Loop-extruders alter bacterial chromosome topology to direct entropic forces for segregation

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Abstract

Entropic forces have been argued to allow bacteria to segregate their chromosomes during replication. In many bacterial species, however, specifically evolved mechanisms, such as loop-extruding SMC complexes and the ParAB*S* origin segregation system, are required for chromosome segregation, suggesting that entropic forces alone may be insufficient. The interplay between and the relative contributions of these segregation mechanisms remain unclear. Here, we develop a biophysical model showing that purely entropic forces actually inhibit bacterial chromosome segregation until late stages of the replication cycle. By contrast, we find that loop-extruders loaded at the origins of replication, as found in many bacterial species, alter the effective topology of the chromosome, thereby redirecting and enhancing entropic forces to enable accurate chromosome segregation concurrent with replication. In addition, our model indicates that locally acting origin separation mechanisms, such as the ParAB*S* system, cannot redirect segregation-inhibiting entropic forces. We confirm our predictions with 3D simulations of a replicating polymer: purely entropic forces do not allow for concurrent replication and segregation; entropic forces steered by loop-extruders loaded at the origin lead to robust, global chromosome segregation; and origin separation alone is effective at early replication stages, but does not fully segregate terminal regions. Together, our results illustrate how entropic forces and loop-extrusion play synergistic roles during bacterial chromosome segregation, whereas locally acting mechanisms serve a complementary function.

1 Introduction

Many bacteria contain a single circular chromosome that is simultaneously replicated and segregated during the cell cycle [1, 2, 3]. The physical segregation of these large DNA polymers ($\sim 1\ mm$) must be achieved rapidly, accurately, robustly, and within the tight confinement of the cell ($\sim 1\ \mu m$), to ensure the viability of daughter cells. Various mechanisms, including entropic forces [4], loop-extruding structural maintenance of chromosomes (SMC) complexes [5, 6, 7, 8], and the origin segregating ParAB*S* system [9, 10, 11], are implicated in the bacterial chromosome segregation process. However, the interplay between and the relative importance of these mechanisms are not fully understood. In this study, we investigate when in the replication cycle, where along the chromosome, and how these different components individually and collectively contribute to bacterial chromosome segregation.

The concept of entropic segregation forces arises from the field of polymer physics. Put simply, two spatially confined polymers with excluded volume interactions will segregate because there are more configurations that they can take when they do not overlap [4, 12, 13]; segregated states are more numerous and therefore have higher entropy. Entropic forces can also cause chromosomal loops to segregate, which has been proposed to affect the organization and segregation of eukaryotic [14] and prokaryotic [15] chromosomes. Previous theoretical work has suggested that entropic segregation forces could largely explain bacterial chromosome segregation [4, 16]. These works theoretically focused on the entropic segregation of two fully replicated chromosomes, whereas *in vivo*, segregation is concurrent with replication [17, 18, 3].

A concentric-shell model was needed to achieve concurrent chromosome replication and segregation in simulations; a newly replicated chromosomal strand was given a larger accessible volume, an assumption that may not hold for all bacteria [16]. Hence, while the theoretical concept of entropic segregation has gained traction, in the absence of direct experimental evidence, the role and importance of entropic segregation forces are still subjects of debate. When in the replication cycle are entropic segregation forces effective? And how do they act in conjunction with dedicated chromosome segregation mechanisms?

Two widely spread chromosome segregation mechanisms in prokaryotes are loop-extrusion by SMC complexes [19, 8] and origin segregation by the ParAB*S* system [20, 21]. Loop-extrusion is said to "individualize" newly replicated chromosomes, but it remains unclear how this biophysically promotes spatial segregation [3]. The ParAB*S* system, on the other hand, is better understood, at least in *Caulobacter crescentus*. A protein ParB forms a complex around each replicated origin of replication, and one origin stays tethered near the cell pole, whereas the other follows a gradient of ParA towards the opposite pole [22]. This mechanism can be modelled as a locally acting force that separates origins of replication [23, 24, 25]. In some bacteria, such as *C. crescentus*, the ParAB*S* system is essential for chromosome segregation [11] whereas loop-extrusion is not needed in slow growth conditions [26]. By contrast, *Escherichia coli* and *Streptococcus pneaumoniae* have no ParAB*S* system, and instead rely on loop-extrusion for chromosome segregation [1, 21]. Since either loop-extrusion or origin segregation seems to suffice for these bacteria, these mechanisms might play partially over-lapping roles during bacterial chromosome segregation. On the other hand, in fast growth media, *C. crescentus* [27, 28] and *Bacillus subtilis* [29, 30, 31] depend on both a ParAB*S* system and loop-extrusion to achieve accurate chromosome segregation. Therefore, in all these species the relative contributions of these biophysical mechanisms to chromosome segregation, as well as their interplay with entropic forces, remain unclear.

Here, we start by revisiting the theoretical question of whether entropic forces alone can segregate bacterial chromosomes. We develop a theoretical and a computational model for entropic segregation that takes the geometry and topology of a replicating chromosome into account. We show that, until late replication stages, purely entropic forces cause the alignment of replicated chromosome strands, and therefore actually inhibit chromosome segregation. Remarkably, our models also demonstrate that topological constraints due to origin-proximally loaded loop-extruders transform these segregation-inhibiting entropic forces into "topo-entropic" forces that efficiently drive segregation. By contrast, we find that ParAB*S*-like local forces do not affect the direction of entropic forces, and can be insufficient for global chromosome segregation. Our models explain results from existing knock-out experiments, makes novel, testable predictions, and provides a conceptual framework for understanding how different bacterial chromosome segregation mechanisms operate in unison.

2 Theory

We start by developing a minimal model for how entropic forces act on the global organization of a replicating bacterial chromosomes. Unlike in previous theoretical work [4], we consider the extent of replication as a parameter controlling the geometry of the chromosome and thereby the direction of entropic forces, and we do not assume any constraints on the separation between the replication forks. Using this model, we investigate how entropic forces are affected by loop-extruders, which can alter the effective topology of the chromosome, as well as ParAB*S*-like locally acting *ori* segregation mechanisms.

2.1 Entropy does not segregate partially replicated circular chromosomes

We describe the chromosome as a circular polymer with of N coarse-grained monomers of radius b – larger than the persistence length of DNA – at a time point where R monomers have been replicated. This partially replicated chromosome is confined to a cylinder of diameter d and length L. Chromosomes are highly compressed: without confinement, they expand drastically [34, 35]. In this highly compressed limit ($Nb^{1/\nu} \gg L^{1/\nu}$, where ν is the Flory exponent), we can model chromosomal strands as compressed entropic springs.

We start by decomposing a partially replicated chromosome into the largest possible ring at a given replication stage and a remaining linear segment connecting the replication forks (Fig. 1A, inset in arrow). In the first half of the replication cycle ($R < N/2$) the largest ring is given by a union of one replicated strand and the unreplicated strand of the chromosome, together of length N. In the second half of the replication cycle ($R > N/2$), the largest ring is given by the union of the two replicated strands, together of length $2R$. We seek long-axis configurations that maximize the entropies of the ring and the linear segment, under the constraint that they are conjoint at the replication forks. Initially, we neglect excluded volume interactions between these segments, but subsequently we show that including these interactions does not alter the entropically favored global chromosome configurations.

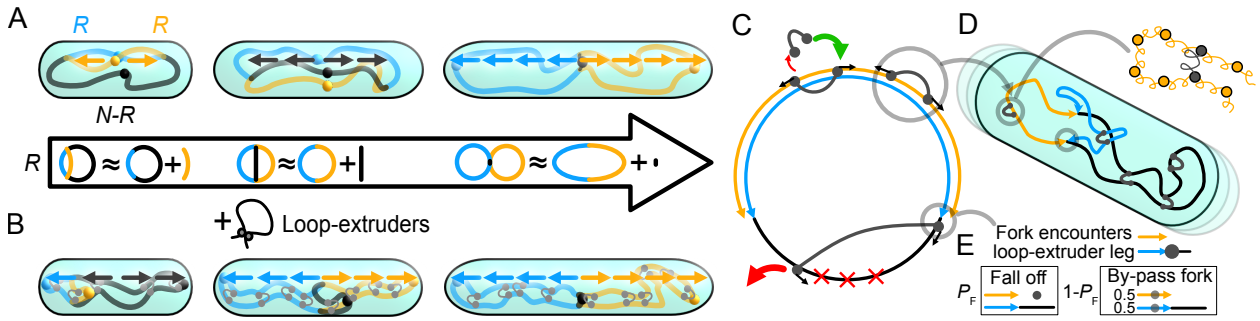

Figure 1: **Schematic of minimal and computational biophysical segregation model A** We consider a circular chromosome of N monomers, with R monomers replicated, confined to cell. At each replication stage (see large white arrow), the partially replicated chromosome is decomposed into the largest possible ring, and a linear segment. Orange and blue lines are newly replicated chromosomal strands, the black line is the unreplicated strand. The entropically favored global configuration is sketched for three distinct replication stages, and the colored arrows indicate how purely entropic forces act. Filled circles mark *ori*s (blue and orange) and the *ter* (black). Until late replication stages, we hence expect fork-segregated left-*ori*-right configurations, and at late replication stages, a segregated *ori-ter-ori* configuration. **B** When bidirectional loop-extruders are loaded at *ori*s, the opposite arms of the chromosome are effectively tied together. The largest ring and the linear segment hence act as effectively linear polymer strands with the *ori*s at their ends. Under these topological constraints, the entropically favored global configurations are sketched at different replication stages and the colored arrows overlaid indicate how topo-entropic forces act. For $R < N/2$, we hence expect an *ori-ori-ter* organization, and for $R \geq N/2$, a segregated *ori-ter-ori* configuration. **C** Bidirectional loop-extrusion is simulated in 1D on a replicating bacterial chromosome [32, 33]. The replication forks move forward independently, creating new sites where loop-extruders can move and be loaded. Loop-extruders are preferably loaded at the *ori*s, and the terminal region has an enhanced off-loading rate. See SI for details. **D** From these 1D simulations, loop-extruder and replication fork positions are extracted at a given time point. These data are used to constrain 3D bead-spring polymer simulations, where loop-extruders act as springs between monomers. The replicating polymer is confined to an exponentially growing cylinder. The integrated 1D and 3D simulations constitute our computational model. **E** When a replication fork encounters a loop-extruder leg, the loop-extruder can either fall off, or by-pass the fork by stepping onto either strand on the other side of the fork.

The configuration entropy of the largest ring is maximized when it extends to its maximum possible length: the length L of the cell. But the ring has no entropically preferred orientation; for any given configuration, shifting the monomer labels gives a different orientation. The entropically preferred orientation of the partially replicated chromosome is hence determined by the remaining linear segment.

In the first half of the replication cycle, once the replicated segment is long enough ($R \gg (d/b)^{1/\nu}$), the linear segment will entropically prefer to extend along the long-axis of the cell. Hence the replication forks are pushed apart, resulting in a "fork-segregated", left-*ori*-right configuration, where the newly replicated chromosome segments lie parallel to each other (Fig. 1A, first cell). For the first half of the cell cycle, purely entropic forces thus inhibit chromosome segregation.

In the second half of the replication cycle, as long as the unreplicated strand is long enough ($N - R \gg (d/b)^{1/\nu}$), the linear segment's entropy is again larger when the replication forks are pushed apart along the long-axis of the cell. The chromosome hence still prefers a fork-segregated left-*ori*-right orientation (Fig 1A, second cell). We thus predict that for most of the cell cycle, purely entropic forces do not enable chromosome segregation.

Once replication is nearly complete ($N - R(d/b)^{1/\nu}$), the limit of two conjoined circular chromosomes is reached. The unreplicated linear segment is now so short that it ties the replication forks together, resulting in an *ori-ter-ori* configuration, and we finally achieve long-axis entropic segregation, as previously argued [4] (Fig 1A, third cell).

So far, excluded volume interactions between the largest ring and the linear segment have been neglected. We now argue that minimizing these interactions by segregating these two segments along the long-axis of the cell does not give rise to higher entropy states. To this end, we decompose the partially replicated chromosome in three linear elements conjoint at the replication forks. Long-axis segregation of two of these elements implies that the replication forks colocalize along the long cell axis. If the third remaining element is long enough ($\gg (d/b)^{1/\nu}$), it forms an effective loop from the replication forks that overlaps with one of the two segregated elements. In such chromosome segregated states four chromosomal strands thus overlap along the long-axis of the cell, and there is an additional entropic cost

for folding the third element into a loop. In fork-segregated states, by contrast, only three strands overlap along the long-axis of the cell, and the third element is in an entropically favored extended state. In summary, fork-segregated states are therefore still entropically preferred in the presence of excluded volume interactions between the largest ring and the linear segment (Fig 1A). Using this model, we thus predict that, until late stages of replication, purely entropic forces drive fork segregation, and thereby inhibit sister chromosome segregation. This implies that bacteria must rely on dedicated mechanisms, such as active loop-extrusion, to start segregation earlier in the cell cycle.

2.2 Loop-extrusion redirects entropic forces enabling segregation during replication

We now consider the effect of bidirectional loop-extruders primarily loaded at the origins of replication, such as condensin in *B. subtilis* [8], *S. pneumoniae* [36], or *C. crescentus* [37]. The loop-extruders tie the opposite arms of the chromosome together, effectively changing the chromosome topology. This implies that, in the presence of enough loop-extruders, the largest chromosomal ring at a given replication stage is effectively linearized, with the loop-extruder loading sites acting as "chain ends". This linearized segment entropically prefers to extend across the long-axis of the cell, so that the loop-extruder loading site is near a cell pole. The replication forks are held together by loop-extruders, and the remaining linear segment is also folded double. For the first half of the replication cycle, the preferred chromosome configuration is then *ori-ori-ter* (Fig. 1B, first cell), and for the second half, *ori-ter-ori* (Fig. 1B, second and third cell). Conceptually, the entropic forces are modified by topological constraints imposed by loop-extruders, into what we will call "topo-entropic" forces. In contrast to purely entropic forces, such topo-entropic forces enable chromosome segregation concurrent with replication.

2.3 Origin segregation competes with entropic forces

Instead of loop-extruders, we can consider a ParAB*S*-inspired localized force that pulls the *ori* to the pole of the cell and breaks the rotational symmetry of the largest ring (Fig. 1A). Such a force makes unreplicated chromosomes favor an *ori-ter* configuration. However, once replication begins without loop-extruders, it is still entropically favorable to stretch out the linear segment connecting the replication forks. In this case, we thus expect a competition between locally applied and entropic forces; whereas forces separating the origins promote sister chromosome segregation, entropy promotes fork segregation and alignment of the replicated chromosomal strands.

In summary, we have constructed a minimal biophysical model showing that, unlike previously proposed [4], entropic forces inhibit rather than promote bacterial chromosome segregation for most of the replication cycle. Importantly, our model instead ascribes concurrent replication and segregation to how loop-extruders redirect entropic forces by changing the effective chain topology. Finally, our model further indicates that locally acting forces, such as ParAB*S*(-like) mechanisms, do not prevent entropically driven fork segregation.

3 Computational model

To test our predictions, we construct a simulation scheme for loop-extrusion on a replicating bacterial chromosome, by adapting a previously published algorithm for a single bacterial chromosome [32, 33], with system parameters modelled after *C. crescentus* [38, 39, 40, 41] (SI Appendix). Briefly, the 1D simulations for loop-extrusion are set to track the positions of independently moving replication forks (Fig. 1C), and the loop-extruder and fork positions are then used to constrain 3D bead-spring polymer molecular dynamics simulations (Fig. 1D). When a loop-extruder leg encounters a replication fork, it either falls off with a probability P_{F}, or steps to either DNA strand on the other side of the fork with equal probabilities (Fig. 1E). Similar rules are defined for cases where loop-extruders overtake replication forks (Fig. S1C). We find that increasing P_{F} slightly improves segregation, but does not qualitatively impact 3D chromosome configurations (Fig. S2), and hence a value of $P_{\mathrm{F}} = 0$ is used unless otherwise stated. Similarly, the number of loop-extruders does not qualitatively affect our results over a broad range of values (SI Appendix, Fig. S3). The replicating chromosome is confined to an exponentially growing cylinder, and we add origin-pulling forces to qualitatively model the ParAB*S* system. This simulation model allows us to test how different mechanisms affect the dynamics of replicating chromosomes individually and together.

3.1 Simulations without loop-extrusion converge to fork-segregated states

The efficiency of chromosome segregation depends on two factors: the directions of the relevant forces, which determine the steady-state configuration a partially replicated chromosome would relax to given enough time; and the speed at which the system relaxes. To test our predictions for the directions of entropic forces with or without loop-extrusion, we start by investigating steady-state simulations with a given replicated length R. We initialize simulations with

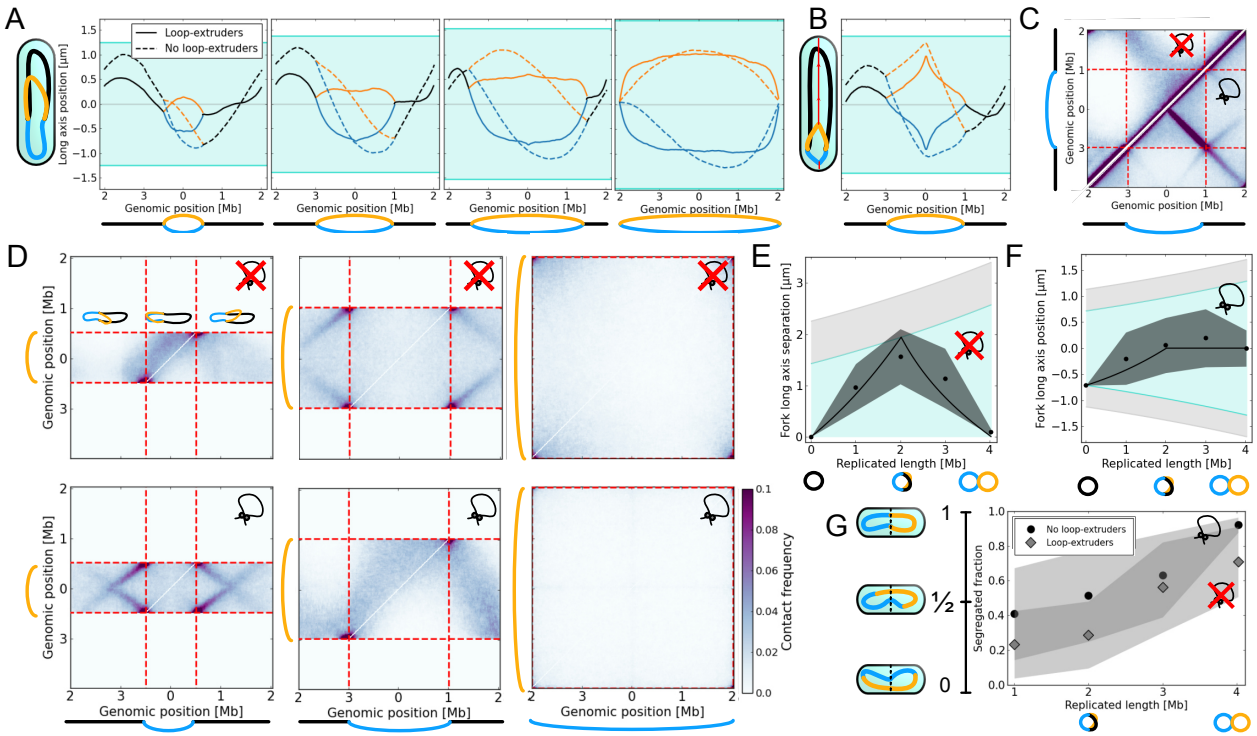

Figure 2: **Steady-state simulations of chromosome organization at different replication stages A** Steady-state mean long-axis positions of chromosomal regions as a function of genomic position when $R = 100$, 200, 300, or 402, for a total chromosome length of $N = 404$. Without loop-extruders, replication forks are pushed apart, whereas with loop-extruders, they occupy the same mean long-axis position. **B** When *ori*-pulling forces are added in replication stage $R = 200$, steady-state configurations without loop-extruders still exhibit strong fork segregation, even though origin-proximal regions segregate. With loop-extrusion, *ori*-proximal regions are better segregated. See Fig. S5 for other replication stages. **C** Intrachromosomal contact maps at $R = 200$ without loop-extruders (top-left) show that the chromosomes arms fold starting from a point near mid-arm of the chromosome, consistent with a fork-segregated left-*ori*-right orientation of the chromosome. With loop-extruders (bottom-right), the arms fold starting from the origin of replication, consistent with an *ori-ter* organization. The weaker lines from the replication forks arise from the unreplicated chromosome segment folding towards the origin of replication. Color bar in Subfigure D. **D** Inter-chromosomal contact maps do not show significant chromosome segregation until $R \approx N$ without loop-extruders (top row, $R = 100$, 200, 402). With loop-extrusion (bottom row), inter-chromosomal contacts are diminished implying better segregation. Inset in top-left contact map illustrates which chromosome configuration should give contacts for each section of the map. **E** Steady-state simulation data for the average long-axis separation between replication forks without loop-extruders. Black line gives a prediction calculated by assuming that the distance between the forks scales with the shortest genomic length between them, and that the maximum distance between forks is given by $L - d$. The turquoise area indicates $L - d$ as a function of replication stage, and the gray area indicates L. **F** Steady-state simulation data for the mean long-axis position of the replication forks with loop-extruders. The distance between *ori* 1 and the replication forks is predicted to scale with $R/2$ for $R < N/2$, after which the forks should stay at mid-cell. **G** The segregated fraction is defined as the fraction of replicated monomers in the correct cell half minus the fraction of replicated monomers in the incorrect cell half. In steady-state simulations, loop-extrusion gives better segregation at all replication stages.

unsegregated, overlapping *ori-ter* configurations. Simulations are run long enough that the mean long-axis positions of various loci converge to steady-state values (Fig. S4). Since the two replicated strands are identical, averaging over sampled configurations yields the same statistics for both replicated strands, as well as for both replication forks. We hence orient all chromosome configurations to break these symmetries (Materials and Methods). This orientation allows us to look for indicators of chromosome and fork segregation in the data.

Mean long-axis positions of monomers show that, without loop-extruders, for $N/4 \leq R < N$, the replication forks are on average in different cell halves (Fig. 2A). The replication forks are hence clearly separated along the long-axis of the cell, and the replicated chromosomal strands are mostly aligned in slightly shifted left-*ori*-right configurations.

By contrast, with loop-extruders the chromosomes are in *ori-ori-ter* ($R < N/2$) or *ori-ter-ori* (R>N/2) configurations, as predicted by our minimal model. Furthermore, alignment of the chromosomal arms implies that the replication forks coincide at roughly the same long-axis coordinate, consistent with experimental evidence that replication forks colocalize in *C. crescentus* [42]. Addition of local origin-pulling forces does not significantly alter fork positioning in our simulations, although *ori*-proximal regions are pulled to opposite poles of the cell (Fig. 2B, Fig. S5). This indicates that, in the absence of loop-extruders, entropic forces cause fork segregation, even if locally acting mechanisms constrain the origins of replication. By contrast, loop-extruder steered topo-entropic forces are sufficient to drive segregation with or without an *ori*-pulling mechanism.

The preferred chromosome orientations can also be seen from simulated contact maps, which measure how frequently two genomic regions are spatially close to each other. The cis-contact maps of individual chromosomes (Fig. 2C, Fig. S6) without loop-extrusion show an off-diagonal line of enhanced contacts starting from mid-arm of the chromosome, indicative of a left-*ori*-right organization. With loop-extruders, by contrast, the off-diagonal line starts from the origin of replication, indicative of an *ori-ter* configuration. We also find that trans-contact maps show less contacts between replicated chromosomal strands with loop-extruders (Fig. 2D, Fig. S6), further illustrating how loop-extrusion enhances segregation.

To better understand fork segregation in the absence of loop-extruders, we analyze the mean long-axis separation between replication forks. We find that the fork separation can be predicted by presuming that the polymer strands are all equally compressed along the long-axis of the cell (SI Appendix, Fig. 2E). In the presence of loop-extruders, a similar argument can be used to predict the average long-axis separation between replication forks and the *ori* (Fig. 2F). Our findings indicate that, as replication progresses in the absence of loop-extruders, the forks approach each other as the unreplicated segment between them shrinks, so that there is a smooth transition from fork-segregated to chromosome-segregated states. With loop-extruders, on the other hand, we expect a smooth transition towards segregated states half-way through replication.

To quantify the long-axis segregation of the chromosomes, we calculate the fraction of monomers segregated to opposite cell halves (Fig. 2G). For all simulated values of R, we find that the the segregated fraction at steady-state is larger in the presence of loop-extruders . Strikingly, for fully replicated chromosomes with loop-extruders, near-perfect segregation is achieved (mean 0.92, standard deviation 0.02), much better than without loop-extruders (mean 0.71, standard deviation 0.20). This effect could be due to repulsion between chromosomal loops [14] caused by off-target loop-extruder loading. This indicates that even if purely entropic forces can segregate fully replicated chromosomes, loop-extruders additionally enhance the segregation of terminal regions (Fig. 2A, D), leading to more reliable segregation.

Together, these results show that for most of the replication process, purely entropic forces push chromosomes towards a fork-segregated left-*ori*-right configuration, and hence inhibit segregation, whereas topo-entropic forces directed by loop-extruders enable chromosomes to segregate both earlier and more efficiently. Once replication is nearly complete, purely entropic forces do start to drive long-axis segregation, but loop-extrusion still reduces overlap between sister chromosomes.

3.2 Loop-extrusion is sufficient for simultaneous segregation and replication

Our simulations at fixed replication stages indicate the direction of (topo-)entropic forces on partially replicated chromosomes, but it is unclear how these entropic forces affect the dynamics of rapidly replicating chromosomes. We now test whether chromosomes undergoing dynamic replication in growing cells, where the polymer does not have time to fully relax, will display similar global organization as in the converged steady-state simulations. All simulations are initialized with a single chromosome in an *ori-ter* orientation, since this is the alignment of a *C. crescentus* chromosome at the start of replication. The time-scale of the simulations is set such that the diffusivity of the origin of replication resembles experimental data [43] (SI Appendix, Fig. S7).

Strikingly, animations from our simulations readily show that simulated chromosomes do not segregate without loop-extruders (Fig. 3A, Supplementary Video 1). Simulations with loop-extruders, by contrast, show clear transitions to segregated states roughly half-way through replication (Fig. 3B, Supplementary Video 2). This difference is striking: we find that the segregated fraction plateaus at roughly 0.2 in the absence of loop-extruders, but reaches values of 0.7 with loop-extruders (Fig. 3B). This suggests that even in dynamically replicating systems in growing cells, purely entropic forces do not allow for simultaneous replication and segregation, whereas topo-entropic forces do.

Despite starting from an *ori-ter* configuration before replication, chromosomes without loop-extruders rotate towards fork-segregated states during dynamic simulations, as can be confirmed from the average long-axis positions of monomers (Fig. 3C). With loop-extruders, on the other hand, the chromosomes maintain clear *ori-ter* configurations throughout the replication cycle, as seen in *C. crescentus* [18]. This suggests that loop-extrusion ensures that replicating chromosomes maintain their orientation.

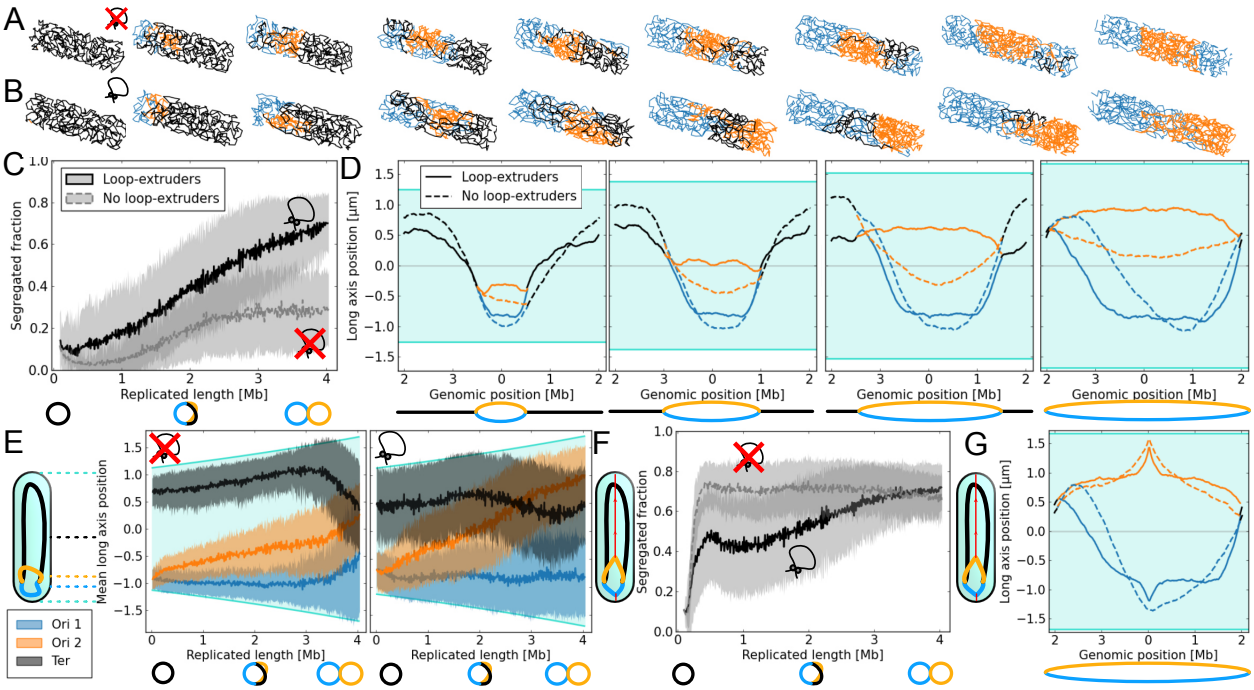

Figure 3: **Dynamic simulations of chromosome organization at different replication stages A** Snapshots from simulations of a replicating chromosome without loop-extruders or *ori*-pulling forces, with replication progressing from left to right. Orange and blue lines are newly replicated chromosomal strands, the black line is the unreplicated strand. **B** Snapshots from simulations with loop-extruders but no *ori*-pulling forces. Roughly half-way through replication, the replicated strands start to segregate along the long-axis of the cell. **C** The segregated fraction, defined as the fraction of replicated monomers in the correct cell half minus the fraction of replicated monomers in the incorrect cell half. In dynamic simulations, the segregated fraction increases at a much faster rate in the presence of loop-extruders as compared to without loop-extruders. **D** Mean long-axis positions of chromosomal regions as a function of genomic position from dynamic simulation at different replication stages (as in Fig. 2B). Despite starting from an *ori-ter* organization, without loop-extruders dynamic simulations exhibit fork segregation. Regions around the replication forks appear intermingled. With loop-extruders, segregation is more robust. **E** Mean long-axis position of genomic regions *ori* 1, *ori* 2, and *ter* across the replication cycle. Without loop-extruders, the origins of replication follow similar paths towards mid-cell, consistent with a transition towards a fork-segregated, left-*ori*-right organization. With loop-extruders, half-way through replication we observe the terminal region move towards mid-cell, indicating the predicted transition from an *ori-ori-ter* to an *ori-ter-ori* configuration. **F** Same as C with *ori*-pulling forces that tether one copy of the *ori* in place and pull the other across the cell. **G** Mean long-axis positions of chromosomal regions at the end of replication with origin-pulling forces, showing that without origin-proximal regions are well segregated while terminal regions appear unsegregated.

Tracking the *ori*s and the *ter* over time reveals that, without loop-extruders, both origins of replication slowly move towards mid-cell, as expected for left-*ori*-right configurations (Fig. 3E). By contrast, in the presence of loop-extruders, the origins of replication separate at a steady rate, as expected if their mean separation is proportional to the genomic distance between them. The terminal region starts to move towards mid-cell when $R \approx N/2$, corresponding to the predicted transition from an *ori-ori-ter* configuration to an *ori-ter-ori* configuration. Overall, our results show that even if replication proceeds rapidly compared to the relaxation of the chromosome, topo-entropic forces directed by loop-extruders drive effective chromosome segregation concurrent with replication.

3.3 Origin-pulling alone does not segregate terminal regions

Several species, such as *C. crescentus*, rely on the ParAB*S* system during chromosome segregation [11]. We therefore test whether locally applied *ori*-pulling forces without loop-extrusion are sufficient for segregation in our dynamic simulations. We find that early on in the replication process, pulling origins apart allows for faster segregation with or without loop-extruders (Fig. 3A, F; Supplementary Videos S3, S4). However, at later replication stages, the segregated fraction of monomers decreases in the absence of loop-extruders, suggesting that forces exerted at the *ori*s do not

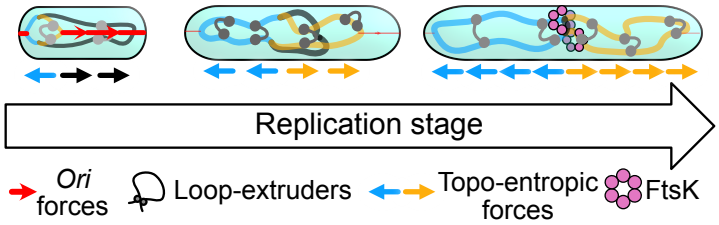

Figure 4: **Schematic model of the relative contributions of different bacterial chromosome segregation mechanisms.** At initial replication states, *ori* segregation enables fast separation of newly replicated regions. Around half-way through replication, topo-entropic forces give the most important contribution. Finally, near the end stages of replication, purely entropic forces start to drive chromosome segregation, and factors such as loop-extrusion and FtsK help resolve terminal regions.

propagate all the way to the terminal regions. This is also visible in the mean long-axis positions of the chromosomal regions (Fig. 3G): although most genomic regions are clearly segregated to opposite cell halves without loop-extrusion, *ter*-proximal regions are intermingled. We hence find that ParAB*S*-like forces alone can be sufficient to maintain chromosome orientation and to largely segregate bacterial chromosomes, but loop-extruders are still needed to segregate terminal regions. In the presence of loop-extruders, segregation is equally accurate with or without origin-pulling.

4 Discussion

In this work we have developed a simple biophysical model and detailed simulations to conceptually understand how distinct bacterial chromosome segregation mechanisms work in unison, when during the replication process they are effective, and whether they allow for global or local segregation (Fig. 4). Our theory reveals that purely entropic forces cause partially replicated circular chromosomes to adopt unsegregated left-*ori*-right configurations. In accordance, our simulations without loop-extrusion did not exhibit segregation until very late replication stages. Our findings contrast previous simulation results [4], where the authors used a concentric shell model and constraints on the distance between replication forks to achieve concurrent replication and segregation. Unlike this previous model, our work explains why mechanisms such as loop-extrusion or the ParAB*S* system are necessary to achieve concurrent chromosome replication and segregation: they are needed to overcome segregation-inhibiting entropic forces during replication. Finally, our model elucidates how loop-extrusion physically enhances segregation: topological constraints caused by loop-extruders turn segregation-inhibiting entropic forces into segregation-driving topo-entropic forces.

Our simulation results further indicate that, whereas locally acting origin segregation forces are an effective segregation mechanism at early replication stages, they do not lead to effective segregation of the terminal regions without loop-extruders, as seen in *B. subtilis* [6]. ParAB*S*(-like) systems hence need to be complimented by other mechanisms, such as SMC-mediated topo-entropic forces, repulsion between SMC-loops [14], or the highly conserved FtsK translocase that segregates terminal regions before cell division [44]. Thus our model can explain why simultaneously inhibiting FtsK and loop-extrusion has been found to be lethal in *C. crescentus* [45] and *B. subtilis* [46]. Additionally, at late replication stages even purely entropic segregation forces become effective, especially if segregation has already been initiated [47], suggesting that when loop-extrusion is inhibited in species such as *C. crescentus* in slow growth conditions, origin segregation could be responsible for initiation, and entropic forces together with FtsK for completion of segregation. Loop-extrusion directed topo-entropic forces, by contrast, appear to facilitate chromosome segregation globally, and can be sufficient without origin segregation forces, as observed in *S. pneumoniae* [21].

Our work gives several additional experimentally testable predictions: first, in the absence of loop-extruders loaded at the origin, replication fork separation should be observable; second, as the "ends" of effective linear segments, loop-extruder loading sites should be the first loci to segregate after replication; and third, in systems with sufficiently efficient loop-extrusion and an origin segregation system, loop-extrusion might be sufficient for chromosome segregation, whereas locally acting origin segregation forces should always be complemented by a mechanism for terminus-segregation. Further work could also explore whether entropic fork segregation plays a role in establishing left-*ori*-right chromosome states in some bacteria, such as in *E. coli* [48] and transiently in *B. subtilis* [49].

Our work also provides insight into possible mechanisms of loop-extrusion in bacteria. In our model, we employed nontopological loop-extruderscapable of by-passing obstacles [33, 50, 51], as opposed to topological loop-extruders that trap a chromosomal strand in a ring [52]. When we repeated our simulations with topological loop-extruders, we found that chromosomal segregation was drastically inhibited (SI Appendix, Fig. S8). This suggests one possible reason why nontopological loop-extrusion might be preferred by bacteria.

The physical principles we have elucidated — on how entropy, loop-extrusion, and locally acting forces act together to segregate and organize bacterial chromosomes — could be used to design segregation mechanisms for synthetic cells [53] or "genomes-in-a-box" [54]. Finally, although our work has focused on SMCs loaded at the origins of replication on a circular chromosome, our model for how topo-entropic forces orient polymers could be adapted for prokaryotic or eukaryotic systems with different patterns of loop-extruder loading.

Methods

Orienting sampled chromosome configurations

Since the two replicated strands and two arms on a partially replicated chromosome are indistinguishable, the mean positions of both replication forks and both *ori*s are equal. Hence, to distinguish whether chromosomes or replication forks segregate, each simulated configuration is oriented so that these symmetries are broken. We define Strand 1 such that the center of mass of Strand 1 and the unreplicated segment is closer to mid-cell than Strand 2 and the unreplicated segment (Fig. S9A). Pole 1 is defined as the pole closer to Strand 1. Arm 1 is defined as the arm of Strand 1 and the unreplicated segment that is closer to Pole 1 (Fig. S9B). When *ori*-pulling forces are simulated, the strands are not renamed, since the applied forces distinguish the two replicated strands.

In replicating simulations, the direction of replication distinguishes the terminal region. In steady-state simulations with loop-extruders, the terminal region lacks a loop-extruder loading site. However, in steady-state simulations without loop-extrusion, when $R = N/2$, all three chromosomal strands are indistinguishable. In this case, we label Strand 1 as the one with a center of mass closest to a pole, Strand 2 as the strand closest to mid-cell, and Strand 3 as the strand closest to the other pole.

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6 Data Availability

The simulation code can be accessed at github.com/PLSysGitHub/loop-extrusion_with_replication, and the code used for analysis, as well as generating figures, at github.com/PLSysGitHub/loop-extrusion_with_replication_analysis.

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