

Splitting up: A Time and Place for it in Bacteria

Margaret D. Migocki and Elizabeth J. Harry

School of Molecular and Microbial Biosciences, The University of Sydney, NSW 2006

Note: figures for this article are reproduced in colour on the inside back cover of this issue.

The way in which living organisms divide and grow has fascinated scientists for centuries. A dividing cell needs to ensure that each daughter cell receives a complete copy of the genome. Thus division needs to be coordinated in time and space with chromosome replication. Advances in the field of eukaryotic cell division have revolutionised our understanding of how cells coordinate key cell cycle events like DNA replication and cell division. Despite the relative simplicity of prokaryotic cells, the way in which bacteria coordinate these events and how division itself is regulated remains a mystery.

The recent development and popularisation of fluorescence microscopy and novel genomic approaches in bacteria has, however, made an immense contribution to our understanding of these cell cycle processes. Fluorescence microscopy specifically has led to some revolutionary discoveries in bacteria. These findings include the following: the existence of a bacterial cytoskeleton; the identification of bacterial homologues of the eukaryotic Structural Maintenance of Chromosome (SMC) proteins which mediate the dynamics of chromosome structure and the vital role that protein localisation plays in regulating biological processes, including cell division and DNA replication. Understanding how such a basic process like bacterial cell division is regulated will facilitate the identification of novel targets for antimicrobial agents, a necessary development in terms of overcoming the increasing numbers of antibiotic-resistant disease-causing bacteria.

Cell division and FtsZ

Cell division in bacteria has been studied mainly in the model rod-shaped organisms *Escherichia coli* and *Bacillus subtilis*. Both are well characterised genetically and morphologically. Division in these organisms involves the invagination of the cell wall and membrane layers to form a septum at the centre of the cell, resulting in two identical daughter cells (**Fig. 1**). A protein complex, located at midcell and consisting of at least eight different proteins, drives division. The first protein known to localise to the future division site and to be crucial for the assembly of the division complex is FtsZ. This is an intensively studied, essential protein, highly conserved amongst virtually all eubacteria and archaea; it exhibits remarkable structural and biochemical similarity to eukaryotic tubulin (see refs 1,2). The localisation of FtsZ at midcell is highly dynamic during the cell cycle. Prior to septum formation the protein undergoes a GTP/GDP-dependent polymerisation from a nucleation site on the inner surface of the cell membrane, forming a Z ring (3). The Z ring then contracts with the ingrowth of the cell envelope and remains with the leading edge of the septum (4); the ring is subsequently disassembled. As Z ring formation appears to be the earliest event in bacterial cell division, it is likely that any regulation concerning where and when division takes place occurs at the level of Z ring assembly. Previous studies in both *E. coli* and *B. subtilis* have indicated Z ring formation is not primarily controlled at the level of *ftsZ* transcription but rather at the level of FtsZ polymerisation.

How is the Z ring positioned?

Midcell placement of the Z ring is very precise in rod-shaped bacteria. In *B. subtilis* and *E. coli* Z rings form with a standard deviation of about 2.4% off centre (5,6). The Z ring is also capable of assembling anywhere in the bacterial cell under conditions that remove key regulators of its assembly, indicating that there is not just one nucleation site at midcell (see ref. 1). What ensures the high precision of midcell placement of the Z ring? It has been known for some time that nucleoid occlusion and the Min system influence division site placement. These two distinct mechanisms, outlined below, negatively regulate Z ring assembly by preventing Z rings from forming in the wrong place.

Nucleoid occlusion

The nucleoid occlusion effect prevents septum formation from occurring in regions occupied by the chromosome or nucleoid (7). More recently it has been shown that the nucleoid exerts this negative effect at the level of Z ring assembly (5,8,9) although the molecular mechanism of this effect remains unknown. This model proposes that septum formation becomes possible only when the chromosomes have segregated enough for a DNA-free space to become available within the central region of the cell. However, although complete separation of nucleoids is possibly required for a division septum to form between them, there is no clear evidence to suggest that this is a requirement for midcell Z ring formation. Furthermore, nucleoid occlusion can be overcome in some situations. Z rings and septa have been shown to form around or bisect nucleoids that have not yet been segregated in *E. coli* and *B. subtilis* (8,10,11). These findings suggest that there are probably additional mechanisms guiding Z rings to assemble at midcell.

The Min system

min mutants of *E. coli* and *B. subtilis* are viable but form minicells that lack DNA (see ref. 12). These minicells are a result of divisions occurring at the cell poles rather than at the cell centre. Presumably these polar divisions are occurring as a result of DNA-free regions at the poles. Clearly one of the roles of the Min system is to prevent division from occurring at the poles. The Min system exerts its effect directly at the level of Z ring assembly. The *E. coli* Min system consists of three proteins: MinC and MinD, which form a membrane-bound complex that inhibits Z ring assembly, and MinE, which topologically regulates this complex to relieve MinCD inhibition in the central region of the cell. It has been shown very recently that these proteins exhibit very dynamic localisation in the cell. MinE, and consequently the MinCD complex, has been shown to oscillate rapidly from cell pole to cell pole (13,14). The Min system in *B. subtilis* does not oscillate, lacks the MinE protein and instead includes DivIVA, which anchors MinCD at the cell poles (15). The overall effect in both organisms is the same: the concentration of the MinCD complex is highest at the cell poles and lowest in the central region of the cell, enabling Z ring formation in this region. In *E. coli* this is a time-average effect of the oscillating MinCD inhibitor.

It has recently been proposed, and also supported by mathematical models, that the Min system in *E. coli* is capable of precisely placing a Z ring at midcell. The lowest concentration of the MinCD inhibitor would coincide with the site equidistant from both cell poles, allowing a Z ring to assemble at this site. Is the Min system actually responsible for the highly precise positioning of a Z ring at midcell? We recently answered this question by examining the position of the first Z ring to form in cells growing out from spores of *B. subtilis*, in the presence or absence of the MinCD inhibitor. The *B. subtilis* spore outgrowth system is ideal for these experiments because the system is not complicated by previous cell cycle events. So, unlike vegetatively growing cells of MinCD⁻ strains, one can ensure that the possible formation and removal of a minicell from one end has not influenced the relative position within the rod of the first midcell Z ring. Surprisingly, in the absence of the Min system in *B. subtilis*, the highly precise positioning of the Z ring at midcell is completely maintained (6), suggesting that the Min system is simply preventing Z rings from assembling in the nucleoid-free polar regions of the cell. An example of a polar Z ring is shown in **Fig. 2A**.

The Min system is not essential for bacterial survival nor, as we have shown, is it necessary for the precise placement of a midcell Z ring. It is also not present in some bacteria, for example in *Caulobacter crescentus* and *Haemophilus influenzae*. This strongly suggests that the Min system is not sufficient for the precise midcell placement of the Z ring in all bacteria.

Coordinating chromosome replication with cell division via the Z ring

Recently a third factor has been identified that influences Z ring placement in the cell. This factor is chromosome replication. Could the Z ring be the link that coordinates chromosome replication with cell division? It has been previously shown that termination (completion) of the first round of replication is not an obligatory requirement for midcell division (16). So although a central Z ring may normally form near the end of a round of replication, the signal for it to form is possibly present significantly earlier in the round.

More recently, we examined the possibility that chromosome replication and midcell Z ring assembly are linked. By studying a synchronous cell cycle using the *B. subtilis* spore outgrowth system and immunofluorescence microscopy, we addressed how the timing of assembly and positioning of the Z ring is affected when the first round of replication is inhibited at various stages. Normally Z rings form toward the end of a round of replication. We found that blocking initiation of replication blocks midcell Z ring formation (9). Instead, acentral Z rings form under these conditions (**Fig. 2B**). Allowing initiation and a very small amount of DNA synthesis (10-20% of the chromosome) still blocks midcell Z rings from assembling (11). These findings suggest the existence of a cell cycle checkpoint. This checkpoint prevents Z rings from assembling at midcell too early, before the chromosomes have been sufficiently replicated, and is relieved toward the end of the round.

A clue as to how this checkpoint might function was revealed (11) when assembly of midcell Z rings occurred when initiation of replication was allowed but elongation of the DNA chain was prevented (**Fig. 2C**). Somehow the checkpoint was being relieved prematurely under these conditions. Intriguingly, unlike the wildtype situation, we

discovered that specific degradation of the origin region of the chromosome (*oriC*) occurred under conditions that were allowing this premature midcell Z ring assembly (11). Fluorescence *in situ* hybridisation (FISH) experiments have shown that, prior to completion of the initiation stage of DNA replication, *oriC* is located predominantly at midcell (11). The replication apparatus (replisome), which binds to *oriC* and subsequently facilitates DNA chain elongation, is also positioned predominantly at midcell in *B. subtilis* (17).

Based on these findings, we have formulated a model that proposes a new, additional mechanism that ensures a Z ring assembles at the right place and at the right time (**Fig. 3**). This mechanism incorporates the notion that the midcell localisation of the replisome directly controls the utilisation of the midcell Z ring nucleation site. The model suggests that the completion of initiation of replication is involved in the formation of this nucleation site. During this early stage of the round of replication, the nucleation site is masked by the midcell replisome. The site becomes available for Z ring assembly much later in the round (following 70-80% completion of replication) when each replisome (one on each replication fork) moves away from midcell with the segregating chromosomes. We are proposing that this is a replisome-mediated checkpoint established by the cell to coordinate cell division with chromosome replication.

Of course, the replisome-mediated blocking and unblocking of the nucleation site is the most speculative aspect of the model and this is currently being tested. The model also incorporates nucleoid occlusion, preventing Z rings from forming in regions that the chromosome occupies, and the Min system inhibiting Z ring assembly at the nucleoid-free polar regions of the cell. The early stages of chromosome replication have also recently been shown to be linked to midcell Z ring assembly in the distantly related marine bacterium *C. crescentus*, indicating that, not surprisingly, this link will be conserved in all bacteria (18).

This model introduces the possibility of the existence of a midcell structure to which FtsZ and possibly other elements are recruited. What is targeting these proteins? Differences in composition of the membrane bilayer or membrane proteins serving as an anchor at midcell are possibilities. We are currently investigating the likelihood of the existence of such a membrane or membrane-bound target and are dissecting how the proposed cell cycle checkpoint functions.

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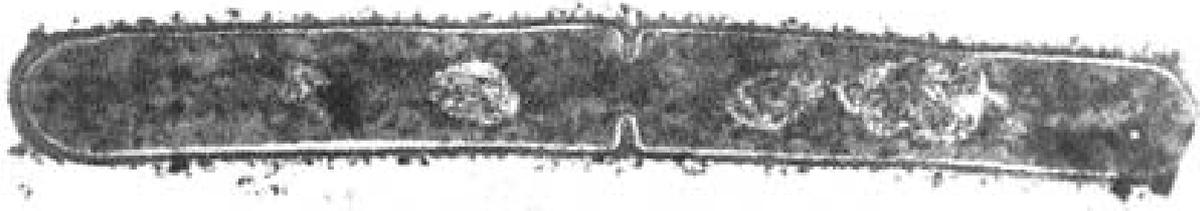


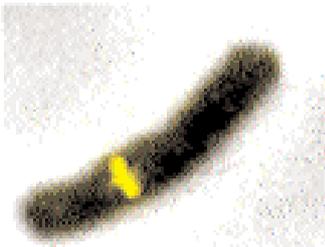
Fig. 1. Electron micrograph of vegetative *Bacillus subtilis* cell undergoing septum formation.

Septum formation occurs at the middle of the cell and involves ingrowth of the cell membrane and cell wall layers. Scale bar is 1 μm .

A



B



C

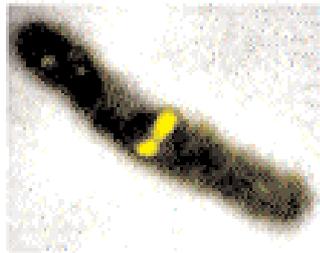


Fig. 2. Z ring positioning under various conditions in outgrown spores of *B. subtilis*.

A. A polar Z ring visualised as an FtsZ-yellow fluorescent protein fusion in live cells in the absence of the Min system.

B and C. Z rings immunostained with fluorescein. An acentral Z ring is observed under conditions that block the initiation stage of DNA replication (**B**) and a midcell Z ring is observed when initiation is allowed but DNA elongation is prevented (**C**). Scale bar is 1 μm .

Splitting up: a Time and Place for it in Bacteria,

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[For main text, see pages 4-5,13]

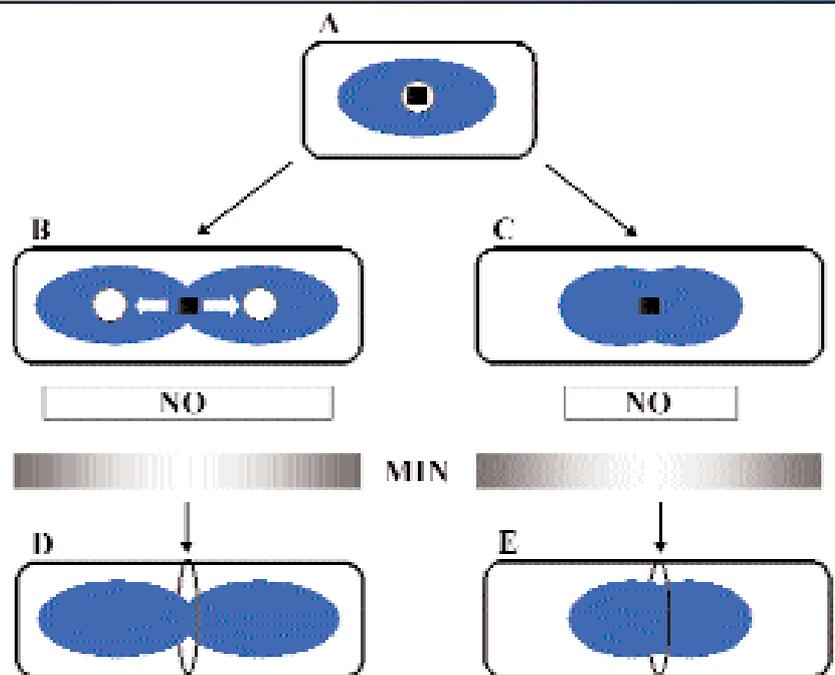


Fig. 3. Model linking chromosome replication with midcell Z ring assembly.

The black square and the open circle represent the midcell Z ring nucleation site (NS) and the replisome respectively. The nucleoids are shaded ovals. **A.** Prior to the elongation stage of DNA replication the NS is masked by the midcell replisome. **B.** Following replication of the chromosome to ~80% completion, with the expanding nucleoid filling most of the cytoplasm, the replisomes move away from the midcell NS, allowing Z ring assembly at the exposed site (**D**). In the absence of thymine (**C**), the elongated cell contains an unreplicated nucleoid and extensive DNA-free regions on either side of it. The replisome is unstable under these conditions and dissociates from *oriC*, exposing the midcell NS for Z ring assembly (**E**). In **B** and **C**, the regions of the cell where Z ring assembly is prevented by nucleoid occlusion (NO) and the Min system (more effective near the cell poles) is shown. Figure adapted from ref. 12.

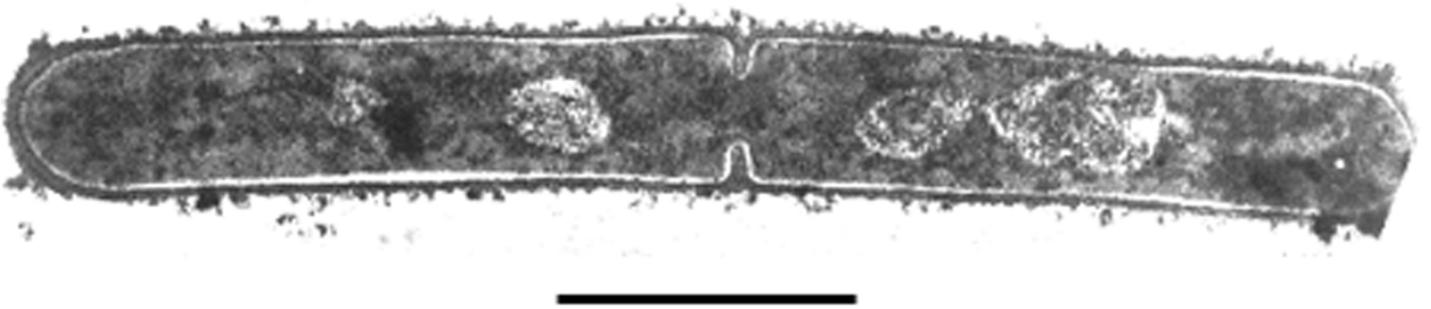
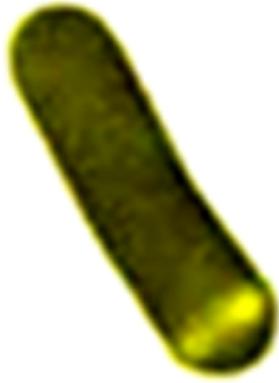
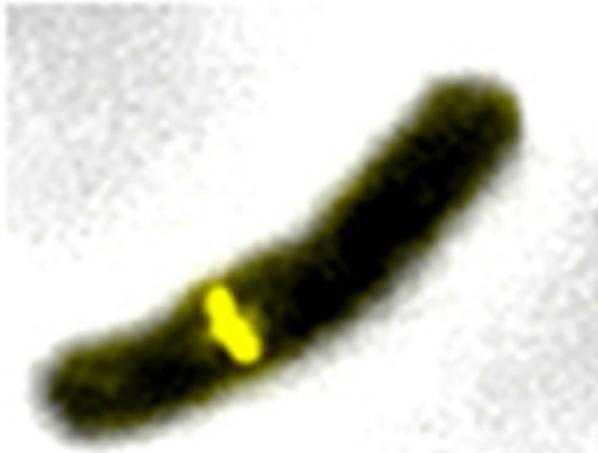


Fig. 1

A



B



C

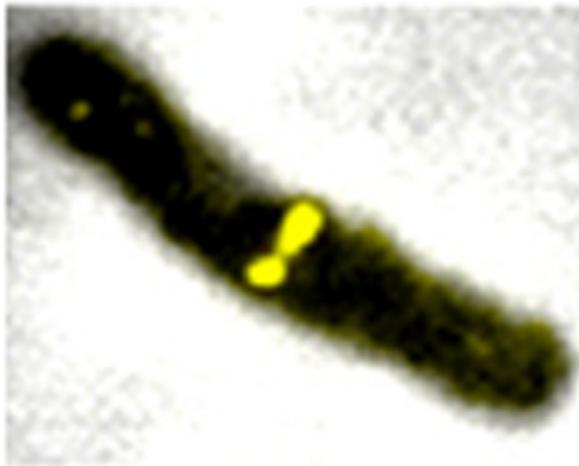


Fig. 2

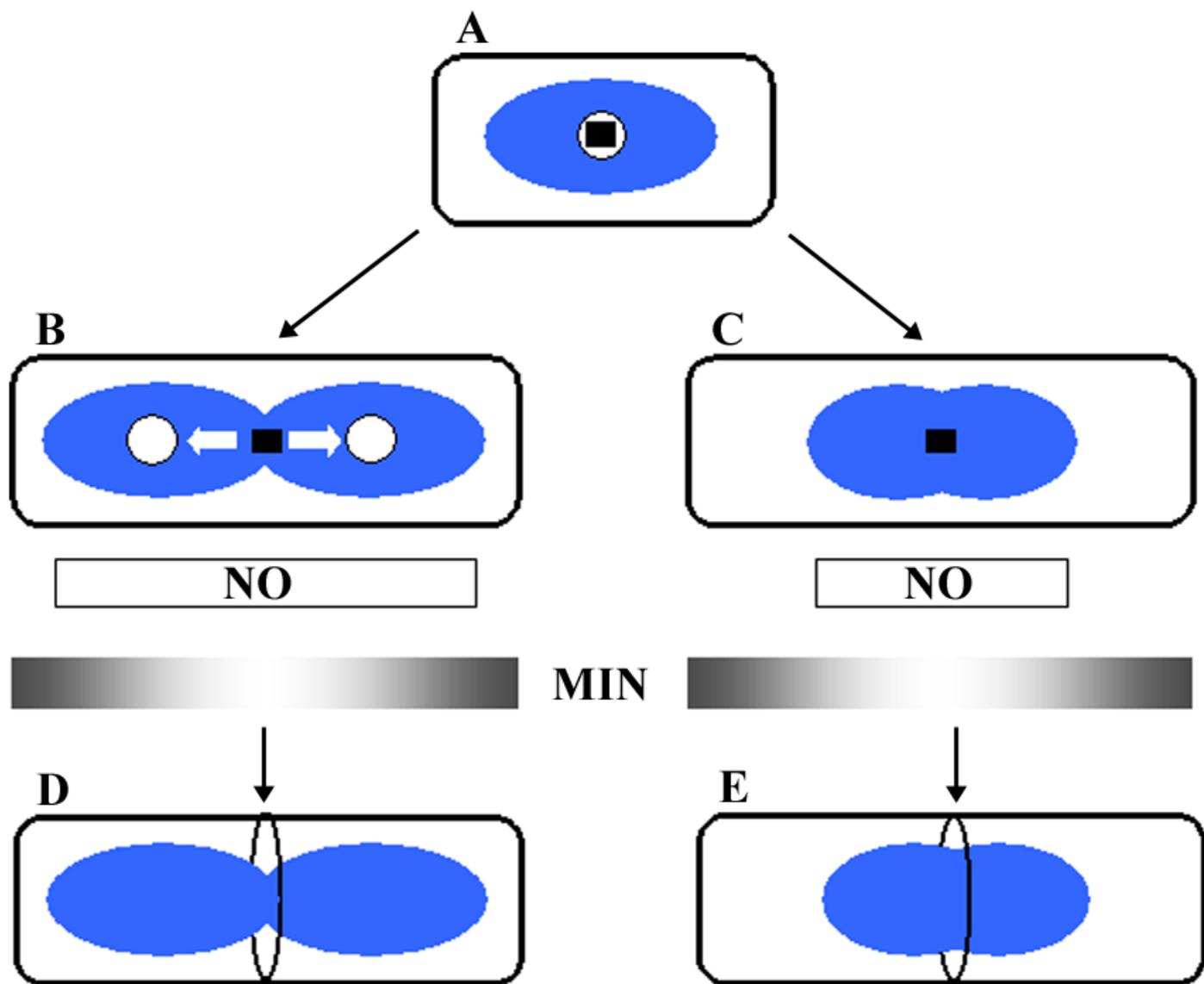


Fig. 3