Towards understanding the molecular basis of bacterial DNA segregation

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Bacteria ensure the fidelity of genetic inheritance by the coordinated control of chromosome segregation and cell division. Here, we review the molecules and mechanisms that govern the correct subcellular positioning and rapid separation of newly replicated chromosomes and plasmids towards the cell poles and, significantly, the emergence of mitotic-like machineries capable of segregating plasmid DNA. We further describe surprising similarities between proteins involved in DNA partitioning (ParA/ParB) and control of cell division (MinD/MinE), suggesting a mechanism for intracellular positioning common to the two processes. Finally, we discuss the role that the bacterial cytoskeleton plays in DNA partitioning and the missing link between prokaryotes and eukaryotes that is bacterial mechano-chemical motor proteins.

Keywords: Soj; Spo0J; *ParAB*; MinCDE; partitioning system; chromosome segregation

1. INTRODUCTION

In contrast to our understanding of eukaryotic mitosis, the principles that underlie the partitioning of DNA in prokaryotes are still largely unknown. Several models have been put forward. Forty years ago, Jacob *et al.* (1963) postulated the involvement of the elongating cellular envelope as a means of DNA segregation. However, recent experiments revealing that the origins of bacterial chromosomes rapidly separate, in a manner independent of cell elongation, have rendered this model invalid. The finding that DNA replication in *Bacillus subtilis* and *Escherichia coli* probably occurs at a stationary, centrally located replication factory has led to the proposal that bi-directional extrusion of newly replicated DNA from the replication factory followed by DNA condensation might constrain the motion of sister nucleoids to opposite sides of the division plane (Gordon & Wright 2000; Koppes *et al.* 1999; Lemon & Grossman 1998, 2000, 2001; Onogi *et al.* 2002; Sawitzke & Austin 2001). Transertion (coupled transcription, translation and insertion) of membrane proteins has been speculated to have a role in chromosome segregation (Norris 1995; Woldringh 2002) and more recently, Dworkin & Losick (2002) have proposed RNA polymerase as a novel candidate for driving the poleward motion of segregating bacterial chromosomes. The fifth, most radical model involves the existence of a prokaryotic mitotic-like machinery that actively transports plasmid and, possibly, chromosomal DNA into each daughter cell (Møller-Jensen *et al.* 2002).

2. DNA PARTITIONING OF THE LOW COPY NUMBER PLASMIDS

The field of bacterial DNA partitioning has largely focused on studies of the low copy number plasmids P1, F factor and R1 of *E. coli* and the broad host range plasmid RP4. These model plasmids contain several different types of systems that prevent plasmid loss at cell division (Nordstrom & Austin 1989). One such type encodes a differentiation system that leads to the killing of plasmid-free cells and thereby confers plasmid stabilization (Gerdes *et al.* 1997; Jensen & Gerdes 1995). Conversely, true partitioning systems stabilize their replicons by actively distributing the plasmid molecules to the daughter cells, and this becomes essential for very low copy number plasmids.

3. *E. coli* PLASMID P1 AND F FACTOR

In general, plasmid partitioning modules encode two trans-acting proteins and a *cis*-acting, centromere-like DNA sequence required for partitioning (table 1). The P1 *par* and F *sop* family of partitioning systems encode: (i) homologous ATPases, ParA and SopA, respectively, which are characterized by the presence of a conserved 'deviant' Walker A motif containing a signature lysine (Koonin 1993); and (ii) site-specific DNA-binding proteins containing helix-turn-helix (HTH) motifs, ParB and SopB, respectively. The centromere-like sites *parS* and *sopC* are located downstream of the genes encoding the partitioning proteins.

In the P1 system, ParB binds like a dimer to the *parS* site in conjunction with the host factor, IHF (Funnell 1988b) to form a higher-order nucleoprotein complex called the partition complex (Davis & Austin 1988; Funnell 1988a, 1991). Additional ParB molecules can bind to the flanking sequences, leading to silencing of nearby genes (Bouet *et al.* 2000; Rodionov *et al.* 1999). Overexpression of both ParA and ParB interferes with partitioning. Overproduction of ParB was found to make all *parS*-carrying plasmids even more unstable

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Table 1. Gene and protein names. (MreB, bacterial actin homologue, forming intracellular helices; FtsZ, bacterial tubulin homologue, forming a ring at mid-cell during division; MinC, inhibitor of FtsZ polymerization, binds to MinD; MinD, ParA/NifH-like ATPase, binds to membrane and oscillates from pole to pole; MinE, MinD binding, enhances MinD ATP hydrolysis rate.)

partitioning system	R1 <i>par</i>	P1 <i>par</i>	F1 <i>sop</i>	RP4	chromosome	sporulation
<i>cis</i> -acting DNA	<i>parC</i>	<i>parS</i>	<i>sopC</i>	O _B 1-12	<i>parS</i>	<i>parS</i>
DNA-binding protein	ParR	ParB	SopB	KorB	ParB	Spo0J
ATPase protein	ParM	ParA	SopA	IncC	ParA	Soj

than if they were randomly distributed at cell division (Lobocka & Yarmolinsky 1996). SopB of F plasmid has also been reported to disrupt partitioning when overexpressed (Mori *et al.* 1986) and, in a similar manner to P1 ParB, appears capable of silencing genes flanking the *parS* analogue *sopC* (Lynch & Wang 1995). ParB contains two multimerization domains including a primary dimerization domain located in the C-terminal 59, amino acids of the protein and a second self-association domain within the N-terminal half of ParB. These observations led to the proposal that the two domains play distinct roles in the formation of the partition complex (Surtees & Funnell 1999). It has long been assumed that the mechanism of partitioning would first involve pairing of the elements to be partitioned before they proceed to opposite poles of a dividing cell, a process not unlike the pairing of sister chromatids during the metaphase stage of eukaryotic mitosis. *In vivo* evidence that ParB can mediate intermolecular pairing of P1 plasmid centromeres (Edgar *et al.* 2001) has given weight to this hypothesis.

The postulated role of ParA is to supply energy for a subsequent step, the obvious one being plasmid movement (Davis *et al.* 1992). ParA has been shown to possess weak ATPase activity, which is stimulated *in vitro* by ParB and non-specific DNA (Davis *et al.* 1992). The determinant for ParB-mediated stimulation of ParA ATPase activity has been localized to the N-terminal 40 amino acids of ParB (Radnedge *et al.* 1998), whereas SopB has been shown to contain a species specific SopA interaction determinant within its N-terminal 45 amino acids (Ravin *et al.* 2003).

Autoregulation is common to the plasmid partitioning modules. Repression of P1 *par* transcription is exerted by the binding of ParA to a sequence that overlaps the *parAB* promoter (Davis *et al.* 1992). Binding of ParA to the operator site requires either ATP (Davis *et al.* 1992) or ADP (Davey & Funnell 1994), and repression is enhanced by ParB (Friedman & Austin 1988). However, studies of SopA of F plasmid have found that no nucleotide is required for its autoregulatory function (Mori *et al.* 1989), suggesting that the ATPase activity of SopA is directly involved in partitioning. Genetic data also show a requirement for ParA in partition, even when its regulatory role is bypassed (Davis *et al.* 1996; Friedman & Austin, 1988). ParA has been found to dimerize in the presence of ADP and ATP (Davey & Funnell 1994), suggesting that ParA binds DNA as a dimer. It has since been shown that ParA assembles onto the partition complex at *parS* and that this interaction

is completely dependent on ATP, whereas the repressor activity of ParA prefers the ADP-bound form. These observations have led to the proposal that an ATP-ADP switch controls ParA entry into two separate pathways in which ParA plays separate roles (Bouet & Funnell 1999).

How is partitioning achieved in the cell? Time-lapse fluorescence microscopy studies have been used to investigate this question. P1 Plasmid copies have been visualized as a single focus at the cell centre or as two focuses at one-quarter and three-quarter positions in *E. coli*. After cell division, these quarter positions correspond to the new cell centres (Gordon *et al.* 1997). The observation that ParB forms focuses at the cell quarter positions in the presence of a P1 plasmid is consistent with a direct role in segregation (Erdmann *et al.* 1999). In the absence of ParA, focuses were formed that failed to localize to the quarter positions (Erdmann *et al.* 1999), and partition was defective. ParA and ParB have now been shown to actively mediate the segregation of P1 copies to the quarter positions in dividing cells. P1 plasmids expressing a dominant negative ParB, Δ 30ParB form a plasmid focus at the cell centre, but never segregate to the cell quarter positions (Li & Austin 2002). In the absence of ParB, a plasmid focus is formed that does not localize to the cell centre, does not divide and is inherited by one daughter cell only. In the absence of ParA, focuses formed and frequently localized to the cell centre, but failed to segregate (Li *et al.* 2004). Thus, ParB appears to be required for plasmid localization to mid-cell, whereas ParA is required for focus division and ejection from the cell centre. Consistent with this, the ParA ATPase active site mutant, K122E, that abrogates nucleotide binding, blocks plasmid partitioning and the mutant M314I show a missegregation defect (Li *et al.* 2004) most probably attributable to a reduced affinity for nucleotide.

4. STRUCTURAL INSIGHTS FROM THE BROAD-HOST-RANGE PLASMID RP4: IncC/KorB (ParA/ParB)

The self-transmissible plasmids of incompatibility group P (IncP) are maintained as stable, autonomously replicating elements in a wide variety of Gram-negative hosts (Sia *et al.* 1995). RP4 is a 60 kbp IncP- α plasmid that contains a partitioning module comprising the ParA-like ATPase IncC, a site-specific DNA-binding ParB-like protein, KorB, and DNA sequences to which KorB bind (Rosche *et al.* 2000; table 1). X-ray crystallographic studies of RP4 KorB have shed light

on the dimerization of KorB and its mode of DNA binding. The C-terminal 62 amino acids of KorB (KorB-C) form a globular domain. KorB-C is a dimer with an extensive, predominantly hydrophobic subunit interface (Delbruck *et al.* 2002). Yeast two-hybrid studies have implicated the C terminus in dimerization of full length KorB (Lukaszewicz *et al.* 2002).

The crystal structure of the central, DNA-binding domain of KorB has been solved in complex with a 17 bp oligonucleotide containing the operator sequence (O_B) (Khare *et al.* 2004). The structure shows two KorB-O (operator-binding domain of KorB) molecules symmetrically bound to two operator half-sites. KorB-O contains the predicted HTH motif commonly found in prokaryotic DNA-binding proteins. In the crystal structure, the operator DNA adopts a standard B-DNA conformation with a straight helix axis and two KorB-O molecules bound in successive major grooves of the DNA. Sequence recognition is not performed by the recognition helix of the HTH motif, but rather by two residues, Thr211 and Arg240, that form hydrogen bonds directly with bases in the operator site and when mutated to alanine, they abolish binding. There is only one protein-protein contact formed by the two KorB-O molecules in the structure between two glutamate residues (Khare *et al.* 2004). Whether this contact is important for dimerization in solution in the absence of DNA, remains to be determined, but KorB-O is monomeric in the absence of DNA.

The crystal structures of the two domains of KorB have led to a model in which KorB-C mediates dimerization of intact, full length KorB and owing to contacts between KorB-O subunits, the operator DNA is completely enclosed within the dimeric KorB. It is believed that KorB-C and KorB-O are connected by a flexible linker, which is required for the protein to undergo a conformational change upon binding that leads to engulfment of the DNA helix. The role of the N terminus remains to be elucidated (Khare *et al.* 2004).

5. THE R1 *par* SYSTEM: ACTIN-LIKE FILAMENTS INVOLVED IN PARTITIONING

Like the P1 *par* and F *sop* systems, the *par* locus of plasmid R1 encodes two genes, *parM* and *parR*, and a *cis*-acting site *parC* (table 1), which, in this case, is located upstream of *parM*. Plasmids containing *par* of R1 also move rapidly from mid-cell to positions near the cell poles in a symmetrical pattern (Jensen & Gerdes 1999). However, the components of the R1 *par* locus differ from the P1 and F systems. ParM of R1 belongs to a superfamily of ATPases that includes actin and its putative bacterial ancestor MreB, which has been confirmed by structural work (Bork *et al.* 1992; Jones *et al.* 2001; Møller-Jensen *et al.* 2002; van den Ent *et al.* 2001, 2002). Importantly, ParM forms actin-like, double helical filamentous structures *in vivo* extending along the longitudinal axis of the cell, and filament dynamics are required for active partitioning of the R1 plasmid. *In vivo*, filament formation and turnover is controlled by the ParR-*parC* complex and the intrinsic ParM ATPase activity. ATP binding is required for *in*

vitro polymerization while hydrolysis is required for depolymerization (Møller-Jensen *et al.* 2002). ParR is a DNA-binding protein analogous, but not homologous, to ParB of P1 and SopB of F plasmids. ParR dimers bind cooperatively to two sets of five direct repeats (iterons) within the 160 bp *parC* site (Breuner *et al.* 1996; Jensen *et al.* 1994, 1998), thereby forming a complex that mediates specific pairing of plasmid molecules *in vitro* (Jensen *et al.* 1998). All 10 repeats in *parC* are required for optimal function in plasmid stabilization and autoregulation of the promoter by ParR (Breuner *et al.* 1996). ParR and ParM interact *in vivo* and *in vitro* (Jensen & Gerdes 1997). ParM filamentation is supported only by ParR-*parC* and not by ParR alone, even when overexpressed, lending support to the notion that ParR-*parC* acts as a nucleation point for the polymerization of ParM (Møller-Jensen *et al.* 2002, 2003).

The observations of ParR-mediated dimerization of R1 plasmids *in vitro*, dynamic filaments of ParM and the *in vivo* abrupt separation and segregation of plasmids from mid-cell to positions near to the cell poles, suggests a model for R1 partitioning that invokes the function of the ParM ATPase. The model assumes that plasmids are replicated at mid-cell by the replication factory. Following replication, the plasmids are paired rapidly by the ParR-*parC* complex (Jensen *et al.* 1998). ParR molecules in the paired complex then serve as nucleation points for ParM polymerization. Force is generated by inserting ParM subunits between the tip of the filament and ParR, and the plasmids are pushed towards the cell poles by a 'polymerization motor'. Such a model would work even if the plasmids were not replicated at, or segregated from, mid-cell since the only requirement would be a minimal separation distance longer than half the dividing cell at cell division (Møller-Jensen *et al.* 2003).

Interestingly, database analyses have revealed that plasmids of both Gram-negative and Gram-positive bacteria encode actin-like proteins (putative ParM orthologues) more closely related to the chromosomal actin-like MreB family of proteins, than to each other. Thus, it is conceivable that plasmids recruited MreB at least twice during evolution to perform the task of DNA partitioning. This then raises the important question of whether the actin-like MreB protein plays a role in prokaryotic chromosome segregation.

6. BACTERIAL CHROMOSOME SEGREGATION

Over the past 40 years, various models have been proposed for the mechanism of chromosome segregation in bacteria, postulating the involvement of the elongating cell envelope, the DNA replication factory, transertion of membrane proteins, chromosome condensation and RNA polymerase. Crucial to recent progress has been the ability to determine precisely the subcellular localization of proteins and DNA within bacterial cells by fluorescence microscopy.

7. ORIENTATION, POSITIONING AND MOVEMENT OF THE BACTERIAL CHROMOSOME

The first indications that the bacterial chromosome might have a distinct orientation within the cell came from studies on sporulation in *B. subtilis*. The formation of the prespore compartment is brought about by assembly of an asymmetric septum near one pole of the cell. At the completion of the asymmetric division that follows DNA replication, both the prespore compartment and the larger, adjacent mother cell each contain a complete chromosome. Differential gene expression pathways in each compartment direct the successful development of the spore. However, during invagination of the septum, only 30% of the prespore chromosome actually resides in the prespore compartment with the remaining 70% in the mother cell. Hence, the closing septum is set to bisect the prespore chromosome. How, then, is the remainder of the chromosome transported into the prespore compartment before septation is complete? The answer lies in the *spoIIIE* gene, mutations in which prevent the completion of DNA segregation into the prespore (Wu & Errington 1994). SpoIIIE is localized to the prespore septum and is proposed to translocate the chromosomal DNA into the prespore compartment as a DNA 'pump'. Biochemical experiments have demonstrated that the conserved C-terminal domain of SpoIIIE possesses an ATPase activity that can be stimulated tenfold by the presence of double stranded DNA. Moreover, SpoIIIE affects the topology of plasmid molecules, consistent with tracking of the protein along the DNA (Bath *et al.* 2000). It has been determined that the DNA located in the prespore of a *spoIIIE* mutant always consists of the same region of chromosome, corresponding to a 500 kb region surrounding the origin of replication, *oriC* (Wu & Errington 1994; Wu *et al.* 1995). SpoIIIE homologues are widespread in non-sporulating bacteria, including the conserved cell division protein FtsK. FtsK probably functions like SpoIIIE to ensure the fidelity of chromosome segregation into the daughter cells prior to septation (Aussel *et al.* 2002), and has been shown in a recent single molecule experiment to act as a DNA translocase *in vitro* (Saleh *et al.* 2004).

Following the discovery that the prespore chromosome adopts a specific orientation in sporulating *B. subtilis*, specific orientation of the chromosome was discovered in *E. coli* and vegetative cells of *B. subtilis* (Gordon *et al.* 1997; Niki & Hiraga 1998; Sharpe & Errington 1998; Webb *et al.* 1997, 1998) by visualization of the origin and terminus regions using the LacI-GFP reporter system. High resolution data have demonstrated that in slow-growing *E. coli*, the origins move to one-quarter and three-quarter positions. Termini stay at mid-cell until very late in the cell cycle and are non-symmetrical with respect to the middle of the mother cell (Lau *et al.* 2003). The one-quarter and three-quarter positions represent predivisional sites in each of the nascent daughter cells. In fast-growing *E. coli* cells, overlapping rounds of replication generate newborn cells in which replication has already been initiated. In these cells, the two copies of the origin are located at or near the one-quarter and three-quarter

positions following DNA segregation. Upon completion of replication at mid-cell, the replication factories reposition themselves at the one-quarter and three-quarter positions where they reinitiate replication at the origins of the new chromosomes prior to the first round of septation. Multiple rounds of overlapping replication may proceed simultaneously with newly replicated origins moving to the one-eighth, three-eighth, five-eighth and seven-eighth positions shortly after replication has been initiated. Subsequent septation at all of these predivisional sites results in eight daughter cells. *B. subtilis* origin placement is similar to that observed in *E. coli* (Sharpe & Errington 1998; Webb *et al.* 1998).

The rapid, bi-directional migration of chromosomal origin regions is reminiscent of *E. coli* P1 and F plasmid partitioning described above (Gordon *et al.* 1997; Niki & Hiraga 1997) and points to the existence of a conserved, mitotic-like apparatus in bacteria. It appears that the origin-proximal region of the bacterial chromosome plays an analogous role to the eukaryotic centromere. However, although *oriC* itself is not sufficient for localization (Gordon *et al.* 2002), regions around *oriC* have been reported to facilitate correct orientation, as will be discussed below (Lin & Grossman 1998; Wu & Errington 2002; Yamaichi & Niki 2004).

8. Par PROTEINS ENCODED BY BACTERIAL CHROMOSOMES—COMPONENTS OF AN ACTIVE SEGREGATION MECHANISM?

Many bacterial chromosomes contain genes for homologues of the plasmid partitioning proteins ParA and ParB, immediately suggesting a role of these proteins in chromosome segregation. However, elucidation of that role has been complicated by the lack of a strong missegregation phenotype in deletion mutants. Chromosomal homologues of ParA and ParB (sometimes denoted as Soj and Spo0J, because of their involvement in sporulation) have been identified in a wide range of both Gram-negative and Gram-positive bacteria (Yamaichi & Niki 2000), covering most of the bacterial phylogenetic tree. The notable exceptions are the enteric bacteria, including *E. coli* and the gamma subdivision of proteobacteria (Yamaichi & Niki 2000). Although Soj/Spo0J were first discovered in *B. subtilis*, because of their role in chromosome segregation during sporulation, many chromosomal homologues of ParA and ParB are found in organisms that are not known to sporulate, suggesting a more fundamental role for these proteins.

Importantly, the chromosomal partitioning proteins can be engineered to function in the same way as plasmid *par* proteins to stabilize an otherwise unstable replicon and direct its correct positioning within the cell (Godfrin-Estevenson *et al.* 2002; Lin & Grossman 1998; Yamaichi & Niki 2000). Furthermore, null mutants of ParB are lethal in *Caulobacter crescentus* (Mohl *et al.* 2001), and mutations in *spo0J* in *B. subtilis* result in the formation of up to 3% anucleate cells (Ireton *et al.* 1994). Spo0J/ParB mutants of *B. subtilis* and *Pseudomonas aeruginosa* are viable but display

perceptible defects in chromosome segregation in both vegetative and sporulating cells (Godfrin-Estevenon *et al.* 2002; Ireton *et al.* 1994; Lewis *et al.* 2002; Sharpe & Errington 1996). During vegetative growth, deletion of the *parAB* genes of *Pseudomonas putida* slightly increases the formation of anucleate cells, but during both the transition from exponential to stationary growth conditions and during overexpression of *parAB*, high levels of anucleate cells were formed (Godfrin-Estevenon *et al.* 2002). Deletion of *parAB* in *Streptomyces coelicolor* had no detectable effect in vegetatively growing cells, but also resulted in the production of significant numbers of anucleate spores (Kim *et al.* 2000).

The identification and characterization of a bacterial chromosome partitioning site has revealed 10 pseudo-palindromic 16 bp sequences found in the origin-proximal 20% of the *B. subtilis* chromosome (Lin & Grossman 1998). Research has shown that 8 of the 10 sequences are bound by Spo0J *in vivo*, and that the presence of one such site on an otherwise unstable plasmid stabilizes the plasmid in a Soj- and Spo0J-dependent manner, demonstrating that the site, *parS*, can function as a partitioning site (Lin & Grossman 1998). Moreover, it has since been shown that both the *B. subtilis* and *P. aeruginosa* partitioning machineries can function in *E. coli* (Godfrin-Estevenon *et al.* 2002; Yamaichi & Niki 2000).

The 16 bp *parS* sites identified in *B. subtilis* have since been identified in other bacterial species including *P. aeruginosa* and *P. putida* (Godfrin-Estevenon *et al.* 2002) and *Thermus thermophilus* (Nardmann & Messer 2000). More recently, a putative chromosomal partitioning site, *migS*, has been identified in *E. coli* (Yamaichi & Niki 2004). The *migS* site is located origin-proximally and is required for bipolar placement of *oriC*. When placed elsewhere on the chromosome or on a plasmid, *migS* mediates poleward localization of its new DNA context. The consensus sequence, 5'-tGTTTCACGTGAAACa-3', shows a dyad symmetry typical of targets for dimeric DNA-binding proteins. The half-palindromes of these sequences (tGTTTCAC, GTGAAACa) are similar to ParB box A motifs of P1 plasmid centromeres (e.g. A1, ATTTTCAC; A3, GTGAAAT; Bouet *et al.* 2000; Surtees & Funnell 2001).

Complexes of ParB proteins bound to nucleoids have been detected as discrete bipolar foci that coincide with *oriC* in living cells (Glaser *et al.* 1997; Lin *et al.* 1997). Prespore-compartment-specific gene expression assays in *B. subtilis* show that *spo0J* mutants have defects in the orientation of the prespore chromosome (Sharpe & Errington 1996), suggesting that Spo0J is involved in the spatial organization of the origin proximal regions of the chromosome. However, Spo0J is not required for proper positioning or movement of the *oriC* region in *B. subtilis* during vegetative growth (Webb *et al.* 1998). This finding possibly reflects a level of redundancy in the system, which localizes the *oriC* region to the cell poles, at least during sporulation. Recent evidence has shown that the division site selection protein DivIVA has an additional role in positioning the origin region of the *B. subtilis*

chromosome during sporulation (Thomaides *et al.* 2001). Together with the DNA-binding protein, RacA DivIVA plays a pivotal role in anchoring the chromosome at the cell pole during sporulation. RacA mutants often fail to trap prespore DNA prior to asymmetric septation, resulting in the production of anucleate cells. RacA interacts with DivIVA, localizing the origin region at the cell pole prior to spore formation (Ben-Yehuda *et al.* 2003). Furthermore, in the absence of Soj and RacA, a $\Delta divIVA$ -like defect in prespore chromosome segregation is observed and deletion of RacA, Soj and Spo0J results in the elimination of the *oriC*-specificity of orientation of the prespore chromosome altogether (Wu & Errington 2002).

Deletion of *soj* (*parA*) alone does not result in a DNA segregation defect (Ireton *et al.* 1994). However, Soj is required for the stabilization of *parS*-containing plasmids (Yamaichi & Niki 2000). Soj and other ParA proteins are members of a large family of ATPases that include the bacterial cell division regulator MinD, nitrogenase iron protein involved in biological nitrogen fixation and the anion pump, ATPase ArsA (Koonin 1993). Soj has been shown to associate with the promoter regions to inhibit the transcription of several early sporulation genes *in vivo*, an effect that is antagonized by Spo0J (Ireton *et al.* 1994; Quisel & Grossman 2000; Quisel *et al.* 1999). It is also known to play a role in the formation of condensed Spo0J foci on *oriC/parS* (Marston & Errington 1999).

Localization studies of Soj have shown that it oscillates within the nucleoid regions of the cell over a time period of approximately 20 min and to be statically nucleoid-associated in the absence of Spo0J (Marston & Errington 1999). Mutations in the nucleotide binding site of Soj disrupt localization and function, rendering it insensitive to regulation by Spo0J (Quisel & Grossman 2000; Quisel *et al.* 1999). These observations suggest that the non-oscillating form of Soj directs repression of early sporulation gene promoters and that the oscillating form does not. The switch between these two forms is probably linked to Soj ATPase activity, a hypothesis that is supported by MinE regulation of the Soj-related ATPase MinD. The MinD protein of *E. coli* regulates division site selection in conjunction with the division inhibitor, MinC, and the topological specificity factor, MinE, and exhibits an oscillatory pattern loosely related to that of Soj (Raskin & de Boer 1999). Whereas MinD oscillates all the way to the poles, Soj oscillation is restricted to the nucleoid. MinCD oscillation is dependent on MinE in a seemingly analogous fashion to Spo0J-dependent oscillation of Soj (Quisel *et al.* 1999). MinD undergoes ATP- and membrane-dependent self-association by virtue of a conserved C-terminal amphipathic helix which associates with the lipid bilayer (Lutkenhaus & Sundaramoorthy 2003). Members of the ParA family, including Soj, do not contain this helix and do not bind to the membrane, but instead associate dynamically with the nucleoid (Ebersbach & Gerdes 2001; Marston & Errington 1999; Quisel *et al.* 1999).

The *par* system has also been studied in *C. crescentus*. Like *B. subtilis*, the operon containing the *parA* and *parB* genes is near the origin of replication. ParB binds to several *parS* sequences adjacent to the *par* operon and the origin (Mohl & Gober 1997). Subcellular localization experiments have shown that a single focus of ParB is found at one pole of a cell containing a single chromosome (Mohl & Gober 1997; Mohl *et al.* 2001). Initiation of DNA replication results in the rapid formation of a second ParB focus at the opposite pole of the cell. This dynamic localization pattern coincides with the movement of the newly duplicated origin during the cell cycle (Jensen & Shapiro 1999). ParA localizes to the cell poles in *C. crescentus* but has not been assayed for polar oscillation in living cells (Mohl & Gober 1997). The case for the direct involvement of ParA and ParB in the movement of chromosomal DNA towards the cell poles in dividing cells is supported by the fact that both *parA* and *parB* are essential for viability in *C. crescentus*. Overexpression of either gene results in both cell division and chromosome partitioning defects. On the other hand, overexpression of both has only a mild effect on cell division but a relatively severe defect in partitioning, suggesting that the correct balance of the two proteins is essential for normal partitioning and division. In ParB depletion experiments, loss of the protein leads to cell filamentation and eventual death as a consequence of the inability to form FtsZ-rings; an identical phenotype is observed when ParA is overexpressed (Mohl *et al.* 2001).

It has been deduced that ParB of *C. crescentus* acts as a nucleotide exchange factor for ParA, stimulating the rapid exchange of ADP for ATP (Easter & Gober 2002). The N-terminal region of ParB of *C. crescentus*, like that of plasmid P1 ParB and F plasmid SopB, is the determinant for interaction with ParA (Figge *et al.* 2003).

The *par* proteins in both *B. subtilis* and *C. crescentus* are clearly required for efficient chromosome partitioning, but their exact roles in orienting the origin region, directing chromosome movement or regulating a cell cycle checkpoint coupling partitioning to cytokinesis remain unclear. The observation that increasing the expression of either protein in *C. crescentus* also causes a cell division defect could point to a role in coordinating cell division with chromosome movement. ParA and ParB may provide the cell with a mechanism to sense the arrival of the chromosomes at the cell poles, at which time division could proceed. This hypothesis is further strengthened by the homology between ParA/Soj and the cell division regulator MinD. The Min system discriminates between mid-cell and the cell poles through MinCD oscillation, which prevents septum formation at the poles but permits it at mid-cell. ParA/Soj could function to prevent septation in nucleoid occupied regions of the cell, or 'sense' the arrival of the chromosomes at the one-quarter positions of the cell or at the cell poles.

9. A COMMON MECHANISM OF ATP-DEPENDENT DIMERIZATION AND ATPase ACTIVATION SHARED BY MinDE AND ParAB?

The ATPases of the two systems, Soj and MinD, are structurally homologous to the nitrogenase iron protein (Cordell & Löwe 2001; Hayashi *et al.* 2001; Leonard *et al.* 2004; Sakai *et al.* 2001), which, in conjunction with molybdenum iron protein, mediates electron transfer during biological nitrogen fixation (Schindelin *et al.* 1997). Nitrogenase, MinD, Soj and the anion pump ATPase ArsA are members of a family of ATPases characterized by the presence of a deviant Walker A motif containing a conserved 'signature' lysine at the start of the P-loop (Koonin 1993). Nitrogenase iron protein is a dimeric ATPase in which the constituent monomers are covalently linked by a 4Fe:4S cluster. Each monomer binds a single adenine nucleotide. In the presence of ADP·AlF₄⁻, which mimics the transition state, the dimer switches from an 'open' to a more compact, 'closed' conformation, resulting in the rotation of the two subunits by approximately 13° towards the dimer interface. This transition state is then competent for interaction and complex formation with molybdenum iron protein (Schindelin *et al.* 1997). Until recently, all published structures of MinD-like proteins showed them to be monomeric (Cordell & Löwe 2001; Hayashi *et al.* 2001; Sakai *et al.* 2001), even in the presence of a non-hydrolysable ATP analogue, AMPPCP (Hayashi *et al.* 2001). Although the anion pump ArsA is also monomeric, it is twice the size of NifH and MinD and contains two similar domains connected by a short linker, such that each ArsA monomer is functionally a pseudodimer (Zhou *et al.* 2000). The crystal structure of *T. thermophilus* Soj D44A, which is deficient in nucleotide hydrolysis, shows conclusively for the first time that these MinD-like proteins do, indeed, form ATP-dependent nucleotide sandwich dimers (Leonard *et al.* 2004). The 'signature' lysine of each monomer is expected to stabilize the developing negative charge on the β-phosphate during the hydrolysis reaction.

The dynamic behaviours of MinD and Soj are regulated *in vivo* by the topological specificity factors MinE and Spo0J, respectively. MinE drives the pole-to-pole oscillation of the MinCD division inhibitor in many bacteria, restricting its activity to the vicinity of the cell poles (Fu *et al.* 2001; Hale *et al.* 2001; Hu & Lutkenhaus, 2001; Hu *et al.* 2002; Shih *et al.* 2003). The result is that the mid-cell is left free for formation of the division plane. In the Gram-positive bacteria, including *B. subtilis*, a different topological specificity factor, DivIVA, achieves a similar result by recruiting the inhibitor to the cell poles and retaining it there (Cha & Stewart 1997; Edwards & Errington 1997; Marston *et al.* 1998). In the presence of ATP, MinD binds to the division inhibitor MinC and goes to the membrane (Hu *et al.* 1999, 2003; Lackner *et al.* 2003). However, the ATPase is not stimulated and the complex is stable. MinD undergoes ATP-dependent dimerization *in vitro* and is expected to form a stable complex with a dimer of MinC (Hu & Lutkenhaus 2003). Subsequent interaction of this complex with MinE results in ATP hydrolysis and dissociation

of both the complexes MinCD and MinD from the membrane. MinE mutants unable to stimulate MinD ATPase activity are unable to induce MinD oscillation and mutants with decreased ability to stimulate the ATPase have lower MinD oscillation frequencies (Hu & Lutkenhaus 2001), suggesting that the rate of MinE stimulation controls the oscillation period.

Likewise, oscillation of Soj within the nucleoid region, is abrogated both by mutations in the ATPase active site and by deletion of *spo0J* (Marston & Errington 1999; Quisel *et al.* 1999). It is not known how MinE stimulates MinD or how Spo0J stimulates Soj, but the interaction determinants of MinE and Spo0J have both been mapped to the N-terminal 22 and 20 amino acids, respectively (Leonard *et al.* 2004; Ma *et al.* 2003). Furthermore, Leonard *et al.* (2004) show that the activating regions of Spo0J and MinE contain significant sequence homology, including a conserved basic residue (see also Hu & Lutkenhaus 2001), mutation of which in Spo0J abrogates activation of Soj ATPase. Interestingly, the interaction determinants of both MinE and Spo0J also bear significant sequence homology to the conserved C terminus of FtsA, which presently has an unknown function, but may reasonably be expected to fulfil a similar role (Hu & Lutkenhaus 2001; Leonard *et al.* 2004). FtsA is part of the septum and interacts directly with FtsZ.

The emerging theme for members of the deviant Walker A class of ATPases is that binding of ATP drives the formation of an active dimer that has a high affinity for a dimeric binding partner. Thus, a NifH dimer binds the MoFe protein dimer and ArsA binds an ArsB dimer. Most likely, a MinD dimer binds to the dimeric MinC and we speculate that a Soj dimer binds to dimeric Spo0J. The timing of ATP hydrolysis will determine the transient or persistent nature of such complexes since hydrolysis causes their dissociation. In the case of MinD, the MinCD complex exists for a relatively long period, as MinC does not stimulate the basal ATPase activity of MinD. Instead, MinCD dissociation is only brought about by the action of MinE, the rate of which controls the lifetime of the complex.

10. SPECULATION: Spo0J-ASSISTED TREADMILLING OF Soj

The similarities between MinDE and Soj/Spo0J are wide-ranging: (i) MinD and Soj oscillate (jump) between places in the cell; (ii) MinD oscillates by binding to the membrane, Soj, by binding to the nucleoid (DNA most probably); (iii) MinD and Soj have the same three-dimensional structure apart from the amphiphatic helix on MinD that is involved in membrane binding; (iv) MinD and Soj most probably from the same ATP-dependent dimer; (v) MinD and Soj ATPase activity is activated by a short, disordered peptide located on a dimeric binding partner (MinE N terminus, Spo0J N terminus, respectively); (vi) both MinD and Soj bind to extended surfaces (MinD: membrane, Soj: DNA) and binding is regulated by dimerization; (vii) both the MinDE and the Soj/Spo0J system are involved in accurate positioning (MinC and region of the nucleoid, respectively); (viii)

copy numbers of MinD and Soj are high, copy numbers for MinE and Spo0J are low; and (ix) MinD and Soj oscillate by forming 'helical tracks' inside the cell.

We believe that these parallels are strong and that it may be helpful to attempt to predict how Soj and Spo0J position regions of the nucleoid and help with DNA segregation. Central to Soj's function is its ability to polymerize in an ATP-dependent manner onto DNA (Leonard *et al.* 2004). This is analogous to MinD's ability to bind to the membrane once dimerized. By comparing the Soj dimer with nitrogenase iron protein, it is clear that a conformational change will occur upon nucleotide hydrolysis. The structure also shows that the nucleotide is occluded by the protein making nucleotide exchange improbable when dimeric. This leads to a model in which the polymer of Soj on the DNA has two different ends, a polymerizing plus end with an ATP cap and a minus end with the Soj subunit, which either depolymerize by themselves or bind to the DNA in an ADP-Pi state. The bulk ATPase activity of Soj is not enhanced by binding to the DNA, but by interaction with Spo0J, which is similar to MinD ATPase activation by MinE. Spo0J probably does not bind to the ATP dimer form of Soj but rather to the ADP-Pi form and functions as a nucleotide exchange factor for Soj. Therefore, Spo0J will bind preferentially to the minus end of the Soj filament and this will be enhanced by Spo0J's own DNA-binding activity. Spo0J might, thus, chase Soj off the DNA, just as MinE is thought to chase MinD off the membrane; we have termed this model 'assisted treadmilling' (figure 1). This model is somewhat related to the treadmilling of microtubules; however, the difference is that nucleotide hydrolysis is activated using an external factor (Spo0J). If polymerization and depolymerization are cooperative, then a pattern will form with waves of proteins oscillating and this can be modelled *in silico* (Howard *et al.* 2001; Hunding *et al.* 2003; Meinhardt & de Boer 2001). The time-averaged result is a concentration gradient that can be used to obtain positional information, as has been predicted for the MinCDE system. In the case of Soj/Spo0J, this is not as straightforward because oscillation does not extend along the whole length of the cell, but is restricted to the nucleoid, and positioning has to be with respect to the cell. What the system can do is mark the outer extremes of the nucleoid so that they can be recognized by other molecules to drag it towards the poles. We note that the marking could also be achieved by a stationary marker.

An alternative explanation involves using the assisted treadmilling for active DNA transport. The presence of *parS* sites near the chromosomal origin may impose directionality upon Soj polymerization, and detachment from the DNA may be initiated only near these sites. The model functions as follows: Soj polymerizes onto the DNA cooperatively. Spo0J has its highest affinity for the nucleoid at the 10 or so *parS* sites where it binds and starts depolymerizing Soj nucleoprotein filaments. Starting at *parS* means that depolymerization at the shrinking (−) end has a direction along the DNA away from the origins in both directions. Polymerization of Soj occurs at the other, growing (+) end of the filament. We

Figure 1. Model of 'assisted treadmilling' of ParA-like proteins. Soj (ParA) binds to DNA in its ATP-dependent dimeric form. Because the nucleotide is almost completely occluded in the 'sandwich dimer' of Soj, there is probably very little nucleotide exchange in and out of the nucleoprotein filament—a pre-requisite for the two ends of the filament being different. Spo0J (ParB) enhances Soj ATP hydrolysis and, therefore, can chase Soj off the DNA. The result is treadmilling of Soj assisted by the N-terminal residues of Spo0J. The result is a relative movement of Soj/Spo0J against the DNA, and this movement could be used in various ways depending on what is more stationary in the cell. Note that a similar mechanism involving membrane-associated polymerization can be proposed for MinD (replacing Soj) and MinE (replacing Spo0J).

speculate that if there is anything fixed in the cell binding to either Soj or Spo0J during this process of assisted treadmilling, then the DNA will be moved, just as with eukaryotic motor proteins. By extension, the many Min proteins found in some organisms may be used to move cargo, other than MinC, around in the cell in an analogous fashion.

None of these models explain the 'tracks' of MinD and Soj/ParA that can be seen by microscopy, and recent data indicates that there is no interaction with filamentous FtsZ or MreB (Shih *et al.* 2003; Thanedar & Margolin 2004). However, it seems theoretically possible that an interaction between Spo0J/Soj and MreB or FtsZ would explain the helical pattern of these proteins and could be included in the proposed model of assisted treadmilling of Soj/ParA/MinD.

11. COORDINATION OF PARTITIONING AND DIVISION: THE NUCLEOID OCCLUSION MODEL

Actively dividing cells must coordinate septation with accurate partitioning of the newly replicated nucleoids to ensure that each daughter cell receives an undamaged copy of the genetic material. Delays to DNA replication (e.g. for repair) or to chromosome segregation must, therefore, delay cell division until these processes are complete. In *E. coli* and vegetatively growing *B. subtilis*, the timing of division is regulated so that it follows the replication and

segregation of sister chromosomes and division occurs at mid-cell in the DNA-free space between the partitioned nucleoids. The 'nucleoid occlusion' model, first proposed over a decade ago by Woldringh *et al.* (1991), in combination with the Min system could provide a dual mechanism for targeting the division machinery to mid-cell and simultaneously protecting the nucleoid from bisection by aberrantly forming septa. Wu & Errington (2004) have recently reported the identification of a novel nucleoid occlusion protein, Noc, which prevents the division machinery from assembling in the vicinity of the nucleoid. However, *noc* mutants do not exhibit any loss in specificity of division site selection in unperturbed cells, suggesting a level of redundancy in the systems controlling it (Wu & Errington 2004). It may be that the Min system can prevent division at significant distances from the cell pole or that a Noc-independent system biases division toward inter-nucleoid spaces. Interestingly, Noc is 40% identical to the *B. subtilis* ParB homologue, Spo0J. Many bacteria do not contain a homologue of Noc whereas Spo0J is almost universally conserved (Yamaichi & Niki 2000), raising the question of how Noc has arisen. Additionally, many bacteria do not contain a complete Min system so it follows that bacteria may have evolved a range of mechanisms to control the correct timing and proper placement of their division septum.

12. A LINK BETWEEN DNA SEGREGATION AND THE BACTERIAL CYTOSKELETON?

In eukaryotic cells, the forces that drive chromosome segregation are well understood. Mitosis is a highly dynamic process involving a host of motor proteins which, together with cytoskeletal components, mediate the controlled movement of sister chromatids to opposite cell poles (Heald, 2000; Scholey *et al.* 2003). Microtubules of the spindle apparatus undergo alternating phases of polymerization and depolymerization, which, in combination with microtubule associated motor proteins that actively move microtubule filaments relative to one another in a 'sliding filament' mechanism, generates the force required for rapid poleward movement (Howard & Hyman 2003).

It was a long-held belief that bacteria did not possess a cytoskeleton and, therefore, that no such analogous means of segregating newly replicated chromosomes existed. However, the discovery of the bacterial cytoskeleton over the past decade has prompted a revision of our thinking regarding intracellular organization in bacteria. The bacterial tubulin homologue, FtsZ, forms rings at cell division sites (Bi & Lutkenhaus 1991; Löwe & Amos 1998; Mukherjee & Lutkenhaus 1994), whereas the actin homologues, MreB and Mbl, have established roles in cell shape determination (Jones *et al.* 2001; van den Ent *et al.* 2001).

The discovery of a plasmid partitioning determinant (ParM), also homologous to actin and capable of forming F-actin like filaments (van den Ent *et al.* 2002), has raised the question of whether MreB plays a role in chromosome segregation. MreB has recently been shown to be required for chromosome segregation in *C. crescentus* (Gitai *et al.* 2004), *E. coli* (Kruse *et al.* 2003) and *B. subtilis* (Soufo & Graumann 2003). In *E. coli*, depletion of MreB causes cells to segregate their chromosomes in pairs, consistent with their cohesion. Ectopic overexpression of wild-type MreB results in an impairment of cell division but does not affect chromosome segregation. Overexpression of active site mutants of MreB results in inhibition of cell division, abnormal MreB filament morphology and, importantly, induces severe defects in the localization of the origin and terminus regions of the chromosomes (Kruse *et al.* 2003). The striking observation that depletion or mutational inactivation of both actin homologues ParM and MreB result in a DNA segregation defect, raises the possibility that MreB filaments participate directly or indirectly in chromosome segregation. The discovery that MreB filaments *in vivo* are dynamic would satisfy a mechanism in which polymerization of MreB would actively move chromosomal DNA in opposite directions toward the cell poles (Carballido-Lopez & Errington 2003; Defeu Soufo & Graumann 2004). Alternatively, MreB filaments may serve as the tracks along which presently unidentified motor proteins move to drive chromosomal origin regions apart. Another possibility is that MreB filaments help to position the centrally located replication factory, such that, origin regions are extruded in opposite, poleward directions in a manner described by the 'factory model' of replication (Lemon & Grossman 1998). In *B. subtilis*, the bipolar

localization of the origin regions during cell division is lost when MreB is depleted. Depletion of three other cell-shape determining proteins has no effect on chromosome segregation (Soufo & Graumann 2003). These data are consistent with any of the above mechanisms, although it remains a distinct possibility that the depletion of MreB or the overexpression of MreB mutants has an indirect effect on chromosome segregation owing to defects in cell shape that are not always detected given the resolution limits of microscopy. In addition to chromosomal origin regions, four independent cell polarity markers in *Caulobacter*, are mislocalized or become uniformly distributed when MreB is either depleted or overexpressed. This suggests that MreB plays a role in determining the polarity of the cell by delivering or localizing polar proteins to their correct positions within the cell (Gitai *et al.* 2004). Replenishment of MreB in depleted cells restores the polar localization of these proteins in a randomized fashion, suggesting that conservation of MreB filament polarity throughout generations is important. Deletion of the integral membrane protein SetB, which interacts with MreB, causes a delay in chromosome segregation, whereas its overproduction causes nucleoid stretching and disintegration (Espeli *et al.* 2003). This puzzling finding suggests that the chromosome might, indeed, be linked to the membrane as proposed initially, 40 years ago.

13. THE SEARCH FOR BACTERIAL MOTOR PROTEINS

Despite the discoveries of bacterial actin, tubulin and, most recently, an intermediate filament-forming protein in *C. crescentus* (Ausmees *et al.* 2003) that together make up the bacterial cytoskeleton, no motor proteins have been identified for any cytoskeletal element. Furthermore, no nucleation factors besides ParR of plasmid R1 (Møller-Jensen *et al.* 2003) have been identified and the only known bundling proteins are ZipA and ZapA, which both cross-link FtsZ filaments (Hale *et al.* 2000; Low *et al.* 2004). This is in stark contrast to the multitude of cytoskeleton-associated proteins that have been identified in eukaryotes, including the microtubule-associated directional motors of the dynein and kinesin families that mediate organelle movement along microtubules, as well as the actin motor myosin involved in force generation in skeletal muscle and a vast array of other functions. Do motor proteins related to myosin, kinesin or dynein not exist in bacteria? Are bacteria small enough that they do not require directed intracellular addressing or do they achieve it by mechanisms that do not require motor proteins, such as that proposed for the partitioning of the R1 plasmid in *E. coli*?

The discovery of the highly dynamic Min system initially suggested that the mystery of bacterial motors had been solved. The notion that MinD ATPase is a primitive motor protein is further supported by the observations that ParA and Soj (and its homologues involved in plasmid and chromosome partitioning) also display dynamic oscillatory behaviour *in vivo*

(Ebersbach & Gerdes 2001; Marston & Errington 1999; Quisel *et al.* 1999). In addition, ParA is required for the bi-directional extrusion of replicated P1 plasmids from mid-cell towards the cell poles (Li *et al.* 2004). However, MinD and ParA share very little sequence or structural homology with myosin and kinesin that are distantly related in their motor domains. Furthermore, MinD and ParA have been reported not to colocalize with any cytoskeletal track (Shih *et al.* 2003; Thanedar & Margolin 2004). However, it is impossible to imagine the eukaryotic cell without the mechano-chemical motors myosin, kinesin and dynein, and despite the fact that no bacterial motors have been discovered to date, it is not unreasonable to expect that they exist in some form or another.

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