Chromosome Architecture and Segregation in Prokaryotic Cells

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The 3-D structure of prokaryotic chromosomes, their copy number, replication and segregation have gained an enormous interest in the past 15 years, for the most part because the spatial dynamics of chromosomes have become amenable for analysis through fluorescence microscopy techniques and other single-cell-based assays. And, of course, because these issues are of fundamental biological importance and impact on many cellular processes such as global gene regulation and the cell cycle.

This special issue of *Journal of Molecular Microbiology and Biotechnology* deals with various aspects of bacterial and archaeal chromosomes; their arrangement, their dynamics and segregation mechanisms. Several reviews in this issue deal with different aspects of chromosome organization and partitioning in selected model bacteria, and processes such as dimer resolution and important factors such as nucleoid-associated proteins (NAPs), SMC proteins and topoisomerases are discussed in greater detail in the following chapters. Bacteria can have vastly different numbers of their chromosome, which is discussed in one chapter [Soppa (pp. 409–419)], the domain structure of the *Escherichia coli* chromosome [Messer-schmidt and Waldminghaus (pp. 301–315)], and the dynamics of the two chromosomes in *Vibrio cholerae* [Ramachandran et al. (pp. 360–370)] have received their own chapters, because considerable knowledge has accumu-

lated about these model organisms. This review is intended to provide an overview of the mode of chromosome segregation in bacteria in general and on what is known about chromosome dynamics in archaeal cells [see review by Samson and Bell (pp. 420–427)] and to give information on some groups of bacteria that have been studied in greater detail.

In bacteria such as *E. coli* and *Bacillus subtilis*, chromosomal DNA does not extend throughout the entire cytoplasm, as is the case for *Caulobacter crescentus*, but is condensed into a structure called the nucleoid (fig. 1). Compaction is mediated through nonspecific DNA-binding NAPs (which however do have a preference for certain DNA structures) [reviews by Dorman and van der Valk et al., pp. 316–331 and 344–359, respectively] and SMC proteins [Graumann and Knust, 2009]. The separation of duplicated chromosomes is extremely robust: only 1 in 10,000 cell cycle events shows a failure in DNA partitioning in *B. subtilis*, even when cells contain multiple replication forks and contain multiple chromosome copies [Iretton et al., 1994]. It is also possible to integrate an entire cyanobacterial chromosome (3.5 Mbp) into the *B. subtilis* chromosome (4.2 Mbp), and cells still grow fairly well [Itaya et al., 2005], revealing great plasticity of a bacterial genome. A dedicated segregation motor has remained elusive; ParA proteins in *C. crescentus* [Ptacin et al., 2010]

and in *V. cholerae* [Fogel and Waldor, 2006] provide directionality of the segregation process [Ramachandran et al., pp. 360–370]. Inversions of smaller regions on the chromosome are not problematic, but the inversion of an entire arm of the *E. coli* chromosome results in slow growth, generation of anucleate cells, and mispositioning of chromosome regions (see below) [Niki et al., 2000]. This is in part due to the fact that normally, DNA and RNA polymerase frequently collide, because most genes are arranged such that transcription runs away from origin regions, as does replication. Upon inversion of e.g. a quarter of the *B. subtilis* chromosome, DNA polymerase movement is greatly slowed down [Merrikh et al., 2011]. Moreover, bacterial chromosomes have several features, including distinct domains and asymmetrically distributed DNA motifs, which aid in the organization and segregation of the genome, as detailed in this issue.

Most amazing at the time of discovery, bacterial chromosomes are not random coils of DNA, but are arranged in a spatially well-defined manner. The common scheme in several bacterial species is the observation that all loci on the chromosome have a rather defined position within the cell, and that chromosomes are arranged according to their physical structure, so that genes adjacent to each other on the chromosome will also be in close spatial proximity [Niki et al., 2000; Teleman et al., 1998; Viollier et al., 2004] (fig. 1). Factors such as SMC proteins are important for the maintenance of global chromosome arrangement, and are dealt with in two reviews within this compendium [Kleine Borgmann and Graumann, pp. 384–395, and Rybenkov et al., pp. 371–383]. Within the chromosome, relatively independent domains exist that are between 24 and 400 kbp (dependent on the method used), and isolated against neighboring domains. Inside such topological domains, genes show close connections in terms of superhelical density and recombination events [Le et al., 2013; Postow et al., 2004; Staczek and Higgins, 1998]. These domains are thought to be composed of plectonemes, i.e. loops of superhelically twisted DNA, whose formation depends on several factors: (a) active transcription: the borders of chromosome interaction domains (CIDs) in *C. crescentus* are often flanked by highly expressed genes, whose translocation changes CID borders, and whose transcriptional inhibition leads to a major loss of CID formation; (b) condensation activity of SMC and NAPs, and (c) supercoiling, as CID borders become fuzzy during inhibition of topoisomerases [Le et al., 2013]. The existence of topologically isolated chromosome domains is quite handy because a DNA double-strand break would not lead to a genome-wide relaxation

Fig. 1. Arrangement of bacterial chromosomes. Upper panel: fluorescence microscopy of chromosomes (stained in green) and cell membranes (stained in red). White bar = 2 μ m. Drawings illustrate the arrangement of chromosomes in *E. coli*, *V. cholerae* (note that this organism has a second chromosome, which appears to be similar to a ‘bacillus-like’ chromosome), *C. crescentus* and *B. subtilis*. Origins (0°) and termini (180°) are illustrated as circles. Numbers indicate other positions on the circular chromosome.

of supercoils, but only affects a limited region of the chromosome. The domain structure of bacterial chromosomes may also facilitate segregation. In addition to CIDs, so-called ‘macrodomains’ exist, which can have a size of almost a quarter of the chromosome. Four such domains have been identified in *E. coli*, and they are again somewhat isolated from other macrodomains in terms of recombination events, subcellular positioning and segregation dynamics; these chromosomal superstructures are discussed in the reviews by Messerschmidt and Waldminghaus [pp. 301–315] and Muskhelishvili and Travers [pp. 332–343].

In general, chromosomes of mesophilic bacteria have overall negative supercoiling, with a superhelical density (sigma) of -0.06 (meaning 6% underwinding), facilitating the melting of the DNA duplex for transcription and replication initiation. Negative supercoiling is introduced by

Fig. 2. Simplified model for the bacterial cell cycle under slow-growth conditions based on *B. subtilis* (several aspects are different in e.g. *E. coli* or *C. crescentus*, and even in *B. subtilis*, the cell cycle is somewhat more complicated [Wang et al., 2014]). See text for details.

DNA gyrase, a type II topoisomerase, whereas excess negative supercoils are relaxed by topoisomerase I (Topo I). Upon thermal stress, the synthesis of one enzyme is reduced and that of the other increased. For instance, upon cold shock, more negative supercoils are needed to achieve strand opening, and thus the ratio of gyrase versus Topo I is increased [Lopez-Garcia and Forterre, 2000]. Thermophilic genomes are often positively supercoiled, mediated by the action of reverse gyrase, a type I topoisomerase [Valenti et al., 2011]. Positive or relaxed super-

coiling counteracts strand opening, thereby preventing spontaneous melting of DNA strands at high temperature. However, the superhelical state of bacterial and archaeal genomes is more complex, with different degrees of supercoiling coexisting, most likely due to the mentioned chromosome domains.

Chromosome Arrangement and Segregation in Rod-Shaped and Curved Cells

B. subtilis is a Gram-positive rod-shaped bacterium that has been used for many pioneering studies. It was also in this species that it was shown that the chromosome has a preferred arrangement, with origin regions being close to each cell pole [Webb et al., 1997], terminus region(s) close to the cell center, and 90° and 270° positions being positioned in between [Teleman et al., 1998] (fig. 1). It was later shown in much greater detail that several other bacterial genomes also have a defined 3-D layout, which however differs in arrangement (see below). The replication machinery in *B. subtilis* is positioned in the cell center in young cells, and during the later part of the cell cycle, the two replication forks move apart to the quarter sites of cells [Lemon and Grossman, 1998]. Even though closely adjacent to each other, replication forks can move apart and come back to the cell center, showing that the forks are not fixed to any specific site in the cell [Migocki et al., 2004]. Nevertheless, the relatively stationary positioning of replication forks implies that the chromosome moves through the cell center as it is being replicated, which was elegantly shown to be the case [Lemon and Grossman, 2000] (fig. 2). Soon after initiation of replication, origin regions move out towards opposite cell poles [Sharpe and Errington, 1998] through a mechanism that has not yet been elucidated. All other duplicated chromosome regions follow in a sequential manner such that replication and segregation occur concomitantly (fig. 2). Overall, the chromosome is arranged in a helical manner, and newly replicated DNA can be seen to emerge along a helical path from the cell center towards the poles [Berlatzky et al., 2008]. Spaces between the helical arms are filled at a later point of the replication center. A global helical architecture has also been seen for the *E. coli* chromosome [Fisher et al., 2013] and for *Caulobacter* [Umberger et al., 2011], indicating that this may be a preferred way of folding a bacterial chromosome into a tube-shaped cell. Chromosome arrangement in *B. subtilis* is somewhat different from other bacteria: in slowly growing *E. coli* cells, the origin region and the terminus are in

the cell center; the left arm of the chromosome is in one cell half, and the right arm is in the other [Niki et al., 2000; Wang et al., 2006] (fig. 1). Upon initiation of replication, the replication forks separate and move into opposite cell halves [Reyes-Lamothe et al., 2008]; from both forks, duplicated strands are moved into opposite cell halves, such that at the end of the cell cycle, the arrangement of chromosome arms (left in one cell half, right in the other) is identical in the sister cells. This is in contrast to the mirror image of duplicated chromosomes in e.g. *B. subtilis* (fig. 2) and in *C. crescentus*. However, during multifork replication, the orientation of the chromosome changes, and now chromosome arms lie next to each other along the long axis of the cells, rather than in opposite cell halves, and oldest replication forks are in the cell center, while younger forks are positioned outwards [Youngren et al., 2014]. This way, the chromosome is smoothly replicated and segregated via evenly spaced replication forks.

C. crescentus cells can only have two replication forks during the cell cycle, and thus are not able to perform multifork replication, like e.g. *E. coli* or *B. subtilis*. The origin of replication is stably attached to the cell pole at the beginning of the cell cycle; the terminus is at the other pole. The two arms of the chromosome lie side by side along the long axis of the cells, so the chromosome is condensed into an elongated donut (fig. 1). The replication machinery assembles at the polar origin, and slowly moves towards the opposite cell pole as it synthesizes new DNA strands [Jensen et al., 2001]. After duplication, one origin region is moved to the opposite pole, where it is again stably anchored. All duplicated regions are translocated towards opposite cell poles, into a position that mirrors the arrangement of the chromosome in the mother cell [Viollier et al., 2004]. Thus, the bottle brush-like chromosome is also replicated and segregated in highly ordered manner.

In *Myxococcus xanthus*, chromosome arrangement is somewhat similar to that found in *C. crescentus*; however, origin regions are not attached to the poles, but are stably positioned at a defined subpolar location [Harms et al., 2013]. Two replication forks move gradually from one origin towards the other edge of the nucleoid, and one newly replicated origin is translocated across the cell like in *Caulobacter*. The fact that chromosomes have preferred 3-D arrangements within cells in at least 5 bacterial species (the above-mentioned and *V. cholerae* [see the review by Ramachandran et al., pp. 360–370]) suggests that this is a common feature in many if not all bacteria. Interestingly, a modeling study has shown that based on self-avoidance of DNA, the specific positioning of just a few DNA loci

(such as origin and terminus), plus the organization of the chromosome into topological domains by DNA compaction proteins, is sufficient to generate a linear arrangement of the chromosome along the cell axis [Buenemann and Lenz, 2010]. Thus, geometric cues can largely shape a chromosome, taking advantage of intrinsic properties of DNA. For *Caulobacter*, polymeric PopZ protein mediates the stable attachment of origins to the old cell pole, and later in the cell cycle to opposite poles [Bowman et al., 2008; Ebersbach et al., 2008; Schofield et al., 2010]. ParB protein acts as an adaptor between origin regions and the polar anchor protein, which in *V. cholerae* is called HubP [Yamaichi et al., 2012]. In *Corynebacterium glutamicum*, which grows through the addition of new cell wall material at the cell pole, rather than at the lateral cell membrane like non-Actinomycete rod-shaped cells, origins are anchored to the poles via DivIVa, a protein that recognized negative membrane curvature [Lenarcic et al., 2009; Ramamurthi and Losick, 2009], and the adaptor again is a ParB protein [Donovan et al., 2012]. Thus, there are landmark proteins in bacteria that provide attachment points for specific chromosome regions and thereby help ordering the genome in 3-D. In bacteria such as *E. coli* and *B. subtilis*, chromosome origins are not firmly anchored to the cell envelope (except during sporulation in *B. subtilis*, where origins are placed at the cell poles via two dedicated proteins, RacA and DivIVa [Ben-Yehuda et al., 2003; Wu and Errington, 2003]), nor is any other site of the chromosome known to be positioned via a geometric cue from the cell envelope (fig. 1), so it remains to be seen how chromosome arrangement is achieved and maintained in these cases.

Proteins Involved in Chromosome Segregation and Maintenance of Its 3-D Architecture

DNA polymerase can contribute to an initial segregation of DNA because it is a powerful motor and, at least in case of *B. subtilis*, remains at its position for a long time during the cell cycle (fig. 1) [Lemon and Grossman, 1998]. However, DNA will bend at length exceeding 100 bp, so DNA cannot be pushed over a long distance, certainly not from the cell center to the poles in a 2- to 4- μ m cell. RNA polymerase generates strong pushing forces on the transcribed DNA, when the enzyme is immobilized. It has been proposed that RNA polymerase may contribute substantially to chromosome segregation. Evidence for this is seen from experiments showing that inhibition of transcription leads to a defect in segregation [Dworkin

and Losick, 2002; Kjos and Veening, 2014] (under these experimental condition, transcription inhibition did not affect replication). The transcription bias of genes, facing away from *oriC*, and the fact that many origin-proximal genes are heavily transcribed may cause translocation of DNA towards the poles, when the many RNA polymerases (being very large enzyme complexes) act simultaneously on the DNA template, being coordinated in their movement.

Proteins playing an important role in chromosome dynamics are:

(a) NAPs, which form a variety of homo- or heterodimers, are abundant and influence chromosome structure, transcription and translation in various ways; they are covered in two reviews in this issue [Dorman, pp. 316–331; van de Valk et al., pp. 344–359].

(b) ParA (Walker A ATPases) and ParB proteins, which are also important partitioning factors for many plasmids [Gerdes et al., 2004]. In some species, ParA and ParB are essential for segregation (and thus for viability), while in others, only mild segregation and/or replication defects occur upon deletion of *parA* or *parB*, which are always in an operon structure. ParA appears to act as a diffusion ratchet, providing directionality for the movement of replicated DNA segments from one cell half into the other half.

(c) SMC (structural maintenance of chromosomes) and the related MukB proteins, huge (50-nm-long) and widely conserved ring-like ATPases, whose absence in *B. subtilis* leads to severely impaired growth that is entirely blocked above 23°C in rich medium, and to the generation of about 15% DNA-free (anucleate) cells [Kleine Borgmann and Graumann, pp. 384–395, and Rybenkov et al., pp. 371–383].

(d) Topoisomerases I, II (DNA gyrase) and IV, which are important for proper DNA supercoiling and resolution of disentangled chromosomes at the end of replication (fig. 3) [Muskhelishvili and Travers, pp. 332–343].

(e) DNA translocases (FtsK/SpoIIIE like) [discussed in the review by Crozat et al., pp. 396–408] which move DNA left at the division plane into future daughter cells (as ATP-driven hexameric ring motors; fig. 3), and also help position chromosome terminus regions, to facilitate or even activate the work of

(f) proteins achieving chromosome dimer resolution (e.g. Xer proteins). 10–15% of cells undergo an unequal number of recombination events between duplicated sister chromosomes, and are left with a single dimeric chromosome (fig. 3). Usually, two dedicated recombinases act at the *dif* site, positioned at the termini, to mediate resolu-

Fig. 3. Upper panel: segregation is coupled to cell division through DNA translocases, which move DNA away from the central division plane, or move DNA through the closed septum; for more details, see Kaimer and Graumann [2011]. Final steps of segregation also include the decatenation of intertwined sister chromosomes through type II topoisomerases (mainly topo IV, gyrase appears to be important at all times for disentanglement). Lower panel: resolution of chromosome dimers.

tion of chromosome dimers [see the review by Crozat et al., pp. 396–408].

It is of note that the absence of several proteins involved in DNA repair via homologous recombination also leads to segregation defects [Carrasco et al., 2004]. Thus, removal of recombination intermediates arising during DNA replication, likely based on stalling of replication forks, which occurs at a considerable rate during each cell cycle, is important for proper chromosome segregation.

Segregation in Round and Oval Cells

Few groups have taken up the task to answer the important biological question of how a round cell knows where to separate its chromosomes before cytokinesis has been commenced, and thus the division plane has been established. This is especially interesting in cells that change the plane of division each time following one cell cycle, such as *Staphylococcus aureus*. In contrast to most rod-shaped cells, where an *smc* (*mukB*) deletion has a very severe segregation and growth defect, the deletion of *smc* in *S. aureus* or *Streptococcus pneumoniae* has a mild effect, such that the growth rate of mutant cells is not affected.

However, when a DNA translocase is deleted in addition to *smc*, chromosome segregation becomes much more inefficient, and ceases at high temperatures [Yu et al., 2010]. Thus, the translocation of DNA away from the closing (or even closed) division septum through the hexameric translocase rings plays an important role in a coccus. Interestingly, sublethal concentrations of transcription inhibitors lead to a considerable partitioning defect, while it leaves initial separation of origin regions largely unaffected, reminiscent of the effect seen in *B. subtilis*. Deletion of the transcription-processivity factor GreA also leads to the formation of an increased number of anucleate cells. Moreover, *smc* and *parB* mutant Streptococci are hypersensitive to transcription inhibitors, and the deletion of *greA* and *smc* is conditionally lethal [Kjos and Veening, 2014]. These experiments show that transcription (or a transcription-related factor) plays an important role in the separation of the bulk of the chromosome, and support the idea that RNA polymerase may play an additional motor-like function in chromosome segregation in bacteria.

Segregation in Cells with Multiple Chromosome Copies: Cyanobacteria

Cyanobacteria are a fascinating phylum within the bacteria, which for our purpose have one striking feature: many (if not most) species contain multiple chromosome copies, even under slow growth conditions. Thus, besides the ability to perform oxygenic photosynthesis and to undergo multiple different differentiation processes (formation of acinetes, heterocysts and hormogonia), these organisms are polyploid. In fact, many if not most bacterial species are polyploid, which is discussed in the review by Soppa [pp. 409–419].

In an initial study, the unicellular cyanobacterium *Synechocystis* sp. was used to address the question if multiple chromosomes (*Synechocystis* has about 10 copies during exponential growth) are distributed into future daughter cells at random, or in a defined, active manner. *Synechocystis* cells are round and can grow via the utilization of externally supplied glucose, and thus have a robust cell cycle when they do not oscillate between light and dark phases. Staining of the DNA showed that in contrast to *B. subtilis*, in which daughter cells receive DNA with an average deviation of 6% (which is expected to be experimental error), *Synechocystis* daughters show 15% average difference, with extreme cases in which one daughter can receive up to 2.5 times more DNA than the other cell. Thus, chromosome segregation seems to be less stringent in this

cyanobacterium than in *B. subtilis* [Schneider et al., 2007]. A second study employed a more refined method to determine segregation in the filamentous species *Anabaena* sp., using an adapted *parS*/GFP-ParB system. It was observed that differences in fluorescence between integrated GFP-ParB spots in neighboring daughter cells are significantly larger than the variation in intensity of soluble GFP expressed between the cells, also suggesting some degree of randomness in the segregation process in a second cyanobacterium [Hu et al., 2007]. However, two studies of the rod-shaped *Synechocystis elongatus* revealed an astonishing degree of spatial organization of the multiple chromosomes: when origin regions were marked with a *tetO*/TetR-CFP array and terminus regions with a *lacO*/LacI-YFP system, the two signals were mostly observed in pairs, rather than randomly distributed, indicating that each chromosome occupies a certain territory. More intriguingly, at a certain time during the cell cycle, origin regions moved to become relatively evenly spaced along the long axis of cells, visually aligned in a straight line. Thus, chromosomes showed a high degree of spatial distribution, which gives rise to a rather even and accurate distribution of chromosomes before and after division. Interestingly, in mutant cells in which the septum is misplaced at various positions along the longitudinal axis of the cells, rather than precisely at mid cell, chromosomes are segregated unequally. Thus, there does not appear to be an active segregation machinery, but accurate chromosome positioning prevents unequal sister chromosome inheritance. Segregation of duplicated origin regions (and thus presumably their replication) was found to be asynchronous between the chromosomes, and copy number could vary considerably before or after division, suggesting that replication/segregation and cell division are not strongly coordinated. Indeed, a single replication machinery was observed in cells at a given time, such that only one or a subset of chromosomes is replicating at any given time. As this cyanobacterium appears to use chromosome alignment instead of active segregation, as well as ploidy (average of 4–5 chromosome copies), no need for a segregation/cytokinesis checkpoint would exist, and replication can also be nonstringently initiated.

Thus, cyanobacteria are a valuable system to study the segregation of multiple chromosomes, and can also be used to address an even more intricate question: how is chromosome segregation altered and arranged with cell branching? Type V cyanobacteria form cell branches and thus change the plane of chromosome segregation. The question of how the segregation mechanism is rearranged is a fascinating one.

Segregation in Cells with Two or More Different Chromosomes: *V. cholerae*

V. cholerae has two distinct chromosomes, which are segregated by two different mechanisms [Fogel and Waldor, 2005; Srivastava et al., 2007], one involving a ParAB mechanism [Fogel and Waldor, 2006], and another yet unknown mechanism. That being stated, there is an excellent review on this interesting question of how to segregate different chromosomes in this issue of *Journal of Molecular Microbiology and Biotechnology* [see the review by Ramachandran et al., pp. 360–370].

Chromosomes in Streptomyces

Streptomycetes belong to the high GC Gram positives, also called Actinobacteria, which grow in a fungus-like apical manner, and have many other interesting properties. Streptomycetes form branched mycelia, and the model organism *S. coelicolor* has a linear chromosome, whose ends are held together by a dedicated protein, such that genetically, it behaves like a circular chromosome [Yang and Losick, 2001]. Its segregation is extremely sloppy: chromosomes are not condensed into nucleoids, but are unevenly distributed along the hyphae. This is in tune with cell division, which occurs infrequently and at irregular positions along the syncytia. In fact, *S. coelicolor* can even grow in the absence of FtsZ, i.e. the central player in cell division, as one large super-bacterium [McCormick et al., 1994]. In contrast, when nutrients are depleted and Streptomycetes initiate the formation of hyphae that grow into the air, for the ultimate formation of exospores, chromosome arrangement and segregation become highly ordered and coordinated: at the tip of aerial mycelium, about 20 or so discrete, orderly spaced nucleoids are formed, partially due to the function of ParAB, SMC, topoisomerase I and DNA translocase proteins [Dedrick et al., 2009; Jakimowicz et al., 2005; Szafran et al., 2013], such that synchronous septation between nucleoids gives rise to forespores, which upon maturation of the cell walls are released as dormant spores.

Chromosomes in Spirochaetes and in Planctomycetes

Spirochaetes form a phylum within the bacteria, and have an unusual cell structure: they contain their flagella within the periplasmic space and are highly helical. Fla-

gellar rotation leads to a cork screw-like movement, which allows cells to move through viscous environments, and many pathogenic species use this property to squeeze between cells and thereby invade tissue. *Borrelia hermsii* contains 13–18 chromosomes and many plasmids, some of which are linear. The DNA is distributed through most of the cell's length [Kitten and Barbour, 1992]. In *Treponema denticola*, DNA is distributed throughout the cells. However, in *Treponema* cells lacking the filament-forming protein CftA, DNA is highly condensed, and cells show a chaining phenotype [Izard et al., 2001]. It is still unclear how CftA affects chromosome structure and cell division. It would be wonderful if better genetic tools were available for this group of bacteria to gain more insight into their cell cycle.

Planctomycetes belong to the Planctomycetes-Verrucomicrobia-Chlamydiae superphylum, which contains many most unusual species. Starting with the fact that many of these bacteria lack an FtsZ gene for cell division [van Niftrik et al., 2009], which is otherwise present in almost all bacteria and a majority of archaea, they provide many examples of unusual cell shapes and cell division types, including yeast-like budding [Lee et al., 2009]. Many Planctomycetes have a highly unusual cell architecture [Jogler et al., 2011], the details of which are still under debate. Some studies indicate the presence of additional intracellular membranes, which are not connected to the cell membrane. This is well established for the anamoxosomes, in which the unusual anamox reaction (generation of ammonia and nitrate in parallel from nitrogen) is driven by special enzymes, and which contain unusual ladderane lipids in the membrane that tightly seal off the reaction, which includes toxic intermediates [van Niftrik et al., 2009]. The existence of a nuclear-like envelope around the DNA has been reported, but was recently disputed, and instead, a highly invaginated cell membrane has been found for one species using cryo-electron tomography [Santarella-Mellwig et al., 2013]. *Gemmata obscuriglobus* has a highly amorphous nucleoid, or several nucleoids, in between which invaginated membranes can be found. DNA is translocated into the bud some time after its growth has been initiated [Lee et al., 2009], suggesting that an active transport mechanism exists. Genetic tools for relevant species have been established, so descriptions on the detailed structure of Planctomycetes and their cell cycle can hopefully be read by the community in the near future.

Chromosomes in Archaea

Studies on archaeal chromosomes and their segregation are still scarce. The archaeon *Halobacterium salinarum* can be synchronized, and its study revealed that two SMC-like proteins, whose overproduction or depletion led to a delay in cell division, are only expressed late in the cell cycle [Herrmann and Soppa, 2002]. Thus, cell cycle proteins are also transcriptionally regulated in some archaea, like in *Caulobacter*, and contrarily to *E. coli* or *B. subtilis*, where all or most of these proteins are constitutively being produced throughout the cell cycle. *H. salinarum* DNA appears to be replicated in the cell center, where the DNA condenses early in the cell cycle, and sister chromosomes are separated towards quarter sites in a manner similar to the bacterial paradigm. However, archaeal chromosomes can differ notably from those of bacteria, e.g. by having several replication origins, by lacking replication stop (*tus*) sites, or by having histone-like proteins rather than NAPs. Further aspects of the archaeal replication/segregation cycle are discussed by van der Valk et al. [pp. 344–359] and Samson and Bell [pp. 420–427].

Active Motor versus Passive Segregation through Entropy

Separate molecules and polymers tend to disentangle, especially if they have the same charge, based on diffusion. The idea that entropy plays a major role in the segregation of bacterial chromosomes has been introduced a long time ago. It can be shown by computer simulation that two fully replicated chromosomes segregate to opposite sides of a tube (i.e. rod-shaped cells) driven by (charge) repulsion of two self-avoiding polymers [Arnold and Jun, 2007; Jun and Mulder, 2006]. These simulations would argue that there is no need for an active machinery that pulls or pushes chromosome regions apart. In addition, it has been proposed that tethering of the chromosome to the membrane, which extends during the cell cycle, can also provide a force that aids in the separation of sister chromosomes. Such a tethering may be provided through a proposed mechanism called ‘transertion’, where the transcription of a gene coding for a membrane protein is directly coupled with the translation of its mRNA and the insertion of the growing peptide chain into the membrane [Woldringh and Nanninga, 2006]. Indeed, recent simulations show that membrane tethering strongly enhances the efficiency of complete separation of chromosomes driven by self-avoidance, and even more so if there is a polar gradient of

DNA attachment to the membrane [Di Ventura et al., 2013]. It has been proposed that MinD could set up such a gradient: the protein oscillates between the cell poles, driven by an ATPase cycle, and binds to DNA in vitro. In the absence of MinD, which helps positioning the cell division plane at the cell middle and binds to the cell membrane through an amphipathic helix, chromosome segregation has a visual defect. This occurs also when an arginine in MinD is mutated that is essential for DNA binding [Di Ventura et al., 2013]. The details of this DNA-membrane tether still need to be elaborated. In any event, it has been proposed that a purely entropy-driven separation of chromosomes is efficient only in separating the bulk of DNA, but energy drops sharply towards bulk separation, such that complete segregation will become inefficient. Moreover, the fact that chromosomes have highly ordered 3-D structures with preferred architecture argues against an ‘entropy only’-driven process because separation through random diffusion cannot explain this degree of ordering. A more direct segregation mechanism has been demonstrated for *C. crescentus* and for *V. cholerae*: ParA ATPase protein localizes as a cloud-like structure from the polar origin to the opposite cell pole [Ptacin et al., 2010]. This structure retracts, and seemingly guides one duplicated origin region towards the other pole [Ptacin et al., 2014]. ParB induces ATPase activity in ParA, suggesting that origin-bound ParB protein (a specific DNA-binding protein) leads to the ParA retraction towards the opposite pole, mediating a diffusion ratchet-like movement of origins. ParA and ParB are essential in *C. crescentus* [Mohl and Gober, 1997], but not in *V. cholerae* [Kadoya et al., 2011], where ParA tethers origin regions of chromosome I to the poles, or in *B. subtilis*, where their deletion causes very mild segregation and replication defects [Ireton et al., 1994; Murray and Errington, 2008]. Moreover, *E. coli* cells do not possess genuine ParAB proteins, so it is clear that bacteria can use different segregation ‘machineries’ or mechanisms. Of note, low-copy plasmids in bacteria can use a ParAB-like partitioning system, an actin-like (ParM/ParR) pushing mechanism [Becker et al., 2006; Gerdes et al., 2004], or a tubulin/FtsZ-like pushing apparatus [Larsen et al., 2007], revealing a marvelous array of different simple mitotic-like modules that can separate defined DNA segments in bacterial cells.

These beautiful reports notwithstanding, an active molecular machinery has been elusive in model bacteria such as *B. subtilis* and *E. coli* [for a discussion of possible mechanisms of segregation in *E. coli*, see the review by Messerschmidt and Waldminghaus, pp. 301–315], so in its absence, segregation must be assumed to be based on

physico-/chemical mechanisms. I would like to speculate that in many bacteria, chromosome segregation has three layers: (a) several dedicated proteins or protein complexes (such as SMC/MukB) that compact DNA in a nonuniform manner ensure that separated chromosome regions remain in their given space [for more details, see the reviews by Kleine Borgmann and Graumann, pp. 384–395, and Rybenkov et al., pp. 371–383]; (b) sequence information on the chromosome sets up distinct domains via specific DNA binding proteins, possibly a topoisomerase gradient [see the review by Muskhelishvili and Travers, pp. 332–343], and other architectural measures that shape the chromosome and aid in providing its 3-D arrangement within the cell, and (c) a passive segregation motor utilizes in part the power of DNA polymerase, which generates strong DNA pushing forces (over short distances), and entropy, to separate duplicated chromosome regions.

It will become apparent in this issue of *Journal of Molecular Microbiology and Biotechnology* that parts a and b have very interesting and intricate mechanisms, and that the field of bacterial chromosome structure and segregation is an exciting one promising to yield many more surprises in the future and finally insight into a very fundamental biological process.

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