Review

Escherichia coli and its chromosome

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The *Escherichia coli* chromosome is a circular DNA molecule that is ~1000 times compacted in the living cell, where it occupies ~15% of the cellular volume. The genome is organized in a way that facilitates chromosome maintenance and processing. Despite huge efforts, until recently little has been known about how the chromosome is organized within cells, where replication takes place, and how DNA is segregated before cell division. New techniques for labeling genetic loci and molecular machines are allowing the simultaneous tracking of genetic loci and such machines in living cells over time. These studies reveal remarkable organization, yet a highly dynamic flux of genetic loci and macromolecules. It seems likely that the cellular positioning of chromosomal loci is the outcome of the formation of two chromosome arms (replichores) by replication, followed by sequential chromosome segregation, rather than from the presence of cellular positioning markers.

Overview

During the past fifty years the power of molecular biology has revealed the mechanisms of DNA replication, transcription, repair, recombination and how they are controlled. Advances in X-ray crystallography have provided the structures of many key proteins that function in cellular metabolism. In eukaryotes, much has been learnt about how chromosomes are organized and replicated, and the temporally separated process of chromosome segregation.

By contrast, in prokaryotes, little insight has been gained into how bacterial DNA is organized within the cell and what processes maintain nucleoid organization, while allowing DNA replication, transcription and chromosome segregation. No single protein or group of proteins seems to have an essential role in chromosome organization, although several histone-like proteins might contribute to DNA compaction through their transient association with DNA. Similarly, the *Escherichia coli* structural maintenance of chromosomes (SMC) complex, MukBEF and its relatives in other bacteria, have an important role in chromosome organization, by an unidentified mechanism.

The reasons for this lack of insight are that classical molecular genetics approaches have failed to find genes that have key roles in bacterial chromosome organization or segregation, and the small size of the cell and nucleoid and their dynamic properties has challenged cell biological approaches. For example, conventional light microscopy has a resolution of approximately half the wavelength of light, approximately one-third of the diameter of a typical rod-shaped bacterium. Furthermore, different fixation techniques for both light and electron microscopy have given inconsistent information on organization. The fact that bacterial generation times can be shorter than the replication time has also clouded the interpretation of many analyses of cells that accommodate a lifestyle in which a given replication initiation event occurs up to three generations in advance of when the sister chromosomes resulting from an initiation are finally separated into daughter cells.

Here we review results and their interpretations that relate to bacterial chromosome processing (reviewed in Refs [1–4]) and attempt to incorporate them into an integrated view of how the *E. coli* chromosome functions in the context of the living cell. Additionally, we discuss whether these principles are generally applicable to other bacteria.

DNA sequence organization within the bacterial chromosome

Like most characterized eubacterial chromosomes, *E. coli* has a single circular chromosome that is replicated bidirectionally from a unique origin, *oriC*, to create two chromosome arms or replichores. Replication is controlled by strictly limiting initiation by DnaA to once per cell generation (reviewed in Ref. [5]). The two replication forks each travel at 600–1000 bp/s [6]. Replication termination occurs in a broad terminus region that is delineated by redundant groups of replication termination sites. When bound by the terminator protein, Tus, these sites function to ensure that each replication arm (replichore) is ~50% of the chromosome. Most transcription is in the same direction as replication, probably to avoid 'head-on' clashes, which are likely to interfere with both processes [7]. Typically, there is a strand-specific base-composition skew that reverses at the replichore boundaries, thereby allowing one to infer the positions of replication origins and replication terminal sites. This skew probably arises because of different mutational rates on the leading and lagging strand templates, but has been acted on by evolutionary processes that have selected some of these sequences for specific functions. For example, the eight base pair Chi sequences have evolved to be hotspots for homologous recombination and are oriented so that any breakage of chromosome arms arising when replication forks run into single strand discontinuities, can be repaired by RecBCD-RecA-mediated recombination [8]. Similarly, eight base pair KOPS (FtsK orienting polar sequences) act to direct the action of the DNA translocase FtsK [9]. Many key genes are in the *ori*-proximal region, because then their gene copy as a function of cellular mass remains constant independently of growth rate, whereas dosage of genes elsewhere in the chromosome is reduced as growth rate increases.

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Although a single circular bacterial chromosome is common, it is not universal. For example, *Borrelia* and *Streptomyces* have linear chromosomes that have different mechanisms of 'end replication', whereas *Vibrio cholera* has two circular chromosomes that are independently controlled (although one of these contains largely non-essential genes and could be considered a very large plasmid). The archaeal bacterium, *Sulfolobus solfataricus*, has a single circular chromosome with three replication origins, consistent with its shared ancestry with multi-replicon eukaryotes [10].

The genetic map is recapitulated with the living cell

Three methodologies have been exploited to visualize specific chromosomal loci in *E. coli* and other bacteria, FISH, FROS and ParB–*parS* (see Box 1); the latter two methods allow tracking of loci in live cells. Although initial studies visualized just one or a few loci, largely in fast growing *E. coli* [11–16] or *Bacillus subtilis* [17] with overlapping replication cycles, the most revealing studies in *E. coli* have used growth conditions in which a single round of replication is initiated and completed in the same cell [18–21].

The earliest studies revealed that genetic loci are not randomly localized, but it was only when multiple loci were examined that a clear picture of positioning and consequent organization emerged. A pioneering study that used multiple markers in *Caulobacter crescentus* showed an organization with *ori* at one pole and the *ter* at the other, with markers placed in between according to their map position (Figure 1) [22]. Segregation of newly replicated markers occurred sequentially soon after replication. It was therefore surprising to find that in non-replicating *E. coli*, *ori* was positioned close to mid-cell, with the left and right replichores positioned on either side and the *ter* region crossing from one side of the nucleoid to the other [20,21]. Again, marker position broadly corresponded to map position, with the more *ori*-proximal markers being closer to mid-cell. Even more unexpected was the demonstration that most sister chromosomes adopted a <left-right-left-right> organization, rather than a more intuitive bilateral symmetry [20,21] (Figure 1). The position of a given marker is dictated by the replichore to which it belongs; for example, switching the fork that replicates a marker in the terminus region switches its cellular position [20]. The <left-right> organization is retained during multi-fork replication in fast growing cells [20], and seems to be maintained in *E. coli* that have been manipulated to have *Borrelia*-like linear chromosomes [23].

Nucleoid organization and dynamics

Most recent reports indicate that the nucleoid is organized into ~400 independent supercoiled ~10 kb domains *in vivo* [24,25], and a higher level organization into macrodomains has been proposed [12,26]. It is inevitable that domains must be disrupted during their replication and reform soon afterwards. What acts as the restraining component for such domains remains unsolved. Proposed candidates include topoisomerases and transcription machinery.

Although negative supercoiling has a crucial role in the ~1000-fold compaction of the chromosome into a nucleoid,

Box 1. The microscope toolbox

Methods for visualization of chromosomal loci and molecular machines

Traditionally these methods use wide-field epifluorescence, although newer powerful methods are appearing (reviewed in Refs [74,75]). For example, total internal reflectance fluorescence microscopy (TIRFM) allows visualization in a ~100 nm evanescent wave, thereby massively increasing the signal-to-noise ratio and allowing visualization of single molecules [76], which makes it important in experiments that demand high sensitivity or quantitative analysis.

Immunochemistry

Immunochemical methods are used to visualize proteins (e.g. see [17,18]) and newly replicated DNA [40]. These methodologies require fixation of cells with consequent concerns about artefacts. Furthermore, the efficiency of the antibody interaction can lead to an underestimate of focus numbers.

Fluorescent molecular machines

The use of green fluorescent protein (GFP) and its fusions to other proteins in living cells has revolutionized our understanding of cell organization and function [77]. The development of new fluorescent proteins with different emission spectra has made it possible to track multiple components in living cells at the same time [19,20,30,37,78]. Ideally, these proteins are expressed as fusions from the endogenous promoter of their target genes, thereby replacing the wild type allele, so that timing and dosage of the expression is the same as the wild type. Care needs to be taken to make sure fusion does not compromise function of the target protein.

Fluorescence *in situ* hybridization (FISH)

FISH uses nucleic acid hybridization to detect and localize DNA sequences on the chromosome within cells [14,18,20]. DNA probes, usually 2–10 kb in length, are prepared and labeled with fluorophores. These DNA probes are hybridized to fixed and permeabilized cells followed by detection by fluorescence microscopy. This technique enables simultaneous detection of multiple loci and does not require the construction of special strains. However, fixation and permeabilization can perturb the original arrangement of the chromosome and does not allow for real time visualization in living cells. Again, care needs to be taken to maximize labeling efficiency and to have controls to assess this efficiency. Some studies might have underestimated the number of genetic loci present in cells (e.g. see [3,14]).

The fluorescent repressor-operator system (FROS)

FROS was the first method developed to visualize specific chromosomal loci in living cells. GFP variants with different emission spectra that are fused to the LacI and TetR repressors are expressed in cells with tandem copies of *lacO* and *tetO* inserted at defined chromosomal loci. The specific binding of the fluorescent repressors to their cognate operators allows visualization by fluorescence microscopy [11,16,19,20]. This method demands construction of a new strain for each locus, but arrays can be introduced at many random sites by using transposition [22]. Because overexpression of fluorescent repressors and their consequent high occupancy binding to operators can block replication, it is important to prevent such overexpression by having the appropriate inducers present, or maintaining low repressor levels [16,19,20]. FROS allows both snapshot and time-lapse analysis and is most powerful when there is simultaneous tracking of two or three colors.

ParB–*parS* systems

The specific binding of fluorescent fusion derivatives of the P1 plasmid partitioning protein ParB to the 25 bp *parS* sequence also allows chromosome locus visualization in living cells [15]. ParB–*parS* combinations of different specificity allow the simultaneous visualization of two or more loci [21]. This system also requires the construction of strains for each locus and requires that ParB be present at low enough levels to prevent inhibition of DNA replication. It is essential when either this system or FROS is used, to ensure that cell cycle parameters are not disturbed by the assay system. When used appropriately this system and FROS give better resolution than FISH and immunochemical methods.

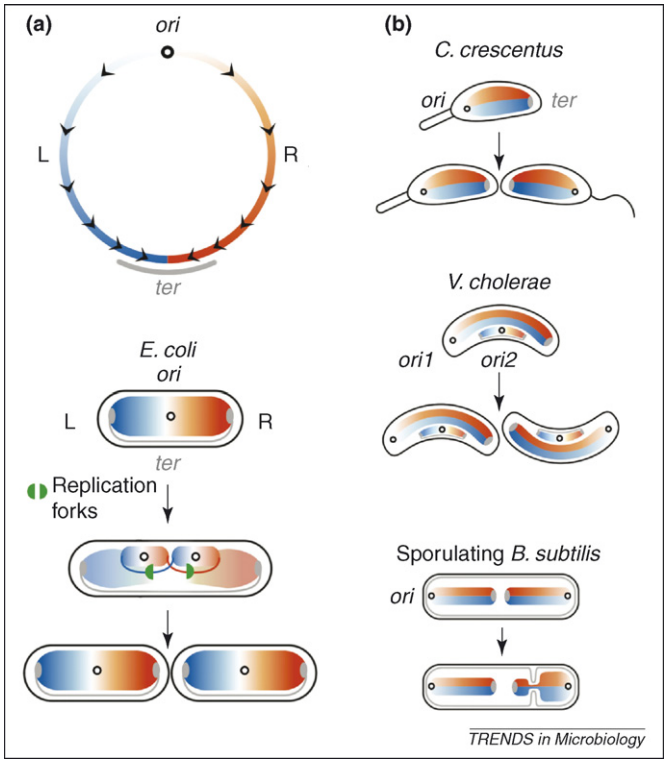

Figure 1. Bacterial chromosome organization. **(a)** (i) Schematic of the bacterial chromosome. The left replichore (L) is colored in blue and right replichore (R) in orange. The color gradient indicates the increasing distance of the loci from the origin. The black arrowheads indicate the direction of the polarized skewed sequences, such as Chi and KOPS. The ~400 kb terminus region is shown as a gray line. The replication origin (*ori*) is indicated. (ii) <Left-right> organization of the *E. coli* chromosome. In slow growth conditions a newborn cell has *ori* close to mid-cell. The left and right replichores are positioned either side of the cell. Most of the terminus region locates to either the left or the right replichore (gray ovals), which leaves a relatively small amount of DNA (<400 kb) that spans the two edges of the nucleoid (thin gray line). During DNA replication, sister origins segregate and the two replication forks (filled green half circles) split to opposite cell halves. By sequential layering of newly replicated DNA (blue and red lines) on either side of the origins, the majority of the dividing cells have <L-R-L-R> structure [20]. **(b)** Proposed cellular organization of the chromosome in other bacteria. (i) In the newborn cell of *Caulobacter crescentus*, *ori* locates to the pole with its stalk and the terminus to the other pole, with the rest of the chromosome localized linearly according to the map. It is not clear whether the two replichores lie on either side of the longitudinal axis, as shown, or intertwine about each other. In either case, we predict that the independent action of sister replisomes will be observed as the movement of the replication machinery from the *ori*-proximal pole to the *ter*-proximal pole. (ii) Possible chromosome organization of the two *Vibrio cholera* chromosomes. The positioning of *ori1* and *ori2* are reminiscent of that of *Caulobacter crescentus* and *Escherichia coli*, respectively [33]. The detailed organization is not known. (iii) *Bacillus subtilis* sporulation: the nucleoid is reorganized and elongated, and the two origins locate to opposite cell poles with the facilitation of DivIVA, RacA, *soj* and Spo0J [52]. SpoIIIE pumps one whole chromosome into the forespore [67].

it is not sufficient to give the observed compaction, unless other factors such as bound proteins, counterions and excluded volume effects stabilize the compacted state. Despite this huge compaction, only ~3% of the nucleoid by mass is DNA. It has been argued that chromosome-associated proteins like Hu, Ihf, Fis and Hns have primarily regulatory roles, and their DNA association is not structural [27]. Nevertheless, we do not see why such proteins should not have both regulatory and structural roles, as do histones in eukaryotes. *E. coli* Dps, which accumulates massively in stationary phase, could have a role in the maintenance of the nucleoid in a quiescent compacted state [27]. If any of the proteins mentioned here function centrally in chromosome organization, their individual roles must be redundant because depletion of any one protein does not apparently lead to gross structural changes in the chromosome. Nevertheless, the recent demonstration that Hu spirals can lead to toroidal negative supercoiling [28] deserves note, because toroidal supercoiling is expected to be more effective than plectonemic supercoiling in DNA compaction-organization (Box 2). Ancient and ubiquitous proteins constitute SMC complexes that have several roles in chromosome organization and processing (reviewed in Ref. [29]). In *E. coli*, the SMC complex, MukBEF, facilitates chromosome organization, apparently by associating with newly replicated *ori* regions [30].

Nucleoid morphology, size and properties are maintained *in vitro* when cells are gently lysed in the presence of polylysine and spermidine [27]; however, it remains to be determined whether the relative positions of genetic markers are maintained *in vitro* where estimates of domain size vary from 6.5 kb [31] to 50 kb [32].

Box 2. Glossary of DNA topology

Plectonemic and toroidal supercoils

Covalently closed DNA with a linkage deficit forms negative supercoils, which can exist in two interchangeable forms. *In vitro*, negative supercoils adopt a right-handed plectonemic conformation in physiological buffers, whereas left-handed toroidal DNA is stabilized by proteins and should give higher levels of compaction-organization [79] (Figure Ia).

Precatenanes and catenanes

Catenanes have two duplexes interlinked and arise during replication as a consequence of the interwound strands of the DNA double helix [79] (Figure Ib). During replication of the circular *E. coli* chromosome, progress of the fork leads to an accumulation of positive supercoiling ahead of the fork, which can be removed by DNA gyrase. If rotation of either of the forks occurs, linkage ahead of the fork diffuses behind the fork so that the two newly replicated duplex sisters become interwound. These links are described as precatenanes. Precatenation links become catenation links upon completion of replication. Topoisomerase IV removes duplex interwrappings in precatenanes and catenanes [79].

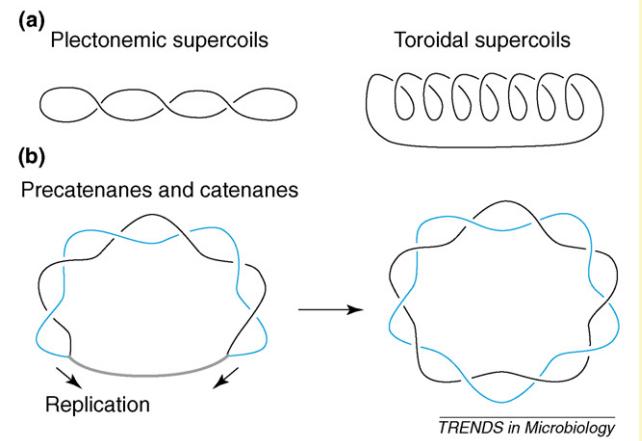

Figure I. DNA topology. **(a)** Schematic contrasting plectonemic and toroidal negative supercoils. **(b)** Schematic showing the interlinking of newly replicated sister duplexes (precatenanes) These arise when rotation of the replication fork allows linkage in right-handed duplex DNA ahead of the fork to form right-handed precatenanes behind the fork. Completion of replication (right panel) converts precatenane links to catenane links. Duplex DNA is represented by single lines.

Chromosomal DNA must be dynamic to be processed. With the advent of improved methodologies for live cell imaging, it has become possible to study this movement and that of associated molecular machines by tracking the positions of genetic loci and molecular machines over short time intervals. These studies show that DNA is highly dynamic, with rapid yet constrained motion even when DNA is neither replicating nor segregating. Furthermore, the dynamics observed in bacteria and in interphase DNA of eukaryotes are similar; typically, genetic loci exhibit apparent diffusion coefficients of $1–4 \times 10^2$ nm^2/s, with step sizes of the order of 0.1 nm/s over short time intervals ([22,33] and refs therein; [34–37]).

Two types of movement in the bacterial nucleoid have been distinguished, one for non-segregating loci (at their 'home' positions) [33] and another when they are undergoing segregation after replication [11,22,33]. At their home positions, diffusional movement of a locus is constrained ('caged'), presumably as a consequence of being in a relatively mobile segment contained with a very large relatively immobile polymer. In *Vibrio cholera* and *E. coli*, the apparent cage size (~400 nm diameter) is larger than the predicted diameter of individual domains (50–100 nm, assuming there are ~400 independent domains) [33,37].

Segregating loci have directed movement imposed on their home dynamics, thereby giving an overall motion greater than that in the home position. For example, in *V. cholera* the difference in locus dynamics at home or when segregating is at least five times for markers close to *ori*, with the rate of directed movement being ~1 nm/s in the long axis [33]. In *E. coli* and *C. crescentus*, the rate of directed segregation of *ori*-proximal markers is even greater [11,22].

Replication and segregation shape chromosome organization

DNA replication and segregation must impose dramatic changes in the organization of the nucleoid. During replication, topological domains must break and reform to allow the transit of the replication machinery associated with each replication fork (replisome), and the growing nucleoid has to accommodate two copies of newly replicated loci and reducing quantities of unreplicated DNA (Figure 2). In fast growing *E. coli*, multiple overlapping rounds of replication and consequent remodeling must be accommodated in the same nucleoid.

The extent to which DNA replication will influence chromosome organization and segregation will depend on how replication is organized. Until recently, thinking about how and where replication occurs has been dominated by the replication factory model [38–41]. The model states that the replication machinery, which constitutes the two sister replisomes derived from a given initiation event, is stationary, and has parental DNA entering it and newly replicating DNA exiting from it. The strictest form of the model has the progress of the two sister forks coordinated.

However, evidence in *E. coli* supports the view that sister replisomes are functionally independent [42]. Furthermore, visualization of replication in living cells, as opposed to fixed cells used in previous reports [40,41], shows that sister replisomes separate bidirectionally from the origin at mid-cell soon after replication initiation, and then track independently along the DNA in each cell half, until termination approaches and they return towards mid-cell [37] (Figure 2). This independent tracking along DNA in separate cell halves provides an explanation for the organization of the *E. coli* chromosome (Figures 1 and 2). Consistent with their tracking along DNA, replisomes exhibit a higher rate of movement as compared with 'home' genetic loci [37]. Furthermore, because replisomes assemble at origins irrespective of their position in the cell [37], in principle, replication machineries will assembly at any 'activated' origins, irrespective of whether they are plasmid, chromosomal or viral, and independently of their cellular position. These observations lead to the view that the replisomes might not be tethered to any cellular structure, and that their positions in cells are determined solely by the segments of chromosomal DNA with which they are associated.

Newly replicated DNA, at least in the early stages of replication, is likely to be excluded from the unreplicated DNA, and might therefore form a concentric outer shell around the unreplicated nucleoid [43]. Sister replisomes appear in separate cell halves approximately five minutes after replication initiation at *oriC*. Some 5–15 minutes later, the two sister origins move to opposite cell halves [37], as part of the two sister elements of this presumed outer shell. Such a relatively brief period of sister cohesion is consistent with other work that shows sequential locus segregation soon after replication [19–21,37,44]. The discrepancy with earlier reports of extensive sister cohesion [18,45] is likely to have resulted from technical limitations of the experiments (see Box 1). We believe that the relative short time and 'patches' of sister cohesion are a direct consequence of the replication process, rather than arising from some dedicated sister chromosome cohesion process, as in eukaryotes. MukBEF does not act in cohesion, because sister cohesion is not reduced in Muk$^-$ cells [30].

Sequential layering of the newly replicated and segregated DNA on both sides of the origin would directly lead to the two sister *ori*s being positioned close to the nucleoid quarters before cell division, thereby leading to *oriC* being close to mid-cell in newborn cells simply as a consequence of the replication–segregation process (Figure 2). The mechanism seems to be independent of the precise chromosomal position of the replication origin and to act instead on a broader *ori*-proximal region [46,47]. MukBEF seems to be an organizing factor that maintains *ori* positioning close to the cell quarters because in its absence sister *ori*s move to the outer nucleoid edge, presumably because all newly replicated DNA is placed inside of the *ori* regions during segregation [30]. Therefore, replication and segregation alone might lead to the observed *ori* positioning at mid-cell in newborn cells; *a priori* there is no need to implicate cellular positioning markers for any genetic locus.

Chromosome unlinking

Effective propagation of the genetic material over generations demands not only that it be faithfully replicated, but that this replication is in concert with cell growth and

division, thereby requiring that control of initiation by DnaA be finely tuned to appropriate cellular signals and that inappropriate re-initiations be avoided [5]. Furthermore, stable propagation requires also that newly replicated sister chromosomes be segregated to daughter cells at cell division, a process that demands that the linkage between the parental DNA strands be reduced to zero. This requires that topoisomerase action reduces linkage as replication

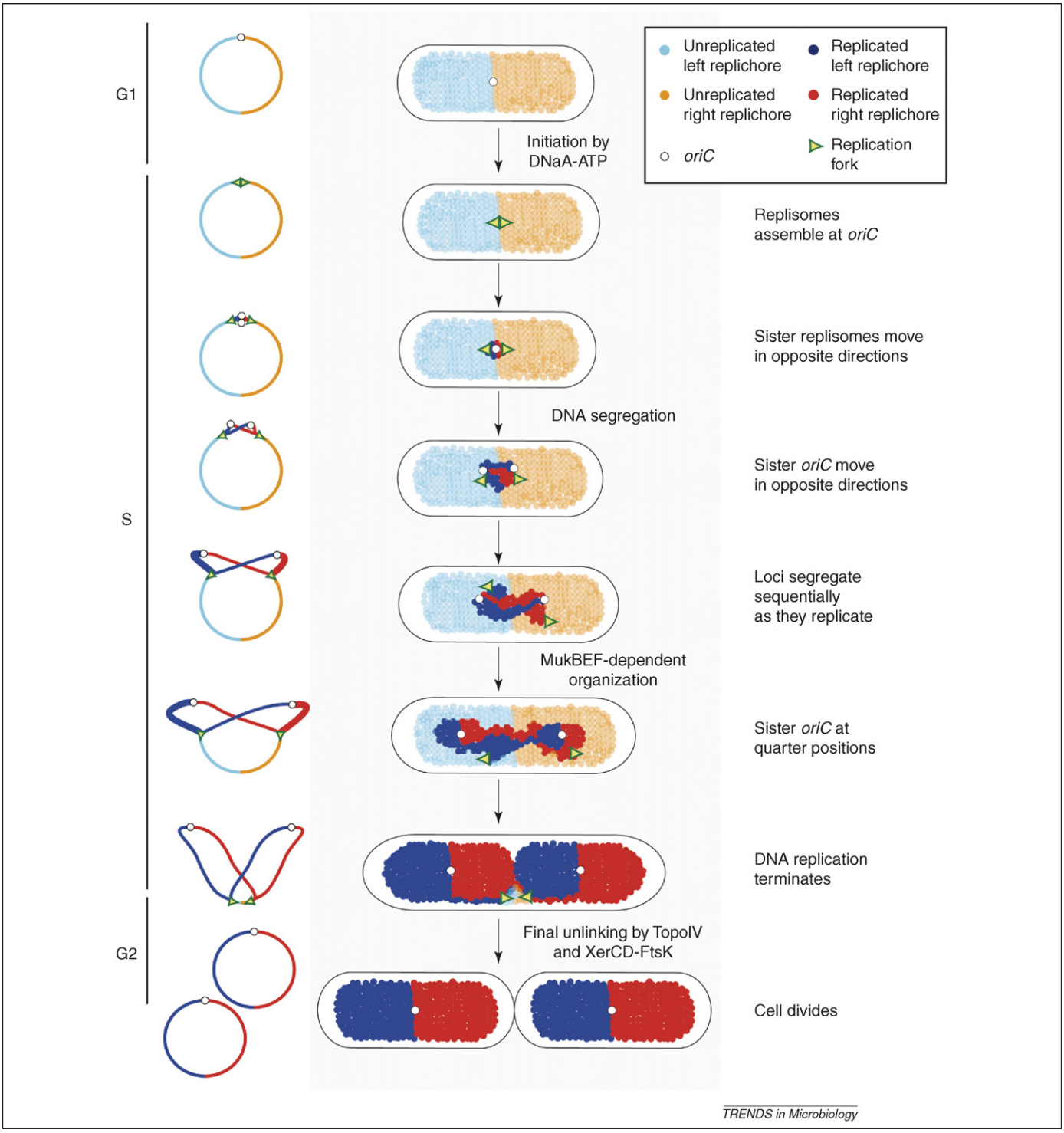

Figure 2. Replication remodels nucleoid organization throughout the cell cycle. *E. coli* nucleoid organization is dictated by the chromosomal replichores (blue and orange), with *oriC* (circle) placed between them. Each replichore is subdivided into many independent topological domains. Under slow growth conditions, cells are born before initiating DNA replication. Initiation will only occur after enough DnaA-ATP is accumulated in the cell, this will trigger the DnaC-mediated loading of the DnaB helicase and the remaining components of the replisome onto *oriC*. Shortly after initiation the two replication forks move in opposite directions, presumably as a result of their migration along DNA, and their location in the cell continues to change as S phase progresses. The two sister copies of *oriC* remain cohesed for a fraction of the S phase and then start to segregate. Downstream loci progressively follow *oriC* as they are replicated. Later in S phase, the sister *oriC*s reach their new 'home' positions, close to the cell quarters, a process that requires the action of MukBEF. This model does not require any cellular marker to position *oriC* at the quarter positions; rather, it results from the progressive layering of the newly replicated DNA on both sides of *ori*, which ends with the formation of two new nucleoids that have the replichore organization of the parent. As the new DNA starts to accumulate, the reorganization of the old and new DNA in a replicating cell might have a role in segregation and shaping of the new chromosomes. Close to the end of replication, the forks move close to mid-cell, replication terminates, and the combined unlinking activity of TopoIV and XerCD-FtsK allows the two nucleoids to complete segregation, thereby allowing the completion of cytokinesis.

proceeds and that any final catenation is removed once replication is complete. DNA gyrase seems to remove most of the ~400 000 *E. coli* links by acting ahead of a progressing fork, where it can remove any positive supercoils and reinstate normal negative supercoiling. Occasional rotation of the fork can lead to some of the linkage ahead of the fork diffusing back to form precatenanes that interwrap the two new sister chromosomes (Box 2) [48]. Formation of precatenanes would seem unlikely if replication occurs at coordinated replisomes in factories, because of the barrier to individual fork rotation. That barrier to precatenane formation disappears if sister replisomes progress independently. Topoisomerase IV (TopoIV) efficiently removes precatenanes and is also likely to remove most of the remaining catenation when replication is complete, although Topoisomerase III, along with the helicase RecQ, can act at the single strand regions of forks as replication termination approaches [48]. Another barrier to unlinking is caused by crossing over during homologous recombination, which produces chromosome dimers approximately once every six generations. Dimeric chromosomes are converted to monomers by XerCD-*dif* site-specific recombination, a reaction that requires the septum-tethered DNA translocase FtsK [49]. The action of XerCD-*dif*-FtsK can also lead to effective decatenation [50].

Chromosome and plasmid segregation

Despite many theories and much work, the mechanism of segregation of the *E. coli* chromosome remains elusive, as does that for other bacterial chromosomes. The earliest model indicated that an inner membrane attachment of the chromosome, followed by outwards growth in the long axis would move apart newly replicated chromosomes [51]. Consistent with this, some work has indicated that the rate of movement apart of newly segregated origins is the same as the rate of growth in the long axis [36]. By contrast, most recent studies have shown that movement apart of newly replicated loci is faster than the average rate of cell elongation ([33] and refs therein). Mitotic-like mechanisms for chromosome segregation have been proposed [17,52–54], as have mechanisms in which extrusion from a stationary replication factory drives segregation [55]. The presence of an apparent centromere-like sequence, *migS*, in the *E. coli* chromosome [56], has not provided new mechanistic insight into segregation; indeed this sequence is not essential for faithful segregation. Processes such as transcription [57], or transertion (insertion of proteins into the membrane as their genes are transcribed and translated) (reviewed in Refs [2,3]), have also been implicated in facilitating chromosome segregation. However, inhibition of transcription with rifampicin does not affect the rate or nature of segregation of newly replicated *E. coli* loci as observed by FROS (X. Wang and D.J. Sherratt, unpublished), which shows that growth in the long axis, transcription and transertion are not directly involved in chromosome segregation. Claims that the actin cytoskeleton has a direct role in *E. coli* chromosome segregation [58] have not been substantiated [59] (X. Wang and D.J. Sherratt, unpublished). Finally, the defects in chromosome segregation in *E. coli*, MukBEF^{-} cells seem to arise indirectly as a consequence of altered chromosome organization [30].

Is the failure to identify the chromosome segregation mechanism a consequence of extensive redundancy in the processes that lead to segregation? We think not, and are attracted by the idea that the driving force for chromosome segregation arises from entropy, which can be stored in, and released from, the compacted bacterial nucleoid [43]. Segmented polymers (e.g. replicating or sister bacterial chromosome) confined to a volume with little free space (the cell) will be strongly self avoiding, thereby leading to spontaneous separation of newly replicated chromosomes; however, it is important to note that during segregation chromosomes not only move apart but move to opposite cell halves. Calculations and modeling of various scenarios indicate that this is a credible mechanism and would explain why plasmids and smaller chromosomes have segregation mechanisms that have been readily identified by classical molecular biological approaches [43]. Although many chromosomes do encode plasmid-related apparent partition systems, these are generally not essential for near-normal chromosome segregation, although their participation is more essential in the specialized segregation that occurs during *B. subtilis* sporulation [53,54].

As indicated, low copy plasmids, some of which approach the size of bacterial chromosomes, inevitably use active ATP-dependent segregation mechanisms mediated by one of two mechanisms that use 'centromere-like' sites and protein that act at these sites (reviewed in Ref. [60]). The most characterized of these mechanisms is the actin-like ParM-ParR system, specified by plasmid R1, in which a growing actin filament leads to separation of sister plasmids [61,62]. The more common Walker type ATPase ParA-ParB-*parS* systems are less well understood mechanistically, although they seem to involve the dynamic oscillation of ParA [63,64]. It is not known whether apparent ParA helices are formed autonomously or by interactions with pre-existing cytoskeletal structures such as MreB [63–65].

The related DNA translocases FtsK and SpoIIIE also participate in chromosome segregation [49,50,66,67], although again it is the role of SpoIIIE in transporting chromosomal DNA into the *B. subtilis* prespore that is an essential function; dissecting the precise role of these proteins in vegetative cells has been more difficult. Nevertheless, it seems clear that both proteins, which are tethered to the septum or prespore septum by their N-terminal domains, use repeated and polarized DNA sequences to direct rapid chromosomal DNA translocation. With *E. coli* FtsK, this translocation will occur when either chromosome dimers or catenanes have led to a delay in unlinking and DNA is trapped in the vicinity of the septum [49,50,67]. Translocation brings the sister termini to this region where XerCD or TopoIV can mediate the final unlinking.

Concluding remarks and future perspectives

E. coli, and bacteria in general, are not the simple bags of enzymes and DNA that the first molecular biologists often imagined. Although supremely convenient tools for extracting DNA, RNA and protein, they are very highly evolved for an adaptable single-cell lifestyle. We note that there are more bacterial cells associated with the average human than human cells in the body of that person, and

the evolutionary potential of a single person's bacteria in one year is likely to exceed that of vertebrate evolution on this planet over the past hundreds of millennia. Bacteria, most of which have never been cultivated, constitute the majority of the earth's biomass and are responsible for most bioconversion and implicate themselves in many processes related to human health and diseases. Bacteria are not only ideal model organisms for studying fundamental life processes, but have their own huge interest and biological importance.

Although our discussion has focused on *E. coli*, the processes that compact and organize bacterial chromosomes are likely to be highly conserved across the eubacteria, with the SMC proteins having a key role in chromosome organization and FtsK family translocases having important roles in the late stages of chromosome segregation. We believe that, like *E. coli* [37], other bacteria (e.g. see Ref. [68]) will assemble replisomes at activated origins, irrespective of their position [69], with sister replisomes derived from a given initiation acting independently [7,42]. In both *C. crescentus* and *B. subtilis* [39,55], sister replisomes seem to be spatially unresolved in cells much of the time, although, in *B. subtilis*, transient separation and re-grouping if sister replisomes has been observed [68,70]. The apparent difference in replisome behavior between *C. crescentus* and *E. coli* could arise from their different chromosome organizations (Figure 1); a high-resolution picture of chromosome organization in exponentially growing *B. subtilis* remains to be determined.

We believe that the principles of chromosome organization, replication and segregation are manifest in the biology of a particular bacterium in a way dependent on its lifestyle. Such lifestyles can incorporate developmental stages, as in *B. subtilis* or *C. crescentus*, or allow (*E. coli*) or do not allow (*C. crescentus*) overlapping replication cycles. In the latter case, there are opportunities to evolve sophisticated cell cycle checkpoint responses. Different morphological shapes can arise without any changes in the ways in which the chromosome is organized, replicated or segregated. Rod-shaped *E. coli* can be converted to viable spherical *E. coli*, which presumably have a spherical nucleoid of precise organization that can be efficiently replicated and segregated [71,72]. Similarly, MukBEF$^-$ *E. coli*, which have the mid-cell positioning of *oriC* replaced by a polar location, are still able to grow [30]. Therefore, neither the shape nor the organization of the nucleoid seems to be essential for the life and propagation of a given cell. Nevertheless, in an analogy to what has been proposed for the bacterial cell shape [73], we believe that the maintenance of a precise nucleoid organization in a given bacterial species is because of selective pressure acting on a function.

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