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The Bacterial Nucleoid: A Highly Organized and Dynamic Structure

Martin Thanbichler, Sherry C. Wang, and Lucy Shapiro*

Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305-5329

Abstract Recent advances in bacterial cell biology have revealed unanticipated structural and functional complexity, reminiscent of eukaryotic cells. Particular progress has been made in understanding the structure, replication, and segregation of the bacterial chromosome. It emerged that multiple mechanisms cooperate to establish a dynamic assembly of supercoiled domains, which are stacked in consecutive order to adopt a defined higher-level organization. The position of genetic loci on the chromosome is thereby linearly correlated with their position in the cell. SMC complexes and histone-like proteins continuously remodel the nucleoid to reconcile chromatin compaction with DNA replication and gene regulation. Moreover, active transport processes ensure the efficient segregation of sister chromosomes and the faithful restoration of nucleoid organization while DNA replication and condensation are in progress. J. Cell. Biochem. 96: 506–521, 2005. © 2005 Wiley-Liss, Inc.

Key words: bacterial chromosome; topological domains; histone-like proteins; SMC; linear ordering of chromosomal loci; DNA segregation; MreB

Bacteria have been intensively studied for more than a century, serving as valuable models to unravel fundamental problems like the function of metabolic pathways, gene regulation and expression, recombination, DNA repair, pathogenesis, and evolution. Until recently, however, little attention was paid to the mechanisms that structurally and functionally organize the bacterial cell and thereby ensure the orderly and coordinated progression of events during the cell cycle. This was partly due to the notion that the ancient evolutionary origin of bacteria, their comparably small genomes, and their tiny dimensions probably obviate a complex cellular architecture. Support for this idea also came from genome sequencing data, which showed that bacteria lacked apparent homologues of known eukaryotic cytoskeletal proteins. On the other hand, the microscopic techniques available did not provide the resolution to dissect the minute structures within prokaryotic cells, which largely frustrated initial attempts to detect subcellular organization in bacteria.

This situation changed dramatically after the development of more powerful light and electron microscopic imaging methods and the concomitant adaptation of cell biological methods to bacterial cells. In particular, the ability to resolve the subcellular localization of defined proteins by fusing them to green fluorescent protein (GFP) or its derivatives opened new doors for investigating cellular processes and considerably advanced the field of microbial cell biology. Thanks to these methodological improvements, bacteria have turned out to be highly organized entities that employ a complex network of regulatory circuits and dynamic cytoskeletal structures to implement their developmental program. Temporal and spatial localization of proteins—long regarded as a hallmark of eukaryotic cells—emerged as a common theme in bacteria, used to establish cell polarity and to create regulatory check points that couple cellular differentiation to cell cycle progression.

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*Correspondence to: Lucy Shapiro, Department of Developmental Biology, Stanford University School of Medicine, Beckman Center B300, 279 Campus Drive, Stanford, CA 94305-5329. E-mail: shapiro@stanford.edu

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The current change in the perception of prokaryotes is significantly influenced by the progress made in understanding prokaryotic chromatin organization and dynamics. Bacteria generally possess one circular chromosome, even though species with multiple circular or linear chromosomes have been described [Volff and Altenbuchner, 2000; Teyssier et al., 2004; Egan et al., 2005]. Unlike eukaryotes, they do not seclude DNA in a separate membrane compartment, but maintain it freely floating in the cytoplasm. Prokaryotic chromatin is, nevertheless, highly condensed and forms a pseudo-compartment that frequently occupies a distinct region within the cell, which is characterized by the absence of ribosomes [Robinow and Kellenberger, 1994]. This chromatin-dense area is functionally equivalent to the eukaryotic nucleus and is, therefore, termed nucleoid.

Despite intensive efforts, electron microscopic analysis of bacterial cells has not given any conclusive indications for organizational patterns within the nucleoid. As, furthermore, the existence of specialized proteins involved in bacterial chromosome condensation has long remained elusive, the nucleoid was generally believed to be an unordered aggregate of randomly coiled DNA. It was, however, puzzling how disordered, entangled chromatin could allow the high replication rates and the exquisite fidelity of chromosome segregation observed for bacteria. This conundrum was solved by recent work, which will be discussed in the following sections, showing that the bacterial chromosome in fact exhibits a clearly defined structure that undergoes dynamic, yet strictly controlled and reproducible changes in the course of the cell cycle.

TOPOLOGICAL STRUCTURE OF THE CHROMOSOME

First insights into the architecture of bacterial chromatin were gained in the 1970s, when Worcel and Burgi [1972] probed nucleoids isolated from *Escherichia coli* and found that the DNA contained therein is compacted by unconstrained supercoiling. In contrast to plasmids, however, which can be relaxed by only one single-strand break, a number of nicks were necessary to completely relax a chromosome. The results obtained by these studies, therefore, suggested the existence of discrete chromosomal domains that are topologically unlinked from each other. A similar conclusion was later drawn from data produced with a different biochemical method [Sinden and Pettijohn, 1981]. At the same time, electron microscopic analysis of *E. coli* nucleoids revealed that the chromosome is organized into plectonemically wound loops of DNA that radially emanate from a central core [Delius and Worcel, 1974]. Interestingly, the number of loops was comparable to the number of domains as defined by biochemical means, which suggests a direct correlation between chromatin arrangement and topology.

Recent findings indicate that this modular design of the chromosome, which has been found in all bacteria studied so far [Bendich, 2001], is highly dynamic. For example, comparing the efficiencies of site-specific recombination between differently spaced $\gamma\delta$ resolvase-binding sites revealed that the boundaries of topological domains are not static structures associated with specific sequences of the *E. coli* chromosome. They rather appear to be placed in a completely random way, so that their positions vary stochastically within a population of cells [Higgins et al., 1996; Deng et al., 2004]. These findings, which were based on probing small, defined regions of the chromosome, were corroborated by a whole genome approach aimed at determining the length and distribution of topological domains in *E. coli* on a global scale [Postow et al., 2004]. To this end, supercoils were relaxed in live cells using an inducible rare-cutting restriction endonuclease. The spread of relaxation resulting from the double strand breaks was then monitored by microarray-based analysis of changes in the transcription of several hundred genes whose expression was known to be influenced by the degree of local supercoiling. Monte Carlo simulations of this experiment showed that the transcriptional profiles obtained could be fitted best by a model that assumed that domain boundaries are fluid and randomly distributed over the chromosome. The length of individual supercoiled domains thereby follows an exponential distribution with a mean value of about 10 kb, which corresponds to approximately 500 domains per chromosome. These computationally derived results are in good agreement with values obtained by analyzing the lengths of individual supercoiled loops on electron micrographs of gently isolated *E. coli* nucleoids [Postow et al., 2004].

Given that these experiments were performed with asynchronous populations of cells, it remains an open question whether the size and position of topological domains are truly random parameters. Alternatively, one could imagine that cell cycle-dependent processes continuously remodel the chromosome in a dynamic but largely reproducible fashion. If so, the positions of domain boundaries would be comparable in cells at the same developmental stage, and yet appear random when averaged over a mixed population.

The existence of small and dynamically positioned topological domains provides several advantages for bacteria [Postow et al., 2004]. First, lesions in the chromosome caused by DNA damage, repair processes, or replication only relax one single domain without affecting the superhelicity of other DNA regions. This design is vital for the cell because the torsional tension of supercoiled DNA is an important reservoir of free energy, which helps to drive all processes requiring the melting of DNA strands, such as transcription [Lim et al., 2003; Peter et al., 2004], replication [Funnell et al., 1986], and recombination [Nash, 1990]. The relevance of limiting the spread of relaxation is underlined by the fact that even slight changes in the overall superhelicity of the chromosome are lethal [Gellert et al., 1976, 1977]. Short distances between domain boundaries might, in addition, facilitate the repair of double-strand breaks because they restrict the movement of the two ends to be joined. By concentrating catenane links and thus increasing the free energy of catenanes, they further improve the efficiency of chromosome unlinking at the end of DNA replication [Postow et al., 2004]. Finally, organization into small supercoiled modules significantly reduces the chromosomal radius of gyration, thereby making an important contribution to the overall compaction of bacterial chromatin [Trun and Marko, 1998].

MECHANSIMS OF NUCLEOID ORGANIZATION

The nature of the factors implicated in structuring the nucleoid is diverse. Topological domains, for example, can be established by any mechanism that prevents a stretch of chromosomal DNA from freely rotating around its long axis. RNA was hypothesized to impose such constraints by associating with and stably interconnecting different supercoiled loops [Pettijohn and Hecht, 1974]. Localized DNA-binding proteins, such as RacA [Ben-Yehuda et al., 2003], Spo0J (ParB) [Lin and Grossman, 1998; Easter and Gober, 2002], and FtsK [Aussel et al., 2002] might, in contrast, prevent the diffusion of supercoils beyond their binding sites by tethering the DNA they interact with to stationary structures within the cell. Topoisomerases are thought to create topological barriers in at least two different ways: on the one hand, they were shown to preferentially bind to juxtaposed DNA helices, which could allow them to generate transient interconnections between different DNA regions [Zechiedrich and Osheroff, 1990]. Accordingly, DNA gyrase was demonstrated to block diffusion of transcription-induced supercoils in *E. coli*, when associated with its catalytic target sites [Moulin et al., 2005]. On the other hand, topoisomerases are responsible for decatenating entangled DNA and controlling the superhelicity of individual DNA segments. This gives them control over the number of topological knots and tangles in chromosomal DNA, which was proposed to be a major determinant of the size of topological domains in exponentially growing cells [Staczek and Higgins, 1998]. Finally, transcription has emerged as an important regulator of nucleoid topology. The RNA polymerase complex is too large to follow the individual helical turns of DNA. Its movement along the template strand, therefore, transiently generates domains of positive and negative supercoiling, respectively, ahead and behind the transcriptional bubble [Wu et al., 1988; Rahmouni and Wells, 1992; Moulin et al., 2005]. These topological changes are significantly stabilized if the transcript encodes a membrane protein [Liu and Wang, 1987; Lodge et al., 1989; Lynch and Wang, 1993], because transcription and co-transcriptional translation are coupled with the insertion of nascent polypeptides into the membrane in bacteria, thereby tethering RNA polymerase to the site of protein translocation [Binenbaum et al., 1999]. This makes the transcription complex an efficient barrier to the diffusion of supercoils between the DNA regions upstream and downstream of the transcriptional bubble. However, it was shown that even transcription in the absence of membrane translocation can induce stable domain barriers, if driven by a strong promoter [Deng et al., 2004].

The factors discussed to this point mostly affect chromosome organization in an indirect way. Their diversity and dynamic mode of action might explain the apparently random organization of topological domains. However, there are also a number of proteins that are specifically designed to modulate the structure of bacterial chromatin.

Many of them belong to the histone-like proteins [Dame, 2005], a heterogeneous family of basic, nucleoid-associated polypeptides that are characterized by their low molecular weights and high intracellular abundance. Out of the twelve members of this group identified in *E. coli* [Azam and Ishihama, 1999], the factor for inversion stimulation (Fis), the integration host factor (IHF), the heat-stable nucleoid-structuring protein (H-NS), and the heat-unstable protein (HU) have been studied in greatest detail.

Fis is the most abundant histone-like protein in exponentially growing cells, whereas its intracellular level decreases dramatically at the onset of stationary phase in *E. coli* [Ali Azam et al., 1999]. It shows preference for a poorly conserved core binding motif, the affinity for which is modulated by its flexibility [Bailly et al., 1995] and sequence context [Pan et al., 1996]. Association with Fis bends DNA by 50–90° [Pan et al., 1996], thereby stabilizing writhe and promoting the extrusion of branches in supercoiled substrates (Fig. 1a) [Schneider et al., 2001]. This activity might play an important role in governing the global dynamics of chromatin reshaping in bacteria. Fis was, however, also identified as a pleiotropic regulator of gene expression [Bosch et al., 1990; Schneider et al., 1999; Hirvonen et al., 2001; Kelly et al., 2004] and site-specific recombination [Koch and Kahmann, 1986; Thompson et al., 1987]. It is thought to act as a topological homeostat that modulates local supercoiling in selected promoter regions in order to maintain conditions that are favorable for transcription. Fis-induced structural changes might thereby reposition promoters at the apices of branches, thus isolating them in small domains of optimally supercoiled DNA and exposing them to the transcription machinery [Rochman et al., 2002; Muskhelishvili and Travers, 2003].

A clear consensus sequence can be defined for the sites preferentially bound by IHF [Engelhorn et al., 1995], although interaction with low-affinity sites was proposed to play a biologically significant role [Ali et al., 2001]. IHF bends DNA by ~160° [Rice et al., 1996; Lorenz et al., 1999], and this ability to induce topological changes might be relevant for nucleoid organization. In fact, a single-molecule study demonstrated that binding of IHF to multiple sites could condense linear DNA into a more compact, but randomly coiled structure [Ali et al., 2001]. IHF was, in addition, found to be a crucial cofactor in a number of regulatory processes [Goosen and van de Putte, 1995]. It might act by binding to specific sites in promoter regions, thereby inducing DNA loops that allows interaction between RNA polymerase and distally bound regulatory proteins [Seong et al., 2002]. By a similar mechanism, it facilitates the assembly of initiation complexes at the replication origins of plasmids [Filutowicz and Inman, 1991; Stenzel et al., 1991] and bacterial chromosomes [Cassler et al., 1995].

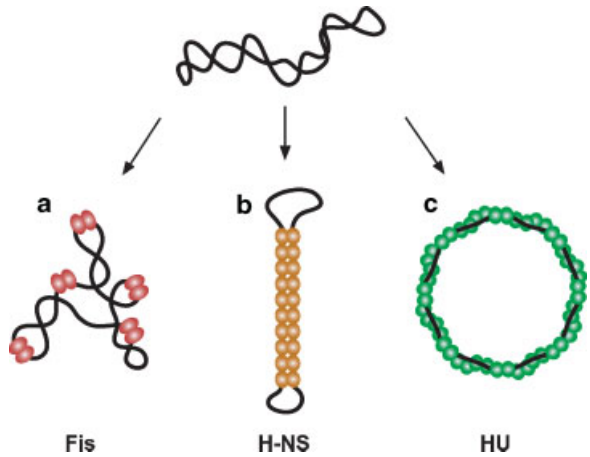

Fig. 1. Effect of histone-like proteins on the conformation of a supercoiled plasmid. **a**: Binding of Fis promotes branching of supercoiled circular DNA substrates. **b**: At elevated concentrations, H-NS assembles into patches of oligomers that interact with each other, thereby converting supercoiled plasmids into dumbbell-shaped structures. **c**: At high concentrations, HU assembles into rigid nucleoprotein filaments that open up supercoiled DNA into a ring-like conformation.

H-NS is thought to interact unspecifically with DNA, although it prefers intrinsically curved binding sites [Dame et al., 2001], and it was shown to act as global regulator controlling the expression of more than 200 genes in *E. coli* [Hommais et al., 2001]. The mechanism by which H-NS influences RNA polymerase activity is not fully understood. Its function as a transcription factor is, however, tightly connected with its ability to oligomerize [Rimsky et al., 2001] and condense DNA into stable

nucleoprotein complexes [Schroder and Wagner, 2000; Dame et al., 2002; Dole et al., 2004; Prosseda et al., 2004], thereby occluding promoter regions or blocking transcription elongation. Recently, atomic force microscopy was used to visualize the effects of H-NS on DNA topology [Dame et al., 2000]: after initial binding at dispersed sites, H-NS assembles into patches of oligomers that interact with each other, thus bridging different DNA regions. Further cooperative polymerization then leads to extension of these bridges into rigid, rod-like filaments (Fig. 1b), which finally collapse into dense globular aggregates. A role in DNA condensation is further supported by the observation that overexpression of H-NS leads to abnormally compacted nucleoids in *E. coli* [Spurio et al., 1992].

The condensing activity of H-NS is thought to be antagonized by HU [Dame and Goosen, 2002], which interacts with DNA in an unspecific manner, but appears to prefer structural distortions such as nicks, gaps, and cruciforms [Castaing et al., 1995; Kamashev et al., 1999]. At low concentrations, HU moderately compacts DNA by inducing flexible bends and negative writhe [Swinger et al., 2003; Sagi et al., 2004; van Noort et al., 2004]. Increased protein levels, however, trigger polymerization of HU into rigid, helical filaments without inducing significant condensation [Sagi et al., 2004; Skoko et al., 2004; van Noort et al., 2004]. Formation of such nucleoprotein complexes could locally reverse H-NS-induced DNA condensation [Dame, 2005]. This hypothesis is supported by the observation that binding of HU opens up supercoiled plasmid DNA (Fig. 1c), whereas H-NS converts circular DNA substrates into compact, dumbbell-shaped structures [Dame and Goosen, 2002]. In agreement with an antagonistic function of these proteins, H-NS-mediated transcriptional repression is enhanced in mutants lacking HU, but alleviated when HU is overexpressed [Manna and Gowrishankar, 1994; Delihas and Forst, 2001]. A general connection between HU-induced architectural changes and gene regulation is further supported by numerous reports that link HU to promoter function, although the molecular details are frequently unknown [Wagner, 2000]. As both the overall concentration and the subunit composition of the protein are subject to growth phase-dependent changes [Claret and Rouviere-Yaniv, 1997; Ali Azam et al., 1999], HU might dynamically modulate chromosome structure to adjust gene expression to the physiological state of the cell.

Finally, although initially identified as components of eukaryotic condensin and cohesin complexes [Hirano, 2002], SMC proteins have emerged as important players in bacterial chromosome organization. They are large polypeptides, characterized by conserved N- and C-terminal regions, which carry a Walker A and B nucleotide-binding motif, respectively. The central part consists of two coiled-coil motifs that are separated by a moderately conserved linker region. These motifs fold back on each other, thus creating a long antiparallel coiled-coil and allowing the assembly of the two terminal segments into a functional ATP-binding domain [Melby et al., 1998; Hirano and Hirano, 2002]. The linker regions of two monomers then interact to form a V-shaped complex with a variable opening angle between 0 and 180° [Melby et al., 1998; Hirano et al., 2001]. A similar arrangement was observed for MukB from *E. coli*, which might represent a highly divergent SMC homolog [Niki et al., 1991, 1992; Melby et al., 1998]. In the presence of DNA, ATP binding promotes end-to-end association of different SMC dimers, which initiates the formation of larger nucleoprotein complexes, but in turn also activates hydrolysis of the bound nucleotides [Hirano et al., 2001]. The mechanistic basis of this phenomenon has recently been clarified by solving the crystal structure of the SMC ATP-binding domain in complex with ATP. It revealed that two molecules of ATP are tightly sandwiched in between two interacting domains, making essential contacts with residues on both sides of the subunit interface. Their hydrolysis is stimulated by double-stranded DNA, which might directly interact with the complex and alter the conformation of the ATPase catalytic site [Lammens et al., 2004]. Both SMC and MukB were shown to cooperate with two accessory proteins, which regulate their function [Hirano and Hirano, 2004] and are essential for their activity in vivo [Yamanaka et al., 1996; Mascarenhas et al., 2002]. Inactivation of either of these complexes leads to temperature-sensitive growth due to a severe disturbance of nucleoid organization and chromosome segregation at elevated temperatures [Niki et al., 1991; Britton et al., 1998; Moriya et al., 1998; Jensen and Shapiro, 1999]. This phenotype

was shown to be suppressed by mutations that cause excessive negative supercoiling, which suggests a role for these proteins in DNA compaction or folding [Sawitzke and Austin, 2000; Lindow et al., 2002a]. Such a function is supported by the global chromosome compaction phenotype observed upon overproduction of SMC in *B. subtilis* [Volkov et al., 2003]. Interestingly, SMC and MukB form discrete clusters within the cell, which are often associated with the replisome or the cell poles [Graumann et al., 1998; Ohsumi et al., 2001; den Blaauwen et al., 2001; Lindow et al., 2002b; Jensen and Shapiro, 2003]. They might, therefore, act in a very early step of chromatin packaging that prepares DNA for further condensation by other systems. It has been suggested that SMC dimers form ring-like structures that embrace two or more DNA strands, thereby stabilizing crossovers and interconnecting different regions of the chromosome [Nasmyth, 2002; Volkov et al., 2003; Hirano and Hirano, 2004].

Apparently, the nucleoid is organized by a number of delicately balanced processes. Macromolecular crowding, supercoiling, and topological constraints imposed by a variety of different proteins act to dynamically condense the chromosome, whereas HU-mediated rigidification of DNA, removal of topological domain barriers during replication, and envelope attachment of DNA by the coupled transcription and translation of membrane proteins counteract compaction. This flexible system allows the cell to reduce the size of its chromosome by about 1,000-fold and concomitantly use DNA condensation as a versatile mechanism to regulate global gene expression in response to a number of physiological inputs.

SPATIAL ARRANGEMENT OF THE BACTERIAL CHROMOSOME

Analysis of site-specific recombination between distant pairs of λ integrase binding sites in *Salmonella typhimurium* [Garcia-Russell et al., 2004] and *E. coli* [Valens et al., 2004] suggests that the movement of DNA within the cytoplasm might be constrained. The results obtained in these studies show that efficient interaction between two sites is only possible within defined chromosomal regions, suggesting the existence of a higher-order structure, in which regions of the nucleoid are spatially separated from each other. More direct evidence for this hypothesis has recently been gained by investigating chromatin organization with cell biological methods. Adapting fluorescence in situ hybridization (FISH) to bacteria made it possible to visualize specific DNA regions in fixed specimens. Fixation, however, bears the danger of disrupting the original arrangement of the chromosome and does not permit the investigation of chromosome dynamics in living organisms. These problems are circumvented by a novel technique, which uses the *lac* or *tet* repressor fused to a fluorescent protein to specifically decorate tandem repeats of the cognate repressor binding site inserted at a chromosomal locus of interest [Robinett et al., 1996; Straight et al., 1996; Michaelis et al., 1997; Lau et al., 2003]. Using these two approaches, it was shown that bacteria possess mechanisms to orient the replication origin and terminus towards opposite poles in newborn cells [Gordon et al., 1997; Webb et al., 1997; Niki and Hiraga, 1998; Jensen and Shapiro, 1999; Fogel and Waldor, 2005]. This finding already indicates some degree of order within the nucleoid. In agreement with this idea, two sites located at the quarter positions of the circular chromosome were found to consistently localize in-between the subcellular regions occupied by the origin and terminus in *B. subtilis* [Teleman et al., 1998]. Moreover, probing twenty-two sites evenly distributed over the chromosome, Niki et al. [2000] showed that genes within ~900 kb origin-proximal and terminal regions, respectively, colocalize with the origin and terminus in *E. coli*, thus constituting two independent macrodomains. Intermediate loci, by contrast, adopted subcellular positions in-between these two fixed points. Interestingly, the macrodomains identified in this study are identical to regions of elevated recombination frequency as defined by Boccard and coworkers [Valens et al., 2004]. The results of the above studies suggest a correlation between the physical position of a locus on the chromosome and its spatial localization within the cell. However, definitive conclusions were complicated by the moderate density of probed sites, the inability to synchronize *B. subtilis* and *E. coli* cultures, and the tendency of these bacteria to accumulate multiple partially replicated chromosomes. Recent study by Viollier et al. [2004] avoided these issues by focusing on synchronized G_1 cells of *Caulobacter crescentus*. Using both targeted

and random, transposon-mediated insertion, a library of 112 strains was created that carried tandem arrays of the *lac* and *tet* repressor binding sites at known positions on the chromosome. After visualization by fluorescence microscopy, the subcellular localization of each of the labeled sites was determined by measuring the position of the fluorescent focus in several hundred cells with the help of automated image analysis. It emerged that every chromosomal locus adopts a specific subcellular position, with its spatial localization along the cellular long-axis showing a linear dependence on its position on the chromosomal map relative to the origin of replication. The existence of macrodomains as suggested for *E. coli* [Niki et al., 2000] was not observed.

These results clearly argue for the existence of a defined chromosome superstructure. They also imply an architecture in which tightly packed loops of adjacent DNA segments are stacked on top of each other in layers that are largely perpendicular to the long-axis of the cell. The two arms of the circular chromosome could thereby either be physically separated, which would result in a ring-like overall structure, or closely intertwined (Fig. 2). The limited resolution of light microscopy does not allow a distinction between these two possibilities. The former, however, is supported by the fact that circular nucleoids were observed in *E. coli* under certain conditions [Niki et al., 2000; Kim et al., 2004]. Other models for chromosome organization, in contrast, such as a random coil structure, layers of loops parallel to the long-axis of the cell, or a rosette of loops protruding from a central core can be eliminated as they are not compatible with the linear layout observed [Breier and Cozzarelli, 2004].

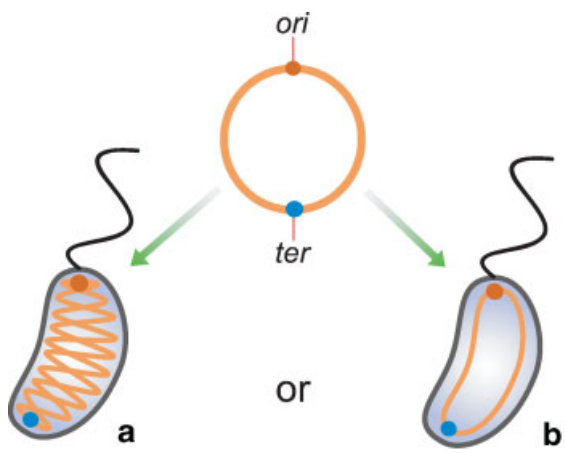

Fig. 2. Model for the arrangement of chromosomal DNA in the *C. crescentus* swarmer cell. Viollier et al. [2004] showed that the subcellular position of chromosomal sites is linearly dependent on their distance on the chromosomal map from the origin of replication. This finding implies that the chromosome is condensed into a self-contained structure, consisting of tightly packed stacks of adjacent supercoiled loops. The two arms of this structure could either be (**a**) intertwined or (**b**) physically separated, adopting a ring-like overall arrangement.

DYNAMICS OF THE REPLICATION PROCESS

The cellular organization of the chromosome, by necessity, changes during the process of of DNA replication.

In *B. subtilis* and *E. coli*, under conditions of slow growth, new-born cells contain a single, fully replicated chromosome, with the origin and terminus being positioned on opposite sides of the nucleoid near the cell poles. Its replication starts with the movement of the origin to the relisome [Webb et al., 1998; Roos et al., 1999; Niki et al., 2000; Li et al., 2002], which is positioned at the center of the cell [Lemon and Grossman, 1998; Smith et al., 2001]. Upon initiation of strand synthesis, the newly synthesized origin regions are separated and moved toward opposite cell poles. In *B. subtilis*, segregation of the daughter strands appears to occur as replication proceeds [Webb et al., 1998]. For *E. coli*, the situation is less clear, as data obtained by FISH analysis suggest that the two sister chromosomes cohere for an extended period of time [Hiraga et al., 2000; Sunako et al., 2001], while other studies on the localization of *oriC* in live cells suggest earlier segregation [Roos et al., 2001; Li et al., 2002; Lau et al., 2003].

In *C. crescentus*, replication is always initiated only once per cell cycle, irrespective of the growth conditions. At the swarmer cell stage, which is characterized by a quiescent chromosome and, therefore, equivalent to the G_1-phase of the cell cycle, the origin and terminus are located at the two opposite poles of the cell. After assembly of the replisome at the polar origin and initiation of DNA synthesis in the newly differentiated stalked cell, the duplicated strands are rapidly segregated, with one origin staying at the original position, while the second moves to the other end of the cell [Jensen and Shapiro, 1999; Jensen et al., 2001; Viollier et al., 2004]. As replication continues, the replisome is gradually displaced from its

initially polar position to the division plane [Jensen et al., 2001]. Examination of the movement and cellular positioning of individual chromosomal loci during the course of replication revealed that duplicated DNA segments are immediately recondensed and rapidly moved in opposite directions to assume their final positions in the incipient daughter cells. Interestingly, DNA segregation appeared to occur in two distinct phases—an initial phase of fast translocation from the replisome to the daughter cell compartments and a subsequent slower phase, which might correspond to the final integration of the arriving segments into the tightly compacted superstructure of the chromosome [Viollier et al., 2004].

In *Vibrio cholerae*, finally, the situation is complicated by the fact that the genome is divided into two replicons. Chromosome I contains most of the essential genes, while chromosome II only carries a few essential functions and is probably derived from a megaplasmid [Heidelberg et al., 2000]. The replication origins of these two chromosomes localize to distinct positions during all stages of the cell cycle [Fogel and Waldor, 2005]. The origin of chromosome I is initially located close to one pole of the cell. After initiation of DNA replication, one of its copies remains fixed at the original position, whereas the other one rapidly traverses the cell to adopt is final position at the opposite pole, as is the case for the single chromosome in *C. crescentus*. The origin of chromosome II, by contrast, starts out at the cell center, and its copies are moved to the quarter positions of the mother cell. Although both chromosomes initiate replication at the same time [Egan et al., 2004, 2005], segregation of chromosome II takes place later in the cell cycle.

CHROMOSOME SEGREGATION

The mechanism underlying bacterial DNA segregation has long been a matter of speculation. Initially, it was proposed that the newly replicated chromosomes are attached to the cell membrane and passively separated by insertion of new lipids in-between them [Jacob et al., 1963]. The speed of origin movement, however, reaches 0.1–0.3 μm/min [Webb et al., 1998; Viollier et al., 2004] and similar values were measured for the segregation of other chromosomal loci [Viollier et al., 2004]. This exceeds the rate of cell elongation by nearly two orders of magnitude and argues for the existence of a system that actively segregates DNA upon release from the replisome. Recently, RNA polymerase has been suggested to provide the force for this process [Dworkin and Losick, 2002]. It is a powerful and abundant motor protein that is thought to translocate the template rather than moving along DNA itself during RNA synthesis. As transcription of highly expressed genes tends to be directed away from the origin of replication, it was hypothesized that the combined and coordinated effects of many transcription events could result in a net displacement of nascent nucleoids towards the poles. The extrusion-capture model, by contrast, suggests that the chromosomes are pushed apart by the bidirectional extrusion of the newly synthesized daughter strands from the replisome, driven by the free energy of DNA polymerization [Lemon and Grossman, 2000]. This process is thought to be assisted by factors that capture and maintain the origin regions at the poles and by proteins that condense DNA, thereby exerting a pulling force that guides replicated DNA into the nascent daughter cells.

It is conceivable that polymerization reactions and DNA condensation, potentially assisted by constrained diffusion of DNA [Woldringh, 2002], act in concert to partition the bulk of the nascent chromosomes. However, these processes can neither account for the exquisite directionality of origin movement nor for the distinct temporal and spatial localization patterns observed for the origin regions of multipartite genomes. Bacteria might, therefore, possess specific mechanisms that position the origins of replication, which then serve to guide subsequent DNA segments into the nascent daughter cell compartments. In agreement with this idea, a short DNA sequence (*migS*) with centromer-like function has been identified close to *oriC* in *E. coli* [Yamaichi and Niki, 2004; Fekete and Chattoraj, 2005]. It is necessary and sufficient for bipolar localization of the origins and acts independent of its position on the chromosome. Moreover, homologs of the plasmid partitioning proteins ParA and ParB are encoded in most bacterial genomes sequenced so far. At present, their exact function is still unclear. It was, however, demonstrated that ParB interacts with conserved binding sites which are clustered in the origin-proximal region [Lin and Grossman,

1998; Easter and Gober, 2002; Bartosik et al., 2004] and that both proteins are necessary for proper chromosome segregation [Glaser et al., 1997; Mohl and Gober, 1997; Marston and Errington, 1999; Kim et al., 2000; Lewis et al., 2002]. With the exception of *C. crescentus*, however, deletion of *parA* or *parB* is not lethal and only results in mild growth phenotypes, which might imply redundancy in the systems that control positioning of the chromosome. Finally, RacA, a protein only expressed in sporulating cells of *B. subtilis*, binds to a number of sites within ~200 kb of the origin, thus forming a large nucleoprotein complex that attaches the chromosome to the cell pole in the fore spore compartment [Ben-Yehuda et al., 2003; Wu and Errington, 2003]. Deletion of the corresponding gene leads to a high frequency of anucleate spores, whereas overexpression during vegetative growth was shown to shift the nucleoids from their central positions to the very poles of the cells [Ben-Yehuda et al., 2003].

Recent findings indicate that the actin homolog MreB is an important component of the bacterial chromokinetic machinery. It is found in a variety of bacteria and polymerizes into spiral-like filaments that span the inner surface of the cytoplasmic membrane. Initially, it was identified as a cytoskeletal element that serves as a track for enzymes involved in peptidoglycan biosynthesis and is responsible for the maintenance of a rod-shaped cell envelope [Jones et al., 2001; Daniel and Errington, 2003; Figge et al., 2004]. Later findings, however, suggested that MreB participates in a number of additional cellular processes. It was implicated in protein localization and the establishment of cell polarity in *C. crescentus* [Gitai et al., 2004]. Apart from that, inactivation or overexpression of the *mreB* gene severely affect polar positioning of the origins and chromosome segregation [Kruse et al., 2003; Soufo and Graumann, 2003; Gitai et al., 2004]. In these early studies, however, manipulations in the MreB cytoskeleton were based on genetic methods, which resulted in delayed expression of the phenotype and concomitant cell shape abnormalities. It thus remained unclear whether the segregation defects observed really were due to a primary function of MreB in chromosome partitioning. In the light of these doubts, it is interesting that deletion of *mreB* in *B. subtilis* can be complemented by high concentrations of magnesium and osmoprotective substances in the growth medium [Formstone and Errington, 2005]. This suggests that the activity of the protein is indeed focused on cell wall biosynthesis in this bacterium. On the other hand, *B. subtilis* possesses two more actin homologs (Mbl and MreBH), which are necessary for efficient chromosome segregation [Defeu Soufo and Graumann, 2004] and might, therefore, be able to compensate for the loss of MreB. More definitive evidence that the actin cytoskeleton actively promotes DNA segregation in at least a subgroup of bacteria comes from recent study performed in *C. crescentus*. Gitai et al. [2005] established that a novel antibiotic, A22 [Iwai et al., 2002], specifically targets the ATP-binding site of MreB, thereby leading to nearly instantaneous disassembly of the spiral-like filaments after addition to the culture. In cells treated with A22, DNA replication initiated and proceeded normally, but the newly duplicated origin regions were not separated. However, if the drug was removed from the growth medium later in S-phase, the cytoskeleton was restored and the origins were immediately moved to opposite poles of the cell. A functional MreB spiral, therefore, appears to be necessary for the initial steps of chromosome segregation. This hypothesis is further supported by the fact that loci in the vicinity of the origin of replication could be crosslinked, directly or indirectly, to MreB in chromatin immunoprecipitation experiments. By contrast, no effect on DNA partitioning was observed, if A22 was added after separation of the origin regions. These results suggest a sequential model for chromosome segregation, where separation of the sister chromatids is initiated by active transport of origin DNA into the incipient daughter cell compartments, thereby setting the corner stones for the establishment of two new daughter nucleoids (Fig. 3). Subsequent DNA segments then follow by different mechanisms, most probably the pulling force generated by DNA condensation.

THE LAST STAGES OF DNA REPLICATION

Bacterial cell division is a highly coordinated process, with many mechanisms in place to ensure proper partitioning of the sister chromosomes to the daughter cells. Among the events involved, segregation of the terminus region represents an especially critical step, which is complicated for several reasons.

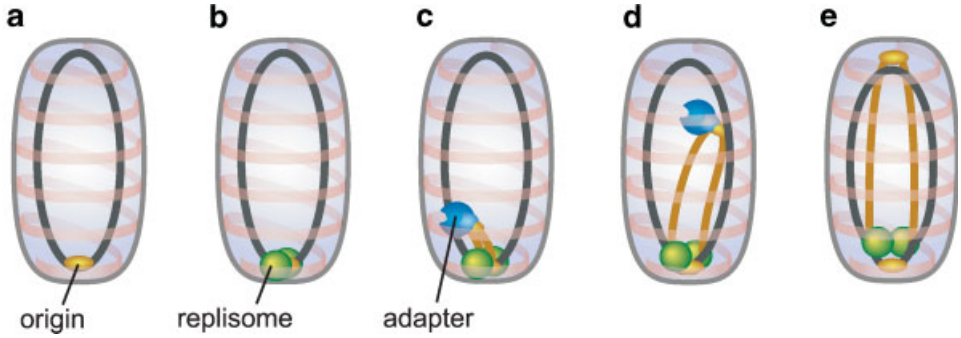

Fig. 3. Model for the rapid segregation of the origin regions in *C. crescentus*. **a**: Prior to DNA replication, the cell contains a single chromosome with the origin located at one of the cell poles. **b**: DNA replication is initiated by assembly of the replisome on the origin region (**c**, **d**). The newly duplicated origin region is recognized by a putative adapter complex and linked to the MreB cytoskeleton. Dynamic movement of the MreB spiral within the cell or migration of the adaptor complex along the MreB filament leads to the rapid and directed transport of the origin to the opposite pole of the cell. **e**: After reaching its target localization, the origin is released and stably positioned at the cell pole.

First, the end of DNA replication poses a topological dilemma, as it leaves two catenated circular chromosomes. This intermolecular link must be resolved by topoisomerase IV before closure of the division septum can occur [Adams et al., 1992; Peng and Marians, 1993]. The importance of this reaction is underscored by the fact that loss of decatenation activity causes filamentation, accumulation of anucleate cells, and cell death in *E. coli* and *Salmonella typhimurium* [Kato et al., 1988, 1990; Springer and Schmid, 1993]. Similar partitioning and cell division defects were observed in *C. crescentus* [Ohta et al., 1997; Ward and Newton, 1997]. A recent study in this organism further showed that topoisomerase IV might play a role in overall nucleoid organization as conditional mutants lost the ability to correctly position the origins of replication at the cell poles [Wang and Shapiro, 2004].

Second, if an odd number of homologous recombination events occurs between the two sister strands, chromosome dimers are formed, which have to be resolved into monomers to allow segregation to be completed. This process is carried out by the combined action of the site-specific tyrosine recombinases XerC and XerD, which have been extensively characterized in *E. coli* [Lesterlin et al., 2004]. These enzymes specifically recognize a short sequence, named *dif*, in the terminal region of the chromosome and introduce an additional crossover, thereby generating two independent DNA molecules [Blakely et al., 1993].

Third, formation of the division septum takes place while DNA replication is still in progress. Immediately before cell division, the terminus regions are often found to be localized asymmetrically in one of the daughter cell compartments, so that DNA becomes trapped in the septal region of the cell [Lau et al., 2003]. Clearance of the division site is catalyzed by FtsK, a bifunctional protein that is comprised of an N-terminal transmembrane domain and a C-terminal ATPase domain, which are separated by a proline/glutamine-rich linker region in *E. coli*. The N-terminus is responsible for targeting FtsK to the division machinery, where it plays an essential role in the septation process [Draper et al., 1998; Yu et al., 1998]. The C-terminus, by contrast, is dispensable for divisiome function and acts as an ATP-dependent DNA translocase [Aussel et al., 2002; Saleh et al., 2004] that pumps DNA away from the septal region of the cells [Liu et al., 1998; Yu et al., 1998]. It is still unclear how FtsK determines the direction its substrate has to be translocated to be positioned in the correct daughter cell compartment. A recent single molecule study, however, demonstrated that the sequence of the terminus region is the sole determinant necessary for guiding the protein towards the *dif* site [Pease et al., 2005]. Directional information could be provided by short repeated sequences identified in the terminal part of the chromosome [Corre and Louarn, 2002], the orientation of which is biased and abruptly changes at *dif*, thereby pointing toward the site of dimer resolution [Salzberg et al., 1998].

In addition, FtsK coordinates the final steps of chromosome segregation with cell division.

It is not only involved in chromosome decatenation by stimulating the activity of topoisomerase IV [Espeli et al., 2003a,b] but also promotes dimer resolution by catalyzing synopsis of the terminal *dif* sites [Capiaux et al., 2002] and activating the XerCD recombination system [Aussel et al., 2002; Massey et al., 2004]. This elegant interplay between different systems at the septal region ascertains that DNA partitioning and cell septation occur at the right place and precisely at the right time in the replication process, thereby ensuring unhindered progression of the cell cycle.

FUTURE DIRECTIONS

The bacterial nucleoid is a complex structure divided into different, hierarchic levels of organization. A number of mechanisms fold chromosomal DNA into discrete supercoiled loops that are dynamically rearranged to fit the needs of the growing cell. These fluid topological domains, might interact with each other to form condensed filaments and loops, as recently suggested by atomic force microscopy of *E. coli* chromatin [Kim et al., 2004] and finally assemble into a defined ring-like superstructure. The mechanisms mediating the establishment of these distinct patterns are still unclear and their identification might necessitate the combination of cell biological and high-resolution electron microscopic approaches. Apparently, histone-like proteins and SMC complexes play an important role in chromosome condensation and organization, but their roles are still poorly defined. Many open questions also concern the issue of chromosome segregation. Meanwhile, it is largely accepted that partitioning of newly replicated origin regions occurs in an active process that, in *C. crescentus*, involves the actin homolog MreB. Nevertheless, it still remains to be established how the chromosome is attached to the bacterial cytoskeleton and what mechanism is responsible for translocation of the putative centromeric region along the helical filaments. However, there is also a considerable number of bacteria that lack an apparent MreB homolog. Different, independent mechanisms might, therefore, have evolved to ensure proper positioning of the origins of replication. Finally, the machinery responsible for segregation of the bulk of chromosomal DNA is still elusive, although many systems have been implicated in this process.

We have just started to understand the cell biology of bacteria. Yet, it has already emerged that many systems previously thought to be typically eukaryotic inventions are evolutionarily or functionally conserved in prokaryotes as well.

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