COMMENTARY

Switching modes of chromosome dynamics in the bacterial cell cycle

Barbara E. Funnell[1]

Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada M5S 1A8

Bacterial cells have no nucleus, and the chromosomal DNA occupies much of the intracellular space in a highly compacted protein–DNA structure called the nucleoid. The genomes of most bacterial species consist of one circular chromosome that is several million base pairs in length. Many studies over several decades have sought to address how so much DNA can fit into a small space in such a way as to be faithfully replicated and segregated in the bacterial cell cycle. Development of sophisticated cell biology tools has allowed researchers to examine how chromosome organization and the DNA replication machinery act to ensure accurate chromosome segregation. In PNAS, Wang et al. address a debate concerning two different types of chromosome orientation that have been reported in different bacterial species (1). They show that both patterns occur in a distinct temporal progression during the *Bacillus subtilis* cell cycle, identify proteins that contribute to this choreography, and develop a model for chromosome segregation that is generally applicable to many species.

The overall shape of the bacterial nucleoid appears as a compressed helical structure, and multiple factors cooperate to compact the chromosome, notably negative supercoiling, small nucleoid binding proteins, and SMC condensins (structural maintenance of chromosomes) (2, 3). The nucleoid is also spatially organized, and the locations of the replication origin (*ori*), terminus (*ter*), and various other loci on the chromosome have been examined during the cell cycle and in relation to the positions of the replication forks. The basic message is that DNA replication and segregation occur concurrently, so that daughter chromosomes are moved away from each other as the DNA is replicated.

Proteins that participate in chromosome orientation and segregation have been identified in different species, but a complete description of the mechanisms involved is still lacking. Although SMC condensins are nonspecific DNA binding proteins that bind throughout the chromosome, they are concentrated at the replication origin and are necessary for origin and replichore separation (4–6). Many, but not all, bacterial species use "ParABS" partition systems that are homologous to those that segregate bacterial plasmids. ParB binds to DNA sites called *parS*, which are generally found in the origin region of the chromosome. The ParB/*parS* complexes are acted on by ParA, an ATPase, which is thought to help organize and position this region of the chromosome. In *B. subtilis*, SMC is recruited to the origin region by Spo0J (ParB), but this is not the only role of the partition system (7, 8). The study by Wang et al. demonstrates an interplay between the actions of SMC condensins and the bacterial partition machinery that determines the specific patterns of chromosome organization during the replication cycle.

Two general patterns of chromosome organization in bacteria have been reported, called *ori-ter* and left-*ori*-right (Fig. 1). In *ori-ter*, the origin is located at one cell pole, the terminus at the other pole, and both left and right chromosomal arms, or replichores, follow the longitudinal axis of the cell. This pattern has been observed in *B. subtilis, Caulobacter crescentus,* and *Vibrio cholera* chromosome I, for example. The left-*ori*-right pattern is essentially rotated 90° with respect to *ori-ter*; the origin is centrally positioned, and the left and right replichores flank the origin in opposite cell halves. The best-studied example of left-*ori*-right is *Escherichia coli*. What determines origin localization and longitudinal versus transverse axis positioning of the left and right chromosomal arms? Are there two fundamentally different mechanisms and machinery at play here, or one with variable outcomes depending on additional factors? The study presented here argues for the latter possibility.

In some *ori-ter* species, such as *Caulobacter crescentus* and *V. cholera* (chromosome I), the origin is tethered at one pole, and following replication initiation, one of the daughter origins is released and translocates to the opposite pole. Termination occurs in the cell center, resulting in symmetrical *ori-ter-ter-ori* arrangement before cell division. *B. subtilis* exhibits an *ori-ter* arrangement during most of the cell cycle, but its origin moves to midcell where initiation has been reported to occur (see below). In *E. coli*, replication initiation occurs at midcell, and the growing left and right replichores are translocated toward opposite cell halves and away from the transverse axis, such that segregation generates an L-R-L-R pattern in dividing cells (9). Removal of MukB (SMC-like condensin) from *E. coli* changes its chromosome pattern from left-*ori*-right to *ori-ter*, implicating condensins in development or maintenance of the former (4). However, all bacteria have SMC or SMC-like proteins, so other factors must be involved in development of the *ori-ter* patterns in the presence of condensins.

Wang et al. are interested in the apparent paradox in *B. subtilis* that its chromosome exhibited the *ori-ter* polar pattern yet DNA

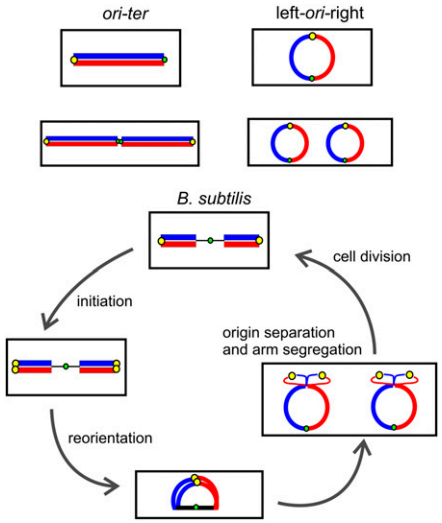

Fig. 1. Simplified cartoons of the patterns of chromosome arrangements in bacterial cells. (*Upper*) *ori-ter* and left-*ori*-right organization in cells with one chromosome and with two completed chromosomes before cell division. The left and right chromosome arms are blue and red, respectively, and *ori* and *ter* are yellow and green circles, respectively. (*Lower*) The alternating choreography of *ori-ter* and left-*ori*-right during a *B. subtilis* cell cycle.

Author contributions: B.E.F. wrote the paper.

The author declares no conflict of interest.

See companion article on page 12877.

[1]Email: b.funnell@utoronto.ca.

replication initiated at midcell as it does in *E. coli*. Through careful cell biology, they make a number of unexpected observations that challenged simple predictions about DNA replication and segregation. They labeled the origin, terminus, and two loci on each of the left and right replichores with different fluorescent tags so that they could follow multiple loci in the same cells. They examined very slowly growing *B. subtilis* in an effort to avoid the complexity of multifork replication when cells are born with replicating chromosomes, which occurs during rapid cell growth. The first surprise was that even when growing slowly, *B. subtilis* cells are born with replicating chromosomes and are thus partially diploid. This is in contrast to the situation in *E. coli,* which contains only one chromosome under these conditions. The second surprise was that when the authors did generate cells with one chromosome (by limiting replication initiation), the pattern changed to a left-*ori*-right orientation. As in *E. coli*, the SMC condensin complex was necessary to maintain the left-*ori*-right pattern because chromosome orientation reverted to *ori-ter* when the complex was removed.

The authors revisited the chromosome organization and segregation patterns in WT cells using time-lapse fluorescence microscopy; that is, on cells born with partially replicating chromosomes (*ori-ter-ori*). The next surprise was that replication initiation did not occur at midcell as had been previously reported. Instead, origin duplication occurred at the poles (or the periphery of the nucleoids), and then the origins moved to midcell. This change coincided with reorientation of the left and right replichores from the longitudinal to the transverse axis (*ori-ter* to left-*ori*-right). Next, the origins moved to the edges of the nucleoid, and the chromosomal arms reoriented to the longitudinal axis following their replication (Fig. 1). Finally, experiments in *soj* (*B. subtilis parA*) mutants implicated the partition system in the *ori-ter* reorganization. Without Soj/ParA, the movement of newly replicated origins toward the nucleoid periphery and the reorganization of the chromosomal arms were perturbed.

Therefore, *B. subtilis* undergoes a complicated choreography of alternating chromosomal orientation in the same cell cycle. The dance requires the participation of SMC condensin complexes, the partition machinery, and interactions between them. The model that emerges from these observations proposes that the switch from *ori-ter* to left-*ori*-right is a signal from replication, directly or indirectly by the duplication of the origin DNA. The role of SMC would be to facilitate origin separation and release the origins from the factors that put them at the nucleoid periphery, setting up left-*ori*-right. One important such factor is ParA/Soj, which acts on ParB to pull the origins apart toward the nuclear periphery to set up the *ori-ter* arrangement. In this model, the left-*ori*-right orientation is the "ground state," maintained by SMC complexes interacting with the origin region of the chromosome. The authors argue that this situation is generally applicable and predict that alternating patterns will be observed in other species with careful time-lapse microscopy.

Wang et al. address a debate concerning two different types of chromosome orientation that have been reported in different bacterial species.

Is the model consistent with the behavior of chromosomes, SMC condensins, and the partition machinery in other species? The answer is yes in several respects. First, SMCs are necessary for separation of origins and of the two chromosome arms (4–6). Second, the action of chromosomal ParA is similar to that proposed for plasmid ParAs, which interact with their ParB/*parS* complexes to move daughter plasmids away from each other over the surface of the bacterial nucleoid during their partition process (10). Third, *C. crescentus* and *V. cholera* require the partition machinery to move newly replicated origins from one pole to the other, and they prevent left-*ori*-right by tethering the origins to polar proteins that interact specifically with Par proteins (11, 12). Therefore, variations in the specific choreography in different species may depend on additional factors that favor or prevent one of the two modes of chromosome dynamics seen in *B. subtilis*.

There are still many questions that remain. The molecular nature of the replication switch and of the opposing actions of ParA and SMC at the origins remain to be identified. How do organisms such as *E. coli* without a ParABS system set to *ori-ter* when condensins are removed? What other factors contribute in these and other species? Recent biophysical analyses have proposed that newly replicated chromosomes are confined polymers that, based on thermodynamic principles, avoid each other, driving segregation (13–15). How these principles in conjunction with proteins such as SMCs and ParA explain the alternating patterns of chromosome organization is an important and intriguing question.

ACKNOWLEDGMENTS. This work was supported by Canadian Institutes of Health Research Grant 133613.

1 Wang X, Montero Llopis P, Rudner DZ (2014) *Bacillus subtilis* chromosome organization oscillates between two distinct patterns. *Proc Natl Acad Sci USA* 111:12877–12882.
2 Reyes-Lamothe R, Nicolas E, Sherratt DJ (2012) Chromosome replication and segregation in bacteria. *Annu Rev Genet* 46:121–143.
3 Wang X, Montero Llopis P, Rudner DZ (2013) Organization and segregation of bacterial chromosomes. *Nat Rev Genet* 14(3):191–203.
4 Danilova O, Reyes-Lamothe R, Pinskaya M, Sherratt D, Possoz C (2007) MukB colocalizes with the *oriC* region and is required for organization of the two *Escherichia coli* chromosome arms into separate cell halves. *Mol Microbiol* 65(6):1485–1492.
5 Gruber S, et al. (2014) Interlinked sister chromosomes arise in the absence of condensin during fast replication in B. subtilis. *Curr Biol* 24(3):293–298.
6 Wang X, Tang OW, Riley EP, Rudner DZ (2014) The SMC condensin complex is required for origin segregation in *Bacillus subtilis*. *Curr Biol* 24(3):287–292.
7 Sullivan NL, Marquis KA, Rudner DZ (2009) Recruitment of SMC by ParB-*parS* organizes the origin region and promotes efficient chromosome segregation. *Cell* 137(4):697–707.
8 Gruber S, Errington J (2009) Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. *Cell* 137(4):685–696.
9 Wang X, Liu X, Possoz C, Sherratt DJ (2006) The two Escherichia coli chromosome arms locate to separate cell halves. *Genes Dev* 20(13):1727–1731.
10 Hwang LC, et al. (2013) ParA-mediated plasmid partition driven by protein pattern self-organization. *EMBO J* 32(9):1238–1249.
11 Yamaichi Y, et al. (2012) A multidomain hub anchors the chromosome segregation and chemotactic machinery to the bacterial pole. *Genes Dev* 26(20):2348–2360.
12 Ptacin JL, et al. (2014) Bacterial scaffold directs pole-specific centromere segregation. *Proc Natl Acad Sci USA* 111(19):E2046–E2055.
13 Jun S, Mulder B (2006) Entropy-driven spatial organization of highly confined polymers: Lessons for the bacterial chromosome. *Proc Natl Acad Sci USA* 103(33):12388–12393.
14 Youngren B, Nielsen HJ, Jun S, Austin S (2014) The multifork Escherichia coli chromosome is a self-duplicating and self-segregating thermodynamic ring polymer. *Genes Dev* 28(1):71–84.
15 Fisher JK, et al. (2013) Four-dimensional imaging of *E. coli* nucleoid organization and dynamics in living cells. *Cell* 153(4):882–895.