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The structure and function of the bacterial chromosome

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Advances in microscopic and cell biological techniques have considerably improved our understanding of bacterial chromosome organization and dynamics. The nucleoid was formerly perceived to be an amorphous entity divided into ill-defined domains of supercoiling that are randomly deposited in the cell. Recent work, however, has demonstrated a remarkable degree of spatial organization. A highly ordered chromosome structure, established while DNA replication and partitioning are in progress, is maintained and propagated during growth. Duplication of the chromosome and partitioning of the newly generated daughter strands are interwoven processes driven by the dynamic interplay between the synthesis, segregation and condensation of DNA. These events are intimately coupled with the bacterial cell cycle and exhibit a previously unanticipated complexity reminiscent of eukaryotic systems.

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Introduction

The complexity of bacteria has escaped our attention for many years, mainly owing to a lack of suitable techniques to visualize the minute structures within prokaryotic cells. With the advancement of high-resolution microscopy and the adaptation of cell biological methods to bacterial systems, however, the realization dawned that bacteria maintain a highly organized interior that is dynamically rearranged to support basic cellular processes such as growth, differentiation, chromosome segregation and cell division. In particular, these developments fundamentally changed our conception of prokaryotic genome organization. Most bacteria possess one circular chromosome, the replication of which initiates at a single origin and proceeds bidirectionally until the replication forks

meet in the terminus region. It is not encased by membranous structures but embedded in the cytoplasm. Nevertheless, it is highly condensed and often occupies a defined region of the cell, characterized by the absence of ribosomes. This chromatin-dense area forms a pseudo-compartment [1], which is functionally equivalent to the eukaryotic nucleus and is, therefore, generally referred to as the nucleoid. The *Escherichia coli* genome comprises about 4.6 Mb of DNA, which would result in a length of about 1.5 mm in the extended state. Fitting a DNA molecule of this size into a cell with a length of approximately 1 μm necessitates extensive condensation and maintenance of a highly organized structure that ensures efficient DNA replication and segregation.

Our understanding of the mechanisms underlying these processes has been advanced significantly by recent findings, which are the scope of this review.

Topological structure of the chromosome

Early biochemical work showed that the *E. coli* chromosome does not behave as a single topological unit but, rather, is composed of independently supercoiled domains (see Glossary), whose topological state is uncoupled from the rest of the chromosome by supercoil diffusion barriers [2,3]. As a consequence, nicking a DNA strand only relaxes a single domain without affecting the superhelicity of neighboring nucleoid segments [4]. This design is vital for the cell because the energy stored in the torsional tension of supercoiled DNA is a major driving force in all cellular processes that require the melting of DNA strands, such as transcription [5,6^{••}], replication [7] and recombination [8], and even slight changes in the overall superhelicity of chromosomal DNA are lethal [9]. Electron microscopic analysis further corroborated the modular architecture of the chromosome [10,11], revealing that nucleoids isolated from *E. coli* had a rosette-like appearance with plectonemically interwound loops emanating radially from a central core containing RNA and proteins. The number of loops roughly equalled the number of topological domains defined by biochemical methods, which suggests that these two entities correspond to each other (Figure 1). Given that similar results were obtained for bacteria from a wide range of lineages [12], the basic mechanism of chromatin organization seems to be conserved throughout the prokaryotic world.

Recently, several attempts have been made to clarify the nature of chromosomal domains. The efficiency of site-specific recombination between differently spaced $\gamma\delta$ resolvase binding sites was used to demonstrate that

Glossary

Supercoiling – In relaxed double-stranded DNA, the two strands twist around the helical axis once every 10.6 base pairs. Changing this relation in a topologically closed segment of DNA creates torsional tension, which can be relaxed by coiling of the double strand. Decreasing the number of bases necessary to make one helical turn overwinds DNA and results in positive, left-handed supercoiling, whereas increasing this number creates underwound DNA with negative, right-handed supercoiling. Two topologically distinct forms of superhelicity can be differentiated. Plectonemic supercoiling results in helical intertwining of the DNA double strand, as exemplified by the transition of circular DNA from an 0-shaped to an 8-shaped conformation. It is typically observed for DNA regions not complexed by proteins. Solenoidal supercoiling, by contrast, is characteristic for DNA wrapped around histones in eukaryotic cells. In this case, the DNA double strand follows a spiral-like path — like the wire in a spring — without ever crossing itself.

Synchronization – The isolation of a homogeneous population of cells, each of them being at the same stage of the cell cycle.

The *Caulobacter* cell cycle – In contrast to many other prokaryotes, synchronized cells can be easily obtained for the dimorphic bacterium *Caulobacter crescentus*, using a density centrifugation protocol that separates flagellated ‘swarmer’ cells from stalked ‘predivisional’ cells. Swarmer cells possess a single chromosome and are arrested in G₁ phase until they eventually undergo a differentiation process that involves shedding of the polar flagellum and growth of a stalk-like protrusion at the same pole. As the cell cycle progresses, the chromosome is duplicated and a flagellum is re-synthesized at the pole opposite the stalk. Cell division then yields two morphologically and physiologically distinct daughter cells: a mobile swarmer cell, whose cell cycle is temporarily stalled in G₁ phase, and an immobile stalked cell, which immediately initiates a new round of DNA replication and cell division.

domain boundaries are not static structures that are confined to specific regions of the chromosome in *E. coli*. On the contrary, they are distributed stochastically and vary in position within a population of cells or even within a single cell over time [13,14^{*}]. Postow *et al.* [6^{**}] employed a whole genome approach to determine the length and distribution of topological domains *in vivo*. Supercoils were relaxed with the use of an inducible restriction endonuclease, which introduced double-strand breaks at specific chromosomal sites. Subsequently, DNA microarrays were used to monitor changes in the transcription of several hundred genes whose expression is known to be affected by the degree of supercoiling. The spread of DNA relaxation as revealed by these transcriptional changes then provided the basis to calculate the position of domain barriers. The results indicate that the establishment of domain boundaries is essentially random throughout the entire nucleoid. Furthermore, they provide evidence that the domain length is highly variable and exhibits an exponential distribution with a mean size of approximately 10 kb — a conclusion that was supported additionally by statistically analyzing electron microscopic images of gently isolated nucleoids.

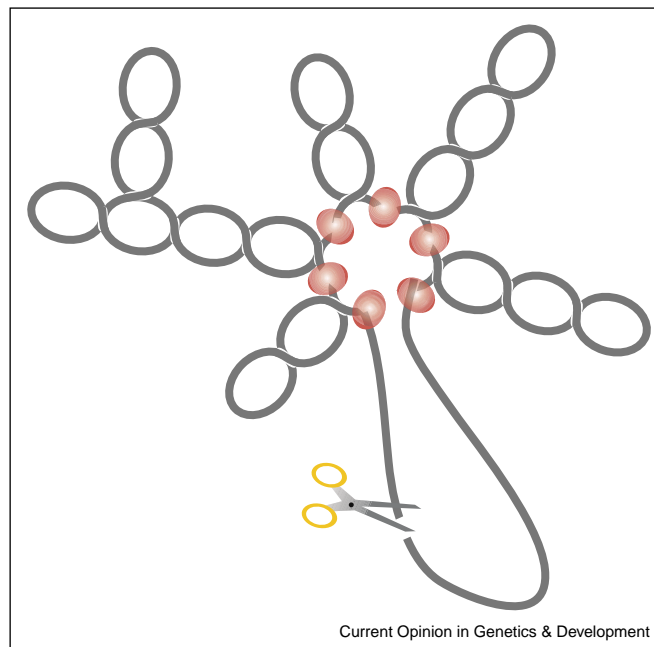
However, the actual identity of domain barriers remains unclear, although several models have been proposed on the basis of *in vivo* and *in vitro* data. In general, topological domains can be generated by any mechanism that anchors

a chromosomal site to a stationary structure in the cell or to another site on the chromosome. This could be accomplished by RNA, which was hypothesized to stabilize the structure of the nucleoid by binding to and interconnecting different supercoiled loops [15]. Moreover, several proteins that dynamically localize to defined positions in the cell, such as ParB/Spo0J [16,17], RacA [18^{**}] and FtsK [19], are known to interact with chromosomal DNA directly. Topoisomerases are thought to establish topological domains by preferentially binding to juxtaposed DNA helices, thus transiently linking two chromosomal segments [20]. By adjusting the degree of superhelicity and decatenating entangled DNA, topoisomerases further modulate the number of topological knots and tangles in chromosomal DNA, which are proposed to be a major restraint to supercoil diffusion in exponentially growing cells [21]. Random segments of chromosomal DNA were found to be stably associated with the cell envelope in *E. coli* [22]. This attachment is possibly mediated by membrane-bound components of the DNA replication or segregation machineries, such as DnaA [23] and SetB [24]. Envelope association might also be based on a process called transertion, which describes the coupling of transcription and co-transcriptional translation of membrane protein genes with the insertion of the nascent proteins into the cell membrane [25]. Actively transcribing RNA polymerase complexes are thereby tethered to the site of protein translocation and prevented from rotating around the DNA as they migrate along their templates. This leads to the transient accumulation of positive and negative supercoils in the vicinity of the transcriptional bubble and efficiently creates new domain barriers in plasmid substrates and presumably chromosomal DNA [26–28]. Even transcription in the absence of membrane translocation is sufficient to create supercoil diffusion barriers *de novo* if driven by a strong promoter, although it is likely that only a small number of genes have high enough expression to cause this effect [14^{*},29].

In the models described to date, barrier formation largely relies on primarily unrelated cellular processes. The dynamic activation and diverse intracellular distribution of these activities agrees with the apparently random placement of domain barriers [6^{**},13]. However, specific proteins — such as H-NS (histone-like nucleoid structuring protein) [30,31] or the bacterial SMC (structural maintenance of chromosomes) and MukB complexes [32^{**}] — might exist that are dedicated to close off topological domains.

Evidently, different mechanisms act in concert to maintain the bacterial nucleoid in an organized, albeit highly dynamic, state. Given that our present knowledge is based entirely on the analysis of unsynchronized cultures, it remains to be clarified whether the observed dynamics reflect a truly random establishment of topological

Figure 1



Topological organization of the bacterial chromosome. DNA is compacted into individual supercoiled domains that are topologically unlinked from each other because of supercoil diffusion barriers (red). Therefore, introduction of DNA strand breaks as a result of cellular processes or DNA damage (indicated by scissors) only relaxes a single domain without affecting the topological state of the remaining chromosome.

domains. Alternatively, it is conceivable that the nucleoid is continuously remodeled by the cell cycle-regulated expression of defined sets of genes to fit the needs of the proliferating cell. In this case, the topological changes could be highly reproducible and identical between different cells at the same developmental stage and yet appear random when averaged over a mixed population.

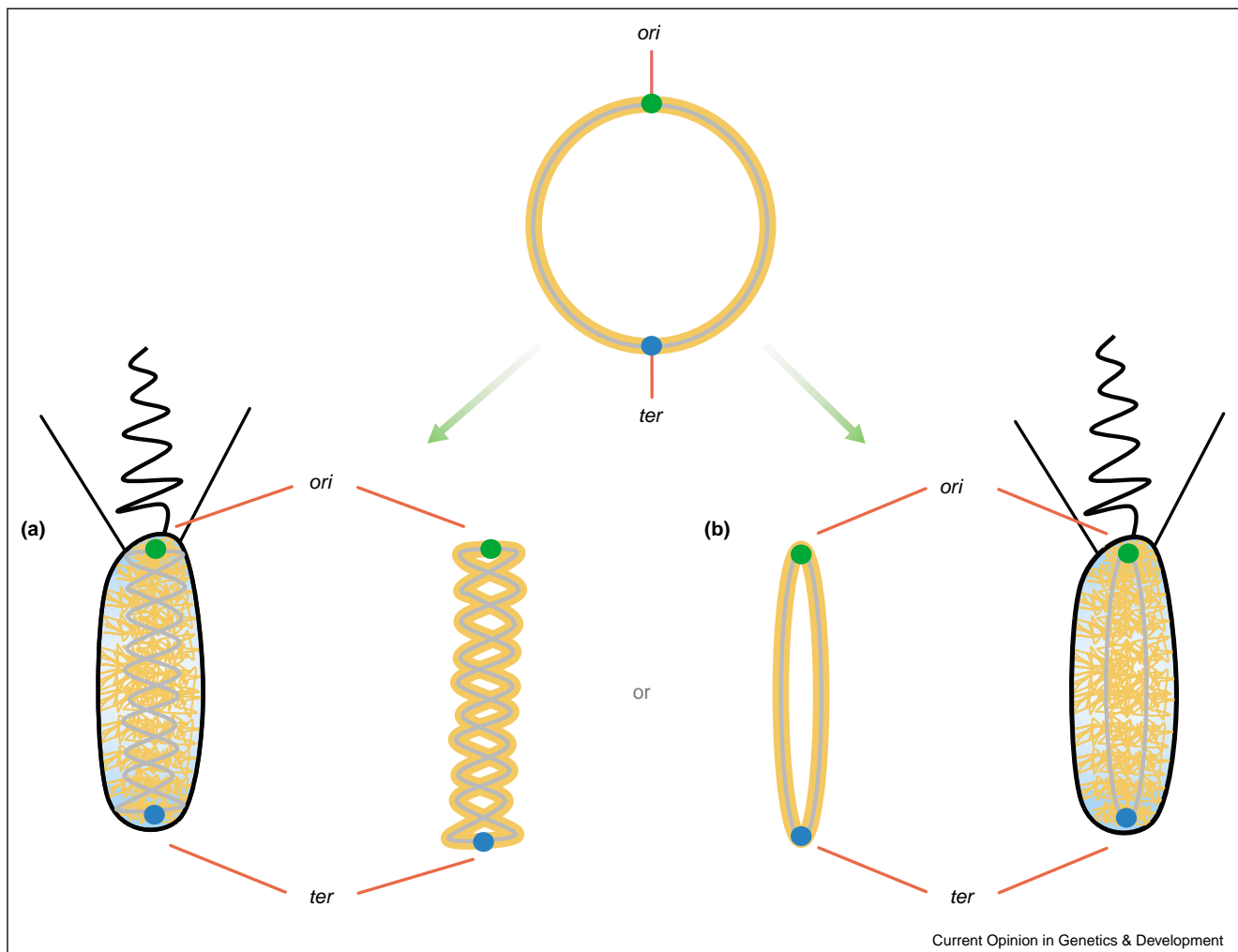
Spatial organization of the nucleoid

The apparent fluidity of the nucleoid at the level of domain barriers raises the question of whether the three-dimensional arrangement of chromosomal DNA in the cell is equally variable or whether it is largely predetermined. Analyzing the efficiency of site-specific recombination between pairs of distant λ integrase binding sites revealed that different regions of the chromosome are not equally accessible to each other in *Salmonella typhimurium* [33] and *E. coli* [34^{*}]. These results indicate that the bacterial nucleoid might not be indefinitely fluid — a hypothesis that has been verified recently by analyzing chromosome organization using cell biological methods. Adapting fluorescence *in situ* hybridization (FISH) to bacteria facilitated the visualization of specific DNA regions in fixed specimens. In addition, a novel technique was established that permits the observation of defined chromosomal sites in living cells. It employs a transcriptional repressor fused to a fluorescent protein in order to specifically decorate tandem repeats of the cognate repressor-binding site inserted at chromoso-

mal loci of interest [35]. Using these two approaches, it was demonstrated that bacteria possess mechanisms to orient the replication origin and terminus towards opposite poles in newborn cells [36–39].

The finding that certain chromosomal segments can adopt a pre-assigned intracellular position supports the idea that the nucleoid adopts a defined higher-order structure. In agreement with this, two genes located at the quarter positions of the circular chromosome were shown to localize consistently in-between regions occupied by the origin and terminus regions in *Bacillus subtilis* [40]. Moreover, Niki *et al.* [41] probed 22 loci evenly distributed over the *E. coli* chromosome and found that the ~900 kb origin-proximal and terminal regions colocalized with the replication origin and terminus, respectively, thus forming two macrodomains. Intermediate sites were arranged in-between these fixed points. Both of these studies imply a correlation between the physical position of a locus on the chromosome and its spatial localization within the cell. However, exact measurements were complicated by the fact that *B. subtilis* and *E. coli* are difficult to synchronize (see Glossary) and tend to accumulate multiple, partially replicated chromosomes. Recent work by Viollier *et al.* [42^{**}] circumvented this problem by focusing on synchronized G₁ cells of *Caulobacter crescentus* (see Glossary). Using both targeted and random transposon-mediated insertion, a library of 112 strains was created carrying tandem arrays of the *lac*

Figure 2



Spatial arrangement of chromosomal DNA in *C. crescentus*. The chromosome is condensed into a highly compacted, self-contained structure in which supercoiled loops of adjacent DNA segments are stacked on top of each other. Recent results [42**] are in agreement with two different models that describe the spatial arrangement of this structure in the cell. The two arms of the molecule could either be (a) intertwined, each following a curved path along the long-axis of the bacterium, or they could be (b) physically separated to adopt an open, ring-like conformation.

and *tet* repressor-binding sites at known positions on the chromosome. Analysis of the intracellular localization of the targeted sites revealed that the position of any given site along the long-axis of the cell showed a perfectly linear dependence on its physical distance from the origin of replication. The existence of macrodomains as suggested for *E. coli* [41] was not observed.

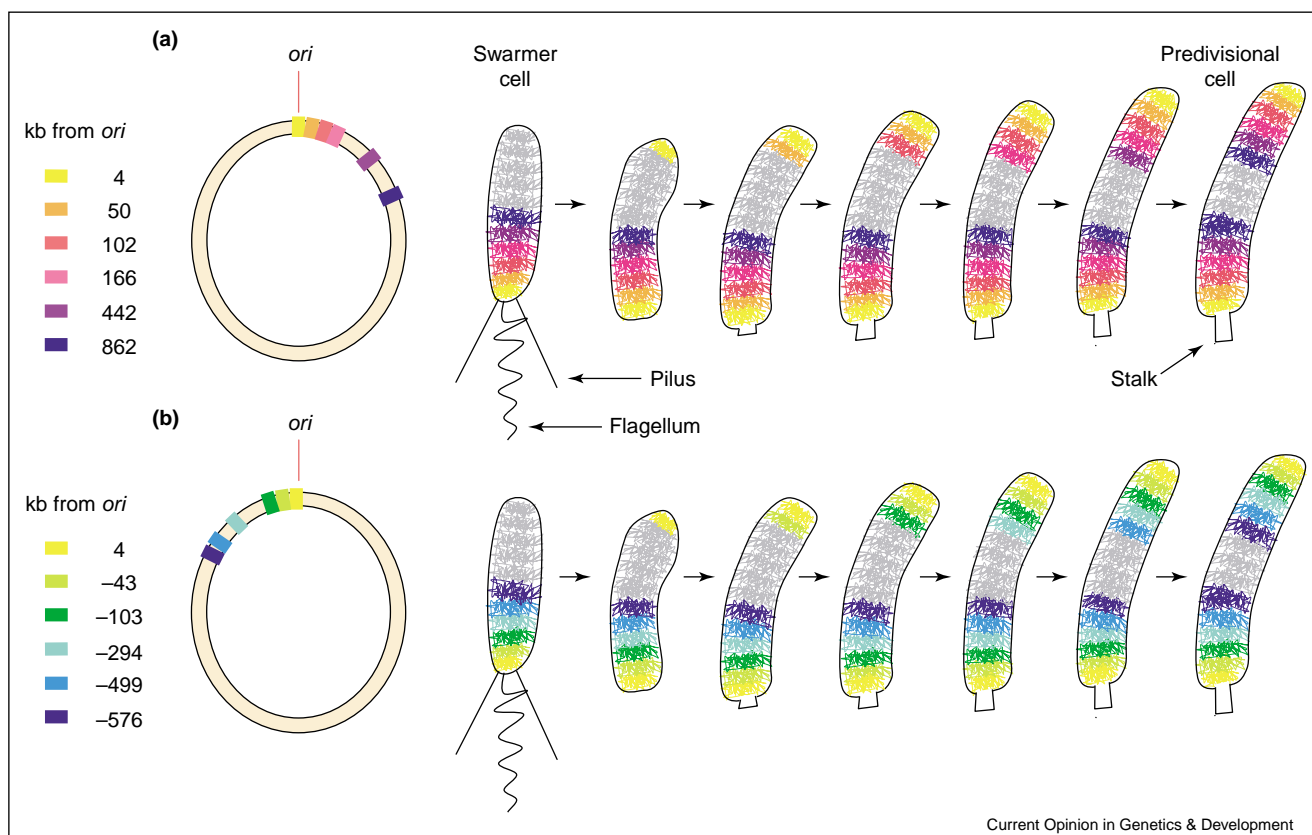
These results unequivocally demonstrate that the spatial arrangement of chromosomal DNA within the bacterial cell is predetermined. They further indicate that the chromosome adopts a folded conformation with loops of adjacent DNA segments stacked on top of each other in layers that are perpendicular to the long-axis of the cell.

In doing so, the two arms of the molecule could either be physically separated, which would result in a ring-like overall structure, or intertwined (Figure 2). The first possibility is supported by the fact that circular nucleoids were observed in fixed *E. coli* cells [41]. Other models for chromosome organization such as a random coil structure, a rosette of loops protruding in various directions, or layers of loops parallel to the long-axis of the cell can be eliminated because they are not compatible with the recent findings [43].

Dynamics of the replication process

Duplication of the chromosome necessitates continuous reshaping of the nucleoid in growing bacteria. At the

Figure 3



Conservation of chromosome structure during DNA replication in *C. crescentus*. In G_1 swarmer cells, the origin region (yellow) is localized at the flagellated pole, whereas the terminus is closely associated with the opposite end of the cell. Intermediate sites are arranged in linear order in-between these fixed points according to their physical distance from the origin. Therefore, chromosomal regions located (a) on the right-hand side of *ori* adopt the same subcellular positions as regions (b) on the left-hand side if their absolute distances from *ori* are comparable. At the beginning of bidirectional DNA replication, the newly synthesized origin region rapidly migrates to the opposite pole of the cell. It is followed by adjacent chromosomal loci, which are successively duplicated and immediately moved to their final positions in the incipient daughter cell compartments, thereby creating two nucleoids with identical three-dimensional organization.

onset of DNA replication, the origin moves to the center of the cell in slow-growing *E. coli* and *B. subtilis* [41,44–46], whereas it remains associated with the stalked pole in *C. crescentus* [39,42^{••}]. These localization patterns are consistent with the intracellular position of the replisome complex at the beginning of the cell cycle [47–49]. After duplication, the newly synthesized origin regions are displaced towards opposite cell poles in a process that was shown to occur instantaneously in *B. subtilis* [44] and *C. crescentus* [42^{••},48]. In *E. coli*, cohesion of the two daughter strands for an extended period of time has been postulated [50,51], but recent studies yielded contradictory results suggesting immediate segregation [46,52[•],53]. As replication continues, chromosomal DNA is successively spooled through the replisome [54], which remains loosely tethered to midcell in *B. subtilis* and *E. coli* [47,49,55], but is slowly displaced from the stalked pole to midcell in *Caulobacter* [48]. At the same time, newly duplicated DNA strands are rapidly separated from each

other and moved in opposite directions to adopt their final positions in the incipient daughter cells (Figure 3) [42^{••},48].

Segregation of the terminus region appears to be temporally and spatially coupled with closure of the cell division septum in *E. coli* [52[•],56[•]]. This observation might be explained by the crucial role of the septum component FtsK in the final steps of chromosome partitioning. The C-terminal part of FtsK is an ATP-dependent DNA translocase [19,57[•]] and has been implicated in clearing the division site of chromosomal DNA [52[•]]. It is further involved in the resolution of chromosome dimers — arising from homologous recombination between the two daughter chromosomes — by catalyzing synapsis of the terminally located *dif* sites at midcell [58] and activating the *dif* site-specific XerCD recombination system [19,59[•]]. FtsK finally promotes the topological unlinking of daughter chromosomes after the end of DNA

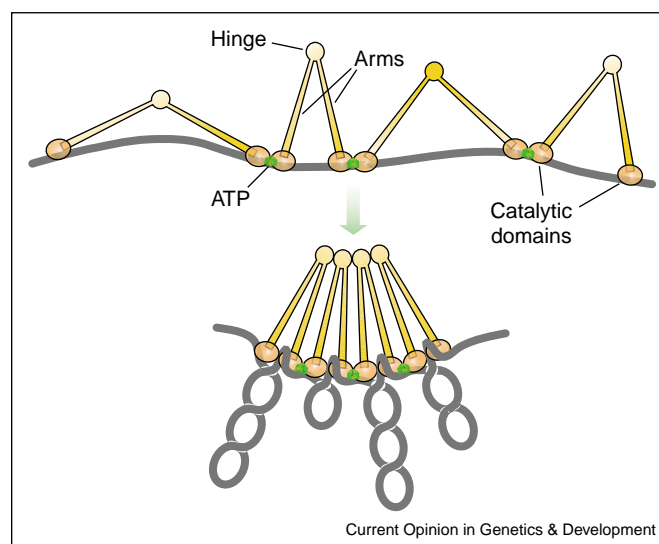
replication by stimulating the activity of topoisomerase IV, the major decatenating enzyme [60[•],61].

The speed of origin movement reaches 0.1–0.3 $\mu\text{m}/\text{min}$ [42^{••},44] and similar values were measured for the segregation of intermediate chromosomal loci [42^{••}]. This exceeds the rate of cell elongation by nearly two orders of magnitude and implies the existence of an active segregation mechanism. The transcription of highly expressed genes tends to be directed away from the origin of replication in many bacteria. This bias could drive chromosome partitioning by exploiting the molecular-motor activity of RNA polymerase, the mobility of which is thought to be largely restricted by interaction with other macromolecular structures. Prevented from migrating along the template strand, the enzyme might, therefore, rather translocate DNA through its active center, extruding it in a pole-ward direction [62]. The extrusion–capture model, by contrast, suggests that the force generated by DNA polymerization pushes newly duplicated daughter strands away from the stationary replisome towards the cell poles [49]. This process is thought to be assisted by traction resulting from the concomitant recondensation of newly replicated DNA into nascent nucleoids.

Several condensing activities are known in bacteria. Introduction of negative supercoils by bacterial DNA gyrase compacts relaxed DNA by converting it into a plectonemic superhelix, the diameter of which is only five times larger than that of a single DNA strand [9]. In

addition, every bacterium contains a varying number of histone-like proteins, which are highly expressed and associated with the nucleoid [63,64]. Members of this family, such as IHF (integrations host factor) [65] and HU (heat-unstable nucleoid protein) [66[•],67[•]], were shown to bend and thereby compact DNA in a sequence-specific or non-specific manner [68]. However, HU also decondenses DNA at high concentrations by polymerizing into a rigid helical filament, which conflicts with the idea of a general role in chromosome condensation [66[•],67[•]]. Another histone-like protein, H-NS, assembles on DNA as patches of oligomers that interact with each other, thus bridging different DNA regions and causing them to condense into compact nucleoprotein complexes [32^{••}]. Finally, bacterial SMC and its analog MukB were demonstrated to be essential for DNA condensation and chromosome structure *in vivo* [39,69–71]. They are V-shaped molecules composed of an amino-terminal ATP-binding domain, two long coiled coils separated by a flexible hinge, and a C-terminal DNA-binding domain [72,73]. ATP binding was shown to promote interaction between different SMC molecules in the presence of DNA [74], and association of the terminal domains by closure of the two arms stimulates ATP hydrolysis [74,75]. Both SMC and MukB form complexes with two accessory proteins, which regulate their function [76[•]] and are essential for their activity *in vivo* [77,78]. A recent single-molecule study clarified the exact mechanism of DNA compaction by the MukBEF trimer. Case *et al.* [32^{••}] demonstrated that binding of the proteins

Figure 4



The mechanism of MukBEF-mediated DNA condensation. MukBEF trimers polymerize on DNA into long filaments that are stabilized by ATP-dependent interactions between the catalytic domains of neighboring MukB molecules. Closure of the two arms subsequently condenses the double strand, thereby creating topologically isolated domains. Only MukB (orange) is depicted in the scheme, because the exact position of the MukEF accessory subunits in the complex and their contribution to the reaction are unknown.

transforms a relaxed DNA strand into an ordered, repetitive structure, presumably by converting it into a series of supercoiled loops that are stabilized and tethered to each other by intermolecular and intramolecular protein-protein interactions (Figure 4).

Polymerization reactions and DNA compaction, possibly assisted by passive processes such as constrained diffusion of DNA [25], might act in concert to drive segregation of the bulk of chromosomal DNA. However, they cannot account for the exquisite directionality of origin movement [42^{••},52[•]]. Specific mechanisms might, therefore, exist to position the origins of replication, which could then serve as a landmark guiding subsequent DNA segments into the nascent cells. This hypothesis is supported by the discovery of DNA regions with centromere-like functions such as *migS*, a short palindromic sequence necessary for polar origin localization in *E. coli* [79[•]]. Moreover, chromosomally encoded homologs of the ParA and ParB plasmid partitioning proteins were shown to interact with sites in the origin-proximal region and to be essential for proper chromosome partitioning in *C. crescentus* and *B. subtilis* [16,17,80–82]. In sporulating *B. subtilis* cells, RacA binds to distinct sites within 200 kb of the origin and forms a large nucleoprotein complex tethering the origin regions to the cell poles in a DivIVA-dependent manner [18^{••},83]. Recent studies have suggested a role for the actin homolog MreB in DNA segregation. In *B. subtilis*, MreB filaments migrate in the cell along helical tracks, thereby generating a potential moving velocity of 0.24 $\mu\text{m}/\text{min}$ [84[•]], which agrees with values reported for the movement of chromosomal sites [42^{••},44]. Moreover, overexpression and inactivation of MreB severely affect polar localization of the origin regions and chromosome segregation [85^{••}–87^{••}]. However, given that the mutations in the MreB cytoskeleton investigated in these studies were accompanied by severe cell-shape abnormalities, it remains unclear whether the segregation defects observed were caused by primary involvement of MreB in chromosome partitioning. Indications for a direct role of MreB in DNA segregation came from recent work performed in *C. crescentus*. Using the small molecule A22, which was shown to specifically, rapidly and reversibly disrupt the helical filaments formed by MreB, Gitai *et al.* [88^{••}] demonstrated that rapid movement of the origin regions to the cell poles was dependent on the presence of a functional MreB spiral. This finding was substantiated by chromatin immunoprecipitation experiments that revealed specific interaction of MreB with origin-proximal sequences. Interestingly, the compound did not affect the partitioning of other chromosomal loci if added after origin segregation. A dynamic actin-like cytoskeleton, therefore, appears to drive the rapid movement of newly replicated origin regions to opposite cell poles, whereas subsequent partitioning of bulk chromosomal DNA is achieved by other mechanisms.

Conclusions

Bacteria developed elaborate mechanisms to organize the nucleoid and thus ensure the ordered and accurate transmission of genetic information to their offspring in the course of cell division. We have only recently started to grasp the intricate network of interactions that couples the dynamic arrangement of the chromosome with cell cycle progression, and we look forward to understanding how replication and continuous transcription of genes are reconciled with the maintenance of domain barriers and the conserved spatial arrangement of the chromosome within the cell.

Although they differ at the molecular level, many of the basic concepts of chromosome biology appear to be conserved in prokaryotic and eukaryotic organisms. This substantiates the increasingly popular view that bacteria are highly organized units, which share many of the systems previously regarded as characteristically eukaryotic inventions.

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This study elegantly clarifies the topological organization of the *E. coli* chromosome. The authors show that chromatin is generally accessible for restriction enzymes *in vivo*. Subsequently, they use transient expression of the restriction endonuclease *SwaI* to introduce double-strand breaks and thereby relax supercoils in the chromosome of live *E. coli* cells. The consequential spread of relaxation is quantified by analyzing changes in the transcription of several hundred genes known to be affected by the level of supercoiling. Comparing the results of Monte Carlo simulations of different models for chromosome topology with the transcriptional profiles obtained, the authors conclude that the barriers of topological domains are fluid and stochastically distributed over the chromosome. The mean length of domains is 10 kb and this varies exponentially. These results are further corroborated by analyzing electron microscopic images of gently isolated nucleoids.

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