Bacterial Chromosome Organization and Segregation

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Bacterial chromosomes are generally ~ 1000 times longer than the cells in which they reside, and concurrent replication, segregation, and transcription/translation of this crowded mass of DNA poses a challenging organizational problem. Recent advances in cell-imaging technology with subdiffraction resolution have revealed that the bacterial nucleoid is reliably oriented and highly organized within the cell. Such organization is transmitted from one generation to the next by progressive segregation of daughter chromosomes and anchoring of DNA to the cell envelope. Active segregation by a mitotic machinery appears to be common; however, the mode of chromosome segregation varies significantly from species to species.

The DNA molecule is a remarkably simple and elegant storage medium for genetic information. However, linear encoding of this kind demands an inordinately long molecule, and so, bacterial chromosomes are much longer than the cells in which they reside. For example, *Caulobacter crescentus* packages a 1.3 mm (4.0 Mbp) genome in a 2 micron cell. This spatial constraint creates a daunting organizational problem that is exacerbated on DNA replication, an act that not only doubles the amount of DNA in the cell, but also creates topologically linked molecules. Watson and Crick published a companion paper to their 1953 DNA structure in which they pointed out that the elegant simplicity of the copying mechanism they proposed was complicated by the fact that the two strands

wind around each other. Hence, to create two separate chromosomes, it is necessary to unlink the original two strands, which, even in the case of a bacterial genome, amounts to unwinding several hundreds of thousands of turns. They wrote: “Although it is difficult at the moment to see how these processes occur without everything getting tangled, we do not feel that this objection will be insuperable” (Watson and Crick 1953). Indeed, in 1971, James Wang purified the first of a class of proteins we now know as DNA topoisomerases (Wang 1971), which solve this particular problem by catalyzing the passage of single strands or duplexes of DNA through each other (Schoeffler and Berger 2008).

Topoisomerases thus provide a solution to the problem of DNA tangling within the cell.

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Other, less biochemically tractable aspects of DNA spatial organization, however, received little attention for a considerable time, partly for technical reasons, but also because bacteria were seen as the proverbial “bag of enzymes,” i.e., small enough for diffusive processes to dominate and thus not requiring any spatial organization. We now know that the bacterial cell is in fact highly organized (Thanbichler and Shapiro 2008), and the chromosome is no exception.

Here, we concentrate on recent findings about the dynamic high-order organization of the bacterial chromosome; that is, how is DNA organized within the cell and how is it segregated faithfully to each daughter cell on division? Our understanding of these two questions has progressed greatly in the past few years, aided in large part by advances in imaging technologies. For the sake of clarity, we explore each question independently. However, as will be clear throughout, DNA segregation and organization are intimately linked.

DNA ORGANIZATION WITHIN THE CELL

The Early Years and the Rosette Model

The very first investigations into the structure of the bacterial nucleoid came in the form of light microscopy of stained specimens (e.g., Feulgen and Giemsa staining), and electron microscopy of thin sections (reviewed in Robinow and Kellenberger 1994). These studies showed that although the nucleoid is mostly separate from the rest of the cytoplasm, it is not bound by a nuclear membrane (Robinow 1956). Some DNA strands extrude from the nucleoid into the cytoplasm like loose pieces in an otherwise tight ball of yarn (Kellenberger 1991), and autoradiography of transcribing genes suggested that these excrescences contain most of the transcriptionally active DNA (Ryter and Chang 1975).

As important as they were, these first studies were based on only limited resolution. In 1971, Stonington and Pettijohn opened a new window by developing a method to gently lyse *Escherichia coli* cells in solutions of high ionic

strength to isolate “folded” chromosomes, i.e., free DNA that does not increase the viscosity of the solution. These isolated chromosomes contained nearly all of the DNA present in the lysate, some protein, and all nascent RNA (Stonington and Pettijohn 1971). Depending on the conditions of isolation, chromosomes were obtained in either membrane-associated or membrane-free form (Kavenoff and Bowen 1976; Kavenoff and Ryder 1976).

When imaged by electron microscopy, isolated chromosomes appeared as unbroken rosettes with a central core from which several tens of plectonemic loops radiate out (Fig. 1) (Delius and Worcel 1974; Kavenoff and Ryder 1976). This organization was sensitive to RNase, suggesting a role for RNA in maintaining the integrity of the core (Kavenoff and Bowen 1976). Isolated chromosomes also showed a biphasic response to increased concentrations of ethidium bromide, indicating that DNA

Figure 1. The “rosette” model of DNA organization. Electron micrograph of isolated membrane-free chromosomes from *E. coli*. The central core, from which several tens of loops radiate, is sensitive to RNase. Single strand cuts to any one loop will only affect the supercoiling of that particular domain, leaving the rest of the chromosome unaffected. Bar = 1 μ m. (reprinted from Kavenoff and Bowen 1976, with permission).

supercoiling was maintained. Consistent with the electron microscopy studies, a few tens of DNase catalyzed nicks are required to obtain a fully relaxed complex (Worcel and Burgi 1972).

The rosette model thus provided some of the first insights into the higher-order structure of the bacterial chromosome. The picture that emerged was one in which the chromosome consists of a central core held together by RNA–DNA interactions. Between 12–80 topologically independent loops—or “domains of supercoiling”—radiate out from this core (Fig. 1). Although all domains have the same amount of superhelicity, a single strand nick in one of the loops eliminates only the superhelicity in that particular domain, leaving the rest of the chromosome unaffected (Worcel and Burgi 1972). Importantly, the existence of domains of supercoiling has been confirmed by *in vivo* studies (Sinden and Pettijohn 1981; Scheirer and Higgins 2001; Postow et al. 2004). The presence of a stabilizing RNA “core,” however, has been subject to certain skepticism because of the possibility of isolation-induced artifacts (Pettijohn 1982) and, to this day, the specific role of RNA in maintaining nucleoid structure is not understood.

Localization of DNA in *Bacillus subtilis* and *Caulobacter*: Specific Orientation

Because chromosomes isolated using the Stonington and Pettijohn method are separated from the rest of the cell, the rosette model does not make any predictions about the subcellular localization of any given DNA sequence. The first indication that the bacterial chromosome has a specific orientation within the cell came from genetic studies in *Bacillus subtilis* (Wu and Errington 1994). Under starvation conditions, *B. subtilis* undergoes a sporulation program that divides the cell into a large mother cell compartment and a small forespore compartment (Barák and Wilkinson 2005). This asymmetric division traps ~30% of the chromosome in the forespore, and segregation of the rest of the chromosome is driven by SpoIIIE (Burton et al. 2007; Ptacin et al. 2008; see also Dubnau 2010). In SpoIIIE mutant cells, DNA

translocation failed to occur and 70% of the chromosome was left stranded in the mother cell. Wu and Errington (1994) discovered that the identity of the stranded DNA was reproducible from cell to cell, suggesting that the chromosome has a specific orientation with respect to the division septum, at least during sporulation.

A few years later, direct visualization of the position of chromosomal loci was achieved. Mohl and Gober (1997) showed that the *Caulobacter* ParB protein, which binds specifically to a site near the origin of replication (termed *Cori* in *Caulobacter* and *oriC* in other bacteria), was localized consistently to the cell poles (Fig. 2A). Simultaneously, Webb and coworkers (Webb et al. 1997) adapted a method originally devised for visualizing DNA loci in eukaryotic cells (Robinett et al. 1996; Straight et al. 1996) to look at the position of *oriC* and the terminus of replication (*ter*) in *B. subtilis*. In this method, a large number (~250) of *lacO* sites, arranged in tandem, are inserted into the chromosome at a site of interest. Subsequent expression of a LacI-GFP fusion results in binding of LacI-GFP to the *lacO* arrays, and creates a localized fluorescent spot that can be visualized with a modern CCD camera. Using this method, Webb and coworkers confirmed the genetic prediction that the *B. subtilis* *oriC* localizes to the cell poles during sporulation. Furthermore, they showed that this localization pattern held true even in vegetatively growing cells, and that *ter* localized to the opposite pole in newborn cells (Fig. 2C,D) (Webb et al. 1997).

It is interesting to note that the total length of these *lacO* arrays is on the order of 10 kb, which, if stretched out, would measure ~3.4 μm ; more than enough to span the length of the cell. Yet, in all cases examined, the arrays appeared as tight diffraction-limited foci. This observation is unlikely to be an artifact of the labeling procedure for two reasons: First, the version of LacI used in these experiments had a truncated carboxyl terminus that prevents dimers from associating with each other and making higher-order structures (Lau et al. 2003); and second, the same result was obtained in fixed cells with fluorescence *in situ* hybridization (FISH) probes of a similar length (Jensen

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Figure 2. Chromosome organization in model bacteria. (A) The *Caulobacter* chromosome is linearly organized, and anchored to the flagellated pole via *parS*/ParB/PopZ. (B) In *Vibrio cholerae*, the origin region of the larger chromosome (chromosome I) is localized to the cell pole, whereas the origin of the smaller chromosome is localized to the cell center. The organization of the bulk of the chromosomes, as well as their separation or intermingling, are currently unknown. (C) Four loci have been localized in vegetative cells of *Bacillus subtilis*, and their organization is reminiscent of the linear order seen in *Caulobacter*. Although the origin region is localized near to one pole, it appears not to be anchored to the cell membrane. (D) Sporulating cells of *B. subtilis*, however, do anchor the origin region, through RacA/DivIVA, to the negatively curved membrane at the pole. RacA also binds all along the chromosome, compacting it into a long “axial filament” before sporulation. (E) The *E. coli* origin localizes to mid-cell, and the two replichores are separated into opposite cell halves. The terminus is broadly localized (arrows), and may be found on either side of the cell center.

and Shapiro 1999). Hence, it appears that in vivo DNA is highly and locally compacted.

The ability to synchronize *Caulobacter* cells played an important role in the next discovery, as it allowed investigators to easily follow the localization pattern of DNA through the cell cycle. Jensen and Shapiro (1999) used FISH to follow the positioning of *Cori* and *ter* in synchronized cells, and saw that *Cori* began the cell cycle at the old pole, whereas *ter* was localized to the new pole. After *Cori* duplicated, it quickly redistributed to a bipolar localization. Meanwhile, *ter* moved away from the pole and relocated to midcell. Near the end of the cell cycle, *ter* itself was duplicated, leaving a mirror image conformation of the chromosome (Jensen and Shapiro 1999).

Jensen and coworkers (2001) also looked at the localization pattern of members of the

replication machinery. They found that the replisome formed near the old pole at the beginning of the cell cycle, close to *Cori*. As replication proceeded, the replisome migrated toward midcell, presumably being displaced from the pole by the accumulation of newly replicated DNA there.

Once it was established that both *Cori* and *ter* have rigorously defined cell-cycle-dependent localization, the question of how the rest of the chromosome is organized became more pressing (Breier and Cozzarelli 2004). Teleman and coworkers (Teleman et al. 1998) labeled four chromosomal positions (359°, 90°, 181°, and 270° on the circular chromosome; *oriC* corresponds to 0°) in fixed *B. subtilis* cells, and concluded that loci at intermediate positions on the chromosome lie between *oriC* and *ter*

inside the cell (Fig. 2C). A similar model was proposed for *Caulobacter* based on pulse labeling of the chromosome in synchronized cells. Early-replicated DNA was shown to localize near the cell poles, whereas late-replicated DNA localized closer to midcell, and intermediate-replicated DNA localized in between the two (Jensen et al. 2001).

These results suggested that in bacterial cells, the entire chromosome was organized, but definitive proof only came in 2004, when Viollier and coworkers (Viollier et al. 2004) developed computational methods to measure the position of fluorescent foci with subdiffraction precision and in a high-throughput manner. Using a transposon-based strategy, they created a set of 112 strains each carrying the *lacO* arrays at a different position, and measured their location in >500 cells. They found a linear correspondence between the position of any given DNA locus on the chromosomal map and its position inside the cell. Thus, a locus that is 1/3 of the distance between *Cori* and *ter* on the chromosome map will be found 1/3 of the way between the old and new poles (Fig. 2A) (Viollier et al. 2004). It is important to remember that bacterial chromosomes are generally circular molecules, with the origin and terminus located at opposite ends of the circle. The two arms of the chromosome, or replichores, run parallel to each other from one cell pole to the other (Fig. 2A). Here, “linear organization” is used to refer to the linear relationship between a DNA locus’s position in base pairs, and its subcellular location.

This striking organization raises the important question of how the chromosome is kept in place. Is there a site—or perhaps several sites—of attachment between the DNA and the cell envelope that ensures proper localization? Indeed, there are. During sporulation in *B. subtilis*, the RacA protein is required to bind the origin region of the chromosome to the cell pole (Ben-Yehuda et al. 2003; Wu and Errington 2003). RacA binds preferentially to 25 sites in a large region around the origin of replication and interacts directly with DivIVA (Ben-Yehuda et al. 2005; Lenarcic et al. 2009). DivIVA, in turn, binds to negatively curved membranes,

such as those that occur on the cytoplasmic side of cell poles (Lenarcic et al. 2009; Ramamurthi and Losick 2009). Hence, polar localization of the origin region of *B. subtilis* results from a pole-DivIVA-RacA-DNA (RacA binding sites) connection (Fig. 2D).

However, RacA is not expressed in vegetatively growing *B. subtilis* and the origin of replication does not appear to be anchored at the cell pole under these conditions (Fig. 2C). Ectopic expression of RacA during vegetative growth caused the chromosome to shift position so that it came in close contact with the pole (Ben-Yehuda et al. 2003). Therefore, the question of how the chromosome is kept oriented during vegetative growth in *B. subtilis* is still unsolved.

The case appears to be more straightforward in *Caulobacter*. Two groups recently showed that the *Caulobacter* chromosome is anchored at the cell pole by the PopZ protein (Bowman et al. 2008; Ebersbach et al. 2008). PopZ is a small, proline-rich protein that self-associates to form a network at the *Caulobacter* cell pole. It interacts directly with ParB, a sequence-specific DNA binding protein that binds to the *parS* sequence (about which, more later), which is only 8 kb away from *Cori*. Thus, in *Caulobacter*, the connection is: pole-PopZ-ParB-DNA (*parS*) (Fig. 2A). Importantly, it was later shown that the polar localization of the origin of replication is not mediated by anchoring of *Cori* itself to the pole. When *Cori* was moved 100kb away from *parS*, its position in the cell shifted away from the pole (Toro et al. 2008). The polar position of *parS*, however, remained unchanged. Therefore, in contrast to previous belief, the determinants of chromosome orientation are not contained within the origin of replication.

Localization of DNA in *E. coli*: Replichore Separation and Nonrandom Segregation

Chromosome organization in *E. coli* appeared, at first, to be similar to that of *B. subtilis* and *Caulobacter*, with *oriC* at one pole and *ter* at the other (Gordon et al. 1997; Niki and Hiraga 1998; Bates and Kleckner 2005). Later work, however, showed a different, more complex

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picture. At the beginning of the cell cycle, slowly growing *E. coli* cells place *oriC* at midcell (Fig. 2E) (Nielsen et al. 2006b; Wang et al. 2006). FISH analysis suggested that the chromosome is linearly ordered in the cell, as in *Caulobacter*, but not along the *ori-ter* axis (Niki et al. 2000). Double-labeling experiments showed that each chromosomal arm, or replicore, localizes to a separate cell half (Nielsen et al. 2006b; Wang et al. 2006). The terminus region is broadly distributed, colocalizing with the left and right replicores in equal proportion (Fig. 2E, arrows) (Wang et al. 2006).

After duplication, the two *E. coli* *oriC* move to quarter cell positions and the bulk of the chromosome assumes a <L-R-L-R> configuration (L and R refer to the Left and Right replicores, respectively) (Fig. 3) (Nielsen et al. 2006b; Wang et al. 2006). Counter-intuitively, this translationally symmetric (as opposed to mirror-symmetric) organization of the replicores predicts mirror symmetry with regards to segregation of the leading versus lagging strands of replication (Wang et al. 2006). To achieve the <L-R-L-R> configuration observed, both leading strands have to be segregated to the distal edges of the cell while the lagging strands stay proximal, or vice-versa (White et al. 2008). Otherwise, the chromosome would toggle between configurations (see Fig. 4 for why this is so). Indeed, White and coworkers showed that leading strands are segregated to the edges of the cell. Using an ingenious method, they induced the specific degradation, in vivo, of the lagging-strand-replicated copy of one locus in the *E. coli* chromosome. By examining the localization of the remaining, leading-strand-replicated copy, they showed that in ~90% of cells, the leading strand is segregated to the outside edges of the cell (Fig. 4B, right) (White et al. 2008).

It should be noted that this leading strand preference for the outer edges of the cell does not necessarily lead to “immortal strand” segregation, which postulates nonrandom segregation of “old” and “new” DNA strands (Lansdorp 2007; Lew et al. 2008). Because of the semiconservative nature of DNA replication, every round of synthesis produces two chromosomes composed each

Figure 3. Chromosome organization in *E. coli*. The origin of replication of the *E. coli* chromosome (*oriC*) is located at midcell, and each arm is kept in a separate cell half (*top*). The terminus region is broadly distributed along the long axis of the cell (not shown). As replication proceeds (*middle*), each daughter *oriC* is segregated to the cell quarters and, when replication is complete, the daughter chromosomes adopt a translationally symmetric <L-R-L-R> configuration (*bottom*).

of a newly synthesized strand annealed to an old strand. Similarly, every round of cell division produces two cells each with a newly synthesized cell pole as well as an old pole. The immortal strand hypothesis postulates that old DNA will be reliably partitioned to the old pole, thus generating a stable and potentially infinite association of one particular pole with one particular strand of DNA. Thus, the origin of the phrase—immortal strand segregation.

The leading versus lagging strand segregation discovered by White and coworkers, then, is slightly different from immortal strand segregation (see Fig. 5 for a visual explanation of this complicated point). Under leading strand segregation, it is possible for a given “old” pole to receive either daughter chromosome. As long as the leading strand of that chromosome is positioned closer to the old pole, White’s law is not broken, even though the immortal strand hypothesis may not be kept. This situation, however, will change the chromosomal configuration in successive generations from

Figure 4. Nonrandom segregation in *E. coli*. (A) A replicating mother cell is shown, highlighting the Left and Right replichores as well as the difference between leading-strand-replicated (solid black lines) and lagging-strand-replicated DNA (dashed black lines). The replication bubble (gray box) is also shown, and repeated above each example in B and C to illustrate the origin of each configuration. (B) To achieve the <L-R-L-R> configuration seen, it is necessary for both lagging strands or both leading strands to be segregated to the distal edges of the cell. Note that in ~90% of cases, leading strand segregation to the distal edges (right) is observed. (C) If random segregation of leading and lagging strands is imposed, the <L-R-L-R> configuration cannot be achieved, and mirror symmetry appears (e.g., <L-R-R-L>).

<L-R-L-R> to <R-L-R-L> (Fig. 5, bottom). Thus, by following two markers in successive generations, it is possible to measure the degree to which *E. coli* undergoes immortal strand segregation, something that Wang and coworkers (Wang et al. 2006) measured: Of 17 divisions observed, 12 kept the <L-R-L-R> configuration, four changed to mirror symmetry (<L-R-R-L> or <R-L-L-R>), and one reversed to <R-L-R-L>. Hence, although ~90% of cells showed nonrandom leading-strand segregation, immortal strand segregation occurred in only ~70% (12/17) of cell divisions.

The immortal strand hypothesis has also been tested in *Caulobacter* (Osley and Newton 1974; Marczyński et al. 1990) and *B. subtilis*

(Errington and Wake 1991), and in both cases it was found that chromosomes segregate randomly to daughter cells. Because neither of these species distinguishes between the Left and Right replichores, nonrandom segregation may be related to the orientation of the chromosome within the cell.

DNA SEGREGATION

The Replicon Model: Cell Growth as the Driver of Segregation

By far, the most influential hypothesis about bacterial segregation was the replicon model proposed by François Jacob, Sydney Brenner,

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Figure 5. “Immortal strand” inheritance versus Leading strand segregation. Two generations of DNA replication and segregation are shown, to illustrate the association of an old DNA strand (colored) with the old cell pole. Note that in all cases, leading strand segregation to the distal cell edges is maintained. (A) After the first generation, both daughter cells carry one “new” and one “old” strand of DNA (black and colored, respectively). (B) During the second round of segregation, two scenarios are possible: (1) immortal strand segregation is kept, and the “old” pole stays associated with the “old” (colored) strand of DNA (top). Note that this is the case observed for *E. coli* cells in ~70% of cases; (2) Immortal strand segregation is not kept, and the “old” pole received two “new” strands of DNA. In this case, the configuration of the chromosome changes from $\langle L-R-L-R \rangle$ to $\langle R-L-R-L \rangle$.

and François Cuzin in 1963 (Jacob et al. 1963). In a small section at the end of a paper dealing mostly with replication control, Jacob and co-workers noted that puzzling observations about the efficiency of conjugation as well as thin section electron micrographs were consistent with the anchoring of specific sections of DNA to the cell envelope. Hence, if sister chromosomes are attached to the cell membrane and growth takes place in between the two attachment sites, DNA segregation could be achieved passively as a by-product of cell elongation.

In order for the Jacob model to be possible, two things must be true: First, the chromosome must be physically attached to the cell wall at a specific site; and second, the cell wall must grow in a nonuniform manner. These

predictions led investigators to look for a specific association of the origin of replication with the membrane, which was found to be present in several species (Sueoka and Quinn 1968; Fielding and Fox 1970; Bowman et al. 2008; Ebersbach et al. 2008), as well as zonal cell wall growth, which was found to be present or absent, depending on the organism (Cole and Hahn 1962; Mobley et al. 1984; Aaron et al. 2007).

Many of the observed associations between DNA and membrane were sensitive to rifampin, a transcription inhibitor (Dworsky and Schaechter 1973), suggesting that they were mediated by transertion (i.e., coupled transcription, translation, and insertion of the nascent polypeptide into the membrane). However, some of the

DNA-membrane interactions were impervious to rifampin, implying that at least some sites on the chromosome, including *oriC*, were specifically anchored to the cell envelope by DNA binding proteins (Jacq and Kohiyama 1980). As mentioned previously, the chromosome-anchoring proteins for *Caulobacter* (ParB-PopZ) and *B. subtilis* (RacA-DivIVA) are now known (Fig. 2).

Two other observations seemed to support the replicon model: First, bacterial nucleoids stained with nonspecific DNA binding dyes appeared to separate gradually, in contrast to what happens in eukaryotes (Van Helvoort and Woldringh 1994); and second, early genetic screens for partitioning mutants in *E. coli* yielded hits involved in resolving topological problems associated with segregation (Hiraga et al. 1989; Adams et al. 1992; Drlica 1992), but no proteins that could provide the force to drive movement (Wheeler and Shapiro 1997). In the absence of force-generating proteins, passive segregation seemed the logical alternative.

It should be noted that the *E. coli* MukB protein, identified in a genetic screen for DNA partitioning mutants, was briefly hailed as the missing molecular motor based on the fact that it existed as a DNA-binding homodimer that could bind ATP/GTP and had a coiled-coil domain, a characteristic of motors like myosin and kinesin (Niki et al. 1992; Wake and Errington 1995). MukB turned out not to be a motor, however. The characterization of the *B. subtilis* SMC condensation complex (Britton et al. 1998; Moriya et al. 1998) later led to the realization that MukB is in fact part of the *E. coli* analogue of SMC (Bartosik and Jagura-Burdzy 2005).

Nondedicated Models of Segregation: Fast, Progressive Segregation in the Absence of a Mitotic Apparatus

Despite the popularity of the replicon model, evidence against it began mounting. Not only did zonal growth appear to be limited to a subset of species, as soon as direct labeling of specific DNA loci in living cells was achieved, it became clear that their speed of movement

was too fast to be accounted for by cell growth (Gordon et al. 1997; Mohl and Gober 1997; Webb et al. 1997; Teleman et al. 1998; Jensen and Shapiro 1999; Viollier et al. 2004). Live tracking experiments also showed that chromosome segregation is progressive; that is, DNA loci were separated while replication was still ongoing (Viollier et al. 2004; Nielsen et al. 2006a). Interestingly, this is not an absolute rule. In *E. coli*, there is evidence that the origin region undergoes a short period of “sister chromatid cohesion,” after which the entire region is segregated as a single unit (Bates and Kleckner 2005; Espeli et al. 2008). Other regions of the *E. coli* chromosome, however, segregate progressively (Nielsen et al. 2006a).

Abrupt DNA separation, coupled to the fact that no force-generating segregation proteins had been found, paved the way for a new wave of “nondedicated” models whose aim was to explain fast segregation in the absence of a mitotic apparatus. Several interesting proposals were put forth. It was hypothesized that the force produced by DNA polymerase (Lemon and Grossman 2001) or RNA polymerase (Dworkin and Losick 2002) could help separate daughter chromosomes. Intriguingly, it was also suggested that DNA segregation may be a self-organizing process, driven by separation of transertion domains (Woldringh 2002), or by entropic exclusion of sister chromosomes (Jun and Mulder 2006). Each of these models is supported by a subset of observations, and it is possible that these mechanisms contribute to bulk DNA segregation (Toro et al. 2008). However, none can fully explain the diverse behaviors observed in vivo.

Dedicated Models of Segregation: A Mitotic-like Apparatus

Around the same time that nondedicated models were being proposed, new observations began to suggest that perhaps a mitotic-like apparatus did exist in bacteria. We have already mentioned the transient suggestion that MukB could be a molecular motor in charge of driving chromosome segregation. A second, more

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promising candidate for force generation is the ParABS system.

Low copy number plasmids like the *E. coli* P1 prophage are inherited with high efficiency despite the fact that as few as two copies of the plasmid may be present before division (Prentki et al. 1977). These observations suggested that such plasmids contained a partitioning system, and deletion of P1 fragments showed that the ParABS locus was necessary and sufficient to confer stability to low copy number replicons (Abeles et al. 1985). This locus contains three components: ParB, a sequence-specific DNA binding protein that binds to the *parS* sequence; and ParA, a Walker type ATPase that has been shown to polymerize and/or oscillate over the nucleoid in several partition systems (Surtees and Funnell 2003; also see Mullins 2010).

Hence, by the early 2000s, it was clear that plasmid partitioning was actively mediated by the ParABS system, and also that the chromosomes of most bacteria (but not *E. coli*) contained homologs of the *par* locus. Based on sequence comparisons of their constituent ParA ATPases, chromosomal ParABS systems are distinct from plasmid ones (Gerdes et al. 2000). Nevertheless, the hypothesis that ParABS systems could indeed drive the segregation of bacterial chromosomes was supported by experiments showing that: (1) deletion/depletion of ParABS components resulted in higher proportions of anucleate cells (Ireton et al. 1994; Mohl and Gober 1997); and (2) addition of various chromosomal ParABS systems to an unstable plasmid increased its efficiency of transmission (Godfrin-Estevenson et al. 2002; Dubarry et al. 2006).

Work in *Vibrio cholerae* has been instrumental in demonstrating the importance of the ParABS system for segregation. *V. cholerae* carries two chromosomes (Fig. 2B), each with their own distinct segregation dynamics and ParABS system, and the larger of which (chromosome I) shows a segregation pattern reminiscent of that observed in *Caulobacter* (Fogel and Waldor 2005). Work by Fogel and Waldor (2006) showed that *parS_I* segregates ahead of *oriC_I*, suggesting that it may act as the chromosomal centromere (i.e., the site of force exertion

during segregation). Toro and coworkers (2008) expanded this observation in *Caulobacter* and found that *parS* invariably segregated ahead of all other loci, regardless of the order of replication. Furthermore, segregation was delayed when *parS* was moved to a site farther away from the origin of replication, indicating that segregation cannot begin before *parS* is duplicated. This showed that, at least in *Caulobacter*, nondedicated models of segregation are unable to initiate segregation.

How might the ParABS system perform its segregation function? Based on time-lapse observations of ParA_I and ParB_I dynamics, Fogel and Waldor proposed a pulling mechanism. In *V. cholerae*, ParA_I forms a fluorescence “cloud” that grows from the pole opposite to *parS_I*. When it reaches *parS_I*, the cloud switches from growth to contraction, and one of the newly duplicated copies of ParB_I follows the receding edge of the cloud until it reaches the opposite pole (Fogel and Waldor 2006). This mechanism is by no means proven, but it is certainly compelling. Work is currently under way to try to characterize the biochemical and structural characteristics of this system (Hester and Lutkenhaus 2007; Castaing et al. 2008).

Despite the promising results outlined above, some important aspects of the ParABS system remain to be explored. New work in *B. subtilis* has shown a link between ParA (Soj) and chromosome replication (Murray and Errington 2008), as well as a direct interaction between ParB (Spo0J) and the SMC complex (Gruber and Errington 2009; Sullivan et al. 2009). Thus, the perverse and often baffling results of *soj* and *spo0J* deletion in *B. subtilis* may be explained by a shift in function.

Compounding the issue, several species (with the exception of *Caulobacter* and *V. cholerae* chromosome II) still segregate their chromosome normally in most cells even when ParABS is inactivated (Ireton et al. 1994; Lewis et al. 2002; Fogel and Waldor 2006; Jakimowicz et al. 2007; Yamaichi et al. 2007). This suggests that there are redundant mechanisms that ensure chromosome segregation in the absence of ParABS. It has been proposed that the actin-like MreB protein contributes

to DNA segregation (Gitai et al. 2005; Kruse et al. 2006; Hu et al. 2007; Karczmarek et al. 2007; Shebelut et al. 2009; see also Gitai 2010). Another candidate is the *migS* sequence found in *E. coli* (recall that *E. coli* does not contain a ParABS system on its chromosome).

migS was found by Yamaichi and Niki by cleverly forcing the cells to break off pieces of the chromosome and using them to create mini-chromosomes (Yamaichi and Niki 2004). Thus, the genome within the cell remains unchanged except for the fact that now a small piece of the chromosome is on a separate molecule. Using this strategy, Yamaichi and Niki found that the presence, in *cis*, of a 25 bp sequence (*migS*) was required for proper separation of *oriC*-proximal DNA, as measured by FISH. *migS* did not stabilize plasmids nor cause them to localize to a particular subcellular location (Yamaichi and Niki 2004). Despite this, there is good evidence that *migS* plays an important role in the segregation of the *E. coli* chromosome (Fekete and Chatteraj 2005).

CONCLUDING REMARKS

What We Know Thus Far

Taking into account all of the above, we can make the following generalizations about chromosome organization and segregation in bacteria: The circular chromosome exists within the cell as a compact structure that is organized into separate superhelical domains. At least one locus, and perhaps several, are specifically positioned within the cell, and the rest of the chromosome is kept linearly organized with respect to those landmarks. DNA is kept highly compacted at the local level by a balance of forces, including supercoiling, compaction by proteins, transcription, transection, and perhaps even RNA–DNA interactions.

Segregation is mediated by an active and dedicated mechanism of which we know very little. In the case of bacteria like *E. coli*, which maintain the origin region at midcell and left and right replichores in separate cell halves, segregation is nonrandom and the leading strand-replicated arms are shuttled to the outer edges

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of the cell. Such distinction is not made in *Caulobacter* or *B. subtilis*, which keep the origin and terminus regions at opposite poles and do not appear to differentiate between the left and right replichores. Progressive segregation, coupled to fast and directed movement of the origin region at the beginning of replication, collaborate to duplicate the organization of the mother chromosome in each daughter cell.

Pressing Matters for the Future

Several critical questions remain to be explored with regards to chromosomal organization in all bacteria. We lack a mechanistic understanding of the requirements for maintaining chromosome orientation inside cells. Is one anchoring point enough to orient the entire chromosome, or do other sites need to be specifically positioned as well? In species like *E. coli* and *B. subtilis*, which apparently do not anchor the origin region to the pole during vegetative growth, are there compensating anchoring sites elsewhere? Indeed, does proper chromosome orientation *always* require an anchoring point to the cell envelope? It is known, for instance, that plasmids are positioned to specific subcellular locations in a self-organizing manner that does not seem to make use of membrane anchors (Ho et al. 2002; Garner et al. 2007). Hence, membrane anchors may be optional. On the other hand, early biochemical experiments in several bacteria—including *E. coli*—showed that multiple chromosomal sites, including not only the region around *oriC* but also the terminus and the site of ongoing replication, are found to associate with the membrane (Ryter 1968; Leibowitz and Schaechter 1975; Hendrickson et al. 1982). Perhaps, then, nonpolar anchoring sites exist in these organisms.

It is also still unclear why the chromosome is kept in a linear order within the cell, and not in the “bowl of spaghetti” configuration assumed in the past. This is a place in which physical modeling of polymers in confined spaces (e.g., Jun and Mulder 2006) may help guide our intuition. For example, linearity may be a simple consequence of progressive segregation

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and DNA compaction, i.e., as the DNA is segregated, it takes up space near the pole, forcing subsequent segments of the chromosome to reside farther away, with a simple linear dependence.

As for segregation, two avenues of investigation are likely to yield exciting results. On the one hand, understanding the mechanism of action of the ParABS and *migS* systems is of obvious importance. On the other hand, the fact that most bacteria seem to live surprisingly happy lives in the absence of ParABS or *migS* implies that there are other mechanisms at work. It is still unclear whether these are dedicated fail-safes, serendipitous consequences of other molecular machines (e.g., polymerases), or self-organizing processes.

Answering all of these questions will require new technologies and ingenious experiments, as well as an understanding of the similarities and differences between model systems. We expect the field of chromosome organization and segregation to be active and exciting in the coming years.

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REFERENCES

- Aaron M, Charbon G, Lam H, Schwarz H, Vollmer W, Jacobs-Wagner C. 2007. The tubulin homologue FtsZ contributes to cell elongation by guiding cell wall precursor synthesis in *Caulobacter crescentus*. *Mol Microbiol* **64**: 938–952.
- Abele AL, Friedman SA, Austin SJ. 1985. Partition of unit-copy miniplasmids to daughter cells. III. The DNA sequence and functional organization of the P1 partition region. *J Mol Biol* **185**: 261–272.
- Adams DE, Shekhtman EM, Zechiedrich EL, Schmid MB, Cozzarelli NR. 1992. The role of topoisomerase IV in partitioning bacterial replicons and the structure of catenated intermediates in DNA replication. *Cell* **71**: 277–288.
- Barák I, Wilkinson AJ. 2005. Where asymmetry in gene expression originates. *Mol Microbiol* **57**: 611–620.
- Bartosik AA, Jagura-Burdzy G. 2005. Bacterial chromosome segregation. *Acta Biochim Pol* **52**: 1–34.
- Bates D, Kleckner N. 2005. Chromosome and replisome dynamics in *E. coli*: Loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. *Cell* **121**: 899–911.
- Ben-Yehuda S, Rudner DZ, Losick R. 2003. RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science* **299**: 532–536.
- Ben-Yehuda S, Fujita M, Liu XS, Gorbatyuk B, Skoko D, Yan J, Marko JF, Liu JS, Eichenberger P, Rudner DZ, et al. 2005. Defining a centromere-like element in *Bacillus subtilis* by identifying the binding sites for the chromosome-anchoring protein RacA. *Mol Cell* **17**: 773–782.
- Bowman GR, Comolli LR, Zhu J, Eckart M, Koenig M, Downing KH, Moerner WE, Earnest T, Shapiro L. 2008. A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. *Cell* **134**: 945–955.
- Breier AM, Cozzarelli NR. 2004. Linear ordering and dynamic segregation of the bacterial chromosome. *Proc Natl Acad Sci* **101**: 9175–9176.
- Britton RA, Lin DC, Grossman AD. 1998. Characterization of a prokaryotic SMC protein involved in chromosome partitioning. *Genes Dev* **12**: 1254–1259.
- Burton BM, Marquis KA, Sullivan NL, Rapoport TA, Rudner DZ. 2007. The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in *Bacillus subtilis*. *Cell* **131**: 1301–1312.
- Castaing JP, Bouet JY, Lane D. 2008. F plasmid partition depends on interaction of SopA with non-specific DNA. *Mol Microbiol* **70**: 1000–1011.
- Cole RM, Hahn JJ. 1962. Cell wall replication in *Streptococcus pyogenes*. *Science* **135**: 722–724.
- Delius H, Worcel A. 1974. Electron microscopic studies on the folded chromosome of *Escherichia coli*. *Cold Spring Harb Symp Quant Biol* **38**: 53–58.
- Drlica K. 1992. Control of bacterial DNA supercoiling. *Mol Microbiol* **6**: 425–433.
- Dubarry N, Pasta F, Lane D. 2006. ParABS systems of the four replicons of *Burkholderia cenocepacia*: New chromosome centromeres confer partition specificity. *J Bacteriol* **188**: 1489–1496.
- Dubnau D. 2010. DNA pumps. *Cold Spring Harb Perspect Biol* **2**: a000406.
- Dworkin J, Losick R. 2002. Does RNA polymerase help drive chromosome segregation in bacteria? *Proc Natl Acad Sci* **99**: 14089–14094.
- Dworsky P, Schaechter M. 1973. Effect of rifampin on the structure and membrane attachment of the nucleoid of *Escherichia coli*. *J Bacteriol* **116**: 1364–1374.
- Ebersbach G, Briegel A, Jensen GJ, Jacobs-Wagner C. 2008. A self-associating protein critical for chromosome attachment, division, and polar organization in *caulobacter*. *Cell* **134**: 956–968.
- Errington J, Wake RG. 1991. Chromosome strand segregation during sporulation in *Bacillus subtilis*. *Mol Microbiol* **5**: 1145–1149.
- Espeli O, Mercier R, Boccard F. 2008. DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome. *Mol Microbiol* **68**: 1418–1427.

- Fekete RA, Chattoraj DK. 2005. A cis-acting sequence involved in chromosome segregation in *Escherichia coli*. *Mol Microbiol* **55**: 175–183.
- Fielding P, Fox CE. 1970. Evidence for stable attachment of DNA to membrane at the replication origin of *Escherichia coli*. *Biochem Biophys Res Commun* **41**: 157–162.
- Fogel MA, Waldor MK. 2005. Distinct segregation dynamics of the two *Vibrio cholerae* chromosomes. *Mol Microbiol* **55**: 125–136.
- Fogel MA, Waldor MK. 2006. A dynamic, mitotic-like mechanism for bacterial chromosome segregation. *Genes Dev* **20**: 3269–3282.
- Garner EC, Campbell CS, Weibel DB, Mullins RD. 2007. Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. *Science* **315**: 1270–1274.
- Gerdes K, Møller-Jensen J, Bugge Jensen R. 2000. Plasmid and chromosome partitioning: Surprises from phylogeny. *Mol Microbiol* **37**: 455–466.
- Gitai Z. 2010. The structure and function of bacterial actin and microfilaments. *Cold Spring Harb Perspect Biol* **2**: a000364.
- Gitai Z, Dye NA, Reisenauer A, Wachi M, Shapiro L. 2005. MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell* **120**: 329–341.
- Godfrin-Estevenon AM, Pasta F, Lane D. 2002. The parAB gene products of *Pseudomonas putida* exhibit partition activity in both *P. putida* and *Escherichia coli*. *Mol Microbiol* **43**: 39–49.
- Gordon GS, Sitnikov D, Webb CD, Teleman A, Straight A, Losick R, Murray AW, Wright A. 1997. Chromosome and low copy plasmid segregation in *E. coli*: Visual evidence for distinct mechanisms. *Cell* **90**: 1113–1121.
- Gruber S, Errington J. 2009. Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. *Cell* **137**: 685–696.
- Hendrickson WG, Kusano T, Yamaki H, Balakrishnan R, King M, Murchie J, Schaechter M. 1982. Binding of the origin of replication of *Escherichia coli* to the outer membrane. *Cell* **30**: 915–923.
- Hester CM, Lutkenhaus J. 2007. Soj (ParA) DNA binding is mediated by conserved arginines and is essential for plasmid segregation. *Proc Natl Acad Sci* **104**: 20326–20331.
- Hiraga S, Niki H, Ogura T, Ichinose C, Mori H, Ezaki B, Jaffé A. 1989. Chromosome partitioning in *Escherichia coli*: Novel mutants producing anucleate cells. *J Bacteriol* **171**: 1496–1505.
- Ho TQ, Zhong Z, Aung S, Pogliano J. 2002. Compatible bacterial plasmids are targeted to independent cellular locations in *Escherichia coli*. *EMBO J* **21**: 1864–1872.
- Hu B, Yang G, Zhao W, Zhang Y, Zhao J. 2007. MreB is important for cell shape but not for chromosome segregation of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. *Mol Microbiol* **63**: 1640–1652.
- Ireton K, Gunther NW, Grossman AD. 1994. spoOJ is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J Bacteriol* **176**: 5320–5329.
- Jacob F, Brenner S, Cuzin F. 1963. On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symp Quant Biol* **28**: 329–348.
- Jacq A, Kohiyama M. 1980. A DNA-binding protein specific for the early replicated region of the chromosome obtained from *Escherichia coli* membrane fractions. *Eur J Biochem* **105**: 25–31.
- Jakimowicz D, Brzostek A, Rumijowska-Galewicz A, Zydek P, Dolzblasz A, Smulczyk-Krawczynszyn A, Zimniak T, Wojtasz L, Zawilak-Pawlik A, et al. 2007. Characterization of the mycobacterial chromosome segregation protein ParB and identification of its target in *Mycobacterium smegmatis*. *Microbiology* **153**: 4050–4060.
- Jensen RB, Shapiro L. 1999. The *Caulobacter crescentus* smc gene is required for cell cycle progression and chromosome segregation. *Proc Natl Acad Sci* **96**: 10661–10666.
- Jensen RB, Wang SC, Shapiro L. 2001. A moving DNA replication factory in *Caulobacter crescentus*. *EMBO J* **20**: 4952–4963.
- Jun S, Mulder B. 2006. Entropy-driven spatial organization of highly confined polymers: Lessons for the bacterial chromosome. *Proc Natl Acad Sci* **103**: 12388–12393.
- Karczmarek A, Martínez-Arteaga R, Baselga RM, Alexeeva S, Hansen FG, Vicente M, Nanninga N, den Blaauwen T. 2007. DNA and origin region segregation are not affected by the transition from rod to sphere after inhibition of *Escherichia coli* MreB by A22. *Mol Microbiol* **65**: 51–63.
- Kavenoff R, Bowen BC. 1976. Electron microscopy of membrane-free folded chromosomes from *Escherichia coli*. *Chromosoma* **59**: 89–101.
- Kavenoff R, Ryder OA. 1976. Electron microscopy of membrane-associated folded chromosomes of *Escherichia coli*. *Chromosoma* **55**: 13–25.
- Kellenberger E. 1991. Functional consequences of improved structural information on bacterial nucleoids. *Res Microbiol* **142**: 229–238.
- Kruse T, Blagoev B, Løbner-Olesen A, Wachi M, Sasaki K, Iwai N, Mann M, Gerdes K. 2006. Actin homolog MreB and RNA polymerase interact and are both required for chromosome segregation in *Escherichia coli*. *Genes Dev* **20**: 113–124.
- Lansdorp PM. 2007. Immortal strands? Give me a break. *Cell* **129**: 1244–1247.
- Lau IF, Filipe SR, Søballe B, Økstad OA, Barre FX, Sherratt DJ. 2003. Spatial and temporal organization of replicating *Escherichia coli* chromosomes. *Mol Microbiol* **49**: 731–743.
- Leibowitz PJ, Schaechter M. 1975. The attachment of the bacterial chromosome to the cell membrane. *Int Rev Cytol* **41**: 1–28.
- Lemon KP, Grossman AD. 2001. The extrusion-capture model for chromosome partitioning in bacteria. *Genes Dev* **15**: 2031–2041.
- Lenarcic R, Halbedel S, Visser L, Shaw M, Wu LJ, Errington J, Marenduzzo D, Hamoen IW. 2009. Localisation of DivIVA by targeting to negatively curved membranes. *EMBO J* **28**: 2272–2282.
- Lew DJ, Burke DJ, Dutta A. 2008. The immortal strand hypothesis: How could it work? *Cell* **133**: 21–23.
- Lewis RA, Bignell CR, Zeng W, Jones AC, Thomas CM. 2002. Chromosome loss from par mutants of *Pseudomonas putida* depends on growth medium and phase of growth. *Microbiology* **148**: 537–548.

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- Marczynski GT, Dingwall A, Shapiro L. 1990. Plasmid and chromosomal DNA replication and partitioning during the *Caulobacter crescentus* cell cycle. *J Mol Biol* **212**: 709–722.
- Mobley HL, Koch AL, Doyle RJ, Streips UN. 1984. Insertion and fate of the cell wall in *Bacillus subtilis*. *J Bacteriol* **158**: 169–179.
- Mohl DA, Gober JW. 1997. Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* **88**: 675–684.
- Moriya S, Tsujikawa E, Hassan AK, Asai K, Kodama T, Ogasawara N. 1998. A *Bacillus subtilis* gene-encoding protein homologous to eukaryotic SMC motor protein is necessary for chromosome partition. *Mol Microbiol* **29**: 179–187.
- Mullins D. 2010. Bacterial cytoskeleton: The function of FtsZ and ParA polymers. *Cold Spring Harb Perspect Biol* **2**: a002550.
- Murray H, Errington J. 2008. Dynamic control of the DNA replication initiation protein DnaA by Soj/ParA. *Cell* **135**: 74–84.
- Nielsen HJ, Li Y, Youngren B, Hansen FG, Austin S. 2006a. Progressive segregation of the *Escherichia coli* chromosome. *Mol Microbiol* **61**: 383–393.
- Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG. 2006b. The *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. *Mol Microbiol* **62**: 331–338.
- Niki H, Hiraga S. 1998. Polar localization of the replication origin and terminus in *Escherichia coli* nucleoids during chromosome partitioning. *Genes Dev* **12**: 1036–1045.
- Niki H, Imamura R, Kitaoka M, Yamanaka K, Ogura T, Hiraga S. 1992. *E. coli* MukB protein involved in chromosome partition forms a homodimer with a rod-and-hinge structure having DNA binding and ATP/GTP binding activities. *EMBO J* **11**: 5101–5109.
- Niki H, Yamaichi Y, Hiraga S. 2000. Dynamic organization of chromosomal DNA in *Escherichia coli*. *Genes Dev* **14**: 212–223.
- Osley MA, Newton A. 1974. Chromosomes segregation and development in *Caulobacter crescentus*. *J Mol Biol* **90**: 359–370.
- Pettijohn DE. 1982. Structure and properties of the bacterial nucleoid. *Cell* **30**: 667–669.
- Postow L, Hardy CD, Arsuaiga J, Cozzarelli NR. 2004. Topological domain structure of the *Escherichia coli* chromosome. *Genes Dev* **18**: 1766–1779.
- Prentki P, Chandler M, Caro L. 1977. Replication of prophage P1 during the cell cycle of *Escherichia coli*. *Mol Gen Genet* **152**: 71–76.
- Ptacin JL, Nollmann M, Becker EC, Cozzarelli NR, Pogliano K, Bustamante C. 2008. Sequence-directed DNA export guides chromosome translocation during sporulation in *Bacillus subtilis*. *Nat Struct Mol Biol* **15**: 485–493.
- Ramamurthi KS, Losick R. 2009. Negative membrane curvature as a cue for subcellular localization of a bacterial protein. *Proc Natl Acad Sci* **106**: 13541–13545.
- Robinett CC, Straight A, Li G, Willhelm C, Sudlow G, Murray A, Belmont AS. 1996. In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J Cell Biol* **135**: 1685–1700.
- Robinow C, Kellenberger E. 1994. The bacterial nucleoid revisited. *Microbiol Rev* **58**: 211–232.
- Robinow CF. 1956. The chromatin bodies of bacteria. *Bacteriol Rev* **20**: 207–242.
- Ryter A. 1968. Association of the nucleus and the membrane of bacteria: A morphological study. *Bacteriol Rev* **32**: 39–54.
- Ryter A, Chang A. 1975. Localization of transcribing genes in the bacterial cell by means of high resolution autoradiography. *J Mol Biol* **98**: 797–810.
- Scheirer KE, Higgins NP. 2001. Transcription induces a supercoil domain barrier in bacteriophage Mu. *Biochimie* **83**: 155–159.
- Schoeffler AJ, Berger JM. 2008. DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. *Q Rev Biophys* **41**: 41–101.
- Shebelut CW, Jensen RB, Gitai Z. 2009. Growth conditions regulate the requirements for *Caulobacter* chromosome segregation. *J Bacteriol* **191**: 1097–1100.
- Sinden RR, Pettijohn DE. 1981. Chromosomes in living *Escherichia coli* cells are segregated into domains of supercoiling. *Proc Natl Acad Sci* **78**: 224–228.
- Stonington OG, Pettijohn DE. 1971. The folded genome of *Escherichia coli* isolated in a protein-DNA-RNA complex. *Proc Natl Acad Sci* **68**: 6–9.
- Straight AF, Belmont AS, Robinett CC, Murray AW. 1996. GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr Biol* **6**: 1599–1608.
- Sueoka N, Quinn WG. 1968. Membrane attachment of the chromosome replication origin in *Bacillus subtilis*. *Cold Spring Harb Symp Quant Biol* **33**: 695–705.
- Sullivan NL, Marquis KA, Rudner DZ. 2009. Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. *Cell* **137**: 697–707.
- Surtees JA, Funnell BE. 2003. Plasmid and chromosome traffic control: How ParA and ParB drive partition. *Curr Top Dev Biol* **56**: 145–180.
- Teleman AA, Graumann PL, Lin DC, Grossman AD, Losick R. 1998. Chromosome arrangement within a bacterium. *Curr Biol* **8**: 1102–1109.
- Thanbichler M, Shapiro L. 2008. Getting organized—how bacterial cells move proteins and DNA. *Nat Rev Microbiol* **6**: 28–40.
- Toro E, Hong SH, McAdams HH, Shapiro L. 2008. *Caulobacter* requires a dedicated mechanism to initiate chromosome segregation. *Proc Natl Acad Sci* **105**: 15435–15440.
- Van Helvoort JM, Woldringh CL. 1994. Nucleoid partitioning in *Escherichia coli* during steady-state growth and upon recovery from chloramphenicol treatment. *Mol Microbiol* **13**: 577–583.
- Viollier PH, Thanbichler M, McGrath PT, West L, Meewan M, McAdams HH, Shapiro L. 2004. Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc Natl Acad Sci* **101**: 9257–9262.
- Wake RG, Errington J. 1995. Chromosome partitioning in bacteria. *Annu Rev Genet* **29**: 41–67.

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- Wang JC. 1971. Interaction between DNA and an *Escherichia coli* protein ω . *J Mol Biol* **55**: 523–533.
- Wang X, Liu X, Possoz C, Sherratt DJ. 2006. The two *Escherichia coli* chromosome arms locate to separate cell halves. *Genes Dev* **20**: 1727–1731.
- Watson JD, Crick FH. 1953. Genetical implications of the structure of deoxyribonucleic acid. *Nature* **171**: 964–967.
- Webb CD, Teleman A, Gordon S, Straight A, Belmont A, Lin DC, Grossman AD, Wright A, Losick R. 1997. Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. *Cell* **88**: 667–674.
- Wheeler RT, Shapiro L. 1997. Bacterial chromosome segregation: Is there a mitotic apparatus? *Cell* **88**: 577–579.
- White MA, Eykelenboom JK, Lopez-Vernaza MA, Wilson E, Leach DR. 2008. Non-random segregation of sister chromosomes in *Escherichia coli*. *Nature* **455**: 1248–1250.
- Woldringh CL. 2002. The role of co-transcriptional translation and protein translocation (transertion) in bacterial chromosome segregation. *Mol Microbiol* **45**: 17–29.
- Worcel A, Burgi E. 1972. On the structure of the folded chromosome of *Escherichia coli*. *J Mol Biol* **71**: 127–147.
- Wu LJ, Errington J. 1994. *Bacillus subtilis* spoIIIE protein required for DNA segregation during asymmetric cell division. *Science* **264**: 572–575.
- Wu LJ, Errington J. 2003. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating *Bacillus subtilis*. *Mol Microbiol* **49**: 1463–1475.
- Yamaichi Y, Niki H. 2004. migS, a cis-acting site that affects bipolar positioning of oriC on the *Escherichia coli* chromosome. *EMBO J* **23**: 221–233.
- Yamaichi Y, Fogel MA, Waldor MK. 2007. par genes and the pathology of chromosome loss in *Vibrio cholerae*. *Proc Natl Acad Sci* **104**: 630–635.

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