Entropy-driven spatial organization of highly confined polymers: Lessons for the bacterial chromosome

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Despite recent progress in visualization experiments, the mechanism underlying chromosome segregation in bacteria still remains elusive. Here we address a basic physical issue associated with bacterial chromosome segregation, namely the spatial organization of highly confined, self-avoiding polymers (of nontrivial topology) in a rod-shaped cell-like geometry. Through computer simulations, we present evidence that, under strong confinement conditions, topologically distinct domains of a polymer complex effectively repel each other to maximize their conformational entropy, suggesting that duplicated circular chromosomes could partition spontaneously. This mechanism not only is able to account for the spatial separation *per se* but also captures the major features of the spatiotemporal organization of the duplicating chromosomes observed in *Escherichia coli* and *Caulobacter crescentus*.

bacterial chromosome segregation | *Caulobacter crescentus* | *Escherichia coli* | polymer physics

Bacteria are arguably the simplest living organisms that are able to reproduce independently. The key step in cellular reproduction consists of the duplication of the genetic material and its subsequent distribution over the offspring. Successful as they are from an evolutionary perspective, bacteria are indeed considered to be highly efficient and accurate in these processes.

What then is the mechanism underlying bacterial chromosome segregation? Perhaps the most influential model so far was proposed by Jacob *et al.* in 1963 (1) in their seminal paper on the replicon model of *Escherichia coli*; if replicating chromosomes are attached to the elongating cell-wall membrane, they can be segregated passively by insertion of membrane material between the attachment points. Although the hypothesis by Jacob *et al.* (1) is intuitive and elegant, it has been undermined by a series of recent visualization experiments. In *Bacillus subtilis* (2) and *Caulobacter crescentus* (3), for example, the measured rate of movement of the replication origin ranges from 0.1 to 0.3 $\mu\text{m}/\text{min}$, almost 10 times larger than the cell elongation rate. In *E. coli*, on the other hand, the movement rate of DNA segments is still a matter of debate [see, for example, Elmore *et al.* (4)]. Nevertheless, all three species, including *E. coli*, show striking dynamics and directed longitudinal movements of chromosome loci during replication; although details vary from organism to organism, typically the duplicated replication origins (*ori*) move toward opposite poles in the cell, and the terminus (*ter*) moves toward the cell center (3, 5–7).

A number of alternative models have been proposed recently to explain the driving force for DNA segregation in bacteria [for recent reviews, see Woldringh (8), Errington *et al.* (9), and Gitai *et al.* (10) and references therein]. A consensus view that seems to emerge from this more recent literature, at least in case of *C. crescentus*, favors the existence of putative “eukaryotic-like” mechanisms (11–13), which would actively push/pull the duplicating bacterial chromosomes apart.

However, Marko and coworkers (14, 15) and, more recently and more specifically, Kleckner and coworkers (7, 16) have drawn attention to the possible role of forces with a purely

physical origin related to the mechanical state of the DNA. A potential source for these forces derives from the fact that DNA as a polymer can both store and release entropy, allowing it to perform mechanical work. Despite this fundamental property, most of the current models for DNA segregation do not take into account the entropy associated with the chromosomal conformations. In fact, we are not aware of any quantitative studies aimed at elucidating these effects. Moreover, the widespread conviction that an active segregation mechanism is in fact a necessity seems to derive in part from the implicit assumption that the duplicated DNA would by itself remain completely intermingled. This assumption, which to our knowledge has never been stated explicitly, still remains to be tested.

In the present work, we therefore address the basic physics behind these biologically relevant questions and explore several aspects of the spatial organization and segregation of strongly confined entropic polymers using computer simulations as well as scaling arguments, whenever possible. Because the duplicating bacterial chromosome can be regarded as an example of a closed polymer of slowly evolving topology, we are then naturally led to the question of what insights polymer physics can provide on the bacterial DNA segregation process. As we shall show below, the combination of confinement and topological constraints that occurs in these systems produces spatially structured equilibrium states of the polymers considered, which bear a striking resemblance to the ones actually found for the bacterial chromosome. Moreover, we also demonstrate how compaction of the bacterial chromosome and conformational entropy alone could direct and facilitate the movement of newly replicated daughter strands of DNA.

Results and Discussion

Role of Entropy in Polymer Segregation in Bulk and in Confinement.

When two polymeric coils consisting of N monomers, each in dilute solution (in a good solvent), are brought together within a distance smaller than their natural size, given by the radius of gyration $R_g \sim N^\nu$ ($\nu \approx 3/5$ is the Flory constant) (17), both coils lose some of their conformational entropy because of the excluded-volume interactions and the chain connectivity and, thus, will resist overlap. This effective entropic repulsion between two chains was first characterized by Flory and Krighbaum in 1950 (18), who by using a self-consistent field theory proposed that, when the two chains completely overlap, the free energy cost ($\mathcal{F}$) of overlap scales as $\mathcal{F}_{\text{Flory}} \sim N^{1/5} k_B T \gg k_B T$,[‡] thus predicting that long coils should behave effectively as mutually impenetrable hard spheres. Much later, however, based on the results of des Cloizeaux (19) and Daoud *et al.* (20), Grosberg *et al.* (21) showed with scaling arguments that in fact $\mathcal{F} \sim k_B T$ and thus is both weak and independent of the chain length N in the

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[‡] $1 k_B T \approx 4 \times 10^{-21} \text{ J} = 10^{-24} \text{ kcal}$ is a typical scale of thermal energy at room temperature.

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Fig. 1. Blob approach to segregation of two partly intermingled chains in a cylindrical pore. $R_{||} \sim ND^{1-(1/\nu)}$ is the longitudinal size of a single chain. The free energy of overlap F_{blob} scales as $k_B T R_{\text{overlap}}/D$.

scaling regime, which was later confirmed by renormalization group calculations (22) and numerical simulations (refs. 23 and 24; for a review, see ref. 25). Indeed, DNA molecules in bulk can easily intermingle.

In the presence of confinement, however, the effective repulsion can increase dramatically. To see this effect, consider two linear chains with excluded-volume interactions, which are initially intermingled and confined in an infinitely long cylinder of diameter $D \ll R_g$ (Fig. 1). The free energy cost of intermingling in this case can be estimated using de Gennes' "blob" model (17). Because the total number of blobs per chain is of order $D^{-(1/\nu)}N$, the overlapping free energy scales as

$$F_{\text{blob}} \sim D^{-\frac{1}{\nu}} N k_B T, \quad [1]$$

where we assumed that the individual blob–blob overlap cost is of order $k_B T$ based on the abovementioned results by Grosberg *et al.* (21). The repulsion between two chains in confinement is thus predicted to be very strong, being proportional to the chain length N , in stark contrast to the almost negligibly weak one in bulk. Because of this entropic driving force, the two chains slide away from each other at a characteristic rate $v^* \sim k_B T/DN$ over a distance comparable with the size of an unperturbed chain $l^* \sim D^{1-(1/\nu)}N$, giving the timescale of segregation as $\tau_{\text{seg}} \sim l^*/v^* \sim N^2 D^{2-(1/\nu)}$. Note that this τ_{seg} is much smaller (faster) than the typical timescale, $\tau_{\text{rept}} \sim N^3 D^{2-(2/\nu)}$, for a single chain in the same cylindrical confinement to diffuse over a distance of its own length by "reptation-like" motion.

Demixing of Chains in a Closed Geometry of Confinement and the Effect of Chain Topology. Although the above analysis in an open cylinder clearly demonstrates the effect of lateral confinement on chain segregation, the biologically more relevant geometry is the one in which also the longitudinal direction is confined as in a generically rod-shaped bacterium. We thus performed Monte Carlo simulations to find the typical equilibrium configuration of two linear chains in a closed cylinder of length L capped by two hemispheres of diameter D , where the aspect ratio $(L + D)/D$ of 6 and the volume fraction of the chain $\approx 10\%$ are chosen to be comparable with the situation in slowly growing *E. coli* near the onset of cell division (26).⁵ The results depicted in Fig. 2A show that the majority of the chains in our limited sampling of initial conditions end up being fully demixed (for further details on the Monte Carlo simulations, see *Supporting Text*, which is published as supporting information on the PNAS web site).

The bacterial chromosome, however, is not a linear polymer but a circular one. In bulk solutions, it is known that there is an additional topological repulsion between ring polymers due to the nonconcatenation condition (27). On the other hand, the circular topology might only have a secondary effect in the presence of strong confinement. Simulations of two ring poly-

Fig. 2. Segregation of two chains of various topology in a rod-shaped box of length L_{tot} (normalized center-to-center distance between two chains vs. Monte Carlo sweeps). (A) Linear. (B) Ring. (C) Branched. In all three cases, we used a box of the same dimensions and repeated each simulation for 10 different initial configurations. The aspect ratio of each graph has been chosen such that the "timing" of the segregation can be compared.

mers, in the same setting as the linear ones, indicate that, if anything, two ring polymers demix more readily than linear ones do in confinement (Fig. 2B).

Finally, the bacterial chromosome is also supercoiled, due to the undertwisting of the DNA-helix. This supercoiling causes the structure of the chromosome to resemble a random branching network composed of plectonemes with a length of ≈ 300 nm. We therefore also considered the mixing behavior of pairs of branched polymers under confinement. The results shown in Fig. 2C suggest that the nontrivial topology of the branched polymers increases the tendency to demix even further.

Although clearly not in any way a systematic study of the effects discussed above, which at the present state-of-the-art would require a major commitment in computational resources, we contend that the trends clearly reveal that both confinement and chain topology of the bacterial chromosome impact the segregation behavior.

Spatial Organization of Polymers Under Confinement. As mentioned above, during the cell cycle, a circular bacterial chromosome undergoes a series of topological and geometrical changes (Fig. 3A). Given the observations on the directed movements of duplicating bacterial chromosome, it is of interest to understand the typical conformation of a highly confined chain in a rod-shaped box, where the chain topology and volume fraction mimic that of the bacterial chromosome in different stages of DNA replication.

The results from our simulations, summarized in Fig. 3B and C, suggest how conformational entropy might lead to an evolution of spatiotemporal organization of duplicating circular chain in an elongating rod-shaped cell: In the early stage of replication, the replication "bubble" is small (asymmetric figure- θ) and extruded from the mother strand that has not yet been replicated. At this stage the duplicated *ori* and *ter* are localized near the opposite poles. The reason for this *ori* localization is a depletion effect; when an object whose size is comparable with or larger than the thickness of a polymer is inserted into a volume occupied by a long polymer chain, the chain "expels" this object to gain more entropy. Thus, at the beginning of the cell cycle, the replication bubble formed by the two daughter strands does not mix with the long mother strand. Moreover, as can be seen in Fig. 3B, bipolarity is induced by the geometry of the rod-shaped box, with both the daughter strands tending to occupy a volume at one of the two poles. Similarly, the midpoint of the mother strand (namely, *ter*) will therefore be located, on average, near the other pole. Next, when the circular chromosome is $\approx 50\%$ replicated, the mother strand and the two daughter strands are all approximately equal in length (symmetric figure- θ); i.e., the chain can be divided into three topologically distinct domains of identical

⁵The confinement in our simulations is moderately strong, e.g., the radius of gyration R_g of each linear chain is ≈ 3.6 times larger than the radius of the cylinder $D/2$.

Fig. 3. Chains of nontrivial topology and their typical conformations in strong confinement. (A) A schematic representation of the slowly evolving topology of circular chromosome during replication. (Note: not to be confused with actual polymer conformations.) (B) Results of two stages of the replication process, indicated by the vertical gray bar in A, namely, the asymmetric and symmetric figure-8 conformations, respectively. For each case, a snapshot of a typical conformation is shown (top of each image) and the spatial distribution of the midpoints of the colored segments (blue, red, and gray) around their average position (bottom of each image). (C) The conformation of a figure-eight chain consisting of a total of 2,047 beads (>120 times the width D of the box) in a rod-shaped box of aspect ratio 6. Note the mirror-like, linear ordering of the chain within the rod-shaped geometry, although each bead shows a positional fluctuation comparable with the size of the confinement width D .

size, which repel each other and are (weakly) compartmentalized inside the box.

Perhaps the most interesting case is the chain with a figure-eight topology, which occurs in the final stage of replication of any bacterium having a circular chromosome. Shown in Fig. 3C is the typical conformation of a figure-eight chain, as well as the average positional distribution and fluctuation of each of its monomers along the long axis inside the cylinder. The first important feature of the conformation of this figure-eight chain in confinement is its mirror-like, linear ordering within a rod-shaped box. The *ter* locus (the link of the two rings) is on average located in the center, whereas the two *ori* loci (the “apex” of each ring) are near the two opposite poles. Connecting these three loci, there is a precise linear correspondence between the clock positions of monomers along the chain and their spatial ordering inside the cylinder.

An important observation in Fig. 3C is that the magnitude of the positional fluctuations along the longitudinal axis is large and comparable with the width of the cylinder, i.e., $\langle \delta z_i^2 \rangle^{1/2} = (\langle z_i^2 \rangle - \langle z_i \rangle^2)^{1/2} \approx D$ (where z_i is the position of the i -th monomer, and the brackets $\langle \rangle$ denote the average of what is inside), reminiscent of the blob picture (e.g., Fig. 1).

Simple Model of a Bacterial Chromosome During Replication. So far, we have shown how the interplay between conformational entropy and chain topology can lead a highly confined polymer to adopt a very specific spatial organization within a rod-shaped geometry. In particular, the spontaneous partitioning of two chains of fixed topology and geometry, as well as the linear organization of the figure-eight chain, strongly suggest that conformational entropy *per se* could be a major driving force underlying chromosome segregation. Naturally, we are then led to ask to what extent these physical principles could in fact be responsible for segregation of duplicating bacterial chromosomes.

For this goal, we need a computational model of a duplicating bacterial chromosome, which not only is simple, so that the consequences of underlying assumptions (be they physical and/or biological) remain transparent, but that also reflects the essential features of the *in vivo* situation. We therefore need to address the properties of the nucleoid, the distinct intracellular domain to which the chromosome is confined.

From the segregation point of view, perhaps the most important aspect of the bacterial nucleoid is its compaction.[†] For instance, as mentioned above, supercoiling can change the global shape of a large circular DNA chain to mimic a more ordered, branched tree-like structure. Moreover, nucleoid-associated and structural maintenance of chromosome (SMC) proteins in bacteria (30), as well as molecular crowding (31), could further condense the DNA and stabilize its spatial organization. Indeed, in an important work, Sawitzke and Austin (32) demonstrated how all these factors, DNA condensation, supercoiling, and SMC proteins, are important for chromosome segregation (see also ref 33); they showed that the failure of proper chromosome segregation in *E. coli* due to mutations of the *muk* genes (structural and functional analogues of SMC family of proteins) can be alleviated by a modest increase in chromosomal supercoiling, supporting the idea that the condensation (and the structure) of the chromosome is critical for the efficiency of the bacterial chromosome segregation.

Based on the arguments above, we introduce a “concentric-shell” model of bacterial nucleoid (see Fig. 4). Our idea is to confine the unreplicated mother strands to a rod-shaped “inner-nucleoid” cylinder of radius R_{in} , at least in the early stage of replication (fraction replicated $f \approx 40\%$). The newly synthesized daughter strands, on the other hand, are free to occupy the entire nucleoid volume (of cylindrical radius R_{out}), which is slightly larger than its inner-nucleoid compartment. Because they can gain entropy by avoiding contact with the constrained mother strand, the daughter strands will preferentially favor the peripheral region of the nucleoid. Below, we show that this simple model of the replicating bacterial chromosome captures a number of major features of the experimental data in both *E. coli* and *C. crescentus*. We note that, without the concentric-shell model, simulations often show a significant delay in separation of daughter strands (bipartitioning along the long axis of the cell) in the early stage of replication (see below), indicating its main role in facilitating the onset of segregation.

[†]The nucleoid compaction has been well studied and documented in the past half-century. The pioneers in the field are Mason and Powelson (28), who had already observed the compact nature of bacterial nucleoid in living cell in 1956. For further discussion on other microscopic observations and physicochemical considerations, see a recent review by Woldringh and Nanninga (29).

Fig. 4. The concentric-shell model of replicating nucleoid, which we employ in the simulation of DNA replication.

Application to Chromosome Segregation in *E. coli*. To simulate DNA replication in slowly growing *E. coli*, we first created and equilibrated a circular chain inside a cylindrical tube, where a typical initial conformation at the onset of replication is linear with *ori* and *ter* near the opposite cell poles (see the typical conformation of figure-eight chain above). The chain is “replicated” during the course of our simulation by adding a pair of monomers at the replication forks each time a fixed number of Monte Carlo sweeps of the whole system is completed (i.e., at each fixed “time” interval), mimicking the linear growth in time of the size of the replication bubble. To take into account the growth of the bacterium, we also increased the length of the concentric shells at the same rate that the replication proceeds. Importantly, we also tested the effect of implementing the presence of a “replication factory,” which assumes a colocalization of the bacterial replication machineries (or the two replisomes, where DNA synthesis takes place) (34, 35). In this case, a figure-eight topology of the replication bubble is imposed by forcing the replication forks to be in each other’s proximity.

In Fig. 5, we present two possible segregation scenarios in the elongating cell of *E. coli* obtained from our simulations and compare them with the recent experimental data of Bates and Kleckner (7). Together with a series of snapshots of the replicating circular chain, we show the trajectories of *ori* and *ter* in the presence (Fig. 5 Left) and in the absence (Fig. 5 Right) of a replication factory. Although one can clearly see that the duplicating chromosomes segregate in both cases, the spatial

distribution of the daughter strands differs significantly in the first half of the replication period; when the two replisomes are forced to stay in close proximity to one another, the replicated DNA adopts a more symmetrical, bimodal distribution already much before the half-replicated stage than when no such constraint is imposed. The origin of this early separation of the duplicated *ori* sites can be easily understood from the repulsion between two closed loops, where the replication factory plays the role of a junction (see *Spatial Organization of Polymers Under Confinement*), which is facilitated by the outer shell of our simulated nucleoid. Despite the differences in the degree of symmetry between the two cases, we note that the pair of *ori* “markers” almost always appears on opposite sides of the cell center, as can be seen by the individual paths (dotted lines) in Fig. 5.

On the other hand, *ter* drifted slowly toward the center of the cell in both cases. As a result of the entropy-driven, global movement of both daughter and mother strands, the *ori* and *ter* paths cross each other during replication, where on average the crossing occurs more clearly in the presence of a replication factory than in its absence. Interestingly, the experimental results by Bates and Kleckner (7) stand between these two extreme scenarios (with and without replication factory). Indeed, the DnaX (replisome) markers in their experiment separated around the halfway stage of replication, suggesting that replisomes that are constrained to localize in the cell center during the early phases of replication may facilitate the segregation of daughter strands (see also Fig. 7, which is published as supporting information on the PNAS web site, for the case of transient replication factory confined to the mid-cell).

Application to Chromosome Segregation in *C. crescentus* and the Principal Linear Ordering of Circular Chromosomes in Bacteria. Although there are major cell biological differences between *C. crescentus* and *E. coli*, the physical aspects of their chromosomes such as the size and the topology are very similar. Nevertheless, we incorporated one major experimental observation specific to *C. crescentus* in our simulation, that one of the origins of replication (say, *ori1*) is associated with (36) and stays at the

Fig. 5. Chromosome segregation in *E. coli*, comparing simulation vs. experiment. (Left) A series of typical conformations of a replicating circular chain (mother strand in gray, two daughter strands in red and blue). We also present two sets of segregation pathways (*ori-ter* trajectories during replication) in the presence (Left) and absence (Right) of a replication factory. See the schematic diagrams of the chain topology; the black pentagons represent the replisomes. The dotted lines show the results of 10 individual simulations, and the solid lines show average trajectories. (Center) We juxtapose the simulations with the published data in Bates and Kleckner (7) in an attempt to capture the main features of the experimental observations (note that, to show the average cell growth, we have scaled back the normalized cell lengths presented in ref. 7, using their data). For comparison, we used the fraction-replicated *f* as our “universal clock”.

Fig. 6. Chromosome segregation of *C. crescentus*, comparing simulation (Left) vs. experiment (3) (Right). The simulated trajectories are the average of 26 individual simulation runs (or “cells”); we show the trajectories of nine representative loci (including *ori* and *ter*) on the right-arc of a circular chromosome for the entire duration of replication (up to 99.9%), whereas experimental data are only available for trajectories up to 50% of replication. For clarity, we only show the trajectories from the onset of replication of each locus. A full trajectory of *ter* is shown, however, to emphasize its slow drift from the cell pole to the cell center during replication, in contrast to the fast, directed diffusion of *ori2* in the nucleoid periphery (on the other hand, we kept *ori1* in the volume near the stalked pole until 10–20% of the chain has been replicated). The final spatial organization after the completion of replication bears an interesting resemblance to the mirror-like symmetry of the figure-eight chain (see Fig. 3).

stalked pole during replication, as well as that the two replisomes always remain in the vicinity of each other (3).

In Fig. 6, we show the average (of 26 simulations) trajectories of a number of loci on our “pseudo” chromosome and compare them with the experimental data by Viollier *et al.* (3). The simulation seems to capture the essential features of the spatio-temporal organization of the replicating chromosome in *C. crescentus*: Newly replicated chromosome loci move rapidly and are deposited sequentially at the cell pole to create the mirror symmetry of bacterial chromosome near the end of replication, which is fully consistent with both the principal linear ordering of bacterial chromosome in bacteria [e.g., *C. crescentus* (3), *B. subtilis* (37), and *E. coli* (38, 39)] and with our result on the typical conformation of figure-eight chain in strong confinement presented above.^{††} However, we expect considerable cell-to-cell variations of this linear ordering, because our theoretical predictions indicate that the size of the positional fluctuations of the chain segments is comparable with the width of nucleoid (see the gray area in Fig. 3C). Nevertheless, if specific loci along the chromosome, in particular the *ori* regions, are tethered to the opposite cell poles after release from the replication factory as hypothesized in the “extrusion-capture” model (41), such fluctuations could be reduced (42).

Another issue of interest in *C. crescentus* is the putative role of the actin-like MreB proteins in segregation. In their recent work, Gitai *et al.* (36) showed that addition of a small molecule A22, which targets MreB and perturbs its function, blocks the movement of newly replicated loci near the origin of replication. In contrast, addition of A22 after the origins have segregated did not affect the separation of the origin-distal loci.

In our simulations, we also observed similar failure or significant delay in origin separation when we completely removed the concentric shells. Thus, because the outer shell facilitates the diffusion and bipartitioning of daughter strands in the early stage

of DNA replication, we favor to interpret the role of MreB in chromosome segregation (if any) as effectively comparable with that of the outer shell in our concentric-shell model, and vice versa. Indeed, to produce the results in Fig. 6, it was enough to confine the *ori* region ($\approx 1\%$ of the total chain around *ori*, corresponding to <400 kb of the *C. crescentus* chromosome) in the volume near the stalked pole (36) only until 10–20% of the chain has been replicated, after which point the overall organization established by entropic effects was self-supporting.

Timing and the Concentric-Shell Model. In the two examples above of replicating circular chromosomes, we have used the fraction-replicated, f , as our universal “clock” to compare simulation and data. Although it is difficult to predict theoretically the actual timing of the segregation process, we can still estimate whether the rate of entropy-driven process is consistent with the measured timescale of diffusive motion of DNA segments of bacterial chromosome.

Recently, Woldringh’s group (4, 43) tracked a specifically labeled DNA region using the Lac-O/Lac-I system. The Lac repressor–GFP fusion protein binds to the DNA section where tandem repeats of the Lac operator are inserted, which allowed Woldringh and coworkers to monitor the motion of the DNA. The movement of such a GFP spot has been followed both inside (*in vivo*) and outside (isolated nucleoids) the cell, where the measured effective diffusion constants are $D_{in} \sim 10^{-5} \mu\text{m}^2/\text{s}$ (4) and $D_{out} \sim 10^{-1} \mu\text{m}^2/\text{s}$ (43), respectively.^{‡‡} For the typical lengthscale of $\approx 1 \mu\text{m}$ of a bacterial cell, these diffusion constants imply a timescale of $\tau_{out} \sim 10$ s and $\tau_{in} \sim 10^5$ s, respectively, based on the mean-square displacement relation $\langle x^2 \rangle \sim D_{eff} t^\alpha$ (D_{eff} is the effective diffusion constant) with α being 1 for ordinary diffusion. Because the duration of complete duplication of the *E. coli* chromosome is of order $\tau_{repl} \sim 10^3$ s, there exists a clear separation of timescales $\tau_{out} \ll \tau_{repl} \ll \tau_{in}$. We interpret this result as indicating the following: (i) in the bulk of the relatively stable organization of the *in vivo* nucleoid, the chromosome does not show large-scale diffusion within the timeframe of the cell cycle ($\tau_{repl} \sim 10^3$ s vs. $\tau_{in} \sim 10^5$ s), and (ii) in the nucleoid periphery region, where the DNA is less dense (e.g., in the outer shell of our simulated nucleoid), the DNA dynamics could in principle be fast enough ($\tau_{out} \sim 10$ s) for the daughter strands to find their equilibrium conformations during replication ($\tau_{repl} \sim 10^3$ s), at least in the early stage of replication.

Indeed, our concentric-shell–based simulation scenarios, presented in Figs. 5 and 6, reflect this separation of timescales. In other words, the entropy-driven process can explain the rapid movement of newly replicated loci (e.g., *ori*), without requiring any “mitotic-like” apparatus often discussed in the literature (44, 11) (see below and also the snapshots in Fig. 5), as well as the slow confined diffusion of *ter* in the experimental data. We further remark that, in our concentric-shell model, the effective diffusion rate of the daughter strand can be controlled by the thickness of the outer shell. For example, as the shell becomes thinner than the thickness of the simulated chain, the movement rate decreases, and the *ori-ter* crossing during replication is gradually delayed (see Fig. 8, which is published as supporting information on the PNAS web site). One may think of this effect in terms of increasing “friction” in the outer-shell region.

Future studies should provide more detailed information of the dynamics and organization of DNA inside bacteria. For instance, it would be highly desirable to see the distribution of differentially labeled daughter strands in the early stage of DNA replication within the cell.

^{††}For *E. coli*, however, we note that recent data from Sherratt and coworkers (40) show a tandem-like left–right-arc symmetry of chromosome. Although the significance and the universality of their result is yet to be understood by more studies, extension of our theoretical approaches should help unravel the cause of similar patterns by further considering, for example, asymmetric constraints that the leading and lagging strands of DNA may experience during replication and transcription.

^{‡‡}More recent measurements using fluorescence correlation spectroscopy reveal a faster motion of DNA segments of isolated nucleoid, where the typical timescale is ≈ 1 ms for motion at the scale of $\approx 0.2 \mu\text{m}$ (O. Krichevsky, personal communication).