

**REVIEW
ARTICLE****Bacterial chromosome segregation**

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Recent years have witnessed a resurgence of interest in how the bacterial chromosome is organized and how newly replicated chromosomes are faithfully segregated into daughter cells on cell division. In the past, the problem with studying bacterial chromosomes was their lack of any obvious morphology, combined with the lack of ability to readily separate DNA replication and segregation functions into distinct stages like those observed in eukaryotic cells. This was due to the overlapping nature of these events in most bacterial systems used in the laboratory. The situation has now changed as new tools have become available that enable chromosomes and specific chromosomal sites to be labelled and monitored throughout the cell cycle, and this has led to rapid progress and the discovery of many unexpected results.

Historically, chromosome segregation was thought to be achieved through passive processes where chromosomes were separated via some type of membrane/cell wall attachment and were moved apart as the cell grew (Jacob *et al.*, 1963). We now know that this is not the case and that there are specific mechanisms to actively partition chromosomes. This review will focus principally on the Gram-positive sporulating bacterium *Bacillus subtilis*, but will also cover work carried out on *Escherichia coli*, in which valuable information has been obtained, and will cover the events that occur on termination of chromosome replication, chromosome decatenation and chromosome separation.

Termination of chromosome replication

In the circular chromosomes of *B. subtilis* and *E. coli*, replication forks arise at a single origin, *oriC*, and move bidirectionally around the chromosome until they meet in well-defined terminus regions (*terC*; Hill, 1996; Wake, 1997). Termination systems comprise a polar-acting DNA-binding terminator protein and its binding sites. Although termination of chromosome replication

appears to occur by a similar mechanism in both organisms, the DNA-binding proteins and their binding sites are unrelated, and so probably arose independently. Replication fork arrest systems have also been reported in eukaryotic cells (Boddy & Russell, 1999), and termination systems may well be a general feature of many/all chromosomes.

On entering the terminus region, replication forks enter a trap designed to prevent their exit. *terC* is almost directly opposite *oriC* and so forks should naturally meet at *terC*, although there are often reasons why this may not occur (e.g. DNA damage repair/recombination) and one fork arrives late (Fig. 1a). Therefore, the replication trap has been designed so that a replication fork can pass through an incorrectly oriented terminator–DNA complex, but cannot pass through one in the correct orientation. In Fig. 1(a), the clockwise-moving replication fork would be able to move through the terminator protein–binding site II complex, but would be arrested at site I. Conversely, the anticlockwise-moving fork would be able to move through site I, but not site II. This ensures that replication fork arrest occurs in a small, well-defined region of the chromosome. Terminator proteins work by preventing further progress of helicases situated at the apex of approaching replication forks in a polar manner. At this point it is still unclear what contribution the physical strength of the terminator–DNA complex and what contribution other ‘specificity features’ (e.g. terminator–helicase interactions) play in this process (Duggin & Wake, 2001).

For years the reason for the existence of a termination system was unclear as the terminus region could be deleted with no observable detrimental effects on the cell (Iismaa & Wake, 1987), so any conferred advantage of a termination system must be rather subtle, at least under laboratory conditions. However, more recent work has revealed an increasingly significant role for a replication terminus in both *B. subtilis* and *E. coli*, and indicates that specific termination sites are important for the efficient resolution of interlinked chromosomes prior to separation (see below; Duggin & Wake, 2001).

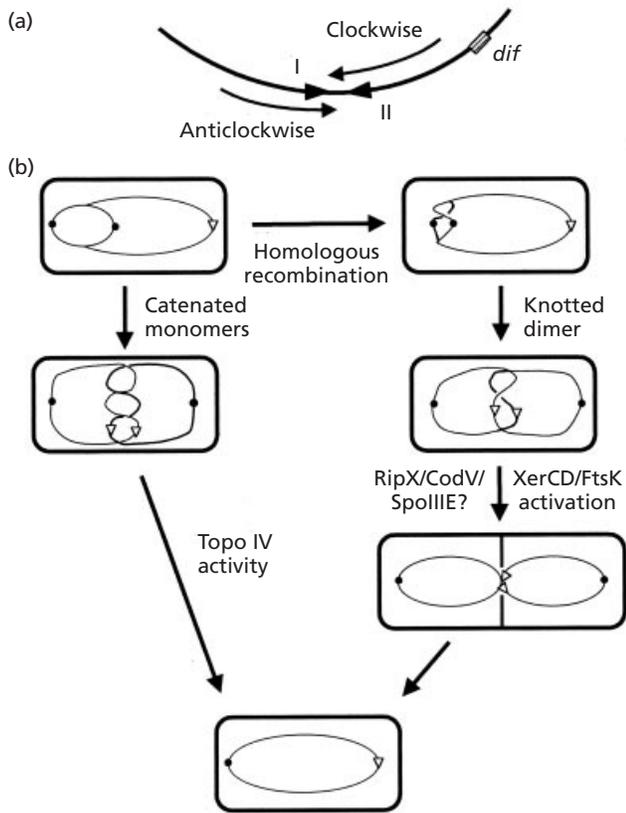


Fig. 1. (a) The replication fork trap. Schematic diagram of the terminus region of the *B. subtilis* chromosome. The direction of movement of the clockwise and anticlockwise replication forks is shown by arrows. I and II represent terminator sequences *terI* and *terII*, respectively. The replication terminator protein (RTP) blocks clockwise replication forks when bound to I, and blocks anticlockwise forks when bound to II, thus creating a replication fork trap in the terminus region. (b) Resolution of chromosome dimers and catenae. The schematic diagram illustrates how chromosome catenae are resolved (left-hand side) and how dimers are resolved (right-hand side) in *E. coli*. Catenae are resolved by the activity of topoisomerase IV (Topo IV), whilst dimers require the activity of XerCD/FtsK, together with a correctly positioned *dif* site. Filled circles represent origins of replication, open triangles represent *dif*/terminus regions.

Resolution of chromosome dimers/catenae

Most of the research into resolution of chromosome dimers and catenae has been carried out in *E. coli*, but the general components and mechanisms appear to be also present in *B. subtilis* and to operate in a similar fashion. The discovery of a site termed *dif* in the *E. coli* chromosome and related sites in some of its plasmids provided the first evidence for a specific locus for resolution of dimers that arise by recombination during the cell cycle (Blakely *et al.*, 1991; Clerget, 1991; Kuempel *et al.*, 1991). The *dif* locus is situated in the terminus region of the *E. coli* chromosome (Clerget, 1991; Kuempel *et al.*, 1991), and a similar site has now been identified in the *B. subtilis* chromosome, also in the terminus region (Sciochetti, *et al.*, 2001). Occasionally

during the cell cycle an odd number of recombination events occur that lead to the formation of chromosome dimers (Fig. 1b). Failure to resolve these dimers leads to a severe segregation defect in about 10–15% of the cells (Steiner & Kuempel, 1998a, b; Perals *et al.*, 2000). In *E. coli*, in addition to the *dif* locus, the XerCD recombinase is required for resolution of chromosome dimers (Blakely *et al.*, 1993). More recently, it has been shown that resolution of chromosome dimers also involves the activity of another protein called FtsK (Recchia *et al.*, 1999; Steiner *et al.*, 1999). FtsK is a bifunctional protein that is involved in both cell division and DNA segregation (Liu *et al.*, 1998; Yu *et al.*, 1998). FtsK is located at the leading edge of the division septum during cell division, and so appears to be able to cooperate with the XerCD recombinase bound to *dif* sites that become trapped in the closing septum (Fig. 1b). The *dif* site is functional when within a 20–30 kb segment called a *dif* activity zone (DAZ) that appears to be defined by the convergence of polar sequences distributed around the chromosome (Cornet *et al.*, 1996; Kuempel *et al.*, 1996; Salzberg *et al.*, 1998; Perals *et al.*, 2000). These converging polar sequences may be important in ensuring the correct placement of a DAZ close to FtsK located on the invaginating division septum (Perals *et al.*, 2000). That the terminus region should be located in the same region of the cell as the closing division septum is not a chance event, but due to specific chromosome organization. Recent studies have revealed that chromosomes are organized with origin regions located towards the cell poles, termini towards midcell, and other sequences distributed between the two extremes (Gordon *et al.*, 1997; Webb *et al.*, 1997; Niki & Hiraga, 1998; Teleman *et al.*, 1998; Niki *et al.*, 2000). In *B. subtilis*, chromosome dimers are resolved by the activity of the XerCD homologues RipX and CodV (Sciochetti *et al.*, 1999). The system may be slightly different as whilst *ripX* mutants give a typical *dif* phenotype, none is observed for *codV* mutants, implying RipX may be able to compensate for *codV* mutations (Sciochetti *et al.*, 1999).

In addition to the formation of chromosome dimers by an odd number of recombination events, interlinked monomeric chromosomes called chromosome catenae are formed during DNA replication, and by an even number of crossovers. These interlinked chromosomes are resolved by the activity of topoisomerase IV (topo IV; Kato *et al.*, 1990, 1992; Lutinger, 1995; Huang *et al.*, 1998; Fig. 1b), and the *dif* locus also appears to be the preferential site for activity of topo IV (Corre *et al.*, 1997; Hojgaard *et al.*, 1999; Fig. 1b). As the replication cycle nears completion, it is likely that catenae become concentrated in the terminus region and so it represents an ideal locus for maximal activity of chromosome-resolving enzymes like topo IV and XerCD.

Sporulation and chromosome segregation

Whilst termination of replication and chromosome decatenation are required to enable physical separation of chromosomes, additional events occur prior to these

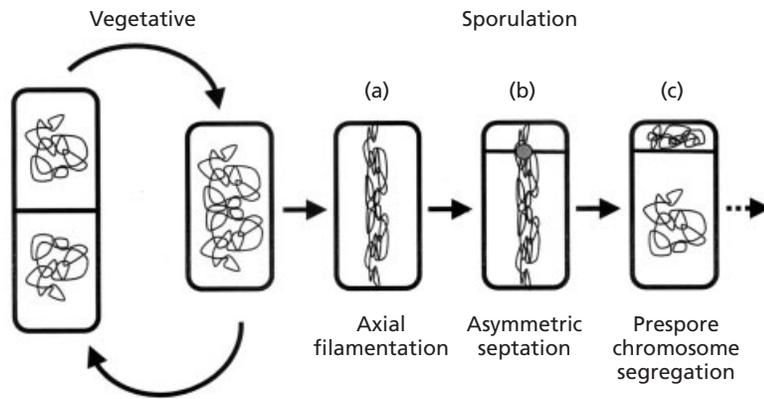


Fig. 2. *B. subtilis* life cycle. Cell walls are shown as heavy lines and chromosomes as the knotted structures inside the cells. The vegetative life cycle is illustrated on the left-hand side where rod-shaped cells grow along their long axis, before dividing across the middle to produce two identical daughter cells. During the sporulation cycle illustrated on the right-hand side chromosomes first form axial filaments (a), prior to the formation of an asymmetric septum (b) that closes around the axial filament. The DNA translocase activity of SpoIII ϵ (shaded circle) ensures that the small prespore and large mother cell each contain a complete copy of the chromosome (c).

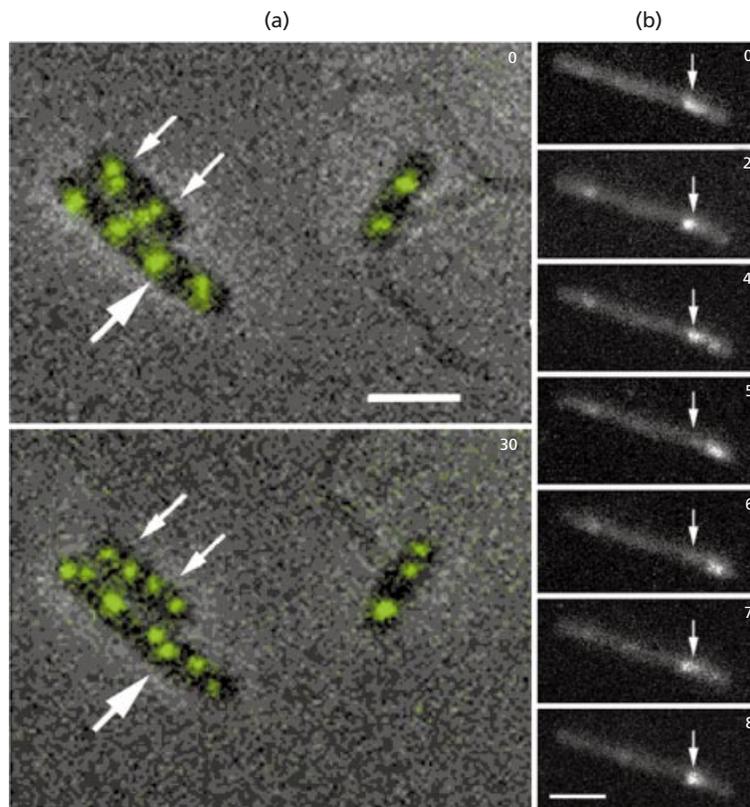


Fig. 3. Spo0J and Soj movement. (a) Phase-contrast/fluorescence overlay images of *B. subtilis* cells labelled with a Spo0J-GFP fusion on initial observation and 30 min later. The large arrow in the top image indicates a single Spo0J focus that has duplicated and segregated in the bottom image. The small arrows indicate newly resolved Spo0J foci in the top image that have fully segregated in the bottom image. Reprinted from Glaser *et al.* (1997) with permission from the publishers. (b) Fluorescence image time course over 8 min of oscillation of a Soj-GFP fusion. The arrow serves as a fixed reference point to help illustrate the dynamic nature of Soj movement. In both panels, the numbers indicate minutes and the scale bars represent 2 μ m.

to separate and organize the newly replicated regions facilitating faithful segregation. Exploitation of the sporulation cycle of *B. subtilis* has been used to shed light on some crucial components of this chromosome segregation machinery. Normally, *B. subtilis* divides when a division septum cleaves the cell in the middle as in other rod-shaped organisms (Fig. 2). However, upon

starvation and the initiation of sporulation, the cell undergoes a highly asymmetric cell division, resulting in the formation of a small prespore and large mother cell compartment (Stragier & Losick, 1996; Fig. 2). Despite this asymmetry, both cells contain a complete copy of the chromosome, and so one chromosome must undergo a dramatic translocation to become packaged into the

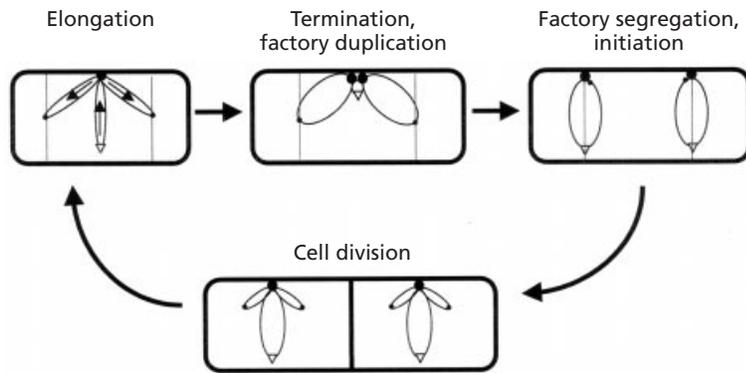


Fig. 4. DNA replication occurs at fixed sites within the cell. Schematic diagram showing the localization of DNA replication in *B. subtilis*. Chromosomes are represented by loops. Small circles, *oriC*; open triangles, *terC*; large circles, DNA replication factories. The direction of movement of different regions of the chromosome prior to and following DNA replication is indicated by arrows. Quarter cell positions are indicated by the dotted vertical lines.

prespore. Much to the surprise of those working with it, a protein called SpoIIIE, which was originally thought to be involved in the regulation of gene expression in the prespore, turned out to be the DNA translocase (Wu & Errington, 1994). Furthermore, this translocation event is a two-step process. First of all, prior to asymmetric septation, chromosomes become elongated (axial filaments; Bylund *et al.*, 1993) and their origin regions become attached (directly or indirectly) at or near the cell poles (Fig. 2a). Upon asymmetric septation, about 30% of a chromosome centred around *oriC* becomes trapped in the prespore (Wu & Errington, 1994, 1998; Fig. 2b). SpoIIIE localizes at the leading edge of the invaginating septum, forms a pore around the trapped DNA and then transfers the remaining 70% of the chromosome through the septum into the prespore (Wu *et al.*, 1995b; Wu & Errington, 1997; Fig. 2b, c). Subsequently, it was shown that SpoIIIE also functions in vegetatively growing cells to rescue any chromosomes that become trapped if they have not fully segregated on completion of cell division (Sharpe & Errington, 1995). Homologues of SpoIIIE have now been found to be present in the genomes of both Gram-positive and Gram-negative bacteria, and it is known as FtsK in *E. coli*. Therefore, the results reported by Sharpe & Errington (1995) suggest SpoIIIE may act in a manner similar to FtsK in the resolution of chromosome dimers.

Another protein first associated exclusively with sporulation in *B. subtilis*, Spo0J, also plays an important role in chromosome segregation. *spo0J* was originally identified as a mutation that prevented cells from sporulating. Originally, it was not thought to have a role in vegetative cells, but careful work by Ireton *et al.* (1994) showed that *spo0J* mutants produced about a 100-fold increase in anucleate cells over wild-type. Although this sounds like a dramatic phenotype, this still only resulted in just over 1% anucleate cells (Ireton *et al.*, 1994). Nevertheless, Spo0J performs an important segregation function. Microscopic examination of Spo0J using immunofluorescence or green fluorescent protein (GFP) fusions showed that it forms foci that localize towards the outer edges of a nucleoid and towards the cell poles (Glaser *et al.*, 1997). These foci were also observed to duplicate and move away from each other, and careful analysis showed that this duplication coincided with the initiation of a new round of DNA replication (Glaser *et*

al., 1997; Fig. 3a). Furthermore, Spo0J-focus segregation was shown to occur independently of cell growth, indicating it was an active intracellular event. Thus, Spo0J was implicated in the segregation of origin regions away from each other, aiding the segregation of daughter chromosomes prior to cell division. Around the same time experiments were also carried out in which origin and terminus regions had been tagged with GFP by binding GFP–LacI fusion proteins to multiple *lacO* sites inserted at specific points in the chromosome in both *B. subtilis* and *E. coli* (Gordon *et al.*, 1997; Webb *et al.*, 1997). These results confirmed the behaviour observed for the duplication and segregation of origin regions and also showed that terminus regions localized far apart from the origins in the middle of the cell. It was subsequently shown that Spo0J foci colocalized with origin regions (Lewis & Errington, 1997; Lin *et al.*, 1997) and that it bound to multiple sites distributed around about 20% of the chromosome either side of *oriC* (Lin & Grossman, 1998). The rate of separation of Spo0J foci has also been measured and found to be biphasic (Sharpe & Errington, 1998). The duplication and resolution of foci represents the slow phase, followed by rapid separation until foci reach a fixed point of maximal separation. Upon reaching this point, movement ceases, ensuring that origin regions remain as far apart from each other as possible within the cell independent of the number of rounds of replication that may be occurring (Sharpe & Errington, 1998).

Spo0J–GFP fusions form one discrete focus per origin region although its binding sites are distributed over nearly 20% of the chromosome. This suggested that separate Spo0J–DNA nucleoprotein complexes aggregate to form a single focus. The gene directly upstream of *spo0J* encodes a protein called Soj. Soj had been shown to antagonize the *spo0J* phenotype resulting in near wild-type sporulation in *soj spo0J* double mutants (Ireton *et al.*, 1994). Single *soj* mutants were not known to cause a phenotype. Soj and Spo0J belong to the ParA/ParB family of proteins that are involved in stable maintenance of low-copy-number plasmids (Motallebi-Veshareh *et al.*, 1990; Marston & Errington, 1999; Quisel *et al.*, 1999). Therefore, it seemed likely that Soj would also complement Spo0J in its chromosome segregation function. In the absence of Soj, Spo0J foci fragment and form multiple microfoci, implying that Soj

plays a role in aggregation of Spo0J–DNA complexes into a large nucleoprotein ‘supercomplex’ (Marston & Errington, 1999). However, localization of Soj using GFP fusions gave highly unexpected results (Marston & Errington, 1999; Quisel *et al.*, 1999). Soj–GFP fusions were found to form large foci that corresponded to between half and the whole of a chromosome in size. These foci were only present in about 50% of the cells and time-lapse microscopy revealed that they oscillated from one end of a cell to the other, although this oscillatory behaviour did not appear to be closely linked to the cell cycle (Marston & Errington, 1999; Fig. 3b). Mutation of the proposed ATP-binding motif in Soj abolished function, implying that it is an ATPase like other ParA proteins (Quisel *et al.*, 1999). Therefore, Soj appears to cause the active aggregation of Spo0J foci. Once formed, these aggregates are probably quite stable as Soj is able to move on to another origin region. Why the level of Soj is limited within cells so that it binds to one or less than one chromosome in less than 50% of the cells remains a puzzle as it is transcriptionally linked to *spo0J*. Also, Soj and Spo0J are not present in *E. coli* and other closely related microbes, although they are utilized by their plasmids, and they appear to be conserved in most of the other available sequenced microbial genomes.

Bulk movement of DNA and chromosome organization

Events involved in segregation that occur near the origin and near the terminus have been examined, but what about all the DNA in between those two extremes? In the absence of any obvious genetic or ultrastructural candidate for a mitotic spindle-type structure, another mechanism for separating the bulk of chromosomes away from each other may operate in bacteria. In general, it was assumed that DNA polymerase holoenzymes and their associated proteins moved bidirectionally around a relatively static chromosome. Work in eukaryotes had shown that this scenario was unlikely to be true and that mobile DNA has to move through relatively fixed DNA replication factories (Cook, 1999). Lemon & Grossman (1998) decided to investigate the localization of DNA replication in *B. subtilis* using GFP fusions to subunits of DNA polymerase III. If replication proteins move around the chromosome, then one, two and one GFP spot(s) should be observed for initiation, elongation and termination, respectively, during a single round of DNA replication. Since the elongation phase occupies the major part of the replication cycle, two spots should predominantly be observed during a single round of replication. If DNA moves through a replication factory, a single GFP spot should be observed throughout the replication cycle. Incredibly, the latter result was obtained. During a single round of replication, a single spot was observed at the midcell position, probably in close proximity to the cell membrane. Around the time of termination, the spot duplicated and moved to quarter cell positions so that on cell division a single factory was once again present at

midcell (Fig. 4). The authors went on to propose that the force of expulsion of newly replicated DNA from the replication factories could play an important role in driving chromosomes apart (Lemon & Grossman, 1998; Losick & Shapiro, 1998).

Extrusion of newly replicated DNA from a replication factory would result in a highly disorganized random coil becoming increasingly tangled as the replication cycle proceeded. However, it appears that the chromosome is not randomly coiled, and is organized so that the linear order of genes is (at least approximately) maintained (Teleman *et al.*, 1998; Wu & Errington, 1998; Niki *et al.*, 2000). Therefore, it is likely that there is a chromosome organizing factor. This role may be played by SMC (or MukB in *E. coli*) that is similar to the eukaryotic structural maintenance of chromosomes family of proteins (Oguro *et al.*, 1996). *smc* mutants show a severe chromosome segregation and condensation phenotype, indicating that they play an important role in maintaining the structural integrity of the chromosome (Britton *et al.*, 1998). Recent work by Sawitzke & Austin (2000) suggests that SMC/MukB acts in conjunction with DNA topoisomerases to condense DNA following extrusion from the replication factory. This condensation serves two roles: first, to compact the DNA and therefore segregate newly replicated regions away from each other, and second, to add a level of structural organization to the nucleoid. It is unlikely that the chromosome is organized in a rigid manner as a level of dynamism must be maintained in order that all genes can be made available for transcription by RNA polymerase.

Conclusion

We can now attempt to put these various strands into context and propose a working model for DNA segregation in bacteria (Fig. 5). First of all, a replication factory needs to form at midcell, although it is not yet known how this occurs. What does seem clear though, is that these factories assemble at midcell independently of the activity of the division protein FtsZ (Lemon & Grossman, 1998), and may provide a nucleation site for Z-ring formation at later stages of the cell cycle. It is also assumed that the replication factory assembles on or directly adjacent to the cytoplasmic membrane due to the known dependence of DNA replication on membrane components (Firshein, 1989). Immediately following the initiation of DNA replication and duplication of *oriC* regions, Spo0J–DNA nucleoprotein complexes form, and become aggregated into a large supercomplex following the activity of Soj. These supercomplexes slowly resolve into two foci, probably due in part to the time needed for them to form as Spo0J-binding sites are distributed over about 20% of the chromosome, and also as a result of the action of newly replicated DNA being extruded from the replication factory forcing them apart. A further mechanism, yet to be described, then moves Spo0J–DNA supercomplexes rapidly apart to quarter and three-quarter positions along the cell.

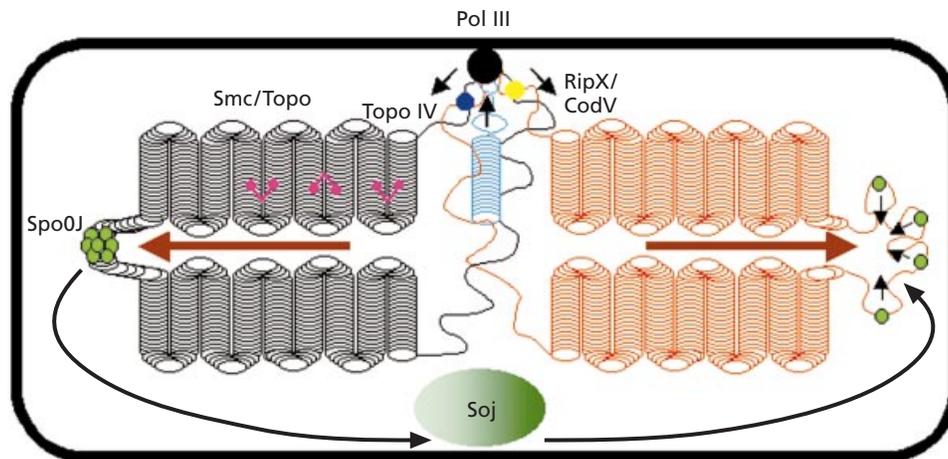


Fig. 5. A model for chromosome segregation. Chromosome segregation is illustrated as involving the concerted effort of a series of different factors. Following initiation of a new round of DNA replication, stable Spo0J–origin region supercomplexes (lime-green circles) are formed by the activity of Soj (shaded green oval). Origins are then segregated in a Spo0J-dependent manner by an unspecified mechanism (brown arrows). Newly replicated strands of DNA (red and black) are forced apart from each other on exit from the DNA replication factory (black circle) where they are condensed, organized and further separated by the activity of topoisomerases and Smc (pink dumbbells). As the terminus region (light blue) approaches the replication factory, topoisomerase IV (dark blue circle) and a RipX/CodV/SpoIIIE-type recombinase system (yellow circle) resolve chromosome catenae and dimers.

This movement may be defined by the DNA replication potential of the cell as the supercomplexes are moved to sites of assembly of new replication factories should a further round of replication be initiated before cell division. If this does not happen, the Spo0J supercomplexes will hold the origin region at the midcell point upon cell division, where a new round of DNA replication would be initiated. As the elongation phase of DNA replication proceeds, topoisomerase and SMC activity causes the chromosome to become condensed and arranged into an organized, but dynamically arranged structure. The force of extrusion of newly replicated DNA from the replication factory may be enough to drive the movement of DNA away from midcell towards the poles, where it is condensed and organized by topoisomerase/SMC activity. As the replication round nears completion, catenae that have arisen from recombination events during the replication cycle become concentrated in the *terC/dif* region. Even numbers of crossovers can be resolved solely by the activity of topoisomerase IV. Odd number crossovers also require the activity of a XerCD/FtsK system in *E. coli* and the paralagous RipX/CodV/SpoIIIE system in *B. subtilis*. This final system appears to have developed to ensure that no DNA is caught in the closing septum, and so presupposes coordination of chromosome replication/segregation and cell division. Indeed, it is well established in *B. subtilis* that cell division will not begin until about 70% of the chromosome replication cycle has been completed (Callister & Wake, 1977; Wu *et al.*, 1995a).

Our knowledge of the mechanisms involved in chromosome segregation has increased rapidly over recent years, but it is clear that we have only just scraped the surface. For example, more detailed information on how bac-

terial chromosomes are organized, and clarification of how factors such as Soj function, is eagerly awaited.

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