Elsevier Editorial System(tm) for Plasmid
Manuscript Draft

Manuscript Number: PLASMID-11-14R2

Title: Prokaryotic ParA-ParB-parS system links bacterial chromosomes segregation with the cell cycle

Article Type: Invited Review Article

Keywords: parABS; chromosome segregation; bacteria; cell cycle

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Abstract: While the essential role of episomal par loci in plasmid DNA partitioning has long been appreciated, the function of chromosomally encoded par loci is less clear. The chromosomal parA-parB genes are conserved throughout the bacterial kingdom and encode proteins homologous to those of the plasmidic Type I active partitioning systems. The third conserved element, the centromere-like sequence called parS, occurs in several copies in the chromosome. Recent studies show that the ParA-ParB-parS system is a key player of a mitosis-like apparatus ensuring proper intracellular localization of certain chromosomal regions such as oriC domain and their active and directed segregation. Moreover, the chromosomal par systems link chromosome segregation with initiation of DNA replication and the cell cycle.

Suggested Reviewers:

Opposed Reviewers:

Cover Letter

Dear Prof. Chattoraj,
Please find enclosed the revised version of the review on ParABS chromosomal systems. The manuscript has been modified according to your suggestions. I removed the controversial “mitotic-like” term from the title and replaced it in the text by mitosis-like. I am hoping you will find this version f the review acceptable for publication.
Yours sincerely. Grazyna Jagura-Burdzy

***Response to Reviews**

The manuscript has been already modified according to reviewers' suggestions in its previous revised version with the exception of "the mitotic-like" term (see below). The controversial "mitotic-like" term has been removed from the title and replaced it in the text by mitosis-like (when appropriate). Additional subtle changes have been introduced according to Editor-in-Chief suggestions.

***Highlights**

The review presents up-to-date evidence on the role chromosomally encoded ParA-ParB-*parS* systems play in the chromosome segregation in different bacterial species highlighting their function in linking chromosome segregation with initiation of DNA replication and the cell cycle.

Prokaryotic ParA-ParB-*parS* system links bacterial chromosomes segregation with the cell cycle

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Running title: chromosomal ParABS systems

Keywords: *parABS*; chromosome segregation; bacteria, cell cycle.

Abstract

While the essential role of episomal *par* loci in plasmid DNA partitioning has long been appreciated, the function of chromosomally encoded *par* loci is less clear. The chromosomal *parA-parB* genes are conserved throughout the bacterial kingdom and encode proteins homologous to those of the plasmidic Type I active partitioning systems. The third conserved element, the centromere-like sequence called *parS*, occurs in several copies in the chromosome. Recent studies show that the ParA-ParB-*parS* system is a key player of a mitosis-like apparatus ensuring proper intracellular localization of certain chromosomal regions such as *oriC* domain and their active and directed segregation. Moreover, the chromosomal *par* systems link chromosome segregation with initiation of DNA replication and the cell cycle.

1. INTRODUCTION

Almost five decade ago, the "membrane attachment" model postulated that newly duplicated origins are tethered to the membrane and the elongating cellular envelope is the motor for bacterial DNA segregation (Jacob et al., 1963). It soon became clear that cell elongation occurs throughout the cell (reviewed by Nanninga, 1998) and cannot be accounted for origins separation, nevertheless the involvement of the membrane in this process has been repeatedly postulated for years (Dingman, 1974; Norris, 1995; van Helvoort and Woldringh, 1994; Woldringh, 1976, 2002). In the recent 20 years, owing to the advanced microscopy techniques and modern cytological studies, it has become clear that the origins of replication of bacterial chromosomes move rapidly and directionally with the speed 10 times faster than cell elongation rate (Bates and Kleckner 2005; Jenal and Stephens, 2002; Viollier et al., 2004; Webb et al., 1998). The extensive studies on the Gram-positive sporulating bacterium *Bacillus subtilis* as well as on the Gram-negative *Caulobacter crescentus* and *Escherichia coli* have revealed that the bacterial nucleoid is orderly structured with a defined orientation and cellular localization of the replication origin (*oriC*), terminus (*ter*) and other chromosomal loci (Hill, 1996; Lau et al., 2003; Niki and Hiraga, 1998; Niki et al., 2000; Sharpe and Errington, 1998; Viollier et al., 2004; Wake, 1997; Webb et al., 1998). The observation that PolIII holoenzyme complexed with other replication proteins, the replisome, has a fixed position in the *Bacillus subtilis* cell until late in the cell cycle, when it moves to new positions before cell division (Lemon and Grossman, 1998), changed the perception of the replication process. The actively moving *oriC* was proposed to follow the docked "replication factory" (Brendler et al., 2000, Jensen et al., 2001; Lemon and Grossman, 2000) and DNA was supposed to be spooled through the stationary replisome. However, some argue in favour of a tracking model instead, in which sister replisomes move along DNA domains (Bates and Kleckner, 2005; Berkmen and Grossman, 2006; Nielsen et al., 2007; Reyes-Lamothe et al., 2008). Regardless of the identity of the stationary component of the replication machinery chromosome starts to replicate from the specifically located *oriC* and segregation of duplicated regions proceeds actively in an orderly fashion to their specific addresses.

In *E. coli* and in vegetative cells of *B. subtillis* a symmetrical segregation of chromosomes has been observed. It was demonstrated that in slow-growing bacteria the newly duplicated origins move rapidly to one-quarter and three-quarter positions. The quarter positions represent predivisional sites in each of the nascent daughter cells. The chromosomal termini stay at mid-cell until very late in the cell cycle (Niki and Hiraga, 1998). In fast-

46 growing cells, overlapping rounds of replication generate newborn cells in which replication
47 has already been initiated. In these cells, the two copies of the origin are located at or near the
48 quarter positions following DNA segregation.

49 In *C. crescentus* (Jenal et al. 1995; Jensen and Shapiro, 1999; Sherratt, 2003) sister
50 chromosomes are segregated unidirectionally. The replication origin of the circular
51 chromosome is localized to the "old" pole of the cell and one of the newly replicated
52 chromosomes is directed to the opposite pole. Similar asymmetrical chromosome segregation
53 is also observed in other alpha-proteobacteria *Agrobacterium tumefaciens* and *Sinorhizobium*
54 *meliloti* which have multipartite genomes with replicons containing different types of origins.
55 In the gamma-proteobacterium *Vibrio cholerae* with the genome divided into two bona fide
56 circular chromosomes each chromosome replicates and segregates differently (Fiebig et al.,
57 2006; Fogel and Waldor, 2005; Srivastava et al., 2006). The larger chromosome I initiates
58 replication at the cell pole and segregates unidirectionally similarly to the chromosome of *C.*
59 *crescentus*, whereas the smaller chromosome II replicates at the centre and segregates
60 bidirectionally to the cell quarter positions like the chromosomes of *E. coli* or vegetative *B.*
61 *subtilis* cells.

62 Despite these differences in the segregation of chromosomes in various bacterial
63 species, the cellular localization of particular regions of the chromosomes as well as the
64 directional movement of the newly replicated DNA to defined cell positions indicate the
65 existence of a conserved, mitosis-like apparatus in bacteria. It appears that the origin-proximal
66 region of the bacterial chromosome plays a role analogous to that of the eukaryotic
67 centromere. While *oriC* itself is not sufficient for localization (Gordon et al., 2002), regions
68 around *oriC* have been reported to ensure correct spatial orientation (Lin and Grossman 1998;
69 Toro et al., 2008; Wu and Errington, 2003; Yamaichi and Niki, 2004).

70 The segregation of bacterial chromosomes is an active, complex process engaging
71 several proteins, although their number is an order of magnitude lower than used in the
72 eukaryotic cell. The most recent studies show that the *par* genes, homologues of the plasmid
73 active partition systems, are directly involved in this process (Ptacin et al., 2010; Toro et al.,
74 2008). However, it is difficult to define their exact roles because of an abundance of
75 segregation factors and the pleiotropic nature of mutants affected in the *par* genes. In *B.*
76 *subtilis*, mutations in *soj*/*spo0J* (*parA*/*parB*) cause only a mild disturbance of chromosome
77 segregation but severely affect sporulation (Autret et al., 2001; Ireton et al., 1994), whereas in
78 *C. crescentus* the *par* genes are essential for survival (Mohl and Gober, 1997). Besides, in

other bacteria depletion of ParA/ParB affects cytokinesis, growth, cell morphology and motility (Bartosik et al., 2009; Godfrin-Estevenon et al., 2002; Jakimowicz et al., 2007b; Lasocki et al., 2007; Lewis et al., 2002; Mohl et al., 2001).

2. ParABS SYSTEM SEGREGATES PLASMID DNA IN A MITOSIS-LIKE MANNER

Low copy number plasmids have developed several specific mechanisms to be stably maintained in the bacterial population (Nordström and Austin, 1989). One of them is represented by *par* loci that secure equal distribution of plasmid copies to the daughter cells at cell division.

A comprehensive review on plasmid partition systems has been published recently (Gerdes et al., 2010) hence only general information will be presented here to help to envisage the role chromosomally encoded Par proteins may play in the cells.

The plasmid partitioning system consists of three components: a weak NTPase (A-component), a DNA-binding protein (B-component) and a *cis*-acting sequence *parC*/*parS*, to which B-component binds specifically. It has been shown that at least some of the partition systems work in a similar manner to the eukaryotic mitotic apparatus used in chromosome segregation. The initial step of plasmid segregation after replication involves binding of *parC*/*parS*, the centromere-like DNA region, by the B-type protein forming a nucleoprotein complex – segrosome, sharing functional similarities with the eukaryotic kinetochore complexes (Tanaka and Desai; 2008). Once the segrosomes are assembled, they may be recognized by the dynamic cytoskeletal filaments built of A-protein, which is either a Walker-A P loop ATPase (ParA, type I), an actin-like ATPase (ParM, type II) (Gerdes et al., 2000), or a tubulin-like GTPase (TubZ, type III) (Larsen et al., 2007). Since the three types of the NTPases involved in plasmid partitioning form different structures, the molecular mechanisms of plasmid segregation seem to differ as well, but eventually produce the same effect of the segrosomes being moved apart.

The crystal structure of the ParM monomer of R1 plasmid reveals that it is related to actin and MreB (van den Ent et al., 2002), however ParM double stranded helix is left-handed opposite to the right-handed actin filaments (Orlova et al., 2007; Popp et al., 2008). ParM polymerizes in the presence of ATP and depolymerizes after ATP hydrolysis (Møller-Jensen et al., 2002, 2003). The polymerization of ParM at the segrosome interface is stimulated by ParR and causes the ParM filaments to extend bidirectionally, pushing the two plasmids apart (reviewed by Gerdes et al., 2010). Subsequently, ATP hydrolysis at the opposite ends of the ParM

filaments leads to the disassembly of the filaments. The dynamic instability of the symmetrical bidirectional growth and unidirectional disassembly of ParM filaments drives plasmid partitioning and resembles instability of microtubules in eukaryotic chromosome segregation (Higuchi and Uhlmann, 2005)

Functioning of the P loop ATPases is poorly understood. They show non-specific DNA binding (Bouet et al., 2007; Castaing et al., 2008; Dunham et al., 2009; Hester and Lutkenhaus, 2007; Leonard et al., 2005b; Pratto et al., 2008) and form a cloud over the nucleoid moving dynamically from pole to pole. Their movements are ATP-dependent and stimulated by the presence of cognate ParB-*parS* segrosome. Earlier *in vitro* and *in vivo* data indicated the ability of deviant Walker-type ATPases to polymerize into filaments and depolymerize after interaction with the segrosome (Barillà et al., 2005; Ebersbach and Gerdes, 2004; Ebersbach et al., 2006; Lim et al., 2005; Ringgaard et al., 2009), suggesting that a pulling mechanism might be responsible for separation of plasmid molecules (Ringgaard et al., 2009). However, recent studies (Vecciarelli et al., 2010) stir some controversy over the role of ParA polymerization and depolymerization in plasmid segregation. The ParA-ATP complex binds non-specifically to the nucleoid with high affinity and the ParB-*parS* complexes interacting with ParA stimulate ATP hydrolysis, causing ParA-ADP release from the nucleoid. A new model "diffusion-rachet" is proposed where a delay is required for ParA to rebind ATP. The delay gives time to ParA to get distributed in the cell, making rebinding of ParA-ATP to the nucleoid in the same place unlikely. This encourages the segrosome to translocate preferably to a new location, where the density of nucleoid bound ParA-ATP is maximal. When two segrosomes are formed (after plasmid replication) they may move away from each other by being repelled from the region of low ParA-ATP density around them. So the uneven distribution and dynamic re-location of ParA-ATP would provide the force for plasmid segregation before cell division.

The recently discovered *par* loci in plasmids of Gram- positive bacteria encode the third type of NTPases involved in the plasmid DNA segregation. The tubulin/ FtsZ- like GTPases designated TubZs are encoded by pXO1 of *B. anthracis* or pBtoxis of *B. thuringensis* (Anand et al., 2008; Tinsley and Khan, 2006). The TubZs assemble into double-stranded microtubule-like filaments *in vitro* (Chen and Erickson, 2008). Dynamic filaments of TubZ of the *B. thuringiensis* plasmid move rapidly *in vivo* along the cell membrane in a treadmilling-like way (Larsen et al., 2007).

The second component of the system, TubR, small DNA binding protein, may be responsible for anchoring of the microtubules to plasmid DNA. However, the dynamic movements of TubZ of pOX1 do not rely on the presence of TubR (Larsen et al., 2007).

Plasmid DNA replication in general is considered not to be coupled with the cell cycle although some controversy exists over e.g. F plasmid (Bogan et al., 2001; Helmstetter et al., 1997; Leonard and Helmstetter, 1988).The segregation of the replicated plasmid copies to positions typical for particular replicon (e.g. polar, ¼ and ¾) may proceed at any time prior to the cell division (Gordon et al., 1997). Moreover, the independence of the plasmid partitioning functions of any other chromosome-encoded components has been demonstrated *in vivo* (Campbell and Mullins, 2007; Ebersbach and Gerdes, 2005) and *in vitro* (Garner et al., 2007).

3. THE ROLE OF *par* LOCI IN CHROMOSOME PARTITIONING

3.1. Chromosomally encoded *par* loci are highly conserved

Among the large number of proteins involved in chromosome segregation, homologues of the plasmid partitioning *parABS* loci are found. They are encoded by the majority of the sequenced bacterial genomes (Livny et al., 2007) with only a few exceptions: members of two families of Proteobacteria, *Enterobacteriaceae* (e.g., *E. coli*) and *Pasteurellaceae* (e.g., *Haemophilus influenzae*), and Firmicutes (e.g., *Mycoplasma* sp.). The first Par homologues were analyzed in *B. subtillis* and designated Soj (ParA) and SpoOJ (ParB) due to their role in sporulation regulation (Ireton and Grossman, 1994; Ireton et al., 1994; Quisel and Grossman, 2000), and further studies confirmed their function in chromosome segregation during sporulation in other bacterial species (Jakimowicz et al., 2005, 2006; Kim et al., 2000). Chromosomal homologues of ParA and ParB are also found in bacteria that do not sporulate, suggesting a more fundamental role for these proteins. Furthermore, homologues of ParAB proteins are encoded in the majority of bacterial chromosomes sequenced in close vicinity to *oriC* (Livny et al., 2007) what also suggests their role in either replication or segregation of chromosomes.

The chromosomal *par* systems are classified as Type I, since the ParA homologues are weak ATPases of type I, which contain Walker A and B motifs, and ParBs belong to the conserved ParB Type I family (Gerdes et al., 2000). Additionally, *parS* sequences which are recognized by ParB have also been identified on bacterial chromosomes (Fig. 1). A striking feature of the chromosomal *parS* sequences (consensus tGTTtCAcGTGAAAAa/g; Lin and

Grossman, 1998) is their high conservation between species (Livny et al., 2007). In the species with multipartite chromosomes these conserved *parS* loci have been found on the primary chromosomes whereas the secondary chromosomes have their own ParABS systems (Livny et al., 2007) demonstrating intra- and interspecies structural and functional diversity (Dubarry et al., 2006; Fogel and Waldor, 2005). The finding that the vast majority of *parS* sites are located in the origin-proximal region of the chromosome suggests that the function of the *par* loci relates to this part of the chromosome and that this function is preserved among diverse bacteria (Livny et al., 2007). The biological function of the few *parS* sites found in origin-distal regions of the chromosome remains to be deciphered.

Importantly, the chromosomal *par* loci can be engineered to function in the same way as a plasmid *par* system to stabilize an otherwise unstable plasmid replicon and direct positioning of its molecules within the cell (Bartosik et al., 2004; Goldfrin-Estevenon et al., 2002; Lin and Grossman, 1998; Yamaichi and Niki, 2000).

Although the enteric bacteria, including *E. coli*, belong to the few exceptions, which do not possess ParA and ParB homologues, it is possible that they have evolved an analogous system for active chromosome partitioning. A putative chromosomal partitioning site *migS*, close to the origin region, has been identified in the *E. coli* chromosome (Yamaichi and Niki, 2004). The consensus sequence of *migS* shows a symmetry typical of targets for dimeric DNA-binding proteins. The half-palindromes of these sequences are similar to the ParB boxA motifs of P1 centromeres (Bouet et al., 2000; Surtees and Funnel 2001). A *migS* sequence has also been found in two different *Shigella flexneri* strains (2a str. 301 and 2a str. 2457T), which do not have any homologues of partition proteins (Fekete and Chattoraj, 2005). It has been suggested that the *migS* site plays the role of a centromere, since it is required for bipolar placement of *oriC* and, when placed elsewhere on the chromosome or on a plasmid, it assures proper localization of its new DNA context (Fekete and Chattoraj, 2005; Yamaichi and Niki, 2004). However, deletion of *migS* has also been shown to have little (Yamaichi and Niki, 2004) or even no effect (Wang and Sheratt, 2010) on overall segregation, suggesting that the sequence is functionally redundant. It is difficult to pinpoint the exact role of *migS* since multiple mechanisms contribute to chromosome segregation (reviewed by Errington et al., 2005; Leonard et al., 2005a) and a defect in any one of those mechanisms may therefore not have a strong effect on chromosome segregation or cell viability. For example, a *mukBmigS E. coli* strain shows a stronger phenotype than strains with single deletions (Yamaichi and Niki, 2004). Also, individual deletions in *par* genes (except in *C. crescentus*) do not severely

influence the cellular functions, but when combined with deletions of other genes such as *smc* (analogue of *mukB* in *E. coli*) they produce serious defects in chromosome segregation (Lee and Grossman, 2006).

Since the *par* function seems to be species specific, the best studied chromosomally encoded *par* genes of *B. subtilis*, *C. crescentus*, *Streptomyces coelicolor*, *V. cholerae* and *Pseudomonas* spp. will be described in more detail.

3.2. Bacteria with complex cell cycle

The chromosomally encoded *par* loci have been extensively studied for almost two decades in bacteria which have developed relatively complex survival strategies, including both Gram-negative and Gram-positives ones such as the sporulating *B. subtilis*, the dimorphic *C. crescentus*, the filamentous *S. coelicolor* and dimorphic *Corynebacterium glutamicum*.

3.2.1. *Bacillus subtilis*

In *B. subtilis*, the *parA* and *parB* homologues *soj* and *spo0J*, respectively, have been shown to be important for chromosome partitioning during both vegetative growth and sporulation (Ireton et al., 1994). The *spo0J* gene was first discovered in a sporulation-deficient mutant (Mysliwiec et al., 1991). The phenotype of the *spo0J* mutant was suppressed by a mutation in the *soj* gene (Ireton et al., 1994). The postulated role of Spo0J in sporulation was to antagonize the ability of Soj to repress expression of early (*spoII*) sporulation-specific genes by binding to single-stranded DNA in the open transcription complex (Cervin et al., 1998; Marston and Errington, 1999; Quisel et al., 1999; Quisel and Grossman, 2000).

It has been shown that in both the *spo0J* (Lee et al., 2003; Ogura et al., 2003) and the *soj* null mutant an over-initiation of replication occurs (Lee and Grossman, 2006). Although Soj is a key regulator of the intiation of chromosome replication acting directly on the initiator protein DnaA assembled on *oriC*, its function is controlled by Spo0J (Scholefield et al., 2011). In the presence of Spo0J Soj-DnaA interactions result in repression of extra initiations (Murray and Errington, 2008). In its ATP-bound dimer form, which accumulates in the absence of Spo0J, Soj stimulates DNA replication *via* DnaA, which in turn activates a sporulation checkpoint protein Sda that blocks sporulation (Burkholder et al., 2001). This data has changed the classical view of how Soj inhibits sporulation and how Spo0J may contradict its action (Murray and Errington, 2008; Scholefield et al., 2011)

Spo0J binds to eight *parS* sequences that are located in the origin-proximal 20% of the chromosome - so called origin domain (Lin and Grossman, 1998) - and two that are far away from the *oriC* region (Breier and Grossman, 2007). Furthermore, subcellular localization experiments have demonstrated that Spo0J-GFP forms bipolar foci at cell quarter positions, coinciding with the *oriC* region both during vegetative growth (quarter cellular positions) and sporulation (Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997). During sporulation, the Spo0J foci move to the extreme poles of the cell to position the *oriC* region and ensure proper orientation of the chromosome entering the prespore compartment of the cell ready for polar septation (Glaser et al., 1997; Sharpe and Errington, 1996). It is postulated (Wu and Errington, 2003) that Soj and RacA cooperate in association of the *oriC* region with DivIVA at the cell pole during sporulation.

Moreover, Spo0J bound to the *oriC* regions of segregated chromosomes relieves Soj-mediated repression. Therefore, Soj/Spo0J operate as a simple signal transduction system that couples chromosome segregation to the initiation of sporulation.

Using fusions of Soj to GFP protein, it was found that Soj localized near the cell poles and upon entry into stationary phase oscillated from pole to pole (Marston and Errington, 1999; Quisel et al., 1999).

While in vegetative cells a lack of Spo0J (ParB) increases the frequency of anucleate cells one hundred-fold, indicating a defect in chromosome partitioning, inactivation of *soj* does not cause an appreciable chromosome partitioning defect on its own (Ireton et al., 1994). It seems that the role of Spo0J may be broader than the one played together with Soj in chromosome partitioning. In a single *B. subtilis smc* mutant (deprived of the Structural Maintenance of Chromosome protein - SMC), about 2% anucleate and "cut" cells with guillotined chromosomes (part of the chromosome trapped by the closing septum) are produced (Lee and Grossman, 2006). SMC is a DNA-binding protein that contributes to chromosome compaction and organization (Lindow et al., 2002). Interestingly, a knockout of either *soj* or *spo0J* individually or a double *sojspo0J* knockout in the *smc* null mutant leads to increased production of anucleate cells (18-19%) and also 8-12% "cut" cells (Lee and Grossman, 2006). It now appears that Spo0J helps chromosomes to segregate properly by recruiting the SMC complex to the origin region (Sullivan et al., 2009). Spo0J seems to be required for the efficient accumulation of SMC not only at chromosomal sites occupied by Spo0J but also at more distant locations, suggesting that it might act as a loading factor rather than a static anchor for SMC on the chromosome (Gruber and Errington, 2009).

Furthermore, the proper subcellular positioning of Spo0J and Soj depends on cell division proteins like DivIB, FtsZ, PBP and MinD (Autret and Errington, 2003; Marston and Errington, 1999; Real et al., 2005). Through such interactions Spo0J and Soj are also involved in regulation of the cell division.

3.2.2. *Caulobacter crescentus*

The dimorphic alpha-proteobacterium *C. crescentus* is a valuable bacterial model system for studying chromosome segregation since its chromosome replication is initiated only once per cell cycle at a well defined time, making it easy to separate and analyze cell cycle events (Jenal et al., 1995). Differentiation is an integral part of the *C. crescentus* cell cycle. In a swarmer cell, which is unable to initiate DNA replication, the origin of replication is located at the flagellated pole (Jensen and Shapiro, 1999). DNA replication initiates when the swarmer cell differentiates into stalked cell and replisome components assemble onto the replication origin at the stalked cell pole (Jensen et al., 2001). A copy of the duplicated origin region moves rapidly to the opposite pole in what is thought to be an active process (Viollier et al., 2004), and as DNA replication proceeds, the replisome progresses from the stalked pole to the division plane (Jensen et al., 2001). When the replication is complete, the replisome disassembles and terminal regions of the sister chromosomes are separated, and finally cell division proceeds.

In *C. crescentus*, homologues of plasmid partitioning genes, *parA* and *parB,* are essential for survival (Mohl and Gober, 1997). They are crucial for DNA segregation and cytokinesis initiation. The *C. crescentus* ParABS system mobilizes one of the two newly replicated *oriC* domains by binding to the origin-proximal centromeric DNA *parS* sequences and moves it unidirectionally from the old pole to the new pole (Toro et al., 2008; Viollier et al., 2004), in contrast to the bidirectional movement observed for plasmid segregation (Gerdes et al., 2010). When the *parS* sequences are manipulated into distant location from the origin, the cell cycle waits for them to be replicated before commencing segregation. Moreover, a lack of the ATPase function of ParA arrests the segregation without affecting replication initiation (which is in stark contrast to the situation in *B. subtilis* where a lack of Soj leads to over-initiation of replication). The chromosome segregation in *C. crescentus* cannot start unless a dedicated *parS* guiding mechanism initiates the movement (Toro et al., 2008). ParB binds to the *parS* sequences adjacent to the *oriC* region hence ParB foci co-localize with the *oriC* region (Figge et al., 2003). Recently, it was reported that the *C. crescentus* ParB/*parS* centromere-like complex (Toro et al., 2008) is anchored to the cell pole by PopZ polymeric

protein and that is required for an effective chromosome segregation and cell division (Bowman et al., 2008; Ebersbach et al., 2008). The ParA ATPase trapped at the new pole by TipN (a pole specific protein) extends to the stalked pole (Ptacin et al., 2010) and pulls one copy of the chromosome by retracting upon association with the ParB/*parS* complex (Fig. 2).

Depletion of ParB inhibits the formation of stable FtsZ rings creating a septum and as a result cytokinesis is blocked and long smooth filamentous cells are produced (Mohl et al., 2001). Furthermore, over expression of ParA results in an almost identical filamentous phenotype, whereas simultaneous over expression of both Par proteins does not cause such cell division defect. Therefore, the intracellular localization and concentration of ParA and ParB seem to be crucial for the initiation of cell division after sister chromosome separation. Previously it was thought (Easter and Gober, 2002) that ADP-bound ParA binds to single-stranded DNA and presumably represses the expression of a critical cell division gene (like in *B. subtilis*). However, a more recent study showed that an ATPase, MipZ, binds directly to ParB and moves with the ParB/*parS* complex and the newly replicated origin to the cell pole (Thanbichler and Shapiro, 2006). MipZ directly interferes with FtsZ polymerization, thereby restricting FtsZ ring formation to the midcell, where the MipZ concentration is the lowest. Thus, in *C. crescentus* the ParABS system and the PopZ, TipN and MipZ proteins involved in anchoring the nucleoprotein ParB/*parS* complexes to a specific cellular location are key components of an integrated temporal and spatial system controlling and coordinating chromosome segregation with the cell cycle progression (Schofield et al., 2010).

3.2.3. *Streptomyces coelicolor*

S. coelicolor is the best-characterized member of the actinomycete genus renowned for its ability to produce secondary metabolites. It is a Gram-positive soil bacterium with a large linear chromosome of about 8.7 Mb and unusual cell division features. *S. coelicolor* is a filamentous bacterium, growing by tip extension and hyphal branching to form a dense mycelia network of vegetative hyphae in which occasional septa separate adjacent multigenomic compartments (Flärdh, 2003). During the development of *Streptomyces* colonies, vegetative hyphae undergo morphological differentiation into aerial hyphae. The growth of the aerial hyphae is accompanied by intensive replication of the chromosomes and even more than 50 non-segregated chromosomes may be present in a single long tip compartment (Ruban-Osmialowska et al., 2006). After termination of the growth, the aerial hyphae tip compartments subsequently develop into exospore chains (Chater, 2001). This process begins with the assembly of a regular ladder of FtsZ rings which are precursors of

sporulation septa (Schwedock et al., 1997). Formation of the sporulation septa is accompanied by chromosome condensation (Flärdh et al., 2000) and chromosome segregation into unigenomic prespore compartments.

The *S. coelicolor* chromosome contains the *parAB* operon whose expression is developmentally regulated, with one of its two promoters strongly upregulated shortly before sporulation (Jakimowicz et al., 2006; Kim et al., 2000). Deletion of either *parA* or *parB* or of the whole *parAB* locus resulted in relatively frequent anucleate spores (15-26%) but did not visibly affect colony growth or viability (Jakimowicz et al., 2002, 2006, 2007b; Kim et al., 2000). ParB binds to 20 *parS* sites near the *oriC* region, and also affects sequences flanking the recognition sites, suggesting nonspecific binding and/or oligomerization of the protein (Jakimowicz et al., 2002). The nucleoprotein complexes may be visualized *in vivo* as ParB-EGFP foci, which behave differently during vegetative growth and sporulation. In vegetative hyphae bright ParB-EGFP signal is detected close to the hyphal tip accompanied by some smaller, irregularly distributed foci through the length of the hyphae. During sporulation, arrays of regularly spaced ParB complexes assemble along the aerial hyphal tip compartment, which form just before DNA segregation and septation and disassemble after these processes are completed (Jakimowicz et al., 2005). It is postulated that ParB assists DNA condensation and segregation by mediating proper organization and/or positioning of the *oriC*-proximal part of the chromosome (Jakimowicz et al., 2005).

ParA of *S. coelicolor* has been found to be required for efficient chromosome segregation and regular septation during sporulation, and shown to have a weak ATPase activity that is needed for these functions (Jakimowicz et al., 2007b). In young aerial hyphae ParA accumulates at the tips and when the hyphae elongate ParA extends back from the tip in the form of helical filaments. Surprisingly, the formation of ParA helical filaments in *S. coelicolor* is not affected by a mutation in ParA eliminating ATP binding, which is in contrast to studies on other ParA homologues, both plasmid and chromosomal, tested so far (Barillà et al., 2005; Ebersbach and Gerdes, 2001; Fogel and Waldor, 2006; Quisel et al., 1999). However, ParA mediates formation of ParB complexes in an ATPase-dependent manner. This is reminiscent of the observations on Soj-Spo0J interactions in *B. subtilis* (Glaser et al., 1997).

Jakimowicz et al. (2007b) proposed a model of the assembly of ParA filaments during development of aerial hyphae in relation to the formation of ParB complexes, Z-rings, septation and DNA segregation (Fig. 3). With the assistance of the ParA filaments, regularly spaced ParB complexes form on the *oriC*-proximal part of the chromosomes, placing these

regions between the sites of future septa. It seems that the ParA filaments themselves, depending on their ATPase activity, act in some way as a "ruler" for the spacing of the complexes and, directly or indirectly, for the positioning of the FtsZ rings. After segregation of DNA the filaments of ParA disassemble and sporulation proceeds. Similar functions in positioning plasmid molecules regularly over the bacterial nucleoid have been proposed by Adachi et al. (2006) and Ebersbach et al., (2006) for SopA of plasmid F and ParA of pB171, respectively.

3.2.4. *Corynebacterium glutamicum*

Another member of the actinomycete genus is *Corynebacterium glutamicum*, which has a characteristic cell morphology and cell division. Corynebacteria are known to lack MreB cytoskeletal structures and a Min system (Letek et al., 2008). Depending on the growth medium *C. glutamicum* is rod-shaped or club-shaped and fast-growing cells often divide into unequal-sized daughter cells, which is in strong contrast to other model bacteria such as *E. coli* or *B. subtilis*.

Besides the *parAB* operon (cg3427 and cg3426) located in the vicinity of the *oriC* region on the *C. glutamicum* chromosome, a *parA*-like gene named *pldP* (cg1610) was found in different location (Donovan et al., 2010). Deletion of either *parA* or *parB* has a drastic influence on growth rate and causes a high increase in the proportion of anucleate cells in a population of fast-growing cells (about 18% in *parA* and about 44% in *parB* mutants). In slow-growing bacteria the loss of ParA or ParB has a minor influence on the growth rate, however, the frequency of anucleate cells in the *parA* strain remains the same (16%), and drops to 12% in *parB*. Interestingly, deletion of the *pldP* gene has no effect on growth and induces only few anucleate cells (about 1%) in both fast- and slow-growing cells. Notably, the *pldP* strain produces significantly longer cells than the wild type, *parA* or *parB* strains. Hence, it seems that ParA and PldP have different cellular roles (Donovan et al., 2010).

Similarly to other bacteria that show polar origin localization (e.g., *C. crescentus*, *V. cholerae* chromosome I), the ParB foci closest to the cell poles are static, which suggests that ParB might be anchored at the cell pole. Mobile ParB foci migrate to the midcell in dividing *C. glutamicum* cells and stay there until division is completed (time-lapse experiments). Using a LexA-based bacterial two hybrid system, an interaction of ParB with FtsZ as well as interactions of both ParA and PldA with ParB have been demonstrated (Donovan et al. 2010). However, only ParA, and not PldA, was found to be necessary for polar ParB localization. The subcellular localization of ParA-CFP reveals large patches over the nucleoid with

stronger signals close to the cell poles. In contrast, PldP-CFP localizes to the site of septation. The intracellular localization of PldP suggests that this protein is engaged in cell division, but no direct interaction *in vivo* with FtsZ has been revealed. Thus, it has been suggested that ParB mediates the putative interaction between PldP and FtsZ.

Interestingly, in another representative of actinomycete genus *Mycobacterium tuberculosis*, two additional ParA homologs (Rv1708 and Rv3213) besides ParA have been found (Maloney et al., 2009). Studies on ParA and its homologs suggest similar roles for all three proteins in nucleoid segregation and possible interaction with ParB (Maloney et al., 2009). It was shown that cells overproducing ParA, Rv1708 or Rv3213, and ParB are filamentous and contain multiple nucleoids indicating defects in cell-cycle division. The co-localization of ParA and ParB at the cell poles indicated that these proteins interact and possibly associate with the chromosomal origin of replication (Maloney et al., 2009).

Transposon library screening has suggested that *parA*, *parB* and *rv1708* are essential in *M. tuberculosis* H37Rv (Sassetti et al., 2003). In another *Mycobacter* species, *Mycobacter smegmatis*, *parB* is not essential. In a rich medium growth of the *M. smegmatis parB* deletion strain was delayed and exhibited a longer lag phase than the wild-type (Jakimowicz et al., 2007a). Besides, 10.3% of the cells were anucleate compared with 0.8% for the wild-type. Thus, the lack of ParB affects segregation of chromosomes and may influence growth of *M. smegmatis*.

3.3. Bacteria with simple cell cycle

Although most of the studies on chromosomally encoded *par* involved bacterial species with fairly complicated survival strategies, some studies have also addressed bacteria with a simple cell cycle, like *V. cholerae* or *Pseudomonas* spp.

3.3.1. Bacteria with multipartite genomes

Still little is known about the mechanisms used accurately to duplicate and segregate multipartite genomes during the bacterial cell cycle. Whether the need to coordinate the movement and positioning of two or more bulky nucleoids demands a degree of precision that only the ParABS systems can provide is the major unanswered question here. Recent studies of chromosome partitioning in *Vibrio* species, which contain two or more chromosomes (Egan and Waldor, 2003; Fiebig et al., 2006; Fogel and Waldor, 2005; Saint-Dic et al., 2006), might give a clearer view of the role of the ParABS systems.

V. cholerae is a curved Gram-negative rod-shaped gamma- proteobacterium that causes the severe diarrheal disease cholera. It has a genome divided between two circular chromosomes. Chromosome I (ChrI; ~3 Mb) contains the majority of genes considered essential for cell growth. Chromosome II (ChrII; 1.1 Mb) harbors the remaining house-keeping genes, which encode ribosomal proteins L22 and L35, a translation initiation factor, and aminoacyl-tRNA synthetases. The presence of essential genes on ChrII classifies this replicon as a bona fide chromosome (Egan et al., 2005). However, the presence of 13 toxin-antitoxin loci, which are often found in plasmids, where they ensure maintenance of plasmids in bacterial population (Gerdes et al., 2005; Hayes, 2003) and the similarity of *oriCII* to *ori* of P1 phage, as well as other features of ChrII (Egan et al., 2005; Egan and Waldor, 2003; Venkova-Canova and Chattoraj, 2011), have led to the suggestion that this chromosome originated as a megaplasmid acquired by an ancestral *Vibrio* species.

Distinct mechanisms appear to mediate the localization and segregation of the two *V. cholerae* chromosomes. ChrI undergoes asymmetric segregation in which one copy of the newly replicated *oriCI* remains near the pole, whereas the other copy is moved rapidly to the opposite cell pole (Fiebig et al., 2006; Fogel and Waldor, 2005, 2006). In contrast, ChrII undergoes a symmetrical segregation in which the two copies of the duplicated *oriCII* rapidly move bidirectionally to the cell quarter positions (Fogel and Waldor, 2005; Fiebig et al., 2006). The localization of the *terI* and *terII* regions is also consistent with an asymmetrical and symmetrical model of chromosome segregation, respectively (Srivastava et al., 2006).

Both *V. cholerae* chromosomes have *parAB* genes near their replication origins (Egan and Waldor, 2003). Interestingly, the ParA and ParB proteins encoded by the ChrI *par* locus (*parABI*) are similar to other chromosomal ParA and ParB proteins, whereas the ParA and ParB proteins encoded by the *parABII* locus of ChrII group with plasmidic ParA and ParB proteins of Type I (Gerdes et al., 2000).

The role of the *parAB* loci in segregation of the two *V. cholerae* chromosomes has been extensively analyzed (Fogel and Waldor, 2006). ParABI seems to be part of the apparatus that mediates the polar localization and asymmetric segregation pattern of the *oriCI* region but does not influence segregation of ChrII (Fogel and Waldor, 2006). Although *oriCI* is mis-localized in a *parAI* mutant, ChrI still successfully segregates to daughter cells, indicating that there are genes other than *parABI* that can drive ChrI segregation (Fogel and Waldor, 2006; Saint-Dic et al., 2006). In contrast, *parABII* is required for both localization and segregation of ChrII. In a *parABII* mutant, *oriCII* is randomly distributed in the cell, and

there is a high frequency of ChrII loss (Yamaichi et al., 2007b). The *parABII* mutant shows no detectable defect in *oriCI* dynamics. Thus, the two *V. cholerae parAB* loci appear to function independently in a chromosome-specific manner.

Three *parSI* sequences, similar to the *B. subtilis parS* sites (Fiebig et al., 2006; Fogel and Waldor, 2005, 2006) are found near *oriCI* on *V. cholerae* ChrI (Saint-Dic et al., 2006). It has been shown that *parABI* can stabilize an otherwise unstable mini F plasmid harboring any one of the three *parSI* sequences (Saint-Dic et al., 2006), and ParBI binding to these *parSI* sites was reported (Yamaichi et al., 2007a). Furthermore, removal of these sites from ChrI results in mis-localization of the origin region of ChrI but not a growth defect (Yamaichi et al., 2007a), phenotypes that have also been observed in a *parAI* mutant (Fogel and Waldor, 2006; Saint-Dic et al., 2006). The studies on GFP-ParBI fusion protein have revealed that this protein forms discrete polar foci that segregate with asymmetric dynamics similar to the segregation pattern of *oriCI* (Fogel and Waldor, 2005, 2006). However, there is a significant difference between the ParBI foci and the *oriCI* region in the extreme polarity of ParBI localization; *oriCI* are rarely observed at the edge of the cell. The extreme polarity of ParBI foci suggests that the ParBI-*parSI* nucleoprotein complex anchors ChrI to the pole, as has been proposed for *C. crescentus* (Mohl and Gobler, 1997). Furthermore, Fogel and Waldor (2006) have shown that ParAI is required for polar positioning and asymmetric segregation of ParBI. ParAI shows a dynamic subcellular distribution and promotes separation of the nucleoprotein complex ParBI/*parSI* (localized 65 kb away from *oriCI*) during chromosome segregation. Mutations in the ATP-binding motif of ParAI abolish its dynamics. Fogel and Waldor (2006) have proposed a model for ParABI- mediated segregation of the origin region of ChrI *via* a pulling mechanism (Fig. 4).

Recent studies (Kadoya et al., 2011) have demonstrated that a lack of ParBI in *V. cholerae* leads to over initiation of DNA replication (similar effect observed in *B. subtilis*). Using a bacterial two hybrid system direct interactions have been demonstrated not only between DnaA and ParAI (like in *B. subtilis*) but also between DnaA and ParBI.

Ten ParBII-binding sites (*parSII*), which show similarity to O_B3 (*parS*) of plasmid RK2/RP4 (Balzer et al., 1992; Williams et al., 1993), have been found on *V. cholerae* chromosome II (Yamaichi et al., 2007a). Most of them are localized in close vicinity to *oriCII*. The ParABSII system stabilized an otherwise unstable mini-F plasmid in a heterologous host, *E. coli* (Yamaichi et al., 2007a, b). The nucleotide sequence of *parSII* differs significantly from that of *parSI*, and the two *V. cholerae* ParB proteins bind only to

their cognate *parS* sequences (Yamaichi et al., 2007a). One of the *parSII* sites is located within *rctA* required for ChrII replication (Egan and Waldor, 2003), suggesting that ParBII binding to this site could influence ChrII replication. Interestingly, one *parSII* site was found near the terminus of ChrI, raising the possibility that ParBII could somehow influence the segregation of ChrI.

In the *parABII* null mutant, ChrII demonstrates a random distribution that results in its frequent missegregation and the generation of cells lacking this chromosome. The cells with only ChrI undergo a highly consistent set of detrimental cytological changes. It has been postulated that the presence of 13 toxin-antitoxin loci on ChrII provides its stability the way plasmid persistence in the bacterial population is ensured, the loss of ChrII and lack of *de novo* synthesis of labile antidotes releases the stable toxins from the control and leads to the cell death (Yamaichi et al., 2007b).

Burkholderia cenocepacia, pathogenic rod-shaped beta-proteobacterium also possesses a multipartite genome, comprising three chromosomes - c1, c2, and c3, respectively-and a low-copy-number plasmid - p1. Within the origin-proximal regions of each replicon are located *parAB* loci. In c1, the *parAB* locus is adjacent to *gidAB, dnaA, rpmH, rnpA*, and *gyrB* genes, as in other typical chromosomal origins (Ogasawara and Yoshikawa, 1992). In c2, c3, and p1, on the other hand, *parAB* is adjacent to elements typical of low-copy-number plasmids: a gene (*repA*/*trfA*) for a plasmid-like replication control/initiator protein and a cluster of directly repeated sequences similar to iterons. Besides a single *parAB* locus, each *B. cenocepacia* replicon also harbors a group of *parS* centromere-like sites. In an *E. coli* plasmid stabilization test, particular *parAB* locus exhibits partition activity only with the *parS* from its own replicon. These ParABS systems thus have the potential to confer specificity and direction to the partition process in their mother organism (Dubarry et al., 2006) but how this potential is used remains unknown.

3.3.2. Bacteria possessing single chromosomes

In ***Pseudomonas putida***, a Gram-negative rod-shaped free-living bacterium, both *parA* and *parB* deletion mutants did not demonstrate significant deficiencies in exponentially growing cultures propagated in rich medium. However, in minimal medium those mutants showed slightly higher frequencies of anucleate cell formation in exponentially growing cultures, and much higher frequencies (5-10%) in the deceleration phase (Lewis et al., 2002). Oversupply of ParA and ParB proteins also increased anucleate cell numbers, specifically in the deceleration phase (Godfrin-Estevenon et al., 2002). It has been proposed that the *par*

system is required to tether chromosomes at cell quarters when partitioning is necessary in the absence of re-initiation of replication, as seen during transition to the stationary phase (Lewis et al., 2002). It seemed to parallel the importance of equivalent genes during sporulation in *B subtilis* and prespore formation in *S. coelicolor*.

In ***Pseudomonas aeruginosa***, a facultative pathogen, neither ParA nor ParB are essential for cell viability, however, their lack causes visible phenotype defects, more severe in the *parA* mutant (Bartosik et al., 2009; Lasocki et al., 2007). In contrast to *P. putida*, the exponentially growing cultures of both *P. aeruginosa par* mutants demonstrate slower growth rate in comparison to WT strain under all growth conditions tested. Stronger effects of *par* genes disruption are observed for cultures growing on minimal medium and/or at lower temperature. Microscopic observations reveal increased cell size, appearance of high fraction of cells with guillotined chromosomes and up to 4% of anucleate cells, that is more than 400-fold increase in comparison to WT strain. The colony morphology is affected and two types of cell motilities: swarming and swimming, are impaired. Null mutants in a *par* gene show increased instability of the partner protein, suggesting that Par proteins interact with each other and are protected from proteolytic degradation when in complex (Bartosik et al., 2009; Lasocki et al., 2007). Our studies showed that whereas both Par proteins are involved in chromosome segregation, ParB on its own may affect other cell functions, e.g., motility, growth and cell-to-cell communications. ParB may act directly as a transcriptional regulator through binding to dispersed *parS* sites, spreading on DNA and silencing the transcription (Bartosik et al., 2004; Kusiak et al., 2011) or indirectly through interactions with other proteins (M. Kusiak and K. Głabski, unpublished). Dimerization of ParB is essential for all its biological functions (J. Mierzejewska, unpublished) since only ParB dimers can bind DNA, oligomerize on DNA and interact with the ParA partner.

CONCLUDING REMARKS

The bacterial chromosome (nucleoid) is a highly organized structure with particular loci occupying defined cell compartments at specific time points of the cell cycle. After replication the nucleoid domains are translocated orderly to their new positions via a multistep process engaging many proteins.

While the mitotic apparatus used by the eukaryotic cell to carry out chromosome segregation is well understood, numerous questions about the mechanisms that mediate chromosome segregation in prokaryotes remain unanswered.

Several models have been proposed to explain the driving force for chromosome segregation in bacteria: DNA polymerase and the replication process as suggested in the "extrusion- capture" model (Lemon and Grossman, 2000; Sawitzke and Austin, 2001), DNA compaction (Gruber and Errington, 2009; Hirano and Hirano, 1998, 2002), RNA polymerase (Dworkin and Losick, 2002), coupled transcription-translation-insertion (transertion) mechanism (Woldringh, 2002), entropic exclusion of sister chromosomes (Jun and Mulder, 2006) and the dedicated mitosis-like process (Gerdes et al., 2004; Møller-Jensen et al., 2002). The redundancy of the mechanisms may be important to ensure that the chromosomes get separated properly even if some of them fail.

The mitosis-like mechanism of prokaryotic DNA segregation has been demonstrated for low copy number plasmids. At cell division these plasmids are faithfully segregated to the daughter cells by active partitioning loci (*par*) encoding three components: an NTPase (A-type protein), a DNA-binding protein (B-type protein) and a centromere-like site (*parC/parS*). Plasmidic systems are characterized by structural variability of A and B proteins but extreme specificity between B-type proteins and *cis*-acting sites they act upon, and between A and B proteins of single *par* system.

On the majority of sequenced bacterial chromosomes the *parA-parB* operons have been found close to *oriC*s. They encode highly similar ParAs and ParBs proteins. The multiple *parS* sites around *oriC* (*oriC* domain) are also conserved between the species (at least in the primary chromosomes). In all tested organisms the chromosomal ParA and ParB proteins together with the centromere-like *parS* sequences participate in the chromosome segregation although to different extent. The rapid and directional movements of *oriC* domains through dynamic interactions of ParA-ATP with ParB bound to *parS* sequences clustered around *oriC* has been shown for *B. subtilis, C. crescentus, V. cholerae, S. coelicolor* and *P. aeruginosa*. The functions of Par proteins go beyond their role in the translocation of *oriC* domains and these functions seem to vary in different organisms.

ParA and ParB may help anchoring *oriC* domains at the specified locations in the cell through interactions with specifically localized cell components (PopZ-ParB and TipN-ParA interactions in *C. crescentus*).

ParB not only recruits SMC complex to the *oriC* region but also is accessory to SMC loading onto the chromosome in other sites providing the chromosome compaction (*B. subtilis*).

The role of ParA(Soj) and ParB(Spo0J) in the control of replication initiation has been demonstrated for *B. subtilis* (through direct interactions of Soj-ATP with DnaA and indirect role of Spo0J by stimulating ATPase activity of Soj) and for *V. cholerae* (both Par proteins seem to interact directly with DnaA in this strain).

ParA and ParB proteins also have been shown to coordinate the segregation of chromosomes with the further cell cycle progression. In *C. crescentus* MipZ, the inhibitor of FtsZ ring formation, interacts with ParB-*parS* nucleoprotein complex and re-locates with it to the pole facilitating the FtsZ ring assembly at the midcell. In *C. glutamicum* a direct interactions of ParB with FtsZ have been demonstrated. In *S. coelicolor* ParA determines the positioning of the FtsZ rings during the sporulation process. In *B. subtilis* the proper positioning of Spo0J depends on FtsZ, DivIVA, PBP and MinD, proteins that are directly involved in cytokinesis. The ParA (Soj) and ParB (Spo0J) interactions couple not only vegetative replication with chromosome segregation but also initiation of sporulation in *B. subtilis*. It has been shown that Soj induced over-initiation of DNA replication via interaction with DnaA, blocks sporulation by activation of Sda protein (sporulation checkpoint). The process is controlled by Spo0J.

An intriguing feature of the chromosomally encoded ParABS systems is that despite the between-species similarity in amino acid sequences of both Par proteins they seem to participate in different cell processes even in the closely related bacteria. The picture emerging so far presents Par proteins as potent cell cycle regulators through their ability to interact with other proteins important for control of the cell cycle progression. It can not be excluded that interactions with the conserved bacterial proteins such as DnaA or FtsZ are universal and will be soon detected in other analyzed systems but also new partners involved in more species-specific processes will be discovered.

Acknowledgments

This work was funded by MNiSW grant 2913/B/PO1/2008/34.

Figure legends

Figure 1. Localization of *parAparB* loci on several bacterial chromosomes described in the text. Black bars mark *oriCs.* Similar loci in different organisms are marked with the same colors. Arrows indicate *parS* sequences in the presented regions (modified after Bartosik and Burdzy, 2005). Genome of *V. cholerae* is divided into two chromosomes (ChrI and ChrII) and only *oriC* adjacent loci on primary chromosome are shown. Information on *oriC* region organization is based on DNA sequence deposition in GenBank: for *Bacillus subtilis* subsp. *subtilis* str.168 (AL009126); *Caulobacter crescentus* str. NA1000 (CP001340); *Streptomyces coelicolor* A3(2) (AL645882); *Vibrio cholerae* O1 str. N16961 (AE003852); *Pseudomonas putida* KT2440 (AE015451) and *Pseudomonas aeruginosa* PAO1 (AE004091).

Figure 2. ParA and ParB localization during cell cycle of *C. crescentus* (according to Ptacin et al., 2010). (A) In a swarmer cell, a single partitioning nucleoprotein complex ParB/*parS* (purple circles) close to the *oriC* is initially attached by PopZ (black bar) to the cell pole containing the flagellum. While a small fraction of ParA (yellow sticks) in the cell interact with the partitioning complex, most ParA is distributed throughout the cell (like a comet tail) with a higher concentration at the opposite pole, where it is tethered by TipN polymers (grey circle). (B) and (C) Before initiation of replication, nucleoprotein ParB/*parS* complex is released from PopZ polymers. Replication of the origin region results in duplication of ParB foci. One nucleoprotein ParB/*parS* complex remains at the stalked pole, whereas the other advances into the vicinity of the ParA "comet tail" and moves to the new pole. Retracting ParA pulls the ParB/*parS* complex towards the opposite pole. (D) and (E) After segregation, the translocated ParB–*parS* complex is anchored by PopZ at the new pole, while TipN is recruited to the division plane to remain at the new poles of the daughter cells, and the ParA structure reorganizes.

Fig. 3. Model of assembly of ParA filaments and ParB complexes during development of *S. coelicolor* aerial hyphae (according to Jakimowicz et al., 2007b). (A) During growth of aerial hyphae ParA filaments (yellow helices) are located in the hyphal tip; DNA is uncondensed and ParB-DNA complexes (purple dots) are irregularly distributed. (B) After stopped growth, the ParA filaments extend along the hyphae. (C) The ParB complexes are then regularly positioned by ParA in ATP-dependent manner and FtsZ rings form (dark grey circles). When septation is in progress the ParA filaments disappear but regularly distributed ParB complexes remain, DNA is segregated, condensed and finally unigenomic compartments are formed (D).

Fig. 4. Model of ParAI-mediated segregation of ParBI-bound *parSI* sequences in close vicinity to *oriCI* in *V. cholerae* (according to Fogel and Waldor, 2006). (A) Predivisional cell contains two almost fully replicated and segregated chromosomes I. The origin region (black circle) of each ChrI is attached to the cell pole by interaction of ParAI (yellow sticks) with unknown cellular component and ParBI (purple circles) is bound to origin-proximal *parSI* sites. (B) Then, probably during the assembly of the cell division machinery, ParAI nucleates at the forming septum and polymerizes bidirectional towards opposite cell poles. During cytokinesis the next round of DNA replication starts and yields two copies of origin region with ParBI-*parSI* complex. One complex is captured by ParAI at the old cell pole. The other complex captured by ParAI extending from the closing septum is pulled across the cell to the new pole by retracting ParAI polymers (C, D).

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Figure1

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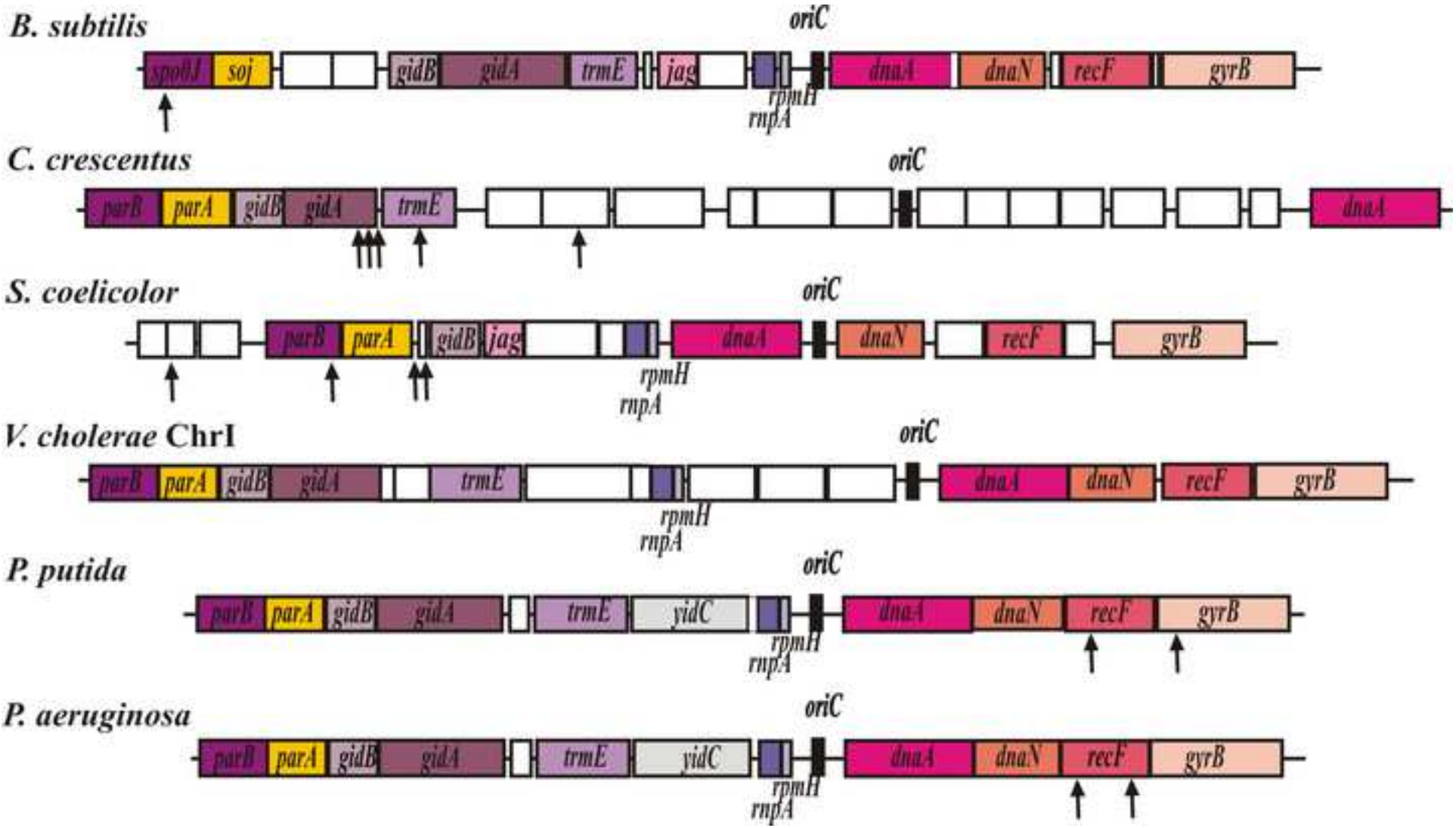

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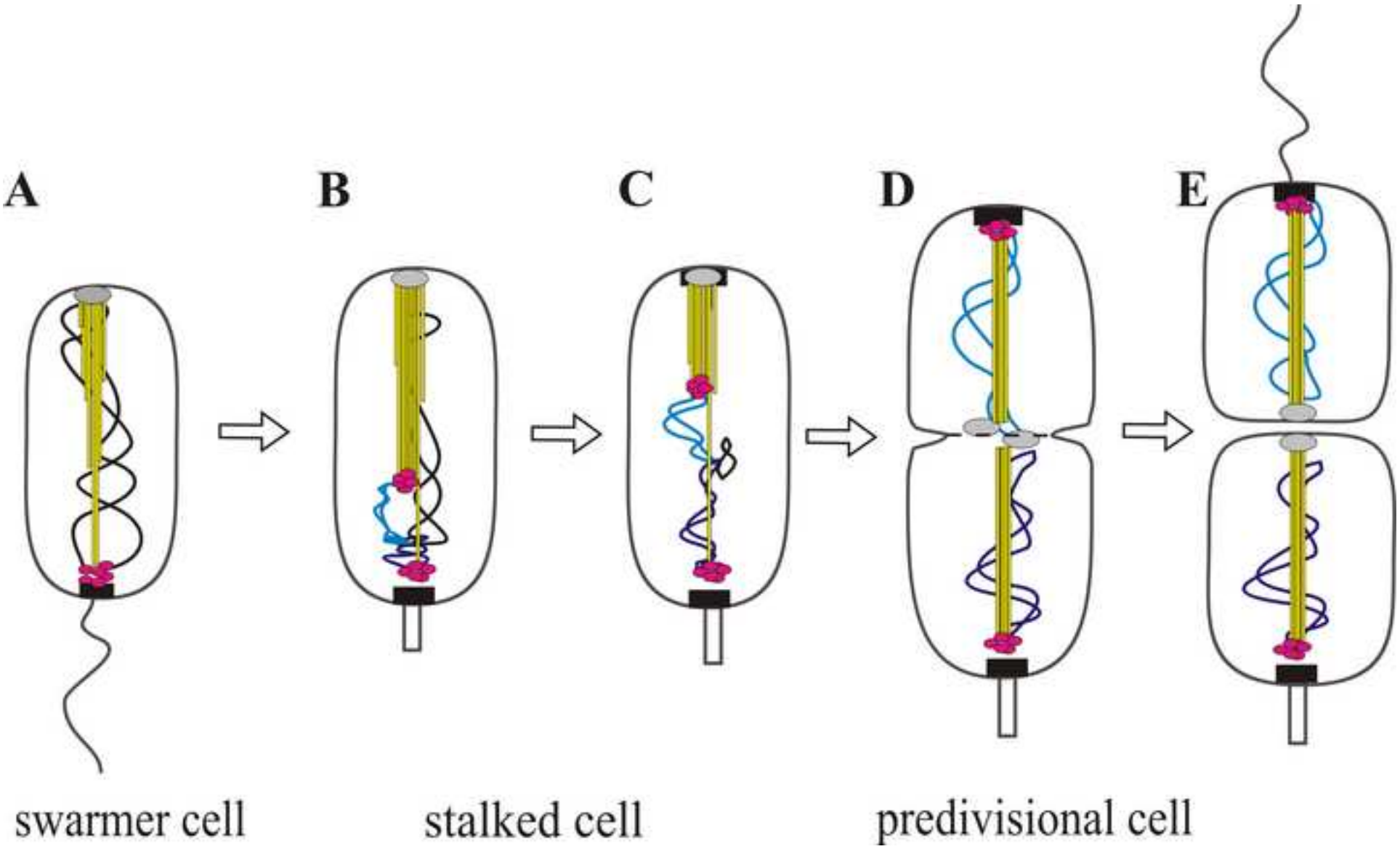

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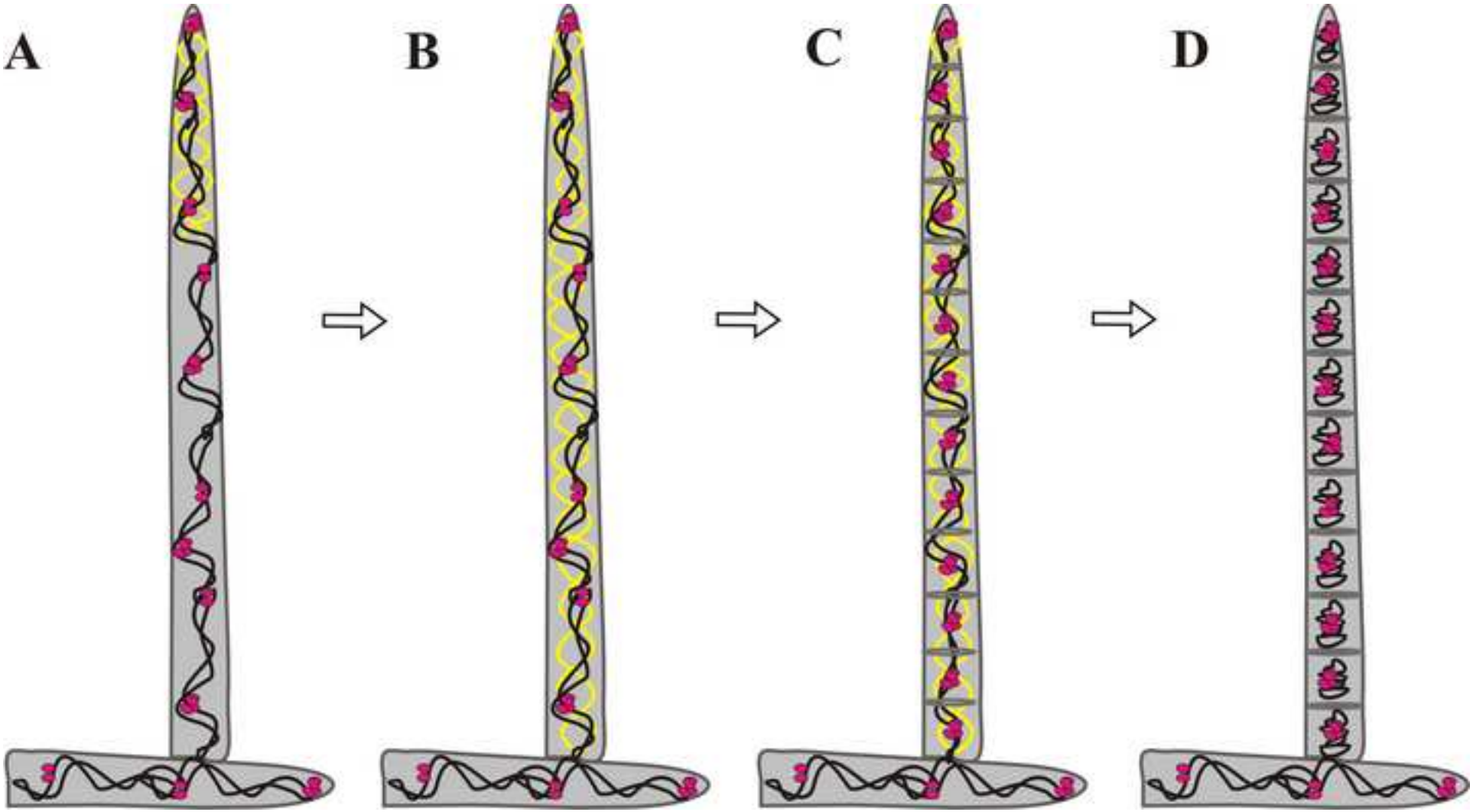

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