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The Bacterial Nucleoid: Nature, Dynamics and Sister Segregation

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Abstract

Recent studies reveal that the bacterial nucleoid has a defined, self-adherent shape and an underlying longitudinal organization and comprises a viscoelastic matrix. Within this shape, mobility is enhanced by ATP-dependent processes and individual loci can undergo ballistic off-equilibrium movements. In *E.coli*, two global dynamic nucleoid behaviors emerge pointing to nucleoid-wide accumulation and relief of internal stress. Sister segregation begins with local splitting of individual loci, which is delayed at origin, terminus and specialized interstitial snap regions. Globally, as studied in several systems, segregation is a multi-step process in which internal nucleoid state plays critical roles that involve both compaction and expansion. The origin and terminus regions undergo specialized programs partially driven by complex ATP burning mechanisms such as a ParAB Brownian ratchet and a septum-associated FtsK motor. These recent findings reveal strong, direct parallels among events in different systems and between bacterial nucleoids and mammalian chromosomes with respect to physical properties, internal organization and dynamic behaviors.

Introduction

Microorganisms have long provided powerful systems for understanding basic principles of chromosome organization, dynamics and function, beginning with the replicon hypothesis [1]. Over the past decade, increasingly sophisticated imaging analysis and insights from physical perspectives have been added to the more traditional tools provided by biochemistry, genetics and molecular biology. An important challenge for current research is to integrate all of these considerations to provide a deeper, more unified view.

The Nucleoid

An important advance in recent years has been appreciation that the circular DNA of the bacterial chromosome, referred to as the “nucleoid”, is a discrete, well-defined physical

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object. Recent fluorescence imaging of living cells and chromosome capture analysis of fixed cells reveal discrete shapes with defined longitudinal substructure [2–6] (Figure 1). Such findings confirm and extend earlier findings from imaging analysis and put to rest models in which the nucleoid comprises a randomly oriented fiber, either linear or topologically branched, which fills up the available cell space.

The nucleoid is self-adhering—Several lines of evidence reveal that there is a strong tendency for overall coalescence of chromosomal material, i.e. that the nucleoid is “self-adherent”. Imaging reveals that virtually all of the chromosomal DNA is part of the nucleoid shape [2,3] Figure 1AB. Also, during the replication/segregation process, elongation can sometimes be seen to involve lobes of protruding newly-replicated material, implying an intrinsic dynamic tendency for coalescence into elongated shapes [3] Figure 1CD. Finally, self-adherence is implied by the finding that individual loci, and pairs of loci, tend to have quite fixed positions relative to one another in resting (G1) nucleoids [7].

Radial, but not longitudinal, confinement—Non-septating cells exhibit chains of discrete nucleoids in the absence of inter-cell boundaries; moreover, the G1 nucleoid does not always extend to the end of the cell. Thus, the nucleoid is a discrete object in the absence of “longitudinal confinement”. In contrast, the nucleoid does touch the inner periphery of the cell in the radial dimension. Given that the shape tends to be helically curved, this contact is not uniform but instead mirrors the helical path. One implication of this configuration is that the nucleoid tends to define a complementary helical space around the cell periphery. Irrespective of molecular links between the nucleoid and the inner cell membrane, it also appears that the nucleoid as a whole tends to “push” outward the cell periphery, i.e. that the shape is “radially confined”. Confinement of the nucleoid in the radial dimension figures prominently in several aspects of chromosome organization, disposition and function (below).

At “G1”: a curved ellipsoidal shape with underlying longitudinal duality—The pre-replication (“G1”) nucleoid, as defined in *E.coli* and *C. crescentus*, tends to be thinner at the ends than in the middle, i.e. to be ellipsoidal, and also to be deformed into a gently curved helical-like shape (Figure 1ABG). Curvature does not necessarily have a specific handedness, implying that the important feature is curvature *per se* [2,3] (Figure 1AB). Also, the nucleoid DNA is denser centrally than radially [3,4]. Underlying this global shape is the fact that the DNA tends to be organized into a pair of parallel bundles that extend longitudinally along the nucleoid length and rotate gently relative to one another to give the gently curved, helical-like nucleoid shape [3–5]. In *C. crescentus*, the two bundles correspond to the left and right replichores [4,5] Figure 1G).

In some circumstances, including late stages of the cell cycle, the nucleoid appears as open ring and/or pairs of well-separated bundles (e.g. [3,6,8] Figure 1FJ). Longitudinal confinement could potentially influence shape in these stages.

Dual radial arrays of plectonemic loops—Chromosome capture analysis in *C. crescentus* suggests that, in that organism, longitudinal duality probably reflects the

existence of two parallel organized “bottle brush” objects, each comprising a radial array of plectonemic loops Figure 1H [4,5,9]. Each loop would be ~15kb in length with a super-organization of ~100kb. A similar underlying organization likely explains duality in *E.coli* [3]. If so, the two identified features might reconcile earlier observations in *E.coli* that variously defined one topologically supercoiled domain per ~50–100kb versus domains of ~10–15kb (discussion in [10]).

Domainal differentiation—For most of the enterobacteria and *vibrionaceae*, the terminus region is highly structured via a dedicated protein, MatP, a plectoneme-linking protein with a preferred binding sequence whose domainal localization is specifically nucleated [8,11,12]. The origin is also embedded in a structured region as shown in *B.subtilis* and *E.coli* [8,13–15]. In *B.subtilis* this structure is created by condensin and nucleated by ParB [13,14]. Origin and terminus domains also occur in *P.aeruginosa* [16]. For *E.coli*, domainal differentiation of interstitial regions has also been suggested ([15], reviewed in [10]). The experimental definitions of these regions may reflect both the segregation process and physical transitions of different macrosegments of the genome [17].

Dynamics

Time lapse imaging has defined local dynamic motions and also has revealed global dynamic behaviors.

Movement of loci over short time scales—Local movement of a fluorescently-tagged chromosomal locus is defined by plotting its mean square displacement (MSD) versus the elapsed time of observation. In a log-log plot, simple diffusive motion gives a linear relationship with a slope of 1. For chromosomal loci in bacteria, the relationship is linear but with a slope of less than 1, implying “sub-diffusive” motion. This behavior results from the viscoelastic nature of the crowded nucleoid: as a locus moves, it pushes against the constraints of the environment, which push back, thereby impeding movement [17,18]. Alternative explanations, i.e. stickiness of the locus to encountered features or impediments from fixed obstacles, are not compatible with the observed patterns [18]. An important sub-component of the apparent diffusion coefficient is nonthermal in nature, with ATP-dependent processes mechanically promoting agitation [19]. No single dominant motor protein has been identified thus far, with DNA gyrase, cell wall biosynthesis and the MreB cytoskeleton excluded as major players and RNA polymerase having only a minor role.

A monitored locus can occasionally exhibit rapid movements that exhibit “near-ballistic” dynamics, implying either an active translocation machinery or effects of stress-relaxation [3,20,21]. Such motions occur for loci throughout the genome, but differently with different genome coordinates. These movements do not correlate strictly with DNA replication, tend to occur along the length of the nucleoid and sometimes accompany chromosome segregation transitions.

Two types of global nucleoid-wide dynamics—Whole-nucleoid imaging in *E.coli* reveals two dynamic behaviors that involve the entire nucleoid [3]. These two behaviors come into play on different time scales. In neither case is the underlying mechanism known.

In both cases, removal of inter-segment tethers (protein-mediated and/or topological, along and between sisters) are proposed to play critical roles, thereby making the nucleoid more “fluid”. Fluidity would facilitate local movements required for diverse chromosomal processes including, for example, displacement of transcribed regions to move to the nuclear periphery for translation (e.g. [22]) as well as the dynamics of replication, sister segregation and organization.

Longitudinal density waves: Total nucleoid density fluctuates along the length of the nucleoid with a periodicity of one-two minutes, probably throughout the cell cycle, with net displacement of ~5% of nucleoid material in every 5s. These waves are proposed to promote internal nucleoid mobility by promoting loss of inter-segment tethers or entanglements that would otherwise create a gel. Such a role would be analogous to that suspected for back-and-forth movements of meiotic prophase chromosomes in correlation with elimination of unwanted entanglements created during chromosome pairing [23].

Cyclic nucleoid extension and shortening: Cell length increases monotonically during growth. In contrast, nucleoid length varies discontinuously, in a cyclic pattern. In each cycle, a five-minute period of nucleoid shortening is followed by a 20min period of elongation. Elongation rate increases for ~10min to a maximum and then decreases, ultimately becoming negative as shortening occurs, followed by the next elongation phase. These kinetics are strikingly consonant with accumulation, release and dissipation of viscoelastic mechanical stress, implying the existence of mechanical stress cycles. Thus far, these cycles are documented for a period that includes, but extends well beyond the period of DNA replication, with hints that it also occurs in G1. Such cycles could comprise a primordial cell cycle that governs the program of chromosomal events and its linkage with the cell division cycle. These global cycles are also implicated specifically in sister segregation (below).

It is proposed that random inter-segment tethers preclude formation of regular nucleoid organization (e.g. radial loop arrays), thus tending to make the nucleoid both larger and less elongated (i.e. wider and shorter) in a high energy (mechanically stressed) state. Eventually the level of stress would become high enough to provoke release of the constraints, thus permitting relief of stress by elongation. This effect would concomitantly be locked in by development of (radial loop) organization.

It might at first seem counter-intuitive that the expansion provided by nucleoid dynamics might be biologically helpful, as it is commonly accepted that chromosome compaction is a key element in chromosome segregation and organization. However one can imagine several scenarios where transient decompaction might help. For example, one should remember that global condensation will not separate sisters and that specifically only length-wise condensation [24] is able to drive segregation. A general proposition is that such a sister-specific condensation (generated e.g. by supercoiling [25]) will be impaired or at least slowed down by molecular cross-links. A local nucleoid expansion might hence decrease the amount of cross-links, subsequently allowing for more efficient length-wise condensation. More generally, there is general agreement that the underlying organization of DNA within the nucleoid is relatively regular (see above and e.g. [7]). Molecular events, and in particular transcription, will perturb that organization, and those perturbations might be either

constrained and/or made permanent by molecular cross-links. Transient expansion waves might again suppress those cross-links, allowing for a reorganization of the nucleoid in its “ideal” state.

Sister Segregation - Part I. Local cohesion and splitting of sisters (one spot - two spot)

Sister “splitting” is defined by appearance of two distinct FISH or fluorescent array foci. In all examined organisms, to a first approximation, splitting occurs progressively with replication.

The details of sister splitting have been studied primarily in *E.coli* [8,26–30]. Bidirectional replication from *oriC* initiates sequentially in the rightward and leftward directions, ~7min apart [26,31]. Most loci transit from one spot to two spots about 7–10min after passage of the replication fork [26], implying that the fork is trailed by a sliding window of juxtaposition of ~300–400kb [27,29,30]. However, some loci exhibit prolonged sister juxtaposition. *oriC* is one such locus, where splitting immediately precedes splitting of left and right replisomes [26,28]. Additionally, in the first half of the right replicore, delayed splitting occurs in several “snap” regions. At these positions, sister loci remain together for an extended period of time and then split contemporaneously in a single concerted transition [26–28] in accord with an earlier study [32]. This transition is critical for effective global segregation of nucleoids [27] (below). Finally, in the terminus region, two sister foci emerge well after completion of bulk replication [10,33]. Each of these delayed-splitting events is temporally correlated with a period of nucleoid elongation [3], consistent with a cause and effect relationship (below).

Colocalization of sisters at *oriC*, and in arm regions, is positively promoted by SeqA protein, which binds the sliding window of hemimethylation that trails the replication fork [27]. SeqA is important at all loci examined and is directly responsible for prolongation of colocalization at snap regions. SeqA is proposed to mediate cohesion via interplay with Topoisomerase IV (TopoIV) and precatenanes (below) and also to act directly, via its inter-molecular bridging activity [27]. In the terminus region, sister chromatid colocalization requires active juxtaposition of sister *ter* domains and direct sister chromatid interaction, both of which are mediated by the domain-specific actor, MatP [15].

Colocalization of sister loci behind the fork is usually thought to involve “precatenanes”, topological intertwining between sisters that would be driven by alleviation of the replication “swivel problem” [34–37]. Catenated sisters have been observed directly on small plasmids and linking of sisters by precatenanes has been inferred for the main chromosome from the fact that absence of TopoIV results in aberrant segregation and delayed locus splitting [27,35,38]. This model does, however, involve certain complexities. (i) Formation of precatenanes involves rotation of the replisome around mother duplex [36]. If the nucleoid is a meshwork of inter-segment links, this could require significant force. Moreover, left and right replisomes are colocalized (and thus presumptively paired) with one another for a short period [26]. During this period they would have to rotate individually despite their association. Precatenanes would arise when leading and lagging strand polymerases track together around the unreplicated DNA duplex. Biochemical studies suggest that this tendency is alleviated by transient dissociation of the lagging strand

polymerase from the fork [39]. (ii) The intrinsic handedness preference of TopoIV for left-handed DNA crossings is the opposite of that required for resolution of precatenanes. A sensible solution to this paradox has been provided [40,41], but it is still not excluded that precatenanes actually do not exist and/or that TopoIV promotes sister separation in other ways. (iii) TopoIV is recruited to specialized sites of action, including the replication origin of replication, by SMC protein MukB [42]; however, *in vitro*, MukB stimulates TopoIV-mediated relaxation of negative supercoils and intra-molecular knotting but not inter-molecular decatenation, leading to the proposal that the MukB/TopoIV interaction is important at the origin for chromosome structuring and not for resolution of inter-sister catenanes [42]. (iv) SeqA exerts its effects in interplay with TopoIV; however, deletion of SeqA alleviates the defect in a *topoIV* mutant at a snap locus but not at a non-snap locus [27]. Thus, the precatenane issue merits further exploration.

Sister Segregation - Part II. Global effects

How do bacteria regularly segregate entire sister chromosomes to daughter cells in the absence of a eukaryotic-like spindle apparatus? Several proposed models now seem unlikely. These include: (i) the original replicon hypothesis in which sister origins were attached to the edge of the cell and driven apart by cell wall biosynthesis [1] (which is incompatible with origin localization and dynamics); and (ii) the idea that sister units are extruded in opposite directions by a replication factory comprising associated left and right replichores (which is excluded by the finding that factories are transient (discussion in [10,43])). Also (iii) the idea that segregation involves entropy-mediated demixing of a self-avoiding random-walk polymer [44], is excluded by evidence that the nucleoid has a specific shape and is not space-filling (above). Finally, (iv) the ParAB system of *C. crescentus* was proposed to drive origin-proximal centromere-like sequences along a continuous filament in a true mitotic-like segregation mechanism [45]. However, there is no extensive, continuous ParB filament and alternative mechanisms for ParAB's ability to move molecular cargo through the cell have emerged [46,47]. Moreover, ParAB is not required for initial stages of segregation ([48] below). New ideas are now emerging in which internal nucleoid state and radial confinement by the cell periphery play important roles (below).

Irrespective of mechanism, what is the phenomenology of segregation? Despite prior emphasis on differences amongst the programs in different organisms, fundamental similarities can also be discerned.

Major Phases—In *E.coli*, replication initiates with the origin at midcell while, in *Caulobacter*, replication initiates when the origin is tethered to one pole. Nonetheless, there are important similarities in the two programs [26,28,48] (Figure 2).

Phase 1: In both organisms, sister origins are initially associated with one another and then split and separate to a significant distance, with one origin moving asymmetrically towards, but not to, a distant pole. In both cases, evolving sisters occupy a joint space near one pole [26,28,48]. In *E.coli*, origin splitting is accompanied by a discrete pulse of nucleoid extension with an accompanying initial tendency for separation of sister units along the

nucleoid length, implying effectively that sisters “push” each other apart (below). An analogous effect could pertain in *Caulobacter*: at this transition, one origin moves away while the other moves back in the opposite direction towards its original pole. This first phase likely permits initial differentiation and positioning of emerging sister units [26,48]. In *E.coli*, leading versus lagging strand identity is important in this process [49].

Phase 2: In the next phase, a transition occurs which sets up a basic symmetric origin-terminus-origin disposition, with origins poleward and the terminus at/towards mid-cell, appropriate for integration of terminus events with cell division (further discussion below). In both cases, this transition involves asymmetric events within the nucleoid. In *E.coli*, following Phase 1, a second abrupt global transition moves one entire sister, including its origin, towards the opposite cell pole, concomitantly placing the mother nucleoid and the terminus region towards midcell, while the other sister remains essentially in place [3,28,29]. This transition involves release of late-splitting inter-sister snaps and a second discrete pulse of nucleoid extension [3,28]. Thus, internal nucleoid dynamics again play a primary role. In *Caulobacter*, in this second phase, the ParAB system moves the partially displaced origin all the way to its opposite pole [48]. ParAB likely functions as an ATP-driven “diffusion ratchet”, proposed to be facilitated by elastic recoil within the nucleoid [46,47]. ParAB-driven origin movement could concomitantly move nucleoid material by “pulling” on chromosomes for segregation, with the ParAB target site serving as a “centromere-like sequence” [48]. However, internal nucleoid transitions could also be involved, as global dynamics have not been defined in this organism. It can also be noted that, in *B.subtilis*, there is direct interplay between SMC-mediated organization of the origin region and the ParAB system, with synergistic roles of the two systems for sister segregation [13,14]. The *E.coli* origin seems to be specifically positioned poleward by an origin-proximal site, *migS* site, but with modest phenotypic effects and implications to be explored [50,51] and dependent on MukBEF [38]. Also, linkage of the origin to the cell periphery just after initiation is suggested by biochemical studies (e.g. [52]).

Phase 3: After Phase 2, replication is accompanied by progressive divergent movement of sister loci in opposite directions. In *E.coli*, this presumably represents incorporation of sister material into the already-developed dual sister nucleoid units. In *C. crescentus*, it is proposed that localization of sister origins to opposite poles is followed by progressive replication and compaction of material towards the poles to give the final outcome [53].

A description of replication/segregation dynamics in *B. subtilis* [54] can be mapped onto the above pattern with relatively minor modifications.

Global nucleoid disposition: Fold-back and linear configurations—Genome disposition: two types of configurations. Studies in *E.coli*, *C. crescentus* and *B. subtilis* all show that the circular bacterial chromosome can exist in one of two configurations within the cell (Figure 3A). Type I is a “folded” configuration in which the left and right replichores are overlapping in parallel, with the origin and the terminus at opposite ends. In G1 cells, the two arms are closely juxtaposed (e.g. Figure 1G). Type II is a “linear” configuration in which the left and right replichores occur to either side of a centrally-positioned origin with the ends linked by an extended terminus region. At this stage, the

nucleoid should be a stiff linear object (e.g. [7]). In some situations, the origin and/or terminus regions are tethered to a cell pole(s) or, possibly for *E.coli*, non-polar regions as discussed above.

In *C. crescentus*, the Type I configuration occurs at the beginning of the replication/segregation program and at the end, with no evidence of a Type II configuration at any stage (Figure 2; Figure 3C).

In *E.coli* the situation is more complex. Recent studies have concluded that the Type II configuration is present at all times in slow-growing cells without overlapping replication cycles [1,30,55,56]. However, we disagree with this view. In our opinion, all current evidence suggests that the replication/segregation program yields a Type I configuration which then transits to Type II prior to the next round of initiation (Figure 3C middle; Figure 4). The first demonstration of two configurations was by Niki and colleagues [8]. That study, using FISH localization of origin, terminus and 20 interstitial loci, over time in the cell cycle of slowly growing cells, directly demonstrated a Type I disposition just before division followed by a progressive transition to Type II disposition before replication (Figure 3B). A subsequent study by Bates and Kleckner ([28] documented the same origin/terminus dynamics as in the earlier study and furthermore: (i) documented by FISH analysis of interstitial loci that a Type I disposition is seen at a critical intermediate stage, for thus-far-replicated loci just after the T2 transition that places emerging sisters at opposite ends of the cell (Figure 4B); (ii) showed that the origin is disposed towards one end of the nucleoid during the period when the terminus is tethered to midcell, inconsistent with an ori-centric Type II configuration and instead matching the Type II configuration as in the study by Niki et al. Figure 4C); and (iii) directly documented the adjustment from this configuration to an ori-centric disposition prior to initiation (Condition1, Figure 4A). Subsequent studies fully define a Type II disposition at G1 and reveal a linear conformation with the peri-ter region extending from end-to-end [7,10,30,43,55,56]. Analysis of the *dif* locus by Niki [8] matches this conclusion. Later studies also show that a Type II configuration exists prominently at later stages of the cell cycle [30,55]; however, this state occurs after an earlier mid-replication transition state [55], and after the same pattern of origin and terminus dynamics, including a final Type I origin/terminus configuration like those in earlier studies ([30,44,55,56]; Figure 4A). The entire constellation of results from all studies can be reconciled by the proposal that the G1 nucleoid is in a Type II state; that sisters evolve mirror-symmetric Type I conformations during the replication/segregation process; and that following completion of events at *ter*, both sisters flip or rotate back to the initial Type I conformation (Figures 3C, 4A). The only apparent difference among different studies is that this transition occurs concomitant with cell division in the earlier studies and well before division in the later studies.

In *B. subtilis*, a complex situation also exists ([54] (Figure 3C right). In this case, with twin nucleoids exhibit a Type I configuration at G1 and rotate/flip to a Type II configuration just after initiation. Emerging replicated nucleoids then assume a Type I configuration, which persists through into the ensuing G1. Interestingly, a Type I configuration is observed in sporulating *B.subtilis* whereas nucleoids are trapped in the transient Type II configuration if

replication initiation is blocked. Also, in *E.coli*, cells with partially overlapping replication cycles appear to exhibit a Type I configuration throughout.

By the above view, in all cases, the primary outcome of the replication/segregation program is a Type I disposition which, in some cases, gives rise to a Type II disposition prior to onset of replication initiation.

It will be interesting to further define nucleoid dispositions in various situations and to determine the relative contributions to the diversity of patterns of origin/terminus tethering, internal nucleoid structure and the physical constraints of radial confinement.

Models and mechanisms: compaction, radial confinement and release of

tethers—Many considerations of sister segregation recognize a critical role for chromosome compaction [3,24,57–59]. At the most basic level, compaction will be required to create well-individualized sister nucleoid shapes. However, individualization *per se* is not sufficient to confer regular sister segregation. Two other problems must be solved.

- First, it is necessary to place sister chromosomes in a disposition suitable for clean segregation to daughter cells. We suggested previously that, for *E.coli*, this requirement is met by radial confinement. If sister units are well individualized and compact, they will tend to exclude one another in space. Given a rod-shaped cell of appropriate dimensions, confinement in the radial dimension will tend to place the sister units in an end-to-end configuration (sister segregation by minimization of radial confinement). This effect would come into play progressively throughout the replication/segregation process. The same effect will pertain in a spiral-shaped cell. Moreover, in a spherical cell of appropriate dimensions, the same effect will position sister nucleoids in a symmetrical arrangement suitable for septum formation between them. Indeed, the tendency to occupy separate spaces will create stress on the cell periphery at the inter-sister interface, thus potentially providing a primordial mechanism for triggering cell division at the appropriate position. Perhaps such a mechanism directs segregation of chromosomes in *B.subtilis* L-form cells which lack a cell wall [60].

Formation of *oriC* domains is proposed to be important for regular sister segregation in *E.coli* [8,59] and *B. subtilis* [13,14,54]. A specific polymer model for *E.coli* sister segregation envisions an initial event involving entropic exclusion of compacted origin domains, perhaps aided by an “entropic spring” of adjacent disordered regions, followed by organization-mediated accumulation of material into distal (terminus-adjacent) regions [59]. A different polymer model has been suggested to explain *Caulobacter* segregation events subsequent to bipolar origin localization [53]. All such models always assume a cell-like cylindrical volume; thus, radial confinement is implicitly present as a central component of segregation.

- Second, it is also necessary to eliminate interlinks between sister chromosomes and, concomitantly, links along chromosomes that impede development of longitudinal organization. Most considerations of this problem envision compaction-mediated loss of links (above). However, we have observed that four

periods of extension defined thus far each corresponds to a time when particular programmed inter-sister tethers are lost with an accompanying discrete increase in longitudinal nucleoid duality, implying an increase in end-to-end sister disposition. In fact, elongation is apparently licensed by release of inter-sister snaps, which are under tension at the time of release [3], further implying an accumulated tendency for sisters to segregate end-to-end followed by release of that accumulated stress. As described above, these cycles may reflect alternation of the nucleoid between more and less chromosome compaction. One effect of such cycles could be to release inter-sister tethers globally, via internal repulsion during the disorganization period, thus permitting ensuing regular organization into a more compacted state, which in turn would drive end-to-end segregation of sisters. Given this progression, compaction-mediated tension between sisters can promote further resolution of inter-sister interlinks, topological and/or protein-mediated.

Specialized events of the terminus region—Events at the terminus region are best defined for *E.coli*. The terminus region, comprising a 1Mb domain organized by MatP, is captured by the septum via a chain of interactions among MatP-ZapB-ZapA and FtsZ interactions [61]. Concomitantly, a specific site in the terminus region, *dif*, is captured by interactions between the XerCD recombinase, which works directly at this site to reduce chromosome dimers, and divisome component FtsK (review in [62]). These two systems work together to ensure TopoIV-mediated decatenation of sisters; to ensure regular, ordered separation of sequences within the terminus domain; and to coordinate completion of events with cell division (via the SOS/SulA checkpoint and the nucleoid exclusion system that blocks septation until the division site is free of nucleoid material) The terminus segregation process includes ordered splitting and movement of the *dif* locus [28] and is mediated by FtsK [63]. Sister splitting in the terminus region can occur well after completion of replication ([15]; above). Split *dif* loci initially occur together on one side of the division site and then move to opposite sides. Sister nucleoids are then displaced away from midcell with an ensuing reorganization to an origin-centric disposition ([8,28], above).

Conclusion: Relationships between bacterial and eukaryotic chromosomes

It has always been envisioned that prokaryotic and eukaryotic chromosome dynamics should somehow be fundamentally similar. Recent observations provide strong support for this proposition and clarify the nature of the relationships. (i) Chromosomes tend to be discrete, coherent units in bacteria (above) as is well known for eukarotes. (ii) The elastic environment that governs short time-scale locus mobility, and its dependence on ATP, is similar in both types of cells [19,65]. So, too, is the observation that individual loci undergo ballistic movements [64,65]. (ii) Chromosomes have a fundamental underlying organization involving a radial array of loops, with hints of higher order organization [66]. Moreover, in both cases, the central “axis” defined by loop bases and associated proteins includes condensin, AT-rich sequences and architectural proteins that bind such sequences [66,67]. (iii) The back-and-forth oscillations of longitudinal density waves seen in *E.coli* have direct parallels in mammalian G1 nuclei [68] as well as in meiotic prophase (above). (iv) Global contraction/extension cycles seen in *E.coli* appear to be closely parallel to global chromosome expansion/contraction cycles seen in the meiotic and mitotic program

programs of eukaryotes, which are also correlated with period individualization of sister chromatids [69]. (v) Sister segregation at anaphase in eukaryotes is preceded by morphological individualization of sisters prior to and independent of spindle forces (examples in [28,69]), in accord with emerging evidence that global internal factors play a central role for sister individualization in bacteria (above).

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Bullet points

- The nucleoid is self-adherent and helical ellipsoidal via radial plectoneme loops.
- The nucleoid is viscoelastic with ATP-mediated enhancement of thermal motion.
- Discovery of nucleoid oscillations and sister segregation-correlated stress cycles.
- Sister dynamics includes local splitting and phased chromosome segregation.
- Bacteria and mammalian chromosomes share basic organization and dynamics.

Figure 1.

Bacterial nucleoids and their organization. (A, B) 3D images of *E. coli* G1 nucleoids illuminated with Hup1-mCherry (A; [3]) or GFP-FIS (B; [2]) illustrating curved ellipsoid shape with variable handedness. (C, D) Elongating *E. coli* nucleoids exhibit thin fingers and progressively protruding ellipsoid ends, implying a strong tendency for chromosomal material to coalesce into defined shapes; imaged as in (A) [3]. Blue arrows in (C) indicate periodic lengthening reflecting the effects of longitudinal density waves in a period of chromosome elongation. (E, F) Complex replicating *E. coli* shapes; note well-separated bundles (arrow in (F)), imaged as in (A); from [3]. (G) *C. crescentus* G1 nucleoid structure defined by chromosome capture analysis: dual longitudinal bundles corresponding to left and right repilchores with variable relationships giving curved ellipsoid shape with variable handedness as in (A). *parS* marks the origin of replication. From [4]. (H, I) Proposed structure of *C. crescentus* longitudinal bundles: radial array of plectoneme loops giving a bottle brush pattern. (H) Each plectoneme is composed of two DNA duplexes running in opposite directions with compaction as indicated in inset. (I) Plectonemes are packed into fibers that are separated by plectoneme-free regions. (details in Supplemental Figure S12 of ref. [5]). (J) Images of *B. subtilis* nucleoids labeled by incorporation of fluorescent nucleotides [6]. Green ball marks Spo0J-GFP that binds to origin(s) of replication.

Figure 2.

Proposed analogy between post-initiation transitions in *C. crescentus* and *E. coli*. Top: *parS* (origin) dynamics in Caulobacter [48]. Bottom: T1 and T2 transitions of *E. coli* defined by origin and nucleoid dynamics [3,26,28]. A first transition separates the two origins at a significant distance. A second transition separates the two origins to distant positions. In both cases, the latter transition sets up the *ori* - *ter* - *ori* disposition that permits integration of terminus events with cell division.

Figure 3.

Two global nucleoid dispositions. (A) Nucleoids can exist in two basic types of configurations. In the fold-back (Type I) configuration, origin and terminus regions are at the ends of the nucleoid and left and right replichores (arms) are parallel. In the linear (Type II) configuration, the nucleoid comprises a single linear object with left and right replichores flanking the origin and terminus material stretched from one end to the other. (B) The first description of Type I and Type II configurations and the transition from Type I to Type II preceding replication initiation in *E. coli* by Niki and colleagues [8]. This transition was proposed to occur by rotation. The Type II configuration was proposed to be a ring, rather than the linear form subsequently described [30,55, 56]. (C) Type I and Type II configuration patterns for three types of bacteria. *C. crescentus* remains in Type I throughout (discussion in [53]). *B. subtilis* cells are multi-nucleate and oscillate between Type I and Type II patterns [54]. For *E. coli* growing with a linear cell cycle (no overlapping rounds of DNA replication) a synthetic proposal is presented to explain patterns observed in two slightly different growth cycles: Condition 1 (from [3,8,26,28]) and Condition 2 (from [30,55, 56]) (see also Figure 4). The two cycles differ with respect to the phasing between the cell division and chromosome replication/segregation cycles. Type I and Type II configurations alternate analogously in both cases; the only difference is whether the transition from Type I to Type II occurs after or before division (Conditions 1 and 2, respectively). Note that in all cases shown, the primary outcome of the replication/segregation program is a **Type I** configuration (in **bold**).

Figure 4.

Proposed synthetic view of *E. coli* nucleoid disposition dynamics in two different conditions with differing relationships between the cell division and chromosome replication/segregation cycles. (A) Summary of proposal. Images for Condition 1 are from live cell imaging of strains fluorescently-labeled at the origin (green) and terminus (red) regions with timing defined by cell length [56]. Data for Condition 2 are from synchronous cell populations examined for origin and terminus disposition by FISH and for nucleoid status by DAPI staining [28]. Individual dots are positions of the indicated loci in independent analyzed cells from each time point. These and other studies agree that a Type II configuration exists at the onset of replication (text). (B, C) For Condition 1, a mirror-symmetric Type I configuration is documented for the indicated intermediate stage by analysis of *oriC* and two right replicore loci, which occur in inverted orientation at this stage (Panel B; Panel A “evolving Type I”). This configuration is inferred to persist until the end of the cell division cycle for three reasons. First, origin and terminus positions are stable from the T2 transition onward (Panel A). Second, disposition of the origin near one end of the nucleoid in about-to-divide cells corresponds to a Type I transition, not a Type II transition where the origin would be located in the middle of the nucleoid (Panel C). Third, the Type I configuration by analysis of interstitial loci in another study of cells with the same Condition [8]. These patterns imply that, in Condition 1, a transition from Type I to Type II occurs immediately following cell division. In Condition 2, cells exhibit the same program of origin and terminus dynamics observed for Condition 1 except that, at the end of

the replication/segregation cycle, the nucleoids switch from Type I to Type II prior to cell division. Analysis of interstitial loci [29,30] documents the Type II configuration and but may be less sensitive for detecting the Type I configuration which, in this Condition, represents a smaller fraction of cells. Thus, the only difference between the two programs would be the timing of cell division relative to the time of the switch from Type I to Type II (see also Figure 3 and text).

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