Minireview

Structural and physical aspects of bacterial chromosome segregation

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Abstract

Microscopic observations on the bacterial nucleoid suggest that the chromosome occurs in the cell as a compact nucleoid phase separate from the cytoplasm. Physical theory likewise predicts a phase separation, taking into consideration DNA supercoiling, nucleoid-binding proteins, and excluded-volume interactions between DNA and cytoplasmic proteins. Specific DNA loci, visualized as oriC-GFP spots in the densely packed nucleoid, exhibit a very low diffusion coefficient indicating that they are virtually immobile and may primarily be moved by overall length growth. Such gradual movement could be effectuated by replication, transertion (combined transcription, translation, and insertion of proteins), and actin- (MreB) directed surface synthesis. Differences in the movement and positioning of gene loci between Escherichia coli and Caulobacter crescentus are discussed. We propose that a low diffusion coefficient could explain the linear positioning of genes in the nucleoid and that differential transcriptional activity could induce different mobilities between either replichi-ores (E. coli) or daughter strands (C. crescentus). The transertion process, possibly in combination with MreB cytoskeletal tracks, could overcome the compaction forces and move specific chromosomal regions and the nucleoid as a whole without invoking a dedicated mechanism.

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1. Introduction

In spite of our detailed knowledge of the enzymology of DNA replication (Kornberg and Baker, 1992) and of the topology of gene expression (Willenbrock and Ussery, 2004; Peter et al., 2004), we do not understand how, on a larger scale, bacterial DNA is organized within cell or nucleoid. Also, in the process of segregation, we hardly know what force(s) move the newly replicated DNA strands faithfully to the prospective daughter cells.

Many groups in the field of bacterial chromosome segregation assume the involvement of a dedicated, “mitotic-like” mechanism as “the process is far too important to leave to chance” (Gitai et al., 2005a). This view is based on the observation of a rapid movement of oriC-GFP spots (Gordon et al., 1997), and on the possible involvement of actin-related proteins (MreB; Kruse et al., 2006) such as those that segregate R1 plasmids (ParM; Garner et al., 2004).

We will discuss the various observations on the dynamics of DNA within the nucleoids of Escherichia coli, Bacillus subtilis, and Caulobacter crescentus. Operationally, we distinguish three stages: (i) initial separation of origins; (ii) separation and positioning of replicated DNA regions; (iii) final separation of the nucleoid. We will first review DNA organization from global microscopic observations and then discuss the physical consequences of macromolecular crowding for chromosome compaction because we consider these issues as the basis for our understanding of DNA segregation.

2. Microscopic observations

An important feature of the structural organization of the bacterial chromosome, we believe, is phase separation of DNA and cytoplasm. An indication for such a phase separation was already present in the time-lapse images of
Mason and Powelson (1956) that showed discrete central structures increasing in size and dividing hand in hand with growth and division of an *E. coli* cell. Due to the limited optical resolution, however, light microscopy cannot resolve how abrupt the phase separation is; in other words what the nature is of the demarcation or surface of the nucleoid. For instance, we do not know to what extent loops of DNA can penetrate into the cytoplasm or whether poly-ribosomal clusters can penetrate the nucleoid surface.

The higher resolution of the electron microscope is of little help as long as the cells are subjected to permeabilization (fixation) and to dehydration which cause leakage of cell components and integral shrinkage, respectively. Nevertheless, it has been claimed that the “coralline” shape of the *E. coli* nucleoid prepared by cryofixation freeze substitution, reflects the dynamic nature of bacterial DNA that is in the process of transcription, translation, and replication (Kellenberger, 1991). However, in this freeze-substitution procedure the amorphous ice in the cells is replaced by acetone containing chemical fixatives (OsO₄, glutaraldehyde, and/or uranyl acetate) at −90°C. Although this substitution occurs at low temperature it still represents a combined dehydration/fixation step, especially when the specimen is brought to room temperature for embedding and thin sectioning. The occurrence of fixation and dehydration artefacts (leakage and shrinkage) are thus not excluded in such freeze-substituted preparations.

At present, perhaps the best electron microscope technique for viewing bacterial structures in their hydrated state, seems to be the procedure of rapid freezing and cryo-electron microscopy of vitreous sections cut at −140°C (Eltsov and Dubochet, 2005). Typical sections obtained from *Deinococcus radiodurans* cells in exponential growth again show a ribosome-free region of different texture, indicative of a structural phase separation. However, the demarcation was not clear-cut and the question about the nature of the nucleoid surface has thus remained.

### 3. Physico-chemical considerations of DNA compaction

When the peptidoglycan layer of *E. coli* is digested with lysozyme and the cell is subsequently lysed either with detergent or by osmotic shock (Cunha et al., 2001a), DNA can be observed to “explode” out of the cell (Odijk, 2000). The liberated nucleoids appear to have expanded 100-fold in volume (Cunha et al., 2005). This explosion suggests that DNA is packed in the cell like a spring in a box. What factors or forces cause its compaction in the living cell?

Important properties that determine DNA compaction are the degree of supercoiling, the concentration and nature of nucleoid-associated proteins (Luijsterburg et al., 2006) and the concentration of soluble cytoplasmic proteins. A deficit in the linking number of bacterial DNA caused by topoisomerases results in a torsional stress that is relieved by the formation of negative supercoils (Holmes and Cozzarelli, 2000). Taking into consideration the ionic strength and the persistence length of the supercoiled DNA, it has been derived that the supercoils form branches (see discussion in Cunha et al., 2001b). Collisions between the branched supercoil segments, depicted in Fig. 1A, represent an expansion force that has to be overcome by compaction forces (see below). Binding of nucleoid-associated proteins influences the diameter of supercoils causing bending and cross-linking of the DNA. In addition, these proteins can induce or repress the global transcriptional activity of chromosome regions (see Luijsterburg et al., 2006). Proteins like H-NS (Dame, 2005) and the MukBEF condensin complex (Hiraga, 1993) have been suggested to constrain the DNA in some 100−200 loops. If interconnected, they could effectively reduce the volume of the DNA supercoil (Hardy et al., 2004; Thanbichler et al., 2005). However, this does not explain the phase separation or the small volume of the nucleoid.

Zimmerman and Murphy (1996) have emphasized that the supercoiled DNA occurs in the cell with a huge number of macromolecules and they proposed that such a high concentration of macromolecules (about 340 mg/ml of RNA and protein; Zimmerman and Trach, 1991) represents a macromolecular crowding force that could compact the DNA into about 20% of total cell volume (Fig. 1B). They noted that this “mandatory condensation” is primarily dependent on the overall concentration of proteins and relatively independent of physiological conditions such as salts, pH, and growth or energy state of the cells. Indeed, bacterial cells show compact nucleoids as long as they have not lost proteins through leakage (Murphy and Zimmerman, 2001) or aggregation (e.g., by aldehyde fixation).

The possibility that polymer- and salt-induced condensation of DNA (Lerman, 1971) might explain phase separation between cytoplasm and nucleoid in bacteria has been invoked before on the basis of microscopic observations (Valkenburg and Woldringh, 1984; Woldringh and Nanninga, 1985). Subsequently, Odijk (1998) developed a semi-quantitative theory in which a thermodynamic equilibrium state for the DNA and proteins in *E. coli* was derived: he defined the self-avoiding interactions between superhelical DNA segments causing expansion of the DNA (Fig. 1A) and the excluded-volume or depletion interactions between DNA and proteins causing its compaction (Fig. 1B). Applying this depletion theory to an *E. coli* “standard cell” (Woldringh and Nanninga, 1985), Odijk (1998) was able to predict the existence of a phase separation and he could calculate a theoretical nucleoid volume that was similar to the observed volume (Valkenburg and Woldringh, 1984; see for a discussion of the theory Woldringh and Odijk, 1999). In an experimental study (Cunha et al., 2001b), the *in vitro* compaction by polyethylene glycol (PEG) of the isolated nucleoid could be described and interpreted in the light of the depletion theory. It appeared that the experimentally derived value for the free energy of the nucleoid was in agreement with theoretical prediction (Odijk, 1998). This result seems to confirm that the nucleoid is composed of a branched plectonemic supercoil (Fig. 1A), partly relaxed through association with DNA-binding proteins and cross-
Fig. 1. Structure of bacterial DNA outside and within the cell. (A) Due to a deficit in linking number caused by topoisomerases the circular chromosome (contour length of 1580 nm) relaxes into a huge branched plectonemic supercoil (contour length of 630 nm). The supercoil segments have a persistence length of 160 nm and interact with each other causing the structure to expand. (B) Chromosome compaction in the intact cell containing a huge number of soluble proteins. The thermal collisions between DNA and proteins represent a compaction energy that overwhelms the expansion energy of the supercoils in (A). This causes a phase separation between the DNA and the cytoplasm.

Fig. 2. (A) Fluorescence images of a relatively large, dividing cell from a population of *E. coli* SG100/pSG20 growing in rich medium (strain obtained from Andrew Wright, cf. Gordon et al., 1997). Within the regions of the DAPI-stained nucleoids, the cell shows two pairs of oriC-GFP spots in the left half and probably four duplicated pairs in the right half, which appears more advanced in the cell cycle. Note the even distribution of the origins and their segregation in the short axis of the cell (not all are in focus). Separated spots are about 500 nm apart. The presumptive replication patterns of the chromosomes have been indicated below the images. (B) Schematic drawing of the chromosome with two replication forks that have travelled for 10 min. Assuming that the chromosome (contour length 1580 nm) is replicated in 70 min (at 30 °C; Elmore et al., 2005), each fork will have synthesized in 10 min two strands of 113 nm DNA. Each daughter strand (blue and red) will have a length of 226 nm DNA. (C) Schematic drawing of an average half cell from the population in (A) with an estimated nucleoid volume of ~0.35 μm³ and containing 1.5 chromosome equivalents (see Figs. 1 and 8 in Elmore et al., 2005). If the two daughter strands become initially packed into two spheres with the same DNA packing ratio as the whole chromosome (i.e., 1580 nm in ~0.23 μm³), each daughter strand will represent a volume of ~0.03 μm³ or a sphere with a radius of ~190 nm. With an optical resolution of about 250 nm (white circle) and assuming a direct and complete separation of the two strands, duplicated spots (blue and red circles) should be visible after about 10 min of replication. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)
linked by a substantial number of physical entanglements and/or proteins.

In view of the above compaction force, what driving forces are needed to separate replicated DNA regions and to move daughter nucleoids apart? We will consider these questions in the following sections.

4. Initial separation of origins

In spite of the slow and gradual movement of bulk DNA as observed in DAPI-stained nucleoids of growing E. coli cells (van Helvoort and Woldringh, 1994) the nucleoid has generally been considered to represent a highly dynamic structure. It was therefore satisfying that tracking of spots representing oriC-lac operator DNA tagged with GFP-LacI indicated rapid movements of this specific DNA region in time-lapse experiments. The interpretation that the GFP spots moved about 10-fold faster (2–8 nm/s) than the rate of cell elongation (0.5–0.9 nm/s; see below) suggested that the origin was moved by an active, “mitotic-like” process (Gordon et al., 1997; Webb et al., 1997). This conclusion was originally inspired by the proposal that the MukB protein could play an active role in segregation (Hiraga, 1993).

Experiments in which the location of origins in relation to cell length was determined by fluorescence in situ hybridization (FISH) have been interpreted in the light of a dedicated segregation mechanism causing the abrupt separation of duplicated spots (review Gordon and Wright, 2000). FISH spots, however, showed large positional variations. These measurements could therefore either be interpreted in favor of a dynamic migration of the origin independent of cell growth (Niki and Hiraga, 1998), or in favor of a gradual movement in line with cell elongation (Roos et al., 1999, 2001). This latter interpretation led to the proposal that confined diffusional motion in combination with attachments of DNA to the protein-synthesizing machinery (translocation; Norris and Madsen, 1995) could represent a growth dependent mechanism for segregation (Woldringh, 2002).

4.1. Cohesion

In a recent analysis, in which the origin was labeled by FISH in synchronized E. coli cells (Bates and Kleckner, 2005), it was concluded that replicated origins remain localized for 14 min after initiation of DNA replication. This result, according to the authors, confirmed earlier suggestions of “cohesion” by Sunako et al. (2001), that contradicted the interpretation from G(C,Y)FP-labeling and time-lapse experiments (Li et al., 2002, 2003; Elmore et al., 2005; Wang et al., 2005) that origin segregation occurred soon after initiation of DNA replication. The problem all these segregation studies have to solve is that of matching the timing of two events derived from different experimental procedures: (i) the time of spot duplication from image analysis of cells classified by length or from time-lapse experiments; (ii) the time of initiation of DNA replication in the cell cycle as calculated from flow cytometric (Boye et al., 1988) or image cytometric (Huls et al., 1999) measurements. Given the fact that different strains have been grown under different conditions (medium and growth temperature) it is not surprising that there are controversies in the literature regarding “cohesion” (cf. Nanninga et al., 2002).

Cohesion is a very complex mechanism in multichromosomal euakaryotic cells. It ensures the bi-orientation of chromatid pairs and the counteraction of forces exerted by microtubules attached to a single centromere by tying sister chromatids together during the process of replication. The ring-shaped cohesin complexes are opened through proteolysis in the meta- to ana-phase transition (for review: Nasmyth, 2002). As cohesins resemble the MukBEF proteins in E. coli, could cohesion play a similar role there? In this respect it should be realized that in bacteria the chromosome is replicated from a single origin so that replication and segregation coincide, without the need of euakaryotic-like cohesion. Moreover, some co-localization of replicated DNA regions is to be expected on the basis of the optical resolution of the light microscope. In Fig. 2B and C it is illustrated that after 10 min of replication oriC-GFP regions can be expected to be visibly separated if it is assumed that the origins move perpendicular to the optical axis and that replicated daughter strands become immediately fully separated. Note that this ideal situation will always tend to create “cohesion” for a replication period of about 10 min under favourable conditions (14 min; Bates and Kleckner, 2005). In the study of the dynamics of oriC-GFP spots (Elmore et al., 2005) the initial separation distance of spots was found to be on average 300 nm. The optical resolution, however, precludes making a conclusion about the size and speed of the initial separation steps.

In their “extrusion-capture” model for bacterial segregation Lemon and Grossman (2001; see also Li et al., 2002) have proposed that newly replicated origins are released from the replication factory to opposite halves of the cell (“extrusion”), where they are held at specific positions (“capture”), suggesting that replisome activity may drive chromosome separation. However, could it be possible that the replicating daughter strands separate through excluded-volume interactions and do not intermingle? (Dill and Bromberg, 2003). Their physical separation could also become enhanced as newly replicated regions have a different conformation (superhelicity; DNA-binding proteins) and perhaps a higher chance to become transcribed.

4.2. Determination of the diffusion coefficient of oriC-GFP spots

Once duplicated the dynamics of the oriC-GFP spots reflects the continued replication and segregation of the DNA daughter strands. To investigate whether the spot movement represented random Brownian motion in step
with cell elongation, or whether the diffusional motion showed an extra drift indicative of a dedicated mechanism, the duplication and dynamics of 124 oriC-GFP spots in 31 E. coli cells growing in rich medium were analyzed in time-lapse experiments (Elmore et al., 2005). Here the same strain and growth conditions were used as described by Wright and co-workers (Gordon et al., 1997; see Fig. 2A). It appeared that duplicated spots showed a very slow, Brownian movement. In this analysis of spatial fluctuations of oriC-GFP loci (Fig. 3A), the lengthwise position of the spots was corrected for the length increase of the cells. From the resulting mean square displacement plots an effective diffusion coefficient has been derived of \( D = 4.3 \times 10^{-5} \text{µm}^2/\text{s} \) (Elmore et al., 2005). This is a very low value as compared with, for instance, the diffusion coefficient of 7.7 µm²/s for the relatively small GFP protein in the E. coli cytoplasm (Elowitz et al., 1999). A low value for the diffusion coefficient seems in accordance with previous observations that DNA reaction rates are much slower in vivo than in analogous in vitro reactions (Hildebrandt and Cozzarelli, 1995) and that chromosomal regions have limited access to each other (Garcia-Russell et al., 2004).

Although the analysis of spots in living cells was limited by the few data points in the trajectories (~20) it was found that the average step size of spot displacements was 110 nm for 3 min intervals. Furthermore, in no trajectory large steps occurred in a systematic way and there was no indication for an additional drift. From simulations it was derived that a drift of 0.5 nm/s should have been detectable (Elmore et al., 2005). Large steps (>500 nm) were sometimes observed and could have resulted from transcriptional or other DNA activities. In an earlier study of expanded nucleoids isolated from the same E. coli strain a diffusion coefficient of

![Fig. 3. Comparison of spot movements in E. coli (oriC-GFP) and in C. crescentus (oriC-CFP) drawn on scale at 3 and 2 min intervals, respectively. (A) E. coli cell taken from Fig. 2 in Elmore et al. (2005). This cell elongates (black dots) at a rate of 0.5 nm/s (1.5 µm in 54 min; growth temperature 30°C). Originally this cell (cf. Fig. 2A) contained two pairs of spots. Of one pair the overall (arrows) and stepwise displacement (trajectories) has been depicted. In this cell one spot ("A") appeared to remain in the polar region of the nucleoid while the other ("B") moved away; its drift velocity is 0.4 nm/s. (B) C. crescentus cell taken from Fig. 3A in Viollier et al. (2004). While the average rate of spot movement was reported to be ~8 nm/s (time interval 2 min), the overall drift velocity of spot "B" in this particular cell is 1.3 nm/s; in this period of 22 min the cell elongates at ~0.1 nm/s (black dots). Note how the spot seems to move along the periphery of this cell.](image1)

![Fig. 4. Differential gene expression in E. coli and C. crescentus. (A) Bidirectional replication from oriC results in two replichores (red and blue) with opposite replication polarity. Arrows at genes “A” and “B”: arrows indicate direction of transcription. Replicosomes are indicated as square white boxes. The ter regions are drawn as in the scheme of Wang et al. (2005): ter2 and ter4 are separated by 400 kb. This gives a maximal linear distance for supercoiled DNA of about 56 µm. Being roughly 10 times longer than the length of a cell the two regions can easily occur at opposite poles. (B) Replichore segregation in E. coli with replisomes at midcell. Schematic representation and cellular position of leading and lagging strands of both replichores (red and blue) and of unreplicated DNA (gray) according to Wang et al. (2005; cf. their Fig. 4C). (C) Daughter-strand segregation in C. crescentus with positioning of newly replicated DNA regions as in Thanbichler et al. (2005). Leading and lagging strands have been assumed not to intermingle.](image2)
0.12 μm²/s was obtained confined to a region of 600 nm diameter (Cunha et al., 2005). From this study it was concluded that the confined movement of the spots reflects their diffusion within a so-called harmonic well, consisting of the viscoelastic network of the nucleoid. This could also apply to the living E. coli cell in which the DNA is virtually immobile and seems to be only moved by growth dependent processes like replication and transcription.

In C. crescentus (Viollier et al., 2004) the situation seems to be quite different. In the cell of Fig. 3B one of the origins remains in the old pole region where the new stalk is being synthesized, while the other origin moves in a directed way towards the other pole with a drift velocity, in this particular cell, of 1.3 nm/s (1.7 μm in 22 min; Fig. 3B). Note that the overall displacement is not very different from that in the E. coli cell of Fig. 3A, albeit that in the latter cell the movement coincides with cell elongation. The average drift velocity found for the C. crescentus cells was 8 nm/s (Viollier et al., 2004). Assuming that the trajectories in the elongating E. coli cell can be explained by DNA activities like replication and transcription, we wonder whether the observed drift in C. crescentus could also be the result of such activities (see below).

5. Separation and positioning of replicated DNA regions

Many authors consider the nucleoid to be a dynamic and ordered structure (Gitai et al., 2005a; Thanbichler et al., 2005; Bates and Kleckner, 2005). Indeed, on the time scale of a generation, in which segregation takes place and hundreds of genes dispersed over the whole chromosome come to expression, the nucleoid can be considered to be highly dynamic. But how can this notion be reconciled with the low diffusion coefficient that causes DNA segments to be virtually immobile? Presumably, the many enzymes acting on DNA like RNA polymerases, topoisomerases and activities like replication, repair, and recombination act as potential motors that are able to move chromosomal regions during the course of the cell cycle. The low diffusion coefficient, on the other hand, could explain the observation in C. crescentus that duplicated genes occur at subcellular positions that correlate with their linear position on the chromosome (Thanbichler et al., 2005).

Different modes of positioning of genes have recently been described in E. coli and C. crescentus. In a study of Wang et al. (2005) of slowly growing E. coli cells it was observed that oriC is localized in the center of the cell and that the individual replicores (Fig. 4A) of each replicated daughter strand occupy distinct nucleoid positions (Fig. 4B). In contrast, chromosomal regions on the two replicores of C. crescentus have been observed (Thanbichler et al., 2005) to occupy the same subcellular location if they occur on similar distances from the origin (Fig. 4C). This positioning requires that the newly replicated daughter strand that harbors the fast migrating origin (“B” in Fig. 3B) must move past the bulk of unreplicated DNA. In the slowly growing E. coli cells studied by Bates and Kleckner (2005; their Fig. 4) the origin and terminus each occupies a polar position in the newborn cell (visualized by FISH). After its duplication, the origin that has to move past the bulk of unreplicated DNA and has to switch position with the terminus, is slowed down in its motion to the pole. A similar asymmetric movement of the origins was observed by Wang et al. (2005). Apparently, another way of motion applies to the situation in C. crescentus (Fig. 4C) where the origin that passes the bulk of unreplicated DNA moves more rapidly.

In vegetatively growing B. subtilis cells DNA movements may be similar to those in E. coli. However, during the asymmetric process of sporulation a large chromosomal region near oriC becomes oriented towards the cell pole, which resembles the situation in C. crescentus (Errington et al., 2005). This positioning is an example of specific proteins that capture and tether DNA at the polar membrane.

5.1. Differential gene transcription

What could be the basis for the different results in DNA movement obtained in E. coli (Wang et al., 2005; Bates and Kleckner, 2005) and C. crescentus (Viollier et al., 2004)? We have seen that volume exclusion interactions between DNA segments and the cytoplasm lead to phase separation and compaction of the DNA. It can be envisaged that such interactions change dramatically when DNA regions become expressed locally. Depending on the transcriptional activity of a chromosomal domain, large regions of DNA could move faster or slower and occupy different nucleoid locations because of changes in superhelicity and/or constraints due to the transertion process.

In their “strand-specific” model for chromosome segregation Rocha et al. (2003) propose that genes transcribed, for instance, from the leading strand of both parental replicores (Fig. 4A) become again expressed, after their replication, on the daughter strand where the leading strand serves as template (Fig. 4A right panel; genes A and B) and not on the daughter strand where the lagging strand serves as template (Fig. 4A, right panel; genes A’ and B’). They explain this phenomenon by assuming a tendency for genes that are physically linked to maintain the status quo in a “hyperstructure” (Norris et al., 2002). Another or additional explanation could be that for some time the lagging strand cannot serve as template because it is involved in the removal of clamp proteins and in ligation of Okazaki fragments. At the replication fork about 10 Okazaki fragments with an average length of 2000 nucleotides occur (O’Donnell, 2006). With a fork movement of 800 nt/s the leading strand could produce the template for some 20 genes, which are not available for transcription on the lagging strand. This unbalance could induce a differential gene expression between the two genetically identical daughter strands and cause different mobilities of leading and lagging strands of the two replicores as proposed by Sherratt and co-workers (Wang et al., 2005). In Fig. 4B their proposal has been depicted by assuming that the leading strands move faster.
towards the outer nucleoid borders, while the lagging strands remain at the nucleoid center; presently, there is no experimental basis for such assumption (Wang et al., 2005).

In Fig. 4C the leading and lagging strand templates of C. crescentus have been assumed to move together without becoming mixed. It may be speculated that, because of the polar localization of origin and replisome and because of the small size of the cell (Fig. 3B), both replichores are forced to move together. As a consequence one daughter strand remains at the stalk pole while the other passes rapidly the bulk of unreplicated DNA. Can the observed rapid movement (Fig. 3B) only be explained by invoking a dedicated mechanism consisting of a cytoskeleton-based mechanism (Gitai et al., 2005b; see below)? As discussed before (Elmore et al., 2005), the asymmetry in daughter strand movement could be ascribed to differential gene transcription between the newly replicated strands if the genes coding for flagellar proteins, for instance, become preferentially co-expressed from only one of the daughter strands. Transcription of functionally similar genes could then induce a transertion-mediated movement of DNA–RNAP–polyribosome–translocase complexes to the swarmer pole through competition for membrane space (Woldringh, 2002). The observation that flagellar gene transcription is spatially restricted to the swarmer compartment of the predivisional cell (Wu and Newton, 1997) seems to support this idea. Whether transertion of flagellar proteins can furnish the observed drift of one strand (Fig. 3B) and whether the other strand is kept at the stalk pole because it is mainly involved in expression of stalk proteins, remains to be investigated.

6. Final separation of the nucleoid

During the cell cycle the cell doubles its length while all (macro)molecules are duplicated. This overall growth process has been depicted in Fig. 5 (long arrows) as a stretching of the cell as would it be a rubber-like body. This causes structures in the cell to become deformed in the same way as the cell as a whole, keeping the relative distances to each other and the cell poles constant. This so-called affine deformation (see Elmore et al., 2005) can account for most of the movement of a DNA region in the nucleoid. However, it cannot explain how DNA regions like the origin come apart or how the duplicated nucleoids can separate. Although signs of nucleoid separation can sometimes already be seen halfway the replication period (Wang et al., 2005; Bates and Kleckner, 2005), completely separated nucleoids are usually evident some time after termination of DNA replication and FtsZ-ring formation (den Blaauwen et al., 1999). It has been shown that upon inhibition of

![Fig. 5. Schematic representation of affine deformation and duplication of spots (oriC-GFP) and nucleoid. For one spot its confined, Brownian motion is indicated by a random walk. Long arrows indicate stretching of the cell by affine deformation through which all relative distances between structures remain constant. Short double arrows indicate duplication and initial separation of spots and separation of the daughter nucleoids. These events cannot be obtained by affine deformation and are ascribed to an interacting framework of transertion and MreB tracks indicated by peripheral spots and helices.](image-url)
protein synthesis separated nucleoids are again merged together (van Helvoort and Woldringh, 1994; Zaritsky et al., 2006). This phenomenon could be ascribed to the depletion forces that compact the nucleoid as discussed in Section 3. How then can it be envisaged that nucleoids separate?

It seems that for the final segregation of nucleoids an additional, active mechanism is required to counteract the depletion forces. Indeed, it has been observed that cell constriction is accompanied by a movement of the sister nucleoids away from midcell (Bates and Kleckner, 2005). This is supported by the observation that inhibition of constriction caused a delay in nucleoid separation as segregating nucleoids in filaments contained more DNA (Huls et al., 1999). In the next section we discuss the possibility for involvement of the actin-like MreB protein in segregation.

7. How does MreB fit into the DNA segregation process?

When discussing the role of MreB in DNA segregation we keep in mind a suggested difference between E. coli and C. crescentus, i.e., in the former segregation takes place on the leading or lagging strand level, whereas this is on the DNA daughter strand level in the latter (Fig. 4). As speculated above this difference might reside in the initially, polar localization of the C. crescentus replication factory, where only one of the newly replicated strands is displaced.

After its initial discovery in B. subtilis (Jones et al., 2003) as an actin-like helical structure underneath the cytoplasmic membrane evidence emerged to show the importance of MreB in maintaining bacterial shape (Daniel and Errington, 2003; Figge et al., 2004). However, it quickly appeared that impairment of MreB also affects DNA segregation in E. coli (Kruse et al., 2003 and references therein), B. subtilis (see, however, Formstone and Errington, 2005) and C. crescentus (Gitai et al., 2004). In E. coli MreB-depletion resulted in cohered terC regions, whereas oriC as well as terC became dislocated (Kruse et al., 2003). The authors suggested that “actin-like MreB filaments actively move chromosomal DNA in opposite directions in coordination with lateral cell wall growth” (Kruse et al., 2003). So far there is no direct proof for this attractive possibility, though it indicates at least that MreB is involved in the maintenance of a (dynamic) cellular framework that facilitates DNA segregation.

Interestingly, SecA and SecY which are components of the membrane-embedded Sec pathway for protein transport appeared to be arranged in a helical fashion in B. subtilis (Campo et al., 2004). This arrangement appeared independent of the helical structures of MreB and Mbl (an MreB-like protein in B. subtilis; Jones et al., 2003) and to depend on active translation, because inhibition of protein synthesis by chloramphenicol and of transcription by rifampicin affected the localization of SecA (Campo et al., 2004). The coupled transcription, translation, and insertion of nascent membrane and exported proteins (transcription) is likewise inhibited by chloramphenicol and rifampicin (Woldringh et al., 1995; Campo et al., 2004). Thus, the association of RNA polymerase with MreB (Kruse et al., 2006) could indicate that the Sec pathway and MreB helix interact, directly or indirectly. As a speculation it can be envisaged that the MreB and SecA helices cross each other. However, it should be emphasized that for E. coli no helical organization of SecA has been demonstrated so far. Nevertheless this does not exclude the possibility that protein export sites occur in the direct vicinity of an MreB helix. Thus, DNA might be indirectly linked to MreB through transcription-active RNA polymerases (Dworkin and Losick, 2002; Woldringh, 2002; Kruse et al., 2006). It implies that in E. coli (and B. subtilis?) MreB is involved in the second stage of DNA segregation, in as much as replicon-specific transcription takes place.

In the case of C. crescentus a distinction has been made between segregation of origin-proximal and origin-distal DNA regions, the former being MreB dependent (Gitai et al., 2005b). So far, no distinction has been made on the replication level of DNA segregation in C. crescentus. As argued above we feel that DNA segregation in this organism occurs on the DNA daughter-strand level. No clear-cut distinction can be made on whether MreB affects the first and/or the second stage of DNA segregation. In any case MreB seems not responsible for the segregation of origin-distal DNA regions (stage two in C. crescentus (Gitai et al., 2005b)). Whether MreB mediates specific DNA regions in E. coli or B. subtilis is not yet known. It would be likewise of interest to know whether MreB and RNA polymerase interact in C. crescentus. If so, this would suggest a role for transcription of origin-proximal DNA regions in DNA segregation (cf. Dworkin and Losick, 2002; Woldringh, 2002).

8. Concluding remarks

8.1. Do prokaryotes have mitosis?

Mitosis (in eukaryotes) refers to the process whereby duplicated chromatids, tied together by cohesins and compacted by condensins, become separated by a dedicated cytoskeletal apparatus. Although in prokaryotes DNA replication and segregation go hand in hand (Mason and Powelson, 1956; Woldringh and Nanninga, 1985) the question remains whether prokaryotic chromosomes possess a mitotic-like apparatus. Two extreme positions can be envisaged: (i) DNA segregation takes place with a dedicated protein machine of which a cytoskeletal element like MreB is one of the components; (ii) DNA segregation is an inherent property of the growing cell. In that case MreB is required for cell growth and therefore for DNA segregation.

8.2. A bacterial chromosome is not an independent structure

A mitotic chromosome is usually a compact structure that is neither involved in replication, nor in transcription
and as such it is basically inert. By contrast, a bacterial chromosome is continuously being transcribed and DNA segregation is not independent of replication. In case of transversion mRNA (polyribosomes) serves as a structural link between DNA and the cytoplasmic membrane. Transversion takes place at many membrane sites (cf. Campo et al., 2004), suggesting that the chromosome represents an entity that is structurally and functionally related to cytoplasmic growth and envelope synthesis. Since depletion forces are thought to create phase separation between nucleoplasm and cytoplasm it is plausible that all functional activities occur at their interface. Physical forces, we believe, also come into play to create a dynamic interface where DNA regions can show rapid movements when they become replicated or transcribed.

8.3. Chromosomal dynamics

Generally, nucleoid size increases in line with cell elongation (van Helvoort and Woldringh, 1994). The segregating chromosome shows bi-polarity because origin-proximal regions move apart. During the replication process subsequent gene regions are possibly pulled through the replication apparatus. In addition, individual segments such as origin and terminus are able to pass each other in spite of a low diffusion coefficient.

How do these observations pertain to the two extreme positions mentioned above? We propose that transversion (including RNA polymerase) and MreB-directed surface synthesis form a dynamic cellular framework that can shape, extend, and move the nucleoid as a whole, thereby counteracting the depletion forces that compact the DNA. In this view MreB does not take part in a dedicated segregation mechanism.

In E. coli different movements for the bulk of leading and lagging strands are proposed to result from differential gene expression on the two replicores (Fig. 4B). Replicore selection thus affects gene localization. In C. crescentus the different movement of the daughter strands (Fig. 4C) could likewise be caused by a differential transcription of flagellar or stalk genes. Of course this remains to be tested.

When we return to mitosis in eukaryotic cells it is striking that cellular structure is radically reorganized to enable chromosome separation. DNA replication and mitosis are temporally distinct processes. In prokaryotes neither cellular reorganization is required nor are special structures made to provide for DNA segregation (Nanninga, 2001). However, what eukaryotes have in common with prokaryotes is that already during replication and before entering mitosis processes of cohesin and condensation take place that cause the disentanglement or individualization of sister chromatids in the absence of microtubules (see discussion Nasmyth, 2002). In both eu- and prokaryotes replicated DNA becomes already separated and bi-oriented in the absence of a mitotic spindle.

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