown that the three-dimensional structures of the two proteins are strikingly similar[97]. Despite this, there is presently no evidence that FtsZ or the FtsZ ring forms microtubular structures, and the molecular organization of FtsZ within the ring has not been established.

can be transcribed and translated. Minicells can therefore be used as convenient factories to identify and study the products of plasmid-encoded genes or heterologous genes that have been introduced into plasmid vectors[2].

In *E. coli*, the original minicell mutation eventually led to the identification of a three-gene operon, the *minCDE* genetic locus[3], sometimes called the *minB* operon (the absence of *minA* and *minB* genes reflects confusion in the early mapping of the minicell mutant). The *minC*, *minD* and *minE* gene products work in concert to prevent septation at potential division sites located near the ends of the cell.

MinC is the division inhibitor in this system. It interacts with the division protein FtsZ to prevent formation of stable FtsZ rings, an essential first step in assembly of the division machinery[4] (BOX 1). However, MinC lacks site specificity and, when overexpressed in the absence of the other Min proteins, causes a global block in cell division and the formation of long filaments[5]. MinE is a topological specificity factor, responsible for giving site specificity to MinC, limiting its activity to sites away from midcell. MinD is a membrane-assembly protein, responsible for the membrane association of MinC and MinE[6–8]. The behaviour of these proteins in intact *E. coli* cells has been intensively studied using endogenous Min proteins that are marked by the covalent attachment of green fluorescent protein (GFP) or one of its derivatives.

MinE gives topological specificity to the system by inducing dramatic redistribution and subsequent pole-to-pole oscillation of the membrane-associated MinCDE proteins (FIG. 1). In the presence of MinE, all three proteins are redistributed into a membrane-associated polar zone that extends from the pole towards midcell[7–10]. The presence of MinC in the polar zone prevents septation from occurring in this region of the cell (FIG. 1). The MinCDE polar zone is prevented from extending past midcell by an annular MinE structure, the E-ring[6], that caps the medial edge of the zone as it approaches midcell[11,12]. A study of *minE* mutants that cannot form the E-ring indicates that an important role of the E-ring is to act as a 'stop-growth' signal, preventing the MinCDE polar zone from extending past midcell and therefore preventing MinC from blocking division at the normal midcell division site[13].

To prevent aberrant septation at both ends of the cell, the *E. coli* polar zone and E-ring undergo a remarkable oscillation cycle, first described by Raskin and de Boer[10], in which the structures disassemble at one pole and then undergo another assembly and disassembly cycle at the opposite end of the cell[4,7,10–12] (FIG. 1). Fluorescence microscopy of intact cells and *in vitro* experiments with purified components indicate that the molecules pass through the cytoplasm as they move from one end of the cell to the other. Further work is required to determine whether they move by simple diffusion, as frequently assumed, or whether they associate with other cellular components to facilitate the pole-to-pole movement. The pole-to-pole oscillation of the MinCDE polar zone has a period of 1–2 minutes per cycle[7] and therefore occurs many times in a single cell cycle, which takes 20 minutes or longer. Therefore, the time-averaged concentration of the MinC division inhibitor is maintained at a high level near the ends of the cell, and at a low level near midcell (FIG. 1). As a result, division is prevented at the ends of cells and is only allowed at sites near midcell. This is the only known mechanism to establish site specificity for midcell septation during normal growth of *E. coli* and related bacteria. It is possible that future work might reveal an additional mechanism that positively marks the midcell site, but so far there is no convincing evidence for this.

This model assumes that accurate division at midcell is a result of the absence of the MinC division inhibitor at the midcell site. The current evidence is also consistent with the possibility that the Min system can also act by specifically inactivating potential division sites that might exist at the cell poles. This has been suggested by studies in *B. subtilis*[14] during the first division after the outgrowth of spores, which indicate that, although the Min system is required to prevent polar divisions, it is not required for accurate placement of the division site at midcell in *B. subtilis*, at least under these circumstances. This implies that accurate midcell placement in these cells might involve another mechanism. The cell poles are derived from septation events that occurred during preceding division cycles; the polar sites, therefore, could result from the residual presence of division

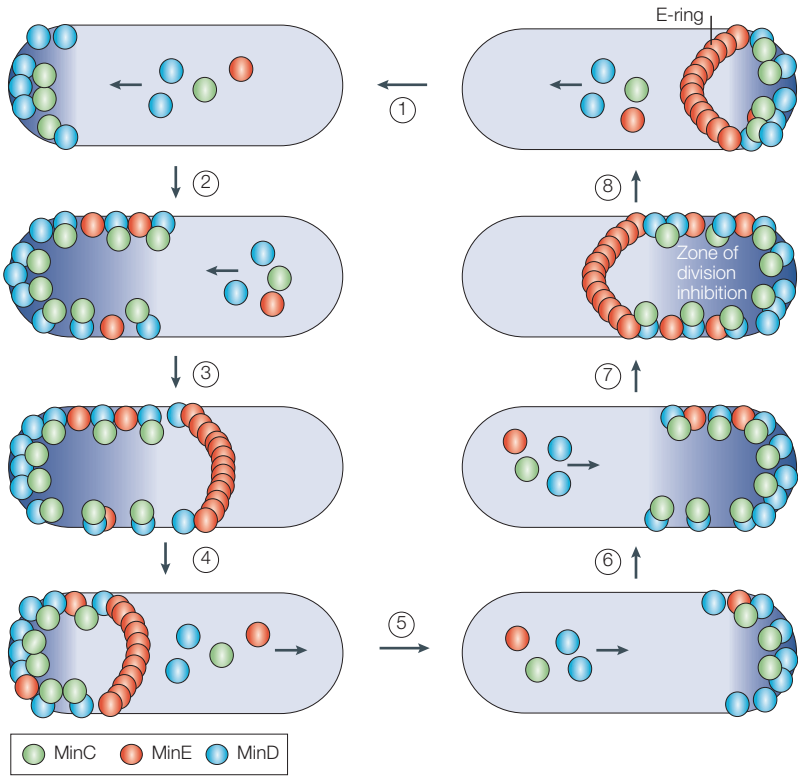

Figure 1| **The MinCDE oscillation cycle.** The MinCDE polar zone begins assembling at a cell pole and grows towards midcell (1–2 and 5–6). The MinE ring then assembles at the leading edge of the polar zone (3 and 7). The polar zone then disassembles, releasing MinC, MinD and MinE molecules, shrinking back to the pole, and finally releasing MinE from the E-ring (4–5 and 8–1). Because of the rapid oscillation, a zone of division inhibition (dark blue shading) is present near the two ends of the cell for a large portion of the cell cycle.

components or factors required for the initiation of assembly of the division apparatus that were present at midcell before the preceding division event.

Cytoskeletal organization of Min proteins. In the polar zone, the membrane-associated MinCDE molecules are organized as a highly ordered cytoskeletal-like coiled structure that winds around the cell, beginning at the pole and extending to the position near midcell where the E-ring is formed[15] (FIG. 2). It is probable that the helical structure comprises parallel arrays of extended MinD polymers, similar to those that have been described *in vitro*[16,17]. Growth of the polar zones probably occurs by the addition of new subunits to the ends of the polymer. It is not known whether individual MinD polymers extend along the entire length of the helical structure or whether several shorter polymers are held together by other proteins or by staggered self-association of the MinD polymers. The helical arrays that comprise the polar zones appear to be part of a longer-range Min helical structure that extends along the entire length of the cell as a permanent pole-to-pole cytoskeletal-like structure. It has been suggested that this could be used as a scaffold for assembly and disassembly of the polar zones during the oscillation cycle[15] (FIG. 2). Much remains to be understood about the formation and molecular organization of these structures.

The same studies have shown that the E-ring is not a free-standing ring but rather a one-coil or two-coil MinE extension of the polar MinCDE helical structure, within which the high concentration of MinE gives the appearance of a ring[15] (FIG. 2). Therefore, the E-ring caps the helical filament, consistent with its ability to block growth of the polar zone past midcell.

The MinD coiled structure was the third helical cytoskeletal-like structure to be described in bacteria, following the description of the MreB (BOX 2) and Mbl cytoskeletal structures of *B. subtilis*[18]. It differs from the *E. coli* MreB cytoskeleton in several ways[15], and the Min and MreB structures seem to be independent entities[19]. As we unravel the organization and biological roles of these and other similarly organized structures that are emerging in bacterial cells[20–23], it is likely there will be many surprises.

The biochemical basis of Min function. The biochemical basis of the assembly and disassembly process and oscillation cycle has been elucidated by *in vitro* studies over the past few years, primarily from the Lutkenhaus laboratory. Based on these and other studies[16,17,24,25], the Min oscillation cycle can be divided into the following stages (FIG. 3). MinD-ATP moves from the cytoplasm to associate with the inner surface of the cytoplasmic membrane[26] near a cell pole. The membrane association is mediated by a short amphipathic helix at the carboxy-terminal end of the MinD protein, which can target the protein to lipid bilayers[17,27–29], although an additional interaction with a specific polar binding site within MinD has not been excluded. MinD–MinD interactions lead to growth of the polar zone by formation of MinD polymers[16,17], which extend from the pole towards midcell. MinC and MinE are brought to the membrane by associating with MinD–ATP. It is not known whether this occurs before or after the membrane association of MinD. When the polar zone approaches midcell, a MinE ring is formed by the addition of MinE molecules at its leading edge[11,13]. MinE in the E-ring activates the ATPase activity of the terminal MinD–ATP molecules. This leads to release of the MinD–ADP product from the membrane, together with the MinC and MinE molecules within the polar zone. The MinE ring is itself not released and remains associated with the medial edge of the shrinking polar zone during the disassembly process until disassembly is completed (FIG. 1). Interestingly, release of the membrane-associated MinC *in vitro* can be catalysed by MinE, even in the absence of activation of the MinD ATPase or the release of MinD[25]. This is consistent with the observation that MinC and MinE interact with the surface of MinD at two overlapping sites, so that binding of one of the proteins to MinD displaces the binding of the other[30]. Therefore, MinE (presumably within the E-ring) has several roles: capping the growth of the MinD polymers, stimulating the release of MinC, and causing the release of MinD by stimulating its ATPase activity and thereby converting MinD–ATP to MinD–ADP. The released MinD–ADP is converted to MinD–ATP, which moves to the other end of the

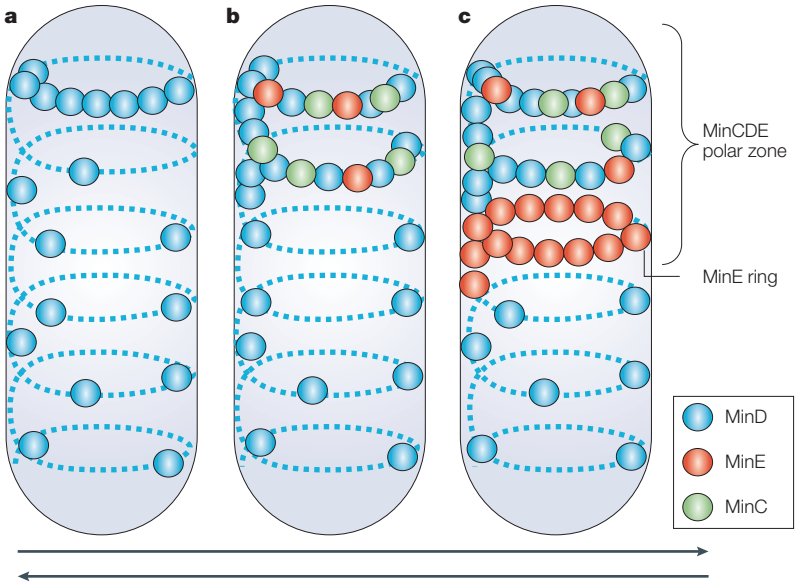

Figure 2 | **The helical organization of the Min system.** MinCDE coils extend from the pole towards midcell, where they are capped by MinE extensions that form the E-ring. It is possible (as shown) that the polar zones assemble on a pre-existing MinD coiled structure that extends between the poles. Assembly of the polar zone and the E-ring proceeds from **a**–**c**, and disassembly occurs in the reverse direction.

cell where the cycle is repeated. A mathematical model based on a polymerization–depolymerization system of this type, including a polar nucleation site, has been proposed that appears to reproduce all of the known characteristics of the *in vivo* system[31].

Although this nicely explains the mechanism of assembly and disassembly of the polar zones during the oscillation cycle, it does not answer the intriguing question of why, during and after disassembly of the polar zone, the released molecules always reassemble at the opposite cell pole. It is possible that there are binding sites at the poles that can act as nucleation sites for the initiation of assembly of the polar zones. The idea that the poles might contain specific protein or lipid binding sites for molecules such as MinD would be consistent with the fact that several other proteins and lipids are specifically located at the poles[32–36]. However, mathematical-modelling studies have shown that a pole-to-pole oscillatory system of this type could be established in the absence of specific polar nucleation sites, based only on diffusional factors, the relative affinities of the proteins for the membrane, and the rate of conversion of the released MinD–ADP to MinD–ATP[37–40]. Although this does not exclude a mechanism that involves polar binding sites, it shows that other mechanisms are possible.

The Min system in other bacterial species. Proteins homologous to the *E. coli* Min proteins are also present in many other bacteria (see Supplementary information S1 (table)). MinC and MinD are present in many Gram-positive and Gram-negative species, with MinD showing the highest degree of sequence conservation. MinE (in Gram-negative bacteria) and its apparent functional homologue DivIVA (in Gram-positive bacteria) are also widely distributed. Homologues of both MinE and DivIVA are present in a few species.

The principles of the *E. coli* MinCDE paradigm, in which a division inhibitor is given topological specificity by another protein to restrict its activity to sites away from midcell, hold true in other organisms in which studies have been carried out[41,42]. Conservation of function between species is shown by the ability of the MinD (MinD*Ng*) and MinE (MinE*Ng*) proteins from the Gram-negative coccus *Neisseria gonorrhoeae* to function when introduced into *E. coli*. Therefore, MinD*Ng* causes a division block in *E. coli*, and MinD*Ng* and MinE*Ng* form polar zones and MinE rings when expressed in *E. coli*. These showed the characteristic pole-to-pole oscillation behaviour of their *E. coli* counterparts[43]. MinC*Ng* and MinD*Ng* also cause a division block when overexpressed in *N. gonorrhoeae* but it is not yet known whether the proteins undergo oscillatory movement within *N. gonorrhoeae* cells.

The use of a pole-to-pole oscillatory system to distribute the division inhibitor to the two ends of the cell is not followed by all species. In *B. subtilis*, the MinCD division inhibitor restricts division to midcell by preventing septation near the poles as it does in *E. coli*, but the proteins remain in position at both poles and do not undergo the characteristic oscillation behaviour seen in *E. coli*[44]. The role of MinE in *B. subtilis* is partially fulfilled by the non-homologous protein DivIVA, which is required for the stable polar localization of MinCD[42]. Functionally, the precise mechanism is probably unimportant as long as the division inhibitor is maintained at the ends of the cell during all, or a large part, of the division cycle.

Interestingly, *B. subtilis* DivIVA also localizes to division sites and cell poles when expressed in *E. coli*, despite its lack of homology to any of the *E. coli* Min proteins[45]. The localization is FtsZ-dependent as it is in *B. subtilis*, indicating that the localization pattern in *E. coli* is probably not fortuitous. Surprisingly, DivIVA also localizes to midcell and the cell poles in the rod-shaped eukaryotic organism *Schizosaccharomyces pombe*[45]. It is not known whether this indicates that DivIVA interacts with a division-related protein or lipid domain in *S. pombe*, which would imply a previously unrecognized relationship between the yeast and bacterial division-related components.

It should be noted that some bacteria, such as *Caulobacter crescentus*, do not contain recognizable Min homologues (TABLE 1; see Supplementary information S1 (table)). In these cases other mechanisms, including nucleoid occlusion (see below) or some as-yet-unidentified mechanism, must exist to ensure the correct placement of the division site.

The Min system in chloroplasts

Among the most interesting discoveries of recent years has been the finding that the Min system also exists and establishes the site of division in plant cell chloroplasts (reviewed in REF. 46). Chloroplasts are believed

Box 2 | **MreB**

MreB is an actin-related bacterial cytoskeletal protein. The *mreB* gene was first identified in *Escherichia coli*[98] as a gene required to maintain the rod shape of the cell. Subsequently, three homologues of *mreB* were found in *Bacillus subtilis*: *mreB*, *mbl* (*mreB*-like), and *mreBH* (*mreB* homologue)[18,99,100]. Evidence that MreB and Mbl are cytoskeletal proteins came from the demonstration that they form long membrane-associated helical structures that coil around the length of the *B. subtilis* cell[18]. The three-dimensional structure of MreB from *Thermotoga maritima*[101] is strikingly similar to the structure of actin, which has a major cytoskeletal role in eukaryotic cells. This lends strong support to the idea that MreB is a prokaryotic equivalent of actin and has a cytoskeletal role in bacterial cells. MreB homologues are present in most rod-shaped bacteria but appear to be absent in spherical bacteria[101]. This is consistent with other evidence that the MreB group of proteins has an essential role in determining and maintaining the shape of rod-shaped bacteria. MreB has been the subject of extensive study during the past few years.

Studies of MreB-depleted cells in several species have shown that MreB is required for other cellular functions in addition to its role in cell-shape regulation. It is not known whether MreB has a direct or indirect role in these events. For example, MreB-depleted cells show defects in chromosome segregation[53,103–105]. This is probably related to the ability of MreB to localize to sequences near the chromosomal origin of replication, as shown by crosslinking and chromatin-immunopreciptiation studies in *Caulobacter crescentus*[106]. The association with the origin region might well involve intermediate linking proteins. MreB also appears to have an important role in regulating cell polarity. Therefore, MreB-depleted cells are defective in polar targeting of several proteins that are normally located at the pole in *C. crescentus*[53,106] and of several *E. coli* proteins[19,54]. Interestingly, despite the fact that MreB is involved in mediating localization of several polar-targeted proteins, these do not include the Min proteins, as shown by the formation of MinD polar zones[19,22] and E-rings[19] in the spherical cells that result from the absence of MreB and their apparently normal spatial relationship to the axis of polarity of the cell, as discussed in this review.

to have originated as bacterial endosymbionts, probably originating from photosynthetic bacteria, and chloroplasts of many plants contain MinD and MinE homologues ($MinD^{CP}$ and $MinE^{CP}$). Studies of the chloroplast proteins suggest that MinD and MinE have similar roles in division-site placement in both bacteria and chloroplasts. Overexpression of $MinE^{CP}$ or loss of $MinD^{CP}$ both lead to misplaced division sites, and overexpression of $MinD^{CP}$ causes global inhibition of chloroplast division[44]. These results resemble those observed with similar manipulations of the *E. coli* Min proteins. Most remarkably, the *Arabidopsis thaliana* MinE protein can apparently carry out some of its functions when introduced into bacterial cells. Therefore, introduction of $MinE^{CP}$ into wild-type *E. coli* leads to a classic minicelling phenotype[44]; $MinE^{CP}$ also counteracts the division inhibition that is induced by overproduction of *E. coli* MinCD (J. Maple and S. G. Møller, cited in REF. 46). In these respects the chloroplast protein mimics the *E. coli* MinE protein. So far, no MinC candidate has been found in chloroplasts, but the analogy to the *E. coli* system indicates that a functional equivalent will be discovered or that perhaps the plant MinD has a division inhibition function. It will be of considerable interest to see whether $MinE^{CP}$ induces pole-to-pole oscillation of the Min proteins in chloroplasts in a manner similar to the behaviour of *E. coli* and *N. gonorrhoeae* MinE in bacterial cells.

Other functions of Min proteins. MinD has homology to a group of proteins that are implicated in the partition of chromosomes[47] and low-copy or single-copy plasmids to opposite sides of the cell[26]. Although there is no convincing evidence that MinD has a direct role in DNA partitioning, it has been reported that the

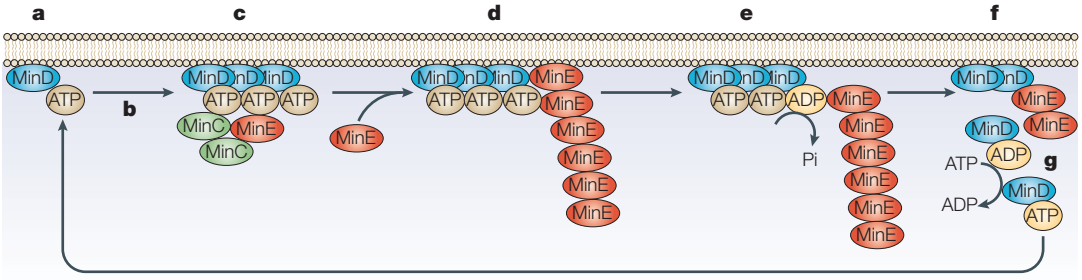

Figure 3 | **The mechanism of polar-zone assembly and disassembly. a** | MinD-ATP associates with the inner surface of the cytoplasmic membrane. **b** | MinD-ATP polymerizes by lateral diffusion and interaction with other membrane-associated MinD-ATP molecules, giving rise to the polar zone. **c** | MinC and MinE associate with MinD-ATP; it is not known whether this occurs at the membrane level, as shown, or within the cytoplasm. **d** | MinE molecules attach to the leading edge of the polar zone, forming the E-ring. **e** | MinE activates the MinD ATPase, leading to conversion of ATP to ADP, and release of phosphate. **f** | MinD-ADP is released from the membrane because of its low affinity for the bilayer. **g** | MinD-ADP is converted to MinD-ATP in the cytoplasm, and diffuses to the other pole where the process is repeated.

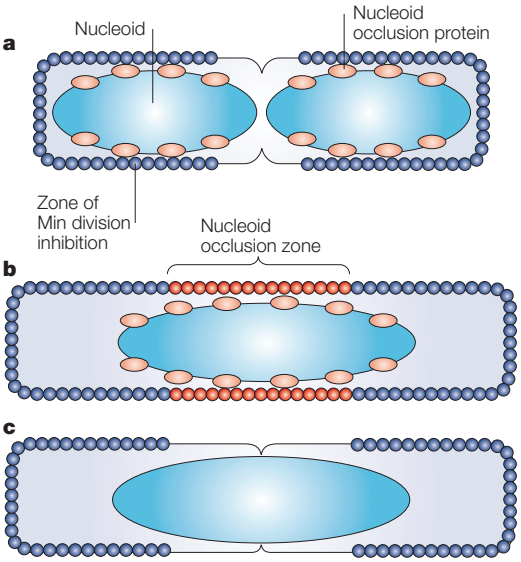

Figure 4 | **The nucleoid occlusion system .** The nucleoid occlusion (NO) proteins (Noc or SlmA, light red ovals) are associated with the nucleoid. **a** | Under normal conditions, the Min system leads to a zone of division inhibition (blue circles) extending from the poles towards midcell. This is apparently sufficient to prevent FtsZ-ring formation and septation over the segregated daughter nucleoids. **b** | When chromosome replication or segregation of daughter chromosomes to the ends of the cell is prevented, the nucleoid remains near midcell and the cells elongate. The NO proteins then prevent FtsZ-ring formation and septation over the nucleoid (the NO zone, red circles). **c** | When the NO protein is absent and chromosome replication or segregation is prevented, the NO effect is lost. The zone of division inhibition induced by the Min system apparently does not extend far enough from the poles to prevent formation of FtsZ rings over the centrally located nucleoid. Figure based on REFS 63,64.

nucleoid-free region at the cell pole is longer than normal in minicell mutants, and that these strains produce a few normal-sized cells that lack nucleoids[48,49]. These observations raise the question of the role of the Min system in nucleoid organization or positioning[50]. It has also been found that *B. subtilis* MinC and MinD are involved in other functions that take place in proximity to the cell poles but are unrelated to division-site placement[51,52]. This indicates that these proteins serve various other functions that remain to be defined.

Cell polarity. The Min proteins are among several proteins that are preferentially located at the poles of rod-shaped bacterial cells. These include proteins involved in locomotion, cell adhesion, cell differentiation and chemotaxis (reviewed in REF. 32). There is evidence that the MreB cytoskeleton is required for the polar localization of several of these proteins, including proteins involved in cell differentiation in *C. crescentus*[53], the Tar chemotaxis protein in *E. coli* (D. Shiomi and I. Kawagishi, personal communication; and REF. 19), and the *Shigella flexneri* virulence protein IcsA[19,54]. However, MreB is not required for the polar localization and oscillatory behaviour of the Min proteins, as formation and oscillation of the Min structures in *E. coli* appear normal in the absence of MreB. The loss of MreB results in conversion of the rod shape of wild-type cells to a spherical shape. Oscillating Min polar zones[19,22] and E-rings[19] are present in spherical Δ*mreB* mutants, and the polar zones undergo apparently normal cycles of oscillation between the two halves of the cell[19]. The formation of oscillating MinD polar zones has also been observed in spherical cells, resulting from loss of the RodA protein[55].

The axis of MinCDE oscillation bears the same spatial relationship to the localization and orientation of the FtsZ ring and septal plane in Δ*mreB cells* as it does in *mreB*$^+$ cells[19], indicating that the Min proteins oscillate between the 'poles' of the cell. This is especially clear in dividing cells, in which the long axis of the cell and the cell poles can be unequivocally identified, with the poles defined as the positions on either side of the division site that are located farthest from the midpoint of the division plane. MinCDE oscillations are also observed in Δ*mreB* spherical cells that have not yet formed visible FtsZ rings[19]. These observations have indicated that the oscillation axis of the Min system is involved in establishing the axis of polarity of the Δ*mreB cells*. Modelling studies have also shown that a stable Min oscillatory system can be initiated in spherical cells[56]. Alternatively, it has been suggested that the initiation of FtsZ-ring formation on one side of spherical cells imparts an asymmetric geometry, and that the Min system uses the long axis of the asymmetric cells to establish its axis of oscillation[55]. In this case, the Min system would not be involved in placement of the FtsZ ring in Δ*mreB* cells although it is in wild-type cells, as described earlier.

Nucleoid occlusion

The Min system is the only system that is known to have an essential role in bacterial division-site placement under normal conditions. However, under abnormal conditions in which chromosome replication or nucleoid segregation is perturbed or delayed, chromosomal DNA remains at midcell at the time when septation normally occurs. In these cases, septal closure at midcell would result in transection of the chromosomal DNA, leading to fragmentation of the genome and the probable demise of the cell. The organism has at least two mechanisms to deal with this type of emergency. First, if the condition is associated with DNA damage or other perturbation of DNA structure, the SOS system is activated[57]. Among other effects, this recruits a global inhibitor of septation, the SulA (SfiA) protein[58], which prevents septation by preventing formation of the FtsZ ring[59]. The SulA division inhibitor lacks site specificity and prevents division over the entire body of the cell.

A second mechanism to prevent septation over nucleoids is called nucleoid occlusion (NO)[60]. The NO system is more specific than the SOS system as it shows site specificity, preventing septation over nucleoids but allowing septation elsewhere. The idea

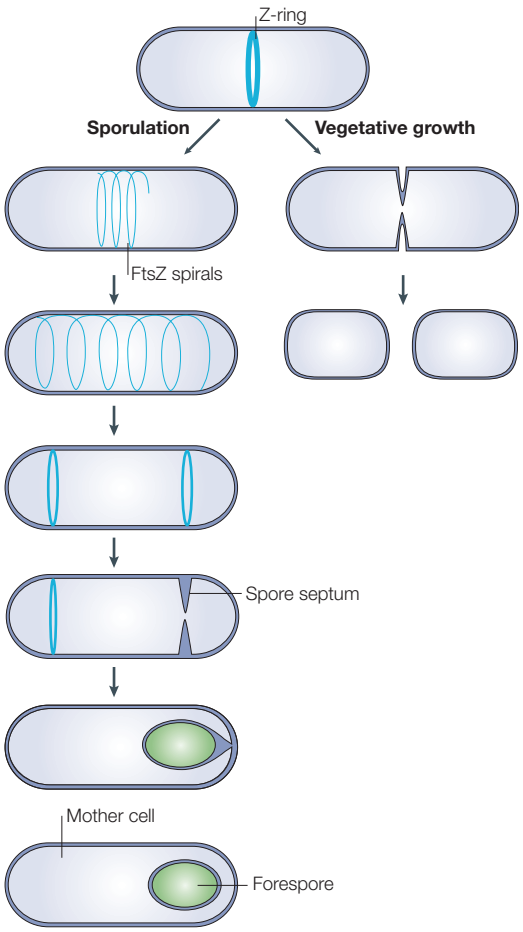

Figure 5 | **The relationship of FtsZ rings and coils to the formation of the polar septum in sporulating cells.** During vegetative growth, the formation of a Z-ring at midcell, and the subsequent assembly of the division machinery and division septum, leads to the formation of two equal daughter cells. By contrast, during sporulation, cell division is asymmetric, and unequal progeny — the forespore and the mother cell — are formed. Asymmetric cell division occurs after the transient formation of FtsZ spirals distributes FtsZ molecules to both poles of the cells, allowing the formation of an FtsZ ring at each pole. Only one of these forms a division or spore septum.

that an NO mechanism exists has come from the observation that division sites tend not to form over nucleoids in some situations in which replication is blocked or nucleoid segregation is prevented[60–64]. Recently, two proteins that have a role in the NO phenomenon have been identified: Noc in *B. subtilis*[63] and SlmA in *E. coli*[64]. These proteins might function in a similar manner although they do not show significant sequence similarity.

SlmA and Noc were identified because they are essential for division in *E. coli* or *B. subtilis* cells in which the Min system is non-functional. Therefore, in the absence of both Min and Noc or SlmA, cells fail to septate. This has been ascribed to the unregulated formation of multiple abortive FtsZ structures over nucleoids, leaving insufficient FtsZ to support division in the internucleoid regions. This idea is consistent with the fact that FtsZ overexpression can suppress the division block that occurs in cells that lack both the Min and NO proteins. As FtsZ structures do not form over nucleoids in cells that contain the Min proteins but lack Noc or SlmA, it seems that formation of FtsZ rings over nucleoids is normally prevented by MinC. If this is correct, under normal conditions of MinCDE expression, MinC can block FtsZ-ring formation along the entire length of the cell except for the region near midcell, and not only near the cell pole (FIG. 4a). The possibility should also be noted that other factors, in addition to the Min and NO systems, might be involved in division-site selection, to explain the high fidelity of division-site placement. The identification of such factors, if they exist, is a challenge for the future.

The best evidence that Noc and SlmA are NO proteins comes from the observation that these proteins are required to prevent assembly of division sites over the unreplicated nucleoids that accumulate near midcell when chromosome replication is blocked by, for example, inactivation of the DnaA protein[63,64] (FIG. 4b). As expected for an NO protein involved in site-specific division inhibition, Noc and SlmA are localized to the nucleoids, and SlmA has been shown to interact with FtsZ[64]. Overexpression of Noc or SlmA causes a global division block leading to filamentation, presumably because the excess protein saturates binding sites on the nucleoid and is then free to prevent division elsewhere in the cell. The proteins are therefore site-specific division inhibitors, the action of which is normally limited to the region of the nucleoid.

Strikingly, so far neither protein appears to be required for proper septal placement or any other detectable cellular function under normal conditions in wild-type cells, whereas the Min system appears capable of preventing septation over nucleoids and at other sites away from the desired midcell site. NO therefore seems to be a fail-safe mechanism, designed to respond to pathological conditions in which chromosome replication or segregation is impaired or significantly delayed. Identification of the two NO proteins represents an important step forward in understanding this phenomenon.

The NO mechanism does not function in several situations where perturbed nucleoid distribution should require it. For example, despite the presence of SlmA, guillotining and fragmentation of nucleoids occurs when *ftsA* or *ftsZ* filaments are allowed to resume division after a period in which DNA replication is blocked by the loss of the DnaA or DnaB proteins in *E. coli*[65]. By contrast, SlmA prevents the division site being placed over nucleoids when DNA replication is blocked by depletion of DnaA in otherwise unperturbed *E. coli* cells[63,64]. A similar guillotine effect is observed during the period of defective nucleoid segregation that occurs in *mukB*[66] or *mreB*[19] mutants in *slmA*$^+$ cells, and NO is also not observed in certain other circumstances[67]. The present evidence suggests that the state of the nucleoid has a role in allowing the NO system to function, and additional components of the system probably remain to be discovered.

Asymmetric or multicentric division

Some bacteria do not divide by septation at midcell (recently reviewed in REF. 68). The best studied are spore-forming bacteria that establish a division septum near the end of the cell during sporulation (see below). In some species, multiple internal division events give rise to multiple spores within the same mother cell. Some non-sporulating species place vegetative septa on one side of the cell, giving rise to a budding division pattern, and in others the cell forms multiple septa along the length of a filamentous parental cell. With the exception of sporulation in *Bacillus* species, little is known about the mechanisms that underlie these unusual division patterns or the genes or gene products that are involved.

During vegetative growth of *B. subtilis*, as in other bacteria, two equal-sized progeny are produced by the formation of an FtsZ ring at midcell, leading to assembly of the division machinery and division septum. However, during sporulation, asymmetric cell division is induced by formation of a division septum near one pole, and progeny of different sizes and developmental fates — the forespore and the mother cell — are produced[69] (FIG. 5). Interestingly, the first event to occur is the formation of FtsZ rings at both poles, only one of which will be used to form the polar spore septum. Formation of the bipolar Z-rings involves the transient appearance of long spiral FtsZ structures that might be intermediates for deploying FtsZ molecules from midcell to the poles[70,71] (FIG. 5). Two factors are necessary to induce bipolar Z-rings[70]: an increase in FtsZ concentration, normally mediated by a burst of *ftsAZ* transcription at the onset of sporulation[72], and the SpoIIE protein, a septum-specific phosphatase that interacts directly with FtsZ and is important for compartmentalization of gene expression during sporulation[73–75]. Increasing the levels of FtsA and FtsZ in *E. coli* also often results in formation of spiral FtsZ structures[76].

Despite the fact that Z-rings are formed at both poles during sporogenesis, ordinarily only one is converted into a division septum. The existence of so-called disporic mutants in which a septum is formed at both poles provides evidence that both of the polar Z-rings can support septum formation. The fact that the two septa form sequentially in disporic mutants[51] indicates that a factor required for cytokinesis[77] might be limiting and can only support the formation of one septum at a time. All disporic mutants carry a mutation in *sigE* or in a gene required for the activation of σ^E or σ^F (REF. 78). The activation of σ^E in the mother cell is dependent on the activation of σ^F in the forespore. These observations imply that formation of a second septum by use of the Z-ring at the distal pole of the cells is normally prevented by a σ^E-controlled gene product that acts at the pole opposite to the spore-forming pole[79,80].

The activation of σ^F, which is necessary for σ^E activation, depends on the formation of the polar spore septum[81–83]. Therefore, the inhibition of septum formation at the distal pole of the cell is delayed until a septum is formed at the forespore-proximal pole. Consequently, the pathway linking σ^E to σ^F and, in turn, σ^F to the polar septum, serves as a morphological checkpoint to ensure that the FtsZ ring at the opposite pole is not inactivated until the spore septum has formed at the other pole.

It is not known how the polar sites are selected during sporulation. To establish a polar Z-ring, the cell must overcome the inhibitory effect of the Min system. Mutations of *minC*, *minD* and *divIVA* have little effect on sporulation[84,85], indicating that the Min system does not have a significant role in selection of the polar site. The MinCD and DivIVA levels fall during sporulation[86] but it is unknown whether this has any role in allowing formation of the polar spore septum. However, the fact that the thin sporulation septum is misplaced from its normal polar site to midcell in a small proportion of *minD* mutants indicates that the Min system or MinD itself could have an auxiliary role in septum placement during sporulation[85,87,88].

Topological memory

Although many interesting and important questions remain to be answered, and unanticipated findings will surely emerge, the main outlines of division-site selection are now becoming clear in cells that divide by binary fission, based primarily on paradigms emerging from studies of the rod-shaped *E.coli* and *B. subtilis*. However, the mechanism of division-site localization in cells that divide asymmetrically is still unknown and should be a fertile field for investigation. In addition, the fact that bacterial cells are often organized in characteristic arrangements that arise from their division patterns represents another virtually untouched area of investigation. In these cases, not only the site of septation but also the orientation of the division septum must be specified.

In rod-shaped cells, the generation of daughter cells of the same size and shape requires that the septum be oriented parallel to the plane of the preceding division event so that both the position and orientation of the division septum are not random. Interestingly, although in spherical cells there are an infinite number of division planes that could generate two equivalent daughter cells, in those cases in which the appropriate studies have been done, division-plane selection also is clearly not random.

In cells such as streptococci, the planes of successive divisions are essentially parallel, giving rise to linear chains of cells. By contrast, in many other coccal species the orientation of the division plane alternates regularly from one division cycle to the next, with each septation event occurring at right angles to the preceding one. In bacteria such as the Sarcinae, the cells form cubical, eight-celled 'packets' that must have arisen by sequential or simultaneous perpendicular divisions in all three planes (X, Y and Z). In *N. gonorrhoeae* and *Staphylococcus aureus*[89,90] it has been directly shown that septation occurs sequentially in three planes (X, Y and Z). Similarly, in spherical cells of *rodA*[91] or *mreB*[19] mutants of *E. coli*, the division plane also alternates sequentially at right angles to the previous division plane. Interestingly, in some organisms that grow on aqueous

surfaces, such as *Lampropedia*[92], the cells appear to use only two of the three possible planes (X and Y) leading to the formation of flat planar arrays of cells.

In all of these examples, selection of the correct orientation of the plane of division depends on knowledge of the division plane used in the previous division cycle. The basis of these intriguing examples of topological memory is unknown.

Concluding thoughts

During the past 50 years, biologists have made phenomenal advances in unravelling the basis of regulation of complicated metabolic and biochemical systems. The concept that cellular life is mediated by linear and branched biochemical pathways was elaborated during the first half of the last century. It then became evident that coordination of these complex pathways required regulatory mechanisms that operate on specific reactions in a pathway. The mechanisms usually involve molecules that act on specific enzyme-catalysed reactions, by affecting the structure and function of individual enzymes. It was also discovered that many essential aspects of cellular development and function required another mode of regulation, in which gene function is modulated by the interaction of regulatory molecules with nucleic acids, rather than with protein enzymes. A great deal is now known about these two modes of regulation.

A third mode of regulation is required to control spatial relationships within the cell, and this requires different paradigms. A prime example of this is the subject of the present review, in which cells require mechanisms to identify the midpoint of the cell or other desired topological sites, so that the biochemical reactions required for cell division occur only at the desired location. As described here, the identification of the midpoint of the cell uses fixed cellular locations — the cell poles — as frames of reference to identify the centre of the cell, which lies midway between the two poles. This is accomplished by the generation of zones of inhibition of septation that extend from the cell poles, with minima at the midpoint of the cell, leaving midcell as the only available site for formation of the division septum. It will be of interest to see whether all schemes of topological identification and spatial regulation also use fixed cellular sites as frames of reference, combined with negative regulatory mechanisms to identify the desired sites.

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Competing interests statement

The authors declare no competing financial interests.

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