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Bacterial cell division: regulating Z-ring formation

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Summary

The earliest stage of cell division in bacteria is the formation of a Z ring, composed of a polymer of the FtsZ protein, at the division site. Z rings appear to be synthesized in a bi-directional manner from a nucleation site (NS) located on the inside of the cytoplasmic membrane. It is the utilization of a NS specifically at the site of septum formation that determines where and when division will occur. However, a Z ring can be made to form at positions other than at the division site. How does a cell regulate utilization of a NS at the correct location and at the right time? In rod-shaped bacteria such as *Escherichia coli* and *Bacillus subtilis*, two factors involved in this regulation are the Min system and nucleoid occlusion. It is suggested that in *B. subtilis*, the main role of the Min proteins is to inhibit division at the nucleoid-free cell poles. In *E. coli* it is currently not clear whether the Min system can direct a Z ring to the division site at mid-cell or whether its main role is to ensure that division inhibition occurs away from mid-cell, a role analogous to that in *B. subtilis*. While the nucleoid negatively influences Z-ring formation in its vicinity in these rod-shaped organisms, the exact relationship between nucleoid occlusion and the ability to form a mid-cell Z ring is unresolved. Recent evidence suggests that in *B. subtilis* and *Caulobacter crescentus*, utilization of the NS at the division site is intimately linked to the progress of a round of chromosome replication and this may form the basis of achieving co-ordination between chromosome replication and cell division.

Introduction

Cell division in rod-shaped bacteria involves in-growth of the envelope layers, cell wall and membrane, forming a septum between two replicated chromosomes. Septum formation is driven by a complex of several proteins, which localize to the division site prior to septal in-growth. During vegetative growth in both *Escherichia coli* and *Bacillus subtilis* the division septum forms at mid-cell and cells divide only once they have reached a critical cell mass. Spatial and temporal regulation of cell division must be maintained and co-ordinated with chromosome replication to ensure equal partitioning of chromosomes into daughter cells. This review focuses on the early stages of cell division and discusses our current understanding of how the division site is positioned at mid-cell and of the possible cues required for its utilization. The well-studied rod-shaped bacteria, *E. coli* and *B. subtilis*, will dominate this review but reference will also be made to the differentiating bacterium, *Caulobacter crescentus*, as valuable information regarding cell cycle control of division has been obtained with this organism.

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FtsZ and the Z Ring

The FtsZ protein has caused unabated excitement in the field of bacterial division over the past decade. Why? FtsZ is the most highly conserved bacterial division protein and it plays a key role in cell division (see Margolin, 2000). Immunoelectron microscopy studies in *E. coli* first demonstrated that prior to septum formation the FtsZ protein forms a ring on the inside of the cytoplasmic membrane. This Z ring appears to subsequently contract while maintaining a position at the leading edge of the developing septum (Bi and Lutkenhaus, 1991). Although a Z ring has not been detected in thin sections by electron microscopy, immunofluorescence and GFP studies have confirmed its presence in bacteria (Addinall *et al.*, 1996; Levin and Losick, 1996; Ma *et al.*, 1996; Wang and Lutkenhaus, 1996) and have demonstrated its dynamic nature during the cell cycle (Addinall and Lutkenhaus, 1996; Sun and Margolin, 1998). As well as its remarkable structural similarity to eukaryotic tubulins (Löwe and Amos, 1998), *E. coli* FtsZ can undergo GTP-dependent polymerization *in vitro* to form protofilaments which resemble those within microtubules (Bramhill and Thompson, 1994; Mukherjee and Lutkenhaus, 1994; Erickson *et al.*, 1996; Yu and Margolin, 1997). The similarity to tubulin and the appearance of FtsZ-spirals and -arcs in some *E. coli* mutants, support

the suggestion that the Z ring forms in response to activation of a nucleation site (NS) at mid-cell, leading to the bi-directional growth of the structure, and subsequently the completed ring (Addinall and Lutkenhaus, 1996). FtsZ is the earliest protein of the division complex to assemble at the division site (see Rothfield *et al*., 1999; Margolin, 2000; Errington and Daniel, 2001 for reviews). How do bacteria regulate the positioning and the timing of assembly of the Z ring?

Positioning the division site

In the past decade, two factors have been considered to be involved in correct placement of the division site in rod-shaped bacteria: the Min system and nucleoid occlusion. Although earlier models have suggested that one or the other factor is sufficient, it is becoming apparent that both factors influence the positioning of the division site in *E. coli* and *B. subtilis*. What specific role do they have in positioning the Z ring?

Min system

min mutants of *B. subtilis* and *E. coli* were identified by their ability to form DNA-less cells, or mini-cells, as a result of division at the cell poles (Adler *et al*., 1967; Reeve *et al*., 1973). In these mutants division also occurs at the 'normal' site resulting in a mixture of mini-cells and longer, nucleated rods of variable length.

The *E. coli* Min system consists of three proteins, MinC, MinD and MinE (de Boer *et al*., 1989). MinC is an inhibitor of division that is activated by MinD. MinE topologically regulates MinC, allowing relief of division inhibition in the central region of the cell (de Boer *et al*., 1989, 1992). How is this achieved? Very recent localization studies using functional GFP fusions have revealed a remarkable oscillatory behaviour for all three proteins. The MinCD-inhibitor complex oscillates rapidly from pole to pole, alternately occupying the membrane in either cell half (Hu and Lutkenhaus, 1999; Raskin and de Boer, 1999a, b). The previously described MinE 'ring' (Raskin & de Boer 1997; Rowland *et al*., 2000) has now been shown, in living cells, to undergo a different rapid oscillation that appears to be coupled to MinD oscillation (Fu *et al*., 2001; C.A. Hale, H. Meinhardt and P. A. J. de Boer, personal communication). The bulk of MinE migrates as a ring from mid-cell towards a pole, then reassembles as a ring at mid-cell again and moves towards the other pole. The overall effect is an MinDE-induced oscillation of MinC, such that over any given period of time the membrane occupancy of the MinC inhibitor is highest at the poles and lowest near or at the cell centre.

Several lines of evidence indicate that MinC-mediated division inhibition in *E. coli* occurs at the level of Z-ring assembly (Bi and Lutkenhaus, 1990a, b, 1993; de Boer *et al*., 1990; Hu *et al*., 1999; Hu and Lutkenhaus, 2000). The possibility has been raised that MinC inhibits division at a stage after Z-ring formation (Justice *et al*., 2000), but this is hard to reconcile with the observations that Z rings are absent at cell poles in wild-type *E. coli* cells yet multiple Z rings form in Δ*minCDE* cells (Yu and Margolin, 1999). The oscillatory behaviour of MinE indicates that it is not MinE which is restricted to the mid-cell by the MinCD inhibitor as previously thought (Raskin and de Boer, 1997). Rather, it has been postulated that FtsZ itself finds the centre by sensing MinC (C.A. Hale, H. Meinhardt and P. A. J. de Boer, personal communication). However, it still remains to be demonstrated whether the Min system in *E. coli* plays a direct role in positioning the Z ring to the division site or whether its main role is to ensure that division inhibition occurs away from mid-cell. In anucleate *E. coli* cells Z-ring formation is restricted to a central region (Sun *et al*., 1998), but its positioning is much less precise compared with that in cells containing a nucleoid (Yu and Margolin, 1999). Assuming that the Min system is functioning normally in these DNA-less cells, this result suggests that in the absence of the chromosome, the Min system can restrict Z rings to a central zone in the cell. It is also possible, however, that the Z ring had formed before the division event that gave rise to the anucleate cell (see Cook and Rothfield, 1999) and therefore did not form at mid-cell. Evidence supporting the argument that the Min system can accurately locate a Z ring to the division site comes from recent replication run-out experiments (Gullbrand and Nordström, 2000) in *E. coli* which allow cell-length extension in the absence of initiation of replication. Under these conditions, Z rings were shown to form at future division sites at the 1/4 and 3/4 positions, not at the cell centre. However, again it remains to be determined whether the Min system is responsible for such positioning under these conditions as proposed (Gullbrand and Nordström, 2000).

The ability of Δ*minCDE E. coli* cells to divide medially, as well as at the poles (de Boer *et al*., 1989; Yu and Margolin, 1999), and the significantly higher concentration of MinC at the cell poles compared with elsewhere in the cell, raise the possibility that the role of the Min system is largely to prevent division occurring at the nucleoid-free cell poles rather than having a direct role in defining the site for Z-ring assembly at mid-cell. The absence of a Min system in at least some bacterial species, such as the closely related bacterium, *Haemophilus influenzae* (Margolin, 2000), and also *Caulobacter* (see Quardokus, 2001), both of which have no nucleoid-free regions (Margolin, 2000; Jensen and Shapiro, 1999), is consistent with this possibility.

In *B. subtilis* the MinCD complex acts as a division inhibitor at the level of Z-ring assembly (Marston *et al*., 1998). DivIVA appears to fulfil the topological regulator

role (Cha and Stewart, 1997; Edwards and Errington, 1997), although it is unrelated to MinE and functions very differently (Edwards and Errington, 1997). All three proteins, MinC, MinD and DivIVA, localize to the division site and the cell poles and there is no protein oscillation involved (Edwards and Errington, 1997; Marston *et al*., 1998; Marston and Errington, 1999). Most notably, DivIVA requires FtsZ for its localization to the division site (Marston *et al*., 1998). In fact various localization-dependency experiments have indicated that DivIVA localizes to the division sites late in their maturation (Edwards and Errington, 1997), and remains at the nascent pole even after division is complete. MinC and MinD show only partial dependence on DivIVA for their localization to the division site but DivIVA is needed to retain them at the completed cell poles (Marston *et al*., 1998; Marston and Errington, 1999), thereby blocking extra divisions at this location and also possibly allowing the mid-cell site to be utilized. This division inhibition system, therefore, appears to have no direct role in recruiting the FtsZ protein to the division site at mid-cell. Rather it appears that its function is to inhibit division occurring at the poles.

In *B. subtilis* another negative regulator of Z-ring formation, EzrA, has recently been identified (Levin *et al*., 1999). It has been proposed that EzrA increases the critical concentration needed for Z-ring formation. Unlike MinCD, EzrA appears to act throughout the cell, not just at the poles (Levin *et al*., 1999).

Potential division sites

Why do some bacterial cells such as *E. coli* and *B. subtilis* have a potential to divide at the poles? It has been suggested that there are only three potential division sites in an undivided cell: one at mid-cell and one at each pole (Lutkenhaus, 1993; Donachie and Begg, 1996; Rothfield and Zhao, 1996). The cell poles were interpreted to be old division sites that remain active in the absence of the Min system (Adler, 1967; Teather *et al*., 1974). However, there are now several lines of evidence that challenge these ideas. First, in outgrowing spores of *B. subtilis min* mutants, polar divisions can occur at poles arising from spore outgrowth, not from division (Coyne and Mendelson, 1974). This is unlikely to be due to the persistence of old division sites present prior to sporulation. Second, acentral Z rings observed in outgrown spores of *B. subtilis*, under conditions which inhibit initiation or progression of the first round of chromosome replication, form in DNA-free regions close to the unreplicated nucleoid, not at the poles (Harry *et al*., 1999). In these outgrown cells DNA-free space is not restricted to the poles because cell extension continues in the absence of DNA replication, giving rise to larger nucleoid-free cytoplasmic regions. Third, in *E. coli* cells in which the entire *min* locus has been deleted, Z rings can form at all positions except those occupied by the nucleoid (see below; Yu and Margolin, 1999), strongly suggesting that positions other than the pole and mid-cell are competent for Z-ring assembly. It is more likely that division occurs near the poles because it is the only nucleoid-free region in wild-type cells. Perhaps this explains why *H. influenzae*, which is closely related to *E. coli*, and has no nucleoid-free areas at the poles, does not have a Min system (Margolin, 2000). Likewise, *Caulobacter* cells do not seem to have nucleoid-free regions at the poles (see, for example, Jensen and Shapiro, 1999) and *minCDE* homologues are also absent in this species (see Quardokus *et al*., 2001). Unlike *E. coli*, where overproduction of FtsZ results in mini-cell formation because of extra polar divisions (Ward and Lutkenhaus, 1985), *Caulobacter* forms additional constrictions which are confined to locations close to the division site, never at the poles (Quardokus *et al*., 2001). Apparently in this organism the poles do not represent potential division sites.

Nucleoid occlusion

The nucleoid occlusion model states that the nucleoid exerts a negative effect on division wherever it occupies space in the cell. It is further proposed that chromosome segregation and the resulting diminished occlusion of the nucleoid at the cell centre allows a septum to form at mid-cell (Woldringh *et al*., 1991). In *E. coli* and *B. subtilis*, nucleoid occlusion also appears to occur at the level of Z-ring assembly (Sun *et al*., 1998; Harry *et al*., 1999; Yu and Margolin, 1999; Gullbrand and Nordström, 2000). However, in some situations Z rings can form directly over the nucleoid in both organisms (Sun *et al*., 1998; Cook and Rothfield, 1999; Gullbrand and Nordström, 2000; Regamey *et al*., 2000). In many but not all of these cases, even though there was no DNA-free region at the site of Z-ring assembly, the concentration of DNA at this site appeared lowered and this may be sufficient to relieve the nucleoid veto. Consistent with this suggestion, mid-cell Z-ring assembly in wild-type *E. coli* cells grown under steady-state conditions did not require complete separation of the nucleoids, although there was a slight decrease in DNA concentration in this region (Den Blaauwen *et al*., 1999). In *B. subtilis* a mid-cell Z ring can be made to form prior to movement of replicated chromosomes away from mid-cell but a decrease in DNA concentration at this site accompanied this situation (Wu *et al*., 1995). Interestingly Z rings can form in a central zone in anucleate *E. coli* cells (Sun *et al*., 1998) although they are not very precisely positioned, suggesting that the nucleoid is required for the normal efficiency of Z-ring positioning at mid-cell (Yu and Margolin, 1999).

The above observations are consistent with a model proposed recently by Margolin (2000), suggesting that nucleoid occlusion of mid-cell Z-ring formation in *E. coli and B. subtilis* is relieved early in chromosome segregation. Presumably this proposed early stage results in at least some decrease in DNA concentration at this site. The molecular mechanism of nucleoid occlusion is unknown. However, if it refers solely to the concentration of DNA, there are several observations that suggest that relief of nucleoid occlusion is not sufficient for the accurate positioning of a Z ring at mid-cell, even if a fully functional Min system is present. First, if progression of the first round of chromosome replication is severely limited by thymine limitation, during spore outgrowth in *B. subtilis*, a mid-cell Z ring is unable to form despite a clear DNA-free gap in this region (see below; Regamey *et al*., 2000). Second, relief of nucleoid occlusion does not appear to apply in *Caulobacter*, in which a Z ring appears to assemble at the future division site during the early stages of DNA replication (Quardokus *et al*., 2001). Third and most intriguingly, Levin *et al*. (1998) found that in the absence of the Min system, vegetatively growing *B. subtilis* showed a dramatic preference for forming a Z ring at mid-cell between segregated nucleoids rather than in the DNA-free spaces near the cell poles. This was particularly noticeable in minimal medium (MM). These observations strongly suggest that placement of a Z ring at the division site is regulated, at least in *B. subtilis* and *Caulobacter*, by an additional, or another in the case of *Caulobacter*, factor or mechanism.

Z-ring assembly and the cell cycle

FtsZ levels and chromosome replication

One way in which Z-ring assembly could be cell-cycle regulated is by linking the cellular level of FtsZ with the progress of a round of replication. However, in *B. subtilis* there appears to be no tight link between chromosome replication and the intracellular level of FtsZ (Rowland *et al*., 1997). It is, therefore, likely that utilization of the NS is the key step in promoting mid-cell Z-ring assembly in this organism. In *E. coli* FtsZ is rate limiting for division (Ward and Lutkenhaus, 1985; Bi and Lutkenhaus, 1990c), and the cellular levels of FtsZ appear to be maintained within certain limits and in the required ratio to other division proteins (Rothfield *et al*., 1999; Margolin, 2000). However, it is not clear whether the actual cellular concentration of FtsZ in *E. coli* is linked to chromosome replication (see Dewar and Dorazi, 2000).

The situation is very different in *Caulobacter* in which FtsZ levels are tightly regulated in a cell-cycle dependent manner (Quardokus *et al*., 1996). Cell division in *Caulobacter* gives rise to two different cell types: a motile swarmer cell, which does not replicate DNA or divide, and a sessile stalked cell, which immediately undergoes a new cycle. FtsZ is absent in swarmer cells. Transcription of *ftsZ* and initiation of DNA replication are co-ordinately negatively regulated (Kelly *et al*., 1998) and begin during differentiation of a swarmer cell into a stalked cell (Quardokus *et el*., 1996; Kelly *et al*., 1998). After initiation of cell division FtsZ is rapidly degraded, probably in both cell types (Quardokus *et al*., 1996, 2001; Kelly *et al*., 1998). Interestingly, if the level of FtsZ is raised in swarmer cells to a level sufficient for Z-ring formation, a Z ring still will not form, and it has been suggested that an early stage of DNA replication is also required for Z-ring formation (Quardokus *et al*., 2001).

When does a mid-cell Z ring form relative to a round of replication?

The high frequency of Z rings observed in *B. subtilis* and *E. coli* cells growing in rich medium has led to the suggestion that a Z ring normally forms very early in the round of replication (Addinall *et al*., 1996; Levin and Losick, 1996) but this may reflect the overlapping rounds of replication that would occur in this situation, making it difficult to determine the timing of Z-ring assembly relative to the round of replication to which it is coupled. In *B. subtilis*, while a mid-cell Z ring can obviously be made to assemble appreciably earlier than termination of a round of chromosome replication, it is not known when this ring normally forms (Wu *et al*., 1995). In wild-type *E. coli* cells grown under steady-state conditions, mid-cell Z-ring formation is approximately coincident with termination of replication (Den Blaauwen *et al*., 1999). In *Caulobacter* Z-ring assembly appears to occur during the early stages of DNA replication (Quardokus *et al*., 2001). At least in *Caulobacter* and *B. subtilis*, utilization of the Z ring NS at the division site can occur appreciably earlier than termination of a round of replication.

Linking mid-cell Z-ring assembly with chromosome replication

It is likely that chromosome replication and cell division are co-ordinated at the level of Z-ring assembly. Although the nature of this link is not known for any bacteria, it has recently been proposed that an early stage of DNA replication is required for Z-ring formation at the division site in *Caulobacter* (Quardokus *et al*., 2001). Recent studies using thymine-requiring outgrowing spores of *B. subtilis* also support the idea that chromosome replication and mid-cell Z-ring assembly are linked and have led to a model proposing a molecular mechanism as to how it occurs (Harry *et al*., 1999; Regamey *et al*., 2000). In these studies it was shown that when initiation of the first round of DNA replication was permitted but DNA synthesis was

completely blocked (no thymine added) mid-cell Z rings formed over an extended but unreplicated nucleoid (Harry *et al*., 1999; Regamey *et al*., 2000; see Harry, 2001). So in the absence of thymine a NS at mid-cell can be utilized much earlier than might be expected with respect to progression into the round of replication. Furthermore, using minimal levels of thymine a correlation was observed between the ability to block mid-cell Z-ring assembly and entry into the elongation phase of replication. The raises the possibility that under normal conditions, a checkpoint control is set up at initiation or soon after entry into the elongation phase which blocks mid-cell Z-ring formation (Regamey *et al*., 2000). Intriguingly, the thymineless conditions used resulted in specific degradation of the *oriC*-region of the chromosome (Regamey *et al*., 2000) suggesting that the status of this region is connected with this 'premature' mid-cell Z-ring assembly. FISH experiments demonstrated that *oriC* is predominantly mid-cell in the outgrowing cell prior to commencement of DNA chain growth (Regamey *et al*., 2000). The conformation of the nucleoid under various conditions of DNA replication inhibition or limitation suggested that, while relief of nucleoid occlusion may be at least partly responsible for mid-cell Z-ring assembly in the absence of thymine, it is not sufficient (Regamey *et al*., 2000).

The above observations and the observation by Lemon and Grossman (1998) that the replisome remains fixed at mid-cell in *B. subtilis* for most (80%) of the round of replication, has led to the suggestion that there is a mid-cell structure that defines a NS for Z-ring formation and this is masked by the replisome complex once it is assembled at *oriC*. It is subsequently unmasked much later in the round of replication, when the two replisomes separate and move away from mid-cell. The latter proposition is consistent with the earlier finding that mid-cell division during spore outgrowth could proceed after 60–70% of the round of replication had completed (Wu *et al*., 1995). In the absence of thymine, this checkpoint breaks down due to the inability of the cell to enter the elongation phase of replication resulting in destabilization of the replisome (and perhaps subsequent *oriC*-region degradation), enabling a Z ring to form at mid-cell. It is not yet known whether completion of the initiation phase of chromosome replication is required for a fully utilizable NS at mid-cell in *B. subtilis* (Regamey *et al*., 2000). The possibility remains that a mature NS can form at the division site independently of the initiation phase of chromosome replication as suggested for *E. coli* (Gullbrand and Nordström, 2000).

B. subtilis model for Z-ring regulation

A new comprehensive model to describe how mid-cell Z-ring assembly is regulated in *B. subtilis* has been proposed (Regamey *et al*., 2000) and is shown in Fig. 1. This model takes into account the observation that NSs for Z-ring formation exist in regions of the cell other than the centre (Harry *et al*., 1999; Yu and Margolin, 1999). It retains nucleoid occlusion of Z rings in the region of the cell occupied by the nucleoid, which prevents rings forming at acentral positions of the cell within this region, and the Min system which inhibits Z-ring assembly at the nucleoid-free regions at the poles. It proposes a replisome-mediated replication 'checkpoint' control over mid-cell Z-ring assembly as the third factor involved in this regulation, and it is this which ensures co-ordination between replication and division (Regamey *et al*., 2000). This model explains nicely why an acentral Z ring, which forms instead of a central one under various conditions of DNA-replication inhibition, is restricted close to the edge of the largely unreplicated nucleoid (Harry *et al*., 1999). In this situation, the mid-cell NS is still masked by the replisome and the Z ring forms in the area least inhibited by both the nucleoid at the cell centre and the Min system at the pole.

The idea that the replisome blocks and then unblocks mid-cell Z-ring assembly in *B. subtilis*, although consistent with experimental observations, is the most speculative aspect of the model in Fig. 1 as there is no direct evidence for such a role. Can this aspect of the model be suitably proposed for other bacteria? In *E. coli* and *Caulobacter*, it is not known whether the replisome resides predominantly at the division site and this is a necessary condition for its proposed role. Recent experiments performed with *E. coli* strongly suggest that replication of *oriC* occurs at mid-cell (Hiraga *et al*., 2000) which is consistent with localization of the replisome at this site, at least initially. In *Caulobacter*, current evidence suggests that initiation at *oriC* occurs at one pole (Jensen and Shapiro, 1999), so initially the replisome may be localized there. However, because the terminus is located at mid-cell, for most of the round of replication (Jensen and Shapiro, 1999), it is quite conceivable that the replisome resides at this location predominantly throughout the cell cycle.

Does Z-ring formation occur at a specific point on the inside of the cytoplasmic membrane at the division site, or can it form at this position anywhere around the inner circumference of the membrane at this site? The answer to this is unknown but the replisome-mediated checkpoint model would be more compatible with a point, as Z-ring assembly at the division site is proposed to be masked specifically by the replisome.

A mid-cell target for Z-ring formation?

Another important, and as yet untested, aspect of this model is the existence of a specific structure at mid-cell that defines a Z ring NS. That such a site exists is implied by the proposed role of the replisome in blocking and then

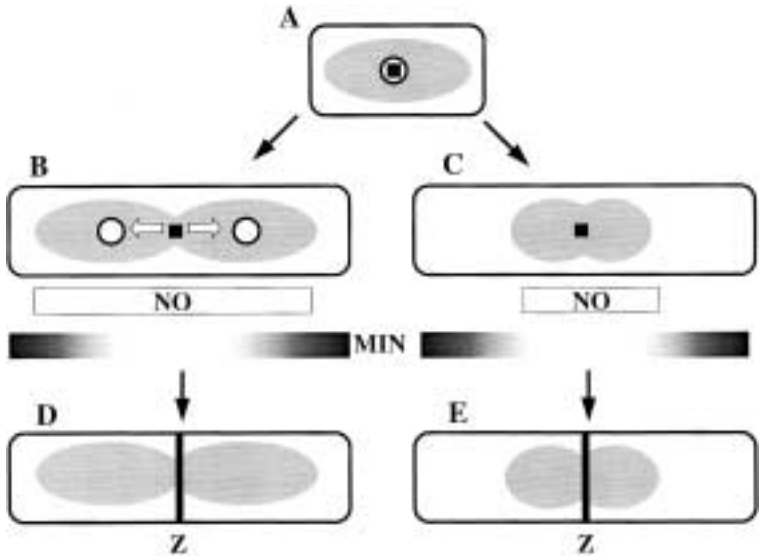

Fig 1. Model for regulation of Z-ring positioning and timing of assembly in *B. subtilis*. The black square represents the mid-cell nucleation site (NS) and the open circle denotes the replisome. In A, both the replisome and the NS are present at mid-cell prior to entry into the elongation phase of replication. The nucleoids are shaded. Utilization of the NS at this stage is masked by the replisome. Once the chromosome has replicated to ~80%, with the expanding nucleoid continuing to fill most of the cytoplasm (B), the replisome resolves into two domains that move away from the mid-cell NS site to allow Z-ring formation (D). The pathway on the right shows the situation in the absence of thymine, resulting in an elongated cell containing an unreplicated nucleoid and more extensive DNA-free regions on each side of it (C). Destabilization of the replisome causes it to disassociate from *oriC*, exposing the mid-cell NS for Z-ring assembly (E). Some outgrown cells in the absence of thymine form an acentral Z ring but the majority form a central Z ring (Harry *et al*., 1999). In B and C, the regions of the cell where Z-ring formation is blocked by nucleoid occlusion (NO) and the Min system (the shading of which is proportional to the level of inhibition) are shown. Modified from Regamey *et al*. (2000).

unblocking mid-cell Z-ring assembly. Consistent with the idea of a site for Z-ring assembly at mid-cell, rather than a central region, is the observation, at least in *E. coli*, that division-site placement is very precise, with a deviation of less than 1% off centre (Trueba, 1982). Z-ring positioning is also highly precise, with the vast majority of rings being located exactly at the cell centre (standard deviation of 2.6%; Yu and Margolin, 1999). The observation that many non-division proteins, such as SeqA of *E. coli* (Hiraga *et al*., 2000) and the replisome in *B. subtilis* (Lemon and Grossman, 1998), and other elements such as low-copy number plasmids (Gordon *et al*., 1997; Niki and Hiraga, 1997), and the terminus region of the chromosome (Webb *et al*., 1998; Lemon *et al*., 2001) also localize to mid-cell, is particularly intriguing. Low-copy-number F and P1 plasmids are capable of localizing to the future division sites in newborn *E. coli* cells, well before Z-ring formation (Gordon *et al*., 1997; Niki and Hiraga, 1997). What positional information are they receiving? Perhaps there is a unique structure or property at the division site, as yet unidentified, that also marks this site for Z-ring utilization in bacteria. This positional marker may be in the form of a protein receptor or a chemical modification on the inner side of the cytoplasmic membrane.

The future

During vegetative growth in *E. coli* and *B. subtilis* Z rings can be made to form at positions other than at the division site at mid-cell, indicating that NSs are not restricted to mid-cell. What enables a cell to utilize a NS at mid-cell so efficiently and precisely and at the right time? In *B. subtilis* and *E. coli*, the Min system and nucleoid occlusion play important roles in regulating the Z-ring position. The main role of the Min system in *B. subtilis* is probably to inhibit division at the nucleoid-free cell poles. In *E. coli*, the Min system may play a more direct role in Z-ring positioning at the division site, but the possibility remains that its main role is analogous to that in *B. subtilis*. While the nucleoid appears to inhibit Z-ring formation in its vicinity, it remains to be determined what nucleoid occlusion actually is and more importantly, what is meant by relief of nucleoid occlusion of Z-ring formation at the cell centre. A nucleoid-free region at mid-cell does not appear to be required for a Z ring to form at this site, although a small decrease in DNA concentration may be necessary. In the differentiating bacterium, *Caulobacter*, the Min system is absent and relief of nucleoid occlusion is not required for a Z ring to form at the division site (see Quardokus *et al*., 2001).

In *B. subtilis* (Regamey *et al*., 2000) and *Caulobacter* (Quardokus *et al*., 2000) it has been suggested that DNA replication is directly linked to NS utilization at the division site. Perhaps the proposed replisome unmasking of a NS at mid-cell in *B. subtilis* is equivalent, in molecular terms, to relief of nucleoid occlusion of Z-ring assembly. This

proposed replisome-mediated checkpoint control of Z-ring formation is attractive and readily testable. Further experimentation with the outgrown spore system in this organism will no doubt continue to contribute significantly to our understanding of the regulation of Z ring at mid-cell and its co-ordination with chromosome replication. It is not known what constitutes a utilizable NS for Z-ring assembly. The observation that many non-division proteins and elements also localize to mid-cell makes it tempting to speculate that there is a unique property or structure at mid-cell that not only provides positional information for these molecules but also defines a Z-ring NS. Elucidation of how the centre of the cell is identified will certainly be exciting challenges for the future.

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References

Addinall, S.G., and Lutkenhaus, J. (1996) FtsZ-spirals and -arcs determine the shape of the invaginating septa in some mutants of *Escherichia coli*. *Mol Microbiol* **22**: 231–237.

Addinall, S., Bi, E., and Lutkenhaus, J. (1996) FtsZ ring formation in fts mutants. *J Bacteriol* **178**: 3877–3884.

Adler, H.I., Fisher, W.D., Cohen, A., and Hardigree, A.A. (1967) Miniature *Escherichia coli* cells deficient in DNA. *Proc Natl Acad Sci USA* **57**: 321–326.

Bi, E., and Lutkenhaus, J. (1990a) Analysis of *ftsZ* mutations that confer resistance to the cell division inhibitor *sulA* (*sfiA*). *J Bacteriol* **172**: 5602–5609.

Bi, E., and Lutkenhaus, J. (1990b) Interaction between the *min* locus and *ftsZ*. *J Bacteriol* **172**: 5610–5616.

Bi, E., and Lutkenhaus, J. (1990c) FtsZ regulates the frequency of cell division in *Escherichia coli*. *J Bacteriol* **172**: 2765–2768.

Bi, E., and Lutkenhaus, J. (1991) FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354**: 161–164.

Bi, E., and Lutkenhaus, J. (1993) Cell division inhibitors, SulA and MinCD, prevent localization of FtsZ. *J Bacteriol* **175**: 1118–1125.

de Boer, P.A.J., Crossley, R.E., and Rothfield, L.I. (1989) A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in E. coli. *Cell* **56**: 641–649.

de Boer, P.A.J., Crossley, R.E., and Rothfield, L.I. (1990) Central role for the *Escherichia coli minC* gene product in two different cell division-inhibition systems. *Proc Natl Acad Sci USA* **87**: 1129–1133.

de Boer, P.A.J., Crossley, R.E., and Rothfield, L.I. (1992) Roles of MinC and MinD in the site-specific septation block mediated by MinCDE system of *Escherichia coli*. *J Bacteriol* **174**: 63–70.

Bramhill, D., and Thompson, C.M. (1994) GTP-dependent polymerization of *Escherichia coli* FtsZ protein to form tubules. *Proc Natl Acad Sci USA* **91**: 5813–5817.

Cha, J.-H., and Stewart, G.C. (1997) The *divIVA* minicell locus of *Bacillus subtilis*. *J Bacteriol* **179**: 1671–1683.

Cook, W.R., and Rothfield, L.I. (1999) Nucleoid-independent identification of cell division sites in *Escherichia coli*. *J Bacteriol* **181**: 1900–1905.

Coyne, S.I., and Mendelson, N.H. (1974) Clonal analysis of cell division in the *Bacillus subtilis divIV-B1* minicell-producing mutant. *J Bacteriol* **118**: 15–20.

Den Blaauwen, T., Buddelmeijer, N., Aarsman, M.E., Hameete, C.M., and Nanninga, N. (1999) Timing of FtsZ assembly in *Escherichia coli*. *J Bacteriol* **181**: 5167–5175.

Dewar, S.J., and Dorazi, R. (2000) Control of division gene expression in *Escherichia coli*. *FEMS Microbiol Lett* **187**: 1–7.

Donachie, W.D., and Begg, K.J. (1996) Division potential in *Escherichia coli*. *J Bacteriol* **178**: 5971–5976.

Edwards, D.H., and Errington, J. (1997) The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division. *Mol Microbiol* **24**: 905–915.

Erickson, H.P., Taylor, D.W., Taylor, K.A., and Bramhill, D. (1996) Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologues of tubulin polymers. *Proc Natl Acad Sci USA* **93**: 519–523.

Errington, J., and Daniel, R.A. (2001) Cell division during growth and sporulation. In *Bacillus Subtilis and its Close Relatives: from Genes to Cells.* Sonenshein, A.L., Losick, R.M., and Hoch, J.A. (eds). Washington, DC: ASM Press, (in press).

Fu, X., Shih, Y.-L., Zhang, Y., and Rothfield, L.I. (2001) The MinE ring required for proper placement of the division site is a mobile structure that changes its cellular location during the *E. coli* division cycle. *Proc. Natl. Acad. Sci. USA*. **98**: 980–985.

Gordon, G.S., Sitnikov, K., Webb, C.D., Teleman, A., Straight, A., Losick, R., *et al.* (1997) Chromosome and low copy plasmid segregation in E. coli: visual evidence for distinct mechanisms. *Cell* **90**: 1113–1121.

Gullbrand, B., and Nordström, K. (2000) FtsZ ring formation without subsequent cell division after replication runout in *Escherichia coli*. *Mol Microbiol* **36**: 1349–1359.

Harry, E.J. (2001) Coordinating DNA replication with cell division: lessons from outgrowing spores. *Biochimie* (in press).

Harry, E.J., Rodwell, J., and Wake, R.G. (1999) Co-ordinating DNA replication with cell division in bacteria: a link between the early stages of a round of replication and mid-cell Z ring assembly. *Mol Microbiol* **33**: 33–40.

Hiraga, S., Ichinose, T., Ichinose, C., Niki, H., and Yamazoe, M. (2000) Bidirectional migration of SeqA-bound hemimethylated DNA clusters and pairing of *oriC* copies in *Escherichia coli*. *Genes Cells* **5**: 327–341.

Hu, Z., and Lutkenhaus, J. (1999) Topological regulation of cell division in *Escherichia coli* involves rapid pole to pole

oscillation of the division inhibitor MinC under control of MinD and MinE. *Mol Microbiol* **34**: 82–90.

Hu, Z., and Lutkenhaus, J. (2000) Analysis of MinC reveals two independent domains involved in interaction with MinD and FtsZ. *J Bacteriol* **182**: 3965–3971.

Hu, S., Mukherjee, A., Pichoff, S., and Lutkenhaus, J. (1999) The MinC component of the division site selection system in *Escherichia coli* interacts with FtsZ to prevent polymerization. *Proc Natl Acad Sci USA* **96**: 14819–14824.

Jensen, R., and Shapiro, L. (1999) The *Caulobacter cresentus smc* gene is required for cell cycle progression and chromosome segregation. *Proc Natl Acad Sci USA* **96**: 10661–10666.

Justice, S.S., García-Lara, J., and Rothfield, L.I. (2000) Cell division inhibitors SulA and MinC/MinD block septum formation at different steps in the assembly of the *Escherichia coli* division machinery. *Mol Microbiol* **37**: 410–423.

Kelly, A.J., Sackett, M.J., Din, N., Quardokus, E., and Brun, Y.V. (1998) Cell cycle-dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter*. *Genes Dev* **12**: 880–893.

Lemon, K.P., and Grossman, A.D. (1998) Localisation of bacterial DNA polymerase: evidence for a factory model of replication. *Science* **282**: 1516–1519.

Lemon, K.P., Kurtser, I., and Grossman, A.D. (2001) Effects of replication termination mutants on chromosome partitioning in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **98**: 212–217.

Levin, P.A., and Losick, R. (1996) Transcription factor SpoOA switches the localization of the cell division protein FtsZ from a medial to a bipolar pattern in *Bacillus subtilis*. *Genes Dev* **10**: 478–488.

Levin, P.A., Shim, J.J., and Grossman, A.D. (1998) Effect of *minCD* on FtsZ ring position and polar septation in *Bacillus subtilis*. *J Bacteriol* **180**: 6048–6051.

Levin, P.A., Kurster, I.G., and Grossman, A.D. (1999) Identification and characterization of a negative regulator of FtsZ ring formation in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **96**: 9642–9647.

Löwe, J., and Amos, L.A. (1998) Crystal structure of the bacterial cell-division protein FtsZ. *Nature* **391**: 203–206.

Lutkenhaus, J. (1993) FtsZ ring in bacterial cytokinesis. *Mol Microbiol* **9**: 403–409.

Ma, X., Ehrhardt, D.W., and Margolin, W. (1996) Colocalization of cell division proteins FtsZ and FtsA to cytoskeletal structures in living *Escherichia coli* cells by using green fluorescent protein. *Proc Natl Acad Sci USA* **93**: 12998–13003.

Margolin, W. (2000) Themes and variations in prokaryotic cell division. *FEMS Microbiol Rev* **24**: 531–548.

Marston, A.L., and Errington, J. (1999) Selection of the midcell division site in *Bacillus subtilis* through MinD-dependent polar localisation and activation of MinC. *Mol Microbiol* **33**: 84–96.

Marston, A.L., Thomaides, H.B., Edwards, D.H., Sharpe, M.E., and Errington, J. (1998) Polar localisation of the MinD protein of *Bacillus subtilis* and its role in selection of the midcell division site. *Genes Dev* **12**: 3419–3430.

Mukherjee, A., and Lutkenhaus, J. (1994) Guanine nucleotide-dependent assembly of FtsZ into filaments. *J Bacteriol* **176**: 2754–2758.

Niki, H., and Hiraga, S. (1997) Subcellular distribution of actively partitioning F plasmid during the cell division cycle in E. coli. *Cell* **90**: 951–957.

Quardokus, E.M., Din, N., and Brun, Y.V. (1996) Cell cycle regulation and cell type-specific localization of the FtsZ division initiation protein in *Caulobacter*. *Proc Natl Acad Sci USA* **93**: 6314–6319.

Quardokus, E.M., Din, N., and Brun, Y.V. (2001) Cell cycle and positional constraints on FtsZ localization and the initiation of cell division in *Caulobacter crescentus*. *Mol Microbiol*. **39**: 949–959.

Raskin, D.M., and de Boer, P.A.J. (1997) The MinE ring: an FtsZ-independent cell structure required for selection of the correct division site in *E. coli*. *Cell* **91**: 685–694.

Raskin, D.M., and de Boer, P.A.J. (1999a) Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc Natl Acad Sci USA* **96**: 4971–4976.

Raskin, D.M., and de Boer, P.A.J. (1999b) MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in *Escherichia coli*. *J Bacteriol* **181**: 6419–6424.

Reeve, J.N., Mendelson, N.H., Coyne, S.I., Hallock, L.L., and Cole, R.M. (1973) Minicells of *Bacillus subtilis*. *J Bacteriol* **114**: 860–873.

Regamey, A., Harry, E.J., and Wake, R.G. (2000) Midcell Z ring assembly in the absence of entry into the elongation phase of the round of replication in bacteria: coordinating chromosome replication with cell division. *Mol Microbiol* **38**: 423–434.

Rothfield. L.I., and Zhao, C.-R. (1996) How do bacteria decide where to divide? *Cell* **84**: 183–186.

Rothfield, L., Justice, S., and García-Lara, J. (1999) Bacterial cell division. *Ann Rev Genet* **33**: 423–448.

Rowland, S.L., Katis, V.L., Partridge, S.R., and Wake, R.G. (1997) DivIB, FtsZ and cell division in *Bacillus subtilis*. *Mol Microbiol* **23**: 295–302.

Rowland, S.L., Fu, X., Sayed, A., Zhang, Y., Cook, W.R., and Rothfield, L.I. (2000) Membrane redistribution of the *Escherichia coli* MinD protein induced by MinE. *J Bacteriol* **182**: 613–619.

Sun, Q., and Margolin, W. (1998) FtsZ dynamics during the cell division cycle of live *Escherichia coli*. *J Bacteriol* **180**: 2050–2056.

Sun, Q., Yu, X.-C., and Margolin, W. (1998) Assembly of the FtsZ ring at the central division site in the absence of the chromosome. *Mol Microbiol* **29**: 491–504.

Teather, R.M., Collins, J.F., and Donachie, W.D. (1974) Quantal behavior of a diffusible factor which initiates septum formation of potential division sites in *Escherichia coli*. *J Bacteriol* **118**: 407–413.

Trueba, F.J. (1982) On the precision and accuracy achieved by *Escherichia coli* cells at fission about their middle. *Arch Microbiol* **131**: 55–59.

Yu, X.-C., and Margolin, W. (1997) Ca^{2+}-mediated GTP-dependent dynamic assembly of bacterial cell division protein FtsZ into asters and polymer networks *in vitro*. *EMBO J* **16**: 5455–5463.

Yu, X.-C., and Margolin, W. (1999) FtsZ ring clusters in *min* and partition mutants: role of both the Min system and the

nucleoid in regulating FtsZ ring localisation. *Mol Microbiol* **32**: 315–326.
Wang, X., and Lutkenhaus, J. (1996) FtsZ ring: the eubacterial division apparatus conserved in archaebacteria. *Mol Microbiol* **21**: 313–320.
Ward, J.E., Jr, and Lutkenhaus, J. (1985) Overproduction of FtsZ induces minicell formation in E. coli. *Cell* **42**: 941–949.
Webb, C.D., Graumann, P.L., Kahana, J.A., Teleman, A.A., Silver, P.A., and Losick, R. (1998) Use of time-lapse microscopy to visualize rapid movement of the replication origin region of the chromosome during the cell cycle in *Bacillus subtilis*. *Mol Microbiol* **28**: 883–892.
Woldringh, C.L., Mulder, E., Huls, P.G., and Vischer, N. (1991) Toporegulation of bacterial division according to the nucleoid occlusion model. *Res Microbiol* **142**: 309–320.
Wu, L.J., Franks, A.H., and Wake, R.G. (1995) Replication through the terminus region of the *Bacillus subtilis* chromosome is not essential for the formation of a division septum that partitions the DNA. *J Bacteriol* **177**: 5711–5715.