Caulobacter requires a dedicated mechanism to initiate chromosome segregation

Esteban Toro*, Sun-Hae Hong*†, Harley H. McAdams*, and Lucy Shapiro*‡

*Department of Developmental Biology, Stanford University School of Medicine, Beckman Center, B300, Stanford, CA 94305; and †Department of Physics, Stanford University School of Humanities and Sciences, Stanford, CA 94305

Contributed by Lucy Shapiro, July 31, 2008 (sent for review July 22, 2008)

Chromosome segregation in bacteria is rapid and directed, but the mechanisms responsible for this movement are still unclear. We show that *Caulobacter crescentus* makes use of and requires a dedicated mechanism to initiate chromosome segregation. *Caulobacter* has a single circular chromosome whose origin of replication is positioned at one cell pole. Upon initiation of replication, an 8-kb region of the chromosome containing both the origin and *parS* moves rapidly to the opposite pole. This movement requires the highly conserved *ParABS* locus that is essential in *Caulobacter.* We use chromosomal inversions and *in vivo* time-lapse imaging to show that *parS* is the *Caulobacter* site of force exertion, independent of its position in the chromosome. When *parS* is moved farther from the origin, the cell waits for *parS* to be replicated before segregation can begin. Also, a mutation in the ATPase domain of ParA halts segregation without affecting replication initiation. Chromosome segregation in *Caulobacter* cannot occur unless a dedicated *parS* guiding mechanism initiates movement.

centromere | parS | ParA

Bacterial chromosomes are highly organized structures with predictable orientation and segregation patterns (1, 2). *In vivo* fluorescence microscopy showed that the speed of segregation of individual loci is too fast to be accounted for by attachment of sister chromosomes to a growing cell envelope (1, 3, 4), as had been proposed (5). These observations led to the suggestion that rapid segregation may be the consequence of nondedicated mechanisms, such as force exerted by the DNA or RNA polymerases (6, 7) or entropic exclusion of sister chromosomes (8), all of which predict that the order of segregation will follow the order of replication. Alternatively, segregation may be driven by a dedicated mechanism acting on a centromeric sequence (9, 10), in which case the first sequence to segregate would be the centromere regardless of when it is replicated.

The *parABS* locus is a large family of plasmid and chromosomal elements composed of a *cis*-acting sequence generally named *parS* and two transacting proteins: ParB, which binds to cognate *parS* sites, and ParA, a MinD-related Walker-type ATPase whose plasmid homologues polymerize *in vitro* and *in vivo* (11, 12). Although chromosomal *parABS* (*chr-parABS*) elements are phylogenetically distinct from those found in plasmids (13), inactivating or overexpressing *chr-parABS* components in several species leads to elevated numbers of anucleate cells (14–16) and introduction of *chr-parABS* stabilizes plasmids in heterologous hosts (17–19). *In vivo* observations of *Vibrio cholerae's* chromosome I dynamics suggested a mechanism by which ParAI (chromosome I's cognate ParA) pulls on the ParBI/*parSI* complex to effect chromosome segregation (20). However, although the absence of *parAI* in *V. cholerae* alters chromosome segregation, growth is not affected (19, 20). Indeed, despite widespread conservation of the *parS* sequence, except for *V. cholerae* chromosome II and *Caulobacter crescentus* (henceforth, *Caulobacter*), the absence of *parABS* elements only mildly impairs growth (18, 21), suggesting the presence of redundant chromosome segregation mechanisms (8, 22–24).

Caulobacter requires an active *parABS* system to live (21) and replicates its single chromosome only once per cell cycle (25), providing a good model organism to study chromosome segregation in its simplest form. Here, we show that the ancestral *parS* sequence is the site of force exertion during the initiation of *Caulobacter* chromosome segregation, and that ParA activity is required for this movement. We also find that in the absence of *parS*-directed movement, segregation of newly replicated loci does not begin. Finally, we demonstrate that *parS* is specifically targeted to the cell pole, and that the subcellular location of two other DNA loci depends on their chromosomal distance from *parS*.

Results

Extra Copies of *parS* Impair Viability of *Caulobacter* Cells. We hypothesized that the site of force exertion for chromosome segregation should show copy-number effects when present in *trans*. If many indistinguishable copies of this site are present, such as when *Caulobacter* is transformed with plasmid-borne extra copies, the cells will segregate a random subset of plasmids and chromosomes to each daughter. This will result in some daughters with zero or two chromosomes, thus causing significantly slower growth. Accordingly, we screened for growth impairment in the presence of extrachromosomal fragments of DNA. We first screened a library of cosmids (each ≈30 kb at five to eight copies per chromosome equivalent) that were tiled across the origin region of the chromosome (Fig. 1). We chose this region because previous experiments had shown that it segregates before the rest of the chromosome (1) and, therefore, should contain the centromeric site. Three cosmids that shared a 6.5-kb region prevented normal growth of *Caulobacter* colonies (Fig. 1*A*). We narrowed this region further by inserting individual subregions into a promoterless plasmid (≈10 copies per chromosome equivalent) and found a 100-bp stretch of DNA that could not be maintained in *Caulobacter* (Fig. 1*B*). This sequence, which lies upstream of the *parAB* genes, contains two ParB-binding boxes (Fig. 1*C*; ref. 26), which comparative genomics analysis identified as the *Caulobacter parS* site (27). Furthermore, previous work had shown that one of these predicted binding motifs is bound directly by *Caulobacter* ParB *in vitro* (28).

To determine whether the ParB-binding sites in *parS* were responsible for the loss of viability seen in our screen (Fig. 1 *A* and *B*), we introduced four point mutations into each site (Fig. 1*C*). These mutations restored the ability of the plasmid to be maintained in cells (Fig. 1*D*), demonstrating that extrachromosomal copies of the ParB-binding boxes impair viability in *Caulobacter*.

Author contributions: E.T. and L.S. designed research; E.T. performed research; S.-H.H. contributed new reagents/analytic tools; E.T. analyzed data; and E.T., H.H.M., and L.S. wrote the paper.

The authors declare no conflict of interest.

‡To whom correspondence should be addressed. E-mail: shapiro@stanford.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0807448105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA

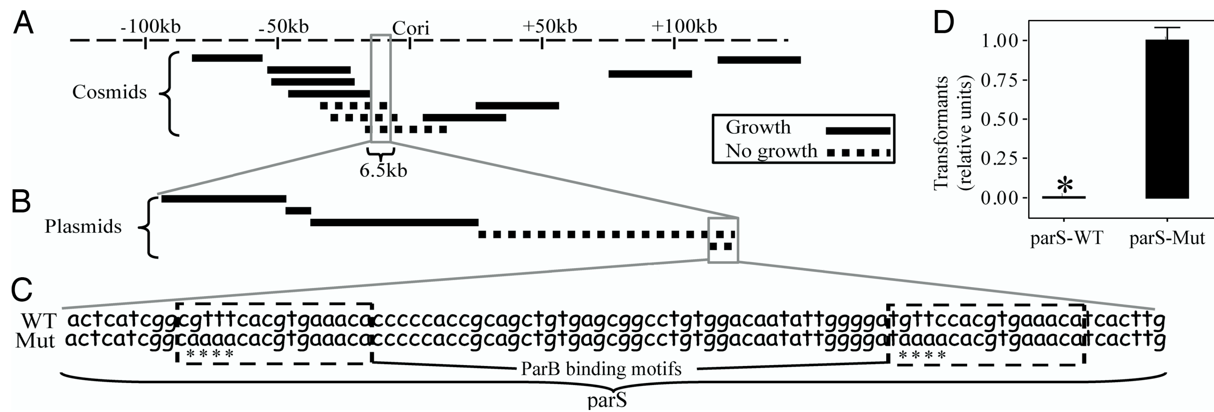

Fig. 1. Extra copies of *parS* DNA impair cell viability. (*A* and *B*) DNA-copy-number screen. Cosmids (*A*) or plasmids (*B*), carrying an antibiotic resistance cassette and a region (lines) of the *Caulobacter* genome, were transformed into cells. Solid lines represent constructs that permitted growth of colonies on selective plates; dashed lines represent constructs that did not allow growth. (*C*) Sequence of the wild-type *parS* site (WT) and *parS* with mutated ParB-binding motifs (Mut). Asterisks denote the location of base-pair changes in *parS*. (*D*) Transformation efficiency (relative number of colonies at day 3) of plasmids carrying either the wild-type (*parS*-WT) or mutated (*parS*-Mut) versions of *parS*. * = 0.003. Bars represent the means of seven separate experiments. Here and elsewhere, error bars represent standard error of the mean (SEM).

Identification of a Chromosomal Region That Contains *parS* as the Site of Force Exertion During Chromosome Segregation. Having established a copy-number effect for the *parS* sequence, we asked whether *parS* is the first part of the chromosome to segregate, as is the case in *V. cholerae* (20). As is common in bacteria, chromosome replication in *Caulobacter* begins at a single origin of replication (*Cori*) [refs. 29 and 30; supporting information (SI) Fig. S1]. We used time-lapse fluorescence microscopy to track the order of segregation of the *parS* region relative to *Cori* and other nearby loci *in vivo*. We followed the cellular position of *parS* using a MipZ-YFP fusion, which binds ParB/*parS* directly (28), under the control of the native *mipZ* promoter. Concurrently, we followed the cellular position of an array of *lacO* operators located at +4 kb (all distances are relative to *Cori*; *parS* is located at −8 kb) using a LacI-CFP fusion driven by a xylose-inducible promoter. As shown in Fig. 2*A* and Movie S1, in 33 of 33 cells examined, *parS* segregated ahead of the *lac* operators.

Next, we inserted a smaller DNA marker (the *lacO* arrays are ≈10 kb in length) between *parS* and *Cori* (Fig. 2*B*). The marker was the *parS* sequence from plasmid pMT1 (≈100 bp). CFP-pMT1Δ23ParB, which binds its cognate *parS* [denoted here as *parS(pMT1)*] was used to follow the cellular position of this locus (31). In agreement with previous reports (31), we did not observe any cross-talk between the *Caulobacter* and pMT1 *parS* systems (Fig. S2 *A* and *B*). Fig. 2*B* and Movie S2 show that in 35 of 35 cells observed, the *Caulobacter parS* sequence segregated ahead of *parS(pMT1)*, despite having been replicated later.

Nondedicated chromosome segregation models predict that the order of segregation follows from the order of replication. When replication begins, entropic exclusion and/or the DNA/RNA polymerases would immediately force the nascent daughter strands apart. It follows then that when two nearby sequences are observed segregating, the one located closest to *Cori* would tend to move first. Our results contradict this prediction (Fig. 2*B*) and show that initial segregation in *Caulobacter* is driven by force exerted on the chromosome at the *parS* site.

To refine the location of the site of force exertion, we constructed the strain shown in Fig. 2*C*, which carries the *lacO* arrays at −64 kb. In this strain, *parS* moved ahead of the *lacO* arrays in all 55 cells observed (Fig. 2*C*), showing that the site of force exertion must be located between −5 and −64 kb.

Separating *parS* from *Cori* Delays Segregation. As is commonly found in bacteria that have *parABS* (27), the *Caulobacter parS* sequence is located near *Cori* (8 kb away in the 4,000-kb genome). We asked whether *parS* would still be the first chromosomal site to segregate when it was moved farther from *Cori*. For this purpose, we used the phage PhiC31 site-specific recombinase (32) to create a strain carrying a chromosomal inversion that separates *parS* from *Cori* by ≈100 kb ("Inverted" in Figs. 2*D* and S3). We used this recombinase, because it has been shown to act unidirectionally (33), so that it will not reinvert the chromosome back to its wild-type configuration after it has catalyzed the inversion reaction (Fig. S3). We also created a similar inversion that excluded *parS*, to control for effects brought about by changes in the orientation of the inverted DNA ("Control Inverted" in Figs. 2*D* and S3).

We then followed the timing of *parS* segregation in synchronized populations of wild-type and the two inversion strains. Fig. 2*E* shows the percentage of cells which have begun segregation (measured as the appearance of two distinct MipZ-YFP foci) as a function of time from synchrony. We found that separating *parS* from *Cori* resulted in a delay in the timing of *parS* segregation (compare green squares and blue diamonds). Inverting a similar fragment of DNA lacking *parS*, in contrast, did not have a noticeable effect compared with wild type (yellow triangles and blue diamonds). The same behavior was observed when we followed the segregation of *lacO* arrays located at +4 kb (Fig. S4). These results show that the location of *parS* relative to *Cori* controls the timing of segregation. When *parS* is close to *Cori*, as in the WT and Control Inverted strains in Fig. 2 *D* and *E*, segregation happens quickly. When *parS* is located farther away, as in the Inverted strain, there is a delay before *parS* is replicated and segregation can begin. Furthermore, this result localized the site of force exertion to a 10-kb region that contains *parS* (−6 to −16 kb in Fig. 2*D*).

A ParAK20R Mutant Halts Segregation *in Vivo* and Induces the Production of Anucleate Minicells. Plasmid *parABS* segregation systems rely on their cognate ParA ATPase to accurately move or position the *parS*/ParB complex (34–36). In *Caulobacter*, overexpressing ParA and/or ParB results in mislocalization of ParB foci and the appearance of 5–10% anucleate cells (16). We therefore asked whether *Caulobacter* ParA is involved in the directed movement of *parS* during segregation. Accordingly, we created a merodiploid strain with both the wild-type *parA* gene at its native position and one of two xylose-inducible genes: wild-type *parA-mCherry* or *parAK20R-mCherry*. Lysine 20 is a

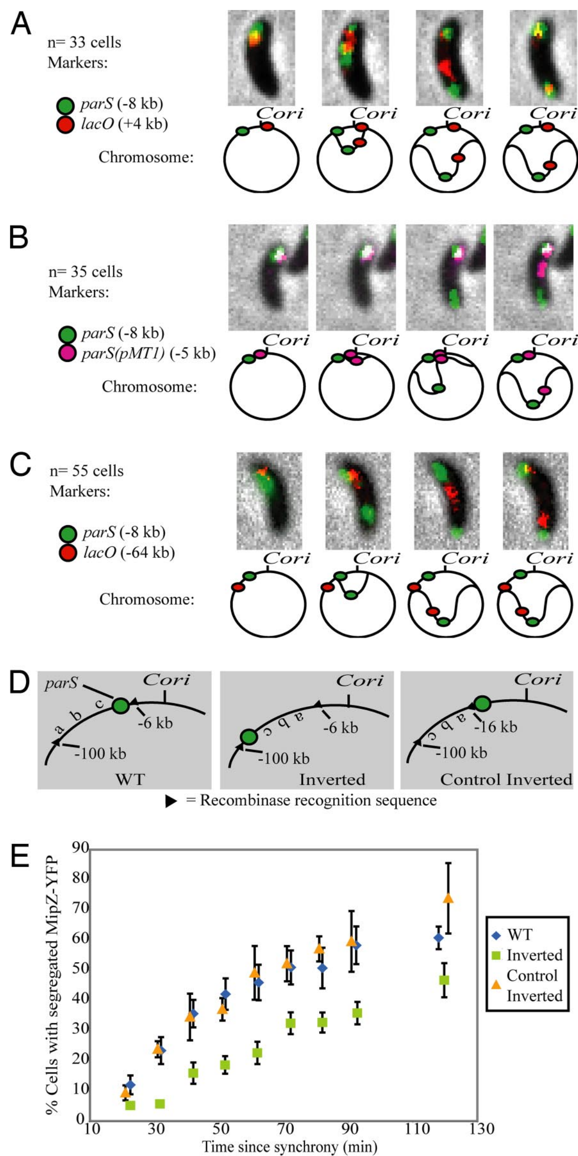

Fig. 2. A 10-kb region including *parS* contains the site of force exertion during segregation. (*A–C*) Segregation pattern of different loci and accompanying schematics of the position of markers used and order of segregation. Note that although micrographs show only one cell, the segregation order shown (*parS* segregating before the other tagged locus) was repeated in all cells observed. In all cases, *parS* visualized with MipZ-YFP. For clarity, schematics are not to scale. (*A*) *lacO* inserted at +4 kb and visualized with LacI-CFP. (*B*) *parS(pMT1)* inserted at −5 kb and visualized with CFP-pMT1Δ23ParB. (*C*) *lacO* inserted at −64 kb and visualized with LacI-CFP. (*D*) Schematic of the chromosomal configuration of inversion strains constructed by site-specific recombination. (*E*) Separating *parS* from *Cori* delays segregation. Plotted are the percentage of cells with two distinct MipZ-YFP foci as a function of time from synchrony. To avoid phototoxicity effects, a new field of cells was imaged for each time point. Chromosome configurations as in *D*. Symbols represent means of three experiments.

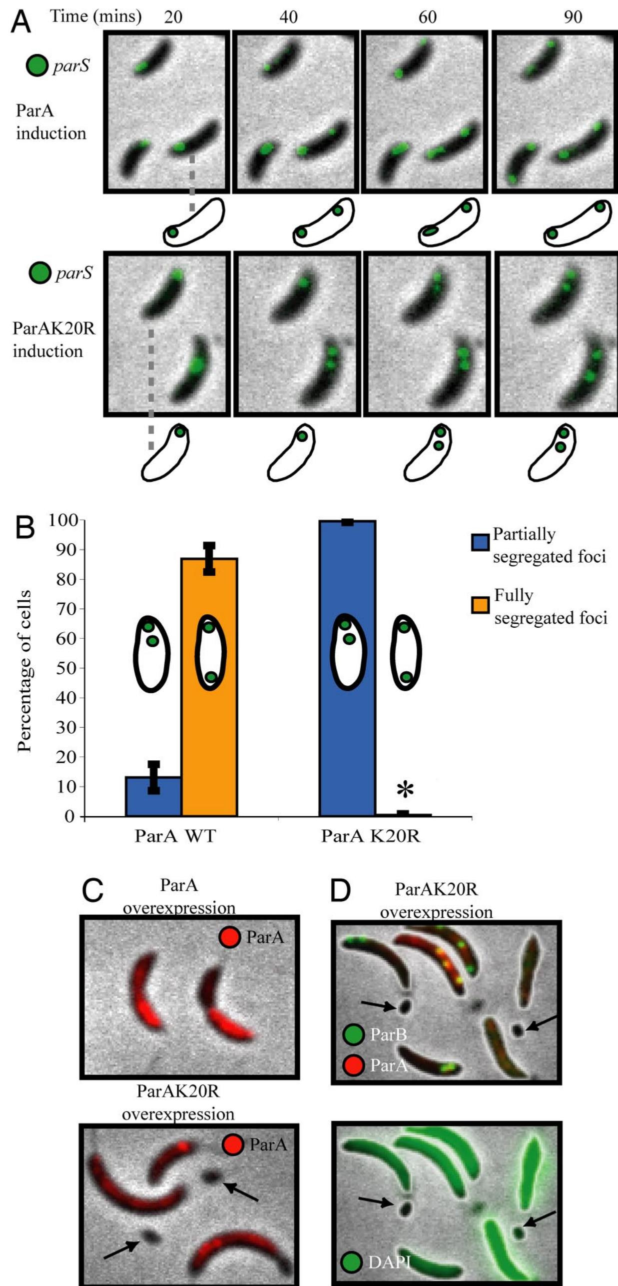

Fig. 3. A mutation in ParA abrogates chromosome segregation and produces anucleate minicells. (*A*) Time-lapse fluorescence micrographs of CFP-ParB in synchronized cells undergoing segregation after 60 min of ParA-mCherry (*Upper*) or ParAK20R-mCherry (*Lower*) induction with 0.03% xylose. (*B*) Percentage of cells with two foci that were either partially (blue bars) or fully (yellow bars) segregated 90 min after synchrony, in cultures treated as in *A*. * = 0.5%. Bars represent the average of two independent experiments, each with at least 150 cells counted for each strain. (*C*) ParA-mCherry (*Upper*) and ParAK20R-mCherry (*Lower*) localization in cells induced for 5 hours with 0.3% xylose. Arrows point to minicells produced only with the mutant ParA. (*D*) Localization of ParAK20R-mCherry and CFP-ParB (*Upper*) and DAPI (*Lower*) in cells carrying ParAK20R-mCherry driven by the xylose promoter and treated as in *C*. Arrows point to minicells that show no CFP-ParB foci and very low DAPI fluorescence. Levels of DAPI fluorescence have been increased to aid visualization of the very low fluorescence in minicells.

highly conserved amino acid in the Walker A ATP-binding motif of *Caulobacter* ParA. It has been shown that a homologous K to R mutation in phage P1 ParA greatly increases plasmid instability (37), so we predicted that it would have a dominant-negative phenotype in *Caulobacter*.

Fig. 3*A* and Movies S3 and S4 show synchronized cells in which we visualized *parS* using CFP-ParB, which binds to and colocalizes with *parS* (28). Under conditions of mild expression, ParA-mCherry did not perturb the segregation pattern of *parS* (Fig. 3*A Upper*). In cells with the ParAK20R-mCherry mutant, however, *parS* was replicated and moved a short distance, but segregation was not completed (Fig. 3*A Lower*, quantified in Fig. 3*B*).

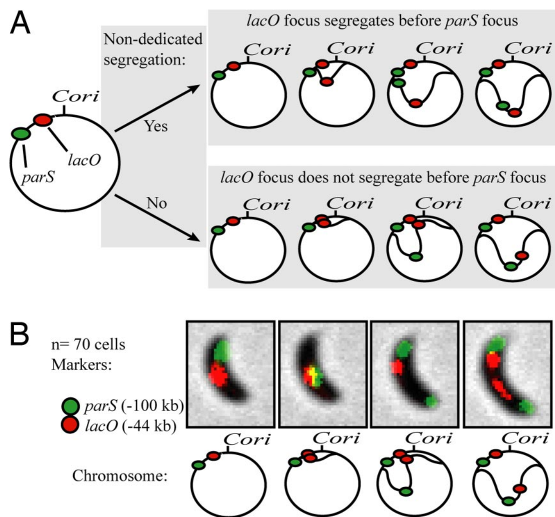

Fig. 4. Replication is not sufficient to initiate chromosome segregation. (*A*) Schematic of the strain constructed to test for the presence of nondedicated segregation mechanisms, and possible outcomes. (*Upper*) Nondedicated segregation occurs. Replicated DNA is moved independently of the *parABS* mechanism, and movement of the *lacO* arrays takes place before *parS* duplication. (*Lower*) Nondedicated mechanisms are not able to initiate chromosome segregation, and *lacO* movement does not take place until after *parS* has begun segregating. (*B*) Results of the experiment outlined in panel A. *parS* was followed by using MipZ-YFP, *lacO* arrays were followed by using LacI-CFP. Seventy cells were observed segregating and in all cases *parS* segregated before the *lacO* arrays.

To determine the long term effects of the presence of ParAK20R-mCherry, we induced its expression with 10-fold higher concentration of xylose for 5 h. Under these conditions, the localization pattern of ParA-mCherry and ParAK20R-mCherry differed slightly (Figs. 3*C* and S5 and S6). Overexpression of both versions of ParA-mCherry caused the cells to grow somewhat filamentous in accord with previous observations (16), but only the mutant produced anucleate minicells (arrows in Fig. 3 *C* and *D*), likely as an indirect result of a *parS* segregation defect. MipZ, which colocalizes with *parS*, acts also as a negative regulator of the FtsZ division ring (28). Therefore, as the cell with ParAK20R-mCherry overexpression grows longer, MipZ remains sequestered at the end of the cell with two copies of *parS*, and the division ring is free to form at the opposite end, creating a DNA-free minicell. Taken together, these experiments show that a ParA mutant prevents chromosome segregation, causes a misplacement of the division ring, and leads to the creation of DNA-free minicells (Fig. 3).

Initiation of Chromosome Segregation Requires *parS*-Directed Movement. The ability to uncouple segregation from replication by moving *parS* away from *Cori* allowed us to determine whether nondedicated mechanisms are sufficient to effect chromosome segregation in *Caulobacter*, as has been suggested (8). The strain shown in Fig. 4*A* carries an insertion of the *lacO* arrays at −44 kb and the same 100-kb inversion described in Fig. 2*D* ("Inverted"), which separates *parS* from *Cori* by ≈100 kb. This inversion creates a significant time interval during which the *lacO* arrays have been replicated and *parS* has not (Fig. 4*A*). We can estimate the duration of this interval by referring to Fig. 2*E*. There, it can be seen that *parS* is replicated ≈20 min later than when it is at its wild-type position. Because the *lacO* arrays are placed roughly halfway between the "WT" and "Inverted" positions of *parS*, we estimate that the *lacO* arrays will be replicated ≈10 min before *parS*.

Nondedicated models predict that newly replicated *lacO* DNA will begin segregating immediately after being replicated, at speeds comparable to the 0.3 μm/min measured experimentally for *Caulobacter* (Fig. 4*A*; refs. 1, 6–8). Assuming the ≈10-min interval discussed above and a segregation speed of 0.3 μm/min, the *lacO* arrays should move across the entire ≈2.0 μm cell before *parS* is replicated (8). However, we never observed this behavior (Fig. 4*B*; Movie S5). In fact, no *lacO* array segregation was ever observed before the beginning of *parS* segregation (Movie S5).

Thus, our results strongly suggest that initial chromosome segregation in *Caulobacter* results from a mechanism that involves ParA exerting force at *parS*/ParB, and that entropy (8) or DNA/RNA polymerization reactions alone are not sufficient to initiate chromosome movement. Importantly, a change in the overall transcription orientation bias of the chromosome would modify the predictions made by the RNA polymerase model (7). This was not the case in our inversion strain. Of 110 genes inverted, 56 are transcribed away from *Cori* and 54 toward, rendering the change in transcription bias insignificant.

***parS* and not *Cori* Is Anchored at the Cell Pole.** In the inverted strain shown in Figs. 4 and 5*A*, *Cori* was located closer to the *lacO* arrays than to *parS* (≈44 and ≈100 kb away, respectively). If *Cori* were the sequence that orients the *Caulobacter* chromosome with respect to the cell pole, we would expect the *lacO* arrays to be found nearer to the pole than *parS*. However, the opposite was true (Fig. 5*A*). This observation suggested that *parS*, and not *Cori*, is specifically anchored at the cell pole. To test this, we constructed the two additional inversion strains shown in Fig. 5*B* and measured the positions of *parS* and *Cori*. Before inversion, *parS* and the *lacO* arrays were located 8 and 4 kb away from *Cori*, respectively (left schematic in Fig. 5*B*), and both loci were found very close to the cell pole (Fig. 5*B*, "wildtype" bars on graph). In the inversion strains, *parS* remained close to the pole (Fig. 5*B*), even though it was now either 100 or 400 kb away from *Cori*. The position of the *lacO* arrays, however, shifted away from the pole proportionally to its distance from *parS* (Fig. 5*B*, red bars). Note that the distance from the *lacO* arrays to *Cori* was unchanged. These results demonstrate that the cellular positions of at least two DNA loci are determined by their relative distance to *parS*, rather than *Cori*.

Discussion

We have shown that *Caulobacter* employs the *parS* sequence as a centromere with which to segregate its chromosome through the action of the essential ParA ATPase. However, in most other bacteria studied, deleting ParA causes only mild segregation defects (14, 15, 17). A possible explanation for these differences is that when the *parABS* system is absent, nondedicated mechanisms of chromosome segregation take over. We have shown that in *Caulobacter*, this is not the case. When the *Caulobacter parABS* system cannot guide directional movement, segregation does not begin (Fig. 4). Earlier work had indicated that the initiation of chromosome segregation depends on the actin-like protein MreB, but our present results establish that the principal driving force for segregation is mediated by the ParABS system. Z, Gitai and coworkers have recently observed that chromosome segregation in *Caulobacter* depends on MreB only under certain conditions (Z. Gitai, personal communication).

It is important to note that the *parS*-driven initiation of chromosome segregation in *Caulobacter* does not preclude a role for forces exerted by the DNA/RNA polymerases (6, 7), compaction from condensins, or entropic exclusion (22), during bulk chromosome segregation. After *parS* has guided the newly replicated origin region to the opposite cell pole, these forces could contribute to the movement of the rest of the chromosome. Indeed, the documented rapid segregation of loci that are far away from *parS* (1) requires such contributions.

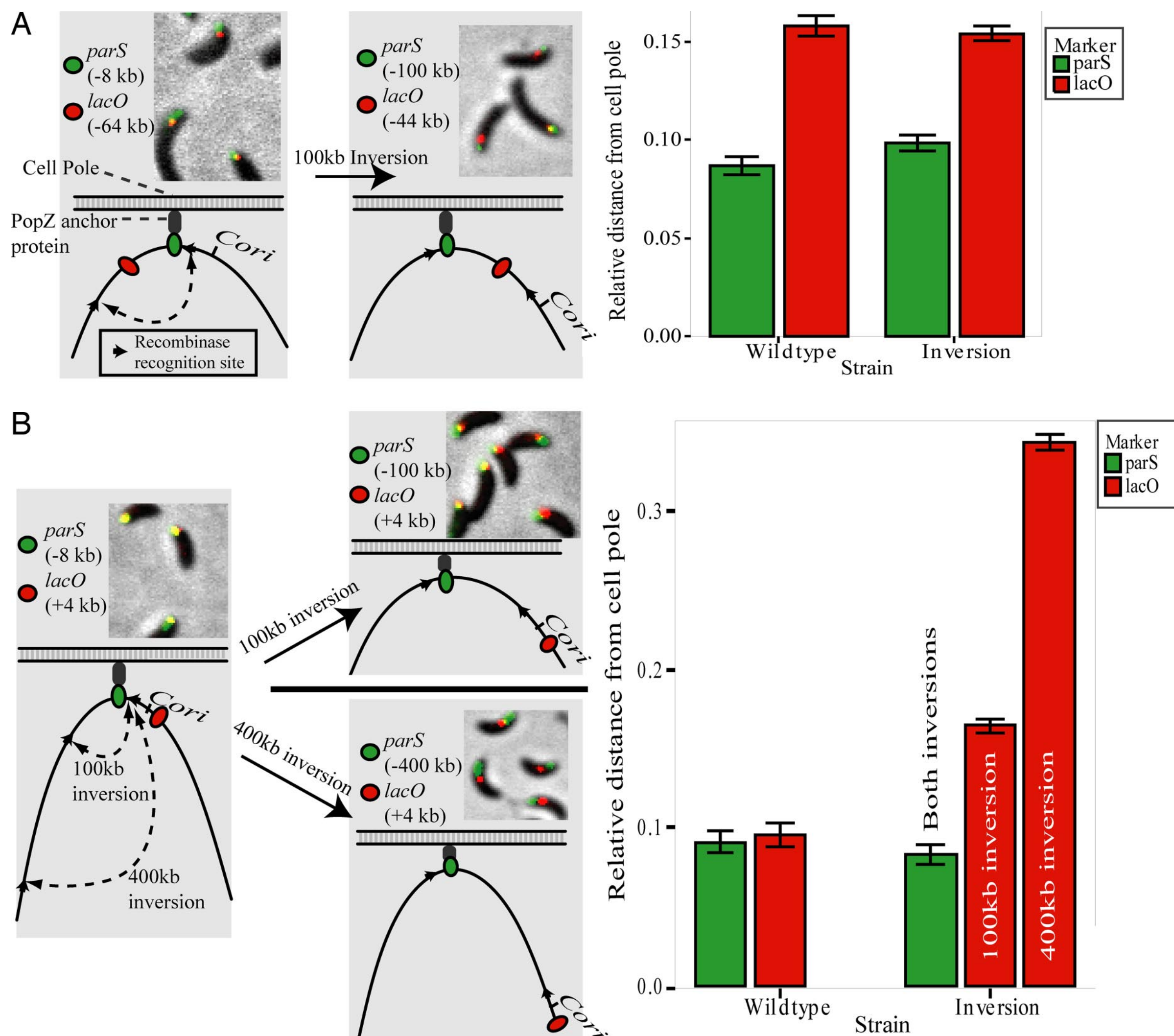

Fig. 5. *parS* is fixed at the cell pole, whereas *Cori* is not. (*A* and *B*) Fluorescent micrographs, schematic, and quantification of the relative positions of *parS* and *lacO* in cells before and after inversion of the chromosome region shown. (*A*) *lacO* originally positioned at −64 kb. (*B*) *lacO* positioned at +4 kb. Bars represent the average of three independent experiments, each with at least 100 cells counted.

The *Caulobacter* chromosome is arranged in a highly organized manner within the cell, as is the case in other bacteria (1, 2, 38), but how this is achieved remains an open question. Recently, it was reported that the *Caulobacter parS*/ParB complex is anchored to the cell pole by the PopZ polymeric protein (Fig. 5*A*; refs. 39 and 40). This polar anchoring is required for effective chromosome segregation and cell division (39, 40). Here, we showed that the orientation of the *Caulobacter* chromosome within the cell appears to be achieved by "clocking" the DNA molecule relative not to *Cori* but rather to *parS*. It remains to be determined whether additional specific targeting sequences exist in the *Caulobacter* chromosome.

The mechanisms through which DNA is localized subcellularly vary considerably among species. During sporulation in *Bacillus subtilis*, for example, the RacA and DivIVA proteins combine to anchor the chromosomal origin region to the cell pole (41). RacA binds to 25 sites spread over a 612-kb region of origin-proximal DNA (42), and DivIVA (which does not share sequence similarity with PopZ) is required for RacA-mediated localization of this region to the pole (41). The *Escherichia coli* chromosome, in turn, does not contain a *parABS* locus, but it has been shown that the *migS cis*-acting sequence affects bipolar localization of the origin region in this species (43). In addition, the *E. coli* chromosome is organized with the left and right arms in separate cell halves (31), an organization that requires the SMC-like condensation protein MukB (44). Clearly, there is a strong selective pressure for bacterial chromosomes to remain organized inside the cell, perhaps to coordinate DNA segregation with cell division. Indeed, RacA-mediated anchoring in *B. subtilis* prevents the formation of DNA-free forespores (41), and loss of polar *parS*/ParB anchoring in *Caulobacter* leads to defects in cell division (39, 40).

In summary, the *Caulobacter parABS* system and its associated proteins initiate segregation, orient and anchor the chromosome, and signal the onset of segregation so that division may begin (28). They are thus emerging as the functional equivalent of a eukaryotic kinetochore.

Experimental Procedures

Tables S1–S3 list all strains and plasmids used in this study, along with a brief description of their construction. We grew all *Caulobacter* CB15N-derived strains in M2G minimal and PYE media at 28°C. Our procedures for ΦCR30 phage transductions and transformation of plasmids into *Caulobacter* (45) and DAPI staining (16) have been described. Detailed descriptions of our synchronization, microscopy, and image analysis methods and the induction of recombination are provided in *SI Text*.

ACKNOWLEDGMENTS. We thank M. Thanbichler (Max Planck Institute, Marburg, Germany), S. Austin (National Cancer Institute), M. P. Calos (Stanford University), and F. G. Hansen (Technical University of Denmark) for providing reagents; Stephanie Weber (Stanford University) for some Matlab code; and P. T. McGrath, A. A. Iniesta, B. Burkholder, and members of the Shapiro and McAdams laboratories for helpful discussions. This work was supported by National Institutes of Health Grants R01 GM51426 R24 and GM073011-04 (to L.S.) and Department of Energy Grant DE-FG02-05ER64136 (to H.H.M.). E.T. was funded by the Smith Stanford Graduate Fellowship.

1. Viollier PH, *et al.* (2004) Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc Natl Acad Sci USA* 101:9257–9262.
2. Niki H, Yamaichi Y, Hiraga S (2000) Dynamic organization of chromosomal DNA in *Escherichia coli*. *Genes Dev* 14:212–223.
3. Fiebig A, Keren K, Theriot JA (2006) Fine-scale time-lapse analysis of the biphasic, dynamic behaviour of the two *Vibrio cholerae* chromosomes. *Mol Microbiol* 60:1164–1178.
4. Bates D, Kleckner N (2005) Chromosome and replisome dynamics in. *E coli*: Loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. *Cell* 121:899–911.
5. Jacob F, Brenner S, Cuzin F (1963) On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symp Quant Biol* 28:329–348.
6. Lemon KP, Grossman AD (2001) The extrusion-capture model for chromosome partitioning in bacteria. *Genes Dev* 15:2031–2041.
7. Dworkin J, Losick R (2002) Does RNA polymerase help drive chromosome segregation in bacteria? *Proc Natl Acad Sci USA* 99:14089–14094.
8. Jun S, Mulder B (2006) Entropy-driven spatial organization of highly confined polymers: Lessons for the bacterial chromosome. *Proc Natl Acad Sci USA* 103:12388–12393.
9. Gerdes K, Moller-Jensen J, Ebersbach G, Kruse T, Nordstrom K (2004) Bacterial mitotic machineries. *Cell* 116:359–366.
10. Gitai Z, Dye NA, Reisenauer A, Wachi M, Shapiro L (2005) MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell* 120:329–341.
11. Pogliano J (2008) The bacterial cytoskeleton. *Curr Opin Cell Biol* 20:19–27.
12. Leonard TA, Butler PJ, Lowe J (2005) Bacterial chromosome segregation: structure and DNA binding of the Soj dimer–a conserved biological switch. *EMBO J* 24:270–282.
13. Gerdes K, Moller-Jensen J, Bugge Jensen R (2000) Plasmid and chromosome partitioning: Surprises from phylogeny. *Mol Microbiol* 37:455–466.
14. Ireton K, Gunther N W t, Grossman AD (1994) spo0J is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J Bacteriol* 176:5320–5329.
15. Lasocki K, Bartosik AA, Mierzejewska J, Thomas CM, Jagura-Burdzy G (2007) Deletion of the parA (soj) homologue in. *Pseudomonas aeruginosa* causes ParB instability and affects growth rate, chromosome segregation, and motility. *J Bacteriol* 189:5762–5772.
16. Mohl DA, Gober JW (1997) Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* 88:675–684.
17. Dubarry N, Pasta F, Lane D (2006) ParABS systems of the four replicons of *Burkholderia cenocepacia*: New chromosome centromeres confer partition specificity. *J Bacteriol* 188:1489–1496.
18. Yamaichi Y, Fogel MA, Waldor MK (2007) par genes and the pathology of chromosome loss in *Vibrio cholerae*. *Proc Natl Acad Sci USA* 104:630–635.
19. Yamaichi Y, Fogel MA, McLeod SM, Hui MP, Waldor MK (2007) Distinct centromere-like parS sites on the two chromosomes of *Vibrio spp*. *J Bacteriol* 189:5314–5324.
20. Fogel MA, Waldor MK (2006) A dynamic, mitotic-like mechanism for bacterial chromosome segregation. *Genes Dev* 20:3269–3282.
21. Mohl DA, Easter J, Jr, Gober JW (2001) The chromosome partitioning protein, ParB, is required for cytokinesis in *Caulobacter crescentus*. *Mol Microbiol* 42:741–755.
22. Arnold A, Jun S (2007) Time scale of entropic segregation of flexible polymers in confinement: Implications for chromosome segregation in filamentous bacteria. *Phys Rev* 76:031901.
23. Woldringh CL, Nanninga N (2006) Structural and physical aspects of bacterial chromosome segregation. *J Struct Biol* 156:273–283.
24. Elmore S, Muller M, Vischer N, Odijk T, Woldringh CL (2005) Single-particle tracking of oriC-GFP fluorescent spots during chromosome segregation in *Escherichia coli*. *J Struct Biol* 151:275–287.
25. Marczynski GT (1999) Chromosome methylation and measurement of faithful, once and only once per cell cycle chromosome replication in *Caulobacter crescentus*. *J Bacteriol* 181:1984–1993.
26. Lin DC, Grossman AD (1998) Identification and characterization of a bacterial chromosome partitioning site. *Cell* 92:675–685.
27. Livny J, Yamaichi Y, Waldor MK (2007) Distribution of centromere-like parS sites in bacteria: Insights from comparative genomics. *J Bacteriol* 189:8693–8703.
28. Thanbichler M, Shapiro L (2006) MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. *Cell* 126:147–162.
29. Dingwall A, Shapiro L (1989) Rate, origin, and bidirectionality of *Caulobacter* chromosome replication as determined by pulsed-field gel electrophoresis. *Proc Natl Acad Sci USA* 86:119–123.
30. Marczynski GT, Shapiro L (1992) Cell-cycle control of a cloned chromosomal origin of replication from *Caulobacter crescentus*. *J Mol Biol* 226:959–977.
31. Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG (2006) The. *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. *Mol Microbiol* 62:331–338.
32. Groth AC, Olivares EC, Thyagarajan B, Calos MP (2000) A phage integrase directs efficient site-specific integration in human cells. *Proc Natl Acad Sci USA* 97:5995–6000.
33. Thorpe HM, Smith MC (1998) *In vitro* site-specific integration of bacteriophage DNA catalyzed by a recombinase of the resolvase/invertase family. *Proc Natl Acad Sci USA* 95:5505–5510.
34. Garner EC, Campbell CS, Weibel DB, Mullins RD (2007) Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. *Science* 315:1270–1274.
35. Derman AI, Lim-Fong G, Pogliano J (2008) Intracellular mobility of plasmid DNA is limited by the ParA family of partitioning systems. *Mol Microbiol* 67:935–946.
36. Saint-Dic D, Frushour BP, Kehrl JH, Kahng LS (2006) A parA homolog selectively influences positioning of the large chromosome origin in *Vibrio cholerae*. *J Bacteriol* 188:5626–5631.
37. Fung E, Bouet JY, Funnell BE (2001) Probing the ATP-binding site of P1 ParA: Partition and repression have different requirements for ATP binding and hydrolysis. *EMBO J* 20:4901–4911.
38. Boccard F, Esnault E, Valens M (2005) Spatial arrangement and macrodomain organization of bacterial chromosomes. *Mol Microbiol* 57:9–16.
39. Bowman G, *et al.* (2008) A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. *Cell*, in press.
40. Ebersbach G, Briegel A, Jensen G J, Jacobs-Wagner C (2008) A multimeric pole-organizing protein critical for chromosome attachment, division and protein localization in *Caulobacter. Cell*, in press.
41. Ben-Yehuda S, Rudner DZ, Losick R (2003) RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science* 299:532–536.
42. Ben-Yehuda S, *et al.* (2005) Defining a centromere-like element in Bacillus subtilis by Identifying the binding sites for the chromosome-anchoring protein RacA. *Mol Cell* 17:773–782.
43. Yamaichi Y, Niki H (2004) migS, a cis-acting site that affects bipolar positioning of oriC on the *Escherichia coli* chromosome. *EMBO J* 23:221–233.
44. 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* 65:1485–1492.
45. Ely B (1991) Genetics of *Caulobacter crescentus*. *Methods Enzymol* 204:372–384.