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Spatial organization of bacterial chromosomes

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Bacterial chromosomes are organized in stereotypical patterns that are faithfully and robustly regenerated in daughter cells. Two distinct spatial patterns were described almost a decade ago in our most tractable model organisms. In recent years, analysis of chromosome organization in a larger and more diverse set of bacteria and a deeper characterization of chromosome dynamics in the original model systems have provided a broader and more complete picture of both chromosome organization and the activities that generate the observed spatial patterns. Here, we summarize these different patterns highlighting similarities and differences and discuss the protein factors that help establish and maintain them.

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Introduction

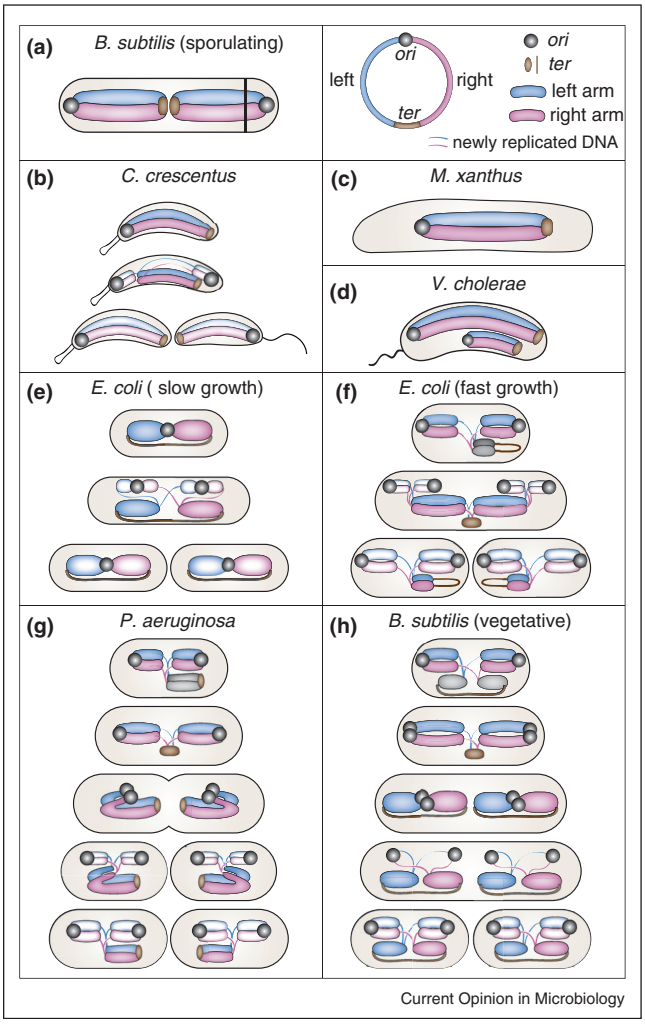
The spatial organization patterns of bacterial chromosomes fall into two broad categories: those in which the chromosome is arranged longitudinally in a so-called *ori-ter* pattern and those in which the chromosome resides in a transverse configuration with the left and right arms (or replichores) present in separate cell halves in a left-*ori*-right arrangement. These patterns were first described in sporulating *Bacillus subtilis* cells [1–3] and vegetatively growing *Caulobacter crescentus* [4] (*ori-ter*) and slow-growing *Escherichia coli* [5,6] (left-*ori*-right). Over the last few years, analysis of chromosome organization in a collection of diverse bacteria has uncovered variations on these themes including one bacterium in which the chromosome alternates between these two patterns. Here, we catalog these patterns and describe the role of homologous and analogous proteins in generating them and ensuring their recapitulation in the next generation.

ori-ter (longitudinal) organization

The most common organization pattern involves the longitudinal arrangement of the bacterial chromosome with the origin present at or near the old cell pole and the terminus near the new one. The left and right arms lie side-by-side between them. This *ori-ter* pattern was first suggested from studies in sporulating *B. subtilis* cells before the dawn of bacterial cell biology. Electron micrographs from the 1960s revealed that the replicated chromosomes of sporulating cells adopt an elongated structure that extends from one cell pole to the other termed the axial filament [7]. Asymmetric division traps ~25% of the chromosome destined for the spore in the smaller pre-spore compartment [8,9]. A DNA translocase then pumps the rest of the genome into the developing spore. In elegant genetic studies that employed a translocation deficient mutant, it was discovered that a spore-specific promoter fused to *lacZ* was expressed if the reporter was inserted at origin-proximal sites but not at loci near the terminus [10,11]. These studies were the first to suggest that a chromosomal locus has a specific address inside the bacterial cell and hinted at a longitudinal arrangement of the mother and forespore chromosomes. Cytological studies using fluorescently tagged DNA binding proteins and operator arrays inserted throughout the chromosome have since confirmed that the axial filament is indeed composed of replicated chromosomes arranged in an *ori-ter ter-ori* pattern [1,3,12•,13] (Figure 1a).

The first systematic analysis of bacterial chromosome organization was undertaken in *C. crescentus* a decade ago [4]. Operator arrays were inserted at >100 loci throughout the genome using of a transposon that harbored the array. Subcellular localization of these loci using fluorescence microscopy established that the *C. crescentus* chromosome is organized longitudinally in an *ori-ter* configuration (Figure 1b). At birth, the origin of replication lies adjacent to the old pole and the terminus resides near the new one with loci along each arm arranged linearly with respect to their position in the genome. More recent genome-wide chromosome conformation capture studies suggest that the two arms are largely spatially distinct entities [14,15]. After replication initiation, one of the two sister origins is segregated to the opposite (new) cell pole [4,16–18]. Newly replicated DNA follows the path of the segregated origins. As replication proceeds, the unreplicated terminus gradually migrates toward mid-cell (Figure 1b). Thus, the *ori-ter* pattern is regenerated in the next generation. In the case of both *C. crescentus* and sporulating *B. subtilis*, the origins are physically tethered to the cell pole [19–22]. But as

Figure 1



Spatial organization patterns of bacterial chromosomes. Replication origins are represented as black balls, terminus as brown lines or a brown oval. The left and right chromosome arms are shown as thick blue and purple lines (or blobs). The chromosome arms are shown with a lighter hue after they are replicated, while newly synthesized DNA is illustrated as thin lines. In cells that are born with partially replicated chromosomes (f–h), the older template DNA is colored gray.

discussed below, the longitudinal organization pattern does not appear to require a physical tether.

Recent systematic analyses of chromosome organization in *Myxococcus xanthus* and *Vibrio cholerae* underscore the prevalence of the longitudinal pattern. *M. xanthus* contains a 9.1 Mb genome that is organized in an *ori-ter* configuration [23•]. Interestingly, this bacterium has large (~1 μm) nucleoid-free regions adjacent to both poles, suggesting that polar tethers may not be critical to generate this longitudinal organization (Figure 1c). *V. cholerae* has a segmented genome with a large (3 Mb) chromosome (called chr I) and a smaller (1 Mb) plasmid-like chromosome (chr II). Cytological analysis of loci on the large and small chromosomes indicates that both adopt *ori-ter* patterns [24•,25,26] (Figure 1d). The larger chromosome is quite similar to that of *C. crescentus* with its origin tethered at the old cell pole and the terminus near the new one. The smaller chromosome lies adjacent to chr I with an untethered origin. Interestingly, chr II resides closer to the new pole and only occupies about half the cell space. What restricts the smaller chromosome to the younger cell half is currently unknown. As with *C. crescentus*, segregation of both *V. cholerae* and *M. xanthus* origins toward opposite poles sets up the *ori-ter* pattern in the next generation. Finally, work in *Mycobacterium tuberculosis* [27] and *Corynebacterium glutamicum* [28] indicates that their replication origins localize to the cell pole suggesting that these two actinobacteria also arrange their chromosomes longitudinally in an *ori-ter* configuration.

Left-*ori*-right (transverse) organization

The *ori-ter* longitudinal pattern is both simple and intuitive, yet systematic analysis of slow growing *E. coli* performed almost a decade ago uncovered a strikingly distinct pattern [5,6]. At birth, *E. coli*'s replication origin resides at mid-cell and the left and right arms are present in separate cell halves; the terminus region (~300 kb) connects the left and right arms to complete the circle (Figure 1e). Thus, the *ori-ter* axis lies perpendicular to the long axis of the cell generating a transverse organization. After the origins are replicated, they are segregated to the cell quarter positions and the newly replicated left and right arms segregate to either side regenerating this transverse pattern in the next generation.

Variations on these themes

Two recent studies of chromosome organization one in vegetatively growing *B. subtilis* [12•] and the other in *Pseudomonas aeruginosa* [29•] have uncovered variations on the two themes described above. Systematic analysis in *P. aeruginosa* indicates that the organization of this bacterium's chromosome has all the hallmarks of a longitudinal arrangement. After their replication, the origins are segregated to the nucleoid periphery in opposite cell halves and at the time of cytokinesis the chromosome is organized in an *ori-ter ter-ori* configuration (Figure 1g). However, careful analysis of the origin region throughout the cell cycle revealed that replication initiates at the cell quarters rather than at the nucleoid periphery. For this to occur, the origin must somehow re-locate from its polar position. The mechanism by which it does so is not yet clear. One possibility is that the origin region moves back toward mid-cell where it is replicated and then segregated to the periphery (Figure 1g). In this model, the *P. aeruginosa* pattern is essentially *ori-ter* with a slight twist (or fold-over) at the time of replication initiation.

Analysis of chromosome organization during the *B. subtilis* cell cycle revealed that this bacterium has an even more

unusual organization pattern: it alternates between an *ori-ter* configuration and a left-*ori*-right pattern [12•]. Even under slow growing conditions, *B. subtilis* cells are born with partially replicated chromosomes, so a description of a 1-N nucleoid was only possible upon inhibition of replication initiation. Under these conditions, the chromosome adopts an *E. coli*-like left-*ori*-right pattern. With the knowledge that this bacterium was capable of a transverse organization pattern, a careful time-lapse analysis was undertaken. At birth, as in the case in *P. aeruginosa*, the origins reside at the nucleoid periphery in opposite cell halves with left and right arms adjacent to each other in a classical *ori-ter-ori* (longitudinal) arrangement (Figure 1h). Interestingly, replication was found to initiate at these polarly localized origins. However, prior to their segregation, the newly duplicated origins move as unit to the middle of the nucleoid. This movement is accompanied by a rearrangement of the unreplicated chromosome such that when the origins arrive at mid-cell, the left and right arms adopt a left-(*ori*)2-right configuration (Figure 1h). The origins are then segregated to the nucleoid periphery in opposite cell halves and the newly replicated DNA follows in a longitudinal arrangement. In this unusual replication-segregation cycle, the template DNA adopts the left-*ori*-right pattern while the newly replicated sisters segregate in an *ori-ter* pattern. It is unclear why *B. subtilis* performs these chromosome gymnastics but one possibility is to allow replisomes to track on spatially resolved arms while segregating the sister DNA to the extreme cell poles. The oscillating patterns observed in *B. subtilis* provide an alternative explanation for the patterns described in *P. aeruginosa*. Future experiments will establish whether or not *P. aeruginosa* also alternates between longitudinal and transverse organizations.

Interestingly, a recent analysis of fast growing *E. coli* undergoing multi-fork replication, suggests that an *ori-ter*-like pattern replaces the transverse configuration [30•]. Under these conditions, polarly localized origins move apart after their replication and the newly replicated left and right arms reside side-by-side (Figure 1f). The localization of the replisome to mid-nucleoid zones in these fast-growing cells [30•] raises the intriguing possibility that a transient left-*ori*-right intermediate might also exist during these overlapping replication cycles. The mechanism underlying the different organization patterns during slow and fast growth in *E. coli* remains unclear but it has been suggested that it is driven by the physical properties of the nucleoid and the degree of radial confinement experienced by the chromosome during the two growth conditions. *E. coli* cells are thinner during slow growth and might not be able to accommodate a side-by-side arrangement of the left and right arms.

In conclusion, the *ori-ter* and left-*ori*-right arrangements serve as the ground states and building blocks for all the patterns observed in bacteria thus far. The observation that both patterns are embodied in the replication cycle of *B. subtilis* and each can be found in *E. coli* depending on growth rate highlights that these seemingly distinct configurations are not so different from each other. Chromosome condensation along contiguous DNA segments generates a compact circular structure that can adopt longitudinal or transverse orientations depending on the proteins that act upon it and the physical properties of the compartment that contains it. In the second half of this review we focus on the protein factors that help establish and maintain these patterns. We begin by discussing the requirement for the condensin complex in setting up the left-*ori*-right pattern. We then focus on the role of the conserved ParABS partitioning system in establishing the *ori-ter* configuration. Finally, we describe the diverse set of proteins that function in anchoring the origins at the cell pole.

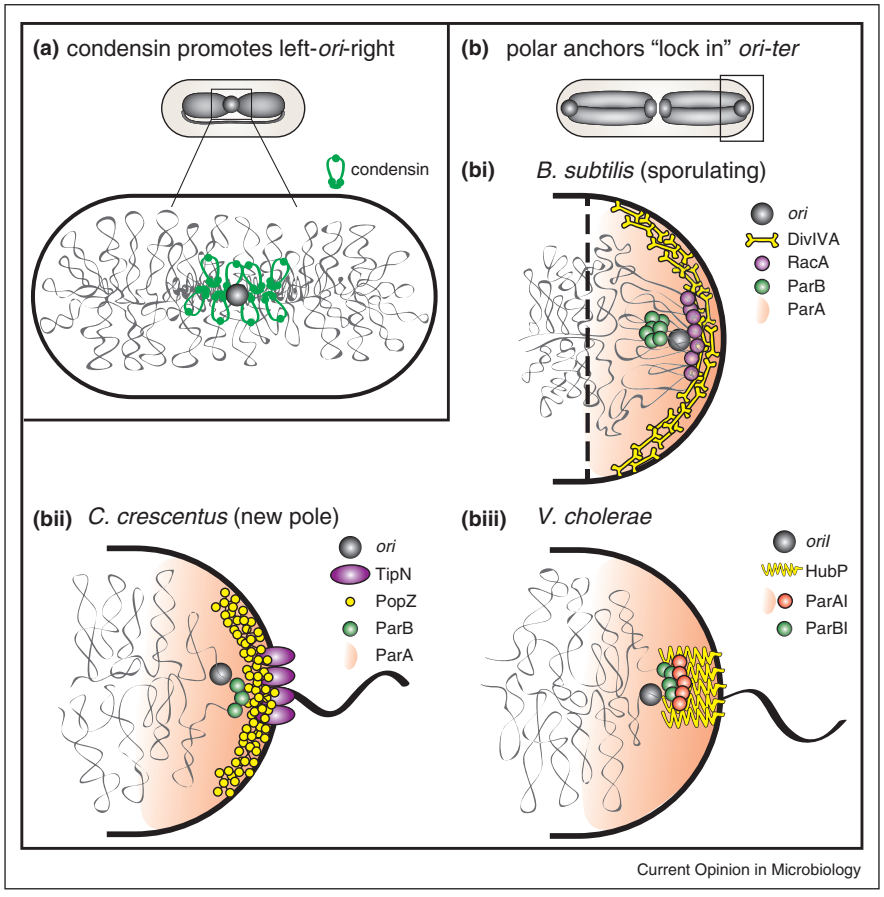
The condensin complex is required for the left-*ori*-right configuration

SMC condensin complexes play a critical role in constraining DNA in both eukaryotes and prokaryotes [31]. The mechanism by which these highly conserved complexes compact and resolve chromosomes remains an outstanding question. Work in both *E. coli* and *B. subtilis* has found that condensin also functions in generating the left-*ori*-right organization pattern [12•,32]. In both bacteria, the complex is essential during fast growth and during slow growth mutants have a high frequency of chromosome loss [33,34]. In slow growing *E. coli*, nucleated cells that lack condensin adopt an *ori-ter* configuration rather than left-*ori*-right [32]. Slow growing *B. subtilis* cells lacking SMC have more heterogeneous nucleoid morphologies, in part owing to a defect in resolution of replicated origins [35,36]. However, depletion of SMC in cells that are restricted to 1-N content through inhibition of replication initiation, have a longitudinal chromosome organization rather than the left-*ori*-right pattern observed in *smc*+ cells when replication is blocked. Importantly, condensin complexes associate with the origin region in both bacteria suggesting that their activity in this region plays an important role in setting up the transverse pattern. We hypothesize that these complexes function to constrain DNA spanning the origin generating a rigid structure that orients along the long axis of the cell. This structure seeds the left-*ori*-right pattern (Figure 2a). It is noteworthy that the condensin complex plays a more minor role in chromosome organization and segregation in the other bacteria in which it has been examined including, *C. crescentus* [15], *V. cholerae* [24•], *P. aeruginosa* [37], and *Streptococcus pneumoniae* [38].

The ParABS partitioning system helps establish and maintain the *ori-ter* pattern

With the exception of *E. coli*, all other bacteria for which we have detailed information about how their genomes are organized, contain the ParABS system. This conserved

Figure 2



Systems that promote longitudinal and transverse configurations. **(a)** The condensin complexes (green) are enriched in the region surrounding the replication origin (gray ball) seeding the left-*ori*-right organization. **(b)** Three examples of polar complexes that anchor the origins to the cell pole locking in the *ori-ter* configuration. ParA is shown as an orange cloud with highest concentration at the cell pole. In sporulating *B. subtilis* (bi), the polar septum is shown as a dotted line. In *V. cholerae* (biii), ParAI is shown as orange balls and an orange cloud.

partitioning module plays a central role in segregating origins toward the cell poles and is the closest analog to a eukaryotic-like mitotic apparatus [39]. The ParB protein binds site-specifically to origin-proximal *parS* sites generating large nucleoprotein complexes. These centromeric complexes are acted upon by the ParA ATPase driving their segregation to the nucleoid periphery. Thus, this system functions to establish and in some cases maintain the longitudinal *ori-ter* organization pattern.

Our understanding of the molecular mechanism underlying ParA-mediated origin segregation has been greatly informed by work on related partitioning systems found on plasmids [40,41]. Analysis of chromosomally encoded Par systems supports and extends this work and indicates that the principles underlying plasmid partitioning and origin segregation are similar. Briefly, ParA(ATP) binds DNA non-specifically throughout the nucleoid. The ParB/*parS* complex interacts with ParA(ATP) bound to the chromosome and triggers ATP hydrolysis and release of ParA(ADP) from the nucleoid. The ParB/*parS* complex then binds to a nearby chromosome-bound ParA(ATP). Repeated cycles of interaction, hydrolysis, and release have been hypothesized to result in movement of the ParB/*parS* complex toward the cell pole leaving a ParA-free nucleoid in its wake. Recent work on the ParABS system in *C. crescentus* suggests that the translocation force in this system is derived from the elastic property of the chromosome [42••]. In this model, ParA(ATP) bound non-specifically to the chromosome function as transient tethers that link the ParB/*parS* complex to the nucleoid enabling the intrinsic spring-like properties of the chromosome to relay the complex from one ParA(ATP) to the next.

Interestingly, although these systems are thought to act similarly in all bacteria, their contribution to origin segregation varies dramatically [18,23•,24•,29•,43]. At one extreme, exemplified by *C. crescentus*, the ParABS system is absolutely critical for segregating origins from one pole

to the other. Induction of a dominant negative allele of ParA was shown to dramatically block origin segregation [18]. At the other extreme, *B. subtilis* mutants lacking ParA (also called Soj) faithfully segregate their chromosomes as assayed by the production of anucleate cells [43]. However, visualization of origin dynamics by time-lapse microscopy in the absence of ParA (Soj) indicates that the partition system facilitates the directed movement of the origins to the nucleoid periphery and helps to establish and maintain the *ori-ter* pattern of the newly replicated DNA [12•]. Furthermore, sporulating cells lacking ParA are impaired in positioning their origins at the extreme cell pole and ~20% of these cells fail to trap their origin in the pre-spore compartment [9]. Thus, even in the situation in which ParA plays a more minor role in chromosome segregation, it contributes to the *ori-ter* longitudinal pattern.

Polar anchors maintain the *ori-ter* pattern

The conserved ParABS module segregates bacterial origins toward the cell pole where, in a number of cases, polar complexes then anchor them in place. Interestingly, three distinct polar anchoring complexes have been identified suggesting that bacteria have evolved independent systems to maintain their longitudinal organization patterns.

DivIVA

In the case of sporulating *B. subtilis* cells, the *ori-ter ter-ori* pattern within the axial filament is locked in place by a DNA binding protein called RacA and an oligomeric protein complex that resides at the cell poles [19,22] (Figure 2bi). RacA is induced upon entry into the sporulation pathway and binds site-specifically to 25 origin-proximal (*ram*) sites [44]. These nucleoprotein complexes are in turn tethered to the pole by DivIVA, a small peripheral membrane protein that self-associates into a meshwork at the cell poles [45–47]. Evidence suggests that negative membrane curvature as is found at the poles provides a geometric cue for DivIVA localization and accumulation [48,49]. Sporulating cells lacking DivIVA or RacA fail to anchor their origins at the cell poles resulting in pre-spore compartments lacking DNA [19,22]. Furthermore, biochemical experiments suggest that DivIVA can recruit RacA to the membrane [48]. Thus, the ParABS partitioning system helps segregate the origins toward the poles where they are anchored through an interaction between RacA/*ram* and the DivIVA meshwork.

Interestingly, pole-associated DivIVA assemblies have also been implicated in directly anchoring ParB/*parS* complexes in actinobacteria [50]. Heterologous expression of DivIVA and ParB homologs in *E. coli* that lacks these proteins revealed that DivIVA from *C. glutamicum* and *M. tuberculosis* (but not *B. subtilis*) can recruit their cognate ParBs to the cell pole. DivIVA has also been found to play a key role in directing polar cell wall synthesis in these bacteria. Intriguingly, ParB mutants have defects in apical growth in *C. glutamicum* suggesting an intimate link between chromosome organization and polar growth in these bacteria [28].

PopZ

In *C. crescentus*, two pole-associated factors (PopZ and TipN) participate in establishing and maintaining the longitudinal organization pattern (Figure 2bii). PopZ is a small protein that forms a self-organizing network at the cell poles [20,21]. Like DivIVA in actinobacteria, PopZ is a key pole organizing protein. In addition to its role in localizing morphogenetic and cell cycle regulatory proteins, PopZ has been shown to directly interact with ParB. Thus, PopZ, like DivIVA 'locks in' the *ori-ter* organization pattern. TipN is a large membrane-anchored protein that localizes to the new cell pole where it organizes flagellar synthesis [51,52]. Analysis of origin dynamics in a TipN mutant indicates that this polar protein also facilitates ParA-mediated origin segregation [16,53]. In cells lacking TipN, progressive and directed origin movement from the old to the new pole is impaired. Interestingly, recent work on PopZ indicates that, like TipN, it also plays an important role in ParA-mediated origin segregation [54•]. PopZ interacts with ParA and mutants in PopZ that abrogate interaction with ParA but only partially impair interaction with ParB have severe defects in origin segregation. Thus, PopZ and TipN appear to act synergistically to help establish the *ori-ter* longitudinal organization. One idea is that these factors help re-generate ParA(ATP) at the new cell pole and thus generate and sharpen the ParA gradient during segregation. Alternatively, they could sequester ParA at the pole after it has been released from the nucleoid by ParB/*parS* to prevent ParA re-binding behind the segregating origin [16,54•].

HubP

Finally, in the case of *V. cholerae*, a large membrane protein called HubP, which bears no resemblance to DivIVA or PopZ was recently shown to serve as the polar anchor for the origin of chr I [55••] (Figure 2biii). HubP interacts with ParA1 and two other chromosomally encoded ParA homologs (ParC and FlhG). ParC participates in localizing chemotactic apparatus while FlhG is involved in flagellar assembly. Each ParA homolog appears to interact with distinct regions of HubP. Thus, HubP, like PopZ and DivIVA is a multifaceted pole-organizing protein. The mechanism by which HubP anchors the origin at the cell pole is still unclear. The current model is a static one in which ParA serves as a bridge between the ParB/*parS* complex and HubP. An alternative possibility is that HubP serves to regenerate ParA activity as has been suggested for PopZ and TipN. In this capacity, HubP would help maintain the longitudinal organization by continuously and dynamically recruiting the origin back to the cell pole.

In conclusion, two chromosome organization patterns, *ori-ter* and left-*ori*-right, serve as the ground states and building blocks for the diverse patterns observed in bacteria. The partitioning system and the condensin complex are the major drivers in setting up these patterns. Interestingly, a robust Par system appears to relax the need for the condensin complex and vice versa. Finally, diverse polar complexes not only function to lock in the *ori-ter* pattern but also promote ParA-mediated origin segregation. The goal for the future is to further define the interplay among these activities and the physical properties of the nucleoid in generating distinct organization patterns.

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