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Bacterial chromosome segregation

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1. ABSTRACT

Dividing cells have mechanisms to ensure that their genomes are faithfully segregated into daughter cells. In bacteria, the description of these mechanisms has been considerably improved in the recent years. This review focuses on the different aspects of bacterial chromosome segregation that can be understood thanks to the studies performed with model organisms: *Escherichia coli*, *Bacillus subtilis*, *Caulobacter crescentus* and *Vibrio cholerae.* We describe the global positionning of the nucleoid in the cell and the specific localization and dynamics of different chromosomal loci, kinetic and biophysic aspects of chromosome segregation are presented. Finally, a presentation of the key proteins involved in the chromosome segregation is made.

2. INTRODUCTION

All dividing cells must have mechanisms to ensure that their genomes are faithfully segregated into daughter cells. While the conserved mitotic apparatus that is used by eukaryotes to direct chromosome segregation is well characterized (1), the mechanisms by which bacterial chromosomes are segregated have remained incompletely understood until recently. An early and influential proposal, the replicon model, suggested that the newly duplicated chromosomal replication origins would attach to the membrane and be separated by zonal growth in between the attachment sites (2). Recently, there have been a number of advances in our understanding of the mechanisms that mediate chromosome segregation in prokaryotes.

The segregation of a single circular chromosome is accurate during vegetative growth in the model organisms *Escherichia coli*, *Bacillus subtilis* and *Caulobacter crescentus*. In wild-type *E. coli*, less than 0.03% of the cells are anucleate (3). Chromosome segregation is also very accurate in more complex scenarios. For example, rapidly growing *E. coli* produce multiple copies of the chromosome (4). Some organisms naturally contain multiple copies of the same chromosome per cell such as cyanobacterial filaments (5), some others including *Vibrio* species (6) and *Burkholderia cenocepacia* (7) contain mutiple distinct chromosomes. Lastly, chromosome segregation must occur faithfully during the asymmetrical division encountered at sporulation in *B. subtilis* (8) and *Streptomyces coelicolor* hyphae (9).

This review summarizes the recent work that has been done to characterize the mechanisms of chromosome segregation in model bacterial organisms. Chromosome segregation encompasses all of the processes that lead to the physical separation of the genetic material into the future daughter cells. In general, DNA replication initiates once per cell division cycle (10) from the chromosomal locus, *oriC* (11), and proceeds bidirectionally to terminate in a defined region opposite the origin, *i.e*., the replication *terminus* (12). The basic components of the DNA replication machinery are highly conserved in bacteria. The chromosome is segregated as it is being replicated. Segregation is a multistep process that begins with the migration of the origin regions. The bulk of the remaining chromosome is then segregated, followed by the separation of the *terminus* regions. Some of the forces and proteins that are involved in the segregation of the origin and *terminus* regions are well characterized; however, those involved in the bulk separation of the chromosome are less well understood.

3. GLOBAL CHROMOSOME POSITIONING

The bacterial chromosome is condensed in the cytoplasm in a nucleoprotein structure called the nucleoid. The nucleoid was first described in 1938 by G. Piekarski as a large mass that stains strongly with HCl-Giemsa (a technique known as the Piekarski-Robinow staining) and is regularly spaced inside the cytoplasm of enterobacteria (13). Later, the nucleoid was observable in live *E. coli and B. cerreus* cells using phase contrast microscopy due to its low refractive index (14). Mason and Powelson observed the nucleoid as a multi-lobed structure with phases of strong deformations during the cell cycle. This experiment was actually the first description of bacterial chromosome segregation.

Recently, several groups have described global nucleoid segregation in live *E. coli* cells and have reported that a variety of nucleoid shapes appear in actively dividing cells in gelatin-containing Luria broth medium. Almost all cells with constrictions at mid-cell were found to have four nucleoids. After the separation of sister nucleoids, their shapes quickly change to resemble lobes or dumbbells. Depending on the cell cycle stage, nucleoids can be elongated or more distinctly lobed (15). Similarly, labeling of chromosomal DNA thanks to a GFP fusion with the HU nucleoid associated protein has revealed that the *E. coli* nucleoid is a very plastic structure (Figure 1A). Using a baby cell machine to synchronize *E. coli* cells, Bates and Kleckner showed that the *E. coli* nucleoid initiates segregation with an abrupt splitting from a cylindrical into a bi-lobed shape (16). This transition appears to be precisely timed during the cell cycle and regulated according to the growth rate. The timing of nucleoid splitting is concomitant with a rapid migration of the *Terminus* region of the chromosome to mid-cell, suggesting that there is a global reorganization of the chromosome. Interestingly, when bi-lobed nucleoids first appear, they are asymmetric, with the ter-side lobe being larger than the non-ter lobe side. Then, as replication proceeds, the two lobes become equal in size and symmetrically dispersed within the cell. This result suggests that unreplicated regions tend to occupy the ter side of the cell; in later stages of replication, this region would be depleted of DNA and newly created sister regions would be incorporated directly into their corresponding domains, which would be established during the nucleoid splitting transition (16).

4. INTRA NUCLEOID ORGANIZATION

4.1 *OriC* and the *Terminus* regions

The study of bacterial chromosome segregation was remarkably improved in the late ‘90s, when tools that were developed in yeast to localize chromosomal markers (17) were introduced into *E. coli* (18) and *B. subtilis* (8). This technique, which was subsequently named FROS (Fluorescent Repressor Operator System), uses an array of *lac* operators bound by a GFP-labeled LacI repressor to determine the position of chromosomal loci. These arrays were integrated into the chromosome near the origin and *terminus* of replication. With this tool, loci can be followed over time during time-lapse experiments. Strikingly, the *oriC* and the *ter* loci were found to have very distinct patterns of localization in both organisms. Since these pioneering studies, the segregation patterns of the *oriC* and *terminus* have been more precisely described in various genetic and environmental contexts for *E. coli (*4, 16, 19-23) (Figure 2).

In *E. coli*, the chromosomal origin of replication appears as a spot that duplicates near mid-cell when the bacteria are undergoing single rounds of replication (slow growth rate). When the bacteria are undergoing two overlapping rounds of replication, the duplication of each origin focus occurs at the ¼ and ¾ positions. The segregation of each focus progresses symmetrically at a speed of (40 nm. min $^{-1}$), which is about two to three times faster than the rate of cell elongation. Once the origins arrive at the future duplication site, they remain at this site, which is frequently called the home position, until the next duplication (18, 23, 24).

Various patterns of motion have been reported for the *terminus* of replication. In early work, various labeling techniques positioned the *terminus* region near mid-cell until just before the onset of septation; then, the focus was observed to duplicate and migrate symmetrically and

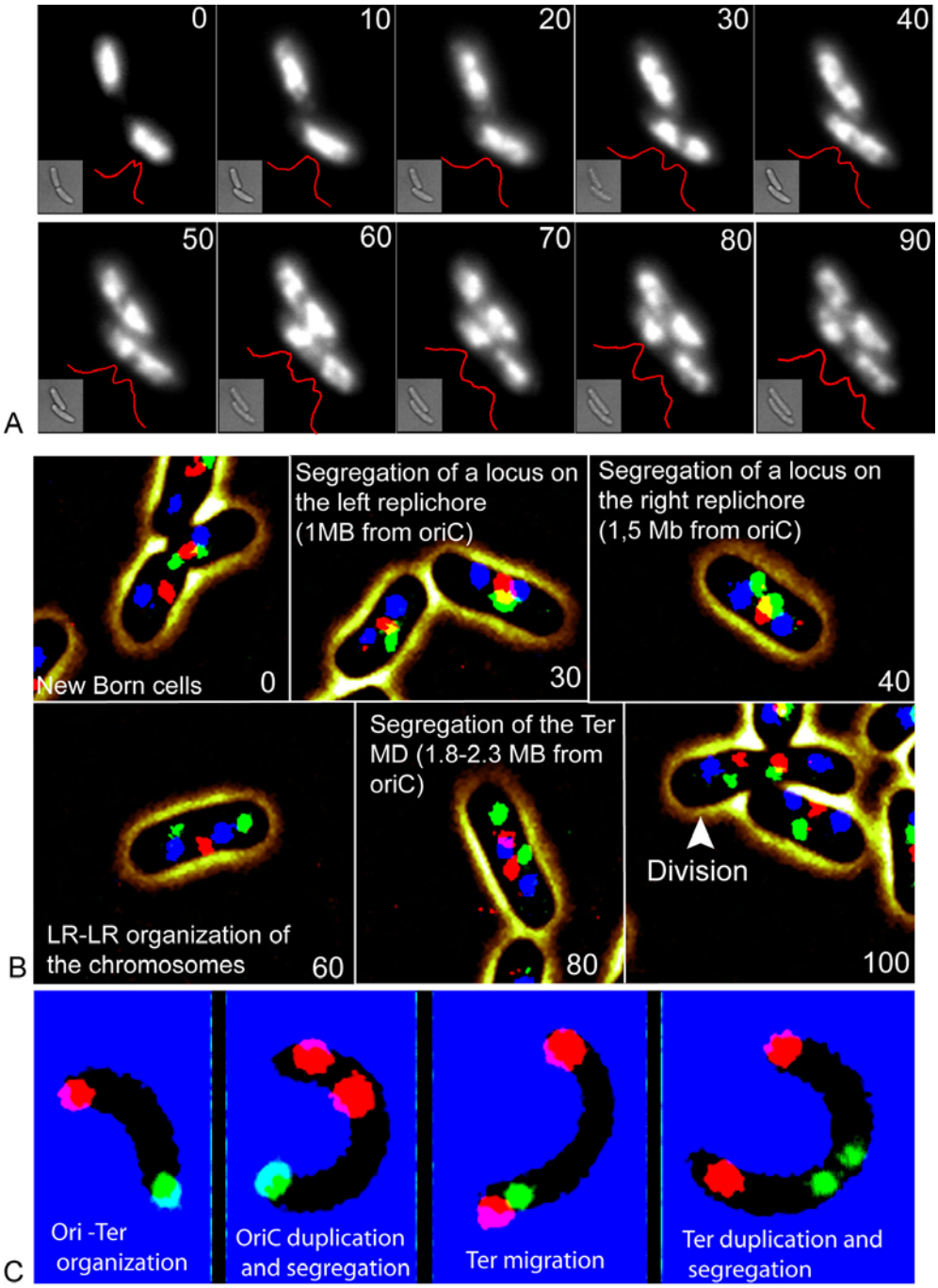

Figure 1. A) The *E. coli* nucleoid is dynamic. Time-lapse experiment of an *E. coli* MG1655 strain containing a pBADHU-gfp plasmid expressing the nucleoid associated protein HU fused to EGFP under the control of the PBAD promoter. Cells were grown in minimal medium supplemented with glucose and casaminoacids on an agarose pad on the microscope stage. Pictures were acquired every 10 minutes. Red traces represent the fluorescence intensity of GFP along the longitudinal axis of the cell. Nucleoid plasticity can be observed as global nucleoid deformation and changes in the local intensity of the GFP signal. Under these conditions, a division is observed every 120 minutes. Nucleoid splitting is observed between time 20 and 30 min for the bottom cell. B) Transverse organization of the *E. coli* chromosome. Snapshots of cells tagged on the Right and the Left macrodomains with *parS*/ParBP1CFP (Blue) and *parS*/ParBpMT1YFP (Green) tags, respectively, and in the Ter macrodomain with the MatP-mCherry fusion (Red). Cells were grown in minimal medium supplemented with glucose and casaminoacids. Cells are organized according to cell cycle stage (estimated timimg after birth is indicated). C) Longitudinal organization of *Vibrio cholerae* chromosome I. The *oriC* region is tagged with a FROS *lacO* array and a LacI-mCherry (Red), and the terminus region is tagged with a *parS*/ParBP1GFP tag (green) (Ariane David and Christophe Possoz, unpublished observations).

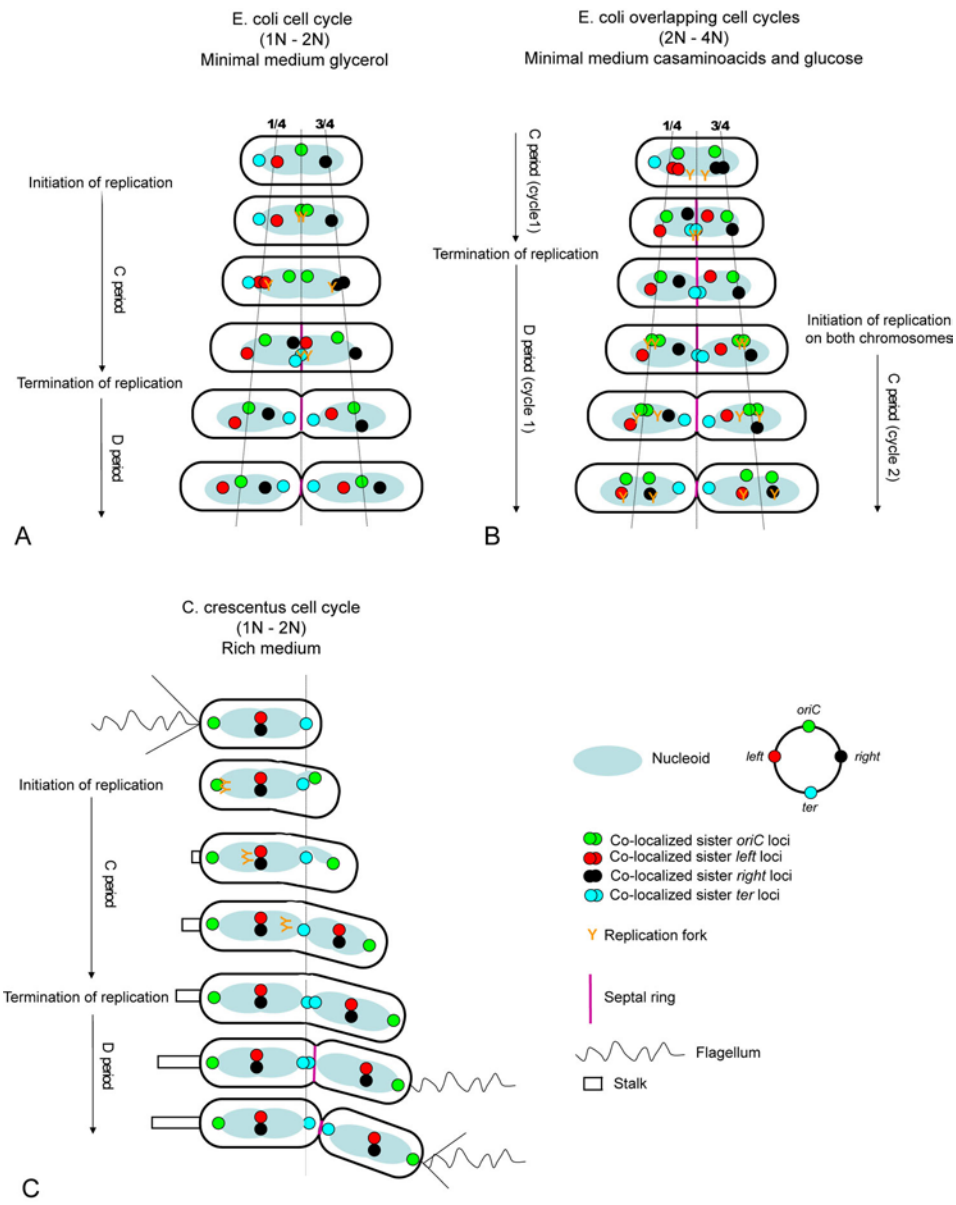

Figure 2. Main steps of chromosome segregation in *E. coli* and *C. crescentus.* A) Segregation of the nucleoid and four tagged loci in *E. coli* grown in conditions favoring an" eukaryotic" like cell cycle. The replication period (C period) is initiated soon after birth on one chromosome and completed before division. The period between the end of replication and the division (D period) is short. The cartoon recapitulates the work by (16, 18, 19, 26, 31, 42). B) Segregation of the nucleoid and four tagged loci in *E. coli* grown in conditions favoring cell cycles with overlapping replication and division. In these conditions, the D period is long. The cartoon recapitulates the work by (4, 19, 23). C) Segregation of the nucleoid and four tagged loci in *C. crescentus.* The cartoon recapitulates the work by (30, 33).

rapidly toward the middle of each daughter cell (Figure 1B and Figure 2) (16, 21, 23). Similar patterns were observed in *B. subtilis* (8) and *Vibrio cholerae* (25). Recently, work by the groups of D. Sherratt demonstrated that in the *E. coli* AB1157 strain, under conditions that support single rounds of replication, foci from the *terminus* region segregate asymmetrically (26). Following duplication, one focus was found to remain close to mid-cell, while the other traveled toward the pole. This pattern suggests that the *terminus* region is stretched across the entire nucleoid length (27). The reason for these contrasting observations is unclear. The MatP-GFP protein, which labels the entire *terminus* region, has a different localization pattern in AB1157 and MG1655 strains (28). It is unlikely that experimental artifacts alone could explain this difference. It is important to note that there are specific experimental conditions for FROS and *parS-ParB* systems that do not affect the normal replication or segregation processes and should be used to avoid artifacts (23, 29). We favor the hypothesis that an

uncharacterized genetic polymorphism exists between these two strains and can influence chromosome organization.

4.2. Replichores and macrodomains

Observing the *ori* and *ter* loci revealed specific dynamic patterns of localization during the cell cycle. However, these two regions have crucial roles for chromosome management and may not be representative of the rest of the chromosome. To date, the organization and segregation of the entire bacterial chromosome has been analyzed in only two systems, first in *C. crescentus* (30) and then in *E. coli (*26, 31).

Most bacteria with circular chromosome present a similar genome organization with two replication arms and a number of elements showing a biased orientation following the origin–terminus axis. This has led to the definition of replichores as accounting for this global organization. Despite presenting the same structure in twin replichores, it was observed that chromosome segregation does not follow the same choreography in *C. crescentus* and in *E. coli*. Using FROS, Viollier and colleagues (30) tagged and analyzed the positions of 120 loci on both replichores. This first "resolutive" image of the whole chromosome showed loci that are sequentially segregated shortly following their replication and that the chromosome is linearly ordered within the new-born cell, from *oriC* near the old pole to the terminus near the new pole. Chromosome segregation appears to be an ordered multistep process in this organism. After replication initiation, the two origins move apart. Once one copy of the origin reaches about 40% of the cell length, the region containing *parS* sites is actively transported by the *parAB* system toward the opposite pole, and the rest of the chromosome follows behind (32). Thus, in the newborn cell, the two replication arms stretch from the origin at one pole to the *terminus* at the other pole (33) (Figure 2).

The first complete picture of this process in *E. coli* was achieved with the analysis of 14 positions on the chromosome by FISH and 8 positions tagged by FROS (26). Later, 14 positions were tagged with the *parS-parB-GFP system* (31). Both reports use pairwise combinations of multiple genetic loci to reveal the same model, which is different from that in *C. crescentus*. In a newborn *E. coli* cell growing at a slow rate, the left and right replichores occupy distinct halves of the nucleoid, with the replication origin in the middle. Sequential replication–segregation regenerates the <left–right> architecture by sequentially layering newly replicated DNA in specific inner and outer edges of the developing sister nucleoids (Figure 1B and Figure 2). Thus, the subcellular position of a given locus depends on its genetic distance from the origin: the closer it is to *oriC,* the closer it is to mid-cell.

From these experiments, we can extract some common themes for the organization and segregation of the chromosome in bacteria. In general, segregation is linear following replication, and the subcellular position recapitulates the genetic map, with two units corresponding to the two replichores. While in *E. coli*, it is clear that a physical distance separates the two replichores (26, 31, 34), we cannot exclude the possibility that the two replichores are intertwined in *C. crescentus*. This difference could potentially be explained by the fact that the origin of the *C. crescentus* chromosome is anchored to the pole, forcing the two arms into a longitudinal arrangement, whereas in *E. coli*, the absence of an anchor could allow the replichores to lie on each side of the origin. The elegant identification of the *migS* sequence revealed that in *E. coli* some unknown factor can influence the bipolar migration of sister chromosomes but perhaps without any anchoring (35). Hence, it is tempting to suggest that the *oriC* in *E. coli* is positioned at mid-cell by default, only because the two replichores are of equal sizes and thereby occupy equal spaces on each side of the origin. This global view has been refined to include macrodomain organization. A macrodomain is defined as a large chromosomal region that is spatially confined and has constrained mobility. Thus, the four macrodomains (MD), Ori, Left, Right and Ter, occupy distinct cellular spaces (cages) without important overlap (23, 34) (Figure 1B). In contrast, two regions have been called NS for non-structured region. These are characterized by an increased mobility and are thought to be positioned in an "open cage" that largely covers the adjacent macrodomain cages (23). The existence of macrodomains in the *C. crescentus* chromosome has not been confirmed experimentally, but in *E. coli* it is tempting to suppose that this organization could participate in chromosome segregation, providing a scaffold for chromosome locomotion. The Ori MD would be the head, the Left/Right MD would be two arms, and the NS would correspond to the flexible joints. The duplicated heads (Ori MD) would be released once the joints (NS) are replicated. The two arms would be repositioned as units on either side of each head. Finally, the tails (Ter MD) would be released only after the two chromosomes are completely untangled, in order to prevent any chromosome breakage that would occur with precocious separation (Figure 1B).

The dynamic behavior of the *Bacillus subtilis* chromosome has not been extensively studied (8). Considering that the origin has been observed at mid-cell during vegetative growth, the left and right replichores could be in a lateral arrangement, as in *E. coli.* Interestingly, during the asymmetric division of sporulation, the chromosome is known to be converted into a longitudinal arrangement called the axial filament (36), with the origin regions at the edges of this filament. Thus, perhaps the specific positioning of the origin region at the pole can modify global chromosome organization.

5. SISTER CHROMATID COHESION

A number of experiments have suggested that following replication, loci from bacterial chromosomes remain in close proximity for a significant period of time near the position where they were replicated (Figure 2). This step, called cohesion or colocalization of the sister chromatids, resembles the well-characterized cohesion step that has been observed for eukaryotic sister chromatids (1). In eukaryotic cells, cohesion is established following S phase due to the binding of the cohesin complex around

sister chromatids (37). Cohesion remains attached until the onset of anaphase.

In bacteria, currently the only evidence for the cohesion of the sister chromatids is the observed colocalization of the two foci (one on each sister chromatid) visualized using the defined fluorescent reporter system inserted at a specific locus of the chromosome (16, 23, 38). Bacterial cohesins have not yet been identified; SMC and MukB have condensation activity but not cohesion activity (39). Nonetheless, it is believed that topological or protein-mediated linking is involved in bacterial cohesion. To study the bacterial cohesion step, an accurate knowledge of the cell cycle stages, particularly the timing of the replication of the considered locus, is required. This requirement can be fulfilled with a combination of two of the following methods: the use of cells with a synchronizable cell cycle, measurement of the DNA content by flow cytometry, measurement of the frequency of markers, direct observation of the replication with SSB-YFP or incorporation of BrdU. One of these techniques should estimate the ratio of unreplicated versus replicated DNA for a given locus, and the other should estimate the number of copies in this given locus.

Most of the work on bacterial cohesion has been performed with *E. coli*. The first description of the cohesion step was described by the group of S. Hiraga (38). Using FROS arrays and bacteria with a synchronized cell cycle, they provided compelling evidence for a long period of cohesion following replication. In their pioneering work, the cohesion of the whole chromosome was found to be broken all at once at a late stage in replication (38). Strong support for the delayed and nonlinear separation of sister chromatids has also been provided by D. Bates and N. Kleckner. They have shown that the first half of the chromosome maintains a cohesive state following replication (i.e., 14 min for a locus close to *oriC*) until the abrupt separation of every locus on each sister (16). This event was called nucleoid splitting. Because only a few FISH probes were used in this study, it was only possible to observe nucleoid splitting on the right replichore. Following nucleoid splitting, the remaining part of the chromosome was assumed to be segregated progressively, with the exception of a locus close to the *terminus*, which presents extensive cohesion, from 15 to 60 minutes according to the extent of the D period (Figure 2). Recently, the sequence of events leading to the *ori* region segregation has been more precisely described (40); at least two large regions inside the Ori macrodomain present a prolonged cohesion compared to rest of the macrodomain. These two regions form intersister snaps that are broken abruptly at the moment of the nucleoid splitting. In agreement with the nucleoid-splitting model, it was shown that the *oriC* locus is not the first to be segregated; a locus called *migS*, which is about 200 kb away from *oriC*, is segregated earlier (15, 41).

Recently, it was shown that the splitting of sister chromatids follows the macrodomain topography of the chromosome (23). Shorter cohesion periods have been observed in the NS regions compared to the macrodomains. This difference could explain the observed nucleoid splitting because the whole Ori macrodomain and the NS regions are segregated in one step about 30 minutes after replication initiation. The *terminus* region of the chromosome also displays an extensive cohesion period. It has been shown recently that the Ter MD structuring protein MatP is involved in the delay of segregation of the two sister Ter MD , see below (28). Interestingly, it was also shown that cohesion length differs between the *oriC* and the *terminus* region in C. crescentus. No cohesion was observed near the *oriC* while a long (30 min) cohesion of the sister *ter* region was observed (33).

In all of the above experiments, long cohesion steps were observed for the *ori*C or *ter* regions of the chromosome. The groups of C. Woldringh, D. Sherratt and S. Austin have claimed, however, that the extent of cohesion has been overestimated and that no locus can maintain more than 10 minutes of cohesion (31, 42, 43). The reasons for these discrepancies are not clear. An equivalent cohesion period was measured in the *Terminus* region with FROS, *parS*/ParB and the MatP-GFP localization reporters (28); therefore, it is unlikely that variations in cohesion times can be derived from artifacts of the chromosome localization technique. We favor the hypothesis that cohesion can be modulated according to the growth rate, the cell cycle and ultimately, the genetic background of the strains (Figure 2). Evidence to support this hypothesis has been provided by the S. Hiraga's group (19).

6. KINETICS OF CHROMOSOME SEGREGATION AND ORGANIZATION

Bacterial chromosomes are slowly moving objects. The 2D diffusion coefficient has been measured for various loci in *E. coli* (23, 24) and *V. cholera*e (44). Chromosomal loci at their home position exhibit a very slow subdiffusive movement, with a diffusion coefficient $D_{(X+Y)}$ of $\sim 10^{-4}$ μm^2/sec. Foci were found to be constrained in ellipsoidal cages, reflecting a greater restriction of movement in the short axis of the cell. In *E. coli*, the macrodomain structure appears to influence the subdiffusive motion of the loci: foci in the macrodomains are constrained in smaller cages than those in non-structured regions (23). The kinetic parameters for foci segregation have been measured in *E. coli (*23, 24), *V. cholerae (*44) and *C. crescentus (*30). The segregation of the origin of replication has been particularly well studied. In *E. coli*, the origin is segregated particularly slowly; the movement of this region was found to progress at a rate that is comparable to the rate of cell elongation, never exceeding the elongation rate by three-fold. This slow segregation rate suggests that the separation of sister chromatids may be supported entirely by cell elongation (24). In *Vibrio cholerae* and *C. crescentus*, a more defined segregation drift has been observed (30, 44). The speed of the migrating origin is relatively fast in *C. crescentus* (80 nm/min), even in the absence of significant cell elongation. An anchoring of the origin region to the MreB helix (45) or the *parABS* system could be responsible for this rapid directed motion (32, 46, 47). The *oriC* regions of both *V.*

cholerae chromosomes are also actively segregated, taking an average step size of 50 nm/min for chromosome 1 and 60 nm/min for the chromosomes 1 and 2. These rates are two- or three-times faster than the rate of cell elongation (44). Fewer measurements have been obtained for other points along the chromosome. In *E. coli,* the segregation speed varies according to the locus (23). The fastest segregation speeds have been observed for the *terminus* region; foci in this region could be segregated away from the mid-cell position at rates of up to 150 nm/min. Very reproducible traces were observed for the segregation of the Ter MD loci when 30 cells were compared (23). The fast and reproducible speed of Ter macrodomain segregation indicates that this process has a non-diffusive, directed mechanism for segregation. Similarly, fast and asymmetric segregation speeds have been measured for loci on the arms of the replichores. Taken together, these observations suggest that continuous cell elongation cannot explain the variety of segregation patterns that have been observed in *E. coli* and other bacteria.

7. BIOPHYSICS OF CHROMOSOME SEGREGATION

Two types of biophysical mechanisms have been proposed to explain the specific positioning of chromosomal loci in space and time. *Active mechanisms*, also called *external mechanisms* (27), use energy to form dynamical protein complexes that can drive loci. Examples of such mechanisms include an actin-like polymerization/depolymerization and growing-membrane anchoring systems. *Passive mechanisms* are *internal* to the chromosomes (27). The emerging patterns result from self-organization phenomena that are based on localized intra-chromosomal interactions (48-50).

The absence of completely characterized active processes (see below : “the players in chromosome segregation “ chapter) and recent results in polymer physics suggest passive mechanisms to be central to chromosomal segregation in bacteria. The most basic intra-chromosomal interaction is the electrostatic repulsion between chromosomal loci: the chromosome is a *self-avoiding* polymer chain. In effect, due to Brownian motion, loci jiggle and produce small effective volumes the other loci cannot enter (51): self-avoidance tends to swollen chromosomes. In contrast, both nucleoid-associated proteins and supercoiling activity tend to condense chromosomes. Yet, polymer numerical simulations have shown that supercoiling condensation can generate exclusion effects between supercoiled microdomains, and hence, can efficiently act as a segregation force (52). Thus, just as for the eukaryotic interphase chromatin (49), condensation effects may drive the spatial organization of bacterial chromosomes.

Segregation of the whole bacterial chromosome as a thermodynamically-driven demixing process has been recently proposed by Jun and co-workers (53-55). The question Jun *& co* have addressed concern the volume conditions that lead to the demixing of two confined self-avoiding polymers (the freshly replicated chromosomes). The problem consists in computing the free energy difference between the mixed and demixed states under strong confinement conditions. If no intra-chromosomal interactions other than self-avoidance come at play, the most stable state is that allowing the highest number of polymer configurations; the mechanism is then said to be entropy-driven (54, 55).

Confinement is *a priori* stronger along the smallest dimension of the cell. Demixing the polymers along the largest dimension can alleviate this. However, this process has an entropy cost since, in the demixed state, each chain occupies only half of the cell volume. Polymer blob theory (51) states that the nature of the most stable state critically depends on the smallest volume in which the polymers can be considered as to be unconstrained. In (53),

Jun *& co* consider that this *structural unit* consists of a few plectonemes and argue that, in small *E. coli* strains, the longitudinal dimension of the nucleoid is sufficiently large for the chromosomes to spontaneously demix. Hence, as in (52), the micro-domain structure of the bacterial chromosome is critically important for the passive segregation process.

Segregation is a dynamic process that starts long before replication ends. Several studies have suggested that replication dynamics are compatible with the static entropy-driven mechanism of segregation (54, 56). Yet, the proposed models rely on assumptions that may be biologically irrelevant. For instance, the simulations in (54) are based on a concentric shell model, in which only the newly synthesized DNA can escape. In (56), the structural unit is rather large (200 kb), and the dynamics mostly neglect Brownian motion.

Entropy-driven mechanisms are robust mechanisms that are expected to come at play in bacterial segregation (55). Several questions remain to be elucidated, though. First, what is the role of the cytoplasm? Using entropy minimization principles, Odijk has shown that nucleoid formation can be understood as a phase-separation between the crowding molecules and the supercoiled protein-bound DNA (57) . What, then, would be the impact of molecular crowding on polymer demixing? Second, what is the role of the internal structure of chromosomes such as the formation of macrodomains? Do the latter have to be considered as structural units? Finally, replication during exponential growth involves several partially replicated chromosomes. This may favour the mixed state since the demixed state would consist of strongly confined chromosomal pieces.

8. THE PLAYERS IN CHROMOSOME SEGREGATION

8.1. Partitioning complexes

Several molecular machines have been implicated in bacterial chromosome segregation. Of these, the *ParABS* module, which is commonly involved in plasmid segregation, has been thoroughly studied (58). This module is encoded in a number of bacterial chromosomes but is

absent from Enterobacteria, including *E. coli*. It was first shown to be involved in chromosome segregation during *B. subtilis* sporulation (59, 60). The *parABS* module is composed of two proteins ParA and ParB and a centromere like sequence *parS*. ParB is a DNA binding protein that recognizes the *parS* site and propagates along the DNA flanking *parS*. ParA forms filaments (cables) along the longitudinal axis of the cell. It also interacts with ParB and hydrolyzes ATP. Cycles of polymerization and depolymerization of ParA are used to promote chromosome segregation. While the *ParABS* module is essential for plasmid segregation, it can be deleted from most chromosomes with only moderate consequences, suggesting that chromosome segregation is achieved through redundant mechanisms. A few examples of essential *ParABS* modules have been described, including those involved in chromosome segregation in *Caulobacter crescentus* (46, 61) and the segregation of *Vibrio cholerae* chromosome II (35). An attractive hypothesis is that the *parABS* system in *C. crescentus* is a bacterial counterpart to the eukaryotic mitotic spindle apparatus that is dedicated to actively segregating the chromosome (32, 46, 47). In a *parA* mutant strain of *C. crescentus*, origin segregation is impeded, suggesting a block in chromosome segregation. However, in this organism, the *ParABS* system is essential for regulating the proper timing and positioning of the FtsZ ring (62). In addition, there are putative connections to different DNA metabolic processes (see below); thus, it is possible that the chromosome segregation phenotype of the *parA* mutation is indirect. In *Vibrio cholerae*, which has a genome that is divided between two chromosomes, the origin of chromosome I is actively transported from one pole to the other by a *parABS (parABS1)* system, as in *C. crescentus*. In this organism, the *parABS1* system is not essential, and the efficiency of chromosome segregation is not significantly affected (very few cells lack chromosome I) (6). However, upon deletion of *parABS1*, the origin of chromosome I now localizes to mid-cell, as in *E. coli*, rather than at the pole. This result is consistent with the idea that the positioning of the origin in *E. coli* represents the default. Therefore a role for the *parABS1* system in the segregation of chromosome I cannot be ruled out, but most likely it functions only to position the origin. The *V. cholerae* chromosome II also contains a *parABS2* system, and this system resembles that encoded by plasmids. The *parABS2* module is essential for chromosome II segregation and therefore for cell viability (35).

Bacillus subtilis has a transverse organization of the chromosome with a chromosomal *parABS* system (SpoOJ, Soj) (59, 63). The role of the *parABS* system during sporulation has been well characterized. During this process, the proximal origin region is condensed into the prespore by Spo0J, Soj, and the *parS* sites and anchored to the pole thanks to an interaction between Spo0J and RacA - DivIVA that are developmentally regulated (64, 65) .

8.2. The influence of replication on segregation

Lemon and Grossmann have proposed that DNA replication alone can serve to actively segregate chromosomes (66). In their model, a DNA factory containing both replication forks would be centrally localized in the *B. subtilis* cell and would promote both the capture of the unreplicated DNA to mid-cell and the expulsion of the newly synthesized strands on each side. This model, called the capture and extrusion model, is supported by the following observations. First, a centrally localized DNA factory has been observed (67). Second, in a synchronous population of cells, a chromosomal region midway between *oriC* and *terC* appears to move to the replisome just before replication (68). Lastly, it has been observed that there is a delay in sister loci segregation following ectopic replication arrests (68).

In *E. coli,* the influence of replication on chromosome segregation has been studied with the insertion of an ectopic Tus-binding site, which forces the *ter* locus to be replicated by the left replication fork. In this strain, the *ter* locus was found to be more frequently associated with the left replichore (22), suggesting that replication influences the spatial organization of the chromosome. Mechanistically, replication could influence the leading and lagging strand differently. The DNA derived from the leading strand would be "exported" from mid-cell to the outer edges of the two developing nucleoids, whereas the lagging strands would remain confined to the inner sides (69). Interestingly, this prediction matches the predominant observation that Left and Right replichores alternate from one pole to the other pole of cells with the two daughter nucleoids. The, so called, <Left-Right><Left-Right> segregation pattern could be altered if only one of the two forks is transiently blocked, suggesting that the pattern is governed directly by the replication. The influence of the replichore organization on the chromosome segregation has been shown thanks to large chromosomal inversions (70, 71). These works suggested that a number of steps of the segregation could be perturbed when the genome organization is not optimum and that checkpoint like processes exist to cope with such alterations. Technological improvements for monitoring the subcellular localization of replicative proteins have allowed researchers to follow the dynamics of the sister replisomes. In these experiments, it was found that these two replisomes can be separated by more than 1 μm at certain points of the cell cycle (16, 42, 72). In a *mukB* mutant that has polarly localized origins, the loading of the replisomes also happens at the pole (73). Moreover, in *C. crescentus* the replisome appeared to be an untethered replication factory that was passively displaced towards the center of the cell by the newly replicated DNA (74). Taken together, these observations suggest that replisomes follow chromosomal DNA rather than capture it. While replisomes may not provide the forces necessary to pull the entire chromosome to mid-cell, the forces that are generated by replication could contribute significantly to sister chromatid segregation (75). Influence of the replication on the pattern of chromosome segregation has been illustrated recently by the group of S. Ben Yehuda (76). They have developed a strategy to fluorescently label newly synthesized DNA in live cells and proposed that when *B. subtilis* initiates a new round of replication, newly synthesized DNA is translocated from mid-cell to the two polar proximal regions along an helical path (76). A second helix intertwined with the first one is then formed with the distal

part of the chromosome. It is not yet known whether replication itself or another subcellular achitecture promotes this helicoidal pattern.

8.3. Transcription, Translation and membrane anchoring

While there are specific machineries dedicated to chromosome segregation, other non-specific processes, for example, transcription and transertion, could contribute to this process. The involvement of these additional processes in chromosome segregation could explain the mild phenotypes of mutations in a number of genes encoding proteins involved in DNA trafficking, including ParAB, MukB, SeqA, and MatP. One of the best characterized molecular motors acting on DNA is RNA polymerase, which can pull on DNA with a force >30 pN, making it one of the strongest motors observed (77). Single molecule assays have shown that immobilized RNA polymerase can drive the translocation of its DNA substrate (78). At a fast growth rate, the movement of RNA polymerase in the cell is restricted to transcription factories that are presumably transcribing ribosomal operons (79). These transcription factories could anchor the RNA polymerase; in these cases, transcription could translocate the chromosome. Consistent with this idea, an inhibitor of RNA polymerases has been shown to reduce the separation of newly replicated *oriC*-containing regions of the B. subtilis chromosome (80). Similar experiments in E. coli produced the opposite result; however, *oriC* segregation is not altered by rifampin-mediated transcription arrests (81). It is unclear whether this discrepancy can be explained by inherent differences between these bacterial organisms. For example, it is possible that a stronger bias in the orientation of genes in *B. subtilis* (82), or under certain growth conditions, could explain these opposite observations.

One hypothesis is that structural heterogeneity in the cytoplasmic membrane plays a role in the spatial and temporal organization of the bacterial chromosome (83). The heterogeneity of the membrane is derived from interactions between the membrane and the nucleoid. The idea is that DNA attaches to the cytoplasmic membrane through RNA protein bridges that result from concurrent transcription/translation and the insertion of integral proteins into the membrane, termed "transertion". Several studies have identified membrane domains in bacteria (84), some of which regulate cell cycle proteins (85, 86). With regard to chromosome segregation, it has been proposed that membrane proteo-lipid domains are created around replicating and transcribed DNA and may determine chromosome shape and partitioning (87). There is not yet any direct experimental support for this theory, however, and thus it has not gained significant support. Transcription and transertion-driven chromosome partitioning could potentially explain the symmetric and bidirectional segregation of the *E. coli* and *B. subtilis* chromosomes from the middle of the cell; however, it is difficult to see how these processes may influence the asymmetric segregation process that occurs in *Caulobacter crescentus* (30) and *Vibrio cholerae* (6).

8.4. MukB /SMC and MatP provide anchoring and structuring mechanisms

Theoretically, two loci that are no more than 5 kb apart can be located to opposite poles of a cell, so the positioning of the origin region should not *per se* imply that the whole chromosome is affected. For that to be the case, compaction of the chromosome structure would have to be important for global chromosome segregation. The MukBEF/SMC complex is a good candidate for this global compaction. This complex was implicated in chromosome segregation (3) when it was discovered in a screen for mutants that produce anucleated cells. The *muk* mutant phenotype is characterized by an inability to segregate chromosomes at 37°C. At 22°C, segregation can occur in this strain; however, there is a global loss of chromosome organization (73). The MukBEF/SMC complexes form discrete foci, and the localization of these foci has long been controversial. It is now generally agreed that this complex colocalizes with the origin regions in *E. coli* and *B. subtilis* throughout the entire cell cycle, independent of active replication (73, 88). In *B. subtilis*, the recruitment of SMC to the origin region appears to be mediated by the *parS*/ParB system, which is localized to this region (88, 89). Thus, during vegetative growth, even if the origin is not polarly localized, the *parABS* system acts by recruiting the SMC protein to the centromere sites near the origin. These data suggest that SMC's activity is crucial for the segregation of the first region of the chromosome. This connection between SMC and the partitioning system could also suggest that the segregation defect that is observed in the *parA* mutants of *C. crescentus* could be caused by an inability to recruit SMC.

The molecular manipulation of DNA by the SMC complex *in vitro* has been largely studied by the laboratory of V. Rybenkov. Condensation activities have been observed (90), but the precise mechanism of action *in vivo* remains unclear. Recently, MukB was shown to stimulate the superhelical DNA relaxation activity of TopoIV (91). Chromosome segregation could be achieved by the compaction of newly replicated DNA from the origin region. DNA compaction could be mediated via the combination of MukBEF/SMC and topoisomerase activities and be propagated in *cis* along the replichores until the *terminus* is reached. Such a mechanism could explain how SMC could organize the whole chromosome into two replichores from a single, unique region. The two nucleoids would progressively exclude each other due to space confinement, ultimately leading to chromosomes segregation.

The macrodomain organization could also facilitate/regulate different steps of the segregation process. The MatP/*matS* complex confers the macrodomain organization to the *terminus* region but also controls its segregation. MatP binds to 23 *matS* sites clustered in a 1 Mb region surrounding the *dif* site in *E. coli.* The *matP* gene is only found in the bacterial species that also contain the *dam* (Dam methylase), *seqA* and *mukBEF* genes among others (i.e., γ proteobacteria). In the absence of MatP, the movement and the timing of segregation change, leading to profound partitioning phenotypes at a fast growth rate (28).

The co-conservation of *matP* and *mukB* and the similar phenotypes observed with *matP* and *mukB* mutants suggest that MatP could serve as counterpart for MukB in the Ter MD.

8.5. FtsK: specialized segregation machinery

FtsK plays a major role in the final step of chromosome segregation: the separation of the termini. FtsK is a DNA translocase that is anchored at the septum via its N-terminal domain (reviewed in (92, 93)). Its activity is restricted to the time of cell constriction (94-96). FtsK functions as the last safeguard, clearing DNA from the division septum before cell fission. DNA could be trapped at the septum in presence of chromosome dimers (97), or in the absence of *mukB* (98) or when replichore symmetry is lost (71). Unlike in *E. coli*, the C *terminus* of FtsK is essential in *C. crescentus*. It is involved in maintaining accurate chromosome partitioning and for the localization of topoisomerase IV, suggesting that FtsK may facilitate chromosome decatenation prior to cell division (99). In addition, FtsK homologs can be required for the developmental process, such as the *B. subtilis* sporulation (65).

The C-terminal domain of FtsK assembles into hexameric rings with a central opening that is large enough to accommodate a DNA duplex (100). These complexes function as pumps that can reach translocation rates of up to 7 kb/s (101).

Molecular signposts ensure proper sorting of the chromosomes for FtsK called KOPS (FtsK-orienting polar sequences). KOPS are short, conserved sequence motifs that are highly overrepresented in the genome. Their orientation is skewed toward the *dif* site in the *terminus* region (102). Thus, when a chromosome is trapped at the septum, FtsK can load in an oriented manner and pump DNA until it reaches the *dif* site. In case of a dimeric chromosome, FtsK controls its resolution by properly aligning the two *dif* sites and then activating XerD strand exchange. The sorting of the *terminus* regions not only clears the division site of DNA but also facilitates directly their decatenation by Topo-IV (103, 104).

8.6. Determinants for sister chromatid cohesion

The molecular determinants for sister chromatid cohesion have not yet been completely identified. Several evidences suggest that at least two proteins modulate the extent of cohesion, as assayed by colocalization of sister loci. First, topoisomerase IV activity is essential for the segregation of two loci (one near oriC and one on the left replichore), and overexpression of Topo-IV reduces the length of the cohesion step of the locus near oriC (105). Topological links between sister chromatids, such as catenanes (106) and precatenanes (107), could require Topo-IV for their separation (108). In a wild-type cell, Topo-IV's access to sister chromatids must be prevented for at least 15 minutes, which is the colocalization period. This time period translates to a genetic distance that is more than 500 kb away from the replication fork. Genetic and cellular evidence suggest that Topo-IV's activity is regulated during the cell cycle, with a peak in activity after S phase to ensure the separation of completely catenated chromosomes (109). Limiting Topo-IV activity could be a way to ensure a significant level of precatenation of the sister chromatids after their replication. Since precatenation of chromosomal DNA has not yet been characterized, it is not known if their density is sufficient to block separation of loci Mb away from the replication forks.

The second protein that has been implicated in sister chromatid cohesion is MatP. It was recently shown that a *matP* deletion or a local alteration of the Ter MD structure with the deletion of selected *matS* sites significantly reduces the extent of the cohesion step in the *Terminus* region of the chromosome. This observation suggests that regional organization of the chromosome can delay the segregation of the sister chromatids, potentially with nucleoprotein complexes that bridge the two sister chromatids. It is also possible, however, that the alteration of the Ter MD structure influences cohesion indirectly by affecting the activity of TopoIV or precatenation.

9. CONCLUSIONS AND FUTURE DIRECTIONS

In recent years, research on bacterial chromosome segregation has been particularly productive. A number of teams have applied cell biology techniques to bacteria to describe the major characteristics of chromosome motion in *E. coli*, *B. subtilis, C. crescentus* and *V. cholerae*. Major conceptual breakthroughs have been made: i) the DNA is organized inside the bacterial cytoplasm; ii) this organization varies by bacterial species; iii) the segregation of any particular locus follows a defined pattern that is reproducible from cell to cell; iv) the replication process directly shapes the chromosome, providing a segregation force and controlling the localization of the replichores and the leading and lagging strands; v) the chromosome segregation is influenced by the organization of chromosome into large subregions (macrodomains, Spo0J domain); vi) molecular machineries are involved in chromosome segregation (i.e., *ParABS*, MatP, MukB/SMC, Topo-IV, and the Replisome). New perspectives on chromosome segregation have also recently been provided by the description of the intrinsic physical properties of DNA polymers. Nevertheless, important questions remain unanswered. We believe that the understanding of this process presents a great challenge for the future. Efforts should be made to characterize the molecular mechanisms governing each of the different segregation steps. The interactions between the different players in chromosome segregation should be investigated. The "raison d'être" for the longitudinal versus transversal organization of chromosomes remains unclear. The role of entropy in chromosome segregation should be tested experimentally. The role of the sister chromatid cohesion step in DNA metabolism should be further explored. Our understanding of bacterial chromosome segregation will also benefit from new emerging technologies, such as *in vivo* single molecule microscopy, superresolution microscopy, and high-throughput genome sequencing applied to chromosome conformation capture. These new tools will undoubtedly bring exciting perspectives to the bacterial chromosome segregation community.

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