Cell cycle regulation in *Caulobacter*: location, location

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Summary

Cellular reproduction in all organisms requires temporal and spatial coordination of crucial events, notably DNA replication, chromosome segregation and cytokinesis. Recent studies on the dimorphic bacterium *Caulobacter crescentus* (*Caulobacter*) highlight mechanisms by which positional information is integrated with temporal modes of cell cycle regulation. *Caulobacter* cell division is inherently asymmetric, yielding progeny with different fates: stalked cells and swarmer cells. Cell type determinants in stalked progeny promote entry into S phase, whereas swarmer progeny remain in G1 phase. Moreover, initiation of DNA replication is allowed only once per cell cycle. This finite window of opportunity is imposed by coordinating spatially constrained proteolysis of CtrA, an inhibitor of DNA replication initiation, with forward progression of the cell cycle. Positional cues are equally important in coordinating movement of the chromosome with cell division site selection in *Caulobacter*. The chromosome is specifically and dynamically localized over the course of the cell cycle. As the duplicated chromosomes are partitioned, factors that restrict assembly of the cell division protein FtsZ associate with a chromosomal locus near the origin, ensuring that the division site is located towards the middle of the cell.

Key words: *Caulobacter*, CtrA, MipZ, Cell cycle, FtsZ

Introduction

The fundamental cell cycle events of a prokaryotic cell are the same as those of a eukaryotic cell: the genome is replicated, duplicated DNA is segregated into daughter compartments and cytokinesis separates the cell into two, all of which are coordinated with cell growth. Accurate execution of each of these steps relative to the others in time and space is crucial for the survival of progeny, necessitating the existence of strict regulatory mechanisms that ensure their fidelity. In eukaryotes, the dramatic metaphase alignment of chromosomes and morphological changes accompanying cytokinesis convinced cell biologists early on that sophisticated programs of spatial regulation must be integrated with those that control the timing of cell cycle events. Analogous processes occur in prokaryotes; yet bacterial cells were long regarded as little more than living test tubes that could probably get by with timing mechanisms and relatively simple self-organizing capabilities. Classic research into cell cycle control in bacteria was focused, therefore, on defining the enzymology and genetic and chemical signals that promote DNA replication, chromosome partitioning and cytokinesis.

The past decade or so has seen a renaissance in bacterial cell biology, with the recognition that the interior of the bacterial cell is highly organized: it is replete with specifically and dynamically localized proteins, DNA and other biomolecules, and possesses a surprisingly diverse cytoskeleton (Ebersbach and Jacobs-Wagner, 2007; Gitai, 2005; Lewis, 2004; Moller-Jensen and Lowe, 2005). This paradigm shift has been spurred on by advanced labeling and imaging technologies that make even the smallest bacterial cells amenable to cell biological probing. Within this context, we have begun to appreciate the importance of the physical choreography of molecules during the bacterial cell cycle. In this Commentary, we discuss cell cycle control in the context of the three-dimensional organization of the bacterium *Caulobacter crescentus* (referred to hereafter as *Caulobacter*), focusing on recent advances in our understanding of the elegant spatial mechanisms that govern the initiation of DNA replication and cell division site selection.

The *Caulobacter* cell cycle

*Caulobacter* is a Gram-negative, aquatic α-proteobacterium that has emerged as the pre-eminent model for analysis of prokaryotic cell cycle regulation. It is easily cultured and manipulated genetically in the laboratory, and has the advantage that one can easily synchronize cells without perturbing their normal physiology: a simple density centrifugation procedure yields a relatively pure population of cells in G1 phase, which progress coordinately through the cell cycle (Evinger and Agabian, 1977). This allows precise monitoring of all aspects of *Caulobacter* cell cycle progression. Additionally, morphological changes that occur over the course of the *Caulobacter* life cycle and are intimately coupled to other cell cycle events serve as faithful visual identifiers of the cell cycle status of any given cell.

*Caulobacter* undergo an asymmetric division each cell cycle to produce morphologically distinct progeny that have different fates: a motile, flagellated swarmer cell and a non-motile stalked cell (Fig. 1A). The swarmer cell is unable to enter S phase until it differentiates into a stalked cell, releasing its flagellum and building a stalk where the flagellum previously resided. Upon this swarmer-to-stalked cell transition...
(analogous to the G1-S transition in eukaryotes), the cell becomes competent to initiate DNA replication, an event that occurs exactly once per cell cycle. Replication initiates at a single origin and proceeds bidirectionally (Dingwall and Shapiro, 1989; Marczynski and Shapiro, 1992), with chromosomal loci being segregated to daughter compartments soon after they are duplicated (Viollier et al., 2004). Concurrently with DNA replication and segregation, the stalked cell elongates and, just as replication is completed, begins to constrict at the incipient cell division site (Jensen, 2006). A flagellum is assembled at the pole opposite the stalk in the predivisional cell, and the completion of cytokinesis yields a swarmer daughter and a stalked daughter. In addition to the differences in polar morphology between swarmer and stalked cells, they also differ in size, with swarmer cells being smaller than stalked cells. The stalked daughter immediately begins another round of DNA replication and cell division, whereas the swarmer undergoes an obligate period of growth and differentiation before beginning the cycle anew.

The temporal coordination of the events described above is mediated in large part by regulated transcription of components that are crucial for the execution of each step. Transcript levels of at least 550 genes (~20% of the annotated open reading frames in the Caulobacter genome) vary over the course of the cell cycle, including those encoding factors required for the initiation of DNA replication, for chromosome segregation, for cytokinesis and for biogenesis of the stalk, flagellum and pili (Laub et al., 2000). Notably, many genes are upregulated precisely in time to perform their prescribed functions. At the core of this cascade are three transcriptional regulators whose levels oscillate over the course of the cell cycle: CtrA, DnaA and GcrA (Fig. 1B). These master regulators peak in abundance out of phase with each other and function successively to promote distinct events, together regulating the transcription of over 200 genes (Holtzendorff et al., 2004; Hottes et al., 2005; Laub et al., 2002). Although the transcriptional control of key elements lays the foundation for the precise timing of cell cycle events, coordinating the physical reorganization of the cell as it progresses through the cell cycle requires spatial determinants and the means to communicate positional information. For example, mechanisms must physically segregate the replicated chromosomes and then signal to the cytokinetic machinery that the DNA has been cleared from the nascent division site. Moreover, the asymmetric nature of Caulobacter division implies the existence of factors that distinguish one predivisional compartment from the other to generate daughter cells that are morphologically and functionally distinct. In the following sections, we describe two examples of how the dynamic localization of proteins and DNA is incorporated into the cell cycle regulatory machinery to ensure accurate, asymmetric cell division in Caulobacter.

**DNA replication initiation: only once and only in stalked cells**

The initiation of DNA replication, or entry into S phase, is a key target of cell cycle regulation in many organisms. In most bacteria, control over replication initiation is principly the responsibility of the conserved AAA+ protein DnaA, which binds to sites proximal to the origin and facilitates the local melting of duplex DNA. This permits the loading of components of the replication machinery, which then duplicates the genome (Mott and Berger, 2007). Unlike bacteria such as Escherichia coli that can undergo numerous rounds of DNA replication per cell division, Caulobacter initiates replication only once per cell cycle (Marczynski, 1999). Replication is cell-type specific, being inhibited in swarmer cells and allowed in stalked cells. The levels of DnaA vary over the Caulobacter cell cycle owing to regulated synthesis and proteolysis, and peak at the swarmer-to-stalked cell transition (Fig. 1B) (Collier et al., 2006; Gorbatyuk and Marczynski, 2005). However, DnaA is detectable at points in the cell cycle when replication initiation is never observed. How, then, is the decision to initiate DNA replication unfailingly made within a finite window in the cell cycle? The answer, in part, lies with a repressor of DNA replication initiation whose spatially regulated deactivation, combined
with oscillating levels of DnaA, results in the pattern of DNA synthesis that we observe. This repressor is the dual-function master regulator CtrA.

CtrA is an essential response regulator that controls the transcription of genes involved in cell division and in other important events (Kelly et al., 1998; Laub et al., 2002; Quon et al., 1996; Reisenauer et al., 1999; Skerker and Shapiro, 2000). It can act as a transcription factor, but its active (phosphorylated) form (CtrA-P) also binds directly to five sites within the origin of replication, thereby silencing initiation of replication (Quon et al., 1998). CtrA-P can be deactivated to relieve this replication block by two redundant mechanisms: dephosphorylation and proteolysis (Doming et al., 1997; Hung and Shapiro, 2002; Iniesta et al., 2006; Jenal and Fuchs, 1998; McGrath et al., 2006; Ryan et al., 2004; Ryan et al., 2002). It is unclear whether CtrA-P is actively dephosphorylated by a dedicated phosphatase, but dephosphorylation occurs at the swarmerto-stalked cell transition (Domian et al., 1997). The second mode of deactivation, targeted proteolysis of CtrA, occurs regardless of its phosphorylation state (Ryan et al., 2002).

CtrA is degraded at the swarmerto-stalked cell transition and in the stalked compartment of the predivisional cell just after compartmentalization (Domian et al., 1997; Judd et al., 2003). The essential ATP-dependent protease ClpXP is required for CtrA proteolysis in vivo (Jenal and Fuchs, 1998), and Chien et al. recently showed that ClpXP can degrade CtrA directly in vitro without a requirement for any other protein cofactor (Chien et al., 2007). In the cell, however, the levels of ClpXP remain constant throughout the cell cycle, indicating that the mere presence of protease and substrate is not sufficient for degradation in vivo (Domian et al., 1997; Jenal and Fuchs, 1998). Instead, the proteolysis of CtrA requires a specific spatial arrangement of the substrate and its regulators within the cell (Fig. 2A,B) (Iniesta et al., 2006; McGrath et al., 2006; Ryan et al., 2004; Ryan et al., 2002).

Fluorescence microscopy has revealed that CtrA is located at the future stalked pole during the swarmerto-stalked cell transition and at the stalked pole of the stalked compartment in predivisional cells immediately prior to CtrA degradation (Ryan et al., 2004; Ryan et al., 2002). Interestingly, the ClpXP protease localizes to the stalked pole at the same time as its CtrA substrate (McGrath et al., 2006). Although the presence of ClpXP at the pole is required for CtrA degradation, it is not sufficient. In the absence of an additional degradation factor called RcdA, CtrA is not localized to the pole and cannot be degraded. RcdA does not appreciably affect the proteolytic activity of ClpXP towards CtrA in vitro, however (Chien et al., 2007), which indicates that RcdA is necessary in vivo because it targets CtrA to the subcellular site of ClpXP function. RcdA transiently localizes at the cell pole together with CtrA and ClpXP to form a protein complex, and RcdA localization depends on ClpXP (Fig. 2B) (McGrath et al., 2006). Conversely, in the absence of RcdA, ClpXP is still able to localize to the pole and degrade its other substrates, such as the chemoreceptor McpA. This mechanism thereby allows cells to regulate the activity of ClpXP with regard to only one, or a limited subset, of its substrates.

Spatially constrained CtrA degradation is integrated with the timing of the cell cycle by multiple mechanisms. First, rcdA transcript levels vary in time over the course of the cell cycle. Microarray and chromatin immunoprecipitation experiments suggest that this cell cycle variance stems, at least in part, from direct positive regulation of rcdA transcription by CtrA-P (Laub et al., 2002; McGrath et al., 2007). This constitutes a negative-feedback mechanism in which CtrA-P causes the accumulation of RcdA, which then targets CtrA-P for degradation. Second, polar targeting of ClpXP is controlled by the single-domain response regulator CpdR (Fig. 2A). The unphosphorylated version of CpdR localizes to the stalked pole and enables the localization and activity of ClpXP. In the absence of CpdR or in the presence of phosphorylated CpdR (CpdR-P), the CpdR-RcdA-CtrA complex does not localize to the pole and CtrA is not degraded (Iniesta et al., 2006). Thus, the localization of the components required for inactivation of CtrA-P by proteolysis depends on the phosphorylation state of CpdR, which changes at specified points throughout the cell cycle. CpdR is in its phosphorylated state in the swarmerto-stalked cell and is dephosphorylated and activated at the swarmerto-stalked cell transition (Fig. 2A). Later, CpdR is phosphorylated and inactivated by the same phospho-signaling cascade that activates CtrA in predivisional cells (Biondi et al., 2006; Iniesta et al., 2006). Together, these integrated spatial and temporal mechanisms leave a small window of opportunity in each cell cycle to initiate DNA replication, in which little CtrA-P exists in the cell and DnaA levels are high: this occurs only in new stalked cells, either
after the swarmer-to-stalked cell transition or in stalked progeny upon cytokinesis.

**Communication between the chromosome and the cell division machinery**

The decision to enter S phase sets in motion the program of events that leads to chromosome segregation and cell division. As in all organisms, the physical coordination of these two processes in *Caulobacter* is required to guarantee that each daughter cell contains a full complement of the genome. If the chromosomes are not completely segregated or the division plane is specified incorrectly, the cell runs the risk of unequally partitioning daughter chromosomes or of cleaving its DNA. Moreover, the future division site in *Caulobacter* serves as a crucial landmark for proteins involved in polarity determination and cell growth (Aaron et al., 2007; Huitema et al., 2006; Lam et al., 2006). Below, we discuss the arrangement and movement of the *Caulobacter* chromosome throughout the cell cycle, and focus on a mechanism of communication between the daughter chromosomes and the division plane prior to cytokinesis.

**Chromosome organization and movement**

In *Caulobacter*, as in most bacterial species that have been examined, the chromosome is spatially well-organized (Fig. 3A) (Thanbichler and Shapiro, 2006a). In newborn cells, the chromosomal origin of replication is localized at the old pole (the pole bearing the stalk or flagellum) and the terminus resides near the new pole (the pole formed at the latest cell division) (Jensen and Shapiro, 1999). By examining the positions of 112 distinct chromosomal loci in living *Caulobacter* cells over the course of the cell cycle, Viollier and colleagues (Viollier et al., 2004) demonstrated that, remarkably, each chromosomal locus tested between the origin and terminus occupies an invariant subcellular address, and the loci are arrayed linearly along the long axis of the cell. Similar trends are observed in other bacteria, which suggests that precise physical arrangement of the chromosome is a common feature of prokaryotic cells (Niki et al., 2000; Telemman et al., 1998; Wang, X. et al., 2006). Almost immediately after duplication of the origin, one copy moves rapidly to the pole opposite the stalk (Gitai et al., 2005; Jensen, 2006; Jensen and Shapiro, 1999; Viollier et al., 2004). Segregation of other chromosomal loci to their destinations in the opposite half of the predivisional cell follows sequentially soon after they are replicated (Viollier et al., 2004).

The detailed mechanisms by which the chromosome is partitioned into daughter compartments are unknown, but it seems to be a multi-step process: rapid origin segregation, systematic movement of the bulk of the chromosome and final separation of terminus regions (Ghosh et al., 2006; Thanbichler and Shapiro, 2006a). In *Caulobacter* and other bacteria, bacterial actin, MreB, has been implicated in chromosome segregation (Gitai et al., 2005; Kruse et al., 2006; Kruse et al., 2003; Soufo and Graumann, 2003), in addition to playing its well-characterized role in cell shape determination (Carballido-Lopez, 2006). Using A22, a small-molecule inhibitor of MreB, Gitai et al. established that MreB is required for segregation of the origin of replication in slowly growing *Caulobacter* cells (Gitai et al., 2005). Moreover, chromatin immunoprecipitation experiments revealed a physical link between MreB and origin-proximal regions of the chromosome (Gitai et al., 2005). These results tempted researchers to speculate that an MreB-based force-generating mechanism that acts on a centromere-like element close to the origin might be at work. Subsequent single-molecule studies of MreB in *Caulobacter* demonstrated that individual filaments are short and are not uniformly oriented in polarity, making it difficult to imagine how they might dictate long-range directed movement within the cell (Kim et al., 2006). Additionally, not all bacteria possess MreB orthologs and, among those that do, not all require it for origin segregation (Formstone and

**Fig. 3.** Chromosome organization and division site specification. (A) Physical arrangement of the ~4 megabase pair (Mbp) *Caulobacter* chromosome over the course of the cell cycle. Regions on the circular chromosome are indicated by color: the origin is shown in yellow, the terminus is shown in red and loci between the two are colored in a gradient according to their position. In swarmer cells, the origin resides near the flagellar/old pole and the terminus resides near the new pole. After initiation of replication, one copy of the origin is segregated to the new pole, and other loci are partitioned as they are replicated. Just before cytokinesis, the terminus regions are separated in the final steps of chromosome segregation.

(B) Localization of ParB, MipZ and FtsZ during the cell cycle. ParB associates with the *parS* sequence adjacent to the origin (see A); MipZ binds to ParB, and both move with one copy of *parS* to the new pole upon origin segregation. The FtsZ-inhibitory activity of MipZ forces FtsZ from the new pole to the midcell, where it polymerizes into the Z ring that marks the cell division site.
Errington, 2005; Hu et al., 2007; Karczmarek et al., 2007). This indicates that MreB is not a universal mediator of prokaryotic chromosome segregation. Nevertheless, MreB remains an attractive candidate to participate in the initial stage of chromosome partitioning in Caulobacter.

Little is known about bulk movement of the chromosome after origin segregation. A number of DNA-modifying enzymes and binding proteins that localize dynamically and might play a role in chromosome positioning have been identified recently in Caulobacter. These include the replisome itself (Jensen et al., 2001); the topoisomerase IV ParC subunit, which is involved in decatenation of daughter chromosomes (Wang and Shapiro, 2004); the DNA translocase FtsK, which mediates the terminal steps of segregation at the division plane (Wang, S. et al., 2006); the structural maintenance of chromosomes (SMC) protein (Jensen and Shapiro, 1999; Jensen and Shapiro, 2003); and the partitioning proteins ParA and ParB (Mohl and Gober, 1997). Each of these factors is important for proper positioning of the origin and/or other regions of the chromosome, but further work is necessary to determine whether their effects are direct. Studies in other bacteria also provide compelling evidence for a role for DNA polymerase activity in chromosome movement (Dworkin and Losick, 2002; Kruse et al., 2006), but this possibility has not yet been addressed in Caulobacter.

MipZ mediates communication between the chromosome and FtsZ

Regardless of the mechanism, movement of the replication origin in Caulobacter is vitally important to division site specification. In bacteria, the future division site is determined by localized polymerization of the tubulin relative FtsZ into a structure called the Z ring. The Z ring acts as a scaffold upon which the divisome (the cell division machinery) is built, and is hypothesized to generate constrictive forces that are required to divide the cell (Margolin, 2005). In Caulobacter, a number of essential divisome components are transcriptionally and proteolytically regulated in time, including FtsZ, FtsA and FtsQ (Kelly et al., 1998; Laub et al., 2000; Martin et al., 2004; Quardokus et al., 1996; Sackett et al., 1998; Wortinger et al., 2000). This does not, however, tell us how the Z ring is assembled at the right place in the cell (Quardokus et al., 2001). In all cases so far described, placement of the Z ring is dictated by factors that inhibit FtsZ polymerization everywhere in the cell except where division should occur. In E. coli and Bacillus subtilis, two complementary systems ensure that the division machinery assembles in the middle of the cell in an area free of DNA: the Min system inhibits polar Z ring assembly, and nucleoid occlusion factors inhibit Z ring assembly over the bulk of the chromosome (Barak and Wilkinson, 2007). Caulobacter does not possess orthologs of either the Min proteins or of the nucleoid occlusion proteins, however. Instead, it imposes a mode of regulation that integrates spatial information from both the cell poles and the chromosome via a factor called MipZ (Fig. 3B).

MipZ is an essential ATPase of the ParA superfamily and was identified in a bioinformatic screen for Caulobacter genes that are cell cycle regulated and conserved in α-proteobacteria (Thanbichler and Shapiro, 2006b). The first indication that MipZ might be involved in cell division came from overexpression and depletion experiments: when either too much or too little MipZ is present, cells become filamentous, indicating a block in cytokinesis. Moreover, as opposed to the single Z ring observed in predivisional cells of wild-type Caulobacter (Din et al., 1998; Thanbichler and Shapiro, 2006b), polar FtsZ foci form when MipZ is overproduced, and multiple aggregates of FtsZ appear throughout the cell upon MipZ depletion. These in vivo results indicate that MipZ might be a regulator of FtsZ polymerization. This hypothesis was validated in vitro, where it was shown that MipZ directly interacts with FtsZ, stimulating the GTPase activity of FtsZ and inhibiting polymerization of this protein (Thanbichler and Shapiro, 2006b).

The FtsZ-inhibitory activity of MipZ is spatially restricted in the cell such that it is lowest near the midcell prior to cell division. This localization is achieved by direct physical interaction of MipZ with ParB. ParB was previously suggested to mediate crosstalk between the chromosome and the cell division machinery, because it is required for both cytokinesis and proper nucleoid partitioning (Mohl et al., 2001; Mohl and Gober, 1997). When followed over the course of the cell cycle, MipZ and ParB each begin as a single focus at the old pole. In both cases, this focus then splits into two. Remarkably, one of these foci then accompanies the origin of replication as it is segregated to the opposite pole (Figge et al., 2003; Mohl and Gober, 1997; Thanbichler and Shapiro, 2006b). ParB is localized by virtue of its ability to bind directly to a DNA sequence motif, parS, that is adjacent to the origin of replication on the chromosome (Mohl and Gober, 1997). In vivo, ParB is required to localize MipZ near the origin and, in vitro, MipZ binds directly to a ParB-parS complex. Interestingly, whereas ParB forms a tight focus at each pole after origin segregation in predivisional cells, MipZ forms a looser focus at each pole that gradually fades towards the middle of the cell (Thanbichler and Shapiro, 2006b). This yields a gradient of FtsZ-inhibitory activity that targets FtsZ polymerization and division site specification to the midcell. Importantly, the subcellular positioning of the origin is crucial for MipZ localization, thereby intimately linking replication and segregation of the chromosome to defining the division site. Because the segregated origins localize to opposite poles of the predivisional cell, this mechanism beautifully explains how FtsZ in Caulobacter senses the positioning both of the duplicated chromosomes and of the cell poles.

One interesting aspect of Caulobacter cell division that is not explained by MipZ is the size difference between swarmer and stalked daughters. The MipZ gradient does not show a positional bias towards one pole or the other (Thanbichler and Shapiro, 2006b). This suggests that asymmetric growth of incipient swarmer and stalked cells is decided after initial assembly of the Z ring, perhaps after cytoplasmic compartmentalization yields biochemically distinct daughter compartments (Judd et al., 2005; Judd et al., 2003). Intriguingly, TipN, a protein involved in defining cell polarity in Caulobacter (Huitema et al., 2006; Lam et al., 2006), has been implicated in the pathway leading to daughter size asymmetry (Lam et al., 2006). TipN localizes to the cell division site in predivisional cells (Huitema et al., 2006; Lam et al., 2006), and deletion of tipN results in a reversed bias in cell size: stalked cells are frequently smaller than swarmer cells upon division (Lam et al., 2006). The molecular mechanism
for this reversal is unknown, but these studies suggest that post-
specific information is relayed to the division plane to dictate differences in growth of the new daughter compartments.

Conclusions and Perspectives

Advances in microscopy over the past two decades, particularly the advent of genetically encodable fluorescent proteins, have led bacterial cell biologists to rethink paradigms of how prokaryotic cells function. It is now clear that Caulobacter, and probably all bacteria, have evolved precisely tuned mechanisms to control spatial aspects of their physiology. In the examples of CtrA and MipZ discussed above, we have described how the spatial communication between these factors and other cell cycle regulators is central to accurate cell cycle progression. Clearly, there are numerous physical properties of the cell cycle that remain beyond our understanding – for example, the elusive mechanism of chromosome segregation. Careful experiments, both in vivo and in vitro, will be required, as they are in eukaryotes, for us to develop a firm grasp of how this task is accomplished. Now that we are convinced that bacteria heavily rely on their precise spatial organization for survival, we can enthusiastically pursue avenues of research directly aimed at dissecting it. Towards this end, researchers are beginning to explore the bacterial cell with high-resolution-microscopy methods that enable visualization of subcellular structures in exquisite detail. In particular, the application of cryo-electron tomography to bacterial cells has recently illuminated structures such as the cytoskeleton-associated inner membrane invaginations in magnetotactic bacteria (Komelii et al., 2006) and the attachment organelle of Mycoplasma pneumoniae (Henderson and Jensen, 2006). Caulobacter is also amenable to investigation by cryo-electron tomography, and this technology has been used to follow the progression of its division (Judd et al., 2005) and to discover numerous, unidentified cytoskeletal filaments (Briegel et al., 2006). Continued application and development of high-resolution-microscopy methods, particularly the tricky task of specifically labeling proteins and other biomolecules in combination with tomographic technologies, are required if we are to develop and validate mechanistic explanations for the physical phenomena driving bacterial cell cycle progression.

Finally, we cannot dismiss the fact that genomic studies have been instrumental in the development of models of global cell cycle regulation, the obvious example being the cell cycle-dependent oscillation of transcriptional regulators in Caulobacter. These and other kinds of systems-level analysis continue to contribute massive amounts of information to be digested by bacterial cell biologists. The challenge facing us now is to incorporate these rapidly accumulating data sets with what we learn about the physical arrangement of molecules within the bacterium to come up with a detailed, comprehensive picture of how a prokaryotic cell cycle operates in three dimensions.

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