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Connecting the dots of the bacterial cell cycle: Coordinating chromosome replication and segregation with cell division

Isabella V. Hajduk[a], Christopher D.A. Rodrigues[b], Elizabeth J. Harry[a,*]

[a] The ithree institute, University of Technology Sydney, Sydney 2007, NSW, Australia
[b] Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Proper division site selection is crucial for the survival of all organisms. What still eludes us is how bacteria position their division site with high precision, and in tight coordination with chromosome replication and segregation. Until recently, the general belief, at least in the model organisms *Bacillus subtilis* and *Escherichia coli*, was that spatial regulation of division comes about by the combined negative regulatory mechanisms of the Min system and nucleoid occlusion. However, as we review here, these two systems cannot be solely responsible for division site selection and we highlight additional regulatory mechanisms that are at play. In this review, we put forward evidence of how chromosome replication and segregation may have direct links with cell division in these bacteria and the benefit of recent advances in chromosome conformation capture techniques in providing important information about how these three processes mechanistically work together to achieve accurate generation of progenitor cells.
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1. Introduction

Survival of any cellular organism relies on the efficient coordination of chromosome replication with cell division to produce viable daughter cells. The ordering of distinct events of cell growth, chromosome replication, chromosome segregation and cytokinesis (cell division) is fundamental to the cell cycle in actively dividing eukaryotic cells and occurs through checkpoints that ensure completion of a prior event before initiation of a subsequent event. However, in the cell cycle of bacteria dividing under high nutrient or high growth conditions, the events of cell growth, chromosome replication, chromosome segregation and the assembly of the division machinery at the division site occur simultaneously. The chromosome begins a subsequent round of replication prior to the completion of the first, a mechanism termed multifork replication. As the round of replication nears completion, the division machinery accumulates and assembles at the division site in preparation for septation, although the subsequent splitting of the daughter cells is held off until midcell has been cleared of DNA. The simultaneous nature of these cell cycle events in bacteria makes advances

* Corresponding author. Tel.: +61 2 9514 4173.
E-mail address: liz.harry@uts.edu.au (E.J. Harry).

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in this area challenging due to the difficulty in separating these processes and, despite intense investigation over several decades, the exact mechanism as to how bacteria spatially and temporally couple septum formation with the replication and segregation of the genetic material is not yet clearly understood. This review focuses on the current evidence of a coordinating link between these processes in *Bacillus subtilis* (Gram-positive model system) and *Escherichia coli* (Gram-negative model system), and the latest advances in chromosome conformation capture techniques to bring us one step closer to answering this fundamental question.

2. DNA replication

DNA replication is a tightly regulated and ordered process divided into three main stages: initiation, synthesis (or elongation) and termination. A key regulatory molecule for ensuring that replication occurs only once per cycle and in synchrony with cell growth and division is initiator DnaA, an AAA+ ATPase (ATPases Associated with various cellular Activities) found in virtually all bacteria [1,2]: levels of DnaA are stringently controlled. Means of its control vary amongst bacteria and have been recently reviewed [1,3,4]. In its ATP-bound form DnaA exists as a helical oligomer that binds to specific AT-rich sequences (DnaA-boxes) within *oriC*. It is the binding of DnaA to the single chromosome origin of replication, *oriC*, positioned at the 0°/360° chromosome position that initiates replication. At these sites DnaA forms a highly ordered nucleoprotein complex, the DNA-unwinding element (DUE) [1,5], effectively melting and unwinding the local double stranded DNA to allow the recruitment of the replication machinery [5,6].

Working together with *oriC*-bound DnaA, in *B. subtilis*, the helicase loader (DnaI) and co-loader proteins (DnaD and DnaB) that are also recruited to this site actively load the helicase (DnaC) to establish the replication fork [7–9]. DNA primase (DnaG), DNA polymerase III holoenzyme (PolC) and the accessory polymerase (DnaE) then bind to the *oriC* region and, together with the other replication proteins, form the replisome thus completing the initiation stage [8]. A similar process, although with differing proteins, occurs in *E. coli*, as reviewed by [10]. [Note, particularly that the DNA helicase in *E. coli* is named DnaB, not DnaC as it is in *B. subtilis* and that there is no homolog of the *B. subtilis* DnaB in *E. coli*.]

Starting at *oriC*, DNA synthesis occurs bi-directionally around the circular chromosome [11–13]. The two replication forks continue bi-directionally until they encounter the terminus region (Ter), at which point the replisome disassembles allowing the decatenation and subsequent complete separation of the newly replicated sister chromosomes [14,15]. Resolution of the chromosomes, when required, is then completed by the combined action of site-specific recombinases located at the terminus (XerCD in *E. coli*, and RipX and CodV in *B. subtilis*) and DNA translocases (FtsK in *E. coli*, and SpoIIIE in *B. subtilis*) [16–21].

3. Chromosome segregation

In *B. subtilis* and *E. coli*, DNA replication and chromosome segregation occur concomitantly. Soon after the origin regions are replicated, they migrate in opposite directions towards the future division sites, located at the cell quarter positions [22]. Most research on chromosome segregation in bacteria has focussed on how these newly-replicated origin regions separate. Separation involves the ParABS system and SMC (Structural Maintenance of Chromosome) condensin complex in *B. subtilis*, and the MukBEF complex in *E. coli*.

Although it is now known to be required for chromosome separation in a number of bacteria, including *B. subtilis*, the ParABS system was first identified by the discovery of two proteins, ParA and ParB, required for effective plasmid partitioning on the P1 plasmid hosted in *E. coli* [23]. ParB was found to bind co-operatively to the *parS* cis-acting site along with ParA, a Walker-type ATPase, to form a large nucleoprotein complex resulting in replicated plasmids segregating bidirectionally to the cell poles [24]. Since its discovery, *par* loci have been subsequently found on the chromosome of over 65% of all sequenced bacterial genomes [25], including *B. subtilis.* In *B. subtilis* however, the components of the ParABS system are known as Soj (ParA) and Spo0J (ParB) because they had been previously observed in *B. subtilis* having an effect on sporulation [26]. Cells lacking Spo0J mislocalise sister origin positions [27], suggesting a role for this protein in separating the newly duplicated chromosome origins in *B. subtilis*. Subsequently it was shown that Spo0J binds to several *parS* sites located within the *oriC* region [28,29], and in cells labelled with Spo0J-GFP, Spo0J co-localises with *oriC* and appears as distinct compact foci positioned at the cell quarters [30,31].

Several elegant studies in recent years have revealed significant insight into the roles of the ParABS system and the SMC condensin complex in chromosome segregation in *B. subtilis.* Following binding to *parS*, Spo0J spreads onto non-specific neighbouring DNA, drawing them together to form a nucleoprotein complex (see Fig. 1B) [30,32]. The method by which Spo0J spreads has been recently proposed by Graham et al., such that Spo0J forms clusters on neighbouring DNA bridging them together, forming DNA loops [33]. The formation of these long-distance DNA loops is suggested to facilitate the condensation and compaction of the origin-proximal region of the chromosome as well as the recruitment and loading of the SMC constituents (Fig. 1B) [33]. The SMC condensin complex (from now on referred to as simply SMC), made up of proteins Smc, ScpA and ScpB, and supplemented by the ParABS system, is then suggested to draw the sister origins away from each other [34]. Essentially, SMC resolves the origins enabling ParABS to actively segregate origins towards opposite poles.

The extent to which Soj actually plays a role in the active segregation of chromosomes is unknown. Interestingly it was shown that the primary role of Soj is likely to be in regulating the initiation of DNA replication (Fig. 1A). It does this by directly interacting with the initiation protein, DnaA [35], inhibiting or promoting DnaA activity. As a monomer, Soj inhibits DnaA activity by preventing formation of its helical oligomer, whereas the Soj dimer relieves this inhibition by allowing DnaA to form oligomers. This ability to switch however is mediated by its interaction with DNA-bound Spo0J (Fig. 1A) [36]. So it is now clear that Spo0J has two separate roles, one in the regulation of DnaA via its effect on Soj self-association, and another in chromosome segregation. These functions have been shown to reside in different domains [37].

The mechanism of chromosome segregation in *E. coli* is more elusive. While the ParABS system is absent in this organism it does possess a distant relative of the SMC complex, MukBEF, a protein complex existing in enterobacteria and some γ-proteobacteria [38,39]. This complex plays a key role in separating newly replicated *oriC* regions [40] and, together with topoisomerase IV (TopoIV), in promoting DNA decatenation [41]. Although it doesn't share any homology with Spo0J, MukB is thought to have a similar bridging function, in that it binds DNA forming a cluster, creating bridges with randomly colliding protein-free DNA. Rybenkov et al. postulates that the formation of these bridges stabilises DNA compaction, potentially assisting in pulling apart the sister chromosomes [42]. It is unlikely these proteins are the sole players in chromosome segregation and there are several hypotheses as to how *E. coli* and other bacteria segregate their chromosomes [12,43,44]. In fact, biophysical models in *E. coli* suggest that chromosome segregation is generated via entropic forces [45]. Application of polymer physics concepts to the bacterial chromosome by Jun and Mulder, resulted in a passive segregation model where the

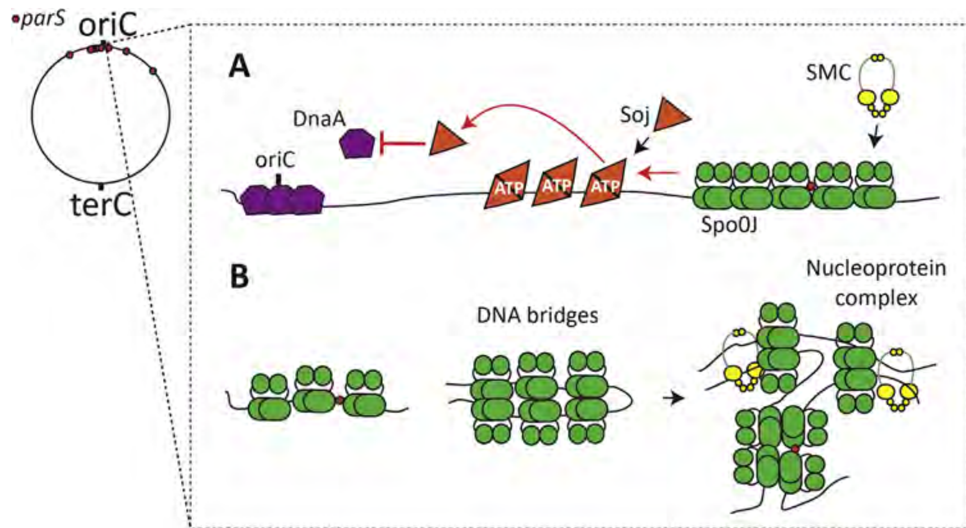

Fig. 1. Roles for Spo0J in chromosome replication and segregation. (A) Role of Spo0J in chromosome replication. DNA-bound Spo0J not only recruits SMC but also regulates the ability of Soj to inhibit DNA replication through DnaA. Spo0J inhibits Soj dimerisation by stimulating its ATPase activity, triggering its monomer form and dissociation from the DNA (red arrows). The Soj monomer is now free to inhibit DnaA by preventing its oligomerisation (red arrows). In its ATP-bound state, Soj binds DNA, thereby relieving the inhibition on DnaA, allowing replication initiation. (B) Model for the role of Spo0J in chromosome segregation by mediating the formation of DNA bridges and large nucleoprotein complexes. Spo0J binds at *parS* sites and spreads on the surrounding DNA forming DNA bridges and trapping DNA loops. Spo0J-mediated recruitment of SMC allows efficient chromosome segregation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

replicated sister chromosomes themselves possess internal forces leading to entropic repulsion or exclusion [45]. However, recent evidence suggests that these entropic forces are insufficient to complete whole chromosome segregation [46,47], highlighting the high complexity of chromosome segregation which requires several different components or modes of action for successful execution.

4. Cell division and regulation of division-site placement

Cell division is dependent on the localisation of numerous specific division proteins to the right place (midcell) at the right time within the cell cycle. The first and foremost of these proteins to localise to midcell is the tubulin-like protein, FtsZ which assembles at the inner face of the cytoplasmic membrane into a ring structure known as the Z ring [48]. The Z ring then facilitates the recruitment of all the other division proteins, together called the divisome [49–51], and provides a contractile force required for the invagination of the envelope layers, or at least the inner cell membrane. Thus, precise recruitment of FtsZ to midcell is central to the regulation of cell division and FtsZ has, over the last 20 years, become one of the most studied bacterial proteins. Much has been elucidated about FtsZ and most, if not all, of the divisome proteins [51], but what yet escapes us is how the assembly of this crucial machinery, not only precisely finds the midcell, but how it does so in concert with the replication and segregation of the chromosome? This question is of utmost importance as the correct timing and positioning of the Z ring between the DNA at midcell is quintessential to the competitive long-term survival of bacteria.

4.1. Negative regulators of Z ring placement

For the past two decades, the positioning of Z ring formation has been described as regulated by the combined action of the Min system and nucleoid occlusion. The Min system prevents the Z ring from forming at the cell poles and nucleoid occlusion prevents the Z ring from forming within the vicinity of the chromosome [52–54]. The overall result is that the two systems prevent the Z ring from forming anywhere other than the cell centre (Fig. 2A).

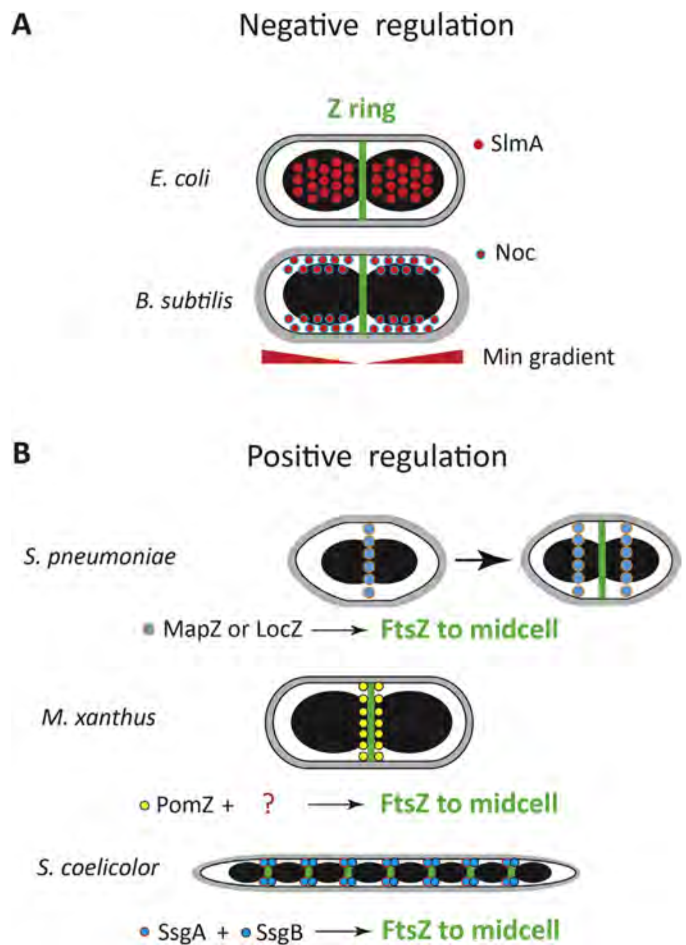

Fig. 2. Regulation of Z ring positioning. (A) Negative regulation of Z ring position by the combined activity of nucleoid occlusion proteins and the Min system gradient. These two systems act by preventing non-productive accumulations of FtsZ at non-medial locations, thereby enhancing efficient and timely Z ring assembly solely at midcell. (B) Positive regulation of Z ring position in diverse bacteria. In all cases a midcell marking protein assembles at midcell prior to FtsZ, thereby defining the midcell accumulation site for Z ring assembly.

Since the discovery of the Min system over 30 years ago [55–57], extensive research has revealed several components of this system that function in a co-operative manner to inhibit polar Z ring assembly and division at the poles. In *E. coli* and *B. subtilis* the Min system consists of two main proteins, MinC and MinD, that function to prevent FtsZ assembly and cell division at the cell poles, and additional Min proteins unique to each organism, that assist in different modes of action. For a complete review of the Min system and its mode of action, the reader is encouraged to read recent reviews [58–60].

Nucleoid occlusion on the other hand, inhibits Z ring formation over the DNA, and is mediated by proteins SlmA (in *E. coli*) and Noc (in *B. subtilis*) [52,53]. Although no sequence homology exists between the two, both SlmA and Noc possess similar characteristics: both proteins bind to specific regions scattered around the chromosome, except for the terminus region, which is largely devoid of these binding sites [61]. This pattern of binding supports the proposal that as chromosome replication nears completion and the terminus region occupies the central position in the cell, SlmA and Noc are no longer present in this region, relieving this area of nucleoid occlusion, thus allowing a Z ring to form there [52]. SlmA and Noc however, have differing modes of action. Recent studies into the activity of SlmA have elucidated two potential mechanisms as to how it inhibits Z ring formation. In the first, SlmA promotes FtsZ depolymerisation [62–64]. When bound to its specific DNA binding sites (SBS), SlmA attaches to the highly conserved C-terminal tail of FtsZ where it competes for binding with other interacting or regulatory partners of FtsZ, including ZipA, FtsZ, ZapD, MinC and ClpX [64]. This promotes further interactions between SlmA and FtsZ, leading to FtsZ protofilament breakage independent of the GTPase activity of FtsZ [64,65]. In a second, alternative hypothesis, Tonthat et al. have suggested that SlmA binds to DNA as a dimer of dimers and spreads along nascent DNA where it forms higher-order nucleoprotein complexes that capture and inhibit FtsZ from coalescing into functional Z rings [66]. Continuing studies into the activity of SlmA are required to elucidate which hypothesis is correct, and, further, to understand how SlmA is able to carry out these membrane-localised functions when tethered to the DNA.

In contrast, Noc in *B. subtilis* mediates its nucleoid occlusion effect by recruiting DNA to the membrane periphery via its newly discovered ability to bind the membrane [67]. Adams et al. propose a model in which Noc mediates its Z ring inhibitory function by physically crowding the available space between the DNA and the membrane periphery such that Z rings are unable to form there [67]. The model raises several questions. Is Noc abundant enough within the cell to mediate this crowding effect on its own or are there other protein players involved? Is this Noc activity coupled with the transertion effect, a theory postulated over 20 years ago, which couples transcription, translation and insertion of membrane proteins? Additionally, what effect does this recruitment of the DNA to the cell periphery have on chromosome organisation and what happens to this organisation in the absence of Noc? Noc could potentially impact chromosome organisation or segregation in a way not previously considered. Noc belongs to the ParB family and shares ∼40% sequence homology with the known chromosome segregation protein, Spo0J [68]. Furthermore, unlike in *B. subtilis*, *Staphylococcus aureus* cells with a *noc* deletion form a significant number of anucleate cells, even during normal, unperturbed growth [69], thus suggesting a role for Noc in chromosome segregation in this organism. This raises the possibility that Noc could also be impacting chromosome organisation or segregation in *B. subtilis* to influence cell division.

Continued study of the Min system and nucleoid occlusion regulatory systems has shown that they cannot be the sole regulators of correct placement of the Z ring at midcell. Under normal growth conditions, when either the Min system or Noc/SlmA in *B. subtilis* or *E. coli* are deleted, cells continue to grow and divide without major changes to cell viability [52,53,70]. However, although division is significantly perturbed in *B. subtilis* and *E. coli* cells devoid of both the Min system and their respective nucleoid occlusion proteins, Z rings nonetheless preferentially form at midcell in internucleoid positions with high precision [53,71,72]. Thus, it appears that the role of the Min system and Noc/SlmA is to ensure there is sufficient FtsZ for Z ring assembly at the desired division site in *B. subtilis* and *E. coli* by limiting the regions in which FtsZ can accumulate. Additionally, a number of bacteria possess only one system, or do not possess either the nucleoid occlusion or the Min protein homologues. Instead, positive mechanisms regulating Z ring positioning have recently been revealed by studies on several of these bacteria, including *Streptococcus pneumoniae*, *Myxococcus xanthus* and *Streptomyces coelicolor*. These are illustrated in Fig. 2B and described below.

4.2. Positive regulators of Z ring placement

A novel protein in *S. pneumonia*, recently described by two independent studies, is named MapZ (Mid-cell Anchored Protein Z) or LocZ (Localising at midcell of FtsZ) [73,74]. MapZ localises to the midcell division site prior to any division proteins, including FtsZ and FtsA. This localisation of MapZ drives the recruitment of FtsZ to its midcell position. Following Z ring assembly, MapZ splits into two rings, which migrate bidirectionally, in tandem with the equatorial rings, to the future division sites [73,74].

Similarly, the ParA-like protein, PomZ (Positioning at Midcell of FtsZ), discovered by Treuner-Lange et al. in *M. xanthus*, localises at the cell centre prior to, and independently of, FtsZ [75]. However, PomZ appears to have a positive spatial and temporal regulatory role. In newborn cells, PomZ is seen to co-localise with the nucleoid. Only once the nucleoid replicates does PomZ migrate to midcell to promote FtsZ recruitment. How it does so is not yet understood. The lack of *in vitro* interaction between purified FtsZ and PomZ, led Treuner-Lange et al. to postulate the existence of interacting partners to mediate PomZ regulatory activity on FtsZ [75].

Interacting proteins positively regulating Z ring placement have also been observed in *S. coelicolor. S. coelicolor* possesses a novel set of proteins unique to Actinobacteria which promote FtsZ recruitment and polymerisation at the correct site. In sporulating *S. coelicolor* cells, the membrane-associated SsgB is localised at midcell by its interaction with SsgA; SsgB then recruits and tethers FtsZ to the division site [76,77].

An outstanding question in these organisms is how do these proteins recognise the future division site? Is there a signal or unidentified marker? And do such positive systems exist in bacteria that possess the Min system and/or nucleoid occlusion proteins? Given that neither the Min system nor nucleoid occlusion are essential in positioning the Z ring correctly in either *E. coli* or *B. subtilis*, it would suggest some other regulatory system exists in these bacteria. A recent positive regulation link has been found between cell division and glycolysis in *B. subtilis.* Monahan et al. describe a model in which PDH E1α (the E1α subunit of pyruvate dehydrogenase, required for the metabolism of pyruvate at the final stage of glycolysis) positively regulates Z ring assembly by co-localizing with the chromosome in a pyruvate-dependent manner [78]. This system may help to coordinate bacterial division with nutritional conditions to ensure the survival of newborn cells. Indeed, increasing evidence is pointing towards aspects of DNA replication and chromosome organisation/segregation influencing cell division in *B. subtilis* and *E. coli*.

5. Coordinating cell division with DNA replication

The first suggestion of a coordinated link between DNA replication and cell division in bacteria came from studies in *B. subtilis*. *B. subtilis* cells are able to begin septation when only 70% of the chromosome has been replicated [79]. Given that Z ring formation precedes septation, this means mechanisms must be at play to trigger this first stage of cell division earlier on in DNA replication. Moreover, blocking the initiation of DNA replication in *B. subtilis* significantly affects Z ring positioning, suggesting a link between these two processes [80,81]. Examining this more closely Moriya et al. examined the effect of different blocks at the initiation stage of DNA replication and found that, the earlier the block in initiation, the less likely a Z ring would form at midcell, with completion of the initiation stage allowing midcell Z rings to form at wild-type levels. Moriya et al. proposed a model, called the Ready-Set-Go model, linking the progression of initiation of DNA replication to midcell Z ring assembly, such that as the initiation phase progresses, midcell becomes increasingly available or "potentiated" for Z ring assembly (Fig. 3). This coincides nicely with the finding that the initiation phase of DNA replication in *B. subtilis* involves several proteins that assemble at *oriC* in a step-wise manner [8]. Most significantly, the "Ready Set Go" phenomenon is independent of Noc [82], and has a positive influence on Z ring placement. What this Z ring potential at midcell actually is, is currently unclear. It is possible that the build-up of the replisome proteins at the medially located *oriC* acts as a beacon for progressive FtsZ accumulation there. Importantly, this study highlighted that Noc activity is insufficient in inhibiting cell division during initiation of DNA replication, suggesting other Noc-independent inhibition strategies must be in place within cells for proper cell division, an idea also supported by studies of Bernard et al. [83].

Linkage between initiation of DNA replication and cell division in *B. subtilis* is further demonstrated in studies by Arjes et al. The authors show that extended inhibition of DNA initiation replication results in an irreversible block to cell division and vice versa. This phenomenon was adequately termed the point of no return (PONR) [84]. What the trigger for the PONR is and why bacteria are unable to resume growth remain outstanding questions. The phenomenon is however independent of the SOS-response and cellular levels of DnaA and FtsZ; and microarray data suggest that the trigger for the PONR may be post-transcriptional [84].

More recently, midcell Z ring assembly has also been linked to DNA replication in *E. coli*. Cambridge et al. found midcell Z ring assembly was inhibited when DNA replication elongation was blocked [85]. Importantly, this occurred in a SlmA-, MinC- and SOS-independent manner. Overall, this finding suggests that DNA replication playing a positive role in Z ring positioning is not exclusive to *B. subtilis*, but is likely to occur in a number of organisms.

6. Coordinating cell division with chromosome organisation and segregation

While the nucleoid occlusion proteins Noc and SlmA are typically associated with coordinating cell division and chromosome segregation, a variety of mutations in chromosome segregation proteins have long been known to lead to incorrect Z ring positioning in both *E. coli* and *B. subtilis* [26,39], providing clear evidence that the two processes are connected. The absence of any of the constituents of the *E. coli* MukBEF complex results in temperature sensitivity, loss of chromosome organisation and condensation, and generation of ~5% anucleate cells at the permissive temperature due to Z ring misplacement [39]. Similarly, *B. subtilis* cells lacking *smc* (under slow growth conditions) or *spo0J* exhibit aberrant positioning, or level of condensation of the nucleoid, and also result in formation of anucleate cells [26,27,86,87]. In minimal media, deletion of both *smc* and *spo0J* enhances this effect whereby the frequency of anucleate cells increases to 19%, with 12% of cells containing nucleoids guillotined by the septum [27]. While these cell division phenotypes of chromosome segregation mutants have been known for a long time, it remains unclear if chromosome segregation and division site positioning are coupled by the chromosome segregation proteins themselves. At the heart of this question is the fact that misplacement of Z rings in chromosome segregation mutants can occur indirectly through nucleoid occlusion: chromosome segregation mutants alter chromosome architecture, thus resulting in improper Noc/SlmA-DNA localisation within the cell and misplaced Z rings. However, it still remains possible that chromosome segregation proteins may actually directly contribute to Z ring placement, independently of their indirect consequences on nucleoid occlusion. One hypothesis is that they may participate directly in establishing the Z ring site; however no direct interaction between chromosome segregation proteins and FtsZ has ever been reported in the literature. A second hypothesis is that through their chromosome-organizing activities, they contribute to an unknown aspect of chromosome organisation that is directly linked to cell division. In favour of this hypothesis is the recent observation that the organisation of a specific region of the *E. coli* chromosome contributes to establishing the Z ring position at midcell. Bailey et al. found that the Ter macrodomain of the chromosome in *E. coli* becomes important for midcell Z ring positioning in the absence of SlmA and the Min system [72]. Specifically, combining the *slmA min* double mutant with a mutant in MatP (the protein that organises the Ter macrodomain) affected midcell Z ring precision. The authors also demonstrated that this effect is mediated through interactions between MatP and the divisome proteins ZapB and ZapA. These interactions were established by Espéli et al. [88]. Thus, these results suggest that the organisation of the Ter macrodomain plays a positive role in Z ring positioning. Intriguingly, in the absence of this link to the Ter macrodomain, SlmA and the Min system, there is still a slight midcell bias for Z ring placement. Thus, these modest effects to Z ring positioning suggest that many levels of control are required for bacteria to accurately coordinate division with chromosome organisation.

In analogy to MatP, and as mentioned above, Spo0J, SMC and MukBEF are suggested to be involved in the overall organisation of the origin region following its replication [33,42,89]. Marbouty et al. and Wang et al. have very recently utilised Hi-C techniques in *B. subtilis* to elucidate the structure of the chromosome in this organism [90,91]. As well as identifying both short- and long-range chromosomal DNA interactions within the *B. subtilis* chromosome, both these studies directly demonstrate the requirement of the ParABS system and SMC complex in origin-region resolution, reformation and segregation following its duplication. Both studies also elegantly shine a light on the intrinsic link between DNA replication and chromosome organisation. Blocking initiation of DNA replication elucidated an effect on these DNA interactions and demonstrated a loss to normal chromosome organisation and segregation. These studies make it intriguing to see which chromosome interaction domains are lost in chromosome organisation mutants resulting in abnormal Z ring formation such as those in the absence of *spo0J*, *smc* or *mukB*. It is possible that changes to chromosome architecture as a result of the action of chromosome segregation proteins is vital for large scale compaction of the origin region to bring together sequence-distal operons to allow for important interactions, for example to form a signal or to localise protein-protein interactions required for proper Z ring formation. Continued studies in this area will surely further lead to the emergence of how these three key processes of DNA replication, chromosome organisation and cell division come together in perfect synchrony.

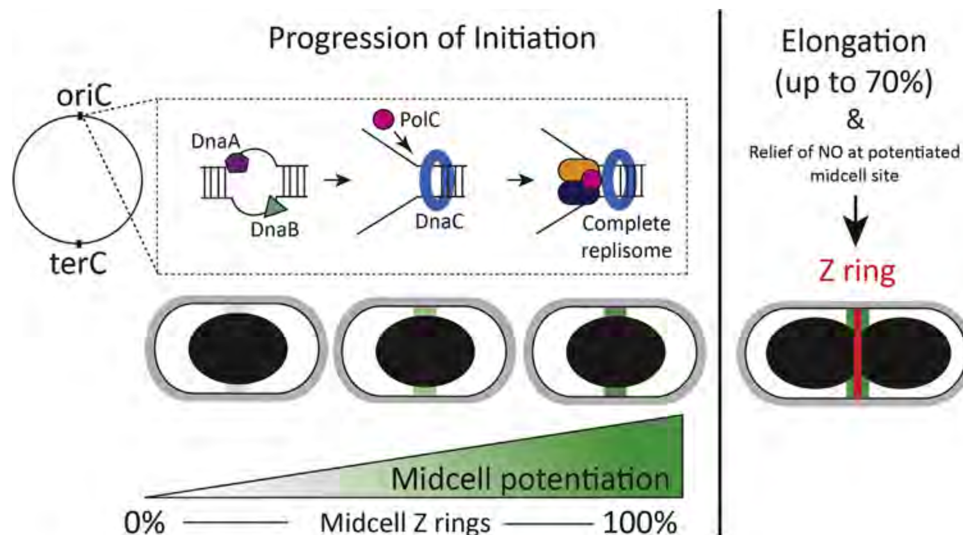

Fig. 3. The Ready-Set-Go model linking DNA replication initiation to midcell Z ring assembly. On the left panel, early in the cell cycle the progression of DNA replication initiation is dependent on the ordered assembly of initiation proteins and replisome components. The midcell position is potentiated for Z ring assembly there as the initiation stage progresses towards complete replisome assembly. Complete replisome assembly allows for 100% midcell Z ring assembly. On the right panel, upon 70% completion of chromosome replication, relief of nucleoid occlusion allows the Z ring to assemble at the midcell potentiated site that was established earlier in the DNA replication cycle.

7. Concluding remarks

Critical to a greater understanding of the spatial and temporal dynamics of cell division and chromosome segregation proteins and chromosomal loci is a greater appreciation of the architecture of the chromosome under various conditions, and the important role this plays in cell cycle regulation. For example, it is not yet clear exactly how or in what way cellular processes such as DNA replication, chromosome segregation or transcription affect chromosome architecture. Isolating the influence of each of these processes on chromosome architecture, and pinpointing cause and effect, will be challenging. Recent advances in technologies to look closer at how the chromosome is compacted and organised will be of great value in this endeavour. Genome-wide conformation capture techniques such as Hi-C and super-resolution microscopy allow us to detect chromosome interaction domains and infer information on the spatial organisation of the chromosome [90–93]. Examining chromosome architecture on a more global scale using these technologies and in different environmental or growth conditions will give us insight into how bacteria coordinate chromosome replication, segregation and Z ring formation under various situations to allow proper daughter cell propagation.

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