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Review

Bacterial chromosome segregation

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In most bacteria two vital processes of the cell cycle: DNA replication and chromosome segregation overlap temporally. The action of replication machinery in a fixed location in the cell leads to the duplication of *oriC* regions, their rapid separation to the opposite halves of the cell and the duplicated chromosomes gradually moving to the same locations prior to cell division. Numerous proteins are implicated in co-replicational DNA segregation and they will be characterized in this review. The proteins SeqA, SMC/MukB, MinCDE, MreB/Mbl, RacA, FtsK/SpoIIIE playing different roles in bacterial cells are also involved in chromosome segregation. The chromosomally encoded ParAB homologs of active partitioning proteins of low-copy number plasmids are also players, not always indispensable, in the segregation of bacterial chromosomes.

In prokaryotes, the essential genetic information is usually carried on a single circular chromosome, although linear (Jakimowicz *et al.*, 2002) and multi-replicon genomes – containing more than one chromosome (Kolsto, 1999; Suwanto & Kaplan, 1992; Jumas-Bilak *et al.*, 1998) – have been found in some bacteria. During the bacterial cell cycle chromosomes are duplicated and segregated into daughter cells.

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Abbreviations: FISH, fluorescence *in situ* hybridization; GFP, green fluorescent protein; PAR, partitioning protein; RAC, remodelling and anchoring of the chromosome; RTP, sequence-specific termination protein; SMC, structural maintenance of chromosome; SSB, single-stranded DNA binding protein.

In eukaryotic cells, chromosome replication, segregation and cell division are separated in time. Chromosomes are duplicated in S phase and remain together during G_2 phase. Partitioning occurs in M phase and then the cell divides after the chromosomes have segregated to opposite halves of the cell. The G_1 phase is the period from the end of cell division to the beginning of the S phase. The eukaryotic cell-cycle nomenclature has been applied to bacteria with some modifications (Helmstetter, 1996). The DNA synthesis phase (S) corresponds to the C period in bacteria and the period from the completion of replication to the end of cell division (G_2-M) is the D period. G_1 is the period from the separation of newly formed daughter cells to the beginning of C phase.

In *Caulobacter crescentus* and in many other slow-growing bacteria the G_1, C and D periods are temporally separated as in eukaryotes (Helmstetter, 1996; Sherratt, 2003). In fast growing bacteria two or more cycles of chromosome replication may overlap (generation time is shorter than the C+D period) and before the newly replicated chromosomes have been segregated to daughter cells the next rounds of replication are initiated. Thus, the newly formed daughter cells have at least two *oriC* replication origins, located in the opposite halves of the cell, and about 50% of the chromosome already replicated (Lau *et al.*, 2003). In contrast to eukaryotic cells, chromosome segregation and cell division in fast growing bacteria occur while chromosomes undergo replication. Nevertheless, DNA replication and segregation ensure faithful transmission of the genetic material to each daughter cell at cell division, therefore both processes have to be highly precise and accurately regulated, so that they are coordinated with the bacterial cell cycle. With the introduction of new techniques to visualize the localization of particular proteins or DNA loci in the bacterial cell (GFP-protein fusions, immunofluorescence and FISH – Fluorescence *In Situ* Hybridization) it was possible to follow chromosome replication, segregation and cell division and to examine the role different proteins play at specified stages of those processes.

BACTERIAL CHROMOSOME REPLICATION

Initiation of chromosome replication

Bacterial chromosome replication starts at a defined time in the cell cycle and at the single, defined site on the chromosome, the replication origin *oriC*. The *oriC* region usually contains binding sites for the initiator protein DnaA (DnaA boxes) and repeats of an AT-rich sequence (13-mers) (reviewed by Thomas & Jagura-Burdzy, 1991). The chromosomal replication origins of different bacteria vary in the number of DnaA boxes (consensus sequence TTNTCCACA) as well as the number of AT-rich repeats. The most highly studied and best understood replication process is that of *Escherichia coli*, so further description of the factors and mechanisms involved will be based mainly on data from this organism. However, data from other organisms will also be discussed where relevant.

Chromosome replication in *E. coli* is initiated by cooperative binding of the DnaA protein (complexed with ATP-DnaA-ATP) to five DnaA boxes (R1–R5) (Messer, 2002) and I2 and I3 sites (McGarry *et al.*, 2004; Leonard & Grimwade, 2005) in the *oriC* region. The origin DNA wraps around the DnaA complex which in the presence of histone-like proteins (HU or IHF) causes unwinding of the DNA double helix at the AT-rich sequences of *oriC* (Leonard & Grimwade, 2005). The hexameric DnaB helicase is then delivered by DnaC protein to the DnaA-unwound DNA complex (Weigel & Seitz, 2002) and the DNA helix is melted further so a primase (DnaG) can act at the open complex to form the first primer which permits sequential loading of the replication machinery.

The initiation of DNA replication is strictly correlated with the bacterial cell cycle and it is important that the chromosome be replicated only once to completion during each cell division cycle (Boye *et al.*, 2000). Initiation takes place whenever the cell reaches a critical mass or volume (Donachie, 2001; Donachie & Blakely, 2003).

Several mechanisms restrict the ability of DnaA to reinitiate DNA replication at *oriC*: the level of available free DnaA in the cell (Messer, 2002; Makise *et al.*, 2002); the inactivation of the active DnaA-ATP form into inactive DnaA-ADP (Kurokawa *et al.*, 1999; Nishida *et al.*, 2002), and sequestration of the newly replicated, hemimethylated *oriC* by SeqA protein (Ogden *et al.*, 1988; Torheim & Skarstad, 1999).

The initiation of replication is followed by loading of the replication machinery onto the DNA at the open complex, which marks the start of the elongation phase of chromosome replication.

The elongation phase of chromosome replication

The complete replication machinery at the replication fork, the replisome, contains primase (DnaG), helicase (DnaB) and the DNA polymerase holoenzyme (Pol III) (Baker & Bell, 1998). Because bacterial chromosome replication is bi-directional, two replication forks work symmetrically in opposite directions starting from *oriC*.

The single Pol III holoenzyme is a ten-subunit enzyme consisting of: (1) two core complexes, each composed of the α (polymerase), ε (proofreading exonuclease) and θ subunits; (2) a τ dimer serving as a bridge between the two core complexes; (3) a clamp containing a homodimer of the β subunit tethering the core complex to the DNA template and (4) a clamp loading complex termed the γ complex consisting of the γ, δ, δ', ψ and χ subunits (Kelman & O'Donnel, 1995). The assembly of individual parts of the replisome on DNA proceeds in an ordered step-wise manner (Baker & Bell, 1998). DnaB helicase interactions with primase at the initiation complex promote primer synthesis on the melted DNA template covered by SSB (single-stranded DNA binding) protein. Then one dimer of Pol III β subunit is chaperoned to the primed DNA in an ATP-dependent reaction catalyzed by the γ complex in the absence of core polymerase. Subsequently the core polymerase joins the complex. The leading-strand polymerase complex remains clamped to DNA to catalyse continuous replication in the 5′–3′ direction, while the lagging strand polymerase complex has to be repeatedly clamped and released from the DNA to synthesize each successive Okazaki fragment.

Within the assembled replisome the DnaB helicase interacts with primase and both proteins interact with the Pol III holoenzyme *via* the τ subunit, which also promotes communication between the two core complexes of the Pol III holoenzyme. These interactions couple the activities of the replisome to ensure coordinated synthesis on the two template strands (Kelman & O'Donnel, 1995).

Termination of chromosome replication — final separation

For circular chromosomes like those of *E. coli* or *Bacillus subtilis* the replication (process) starts at the single *oriC* and proceeds bi-directionally to a site, lying almost directly opposite *oriC*, known as the terminus region *ter* (Hill, 1996; Wake, 1997). In the *ter* region, replication fork arrest occurs (due to formation of the terminator DNA complex and blocking of helicase action) preventing further progress of the replication forks and leading to the dissociation of the replication complex from the DNA. In *B. subtilis* a sequence-specific termination protein, RTP, binds to the terminator sequences (IRI and IRII) creating a terminator complex (Sahoo *et al.*, 1995). In *E. coli* the Tus protein is required for replication fork arrest at the terminus region (Kamada *et al.*, 1996).

The termination of chromosome replication precedes the physical separation of the newly duplicated sister chromosomes prior to cell division. As a consequence of the helical structure of DNA, the products of circular chromosome replication are topologically linked/catenated. In *E. coli*, decatenation of chromosomes is carried out by topoisomerase IV (Kato *et al.*, 1990). This enzyme consists of a heterotetramer formed by a ParE dimer with an ATPase domain, and a ParC dimer containing the DNA binding and DNA cleavage/religation domains. The activity of Topo IV is temporally and spatially regulated: the two subunits of the enzyme reside at different locations in the cell for a significant proportion of the cell cycle (Espeli *et al.*, 2003a). ParC is associated with the replication factory, whereas ParE appears to be distributed in the DNA-free space of the cell. Active Topo IV is formed when the replication factory disassembles (replication fork arrest) after replication is completed. It has been shown that the ParC subunit of Topo IV interacts with the C-terminal domain of the cell division protein FtsK (Espeli *et al.*, 2003b). These interactions concentrate Topo IV at the septal ring where the motor activity of FtsK may prepare DNA for Topo IV action. Thus the action of Topo IV while interacting with FtsK ensures decatenation of newly synthesized circular chromosomes.

DNA replication may also produce chromosome dimers as a consequence of homologous recombination occurring between the newly replicated sister chromosomes. In *E. coli* it has been estimated that chromosome dimers form at least once per every seven cell generations (Steiner & Kuempel, 1998). The chromosome dimers are converted to monomers by the Xer site-specific recombination system, acting at the septum (Recchia *et al.*, 1999). The Xer system of *E. coli* uses two site-specific tyrosine recombinases, XerC and XerD, which act on a 28 bp chromosomal recombination site, designated *dif* and located in the terminus region, to introduce an additional crossing over and thus resolve the chromosome dimer into two monomers (Blakely *et al.*, 1991; Steiner *et al.*, 1999). The Xer recombination activity is controlled by FtsK and requires the specific positioning of the *dif* site in the mid-cell septum region (Steiner *et al.*, 1999). FtsK is located at the leading edge of the division septum and cooperates with the XerCD recombinases bound to the *dif* sites that become trapped in the closing septum. In the presence of chromosome dimers FtsK can load onto DNA close to the *dif* locus and cause a conformational switch in turn activating Xer recombination. The C-terminal domain of FtsK, which functions as a hexameric DNA motor protein affecting DNA topology, is responsible for this activity (Aussel *et al.*, 2002).

Chromosome dimer resolution may lead to chromosome catenation, so the action of XerCD must be followed by that of Topo IV interacting with the C-terminus of FtsK. Thus the action of chromosome-resolving enzymes like XerCD and Topo IV, whose temporal and spatial activity is under control of the cell division protein FtsK, ensures final chromosome separation that clears the division plane in order for cytokinesis and cell division to proceed.

REPLICATION AND SEGREGATION OF THE BACTERIAL CHROMOSOME OCCUR CONCOMITANTLY

Spatial and temporal organization of the replicating/segregating chromosome

Using different fluorescent markers to visualize the replication origin *oriC*, the replication termination region *ter* as well as the replication machinery, microscopic observations have been performed by a number of laboratories (Webb *et al.*, 1998; Lemon & Grossman, 2000; Li *et al.*, 2002; 2003; Lau *et al.*, 2003). The studies have demonstrated that the process of chromosome replication proceeds concomitantly with segregation and created an

image of a dynamic bacterial chromosome which is spatially and temporally organized throughout the cell cycle (Fig. 1).

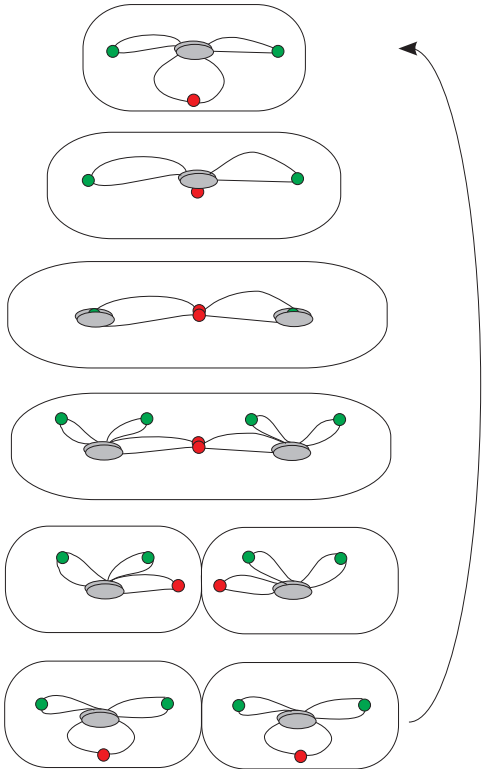

Figure 1. Model representing the dynamic chromosome organization and positioning during the bacterial vegetative cell cycle.

The replication factory is marked by a grey oval; green and red circles indicate the *oriC* and *ter* regions of the chromosome, respectively. After initiation of chromosome replication at mid-cell (fixed replication factory) the duplicated *oriC* regions are rapidly moved close to the quarter positions of the cell. The *ter* region remains at mid-cell until replication is finished and final separation of sister chromosomes (decatenation, resolution of chromosome dimers) and cell division occur. New rounds of replication might be initiated at quarter positions (centres of future daughter cells) before the completion of cell division (fast growth rates).

In fast growing bacterial cultures the chromosome of each newly formed cell is around 50% replicated and its separated origins are close to the quarter positions, while *ter* and the replication machinery are close to mid-cell. The 1/4 and 3/4 positions, to which the origins initially move after replication initiation and separation, become successively occupied by the replisome, FtsZ ring and terminus in the next generations.

It has been proposed that the mechanism which causes the origins to be localized at the 1/4 and 3/4 positions could determine the subsequent positioning of the replisome, the replication terminus and future division site and may act to co-ordinate all these activities (Lau *et al.*, 2003). However, the molecular basis of this mechanism remains unknown. The results of immunofluorescence studies led to the proposal of two opposite models: the factory model and the sister cohesion model, describing the fate of newly replicated DNA and its segregation in bacteria.

The factory (extrusion-capture) model

For years it was believed that the replisomes moved along DNA like trains on a track. Recent immunofluorescence studies have shown that the replisomes are in fact anchored at a specific site in the cell centre and it is the DNA that moves through the replisome (Lemon & Grossman, 1998; 2000; 2001). The factory model for chromosome replication and segregation postulated by Lemon and Grossman (1998) closely resembles one suggested previously by Dingman (1974). The main assumption of the factory model is that DNA movement and segregation is a direct consequence of the replication process and occurs concomitantly with it.

The replication machinery ("factory") consisting of a complex of proteins required for the elongation phase of DNA duplication (see above) is fixed in the middle of the cell at the future plane of cell division (Fig. 2) (Lemon & Grossman, 1998). In some replicons like λ plasmids the replication factory may contain also replication initiation proteins (Wegrzyn & Wegrzyn, 2001; Potrykus *et al.*, 2002). Ongoing DNA replication at the cell centre pushes the newly replicated DNA outwards from the anchored replisome to opposite poles of the cell. Sawitzke and Austin (2001) have proposed that five distinct activities are

engaged in the faithful co-replicational segregation of chromosomes. The newly replicated DNA is pushed, directed, condensed, held and finally separated before the cell division (Fig. 2).

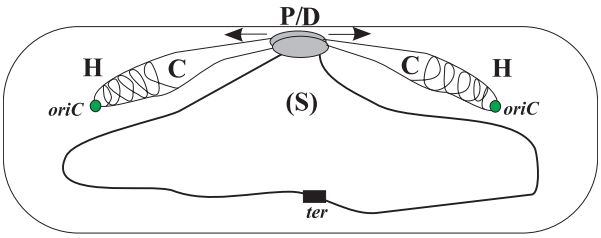

Figure 2. The factory (extrusion-capture) model for bacterial chromosome co-replicational segregation (Lemon & Grossman, 1998; Sawitzke & Austin, 2001).

Duplicated copies of *oriC* (green circles) are extruded (**P,** push) from the centrally located replisome (grey ovals) and move apart (**D,** direct) to quarter cell positions on opposite sides of the replisome where they are captured and held (**H,** hold). Newly synthesized DNA is folded and condensed to organize sister chromosomes (**C,** condense). The terminus remains at mid-cell until it is duplicated and then sister *ter* regions are finally separated (**S**).

The working replication factory provides the driving force for spooling the chromosome through the replisome and also for pushing newly replicated DNA outwards from it (Lemon & Grossman, 2000). It has been postulated that the replication forks are configured in such a way that the products of replication are pushed towards the cell poles in a coordinated fashion (Dingman, 1974; Sawitzke & Austin, 2001) facilitating proper segregation of the newly replicated DNA. It has also been proposed that the origin itself, or sites near the origin, contain information needed for the proper positioning of the segregating chromosomes (see later). As the *oriC* region is duplicated and extruded first, the sister *oriC* regions are directed to the opposite halves of the cell. Visualization studies have demonstrated that the *oriC* regions are separated rapidly to opposite cell poles (Gordon *et al.*, 1997; Webb *et al.*, 1998). The initial action of the replication factory cannot be sufficient to ensure such rapid movement. An unidentified yet active "transport" system is implicated in this rapid relocation of the *oriC* domain. The attachment of the origin sequences to putative cellular structures, followed by DNA condensation may cause the final rapid separation. The role of the ParAB (*parS*) system in *oriC* transport/positioning and chromosome partitioning, by analogy to plasmid partitioning systems, has been postulated for *B. subtilis* and *C. crescentus* (see later) but *E. coli* and closely related species do not encode such a system. In *E. coli* roles for the MinCDE complex in chromosome directing (Shih *et al.*, 2003) and the membrane-binding protein SeqA in tethering the replication forks and organizing newly replicated DNA by forming a channel for segregation have been proposed (Brendler *et al.*, 2000).

After the *oriC* regions have been positioned and fixed at opposite ends of the mother cell (quarter positions), other subsequently replicated regions of the chromosome are pushed and directed towards these locations (Fig. 2). As a consequence of DNA replication the large masses of newly synthesized sister chromosomes have to be organized and condensed to ensure proper segregation. The SMC proteins (Structural Maintenance of Chromosome) in *B. subtilis* and *C. crescentus*, MukB in *E. coli* as well as other non-specific DNA binding proteins such as the histone-like protein HU in *E. coli* or Hbsu in *B. subtilis* are all engaged in chromosome organization and condensation (Graumann, 2000; Niki *et al.*, 1991; Turn & Marko, 1998) (next section).

As new regions of nascent chromosomes are pushed and directed outwards from the replication factory with simultaneously proceeding DNA condensation, a mechanism preventing the DNA from drifting back towards the middle of the cell should exist. This postulated holding activity could be directly connected with positioning and anchoring of the origin sequences by cellular structures/pro-

teins located at the quarter positions of the mother cell. Such an activity could help not only to direct but also to hold the nascent chromosome masses in the appropriate positions to ensure proper segregation during cell division. It has been suggested that the proteins Spo0J of *B. subtilis* and ParB of *C. crescentus* might be involved in the anchoring and holding of nascent chromosomes during the partitioning process since they have been shown to form bipolar foci positioned at the outer borders of the nucleoid close to the poles of the dividing cell (Lin *et al.*, 1997; Mohl & Gober, 1997) (next section). Recently the RacA protein from *B. subtilis* was shown to play an important role in *oriC* anchoring at the cell poles in sporulating *B. subtilis* cells (Ben-Yehuda *et al.*, 2003). It has also been suggested that the transcription and translation of membrane proteins which are produced as DNA is being replicated may play an important role in holding and organizing nascent chromosomes (Woldringh *et al.*, 1995). The transcription and translation of the membrane proteins are coupled and a partially synthesized protein may be inserted into the membrane while its mRNA is still attached to the DNA-coding sequence *via* RNA polymerase. Such coupling may play an important role in holding the nascent chromosomes in place and consequently in their partitioning.

When most of the chromosome has been replicated and its nascent copies, after being pushed, directed, condensed and held, are located at appropriate positions in the cell, the ends of the sister chromosomes often stay together. Chromosome replication produces concatemers or dimers which have to be separated before cell division takes place. The division plane of the cell must be cleared of any DNA to prevent cutting (guillotining) of the chromosome by the invaginating septum. It has been postulated that *E. coli* FtsK is required to translocate any residual DNA from the septum, thus preventing it from being trapped and allowing cell division to go to completion (Aussel *et al.*, 2002). FtsK domain 3 is homologous to the C-terminal domain of SpoIIIE, a protein involved in DNA transfer from the mother cell to the prespore in *B. subtilis* (Bath *et al.*, 2000; Errington *et al.*, 2001). Thus, the FstK protein in *E. coli* and SpoIIIE in *B. subtilis* may play a role in the final separation of the chromosomes at cell division.

All the activities required for co-replicational chromosome segregation in the factory model outlined above involve multiple proteins. For some their role in DNA segregation is well established but for others the functions are only putative. The proteins implicated in chromosome segregation are listed in Table 1 and some will be described in more detail.

The sister cohesion model

There is some discrepancy in the data concerning the timing of *oriC* separation after duplication. Using fluorescence *in situ* hybridization (FISH) Sunako and co-workers (2001) produced evidence that the newly replicated sister DNA regions adjacent to the origin-proximal half of the chromosome remain bound to one another after replication. So the duplicated origins and most other chromosomal regions stay together until late in the replication cycle. Finally, this cohesion is lost and rapid migration of sister *oriC* regions in opposite directions to the future daughter cells occurs (Hiraga *et al.*, 2000), and the sister chromosomes can be reorganized to form two separated folded chromosomes. It has been postulated that chromosome cohesion is independent of DNA methylation by the Dam methylase. The role of the MukEFB complex in chromosome cohesion as well as in chromosome condensation and folding after the loss of cohesion has been confirmed (Hirano, 2000; Sunako *et al.*, 2001). The authors proposed a "sister cohesion model" for *E. coli* chromosome replication and segregation as the alternative to the extrusion/capture model. The "sister cohesion model" under-

Table 1. Proteins implicated in chromosome segregation according to the factory model (extrusion-capture model) for chromosome replication and segregation.

The organism in which the protein function has been studied is given in brackets.

Name	Functions	Putative role according to the factory model (Sawitzke & Austin, 2001)	References
Replisome (several proteins engaged) (*B. subtilis, E. coli*)	chromosome replication – driving force in chromosome segregation	pushing, directing	Lemon & Grossman, 1998; Baker & Bell, 1998
SeqA (*E. coli*)	replication initiation control, tethering the replication forks and DNA channelling during segregation	directing	Brendler *et al.*, 2000; Sawitzke & Austin, 2001
Soj/Spo0J (*B. subtilis*) ParA/ParB (*C. crescentus, P. putida, P. aeruginosa, S. coelicolor*)	chromosome partitioning, cell-cycle regulators	directing, condensing, holding	Ireton *et al.*, 1994; Mohl & Gober, 1997; Lewis *et al.*, 2002; Bartosik *et al.*, 2004; Kim *et al.*, 2000
MukB/SMC (*E. coli/ B. subtilis*)	chromosome condensation/re-condensation, maintaining superhelicity	condensing	Niki *et al.*, 1992; Holmes & Cozzarelli, 2000
Histone-like proteins (HU, H-NS) (*E. coli*)	chromosome organization	condensing	Turn & Marko, 1998
MreB (*E. coli*) MreB/ Mbl (*B. subtilis*)	directional chromosome movement and segregation	directing, holding	Kruse *et al.*, 2003; Jones *et al.*, 2001
SetB (*E. coli*)	chromosome segregation *via* interactions with MreB	directing, holding	Espeli *et al.*, 2003c
MinCDE (*E. coli*) MinCD (*B. subtilis*)	regulation of division site placement	directing?	Shih *et al.*, 2003; Marston *et al.*, 1998
RacA (*B. subtilis*)	chromosome anchoring at the poles, chromosome remodelling	directing, condensing, holding	Ben-Yehuda *et al.*, 2003; Wu & Errington, 2003
Tus (*E. coli*)/RTP (*B. subtilis*)	replication termination, dimer resolution	clearing	Kamada *et al.*, 1996; Sahoo *et al.*, 1995
XerCD/*dif*; Topo IV	dimer resolution, decatenation	clearing	Barre *et al.*, 2001
FtsK (*E. coli*)	dimer resolution, decatenation, DNA translocation through the septum	clearing	Aussel *et al.*, 2002
SpoIIIE (*B. subtilis*)	DNA translocation through septum	clearing	Bath *et al.*, 2000

mines the role of the replication machinery as the driving force for the separation of newly replicated chromosomes towards opposite poles of the cell.

Although the extrusion-capture and sister cohesion models differ in the timing of *oriC* separation and in the proposed role of the replication machinery in this separation, they both imply the existence of an active "partitioning" mechanism, whose nature is still unknown.

PROTEINS IMPLICATED IN BACTERIAL CHROMOSOME SEGREGATION

SeqA

SeqA of *E. coli* is a DNA-binding protein acting at replication origins as a negative regulator of the initiation of chromosome replication. It binds to newly replicated, hemi-methylated DNA at multiple GATC sites thus se-

questering it to prevent premature initiation of replication by DnaA (Lu *et al.*, 1994). SeqA binds also fully methylated GATC sites in the *oriC* region, however, with lower affinity than hemimethylated DNA (Skarstad *et al.*, 2002; Slater *et al.*, 1995). SeqA binding is not exclusive to replication origins; it can bind any DNA containing GATC sites (Slomińska *et al.*, 2001; 2003). Binding studies have revealed (Brendler *et al.*, 2000) that adjacently bound SeqA molecules contact each other to form a complex with the intervening DNA between them looped out. Because chromosome replication produces transiently hemi-methylated DNA it was proposed that tracts of SeqA are associated with the newly synthesized daughter DNA strands at each fork as replication progresses. Because SeqA is also a membrane-binding protein the SeqA tracks might help to tether the forks at the division plane (in accordance with the factory model) and help to organize/compact the newly replicated DNA as it emerges. The existence of a channel created by SeqA interacting with DNA for coordinated segregation of the newly replicated DNA extruded from the replisome has been postulated (Sawitzke & Austin, 2001).

SMC/MukB proteins

SMC proteins were initially described in eukaryotic cells as a large family of proteins engaged in chromosome condensation, cohesion and segregation as well as DNA recombination and repair (Strunnikov *et al.*, 1993; Strunnikov & Jessberger, 1999). Recently homologues of the SMC family have been found in prokaryotes, e.g., *B. subtilis* (Moriya *et al.*, 1998) and *C. crescentus* (Jensen & Shapiro, 1999). Genome sequencing data has revealed the presence of single copy *smc* genes in many bacteria including *Pseudomonas* sp., *Coxiella burnetii, Burkholderia cepacia, Neisseria meningitidis, Agrobacterium tumefaciens, Rhodobacter sphaeroides, Streptomyces* sp., *Mycobacterium* sp., *Lactobacillus lactis, Listeria* sp., *Clostridum* sp., *Staphylococcus aureus* and others.

In a search for segregation mutants in *E. coli,* so-called "mukaku" forms (anucleate cells) were described by Hiraga and co-workers (1989) and later by Niki and co-workers (1991). Disruption of the *mukB* gene in *E. coli* causes temperature-sensitive growth, chromosome decondensation and defects in chromosome partitioning (Niki *et al.*, 1991). A *mukB*-like phenotype was also observed when *mukE* and *mukF* (forming an operon with *mukB*) were disrupted (Yamazoe *et al.*, 1999). It was shown that these three proteins form a complex. It was also revealed that *mukB* encodes an ortholog of SMC proteins. Due to similarities in function and in the predicted three-dimensional structure, these proteins have been classified as one family. Other γ-Proteobacteria like *Vibrio cholerae, Haemophilus influenzae* and *Enterobacteriaceae* such as *Shigella flexneri*, *Yersinia pestis*, *Salmonella* sp. and *Klebsiella pneumoniae* do not encode SMC proteins but only MukB (Herrmann & Soppa, 2002).

The SMC protein of *B. subtilis* was also shown to interact with two additional proteins ScpA and ScpB encoded by genes in close proximity to the *smc* gene (Soppa *et al.*, 2002). A null *smc* mutation in *B. subtilis* or *C. crescentus* causes a similar phenotype to the *mukB* mutant in *E. coli*: temperature-sensitive growth, defects in chromosome condensation and partitioning and abnormal Spo0J assembly at *oriC* (Britton *et al.*, 1998; Jensen & Shapiro, 1999). Interestingly, in a *C. crescentus smc* mutant the defects in nucleoid organization result in cell cycle arrest indicating the presence of a cell cycle checkpoint monitoring and sensing perturbations in chromosome segregation.

All members of the SMC/MukB family have a similar structure which is crucial for their function. They are composed of globular N- and C-terminal domains (heads), separated by two coiled-coil regions connected by a third globular domain creating a flexible hinge

(Graumann, 2001). The N- and C-terminal domains contain conserved ATPase motifs, so-called Walker A and B boxes. The C-terminal domain of SMC has in addition a DA-box motif not found in MukB (Hirano *et al.*, 1995). The prokaryotic SMC/MukB proteins form homodimers of antiparallel configuration (Melby *et al.*, 1998). The MukB dimer has been shown to exist in two conformations, closed and open (V-shaped) with the open form capable of making scissoring movement on DNA. It has been postulated that MukB/SMC molecules bind to two distant DNA locations *via* their head domains and by bending in the hinge region they cause the DNA to "close" and condense (Niki *et al.*, 1992; Graumann, 2001). These proteins also increase the positive writhe of DNA and constrain negative supercoils to counterbalance the action of DNA gyrase (Holmes & Cozzarelli, 2000). It was found that the *mukB* mutation in *E. coli* could be suppressed by topoisomerase I relaxing negative supercoils in DNA (Sawitzke & Austin, 2000).

The SMC protein of *B. subtilis* localizes near the poles (Britton *et al.*, 1998) and within the nucleoid. den Blaauwen and co-workers (2001) demonstrated that MukB in *E. coli* is also preferentially associated with the nucleoid.

One model proposes that bacterial SMC/MukB proteins act after the initial separation and tethering of replicated chromosome origins, facilitating separation of the nascent sister chromosomes by condensation of the strands on opposite sides of the replication factory (Graumann, 2001).

MreB/SetB

MreB of *E. coli,* and MreB and Mbl of *B. subtilis* belong to the actin superfamily of ATPases forming filaments in bacterial cells similar to those of F-actin in eukaryotes (Wachi *et al.*, 1987; Brok *et al.*, 1992; Jones *et al.*, 2001). The filaments formed by MreB/Mbl are part of the bacterial actin-like cytoskeleton required for shape determination, and homologues of these proteins are found in many non-spherical bacteria.

Jones and co-workers (2001) demonstrated that the helical filaments formed by MreB in *B. subtilis* create structures with a short pitch, mainly located at about mid-cell (their number was found to increase with the cell length), which encircle the cytoplasm just under the membrane. It was speculated that these structures are involved in cell width control. Depletion of MreB leads to cell lysis, thus the protein is essential for cell viability. Mbl of *B. subtilis* creates helical structures with a much longer pitch, in comparison to MreB, which run along the whole length of the cell as a double helix, with the two helices intertwined perfectly out of phase. Each helical strand usually makes more than one complete turn around the cell periphery and in longer cells it can be one and a half or two full turns. The Mbl filaments seem to control cell length in *B. subtilis*. Recently it was demonstrated that MreB and Mbl in *B. subtilis* cells are required for bipolar positioning of replication origins (Soufo & Graumann, 2003).

There is no Mbl protein in *E. coli* but its MreB forms helical filaments that extend along the long axis of the cell just beneath the surface of the cytoplasmic membrane in a similar way to the *B. subtilis* Mbl filaments (Kruse *et al.*, 2003). The cells of an *E. coli mreB* mutant are spherical and often segregate their chromosomes in pairs probably due to chromosome cohesion (Kruse *et al.*, 2003), while MreB overexpression inhibits cell division but does not perturb DNA segregation. Overproduction of a mutant form of MreB (mutated at a conserved position of the ATP binding domain) inhibited cell division and led to defects in MreB filament morphology and in chromosome *oriC* and *ter* region segregation. These phenotypes suggest an involvement of MreB filaments in chromosome segregation.

Recently Espeli and co-workers (2003c) demonstrated that MreB in *E. coli* interacts with the integral inner membrane protein SetB which belongs to a family of sugar efflux transporters. Their microscopic observations revealed that SetB also forms helical structures in the cell. Further co-visualization studies revealed that the SetB and MreB helices show spatially coordinated localization. Thus the helical distribution pattern displayed by both proteins could result from their mutual association. Initially the *setB* gene was identified as a suppressor of the partition defect caused by a mutation in *parC* (encoding a subunit of topoisomerase IV). Deletion of *setB* produced subtle chromosome segregation defects with a higher fraction of cells having linked nucleoids, while SetB overproduction led to cell elongation with chromosome stretching and fragmentation. Thus it was proposed that SetB assists anchoring of the MreB helix to the cell membrane and that MreB filaments participate in the generation of an active force promoting/driving chromosome segregation. Interestingly, the plasmid-encoded MreB homologue ParM is engaged in active segregation of R1 plasmid (Moller-Jensen *et al.*, 2002) (see later).

FtsK/SpoIIIE

FtsK of *E. coli* and SpoIIIE of *B. subtilis* are members of a conserved family of proteins involved in chromosome segregation/DNA transport in bacteria. The N-terminal parts of these proteins are the membrane domains, which are separated by variable linker regions to the C-terminal cytoplasmic domains containing Walker-type ATP binding sites (Errington *et al.*, 2001). In *E. coli* the N-terminal domain of FtsK is targeted to the division septum by FtsZ (Yu *et al.*, 1998) and helps to recruit further cell division proteins involved in the closure of the septal ring. The cytoplasmic domain of FtsK functions as a hexameric ATP-driven DNA motor protein, moving along DNA, affecting DNA topology and directly activating resolution of circular chromosome dimers by the XerCD site-specific recombination system (see above). The C-terminus of FtsK also binds to Topo IV at the septal ring where the motor activity of FtsK may mobilize the supercoiled DNA and prepare it for Topo IV action prior to chromosome decatenation (Espeli *et al.*, 2003b). Thus the action of FtsK coordinates the final chromosome segregation with cell division. The ATP-dependent motor activity of FtsK seems to be essential for translocation of any residual DNA through the septum and thus prevents DNA from being trapped and permits cell division to proceed to completion.

FtsK is an essential protein for cell viability in *E. coli*. In contrast, SpoIIIE of *B. subtilis* is dispensable for vegetative growth but plays a crucial role in sporulation (Wu & Errington, 1994). In *B. subtilis* cells, during spore formation, an asymmetrically-positioned (close to the cell pole) division septum is formed before the completion of chromosome segregation which closes around one of the replicated chromosome, trapping only about 30% of it in the forespore. The rest of the chromosome is then actively transported from the mother cell to the forespore through a small pore in the septum in a process performed by SpoIIIE localized at the leading edge of the septum (Bath *et al.*, 2000). The C-terminal domain of SpoIIIE is a DNA-dependent ATPase responsible for active threading of DNA through the septum until the whole chromosome is packed into the forespore. The active DNA transport across the septum is unidirectional due to the specific septum localization of SpoIIIE mediated by its N-terminal region. The specific assembly of SpoIIIE at the leading edge of the septum depends on positional information controlled by the MinCD system (Sharp & Pogliano, 2002) (see later). Recently it has been determined that the SpoIIIE N-terminal domain is also required for membrane fusion during sporulation (Sharp & Pogliano, 2003).

RacA

The *ywkC* gene coding for RacA is one of the most strongly induced genes in sporulating *B. subtilis* cells and is significantly conserved in spore-forming bacteria (Wu & Errington, 2003). RacA was so-named due to its function in remodelling and anchoring of the chromosome. It was found to be required for the formation of axial filaments and anchoring of the origin regions at the cell poles in sporulating *B. subtilis* cells (Ben-Yehuda *et al.*, 2003). The absence of RacA in *B. subtilis* causes a delay in polar septum formation, the presence of stubbing (non-elongation) nucleoids and frequent DNA loss from the forespore (Ben-Yehuda *et al.*, 2003). Expression of a RacA-GFP (green fluorescent protein) fusion creates foci at the extreme poles of the sporangia as well as a haze of fluorescence that co-localizes with the nucleoid. It is assumed that RacA is needed for the formation of an elongated nucleoid whose origin regions localize to opposite poles of the cell, and for efficient trapping of DNA in the forespore. Chromatin immunoprecipitation studies demonstrated that RacA binds preferentially to a region of the chromosome that is located near the origin but which is also found dispersed throughout the chromosome. RacA can directly bind to DNA *via* a putative helix-turn-helix motif located in its N-terminal region. The C-terminal part of the protein possesses two coiled-coil domains, which may play an important role in chromosome remodelling. The polar localization of RacA depends on the DivIVA protein, a cell division factor of *B. subtilis* that is located at the poles (Ben-Yehuda *et al.*, 2003; Wu & Errington, 2003). Soj protein together with Spo0J is also involved in polar *oriC* region localization in *B. subtilis* cells (see below). Results presented by Wu and Errington (2003) suggest that the Soj/RacA/DivIVA system is required for efficient anchoring of the *oriC* region to the cell poles during sporulation in *B. subtilis*.

It has been postulated that RacA is a kinetochore-like protein interacting with a centromere-like element located near the replication origin of the chromosome and (directly or indirectly) with the division protein DivIVA (Edwards & Errington, 1997). It has also been proposed that RacA assembles into an adhesive patch that causes the chromosome origin region to stick to the poles. The dispersed mode of RacA DNA-binding helps to remodel the nucleoid into an elongated structure, the axial filament, which stretches from pole to pole.

RacA may also play a role in polar division. Polar division is normally prevented in growing cells by sequestration of the division inhibitor MinCD (Sharp & Pogliano, 2002) to the cell poles, where it blocks Z ring formation. The division protein DivIVA could be responsible for sequestering MinCD as well as RacA to the poles. The interactions of MinCD and RacA with DivIVA can occur successively and competitively, thereby helping to trigger polar division in *B. subtilis*. It is further postulated that RacA couples two important processes: the tethering of the *oriC* regions at the poles and remodelling of the chromosome prior to proper segregation, and polar cell division in *B. subtilis*.

Par (PARTITIONING) PROTEINS

Two families of bacterial Par proteins, ParA and ParB, were identified and their representatives were found encoded by the majority of bacterial chromosomes (up to date approximatelly 100 species) in the close vicinity of *oriC,* e.g., *B. subtilis* (Ireton *et al.*, 1994; Lin & Grossman, 1998), *C. crescentus* (Mohl & Gober, 1997), *S. coelicolor* (Kim *et al.*, 2000), *P. putida* (Lewis *et al.*, 2002; Godfrin-Estevenon *et al.*, 2002) or *P. aeruginosa* (Bartosik *et al.*, 2004). Par proteins were first described and extensively studied in low copy number plasmids where they were shown to

drive active partitioning of plasmid molecules to the progeny cells. Structural and functional similarities of Par proteins encoded extra-chromosomally and chromosomally seem to implicate the role of the latter in the separation of bacterial chromosomes, although different bacterial species may use them for slightly different purposes. It must be mentioned that at least two evolutionary branches of eubacteria, e.g. *Enterobacteriaceae* and *Pasteurellaceae,* do not possess Par homologs at all. The short summary of the current knowledge on plasmid partitioning presented below is important as an introduction to the studies on chromosomal Par homologs.

par systems of bacterial plasmids as models for bacterial mitotic apparatus

To be stably inherited low-copy number plasmids encode active partitioning mechanisms ensuring their proper segregation at host cell division (Motallebi-Veshareh *et al.*, 1990; Williams & Thomas, 1992). Plasmid active-partitioning systems consist of two partitioning proteins, the A-type and B-type, and the *cis*-acting sequence(s) designated centromere-like sequences to which B components bind. The A-type proteins belong to the ATPase families. Depending on the nature of the encoded ATPase the partitioning loci are divided into two types (Gerdes *et al.*, 2000): *par* loci with Walker-type ATPases (*par* of P1, *sop* of F, IncC/KorB of RK2) belong to type I whereas the presence of actin-like ATPase (*par* of R1 plasmid) is representative for type II. All chromosomally encoded *par* loci known to date encode Walker-type ATPases.

The *parAB* genes form an operon with the *cis*-acting centromere-like sequence (*C*) located downstream or in the promoter of the operon (Moller-Jensen *et al.*, 2002). The A-type protein may bind to the repeated sequences in the promoter region and act as an autorepressor (Mori *et al.*, 1986; Friedman & Austin, 1988). The B protein and the presence of the *C* sequence (*in cis* or *in trans*) act as co-repressors of the system (Mori *et al.*, 1986; Yates *et al.*, 1999; Hao & Yarmolinsky, 2002). In some systems with the centromere-like sequences co-localizing with the promoters, the B-component is the main repressor and A protein plays only an accessory role (Jagura-Burdzy *et al.*, 1999a; 1999b). The tight regulation of the *par* operon ensures that low but sufficient cellular concentrations of the Par proteins are maintained. This is important for their function since it has been shown that an excess of these partitioning proteins decreases the plasmid stability (Kusukawa *et al.*, 1987; Lemonnier *et al.*, 2000).

The A-type proteins exhibit weak DNA-dependent ATPase activities (Davis *et al.*, 1992; Davey & Funnell, 1994; Jensen & Gerdes, 1997) and directly interact with B-type proteins (Ravin *et al.*, 2003; Jensen & Gerdes, 1997; Lukaszewicz *et al.*, 2002). The B-type proteins are DNA-binding proteins recognizing and binding to the specific DNA sequence *C (parS, parC)* and altering plasmid superhelicity (Biek & Strings, 1995; Funnell, 1991; Bouet *et al.*, 2000). The binding of B-protein to its recognition site is affected by the presence of the A-component. Whereas ParA-ADP is involved in autoregulation of the *par* operon, the ParA-ATP form interacts with the ParB–*parS* complex at high concentrations of ParB to play a direct role in plasmid partitioning (Bouet & Funnell, 1999). Interactions of B protein with the centromere-like sequences play a major role in the putative pairing of plasmid molecules followed by their bi-directional separation in which the A-protein is involved.

The centromere-like sequence *C* usually consists of repeated motifs (Mori *et al.*, 1986; Funnell, 1988; Davis *et al.*, 1990), iterons representing the binding sites for protein B (Davis & Austin,1988). A single iteron can promote binding of B to DNA and trigger assembly of a functional nucleoprotein partitioning complex (Biek & Shi, 1994). At least in the case of one subgroup of type I partitioning systems, B-protein binding to *C*-sequence pro-

motes co-operative protein–protein interactions producing a string of B molecules bound to the DNA and causing silencing of genes linked to the centromere (Lynch & Wang, 1995; Rodionow *et al.*, 1999; Rodionov & Yarmolinsky, 2004). The high degree of homology between the protein components of type I partitioning systems leads to the assumption that their mode of action is very similar. The specificity of the systems is provided by the *cis*-acting sites and B-components which directly interact with these sites. The interaction of the B-component with the centromere-like sequence is always modulated by A-components but may also depend on additional host factors (e.g., IHF in P1 prophage).

The genetic and biochemical data on the properties of Par proteins have been recently enriched by visualization of plasmid molecules in bacterial cells and *in situ* monitoring of the dynamic behaviour of Par proteins. The plasmid molecules are located at the mid-cell in newly formed cells and in quarter positions or polar positions after plasmid replication before cell division (Gordon *et al.*, 1997; Niki & Hiraga, 1997; Erdmann *et al.*, 1999; Bignell *et al.*, 1999). The use of time-lapse microscopy demonstrated that the P1 plasmid copies were clustered at the cell centre just before cell division and then rapidly ejected outwards from the central attachment and remained free to move in the daughter cells (Li & Austin, 2002a; 2002b). Later the plasmids were captured at the centre of the newly formed cells to restart the cycle. It was proposed that the attachment of the plasmids to a putative receptor at the cell centre would interfere with septation to delay the final cell division process until plasmid segregation has been completed.

Different plasmids occupy distinct positions in the cells (Ho *et al.,* 2002), determined by the partitioning cassette and the plasmid specific localization requires all three components of the partitioning cassette. Recent immunofluorescence studies on ParA of pB171 demonstrate that ParA oscillates between the poles of the bacterial cells positioning pairs (clusters) of plasmid molecules in the cell centre (Ebersbach & Gerdes, 2004). The helical structures ParA forms around the nucleoid (Ebersbach & Gerdes, 2004) disintegrate at one pole to be assembled at the opposite pole in a short time interval. It is not known if the ParA helices are created by ParA itself or by interactions with a pre-existing cytoskeletal scaffold such as MinD (Shih *et al.,* 2003) or MreB (Kruse *et al.,* 2003) (see above). This dynamic behaviour of ParA is dependent on its ability to hydrolyse ATP and the presence of ParB–*parC* complex. It is postulated that the ParA spirals position plasmid molecules at mid-cell and ParA oscillation is responsible for the movement of the ParB–*parC* complexes to the daughter cells. The exact mechanism of this process awaits elucidation. A clearer picture emerges from studies on ParM of R1 plasmid from the type II *par* systems. This actin-like ATPase may form long filaments threading the bacterial cell from pole to pole (van den Ent *et al.*, 2002; Moller-Jensen *et al.*, 2002). The ParM filaments undergo the cycle of dissociation and association and this dynamic behaviour depends on ATP hydrolysis. The formation of the cable structures is triggered by plasmid pairing by ParR (B-component) *via* the centromere-like sequences (*parC*) in the cell centre. The interaction of ParM with ParR bound to *parC* leads to the insertion of new ParM molecules into the centrally located nucleoprotein complex, ParM polymerization separates the paired plasmid molecules and pushes them apart to the cell poles (Moller-Jensen *et al.*, 2003).

Chromosomal *par* systems — organization of the *oriC* regions of bacterial chromosomes

The bacterial chromosome *oriC* regions are located in the middle of the cell at the beginning of the cell cycle (slow growth rate), and

after duplication the new origins are rapidly segregated to the quarter positions in a similar manner to the actively partitioning plasmids F, P1 or RK2.

Interestingly, homologs of plasmid-encoded *par* genes have been discovered in close vicinity to the *oriC* regions on chromosomes of a wide range of bacterial species including *B. subtilis* (*soj spo0J*), *C. crescentus* (*parA parB*), *S. coelicolor* (*parA parB*), *P. putida* (*parA parB*), *P. aeruginosa* (*parA parB*) and many others (Fig. 3) (Ogasawara & Yoshikawa, 1992; Gal-Mor *et al.*, 1998). The regions around *oriC* contain a cluster of highly conserved genes *rnpA-rpmH-dnaA-dnaN-recF-gyrB*, several of which play key roles in DNA replication (Fig. 3). Also highly conserved is the location of the *gid* and *par* operons downstream of *oriC,* with a few, usually well-conserved, genes in between. In *B. subtilis* the *par* (*soj spo0J)* operon is separated from the *gid* operon by two additional genes, one of which has homology to *spo0J* (Fig. 3). In *E. coli oriC* is linked to *gidA*, which is located about 40 kb away from the *dnaA* region probably as the result of an inversion of the fragment containing those genes. There are no *par* homologues anywhere in the genome of *E. coli* or other members of the *Enterobacteriaceae* family.

Studies on the role of Par proteins, initiated in *B. subtilis* and continued in *C. crescentus*, *S. coelicolor* and *P. putida*, have revealed similarities but also many differences in their function.

SojSpo0J (*parS*) of *Bacillus subtilis*

B. subtilis is a Gram-positive bacterium able to grow vegetatively as well as sporulate (form endospores) during unfavourable conditions. Cell division occurs either in the centre, generating two equivalent daughter cells after chromosome separation, or close to the cell pole, forming two different cell types, a larger mother cell and a forespore developing into the mature endospore enclosed within the mother cell wall (Fig. 4) (Levin & Grossman, 1998). The mature endospore is released from the mother cell and after germination may either begin vegetative growth or enter the sporulation cycle. Sporulating *B. subtilis* cells contain two chromosomes that are condensed in an elongated, serpentine-like structure known as the axial filament. The axial filament extends from pole to pole with the *oriC* regions located at its ends. A septum is formed asymmetrically near one pole trapping about 30% of one chromosome and then the rest of the chromosome is translocated into the forespore by the action of SpoIIIE.

The response-regulator/transcription factor Spo0A plays a key role in the transition from vegetative growth to sporulation, activating the expression of genes required for endospore formation (Trach *et al.*, 1991). The phosphorylation/activation of Spo0A is controlled by several mechanisms involving DNA damage (Ireton & Grossman, 1992), alterations in the initiation of DNA replication (Ireton & Grossman, 1994) and signals related to chromosome segregation (Ireton *et al.*, 1994). The last mechanism requires the *spo0J* (*orf282*) and *soj* (*orf253*) genes (Fig. 3), whose products show homology to members of the ParB and the ParA families, respectively (Ogasawara & Yoshikawa, 1992; Hoch, 1993).

Soj negatively regulates the initiation of sporulation in *B. subtilis* by inhibiting the phosphorylation (activation) of Spo0A, whereas Spo0J antagonizes this inhibitory activity of Soj (Ireton *et al.*, 1994). Spo0J is required for the initiation of sporulation as well as for normal chromosome partitioning during vegetative growth in *B. subtilis* since it has been shown that mutations in *spo0J* lead to an increased number of anucleate cells during the vegetative cell cycle (Ireton *et al.*, 1994). Mutations in *soj* have no significant effect on chromosome segregation in *B. subtilis* although the chromosome becomes decondensed. A *soj spo0J* double mutant is im-

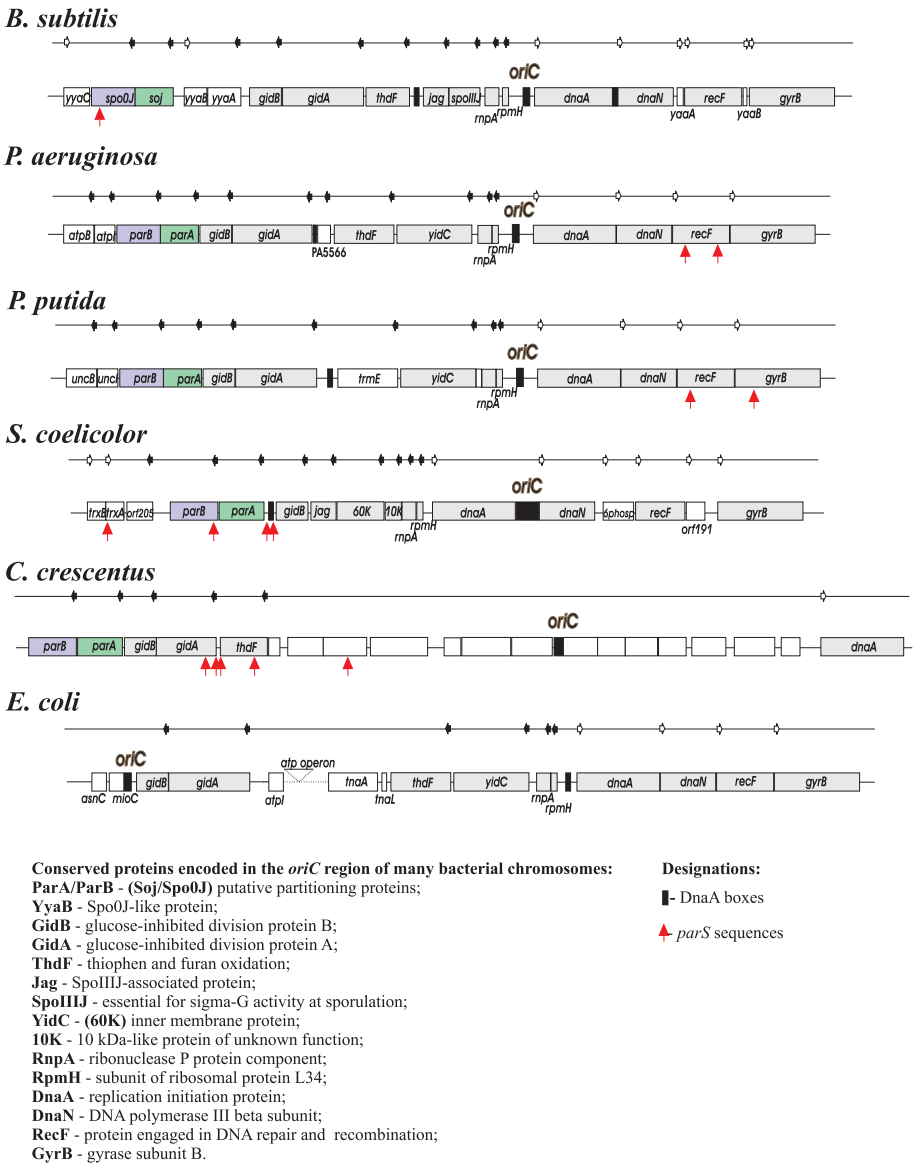

Figure 3. Comparison of gene organization in the *oriC* regions of the chromosomes of *B. subtilis*, *P. putida*, *S. coelicolor*, *C. crescentus* and *E. coli*.

Black rectangles indicate DnaA boxes, red arrows mark putative *parS* sequences (Spo0J/ParB binding sites), *parA/soj* homologues are green, *parB/spo0J* homologues are violet and other conserved genes are shown in grey. Arrows above the genes indicate the direction of transcription.

paired in chromosome partitioning during sporulation (Sharpe & Errington, 1996). It shows a reduced ability to localize and attach the *oriC* region to the prespore and to ensure direction of the translocation process.

Spo0J forms evenly distributed foci in *B. subtilis* cells whose localization and number follow those of the *oriC* region (Fig. 4) (Glaser *et al.*, 1997; Lewis & Errington, 1997). Two Spo0J foci are located near each cell pole (at quarter positions) in newly formed cells during growth on rich medium, while four Spo0J foci are symmetrically situated in older cells during the vegetative cell cycle. Observations in living cells have revealed that the Spo0J foci duplicate at about the same time as *oriC* replicates and then move apart towards the opposite cell poles (Glaser *et al.*, 1997). This suggested the existence of a mitotic-like mechanism that actively segregates the products of chromosome replication to the daughter cells at division.

It has been demonstrated that Spo0J is a site-specific DNA-binding protein recognizing eight 16 bp sequences (called centromere-like sequences or *parS*) located within the 20% of

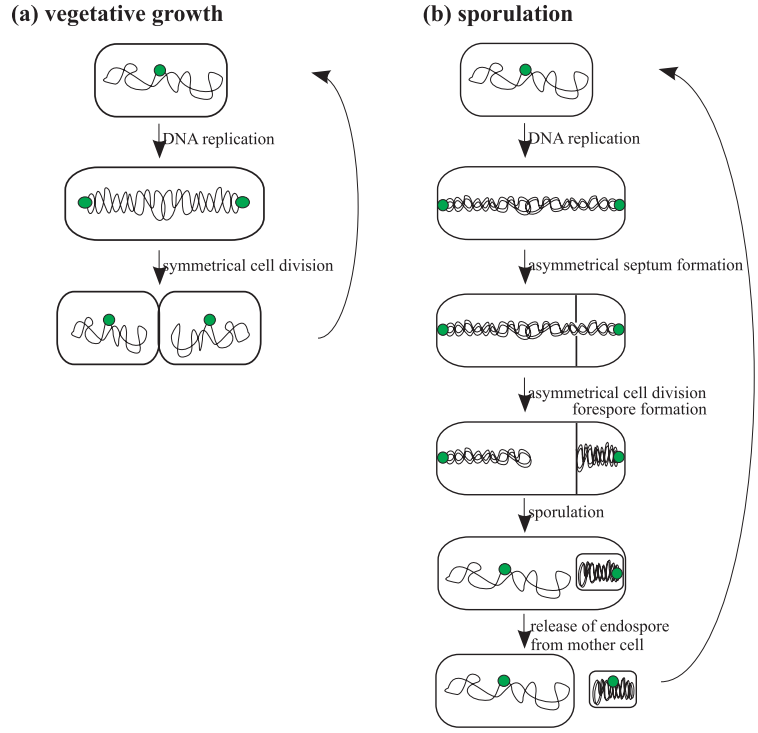

Figure 4. Chromosome organization of *B. subtilis* during (a) vegetative growth and (b) sporulation (Levin & Grossman, 1998).

Green circles mark the *oriC* region of the chromosome. **(a)** During the vegetative cell cycle, after initiation of chromosome replication, the duplicated *oriC* regions are rapidly moved apart towards the opposite halves of the cell. A septum is formed centrally leading to the final separation of daughter chromosomes and cell division. **(b)** During sporulation, replicating chromosomes form the axial filament extending from one pole to the other with the *oriC* regions located at the poles. Asymmetric septum formation creates a larger mother cell and a forespore. The invaginating septum bisects the axial filament and the remaining part of the chromosome is transferred by SpoIIIE action into the forespore. Following engulfment and synthesis of the resistant spore coat, the mature endospore is released from the mother cell.

the chromosome proximal to *oriC*, designated the *ori* domain (Fig. 5) (Lin & Grossman, 1998). The consensus of such *parS* sequences proposed on the basis of comparisons is 5' TGTTNCACGTGAAACA 3'. The detection of similar "*parS*" motifs in other bacterial chromosomal sequences has led to the suggestion that chromosomal *parS* sequences are universal in character (Lin & Grossman, 1998).

The compact foci formed by Spo0J suggest that different *parS* sites bound by Spo0J are co-localized. It has been shown that Soj protein is involved in the condensation and compaction of Spo0J foci and that Spo0J influences Soj behaviour in cells (Marston & Errington, 1999; Quisel & Grossman, 2000). Soj is a dynamic protein that creates patches at one end of the cell or the other and can oscillate from pole to pole in a co-operative manner depending on Spo0J. In the absence of Spo0J, Soj remains evenly distributed across the nucleoid, playing its role as a negative regulator of sporulation.

Initially it was suggested that Spo0J, through its interaction with *parS* sites and Soj, contributes to the correct positioning of the chromosomal *oriC* regions in sporulating as well as vegetative cells and these interactions are engaged in active chromosome partitioning in *B. subtilis* (Fig. 4). Lee and

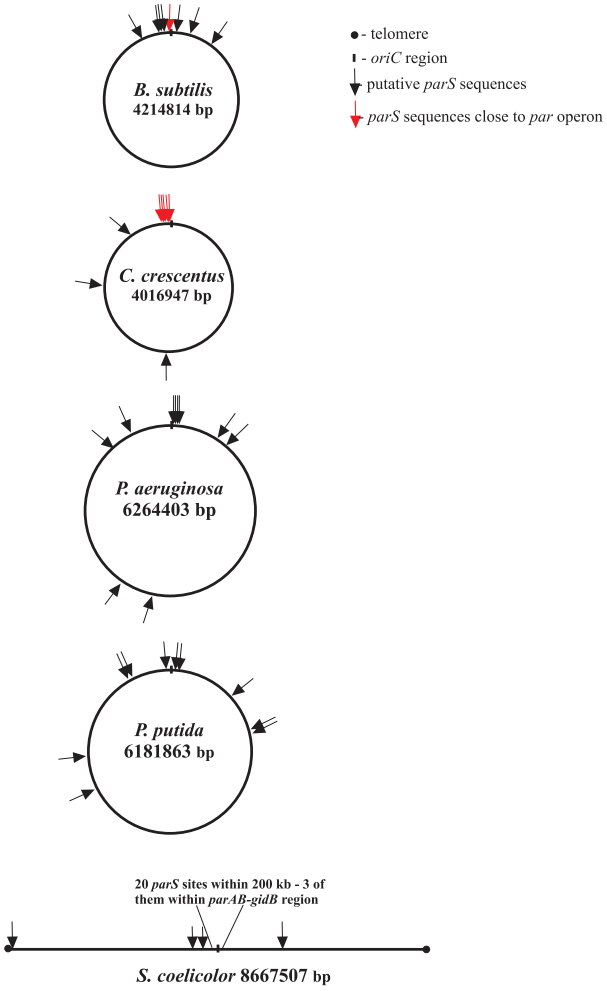

Figure 5. Schematic representation of multiple Spo0J/ParB binding sites (*parS*) (black arrows) within the genomes of different bacterial species.

Red arrows indicate *parS* sequences located close to the *par* operon.

co-workers (2003) recently demonstrated that the subcellular location of Spo0J is indeed a consequence of the chromosomal position of its binding sites (close to the *oriC* region in wild-type cells). The insertion of *parS* at various chromosomal locations in the absence of six of the eight known *parS* sequences revealed that Spo0J was no longer found at the quarters positions where the *oriC* regions are usually located. Thus Spo0J is not sufficient to recruit *parS* sites to specific positions in the cell.

Analysis of *spo0J* null mutant cells (longer than wild-type) showed that many had an increased number of *oriC* regions (chromosome content) and the sister origin regions tended to be located closer together in such cells. The observed phenotypes were the consequence of asynchronous initiation of replication in the *spo0J* mutant cells, and due to defects in chromosome separation (especially their *oriC* regions) during the cell cycle (Lee *et al.*, 2003). Thus Spo0J, by its interactions with *parS* and Soj, helps to compact the *oriC* regions and facilitate their separation, but additional factors must contribute to the correct positioning of the *oriC* regions in the cell. Kadoya and co-workers (2002) identified two separate DNA sequences within the *oriC* domain which participate in chromosome segregation in *B. subtilis*. These two regions do not contain Spo0J binding sites. Ben-Yehuda and co-workers (2003) determined that the RacA protein is responsible for anchoring the chromosomes to the cell poles during sporulation in *B. subtilis* while also playing a role in chromosome remodelling. It seems that different mechanisms may be engaged in proper chromosome organization and movement during the cell cycle to ensure their faithful segregation and the lack of one may be compensated by action of other(s). A close homologue of *spo0J* named YyaB is also encoded by the *B. subtilis* chromosome (Sievers *et al.*, 2002) (Fig. 3). This protein may play a regulatory role in sporulation but its function in genome partitioning is unknown.

The increased rates and asynchrony of the initiation of replication caused by *spo0J* mutations suggest an involvement of this protein in the regulation of chromosome replication initiation. It has been postulated that Spo0J, by interaction with its binding sites (located close to *oriC*), sequesters origins and prevents/delays the initiation of replication (Fig. 5) (Lee *et al.*, 2003). This would be similar to the action of the SeqA protein in the chromosome replication of *E. coli* (Lu *et al.*, 1994; Brendler *et al.*, 2000), where a homologue of Spo0J has not been identified.

Interestingly, an excess of Soj causes early and asynchronous initiation of chromosome

replication similar to that seen in *spo0J* mutant cells (Ogura *et al.*, 2003). Thus an increased ratio of Soj to Spo0J and the association of Soj with the nucleoid (Soj repressor activity?) seem to be responsible for uncontrolled replication initiation. In contrast, overproduction of both proteins leads to delays in the initiation of replication in *B. subtilis*. These observations demonstrate an additional role for the Soj–Spo0J–DNA interactions in controlling replication initiation in *B. subtilis*. Thus, Soj/Spo0J couple the activities involved in chromosome segregation to the initiation of sporulation, which provides an effective way of ensuring that cells do not attempt to form spores unless efficient and proper chromosome separation can occur.

In spite of the regulatory processes in which Soj and Spo0J proteins are involved it has been shown that they can act together with a single Spo0J binding site as an efficient partitioning system, similar to their plasmid homologues (see above). The presence of a single *parS* can stabilize an otherwise unstable plasmid in a Spo0J Soj-dependent manner (Lin & Grossman, 1998). This stabilization was observed even in the heterologous host *E. coli* (Yamaichi & Niki, 2000). The *soj spo0J parS* system was sufficient for positioning duplicated plasmids at the cell quarters in a similar manner to plasmid *par* systems (*par/sop* of P1/F). This indicates functional homology between plasmid and chromosomal *par* systems. However, in the context of the whole chromosome, Spo0J was not sufficient to position ectopic chromosomal *parS* sites at the cell quarters (Lee *et al.*, 2003), which suggests that much more complex mechanisms are involved in chromosome positioning/partitioning in *B. subtilis*.

ParAParB (*parS*) of *Caulobacter crescentus*

C. crescentus is a dimorphic bacterium forming two types of cells during its cell cycle. A pre-divisional cell possesses a stalk attached to one pole and a flagellum at the other, and cell division produces a stalk and a swarmer cell, respectively. Only the stalk cell is able to replicate DNA and enter into cell division so eventually the swarmer cells transform into the stalk cells to continue the cell cycle (Fig. 6) (Gober & Marques, 1995).

In *C. crescentus* homologues of the plasmid partitioning genes *parA* and *parB* were also identified near the origin of replication as part of the *gidAB parAB* operon regulated by ParB (Fig. 3) (Mohl & Gober, 1997). Unlike their *B. subtilis* homologues *soj spo0J*, both *parA* and *parB* are essential for *C. crescentus* viability.

An initial analysis demonstrated that ParB binds specifically to DNA sequences located downstream of the *parAB* operon (Fig. 3) (Mohl & Gober, 1997). Easter, Figge and Gober (personal communication) have demonstrated that at least five *parS* binding sites for ParB exist within 20 kb of the origin in the *C. crescentus* chromosome (Figs. 3 and 5). ParB interacting with the *oriC* region creates a specific pattern of subcellular distribution coinciding with the chromosome movement (Fig. 6). In swarmer cells, which do not replicate DNA, ParB is dispersed within the cytoplasm, although it was also found at the mid-cell or polar positions in a small fraction of cells (Mohl & Gober, 1997). In early pre-divisional cells (stalked cells) ParB is visible as a single focus localized preferentially at the cell pole. As the cell cycle progresses another ParB focus appears at the opposite cell pole. ParA is also localized to the poles in *C. crescentus* pre-divisional cells. The polar localization of both proteins is dependent on DNA replication and reflects movement of the newly replicated chromosomes (initially the *oriC* regions) towards opposite cell poles. It was determined that the presence of ParB is not required for origin movement, but ParB could play a role in the organization and localization of the chromosome during cell division.

The cellular levels of ParA and ParB in *C. crescentus* cells remain constant throughout the cell cycle and their synthesis in stoichiometric amounts seems to be critical for their function (Mohl & Gober, 1997). Overproduction of either ParA or ParB leads to defects in cell division (cell filamentation), ParB mislocalization as well as affecting chromosome partitioning. Mohl and co-workers (2001) revealed that ParB depletion leads to cell filamentation due to the inhibition of FtsZ ring formation at the mid-cell. In cells with depleted ParB the level of ParA increases due to the absence of autoregulation of the *parAB* operon. Thus ParB depletion and/or ParA excess inhibit cell division.

It has been demonstrated that the formation of bipolar ParB foci precedes FtsZ ring assembly and that ParB action stimulates the initiation of FtsZ ring formation and cell division (Fig. 6). It is believed that the ParB protein and also ParA are required for proper cytokinesis in *C. crescentus* and the coupling of chromosome partitioning to cell division.

The ParA protein of *C. crescentus*, like Soj of *B. subtilis*, shares homology with a large family of Walker-type ATPases (Motallebi-Veshareh *et al.*, 1990). Easter & Gober (2002) demonstrated that ParA is an ATPase whose activity is regulated by ParB (ParB promotes ParA nucleotide exchange). ParA-ADP is able to bind to single-stranded DNA probably causing repression of an essential cell division gene(s). ParA-ATP interacts with the ParB–*parS* complexes and promotes dissociation of ParB from DNA. It might also influ-

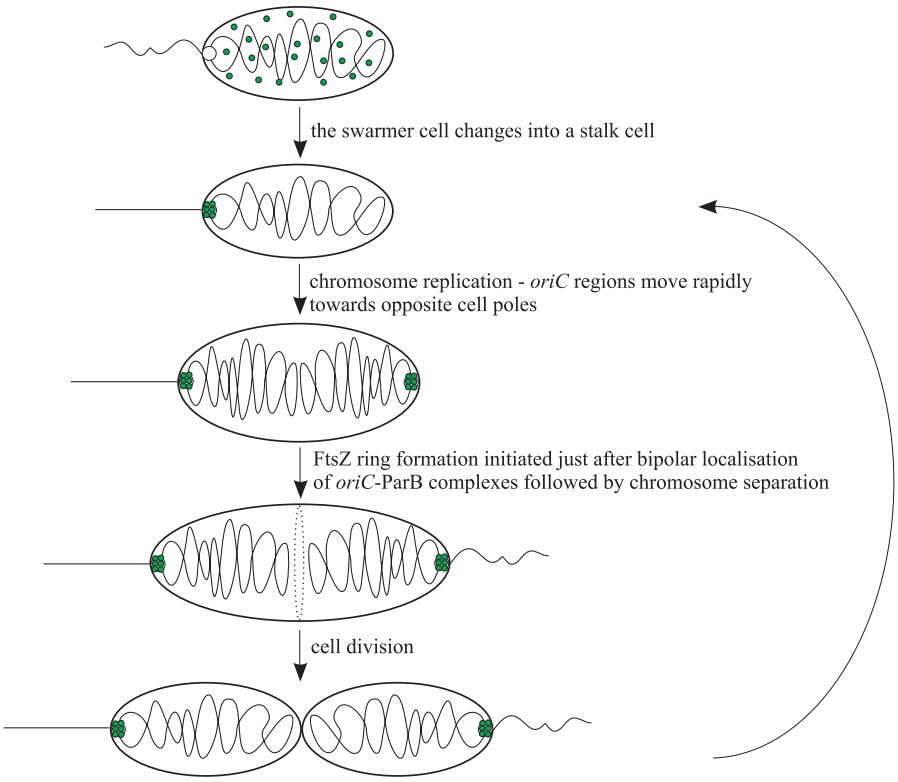

Figure 6. *C. crescentus* chromosome organization during the cell cycle.

White circle – the *oriC* region of the chromosome (Gober & Marques, 1995). A broken oval line marks the FtsZ ring. A swarmer cell is not able to replicate the chromosome, therefore it changes into a stalk cell comprising a single chromosome with a single *oriC* region at the stalk pole and dispersed ParB (green circles). After initiation of chromosome replication, the *oriC* regions are coupled with ParB interacting with the *parS* sites and one *oriC* region is rapidly moved toward the opposite cell pole followed by initiation of FtsZ ring formation at mid-cell. ParA interacts with ParB–*parS* complexes facilitating chromosome partitioning. Finally, chromosome replication is completed and cell division creates two types of cells.

ence the transcription of the *gidAB parAB* operon since it was found that ParB binding to *parS* sites located upstream of *gidA* represses this transcription while a ParA excess increases the transcriptional activity of the *gidAB parAB* promoter (Fig. 3) (Easter & Gober, personal communication).

Figge and co-workers (2003) demonstrated that ParB interacts with ParA *via* its N-terminus. ParB mutants lacking the N-terminal 40 aa or 80 aa failed to interact with ParA and their overproduction resulted in the inhibition of cell division. The inability of these truncated ParB derivatives to interact with ParA leads to an inversion of the ratio of ParA-ATP to ParA-ADP within the cell. Thus, the N-terminal part of ParB is required to regulate the nucleotide exchange on ParA and in this manner it regulates the initiation of cell division. ParB of *C. crescentus* interacts with *parS* sites localized within the chromosomal *oriC* region, and like other DNA-binding proteins it possesses an HTH motif, the DNA binding domain responsible for recognition and binding to *parS* sites. The central residues of *C. crescentus* ParB form the putative HTH motif as is also the case for other ParB homologues. Efficient ParB interactions with DNA also require the C-terminal region where the dimerization domain is located. Thus, ParB is composed of three domains: the N-terminal domain of interaction with ParA, the central DNA-binding domain (HTH motif) and the C-terminus involved in ParB dimerization. A similar three-domain structure was observed for the ParB homologues ParB/SopB encoded by the P1/F plasmids.

ParAParB (*parS*) of *Streptomyces coelicolor*

Another bacterium in which ParAParB proteins have been studied is *S. coelicolor*. This mycelial bacterium undergoes complex morphological differentiation and possesses a large linear chromosome. Germinating spores form a vegetative mycelium of multigenomic hyphae with aerial branches developing into chains of unigenomic spores (Kim *et al.*, 2000). The multigenomic hyphae are the result of uncoupling of DNA replication and separation during vegetative growth of *S. coelicolor*. The formation of spores in aerial hyphae requires synchronized multiple septation of a multigenomic mycelium coupled with DNA condensation and its proper segregation into the spore compartments.

The large linear chromosome of *S. coelicolor* has telomeres at both ends and replicates bi-directionally starting from the *oriC* located at the centre of the chromosome.

The mechanism by which *S. coelicolor* chromosomes segregate during spore formation has yet to be defined but it has been postulated that the *parA* and *parB* genes encoded in the vicinity of *oriC* may be involved in this process (Fig. 3) (Kim *et al.*, 2000). Deletion of either *parB* or both *parA* and *parB* genes had no effect on the growth of mutant cells but the mutations caused defects in DNA partitioning (localization) during septation of the multigenomic aerial hyphae into long chains of spores. The *parA* and *parB* genes form an operon whose transcription may proceed from two promoters. The stronger promoter is significantly activated at about the time of sporulation, which reinforces the idea that these Par proteins play an important role during the sporulation process. Initially, 18 copies of a 14 bp inverted repeat, the putative ParB binding sites (*parS*) were found in the partially sequenced *S. coelicolor* genome (Figs. 3 and 5) (Kim *et al.*, 2000). Recent studies have revealed the existence of six more *parS* sites (consensus sequence 5' tGTTTCACGTGAAACa 3') in the fully sequenced *S. coelicolor* genome, on the basis of homology with *B. subtilis parS* (Jakimowicz *et al.*, 2002). Twenty *parS* sites are located within a 200 kb region around *oriC* and most are recognized and bound by ParB. The affinity of ParB to its binding sites depends on the *parS* nucleotide sequence (the core of the sequence is most important for binding) and its chromosomal lo-

cation (promoter, intergenic or intragenic regions). Two ParB binding sites are located upstream of the *parAB* operon (Fig. 3) which suggests ParB involvement in autoregulation of *par* gene expression (Kim *et al.*, 2000; Jakimowicz *et al.*, 2002). Interestingly, within the same region two DnaA boxes are present so that regulation of the expression of the *par* operon may be coupled with DNA replication.

ParB interaction with the *parS* sites within the *oriC* region probably leads to the formation of a large nucleoprotein complex which is likely to be engaged in chromosome positioning-partitioning during *S. coelicolor* spore formation. The role of ParA and other cellular factors in this process has not yet been defined.

ParAParB (*parS*) of *Pseudomonas putida* and *P. aeruginosa*

The *parA* and *parB* genes have also been identified in the chromosomal *oriC* region of the free-living non-sporulating bacteria *P. putida* and *P. aeruginosa* (Fig. 3) (Ogasawara & Yoshikawa, 1992). It was revealed that *parA* and *parB* mutant cells of *P. putida* do not show significant defects in growth or genome partitioning when propagated in rich medium (Lewis *et al.*, 2002; Godfrin-Estevenon *et al.*, 2002). However, in cultures of *parA*, *parB* or double *parAB* mutants grown in minimal medium, a large fraction (5–10%) of anucleate cells were observed during the transition from exponential growth to stationary phase. *P. putida* cells are sensitive to an excess of ParA or ParB proteins, showing defects in chromosome partitioning and abnormal cell morphologies during the deceleration phase of growth, independent of the medium used (Godfrin-Estevenon *et al.*, 2002). It was suggested that the Par proteins in *P. putida* are particularly important for chromosome partitioning under specific physiological states (transition to stationary phase, slow growth during starvation) when the bacteria are undergoing cell division in the absence of ongoing DNA replication (Lewis *et al.*, 2002; Godfrin-Estevenon *et al.*, 2002). It has been proposed that the *par* system in *P. putida* is required to tether the chromosomes at the cell quarters at cell division in the absence of re-initiation of replication (Lewis *et al.*, 2002). Studies on *P. aeruginosa parB* gene (Bartosik, unpublished) have revealed that ParB plays a significant role in chromosome segregation even in actively growing cells. Mutants in *parB* produce up to 10% of anucleate cells in exponentially growing cultures even on rich medium. The difference in the phenotype of *parB* mutants in such closely related *Pseudomonas* species is remarkable especially that a strong effect of growth inhibition combined with missegregation of chromosomes is observed in both *P. putida* and *P. aeruginosa* strains overproducing $ParB_{P.a.}$ protein (Bartosik *et al.*, 2004). No such effect is observed in *E. coli*.

Godfrin-Estevenon and co-workers (2002) identified three sequences similar to *parS* sites (Spo0J binding sites) of *B. subtilis* within the *oriC-parAB* region of the *P. putida* chromosome. Ten such sequences have been found in the *P. aeruginosa* chromosome (Bartosik *et al.*, 2004; Fig. 5) with a cluster of four *parS* sites around the *oriC* domain. A single *parS* site of *P. putida* or *P. aeruginosa* inserted into an unstable plasmid could stabilize it in *E. coli* cells in the presence of corresponding ParA and ParB (Godfrin-Estevenon *et al.*, 2002; Bartosik *et al.*, 2004). This finding confirms that the chromosomal *par* loci may act as active partitioning systems, recognizing universal host factor(s) involved in this process. *In vitro* ParB of *P. aeruginosa* demonstrates the highest affinity towards those four *oriC* proximal *parS* sites. It has been also demonstrated that chromosomal ParB (Bartosik *et al.*, 2004) is able to silence transcriptionally the genes close to *parS* by spreading and that the silencing is dependent on the *parS* sequence and its orientation. It is then feasible that ParB may be involved in the regulation of expression of operons close to

oriC. It is remarkable that the genes adjacent to *oriC* code for proteins involved in DNA replication, segregation, cell division and morphological development and vital to cell survival.

In summary, most bacterial chromosomes studied to date, excluding *E. coli* and closely related species, contain homologues of the *parA/soj* and *parB/spo0J* genes.

The *par* genes are located in close vicinity of the *oriC* regions of bacterial chromosomes and show significant homology to plasmid-encoded *par* systems of type I (see above). *soj-spo0J-parS* of *B. subtilis* and *parA-parB-parS* of *P. putida* and *P. aeruginosa,* like their plasmid homologues, are able to form an active partitioning system for unstable plasmids and stabilize them even in a heterologous host (e.g., *E. coli*).

With the exception of *C. crescentus,* the chromosomal *parA parB* (*soj spo0J*) genes are not essential for cell viability (e.g., *B. subtilis, P. putida, P. aeruginosa, S. coelicolor*). However, mutations in the *soj*(*parA*) and *spo0J*(*parB*) genes lead to defects in the sporulation of *B. subtilis* and *S. coelicolor,* and in vegetative chromosome partitioning of *B. subtilis* and *P. aeruginosa,* as well as in *P. putida* but in this case depending on the growth medium and phase of growth. The appropriate level of Par proteins' expression seems to be crucial for their function since overproduction of one or the other causes defects in chromosome partitioning and cell division in all species studied.

In the studied bacteria the *par* operons and the multiple Spo0J/ParB binding sites *parS* are located in the so-called *ori* domain (20% of the chromosome around *oriC*) (Figs. 3 and 5). The Spo0J/ParB interactions with *parS* sites and Soj/ParA influence the organization of the *oriC* region of the chromosome. The *parS* sequences from different bacteria show very high homology to the *B. subtilis parS* consensus.

The Spo0J/ParB proteins, interacting with *parS* sequences and Soj/ParA are postulated to be involved in organization, positioning and partitioning of newly replicated *oriC* regions towards opposite halves of the cell (future daughter cell). However, it has been demonstrated that the Spo0J/ParB proteins are not sufficient to localize the *oriC* regions of sister chromosomes to the quarter positions in *B. subtilis* or to the polar positions in *C. crescentus*. Thus, additional factors are required for faithful genome partitioning in bacteria. The participation of the replication machinery, SMC proteins, cytoskeletal MreB-like proteins and the Min system as potential players in bacterial genome partitioning is postulated. They all might help in the active positioning of chromosomes at specific regions of the cell prior to cell division. However, it remains unclear which host factors mark the cellular locations at which proteins and chromosomes are positioned, e.g., the quarter positions of *oriC*/Spo0J complexes.

Test plasmids carrying the *B. subtilis* chromosomal *par* system were localized at quarter positions even in the heterologous host *E. coli*. Thus the positional information for chromosome partitioning and the proteins engaged in this process appear to be universal among bacteria, even in species lacking the *par* system.

Besides their putative role in chromosome partitioning, the Par proteins seem to be involved in the regulation/coordination of important cell survival processes including cell division (the initiation of cytokinesis) in *C. crescentus,* sporulation in *B. subtilis* and *S. coelicolor,* and proper chromosome segregation in special physiological states marked by the absence of ongoing DNA replication in *P. putida*. The recently demonstrated role of Soj/Spo0J proteins in the regulation of DNA replication initiation revealed another vital function of Par proteins; to couple genome partitioning with DNA replication. Thus the role of Par proteins in the bacterial cell cycle could be much more complex than previously thought. They might serve as bacterial cell cycle coordinators. Genes encoding Soj/ParA

and Spo0J/ParB homologues have been identified in the majority of sequenced bacterial genomes. Such widespread and remarkably high conservation of these genes indicates that they play a significant role in cell survival and facilitate bacterial development/adaptation to changing environments.

CHROMOSOME SEGREGATION AND CELL DIVISION

In bacterial cells vital processes such as chromosome replication, segregation of the duplicated genome to opposite halves of the cell and final division of the mother cell have to be precisely coordinated in time. Septum formation between the two replicated chromosomes is a critical point of the cell cycle; when the septum is completely closed there is no chance to correct any mistakes in chromosome segregation. Thus septum formation and cell division have to be spatially and temporally regulated in coordination with chromosome replication and segregation.

Cell division

During vegetative growth, bacterial cell division is symmetrical. The septum, formed precisely in the middle of the mother cell to ensure an equal division of the cell contents, is created by a complex of several proteins comprising the FtsZ ring (Bi & Lutkenhaus, 1991). The Z-ring is composed of filaments of FtsZ, an ancestral homologue of eukaryotic tubulins (Lutkenhaus, 2002). GTP hydrolysis by FtsZ filaments results in bending and shortening of the polymers which probably products the constrictive force for septum contraction and closing (Lu *et al.*, 2000). At least nine other proteins (FtsA, ZipA, FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI, FtsN) are engaged in septum formation, interacting with each other and with FtsZ to coordinate invagination of the cell membrane, inward growth of the peptidoglycan layer and to promote final separation of the daughter cells (Buddelmeijer & Beckwith, 2002).

The most intriguing aspect of septum formation is its proper placement within the cell. In *E. coli* and *B. subtilis* it seems that at least two factors are involved in controlling the division site location: nucleoid occlusion and the Min system (Harry, 2001).

In the nucleoid occlusion model it is proposed that the presence of the chromosome mass in the middle of the cell exerts a negative effect on division. Chromosome segregation results in a chromosome-free zone at the cell centre and allows the septum to form at the mid-cell. This model is not adequate to describe the situation in sporulating *B. subtilis* cells in which a polar septum is formed before chromosome segregation occurs, or in *C. crescentus* in which a Z ring appears to assemble at the future division site during the early stages of DNA replication (Levin & Grossman, 1998; Quardokus *et al.*, 2001). Thus different mechanisms must also be engaged in correct division site placement. One such mechanism which has been quite thoroughly studied is the Min system.

Min system

The designation *min* comes from the production of minicells due to Z-ring formation at the poles of cells in mutant strains of *E. coli* or *B. subtilis* lacking the Min system (Adler *et al.*, 1967; Reeve *et al.*, 1973). The *E. coli* Min system consists of three components: MinC, MinD and MinE (de Boer *et al.*, 1989). In the presence of ATP, MinD, a Walker-type ATPase is able to dimerize and bind to MinC (an inhibitor of division) and the membrane, resulting in assembly into filaments (Hu *et al.*, 2003). The MinCD complex forms a membrane-associated polar cap at one end of the cell. MinE forms a ring at the edge of the MinCD polar zone which moves towards the pole ejecting MinCD from the membrane. MinE stimulates MinD ATPase activity leading to the release of ADP and dissociation of

the complex. After MinD nucleotide exchange the MinCD complex is assembled at the opposite pole of the cell and then MinE forms another ring which moves towards the pole, ejecting MinCD and the cycle is repeated, each time at the opposite pole of the cell. These reversible interactions of the Min proteins with the membrane lead to the rapid movement of the proteins between the two halves of the cell. The overall effect of this pole-to-pole oscillation of Min proteins is to produce the highest MinC concentration at the poles and lowest at the cell centre where the Z-ring may then be formed. It has been suggested that the main role of the Min system is to prevent division occurring at the nucleoid-free cell poles rather than having a direct role in defining the site for Z-ring assembly at mid-cell (de Boer *et al.*, 1989). *C. crescentus* and *H. influenzae* do not possess a Min system but these bacteria have no nucleoid-free regions (Margolin, 2000; Quardokus *et al.*, 2001).

In vegetative *B. subtilis* cells the MinCD complex interacting with DivIVA (not homologous to MinE) also acts as a division inhibitor at the step of Z-ring assembly and in blocking the division at the cell poles (Marston *et al.*, 1998). Shih and co-workers (2003) have recently shown that the Min proteins in *E. coli* are organized into extended membrane-associated coiled structures that wind in a spiral around the cell between the two poles. The rapid oscillations of the proteins from pole-to-pole appear to occur by the dynamic redistribution/rebuilding of the Min proteins within this coiled framework. Similar extended coiled/helical structures are also created by the MreB/Mbl proteins in *E. coli* and *B. subtilis* (Kruse *et al.*, 2003; Jones *et al.*, 2001) (see above). Thus MinCDE, like MreB, might be a part of the bacterial cytoskeleton undergoing dynamic changes and playing important role not only in the regulation of division site placement but also in chromosome segregation since it has been shown that in Δ*minCDE* mutant strains chromosome partitioning is impaired (Mulder *et al.*, 1990). MinD shares homology with the ParA family of proteins involved in chromosome and plasmid partitioning in bacterial cells (Motallebi-Veshareh *et al.*, 1990). It has been postulated that the MinD helical tracks might help to transfer duplicated *oriC* regions from the mid-cell to the cell quarters during the process of chromosome partitioning. The Min system could therefore coordinate two important cell cycle processes, chromosome segregation and cell division.

CONCLUSIONS

During evolution bacteria have developed precise mechanisms to ensure faithful and efficient chromosome replication and segregation coordinated with cell division. Many proteins engaged in these processes appear to be universal among bacteria. The replication machinery, the proteins involved in final chromosome separation, and FtsZ appear to be common to many different bacteria. The mechanisms and factors involved in chromosome partitioning are more varied. There is no one general mechanism ensuring proper chromosome segregation; several different mechanisms participate in this process. The MreB/Mbl actin-like filaments are found in many non-spherical bacteria and their role in generating the motive power for chromosome segregation has been postulated. The Min proteins, which also form coiled structures, are common among bacteria, being found in all but a few species (*C. crescentus*, *H. influenzae*). They play a role in proper septum placement, but the helical tracks formed by MinD might also help in chromosome partitioning. In *Enterobacteriaceae* and *H. influenzae* or in *Vibrio* sp. belonging to the γ-Proteobacteria the MukB proteins play a role in chromosome condensation and partitioning. In these bacteria the SeqA protein is also found, negatively regulating initiation of chromosome replication, organizing the

newly replicated DNA and probably assisting in its partitioning. However, the MukB and SeqA proteins have not been found in many other Eubacteria including other members of the γ-Proteobacteria (e.g., *Pseudomonas* sp.). These bacterial species encode SMC proteins that play a similar role to MukB in genome partitioning. They also encode Par systems, which in plasmids are involved in active partitioning.

Chromosomal Par proteins by their homology to plasmid *par* systems were thought to be the best candidates for the active partitioning system of bacterial chromosomes. The *soj-spo0J-parS* of *B. subtilis* and *parA-parB-parS* of *P. putida* and *P. aeruginosa*, like their plasmid homologues, are able to form an active partitioning system for unstable plasmids and stabilize them even in a heterologous host (e.g., *E. coli*). Studies in different bacterial species showed, however that Par proteins are not sufficient for proper chromosome segregation. They seem to play an important role in organizing the *oriC* regions of the chromosomes, helping to create the compact nucleoprotein structures which could be efficiently partitioned to their places of destination – opposite halves of the cell. The Par proteins may assist in active partitioning of such nucleoprotein structures but its not known what additional proteins supply energy and "tracks" for this process (MinD, MreB). They also may be involved in marking the positions to which newly replicated chromosomes are segregated – 1/4 and 3/4 or 1/8, 3/8, 5/8 and 7/8 depending on the rate of growth. The positions to which the *oriC* regions of the chromosomes after replication are segregated during vegetative growth seem to be similar in all rod-shaped bacteria studied, even in species lacking the *par* system. Thus the positional information for chromosome partitioning and the proteins engaged in this process appear to be universal among bacteria and Par proteins might be one of the players which are able "to read" positional signals. The plasmid *par* systems may rely on the same signals or create an independent network. In bacteria with a more complicated cell cycle like *B. subtillis* additional factors have been discovered which are involved in specific *oriC* regions' positioning and chromosome organizing during sporulation, e.g., the RacA protein.

Interestingly, the positions to which the *oriC* regions of bacterial chromosomes are segregated in vegetatively growing bacteria become the positions of the replication machinery and the division plane in future generations. Thus positional information seems to play a crucial role in organizing/controlling vital processes of the bacterial cell cycle: chromosome replication, segregation and cell division, and could provide basis for precise coordination of these processes.

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