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REVIEW

The Bacterial Cell Cycle: DNA Replication, Nucleoid Segregation, and Cell Division

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Abstract—Data on the bacterial cell cycle published in the last 10–15 years are considered, with a special stress on studies of nucleoid segregation between dividing cells. The degree of similarity between the eukaryotic mitotic apparatus and the apparatus performing nucleoid separation is discussed.

Key words: DNA, chromosome, nucleoid, cellular septum, cell division.

Research in the fields of bacterial cytology and caryology goes back more than a hundred years. Adjustments to the hypotheses on nucleoid structure and on the mechanisms of segregation of nucleoids prior to cell division reflected the progress made in microscopic technique, in methods of fixation and staining, and finally, in the techniques for vital observation of dividing bacteria. In 1963, F. Jacob's hypothesis [1], according to which nucleoids are attached to the cytoplasmic membrane, and, therefore, in the course of bacterial elongation prior to division, they move to the cell poles passively, being pulled apart by this elongation, became popular. The cellular septum, which grows between them, additionally pushes them apart. Together with Jacob's authority, numerous studies in which the spatial relation between the nucleoid and the cell membrane was established beyond doubt seemed to support this hypothesis. This hypothesis, however, had to undergo alteration and additions due to the development of new microscopic techniques and to the results of studies of numerous mutants with various cell division dysfunctions. The concept that the process of nucleoid segregation had an active, rather than passive, nature became prevalent. Specialized proteins were shown to take part in this process, which, to a certain degree, resembled eukaryotic mitosis. The role of the cytoplasmic membrane in chromosome replication and nucleoid segregation has never been disputed; the picture just became more complex, and other points were stressed.

The goal of the present review is to provide a generalized view of the bacterial cell cycle based on studies carried out over the last 10–15 years, with the most attention given to the behavior of the bacterial nuclear apparatus. These ideas are very likely to change in the future; however, the studies performed in this period can be considered as of special importance due to the multitude of newly introduced techniques. The related articles are too numerous for exhaustive citation; therefore, in many cases, only reviews will be mentioned (which are also rather numerous). A short introduction describing the main varieties of bacterial phenotype exhibiting cell division dysfunctions, caused by mutations or external influences, seems to be called for, as is some mention of the principles underlying the techniques related to fluorescence and immunofluorescence microscopy, which is currently the most advanced field in bacterial cytology.

A terminological problem should be settled from the very beginning. The terms *nucleoid* (a morphological concept) and *chromosome* (more of a genetic term in the case of bacteria) will be used synonymously in this review. The chromosome can be thought of as an unwound nucleoid; a nucleoid, as a chromosome compacted by special proteins [2]. Although this is, to some degree, a simplification, in the author's opinion, it should make the review more readable.

1. Phenotypic Manifestation of Mutations Impairing the Bacterial Cell Cycle

Severe impairments of the bacterial cell cycle inevitably result in cell death, whatever their cause. In order to bypass this difficulty, strains with conditionally lethal, and, usually, temperature-sensitive, mutations are used. Such mutations are manifested at elevated incubation temperatures (e.g., above 45°C), while, at 37°C or below, normal or partially affected growth occurs. Even under these conditions, it is often difficult to differentiate between manifestations of mutations in different genes. This circumstance is especially true for cell morphology. A wide range of mutations affecting DNA replication and cell division, even if they are not lethal, have a pleiotropic manifestation: they lead to the formation of threadlike cells (filamentous phenotype),

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cells deprived of a septa (sep phenotype), disorderly distributed nucleoids (par phenotype), and small cells deprived of nucleoids (min phenotype), as well as decelerating growth and decreasing viability; often, all these manifestations occur together. Speaking medically, the symptomatology is blurred. The same can be said about the responses of a normal cell to factors affecting DNA replication and cell division. These responses are morphological phenocopies of the abovementioned pleiotropic mutations, and the relations between cause and effect are not always clear. It is not always possible, even for the most experienced cytologist, to notice the difference between diffused and condensed nucleoids or between serrated and smooth undeveloped cell septa, and the quantitative boundaries are often blurred. However, it is safe to state that, by the 1990s, a breakthrough occurred that led to a more precise understanding of the behavior of a cell's nuclear apparatus and of the role of various proteins at different stages of the cell cycle. This breakthrough was the result of progress in fluorescence microscopy and of the development of new methods of selectively staining specific DNA fragments and aggregates of specific proteins. The basics of the related techniques will be listed in the next paragraph.

2. The Principles Applied in Fluorescence Microscopy of Bacteria

Over 70 or 80 years, different modifications of the Romanowsky–Giemsa staining technique, first used at the end of the 19th century to stain malarial plasmodia in erythrocytes (see Peshkov's monograph [3]), were the most popular methods used to stain bacterial nuclear apparatus for light microscopy. Although this and other staining techniques, together with a range of other methods used in bacterial cytology (vital phase-contrast and interference microscopy and diverse electron microscopic techniques), contributed greatly to our knowledge of the morphology of bacterial nucleoids, they have, in a sense, reached the limit of their potential. In recent decades, staining with DAPI (4,6-diamidino-2-phenylindole) has often been used for studies involving fluorescence microscopy of nucleoids, which, after DAPI staining, appear as bright blue bodies on a background of dimmer cytoplasm. This method can easily be combined with other special staining techniques. For instance, specific bacterial proteins can be made selectively visible by staining them with various fluorophors, such as fluorescein isothiocyanate. In order to do this, the so-called primary antibodies specific to a particular protein, e.g., mouse monoclonal antibodies, are obtained. Bacteria are then treated with these antibodies. A treatment with secondary antibodies follows, e.g., rabbit antimouse antibodies conjugated with a fluorophor. Aggregates of the specified protein in the cell can be discerned as luminous orange or red point against the bluish background of the DAPI-stained structures, including the nucleoids.

Another way to visualize specified proteins is to fuse the gene encoding this protein with the *gfp* (green fluorescent protein) gene. This is a gene of an *Aequorea vicroria* jellyfish protein that emits greenish light. A bacterial gene fused with *gfp*, which acts as a reporter gene, encodes the formation of a hybrid fluorescent protein. This gene can be attenuated to an external promoter, such as lactose promoter, with the result that its action can be induced at will.

In order to visualize the DNA sites corresponding to a specific nucleoid site, fluorescent in situ hybridization (FISH) is used. The genes located at the site of interest are cloned, and the cloned DNA is then amplified by a PCR. The amplification product is labeled by a fluorophor (e.g., fluorescein 11dUTP), and the labeled DNA is fragmented and denatured. When fixed bacterial cells are treated with this DNA, it hybridizes and, thus, binds to the homologous site in the nucleoid. This site then becomes fluorescent.

It should be noted that these techniques were initially developed for the investigation of eukaryotic cells and chromosomes and were only later used, with modifications, in bacterial cytology. Numerous difficulties had to be overcome, for instance, the size of the nucleoid in typical bacteria like *E. coli*, which is as short as ca. 1 μm (whereas the metaphase chromosomes of most eukaryotes are 7–9 μm long).

3. An Overview of the Process of Bacterial Chromosome Replication

The whole of the bacterial cell cycle can be, albeit somewhat arbitrarily, subdivided into two coordinated stages: (1) chromosome replication and separation (segregation) of sister nucleoids and (2) cell division proper (cytokinesis). The moment when the sister cells have just formed a septum between themselves is taken as the zero point, when the cell is considered newborn (see reviews [4–6]). The newborn cells may share a common cell wall; however, completely separated cells are more convenient to work with (see below). For the sake of convenience, a new born rod-shaped cell is schematically subdivided into four equal parts; two of these (regions 1 and 4) are close to the poles, and two (regions 2 and 3) are located in the middle. Region 4 corresponds to the cell pole that was adjacent to the septum prior to division (Fig. 1). The position of the nucleoid in the newborn cell will be discussed further below.

Replication of chromosomal DNA starts after a certain period following cell separation. A great number of articles and reviews discussed chromosome replication; however, their analysis is beyond the scope of this paper. Generally, replication is initiated in response to the concentration of DNAA protein reaching a certain critical level [7, 8]. This event probably coincides with the total mass of the newborn cell reaching a certain level [9, 10]. DNAA protein binds with so-called DNA boxes, specific sequences in the origin site (oriC). DNA

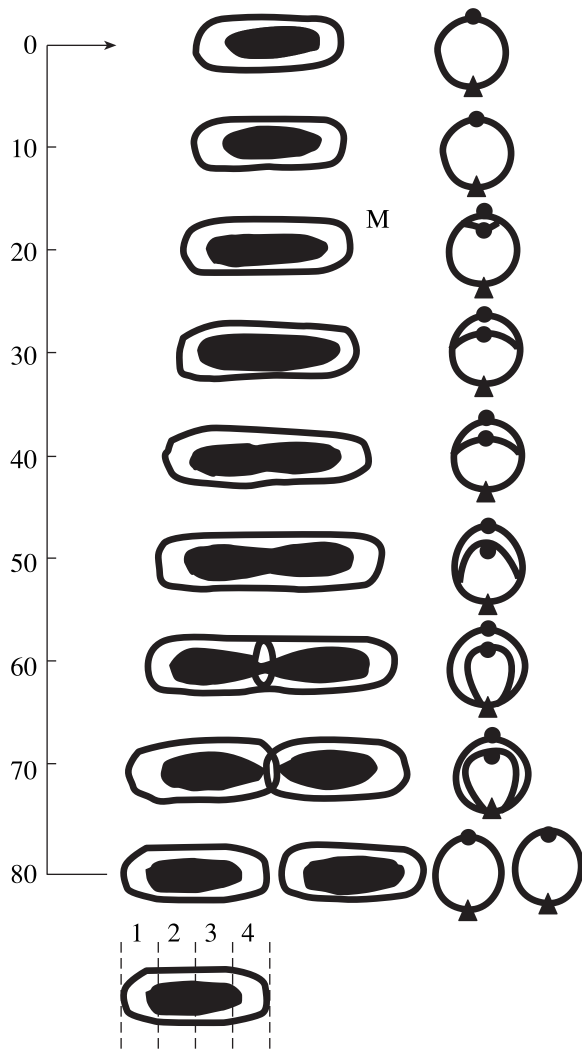

Fig. 1. Division of a bacterial cell, duplication of the nucleoid, and replication of the chromosome (parallel timelines). The scheme presents the life cycle of *E. coli* during decelerated growth (life cycle duration of 80 min). The nucleoid is shown as a black oval body inside the cell (on the left); in the replicating chromosome, the origin and terminus are shown as circles and triangles, respectively (on the right). M (20th minute of the life cycle) is the moment when the cell has achieved the critical mass and the critical amount of DNAA protein for the replication to start. By the 60th minute, the formation of the Z ring (septal ring) is completed; by the 70th minute, growth of the intercellular septum is completed. The scheme is a modification of the schemes presented by Hiraga [97] and Wheeler and Shapiro [103]. In the bottom, a newborn cell conditionally subdivided into four regions (see text) is shown.

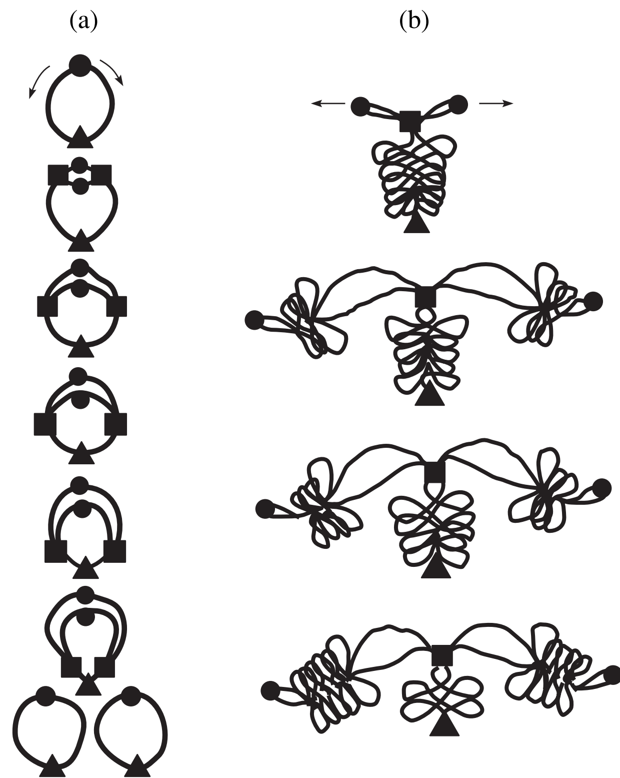

Fig. 2. Models of chromosome replication in bacteria: (a) Replisomes move along the branches of the chromosome in opposite directions and perform DNA replication; (b) chromosome branches move through an immobile replisome and are replicated at the moment they pass through it. The origins and terminuses of the replication are marked with circles and triangles, respectively; the replisomes are marked with squares. The schemes are a modification of schemes presented by Draper and Gober [6] and Hiraga [97].

helicase is then bound to this complex, untwisting the double-stranded DNA and thus enabling replication. DNA polymerase III, together with a complex of other enzymes called replisome, performs replication. For a long time, it was accepted without question that both replisomes move away from the origin, travel along the branches of the chromosome ring, and meet at the termination point (terC). They leave behind them replication forks, i.e., duplicated branches of the chromosome (Fig. 2a). Fluorescence microscopy, however, revealed that the replisome is always located in the central part of the nucleoid; it does not move [11, 12] and is probably attached to the cytoplasmic membrane. Another scheme was therefore suggested according to which the replisome is fixed whereas both branches of the chromosome duplicate while moving through it and leave it in the form of a replication fork (Fig. 2b).

The time taken for chromosome replication depends on the growth conditions of a bacterial culture; slowly growing cells are more convenient for cytological research. In the case of the prolonged (80 min) cell cycle in *E. coli*, preparation for replication and the replication itself take about 60 min (Fig. 1). Then, the nucleoids separate and move apart, and, at the same time, cell division starts. The process takes about 20 min, and, as a result, two individual newborn cells are produced.

This picture is certainly an oversimplification. First, as was mentioned above, a certain fraction of the cells do not separate after division but continue their existence as a bacterium with two or even four nucleoids

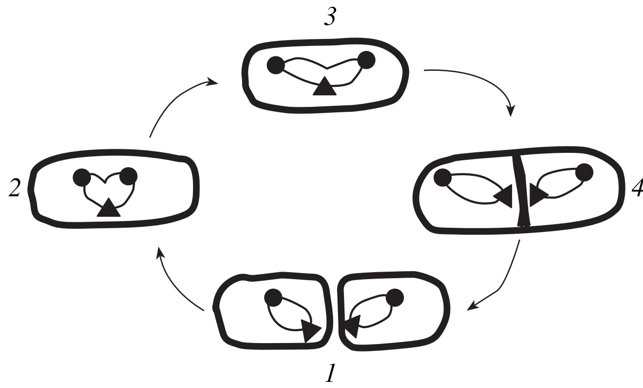

Fig. 3. Migration of various sites of the nucleoid at different stages of a bacterial life cycle. The origins and terminuses of replication are marked by circles and triangles, respectively. (*1*) In a newborn cell, the terminus is turned to the cell pole corresponding to the newly formed septum, and the origin is turned in the opposite direction. Then, (*2, 3*) the nucleoid moves to the center of the cell; the origins and adjoining sites duplicate and start moving apart and towards the poles. (*4*) The terminus with adjoining sites duplicates, and the septum is formed. Two newborn cells are formed, and the cycle is completed (*1*).

separated by partitions but sharing a common cell wall. Second, before the termination of the initial tour of DNA replication, a supplementary tour may start, also in the oriC region. This process doubles the number of replication forks per cell. Special mechanisms exist to prevent the beginning of a new tour, or at least to postpone it until a certain specified time. Lack of methylation of newly synthesized DNA for approximately one-third of the replication cycle is one such mechanism in *E. coli*. Only the old template DNA remains methylated, and the nonmethylated newly synthesized DNA cannot serve as a template in the next tour [13]. The process preventing methylation and, thus, the onset of an extra tour of replication is called sequestration; in *E. coli*, it requires the interaction of Seq protein with DNA A protein. In seqA mutants, the oriC region undergoes repeated duplication and has a multifork structure [14]. This sequestration mechanism can also sometimes fail in normal cells; then, more than one fork exists in the origin region. In the course of research into the bacterial cell cycle, efforts should be made to avoid this situation, since the superposition of replication waves blurs the overall picture. The consequences of such a superposition will not therefore be discussed here, although it is a rather common phenomenon.

In general, any impairments of the replication cycle, both in mutants and in normal cells under the influence of external factors, lead to the production of threadlike forms with impaired nucleoid segregation. Irregularities in cytokinesis will be discussed further in the section of this review devoted to cell division. Now, the data on chromosome orientation and separation will be considered.

4. Spatial Orientation of Chromosomes and Separation of Nucleoids

It has long been suspected that newly formed nucleoids are specifically oriented prior to their partitioning between sister cells; moreover, in a number of instances, similarity between the processes of nucleoid segregation and chromosome separation during mitosis was suggested (see reviews [15, 16]). However, the orientation of different parts of the bacterial chromosome relative to the cell poles during replication was experimentally demonstrated only at the very end of the 20th century and in the beginning of the 21st century. These data were obtained almost simultaneously for three bacteria, *E. coli, B. subtilis*, and *Caulobacter crescentus.* Since each of the three models has its own particular features, these results should be considered separately.

The work on *E. coli* was carried out mostly by Japanese and American researchers [17–22]. In a subpopulation of growing bacteria that contained small mononuclear cells, most probably newborn ones, the migration of the nucleoid as a whole and the orientation of its different parts were monitored. The growth conditions were adjusted so that the cell cycle could be artificially extended to, e.g., 80 min. The oriC and terC sites, the adjacent sites, and the other sites in the chromosome were made fluorescent by various means. With the help of a flow cytometer, a great number of cells were examined and sorted in the course of incubation, and the location of the fluorescent points was recorded.

In the newborn cells, the nucleoid was visible in the central part. The previous stage, probably the initial one (migration of the nucleoid from a pole (position 4) to the cell center), was also sometimes observed [19]. In a centrally positioned nucleoid, fluorescent labels corresponding to the oriC and terC sites were oppositely oriented. The following two events then occurred simultaneously: (1) the nucleoid turned so as to be positioned across the cell (the labeled terC still remained in the center of the cell), and (2) oriC duplicated (two fluorescent points were formed), and both of oriC moved towards the poles and stopped in a short distance from them (in positions 1–2 and 3–4). Then, duplication of terC occurred. The duplicated nucleoids moved to the cell poles without changing their orientation, and the intercellular septum was formed (Fig. 3). Another scenario, not shown in Fig. 3, was also possible: terC could remain near the cell pole in position 4, while the duplicated oriC moved to the opposite sides. In this case, terC was, for some time, located asymmetrically in relation to them [23].

The fluorescent mark was introduced not only into the oriC and terC sites but also into other parts of the chromosome (a total of 22 sites of 230 kb each; these sites covered the entire chromosome). In the course of replication, a relatively big region of the chromosome termed the ori domain (about 900 kb; 20% of the whole chromosome) was found to move together with oriC. A region of the same size was found to be located around terC (the ter domain). The rest of the nucleoid was

shown to be located in the central part of the cell; only after the replication is finished, does it separate into two parts that move in opposite directions [19]. In another study [21], the authors used a strain with the bacterial oriC removed from the chromosome; instead, plasmid replication origins from the F factor were introduced into different parts of its chromosome. This strain often produced anucleate and filamentous forms; its growth was slow and poor but still occurred. The part of the nucleoid oriented to the cell pole was shown not to be the plasmid origin (the site from which replication now began) but the region from which the natural oriC had been removed. Thus, the point of attraction to the pole was located in the ori domain and not in the oriC proper. In another, rather elegant, study [22], sites in the ori domain were sequentially excised from the chromosome by recombination with plasmids. Finally, a strain was obtained missing a small, 25-bp region located 211 kb from oriC (clockwise). In this strain, the nucleoid orientation existing during replication was lost. This region was an imperfect inverted repeated sequence and belonged to a gene not related to encoding proteins involved in the cell cycle. This site was named migS (migration site); when transferred to the ter domain, it caused inverse orientation of the nucleoid and its movement in an unnatural direction. Some protein that is responsible for the orientation of oriC toward the cell poles probably attaches to the migS and to the adjoining regions. The migS site acts only *in cis*; i.e., it does not encode any soluble product and serves only as a "reference point." This site can be compared to the eukaryotic centromere. A protein capable of attaching to it has not yet been found in *E. coli.* In the studies on *B. subtilis*, however, more progress has been achieved, as will be discussed below.

Studies of the same topic, but carried out with *B. subtilis*, were published almost simultaneously with those conducted using *E. coli*, or even a bit earlier, and, since then, these investigations have followed parallel courses. The studies on *B. subtilis* were performed at J. Errington's laboratory (United Kingdom) and by several American teams [24–33]. Since *B. subtilis* is a spore-forming bacterium, and migration of the nucleoid or its parts can be monitored during sporulation, it has some advantages as a model. At one of the early stages of spore formation, the replicating nucleoid extends through the cell, forming the so-called axial filament. At the same time, a membrane partition is formed closer to one of the poles and unevenly divides the cell into a sporangium (mother cell) and a small forespore (the sporangium subsequently dies off, and the forespore turns into a spore). An incomplete septum contains a pore through which one of the sister nucleoids can pass; the second one remains in the sporangium. At the beginning of the second stage of sporulation, not more than one-third of the future chromosome of the spore is contained in the forespore. The fact that some genes can be transcribed only in the forespore (for example, sigma F, one of the sigma factors for *B. subtilis* RNA polymerase, starts functioning there) and that this is the stage at which the process of sporulation is blocked in some of the spo mutants is important. As well as experiments with sporulating bacteria, experiments with vegetative cells of *B. subtilis* can also be performed.

One of the sporulation genes, *spoOJ*, has been shown to be located very closely to oriC. In subsequent studies, the *gfp* reporter gene was attached to the *spoOJ* site. In living cells, the fluorescent point, and, therefore, oriC, was always located in the forespore, close to the pole located distally relative to the membrane partition. Immunofluorescence experiments have confirmed this location of oriC. In vegetative cells, labeled oriC also migrated to the pole [25, 27, 28]. The polar location of the oriC site has also been demonstrated in purely genetic experiments on the determination of forespore DNA transformation activity in relation to the markers located in that region (purA, amyE, etc.). Forespore DNA was capable of transforming competent cells with these markers but not with those located closer to terC [29]. Various refinements were later made. A number of inversions in the oriC locus were obtained, and the effect of these inversions on nucleoid migration into the forespore was monitored. The general strategy of these experiments was similar to that of the above-mentioned experiments on the effect of deletions on the oriC migration in *E. coli.* The region where the migration started, i.e., the polar localization region (PLR), did not completely coincide with oriC; instead, it was located 150–300 kb to the left in the chromosome map. Of course, the migrating PLR also carried away oriC. Moreover, this region is not a small DNA fragment but occupies about 100 kb. Even after being split by inversions, it partially retained its ability to lead the nucleoid separation; however, cells with inversions developed poorly, showing delayed growth and partial impairment of their sporulation. Migration of the PLR into the forespore was monitored not only by fluorescence but also by the activity of the β galactosidase reporter gene, attenuated to sigma F [30]. The reporter gene was therefore able to express itself only in the forespore (see above).

Thus, a chromosome region adjacent to oriC and functionally similar to the eukaryotic centromere was found in *B. subtilis* as well. Since eukaryotic chromosomes carry proteins forming a specific kinetochore structure, to which the threads of the spindle are attached, an attempt was made to find something similar in bacilli. SpoOJ was the first candidate for the role of kinetochore. It could bind to ten imperfect inverted 16-nucleotide repetitions (5'-TGTTCCACGTGAAACA-3') scattered to the left and right of the oriC site and partially occupying the PLR. These repetitions were named parS. If a parS fragment was introduced into a plasmid exhibiting impaired segregation between the daughter cells, plasmid segregation became regular [31, 32]. The opinion prevailed, however, that the role of this protein, together with the protein SOJ, is to make the oriC site more compact during its passage through

the hole in the forespore partition, as well as to generally compactize the whole nucleoid [30, 32, 34]. In another recent study [33], kinetochore-like action was attributed to the protein RacA, which participates in anchoring the oriC site near the cell pole. It binds to a region near oriC. This binding is somewhat disperse and occurs at small isolated sites located within a region of 60–80 kb. This protein probably acts as a "bridge" between the oriC site and the protein DivIA, which is located at the cell pole. Sometimes the DivIA protein is even considered to be the main agent in the active movement of oriC to the poles [35, 36]. It also plays a role in the interaction with MinCD proteins during cell division (see below).

Data on the orientation of the oriC sites relative to the cell poles during chromosome separation have also been obtained in the studies involving *Caulobacter crescentus* [37, 38]. This bacterium is very convenient for such research due to the regular changes exhibited in the physiological state of the cells. Immediately after division, one of the cells is nonmotile, while the other is motile but incapable of DNA replication and division. Then, it looses its flagellum, becomes nonmotile, and divides, producing two nonequivalent cells, a motile and a nonmotile one. The motile cell looses its flagellum and divides, and the cycle repeats [39]. These phase changes are very regular, so a fraction of motile cells can be used to obtain a synchronously dividing culture.

In *C. crescentus*, proteins are present that are homologous to the Par proteins of some of the big plasmids, such as F factor and R_1. These proteins are responsible for the regular distribution of plasmids between the daughter cells (see below). At the early stage of synchronous culture development, the labeled bacterial homologues of these proteins have been shown to be distributed diffusely; subsequently, they gather in the center and then move to the poles. Furthermore, their association with the chromosomal oriC has been demonstrated. This result means that the replication origins are also initially located in the cell center and move away from the center after replication.

Thus, very similar results have been obtained for at least three bacterial species. At the early stages of chromosome replication, the origin sites of the nucleoids were oriented toward the poles; subsequently, the nucleoids moved apart in the same directions. The leading site was located in the same region as the origin but only partially coincided with it. Proteins binding to the leading site were found in *B. subtilis* and *C. crescentus* and possibly act as a connecting link between the leading sites of the nucleoids and the membrane of the polar regions of the cell.

The last site to replicate, the terminus, or terC, is no less critical than oriC. As was previously mentioned, in newborn cells, it moves to the center (positions 2–3) and remains there until the end of chromosome segregation [18, 19]. It is difficult to say whether the terC site moves independently or if the movement of the fluorescent point corresponding to terC is simply a result of the movement of the nucleoid as a whole. This, however, also applies the migration of oriC (see below). The terC site is a relatively big region (in *E. coli*, it is 280 kb on each of the chromosome branches) that contains several noncoding 22-bp sequences with a conservative nucleus of 13 nucleotides and variable fragments [40]. In the center of terC, lies the so-called dif site, which is 28 bp. Deletions in this site lead to a complex of distortions of the normal phenotype: changes in the nucleoid morphology, errors in the chromosome segregation between cells, dysfunctions in the cell division, emergence of elongated thread-like cells, death of dividing cells at nonpermissive temperatures. The very name of the site, *deletion-induced filamentation*, is due to one of these syndromes. The daughter chromosomes are, for some time, an interwound catenated dimeric structure, and their decatenation occurs in the terC region. Separation of the chromosomes, the final stage of decatenation, occurs at the dif site. Chromosome segregation, however, can be successful only if this site is surrounded by the so-called dif active zone (DAZ), stretching more than 8 kb on each side of terC [41, 42]. This zone probably contains a number of sequences oriented in a certain direction. This conclusion is based on the results of experiments involving the insertion of foreign DNA fragments in this region [41, 42]. Some of these insertions, although located outside the dif site, impaired chromosome segregation, whereas others had no effect when directly oriented but prevented chromosome segregation if oriented reversely. The composition of these sequences is still unknown.

DNA gyrase; the resolvases XerC and XerD; topoisomerase IV; one of the domains of the cell division protein FtsK (see below); and homologous recombination enzymes [18, 43] are the main participants in the processes of decatenation and chromosome segregation (in addition, see reviews [6, 16]). When *E. coli* ts gyrase mutants undergo mild lysis at nonpermissive temperatures, mainly dumbbell-like duplicated nucleoids with a short bridge between them are isolated. The bridge corresponds to the catenated region, where the filaments of the daughter chromosomes are interwound. In vitro treatment of such structures with gyrase leads to their decomposition into individual nucleoids [44, 45]. In some parts of terC, the frequency of homologous recombination is highly elevated. These parts, which are of about 8 kb in size, are located in seven loci of this region and bear xi sites [46]. Thus, the ter region in the bacteria is subject to constant rearrangements.

Termination of the movement of the nucleoids to the cell poles occurs very quickly: they, literally speaking, jump away, as has been shown by sequential filming of living *B. subtilis* cells [47]. The cell starts its division at this very moment. The rate of nucleoid segregation tenfold exceeds the rate of cell elongation. Their separation cannot therefore be explained as a mechanical pulling apart of the moving cell poles. The DNA–SeqA protein complex moves together with the nucleoids [48],

and this complex prevents a premature tour of replication (see above).

Nucleoid segregation between cells occurs at a high level of accuracy, with the result that only about 0.03% of wild genotype *E. coli* cells are anucleate after division [49]. The numerous mutations affecting all the stages of DNA replication distort this ideal picture.

The question arises as to the nature the forces generating migration of the parts of a nucleoid and their segregation between sister cells. Are the oriC and terC migrations at the very beginning of replication simply a result of the movement the nucleoid as a whole (similar to the interconnected movement of the two poles of a rotating egg), or are they independent movements of these particular chromosome sites? The second alternative is possibly the valid one. The nucleoid consists of several dozens of supercoiled domains of about 100 kb each; moreover, the torsion tension within each one does not depend on the conditions in the other domains [50]. Initial migration of the domains containing the PRL and DAZ regions (see above) can be assumed, and they are only later followed by the movement of the rest of the nucleoid. What, then, is the engine driving this process?

Bacterial proteins analogous to centromeres and kinetochores were mentioned above. Some of these proteins, e.g., *B. subtilis* DivIYA, possibly have functions similar to those of the eukaryotic chromosome-pulling spindle. An important role in nucleoid segregation is also attributed to the torsion forces created by the supercoiled state of the DNA. Supercoiling (and condensation in general) of the DNA in the nucleoid is promoted, among other factors, by the so-called structural maintenance of chromosomes (SMC) proteins. These proteins are present in both pro- and eukaryotes [51]. A similar big protein (170 kDa), MukB, encoded by a gene of the same name, is present in *E. coli.* At nonpermissive temperatures, mutations of this gene result in the formation of nonviable cells of a normal size, although they are deprived of nucleoids [51–53]. The name of this group of mutations is derived from the Japanese word *mukaku*, meaning deprived of a nucleus [54]. A protein belonging to the SMC family is also present in *B. subtilis* [55]. For a description of the characteristics of these and similar proteins, see review [56]. The SMC proteins participate in DNA condensation and, thus, possibly promote the torsion forces that give an additional push [6] to the separating nucleoids [57]. The forces created by DNA coiling–uncoiling are considered to be highly important in the so-called factory model of replication [11, 58] (in addition, see review [59] for a discussion of this model as applied to nucleoid segregation). The participation of other proteins not only in DNA packing but also in the nucleoid migration to the cell poles is assumed [60–62].

Chromosome segregation and separation are closely related to the beginning of cell division and occur almost at the same time; therefore, it is difficult to differentiate between the end of one event and the beginning of another. For convenience, let us assume that, after nucleoid segregation, the next stage of the bacterial cell cycle, cell division and separation of the daughter cells, follows.

5. Cell Division

Cell division starts almost simultaneously with nucleoid separation. A large number of proteins (and a respective number of genes) are involved in this process. These proteins are primarily those belonging to the Fts group. In *E. coli*, all the *fts* genes, except FtsK, are located in one big cluster [63]. As well as these genes, the cluster contains genes for the biosynthesis of peptidoglycan precursors and for the transport of these precursors across the cytoplasmic membrane. Although the cluster contains at least 20 genes, they share a common terminator; the transcription of all the genes goes in the same direction. The close location and common regulation of the *fts* genes and the genes of peptidoglycan biosynthesis enable strict coordination of the different stages of cell division (see review [4]). Some of the proteins encoded by the genes of this group, e.g., FtsK, were mentioned above. Their name is derived from the initial name of the corresponding mutations in *E. coli* (filamentation temperature sensitive). These mutants have similar phenotypes: long threadlike cells appear at nonpermissive and, sometimes, even at permissive temperatures. Unlike mutants with the par defect, the nucleoids of fts mutants do segregate, but the cellular septum sometimes does not form between them.

The main protein involved in cell division, FtsZ, has GTPase activity and is similar to eukaryotic tubulins [64–66]. About 20000 FtsZ molecules are present in one cell, and, during the first stage of the cell cycle, they are diffusely distributed in the cytoplasm. At the moment of nucleoid segregation, however, the protein molecules gather in the cell center and polymerize, producing the so-called FtsZ ring (or Z ring). This structure has been clearly revealed by immunoelectron microscopy [67]. Other proteins, FtsZ receptors, are located in the region where the Z ring condenses into a united structure. These are mainly MinE proteins. The Min protein group was so named because the respective mutants form very small nonviable cells deprived of chromosomal DNA or mini cells (Min phenotype, see below), which were originally described in 1967 [68]. Apart from MinE, at least two other Min proteins, MinC and MinD, are present in the cell. These two proteins are antagonists of FtsZ. By the moment of cell division, however, only MinE remains on the cytoplasmic membrane, where it forms a "belt," the binding place for FtsZ. The other Min proteins move to the cell poles [69]. The DivIYA protein of *B. subtilis* (see above), although not homologous to MinE, functionally resembles it [70].

In addition to FtsZ, the protein ZipA participates in formation of the central ring. This protein is vitally

important to *E. coli* [71]. About 2000 of its molecules are present in each cell. It possibly marks the place for FtsZ polymerization on the cytoplasmic membrane; unlike MinE and FtsZ, it is localized as impregnations in the membrane and does not form a continuous belt. The mutants with impaired ZipA synthesis form long threadlike cells with numerous nucleoids that are not separated by septa. Experiments with GFP revealed ZipA fluorescence in the same part of the cell where FtsZ was located. The presence of excessive ZipA in the cells (after the introduction of a plasmid with a cloned ZipA gene) also leads to aberrations in the cell division, with the resulting cells resembling those of ZipA mutants. Review [5] gives more information on this protein.

The role of FtsZ is to prepare for the formation of the cell septum separating the daughter cells. After cell division, the polymerized proteins of the Z ring disintegrate into individual molecules distributed throughout the cytoplasm, and MinE forms a belt around the equator of each daughter cell.

Mutations in both the *ftsZ* gene and the other genes affect the formation of the Z ring. In *E. coli* mutants with spherical cells, the Z ring has an arched shape [72]. In *E. coli* cells with the $FtsZ_3$ mutant protein, cell growth is stopped, since this protein does not polymerize and cannot form Z rings. Among other genes antagonistic to Z ring formation, the *sfiA* (former name, *sulA*) gene should be noted first. This gene, normally silent, belongs to a large group of genes that are derepressed in the course of an SOS response of the cell to damage of its DNA. In a derepressed state, it encodes the formation of a small polypeptide (18 kDa) that prevents the formation of the Z ring and, consequently, division, until the damage to the DNA is repaired [73–75] (in addition, see review [76]). The blocking of Z ring formation is due to the blocking of FtsZ polymerization [77]. The system of MinC–MinD proteins prevents Z ring formation in improper places. These proteins are located close to the cell pole. This localization is controlled by the MinE protein, which, as was mentioned above, occupies the central position in the cell. The role of MinCD proteins is to prevent Z ring formation near the cell poles. In mutants with impaired regulation of this system, additional Z rings (and, subsequently, additional septa) are formed close to the poles. The introduction of a plasmid with a cloned *ftsZ* gene causes a two- to sevenfold FtsZ overdose and gives the same result. Very small (10–20 times smaller than normal), bubblelike cells without nucleoids are produced [78–82]. *B. subtilis* also has a MinCD protein system homologous to that of *E. coli*; in this case, it regulates the division of vegetative cells and germinating spores [70, 83, 84]. Evidence of the role of the MinD protein in nucleoid segregation in *B. subtilis* has recently been reported [85].

The protein FtsZ is possibly universal in prokaryotes. It has been found in some gram-positive cocci, and it is present in mycoplasmas, archaea, and even *Arabidopsis thaliana* chloroplasts [86] (plant chloroplasts are commonly believed to be of bacterial origin). The main role of FtsZ is probably to promote the invagination of the cytoplasmic membrane during the process of septum formation. In bacteria that have peptidoglycan, invagination of the cytoplasmic membrane stimulates the synthesis of peptidoglycan septa. After FtsZ polymers have formed the Z ring, FtsA protein molecules are incorporated into it [87–89]. Up to 500 molecules of this 55-kDa protein are present in one *E. coli* cell. It is localized at the internal surface of the cytoplasmic membrane and takes part in septum formation. It is partially homologous to the ATP binding domain of certain ATPases, including actin. FtsA interacts with FtsZ and, also, somehow coordinates peptidoglycan synthesis [90]. The respective mutants lack developed septa. Unlike FtsZ mutants, their filaments are not smooth but "incised," since completion of the septa is blocked only after their formation has been initiated. Cells with certain forms of a mutant FtsA protein (e.g., $FtsA_3$) have septa, though they are underdeveloped.

Another protein belonging to the Fts group, FtsI, also known as PBP_3, participates in synthesis of the peptidoglycan layer of the septum [91]. It has transpeptidase and transglycosylase activity. The corresponding mutants also form filaments without septa. The protein FtsK also participates in the septation process. It has two domains [92, 93], one of which, the C-terminal domain, participates, as was mentioned above, in the segregation of chromosome dimers after replication while the other domain, the N-terminal, is attached to the cytoplasmic membrane. This domain is included in the Z ring and participates in septum formation.

The functions of the protein products of other *fts* genes (O, L, N, W, E, Y, X, and H) are not completely clear. The first three proteins are connected to the internal membrane. The number of their molecules per cell is low (about 50). They are transmembrane proteins, and cell division is impossible in the absence of the protein FtsL, which is probably involved in septation. The protein FtsN forms a complex with FtsA and FtsI, which are also located close to the septum (see review [4]). There is even less information concerning the functions of the other five proteins. One of them, the product of the *ftsW* gene, is homologous to the protein RodA, which participates in maintenance of the rod-shaped cell form. In general, all of these proteins, or most of them, participate in formation of the Z ring (see more details in review [4]). This structure, due to its complex composition and role in the life of the cell, is sometimes considered as a cellular organelle.

Formation of a septum in the region of the developed Z ring is considered to be the initial stage of cytokinesis, i.e., segregation of the daughter cells. It is related to changes in the topology of synthesis of the peptidoglycan (murein) layer, which have been studied by monitoring the incorporation of labeled diami-

nopimelic acid, a peptidoglycan precursor, into different parts of the *E. coli* cell wall. In nondividing cells, the label was incorporated diffusely over the entire cell length; however, during division, the incorporation occurred in the middle of the cell, i.e., in the growth point of the septum. There are no differences between the peptidoglycan at this point and the peptidoglycan in other cell loci; only the topology of its synthesis changes [94]. Development of the septum separating the daughter cells is a complex process. In gram-negative bacteria, all three layers of the cell envelope are involved: the cytoplasmic membrane, peptidoglycan, and outer membrane. Cytokinesis possibly starts with compression of the Z ring. This compression leads to contraction of the cytoplasmic membrane from inside and serves as the signal to initiate peptidoglycan synthesis in the septum. However, it has been suggested that it is peptidoglycan synthesis that serves as the primary push for invagination of the cytoplasmic membrane by pressing the underlying cytoplasmic membrane into the cytoplasm (see review [5]). The role of the cell wall is rather passive; in IkyD mutants with impaired attachment of the peptidoglycan layer to the outer membrane, invagination of the cell wall can be independent of that of the membrane [95]. Some other mechanism of deciding whether peptidoglycan synthesis will be of an elongation or septation type is possibly present in the cell.

In *E. coli*, the *envA* gene participates in cell separation. The respective mutants form threadlike chains of cells that do not break into individual sections. Although many bacteria possess hydrolytic enzymes (lytic transglycosylases, endopeptidases, *N*-acetylmuramyl-L-alanine amidases, etc.), mutations in any one of these enzymes do not lead to noticeable cell division distortions (see review [4]). Many of these enzymes are probably interchangeable during cell division.

6. Mitosis? No Rather Than Yes

In the last decade, it has almost become a rule for publications on bacterial caryology to mention the similarity between the mechanism of chromosome segregation in dividing prokaryotic cells and eukaryotic mitosis. How far does this similarity stretch?

There is no doubt that the mechanisms producing nucleoid segregation have reached a high level of perfection. In wild-genotype *E. coli*, as was already mentioned, only one cell out of every three thousand is left anucleate [49]. The firmly established fact of the ordered oriented movement of chromosomes (nucleoids) to the cell poles prior to cell division indicates similarity between these mechanisms and the mitosis mechanisms, as does the way the orientation is achieved, i.e., the existence of a specialized chromosome site analogous to centromeres. The existence of specialized proteins attached to this site (kinetochore analogues) is probably also a reality. It is, to some degree, possible to speak of specific proteins located in "attraction centers" for nucleoids at the cell poles. The term *spindle* seems appropriate, but no one has yet reliably reported the presence of this highly important component of the mitotic apparatus in bacteria. However, the difference between pro- and eukaryotes is quite pronounced when the states of the nuclear apparatus are compared in dividing and nondividing cells. In a eukaryotic interphase nucleus, the chromosomes are maximally uncoiled and become coiled before mitosis. The differences between bacterial nucleoids occurring in different states, at least the morphological ones, are not great. In the *E. coli* nucleoid, the density of packed DNA in actively dividing log-phase cells is similar to that in an interphase eukaryotic nucleus [96]. Nucleoids may not be able to reach the degree of coiling achieved by metaphase chromosomes. The degree of DNA packing in a compacted nucleoid is nevertheless quite significant. An unwound DNA molecule is approximately 3 orders of magnitude longer than a bacterial cell and 2000- to 2500-fold longer than the nucleoid. In *B. subtilis*, mutants with impaired synthesis of the SMC protein, which condenses DNA, die at nonpermissive temperatures, whereas, at permissive temperatures, approximately 10% of nonviable anucleate cells are formed and the remaining cells, with distorted (loose and elongated) nucleoids, grow very slowly [62]. The mechanisms of DNA compactization and segregation of nuclear structures are most probably different in pro- and eukaryotes. Even over the last few years, although the analogy between the distribution of nuclear material in bacteria and eukaryotes has become almost common knowledge, conclusions to the contrary have still been published. For instance, in a review by Sawitzke and Austin, the conclusion was as follows: "The picture that is emerging is quite distinct from DNA segregation during mitosis in eukaryotes. Rather, it may be more closely analogous to the formation of separate sister chromatids during eukaryotic chromosome replication" [59, p. 792]. It was stressed in the same review that a number of the single mutations that impair different stages of replication and segregation of bacterial chromosomes are not lethal; rather, cells with such mutations remain viable, although they grow more slowly and exhibit other distortions. This factor probably indicates a high "safety factor" and the interplay of the individual components of the nucleoid segregation apparatus. A combination of two mutations, for example, in *seqA* and *mukB*, usually leads to cell death.

Every discussion on the mechanism of nucleoid segregation must mention, at least tangentially, the distribution of big plasmids in the cells. Since only one copy of such plasmids per cell is usually present, accurate segregation is highly important in this case. Many publications and reviews exist on this subject (among the latest are [16, 97]). Two types of segregation system have been most thoroughly studied: type I (plasmid F and prophage P_1) and type II (plasmid R_1). Both systems have three components: a centromeric DNA region and genes for two proteins. In system I, these

proteins are ParA and ParB, and, in system II, ParM and ParR. The proteins ParA and ParB are homologous, respectively, to the *B. subtilis* Soj and SpoOJ proteins (see above). Only the most recent studies on R_1 plasmid segregation will be discussed below.

The system of postreplicational segregation of the two copies of R_1 consists of a small region parC, acting as a centromere, and two genes, *parM* and *parR*, encoding proteins of the same name. In parC, there are 10 direct repetitions of 11 nucleotides each, and, in the middle of this region, there is a promoter site that controls the work of the *parM* and *parR* genes. The product of the *parR* gene is a protein that binds to the centromere site. The resulting nucleoproteid complex interacts with the product of the *parM* gene, a filamentous protein with ATPase activity. This protein is similar to actin. It is activated by the parC–ParR complex and polymerized. Binding with ATP is necessary for polymerization of ParM filaments, and ATP hydrolysis is required for depolymerization. Polymerized ParM molecules form a rod-shaped structure that is located between the centromere–ParR protein complexes of replicated plasmids and expands the plasmids in a springlike manner in the course of its lengthening. Immunofluorescence microscopy has revealed that the sister plasmids are located at the ends of this spring. After the plasmids have moved away from the cell center, the ParM molecules depolymerize [98, 99]. The ParM protein is a component of the cytoskeleton and pushes apart the replicons, unlike the spindle with its pulling apart action.

Thus, at least for some big plasmids, a centromere DNA site, a kinetochore analogue (the ParR protein), and the moving force behind active segregation of replicated DNA molecules (polymers of the ParM protein) have been found. These are the components that the researchers attempting to explain the mechanism of nucleoid segregation try to find in bacteria. This scenario does not require the torsion forces caused by DNA compactization as a segregation engine. The corresponding processes in plasmids are probably independent of nucleoid segregation. At least, plasmid F is successfully segregated in mukB mutant cells [54].

Concerning the similarities and differences between the segregation of chromosomes and big plasmids in bacteria on the one hand and mitosis on the other, it should be added that studies have so far been performed on relatively simple models, i.e., *E. coli* and *B. subtilis.* Bacteria possessing several chromosomes and/or the so-called megaplasmids, linear chromosomes, or plasmids of mixed composition have not yet been studied (see review [99]). Widening the range of models will certainly make our knowledge of the bacterial cell cycle more complete and complex.

The studies cited in this review have contributed greatly to our understanding of the composition and behavior of the bacterial hereditary apparatus, no matter how close the bacterial mechanisms of segregation of nucleoids and big plasmids are to the mechanisms of classical eukaryotic mitosis. For instance, one of the conclusions of [19] (cited above) can be mentioned. In that study, the migration of 22 DNA sites (in addition to oriC and terC) that were uniformly distributed along the *E. coli* chromosome was investigated. Good correlation was found between the positions of each site on the genetic map relative to oriC and terC and their positions in the dividing cell relative to the poles of the nucleoid. These results led to the conclusion that the nucleoid is a closed ring-shaped structure with labeled sites located in the same sequence as the genes on the genetic map. In micrograph form, the figure visibly resembled an oval bagel with a hole. Confirmation of this finding would mean the settling of the long discussion on the true configuration of the nucleoid [2, 96].

Among the publications not cited in the text of this article, the reviews [101–104] should be mentioned.

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REFERENCES

1. Jacob, F., Brenner, S., and Cuzin, F., On the Regulation of DNA Replication in Bacteria, *Cold Spring Harbor Symp. Quant. Biol.*, 1963, vol. 29, pp. 329–348.
2. Prozorov, A.A., The Bacterial Genome: Nucleoid, Chromosome, and Nucleotide Map, *Mikrobiologiya*, 1998, vol. 67, no. 4, pp. 437–451.
3. Peshkov, M.A., *Tsitologiya bakterii* (Cytology of Bacteria), Moscow: Akad. Nauk SSSR, 1955.
4. Lutkenhaus, J. and Mikerjee, A., Cell Division, *Escherichia coli and Salmonella typhimurium*, 2nd ed., Neighard, F.C., Ed., Washington DC: Am. Soc. Microbiol., 1996, pp. 1615–1626.
5. Rothfield, L.I. and Justice, S., Bacterial Cell Division: The Cycle of the Ring, *Cell*, 1997, vol. 88, no. 3, pp. 581–584.
6. Draper, G. and Gober, J., Bacterial Chromosome Segregation, *Annu. Rev. Microbiol.*, 2002, vol. 56, pp. 567–597.
7. Moriya, S., Kato, K., Yoshikawa, H., and Ogasawara, N., Isolation of a dnaA Mutant of *Bacillus subtilis* Defective in Initiation of Replication: Amount of DNAA Protein Determines Cells Initiation Potential, *EMBO J.*, 1990, vol. 9, no. 9, pp. 2905–2910.
8. Zyskind, J. and Smith, D.W., DNA Replication, the Bacterial Cell Cycle, and Cell Growth, *Cell*, 1992, vol. 69, no. 1, pp. 5–8.
9. Donachie, W.D., Relationship between Sell Size and Time of Initiation of DNA Replication, *Nature*, 1968, vol. 219, no. 5158, pp. 1077–1079.
10. Donachie, W.D., Begg, K., and Vicente, M., Cell Length, Cell Growth and Cell Division, *Nature*, 1976, vol. 264, no. 5584, pp. 328–338.

11. Lemon, K. and Grossman, A.D., Localization of Bacterial DNA Polymerase: Evidence for a Factory Model of Replication, *Science*, 1998, vol. 282, no. 360, pp. 1516–1519.

12. Lemon, K. and Grossman, A.D., Movement of Replicating DNA through a Stationary Replisome, *Mol. Cell*, 2000, vol. 6, no. 4, pp. 1321–1330.

13. Russell, D. and Zinder, N.D., Hemimethylation Prevents DNA Replication in *Escherichia coli, Cell*, 1987, vol. 50, no. 7, pp. 1071–1079.

14. Liu, M., Campbell, J., Boye, E., and Kleckner, N., SeqA: A Negative Modulator of Replication Initiation in *Escherichia coli, Cell*, 1994, vol. 77, no. 3, pp. 413–426.

15. Wake, R. and Errington, J., Chromosome Partitioning in Bacteria, *Annu. Rev. Genet.*, 1995, vol. 29, pp. 41–67.

16. Gordon, G. and Wright, A., DNA Segregation in Bacteria, *Annu. Rev. Microbiol.*, 2000, vol. 54, pp. 681–708.

17. Gordon, G., Sitnikov, D., Webb, C., Teleman, A., Straight, A., Losick, R., Murray, A., and Wright, A., Chromosome and Low Copy Plasmid Segregation in *Escherichia coli*: Visual Evidence for Distinct Mechanisms, *Cell*, 1997, vol. 90, no. 5, pp. 1113–1121.

18. Niki, H. and Hiraga, S., Polar Localization of the Replication Origin and Terminus in *Escherichia coli* Nucleoids during Chromosome Partitioning, *Genes Develop.*, 1998, vol. 12, no. 8, pp. 1036–1045.

19. Niki, H., Yamaichi, Y., and Hiraga, S., Dynamic Organization of Chromosomal DNA in *Escherichia coli, Genes Develop.*, 2000, vol. 14, no. 2, pp. 212–223.

20. Yamaichi, Y. and Niki, H., Active Segregation by the *Bacillus subtilis* Partitioning System in *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, no. 18, pp. 14656–14661.

21. Gordon, G., Shivers, R., and Wright, A., Polar Localization of the *Escherichia coli* oriC Region Is Independent of the Site of Replication Initiation, *Mol. Microbiol.*, 2002, vol. 44, no. 2, pp. 501–509.

22. Yamaichi, Y. and Niki, H., migS, a cis-Acting Site That Affects Bipolar Positioning of oriC on the *Escherichia coli* Chromosome, *EMBO J.*, 2004, vol. 23, no. 1, pp. 221–223.

23. Lau, I., Filipe, S., Soballe, B., Okstad, O.-A., Barre, F.-X., and Sherratt, D., Special and Temporal Organization of Replicating *Escherichia coli* Chromosomes, *Mol. Microbiol.*, 2003, vol. 49, no. 3, pp. 731–743.

24. Sharpe, M. and Errington, J., The *Bacillus subtilis soj–spoOJ* Locus Is Required for a Centromere-Like Function Involved in Prespore Chromosome Partitioning, *Mol. Microbiol.*, 1996, vol. 21, no. 3, pp. 501–509.

25. Glaser, P., Sharpe, M., Raetner, B., Perego, M., Olsen, K., and Errington, G., Dynamic, Mitotic-Like Behavior of a Bacterial Protein Required for Chromosome Partitioning, *Genes Develop.*, 1997, vol. 11, no. 9, pp. 1160–1168.

26. Lewis, P. and Errington, J., Direct Evidence for Active Segregation of oriC Regions of the *Bacillus subtilis* Chromosome and Co-Localization with the SpoOJ Partitioning Protein, *Mol. Microbiol.*, 1997, vol. 25, no. 5, pp. 945–954.

27. Lin, D., Levin, P., and Grossman, A.D., Bipolar Localization of a Chromosome Partition Protein in *Bacillus subtilis, Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, no. 9, pp. 4721–4726.

28. Webb, Ch., Teleman, A., Gordon, S., Straight, A., Belmont, A., Lin, D., Grossman, A.D., Wright, A., and Losick, R., Bipolar Localization of the Replication Origin Regions of Chromosomes in Vegetative and Sporulating Cells of *Bacillus subtilis, Cell*, 1997, vol. 88, no. 5, pp. 667–674.

29. Wu, L. and Errington, J., Use of Asymmetric Cell Division and SPOIIIE Mutant To Probe Chromosome Orientation and Organization in *Bacillus subtilis, Mol. Microbiol.*, 1998, vol. 27, no. 4, pp. 777–786.

30. Wu, L. and Errington, J., A Large Dispersed Chromosomal Region Required for Chromosome Segregation in Sporulating Cells of *Bacillus subtilis, EMBO J.*, 2002, vol. 21, no. 15, pp. 4001–4011.

31. Lin, D. and Grossman, A.D., Identification and Characterization of a Bacterial Chromosome Partitioning Site, *Cell*, 1998, vol. 92, no. 5, pp. 675–685.

32. Marston, A. and Errington, J., Dynamic Movement of the ParA-Like Soj Protein of *Bacillus subtilis* and Its Dual Role in Nucleoid Organization and Developmental Regulation, *Mol. Cell*, 1999, vol. 4, no. 3, pp. 673–682.

33. Ben-Yehuda, S., Rudner, D., and Losick, R., RacA, a Bacterial Protein That Anchors Chromosomes to the Cell Poles, *Science*, 2003, vol. 299, no. 5606, pp. 532–536.

34. Autret, S., Nair, R., and Errington, J., Genetic Analysis of the Chromosome Segregation Protein SpoOJ of *Bacillus subtilis*: Evidence for Separate Domains Involved in DNA Binding and Interaction with Soj Protein, *Mol. Microbiol.*, 2001, vol. 41, no. 3, pp. 743–755.

35. Thomaides, H., Freeman, M., El Karoui, M., and Errington, J., Division Site Selection Protein DivIA of *Bacillus subtilis* Has a Second Distinct Function in Chromosomal Segregation during Sporulation, *Genes Develop*, 2001, vol. 15, no. 8, pp. 1662–1673.

36. Hamoen, L. and Errington, J., Polar Targeting of DivIA in *Bacillus subtilis* Is Not Directly Dependent on FtsZ Or PBP_2B, *J. Bacteriol.*, 2003, vol. 185, no. 2, pp. 693–697.

37. Mohl, D. and Gober, J., Cell Cycle-Dependent Polar Localization of Chromosome Partitioning Proteins in *Caulobacter crescentus, Cell*, 1997, vol. 88, no. 5, pp. 675–684.

38. Mohl, D., Easter, J., and Gober, J., The Chromosome Partitioning Protein, ParB, Is Required for Cytokinesis in *Caulobacter crescentus, Mol. Microbiol.*, 2001, vol. 42, no. 3, pp. 741–755.

39. Rizzo, M., Shapiro, L., and Gober, J., Asymmetric Expression of the Gyrase B Gene from the Replication-Component Chromosome in the *Caulobacter crescentus* Predivisional Cell, *J. Bacteriol.*, 1993, vol. 175, no. 21, pp. 6970–6981.

40. Hidaka, M., Kobayashi, T., and Horiuchi, T., A Newly Identified DNA Replication Terminus Site, terC, on the *Escherichia coli* Chromosome, *J. Bacteriol.*, 1991, vol. 173, no. 1, pp. 391–393.

41. Perals, K., Cornet, F., Merlet, Y., Delon, I., and Louarn, J.-M., Functional Polarization of the *Escherichia coli* Chromosome Terminus: The dif Site Acts in Chromosome Dimer Resolution Only When Located between Long Stretches of Opposite Polarity, *Mol. Microbiol.*, 2000, vol. 36, no. 1, pp. 33–43.

42. Perals, K., Capiaux, H., Vincourt, J.-B., Louarn, J.-M., Sherratt, D., and Cornet, F., Interplay between Recom-

bination, Cell Division and Chromosome Structure during Chromosome Dimer Resolution in *Escherichia coli, Mol. Microbiol.*, 2001, vol. 39, no. 4, pp. 904–913.

43. Ip, S., Bregu, M., Barre, F.-X., and Sherratt, D., Decatenation of DNA Circles by FtsK-Dependent xer Site–Specific Recombination, *EMBO J.*, 2003, vol. 22, no. 23, pp. 6399–6407.
44. Steck, T.R. and Drlika, K., Bacterial Chromosome Segregation: Evidence for DNA Gyrase Involvement in Decatenation, *Cell*, 1984, vol. 36, no. 5, pp. 1081–1088.
45. Lobner-Olsen, A. and Kuempel, P.L., Chromosome Partitioning in *Escherichia coli, J. Bacteriol.*, 1992, vol. 174, no. 24, pp. 7883–7889.
46. Horiuchi, T., Fujimura, H., Kobayashi, T., and Hidaka, M., The DNA Replication Fork Blocked at the ter Site May Be an Entrance for the RecBCD Enzyme Into Duplex DNA, *J. Bacteriol.*, 1994, vol. 176, no. 15, pp. 4656–4663.
47. Webb, Ch., Graumann, P., Kahana, J., Teleman, A., Silver, P., and Losick, R., Use of Time-Lapse Microscopy To Visualize Rapid Movement of the Replication Origin Region of the Chromosome during the Cell Cycle in *Bacillus subtilis, Mol. Microbiol.*, 1998, vol. 28, no. 5, pp. 883–892.
48. Hiraga, S., Ichinose, Ch., Niki, H., and Yamazoe, M., Cell Cycle–Dependent Duplication and Bidirection Migration of SeqA-Associated DNA–Protein Complexes in *Escherichia coli, Mol. Cell*, 1998, vol. 1, no. 2, pp. 381–387.
49. Hiraga, S., Ogura, T., Niki, H., Ishinose, Ch., and Mori, H., Positioning of Replicated Chromosomes in *Escherichia coli, J. Bacteriol.*, 1990, vol. 172, no. 1, pp. 31–39.
50. Pettijohn, D., Histone-Like Proteins and Bacterial Chromosome Structure, *J. Biol. Chem.*, 1988, vol. 263, no. 10, pp. 12793–12796.
51. Holmes, V. and Cozzarelli, N., Closing the Ring: Links between SMC Proteins and Chromosome Partitioning, Condensation, and Supercoiling, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, no. 4, pp. 1322–1324.
52. Hiraga, S., Niki, H., Ogura, T., Ishinose, C., Mori, H., Ezaki, B., and Jaffe, A., Chromosome Partitioning in *Escherichia coli*: Novel Mutants Producing Anucleate Cells, *J. Bacteriol.*, 1989, vol. 171, no. 3, pp. 1496–1505.
53. Niki, H., Jaffe, A., Imamura, R., Ogura, T., and Hiraga, S., The New Gene *mucB* Codes for a 177 Kb Protein with Coiled-Coil Domains Involved in Chromosome Partitioning of *Escherichia coli, EMBO J.*, 1991, vol. 10, no. 1, pp. 183–193.
54. Hiraga, S., Chromosome and Plasmid Partition in *Escherichia coli, Annu. Rev. Biochem.*, 1992, vol. 61, pp. 283–306.
55. Melby, T., Ciampaglio, Ch., Briscoe, G., and Erickson, H., The Symmetrical Structure of Structural Maintenance of Chromosomes (SMC) and MukB Proteins: Long, Antiparalled Coiled Coils, Folded at a Flexible Hinge, *J. Cell Biol.*, 1998, vol. 142, no. 6, pp. 1595–1604.
56. Strunnikov, A.V. and Jessberger, R., Structural Maintenance of Chromosomes (SMC) Proteins: Conserved Molecular Properties for Multiple Biochemical Functions, *Eur. J. Biochem.*, 1999, vol. 263, no. 1, pp. 6–13.
57. Lindow, J., Kumano, M., Moriya, S., and Grossman, A.D., Subcellular Localization of the *Bacillus subtilis* Structural Maintenance of Chromosome (SMC) Protein, *Mol. Microbiol.*, 2002, vol. 46, no. 4, pp. 997–1009.
58. Lemon, K. and Grossman, A.D., The Extrusion–Capture Model for Chromosome Partitioning in Bacteria, *Genes Develop*, 2001, vol. 15, no. 16, pp. 2031–2041.
59. Sawitzke, J. and Austin, S., An Analysis of the Factory Model for Chromosome Replication and Segregation in Bacteria, *Mol. Microbiol.*, 2001, vol. 40, no. 4, pp. 786–794.
60. Graumann, P., Losick, R., and Strunnikov, A.V., Subcellular Localization of *Bacillus subtilis* SMC, a Protein Involved in Chromosome Condensation and Segregation, *J. Bacteriol.*, 1998, vol. 180, no. 12, pp. 5749–5755.
61. Moriya, S., Tsujikawa, E., Hassan, A., Asai, K., Kodama, T., and Ogasawara, N., A *Bacillus subtilis* Gene-Encoding Protein Is Necessary for Chromosome Partition, *Mol. Microbiol.*, 1998, vol. 29, no. 1, pp. 179–187.
62. Britton, R., Lin, D., and Grossman, A.D., Characterization of a Prokaryotic SMC Protein Involved in Chromosome Partitioning, *Genes Develop.*, 1998, vol. 12, no. 4, pp. 1254–1259.
63. Mukherjee, A. and Donachie, W., Differential Translation of Cell Division Proteins, *J. Bacteriol.*, 1990, vol. 172, no. 10, pp. 6106–6111.
64. De Boer, P., Crossley, R., and Rothfield, L., The Essential Bacterial Cell Division Protein FtsZ Is a GTPase, *Nature*, 1992, vol. 359, no. 6392, pp. 254–256.
65. Mukherjee, A., Dai, K., and Lutkenhaus, J., *Escherichia coli* Cell Division Protein FtsZ Is a Guanine Nucleotide Binding Protein, *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90, no. 3, pp. 1053–1057.
66. Erickson, H., Taylor, D., Taylor, K., and Bramhill, D., Bacterial Cell Division Protein FtsZ Assembles into Protofilament Sheets and Minirings, Structural Homologs of Tubulin Polymers, *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, no. 1, pp. 519–523.
67. Bi, E. and Lutkenhaus, J., FtsZ Regulates Frequency of Cell Division in *Escherichia coli, J. Bacteriol.*, 1990, vol. 172, no. 5, pp. 2765–2768.
68. Adler, H., Fisher, A., Cohen, A., and Hardigree, A., Miniature *Escherichia coli* Sells Deficient in DNA, *Proc. Natl. Acad. Sci. USA*, 1967, vol. 57, no. 2, pp. 321–326.
69. Raskin, D.M. and de Boer, P., The MinE Ring: An FtsZ-Independent Cell Structure Required for Selection of the Correct Division Site in *Escherichia coli, Cell*, 1997, vol. 91, no. 5, pp. 685–694.
70. Marston, A., Thomaides, H., Edwards, D., Sharpe, M., and Errington, J., Polar Localization of the MinD Protein of *Bacillus subtilis* and Its Role in Selection of the Mid-Cell Division Site, *Genes Develop.*, 1998, vol. 12, no. 21, pp. 3419–3430.
71. Hale, C. and de Boer, P., Direct Binding of FtsZ to ZipA, an Essential Component of the Septal Ring Structure That Mediates Cell Division in *Escherichia coli, Cell*, 1997, vol. 88, no. 2, pp. 175–185.
72. Addinall, S. and Lutkenhaus, J., FtsZ-Spirals and -Arcs Determine the Shape of the Invaginating Septa in Some Mutants of *Escherichia coli, Mol. Microbiol.*, 1996, vol. 22, no. 2, pp. 231–237.

73. Huisman, D., D`Ari, R., and Gottesman, S., Cell-Division Control in *Escherichia coli*: Specific Induction of the SOS Function SfiA Protein Is Sufficient To Block Septation, *Proc. Natl. Acad. Sci. USA*, 1984, vol. 81, no. 14, pp. 4490–4494.

74. Jones, C. and Holland, I., Role of the SfiB (FtsZ) Protein in Division Inhibition during the SOS Response in *Escherichia coli*: FtsZ Stabilizes the Inhibitor SfiA in Maxicells, *Proc. Natl. Acad. Sci. USA*, 1985, vol. 82, no. 18, pp. 6045–6049.

75. Jaffe, A., D`Ari, R., and Norris, V., SOS-Independent Coupling between DNA Replication and Cell Division in *Escherichia coli, J. Bacteriol.*, 1986, vol. 165, no. 1, pp. 66–71.

76. De Boer, P., Cook, W., and Rothfield, L., Bacterial Cell Division, *Annu. Rev. Genet.*, 1990, vol. 24, pp. 249–274.

77. Mukherjee, A., Cao, C., and Lutkenhaus, J., Inhibition of FtsZ Polymerization by SulA, an Inhibitor of Septation in *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 1998, vol. 95, no. 6, pp. 2885–2890.

78. Ward, J. and Lutkenhaus, J., Overproduction of FtsZ Induces Minicells in *Escherichia coli, Cell*, 1985, vol. 42, no. 3, pp. 941–949.

79. Bi, E. and Lutkenhaus, J., FtsZ Ring Structure Associated with Cell Division in *Escherichia coli, Nature*, 1991, vol. 354, no. 6349, pp. 161–164.

80. Hu, Z., Mukherjee, A., Pichoff, S., and Lutkenhaus, J., The MinC Component of the Division Site Selection System in *Escherichia coli* Interacts with FtsZ and Prevents Polymerization, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, no. 26, pp. 14819–14824.

81. Hu, Z. and Lutkenhaus, J., Topological Regulation of Cell Division in *Escherichia coli* Involves Rapid Pole to Pole Oscillation of the Cell Division Inhibitor MinC under the Control of MinD and MinE, *Mol. Microbiol.*, 1999, vol. 34, no. 1, pp. 82–90.

82. Hu, Z., Saez, C., and Lutkenhaus, J., Recruitment of MinC, an Inhibitor of Z-Ring Formation, to the Membrane in *Escherichia coli*: Role of MinD and MinE, *J. Bacteriol.*, 2003, vol. 185, no. 1, pp. 196–203.

83. Beall, B. and Lutkenhaus, J., FtsZ in *Bacillus subtilis* Is Required for Vegetative Septation and for Asymmetric Septation during Sporulation, *Genes Develop.*, 1991, vol. 5, no. 3, pp. 447–455.

84. Harry, E. and Lewis, P., Early Targeting of Min Proteins to the Poles in Germinating Spores of *Bacillus subtilis*: Evidence for Division Apparatus—Independent Recruitment of Min Proteins to the Division Site, *Mol. Microbiol.*, 2003, vol. 47, no. 1, pp. 37–48.

85. Autret, S. and Errington, J., A Role for Division-Site-Selection Protein MinD in Regulation of Internucleoid Jumping of Soj (ParA) Protein in *Bacillus subtilis, Mol. Microbiol.*, 2003, vol. 47, no. 1, pp. 159–169.

86. Osteryoung, K. and Vierling, E., Conserved Cell and Organelle Division, *Nature*, 1995, vol. 376, no. 7352, pp. 473–474.

87. Lutkenhaus, J. and Donachie, W., Identification of the *ftsA* Gene Product, *J. Bacteriol.*, 1979, vol. 137, no. 3, pp. 1088–1094.

88. Sanchez, M., Valencia, A., Ferrandiz, M.-J., Sander, C., and Vicente, M., Correlation between the Structure and Biochemical Activities of FtsA, an Essential Cell Division Protein of the Actin Family, *EMBO J.*, 1994, vol. 13, no. 20, pp. 4919–4925.

89. Ma, X., Ehrhardt, D., and Margolin, W., Colocalization of Cell Division Proteins FtsZ and FtsA to Cytoskeletal Structures in Living *Escherichia coli* Cells by Using Green Fluorescent Protein, *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, no. 12, pp. 12998–13003.

90. Tormo, A. and Vicente, M., The *ftsA* Gene Product Participates in Formation of the *Escherichia coli* Septum Structure, *J. Bacteriol.*, 1984, vol. 157, no. 3, pp. 779–784.

91. Spratt, B.G., Distinct Penicillin-Binding Proteins Involved in the Division, Elongation and Shape of *Escherichia coli* K-12, *Proc. Natl. Acad. Sci. USA*, 1975, vol. 72, no. 18, pp. 2999–3003.

92. Begg, K., Dewar, S., and Donachie, W., A New *Escherichia coli* Division Gene, *ftsK, J. Bacteriol.*, 1995, vol. 177, no. 21, pp. 6211–6222.

93. Wang, L. and Lutkenhaus, J., FtsK Is an Essential Cell Division Protein That Is Localized to the Septum and Induced as Part of the SOS Response, *Mol. Microbiol.*, 1998, vol. 29, no. 3, pp. 731–740.

94. Nanniga, N., Cell Division and Peptidoglycan Assembly in *Escherichia coli, Mol. Microbiol.*, 1991, vol. 5, no. 3, pp. 791–795.

95. Fung, J., MacAlister T., and Rothfield, L., Role of Murein-Lipoprotein in Morphogenesis of the Bacterial Division Septum; Phenotypic Similarity of lkyD and lpo Mutants, *J. Bacteriol.*, 1978, vol. 133, no. 10, pp. 1467–1471.

96. Kellenberger, E., Intracellular Organization of the Bacterial Genome, The Bacterial Chromosome, Drlica K. and Riley, M., Eds., Washington: ASM Press, 1990.

97. Hiraga, S., Dynamic Localization of Bacterial and Plasmid Chromosomes, *Annu. Rev. Genet.*, 2000, vol. 34, pp. 21–59.

98. Moller-Jensen, J., Jensen, R., Lowe, J., and Gerdes, K., Prokaryotic DNA Segregation by an Actin-Like Filament, *EMBO J.*, 2002, vol. 21, no. 8, pp. 3119–3127.

99. Moller-Jensen, J., Borch, J., Dam, M., Jensen, R., Roepstorff, P., and Gerdes, K., Bacterial Mitosis: ParM of Plasmid R_1 Moves Plasmid DNA by an Actin-Like Insertional Polymerization Mechanism, *Mol. Cell*, 2003, vol. 12, no. 6, pp. 1477–1487.

100. Prozorov, A.A., Structure of the Bacterial Genome: Uniformity or Diversity?, *Genetika*, 1995, vol. 31, no. 6, pp. 741–752.

101. Nordstrom, K., Bernander, R., and Dasgupta, S., The *Escherichia coli* Cycle: One Cycle or Multiple Independent Processes That Are Coordinated?, *Mol. Microbiol.*, 1991, vol. 5, no. 4, pp. 769–774.

102. Rothfield, L., Bacterial Chromosome Segregation, *Cell*, 1994, vol. 77, no. 7, pp. 963–966.

103. Wheeler, R. and Shapiro, L., Bacterial Chromosome Segregation: Is There a Mitotic Apparatus?, *Cell*, 1997, vol. 88, no. 3, pp. 577–579.

104. Donachie, W., Co-Ordinate Regulation of the *Escherichia coli* Cell Cycle, or the Cloud of Unknowing, *Mol. Microbiol.*, 2001, vol. 40, no. 4, pp. 779–785.