Permissive zones for the centromere-binding protein ParB on the *Caulobacter crescentus* chromosome

Ngat T. Tran\(^1\), Clare E. Stevenson\(^2\), Nicolle F. Som\(^1\), Anyarat Thanapipatsiri\(^1\), Adam S. B. Jalal\(^1\), and Tung B. K. Le\(^1\)*

\(^1\)Department of Molecular Microbiology
\(^2\)Department of Biological Chemistry
John Innes Centre, Norwich, NR4 7UH, United Kingdom

\(^\dagger\)Co-first authors
\(^*\)Correspondence: tung.le@jic.ac.uk

**ABSTRACT**

Proper chromosome segregation is essential in all living organisms if daughter cells are each to inherit a full copy of genetic information. In *Caulobacter crescentus*, the ParA-ParB-parS system is required for proper chromosome segregation and cell viability. The bacterial centromere-like parS DNA locus is the first to be segregated following chromosome replication. parS is recognized and bound by ParB protein, which in turn interacts with ParA to partition the ParB-parS nucleoprotein complex to each daughter cell. In this study, we investigated the genome-wide distribution of ParB on the *Caulobacter* chromosome using a combination of *in vivo* chromatin immunoprecipitation (ChIP-seq) and *in vitro* DNA affinity purification with deep sequencing (IDAP-seq). We confirmed two previously identified parS sites and discovered at least three more sites that cluster ~8 kb from the origin of replication. We showed that *Caulobacter* ParB nucleates at parS sites, then associates non-specifically with flanking DNA to form a high-order nucleoprotein complex that occupies an extensive ~10 kb DNA segment on the left chromosomal arm. Lastly, using transposon mutagenesis coupled with deep sequencing (Tn-seq), we identified a ~500 kb region surrounding the native parS cluster and a ~100 kb region surrounding the terminus of the *Caulobacter* chromosome that are tolerable to the insertion of a second parS cluster without severely affecting cell viability. Our results demonstrate that the genomic distribution of the bacterial centromere-like parS is highly restricted and is crucial for chromosome segregation in *Caulobacter*. 
INTRODUCTION

Proper chromosome segregation is essential in all living organisms if daughter cells are each to inherit a full copy of the genome. In eukaryotes, chromosome segregation during mitosis starts with sister chromosome condensation, followed by the formation of spindle fibres that attach to the kinetochore to pull sister chromatids apart. The kinetochore is the protein structure that assembles on the centromere and links each sister chromatid to microtubules polymers from the mitotic spindle. Unlike in eukaryotes, bacterial chromosome segregation happens without a dedicated spindle-like apparatus (Lim et al., 2014; Vecchiarelli et al., 2013, 2014). Nevertheless, this process is highly organized and also involves protein-based components (reviewed in Badrinarayanan et al., 2015). The first segregated segment of the chromosome is usually proximal to the origin of replication (ori) (Lagage et al., 2016; Lin and Grossman, 1998; Livny et al., 2007; Toro et al., 2008). In many bacteria, this region is segregated by the tripartite ParA-ParB-parS partitioning system (Fogel and Waldor, 2006; Ireton et al., 1994; Lin and Grossman, 1998; Mohl et al., 2001). parS is a centromere-like DNA sequence that most often locates near ori. ParB is a DNA-binding protein that nucleates on a parS sequence. ParB, via its N-terminal domain, binds DNA non-specifically to spread along the chromosome from its cognate parS nucleation site (Graham et al., 2014; Lin and Grossman, 1998; Murray et al., 2006; Taylor et al., 2015). ParB might also bridge distal DNA together to coalesce into a large nucleoprotein complex (the “spreading and bridging” model) (Breier and Grossman, 2007; Graham et al., 2014; Murray et al., 2006; Taylor et al., 2015). Similarly, the formation of the nucleoprotein complex for plasmid-borne ParB-parS was proposed to happen via a “nucleation and caging” mechanism where the nucleation of ParB on parS creates a high local concentration of ParB, thereby caging ParB dimer-dimer together with non-specific DNA surrounding parS (Sanchez et al., 2015). Following ParB binding to parS, ParA, a Walker-box ATPase protein, interacts with ParB and powers the segregation of the ParB-DNA nucleoprotein complex to partition replicated chromosomes to each daughter cell (Easter Jr. and Gober, 2002; Leonard et al., 2005).

In Caulobacter crescentus, the ParA-ParB-parS system is essential for viability (Mohl and Gober, 1997; Mohl et al., 2001). In G1-phase Caulobacter, parS/ori reside at one cell pole, the terminus (ter) is near the opposite pole, and the two chromosomal arms run orderly in parallel down the long axis of the cell (Le et al., 2013; Vioillier et al., 2004). After replication, the duplicated parS sites are released from the pole and separated slightly from one another before one parS site is translocated unidirectionally to the opposite cell pole. Toro et al (2008) identified two parS sites located ~8 kb from the ori on the left arm of the Caulobacter chromosome, while other works predicted six parS sites bioinformatically but did not report their sequences nor verify them experimentally (Bergé et al., 2016; Figge et al., 2003; Mohl and Gober, 1997). Furthermore, it is not yet known whether Caulobacter ParB spreads non-specifically on DNA, and if it does, how far it spreads along the chromosome from the parS nucleation site. Regarding the genome-wide distribution of parS sites, a comparative genomic study suggested that parS sites are not distributed randomly on bacterial chromosomes, rather they are found almost exclusively near the ori (Livny et al., 2007). Notably, in Pseudomonas aeruginosa, parS sites must be located within a ~650 kb region surrounding the ori for the chromosome segregation to proceed correctly (Lagage et al., 2016).

In this study, we used genome-wide techniques (ChIP-seq and IDAP-seq) together with in vitro biochemical characterization to clarify the number and locations of parS sites in Caulobacter. We show that there are at least five parS sites clustered closely near the ori of Caulobacter chromosome, and that ParB occupies ~10 kb of DNA on the left arm of the chromosome. We also show that Caulobacter ParB nucleates on parS, then spreads to flanking DNA independent of the location of parS on the chromosome. Moreover, using transposon mutagenesis coupled with deep sequencing (Tn-seq), we define a ~500 kb region surrounding the native parS cluster, and ~100 kb region surrounding ter of the...
Caulobacter chromosome that are tolerable to the insertion of a second parS cluster without severely affecting cell viability. Our results demonstrate that the genomic location of parS is highly biased and crucial for proper chromosome segregation.

RESULTS AND DISCUSSION

ParB occupies a 10 kb DNA region near the origin of replication

To define the distribution of ParB on the chromosome, we performed chromatin immunoprecipitation with deep sequencing. We fused the flag tag to the ParB-encoding gene at its 5’ end and placed this allele downstream of a vanillate-inducible promoter (P\textsubscript{van}), at the chromosomal vanA locus. The vanillate-inducible flag-parB was then transduced to a Caulobacter strain where the native and untagged parB was under the control of a xylose-inducible promoter (P\textsubscript{xyl}). Caulobacter cells were depleted of untagged ParB by addition of glucose for 5 hours, then vanillate was added for an additional hour before cells were fixed with 1% formaldehyde for ChIP-seq (Fig. 1A). Caulobacter cells depleted of native ParB while producing the FLAG-tagged ParB version are viable, indicating that the tag does not interfere with ParB function (Fig. S1A). For ChIP-seq, DNA-bound to FLAG-ParB was pulled down using α-FLAG antibody coupled to sepharose beads. The immunoprecipitated DNA was deep sequenced and mapped back to the Caulobacter genome to reveal enriched genomic sites (Fig. 1A). As a negative control, we performed α-FLAG ChIP-seq in a Caulobacter strain that produces FLAG-tagged YFP, a non-DNA binding protein (Fig. 1B). The ChIP-seq profile of FLAG-ParB showed a clear enrichment in the DNA region on the left chromosomal arm, ~8 kb away from the origin of replication. No other significant enrichment was observed elsewhere on the chromosome or in the negative control (Fig. 1A-B). A closer examination of the ori-proximal region revealed an extended ~10 kb region with significant enrichment above background and four defined peaks (Fig. 1A). To independently verify our results, we repeated the ChIP-seq experiment using α-GFP antibody to pull down DNA from a Caulobacter strain that produces a CFP-ParB fusion protein from its native location as the only source of ParB in the cell or using a polyclonal α-ParB in a wild-type Caulobacter (Fig. S1B). For all cases, we retrieved very similar ChIP-seq profiles to that of FLAG-ParB, suggesting the extended DNA region associating with ParB is not an artefact of tagging but a property of Caulobacter ParB itself.

The extensive 10-kb ParB-binding DNA region cannot be explained by the length of DNA fragments that were sheared as part of a ChIP-seq protocol. We sequenced immunoprecipitated DNA from both ends to determine their exact size distribution (Table S1). Pulled-down DNA averages around 150 bp, much smaller than the size of ChIP-seq peaks in our study. However, the extended ParB-binding DNA region can be most easily explained by the non-specific binding of ParB to DNA outside of the parS nucleation site, either by a “spreading and bridging” or “caging” mechanism. If so, Caulobacter ParB mutants that are impaired in binding to non-specific DNA are predicted to spread less. To identify such mutants in Caulobacter, we mutated the highly-conserved N-terminal Box II motif which was shown to be important for the non-specific DNA-binding activity of B. subtilis ParB (Fig. S2A) (Breier and Grossman, 2007; Graham et al., 2014). Four variants were constructed parB (G101S), parB (R103A), parB (R104A), and parB (R106A). We introduced the flag-tagged parB mutant allele at the van locus, in the P\textsubscript{xyl}-parB genetic background, then employed α-FLAG ChIP-seq to assess the distribution of mutated ParB on the chromosome. Two mutants, ParB (G101S) and ParB (R104A), were found to produce well-defined and symmetrical peaks (~400 bp in width) that are typical of site-specific DNA-binding proteins (Fig. 1B and Fig. S1B). On the contrary, wild-type ParB peaks are much wider and asymmetrical (Fig. 1A). These data suggest that Caulobacter ParB, similar to B. subtilis and P. aeruginosa ParB, also binds DNA non-specifically to spread along the chromosome from its parS nucleation sites. Lastly, we noted that DNA enrichment in ChIP-seq experiments with ParB (G101S) or ParB (R104A) is ~5 fold less than that of wild-type ParB (Fig. 1 A-C), despite the fact that ParB variants nucleate equally well on DNA in vitro (Fig. S2B). This is
most likely because ParB (G101S) and ParB (R104A) are less stable than wild-type ParB in vivo (Fig. S2C).

Identification of parS sites and correlating ParB-parS in vitro binding affinities to their in vivo ChIP-seq enrichment

Since the large width of ChIP-seq peaks obscures the exact position of parS, we employed in vitro DNA affinity purification with deep sequencing (IDAP-seq) (Belitsky and Sonenshein, 2013) to pinpoint parS sequence to near single-nucleotide resolution. Purified ParB-(His)₆ was incubated with randomly-fragmented Caulobacter genomic DNA, then ParB-DNA complexes were pulled-down using immobilized Ni²⁺ beads. ParB-bound DNA fragments were eluted out and sequenced en masse. The sequencing reads were mapped back to the Caulobacter genome (Fig. 1D and Fig. 2). Analysis of the strand-specific coverage map allows identification of seven 16 bp putative parS sites (see Fig. 1D and Fig. S3 for the methodology of IDAP-seq data analysis). These included the two parS sites (sites 3 and site 4) that were first discovered in Toro et al (2008) but revealed five more putative sites (sites 1, 2, 5, 6 and 7).

To correlate the sequence conservation to the binding affinity of ParB, we measured the equilibrium dissociation constant (K_d) of ParB binding to 24-bp double-stranded oligonucleotides containing individual putative parS sites by Surface Plasmon Resonance (SPR) (Fig. 3 and Fig. S4). The double-stranded oligonucleotides was tethered to a chip surface within an SPR flow cell. Purified ParB-(His)₆ was flowed over the test DNA. ParB binding was recorded by measuring the change in response units during ParB injection. After injection the chip was washed with buffer and subsequently with high salt buffer to remove any bound ParB. This cycle was repeated for an increasing concentration of ParB dimer to enable the estimation of K_d (Fig. 3 and Fig. S4). Note that the length of the double-stranded oligonucleotides was limited to 24 bp so that only the nucleating event of ParB on parS was observed, and not the interaction with DNA flanking parS. We observed that sites 2, 3, 4, 5, and 7 have low nM K_d values (Fig. 3), consistent with their high ChIP-seq peaks (Fig. 1). On the other hand, ParB binds to the putative sites 1 and 6 weakly in vitro, albeit more than to a scrambled parS control (Fig. 3), suggesting that sites 1 and 6 are perhaps unlikely to be significant in vivo.

ParB spreads to a maximum of 2 kb around individual parS site

The extended ChIP-seq peak of ParB around parS is consistent with ParB spreading from the parS nucleation site by associating with neighbouring DNA. Since parS sites are located within essential genes or genes that have a high fitness cost, we were not able to ablate individual parS site to investigate the spreading of ParB in Caulobacter. Instead, we investigated the spreading of ParB from individual parS sites by expressing the Caulobacter ParB/parS system in Escherichia coli. Since E. coli does not possess a ParB homolog nor a Caulobacter parS-like sequence, it serves as a suitable heterologous host for this experiment. We inserted individual parS sites onto the E. coli chromosome at the ybbD locus (Fig. 4). The ParB protein was expressed from an IPTG-inducible promoter as a C-terminal fusion to the T18 fragment of Bordetella pertussis adenylate cyclase. The T18-ParB is fully functional in E. coli as judged by its interactions with their known partners such as ParB itself, ParA, and MipZ in a bacterial-two hybrid assay (Fig. S5A). We induced exponentially-growing E. coli cells at 28°C with 500 µM IPTG for an hour before fixing with formadehyde for ChIP-seq. DNA bound to T18-ParB was immunoprecipitated using α-T18 conjugated sepharose beads. A scrambled parS site 3 was also inserted at the ybbD locus to serve as a negative control. As expected, the strong parS sites (sites 2, 3, 4, 5, and 7), on their own showed a high level of DNA enrichment, in agreement with their in vitro ParB binding affinity (Fig. 4). The weak putative parS sites (site 1 and 6) show little to no enrichment above background (Fig. S5B). Most importantly, we observed that ParB in an E. coli host spreads to a maximum of ~2 kb around each parS site (Fig. 4). Next, we repeated the ChIP-seq
experiment but with a spreading-defective ParB (G101S). This revealed symmetrical peaks with a ~400-bp width, confirming that Caulobacter ParB can spread to any neighbouring DNA and that non-specific interaction with DNA is mainly dependent on an initial ParB-parS nucleation event. Lastly, we noted that the spreading of wild-type ParB is not equal on both sides of parS. It is likely that the non-specific association of ParB with neighbouring DNA might be influenced by on-going transcription or other nearby DNA-binding proteins. This asymmetrical spreading has been observed previously with ParB homologs from other bacterial species (Attaiech et al., 2015; Breier and Grossman, 2007).

Since Caulobacter ParB associates maximally with ~2 kb DNA surrounding individual parS site, the clustering of parS sites might serve to enable a higher concentration of DNA-bound ParB near ori than is possible with a single site. A previously study estimated that ~80% of the total cellular ParB is bound at parS sites in Caulobacter (Lim et al., 2014). Caulobacter ParA was also found to require a high concentration of DNA-bound ParB to activate its ATPase activity, an essential step for chromosome segregation by the ParAB-parS system (Lim et al., 2014). Furthermore, it is known that Caulobacter ParB interacts with MipZ, which in turns binds PopZ to anchor the ori-proximal DNA to the cell pole (Bowman et al., 2008; Ebersbach et al., 2008; Thanbichler and Shapiro, 2006). A high local concentration of DNA-bound ParB would enable a robust anchorage of the ori DNA domain to the cell pole. Since high-affinity parS sites might serve to enable a higher concentration of DNA-bound ParB affects the chromosome segregation or the anchorage of the ori-proximal domain. Nevertheless, we noted that the nucleation-competent but spreading-defective ParB (G101S) or ParB (R104A) variants are unable to support Caulobacter growth, implying that interactions with non-specific DNA is required for cell viability (Fig. S1A). In line with our study, B. subtilis or P. aeruginosa engineered with a single parS are defective in chromosome segregation, resulting in elevated numbers of anucleate cells (Breier and Grossman, 2007; Jecz et al., 2015; Lagage et al., 2016).

**Extra copies of parS can reduce the fitness of Caulobacter depending on their genomic locations**

Additional copies of parS, for example when is placed on a multi-copy number plasmid, can be lethal for cells because plasmid DNA can be segregated instead of the chromosome, resulting in daughter cells with either zero or two chromosomes (Toro et al., 2008). Indeed, we found the presence of a parS-carrying plasmid caused growth impairment in Caulobacter, and the fitness cost correlates well with the ParB-parS binding affinity (Fig. 5). Plasmid-borne sites 3 and 4, which are the strongest parS sites, reduced cell viability by ~1000 fold compared to a negative control (scrambled site 3). Extra copies of sites 2, 5 and 7 reduced cell viability by ~100 fold compared to a control, while the weaker parS sites 1 and 6 did not impact cell viability when present on a plasmid.

We reasoned that if the toxicity of a plasmid-borne parS site was due to the segregation of plasmids instead of the chromosome then having extra parS sites on the chromosome should eliminate the toxicity. Indeed, we were able to engineer a 260-bp DNA segment containing both strong parS site 3 and site 4 at various positions from ori to ter on both arms of Caulobacter chromosome. On the contrary, a plasmid containing both parS sites 3 and 4 are completely lethal to Caulobacter cells (Toro et al., 2008). Nevertheless, we noted a variation in the fitness of Caulobacter with extra chromosomal parS sites, depending on the location of the ectopic parS (Fig. 6). An extra parS$^{3+4}$ inserted at +200 kb (near ori) or at +1800 kb (near ter) did not impact the fitness of the cell dramatically as judged by a normal cell length distribution and a 6-fold increase in the number of anucleate cells (Fig. 6B and Fig. 6D). On the contrary, parS$^{3+4}$ inserted at +1000 kb (middle of the right arm of the chromosome) caused a more severe fitness defect. The cells were more elongated (4.74 ± 3.3 µm) compared to WT (2.97 ± 0.77 µm) (Fig. 6). Furthermore, the number of cells with no or more than two CFP-ParB foci were elevated ~11 fold in comparison to strains without an
ectopic parS3+4 (Fig. 6C). Our data suggest that the genomic location of an extra chromosomal copy of parS is important for the cell fitness.

**Systematic identification of a permissive zone for parS insertion by transposon mutagenesis with deep sequencing (Tn-seq)**

Previously, a comparative genomics study surveyed and predicted the positions of parS sites over a wide range of bacteria and found that most parS sites are located close to the ori on the chromosome (Livny et al., 2007). Here, in Caulobacter, we have found that a second parS cluster, depending on its location on the chromosome, can affect chromosome segregation and cell fitness. To investigate this positional bias systematically, we employed a genome-wide transposon mutagenesis with deep sequencing (Tn-seq) approach. Briefly, a Tn5 transposon carrying parS sites 3, 4 and 5 was used to insert these strong parS sites randomly around the chromosome. A library of approximately half a million of single colonies were generated and the genomic locations of the inserted parS cluster was then determined en masse by deep sequencing. As a control, we generated an insertion library using a transposon that does not carry parS. Wild-type Caulobacter cells were first mutagenized with parS or parS transposon, and the number of insertions was binned to 10-kb segments along the Caulobacter chromosome. The ratio of the frequency for the parS transposon and that of the parS transposon was plotted as a log10 scale against genomic position (Fig. 7A), and used as a proxy to determine the genomic preference for an extra cluster of parS. We observed that a second parS cluster is most tolerated within ~500 kb surrounding ori (Fig. 7A and Fig. S6A). In contrast, an ectopic parS is strongly disfavoured near the middle of each chromosomal arm (Fig. 7A and Fig. S6B), consistent with our observation that parS3+4 at +1000 kb caused cell elongation and chromosome segregation defects. A limited zone of parS enrichment was also found within ~100 kb around the ter (Fig. 7A and Fig. S6C).

Lastly, we also note the presence of two parS insertion “hot spots”. The first hot spot locates near the native parS cluster (Fig. 7B), likely strengthening the existing native ParB binding area on the left arm of the chromosome. The second hot spot encompasses the recF, gyrB and CCNA0160 genes (Fig. 7C). One possibility is that a parS insertion in the vicinity of gyrB is preferred because it alters the global supercoiling level. However, we found that the gyrB transcription was unchanged compared to wild-type cells or cells with an extra parS elsewhere on the chromosome. The mechanism responsible for the gyrB “hotspot” therefore remains unknown.

We noted that parS insertion frequency decreases gradually from ori to the mid-arm without a clear boundary, suggesting that the parS permissive zone is perhaps dependent on the genomic distance away either from ori or from the native parS cluster. To test this hypothesis, we employed a Flip 1-5 strain where the native cluster of parS sites were relocated ~400 kb away from ori through an inversion between +3611 kb and +4038 kb (Fig. 7D) (Tran et al., 2017). The Tn5 transposon with or without the parS cluster was again used to randomly mutagenize the Flip 1-5 strain. As a control, we also transposon mutagenized another inversion strain (Flip 2-5) where the native parS cluster remains at its original location but a similar chromosome segment (between +3611 kb and +4030 kb) was inverted (Fig. 7D). Results showed that the permissive zone for insertion of an extra parS cluster in Flip 1-5 was now centred near the relocated parS site at +3611 kb, while the permissive zone remains centred at the native parS in the control Flip 2-5 strain (Fig. 7D) (Tran et al., 2017). Altogether, our results suggest that the genomic distance from the original parS cluster, not the distance from ori, is likely the main determinant of the permissive zone for the insertion of a second parS cluster.

Most bacterial species with a ParAB-parS system have more than one parS site (Livny et al., 2007), and some species such as Streptomyces coelicolor and Listeria innocua have accumulated 22 parS sites near their origin of replication (Jakimowicz et al., 2002; Livny et al., 2007). How the bacterial centromere-like region expands and what drives its extension over time are interesting biological questions. Our finding that new parS sites can locate
near the native parS cluster but not elsewhere could potentially explain the clustering of parS sites on bacterial chromosomes over time. New parS sites preferentially locate near the original parS cluster because it is the least disruptive to chromosome segregation and cell viability (Fig. 6 and 7). In Caulobacter, parS, not ori, is the site at which force is exerted during chromosome segregation (Toro et al., 2008). ParA forms a gradient emanating from the opposite pole to the ParB-parS cluster. A ParA gradient retracts upon contacting ParB-parS and this nucleoprotein complex moves in the retreating gradient of ParA to the opposite cell pole. ParA-ParB-parS are only required for the segregation of parS-proximal DNA, but not of the distal DNA loci (Badrinarayanan et al., 2015b). Once the parS-proximal DNA is properly segregated by ParA-ParB-parS, distal DNA regions follow suit, driven by separate molecular machinery, or more likely without the need of a dedicated system (Badrinarayanan et al., 2015b). It is, therefore, foreseeable that expanding the parS region by adding new parS sites near the native cluster is least disruptive to chromosome segregation since the parS-proximal DNA remains the first locus to be segregated. Similarly, in P. aeruginosa, parS is also the first segregated locus and it is preferable for cell viability that parS segregates soon after DNA replication (Lagage et al., 2016).

In this study, we also discovered that new parS sites are also tolerated near the ter region, albeit with less preference than near the native parS cluster. In P. aeruginosa or B. subtilis, insertion of parS near the ter region is strongly discouraged, presumably due to the recruitment of the Structural Maintenance of the Chromosomes (SMC) complex away from ori (Lagage et al., 2016; Sullivan et al., 2009). SMC is a prominent protein involved in bacterial chromosome organization and segregation (Gruber and Errington, 2009; Minnen et al., 2011; Schwartz and Shapiro, 2011; Sullivan et al., 2009; Tran et al., 2017). To test if SMC might contribute to shape the distribution of ectopic parS sites in Caulobacter, we transposon mutagenized the Δsmc Caulobacter strain (Fig. S6D). In Δsmc cells, the pattern of parS permissive zones does not change dramatically. New parS sites remain disfavoured near mid-arms, although they are less favoured near ter compared to wild-type cells (Fig. S6D). Our previous study showed that Caulobacter SMC are recruited to the ter-located ectopic parS and cohesin flanking DNA together, nevertheless the global chromosome organization remained largely unchanged with ori and ter at opposite poles and two chromosomal arms running in parallel down the long axis of the cell (Tran et al., 2017). All together, we conclude that SMC contributes to the determination of parS permissive zones but cannot solely explain some of the preference for the ter region and the disfavour for mid-arm regions in Caulobacter crescentus. Further investigation into the molecular mechanism that gives raise to the permissive zones of parS will undoubtedly improve our understanding of bacterial chromosome segregation and organization.

MATERIALS AND METHODS
All experimental procedures are reported in the Supplementary Information

AUTHOR CONTRIBUTIONS
Conceptualization, N.T.T. and T.B.K.L.; Investigation and Data analysis, N.T.T., C.E.S., N.F.S., A.T., A.S.B.J., and T.B.K.L.; Writing, N.T.T., C.E.S., and T.B.K.L.; and Funding Acquisition, T.B.K.L.

ACCESSION NUMBERS
The accession number for the sequencing data reported in this paper is GEO: GSE100233.

ACKNOWLEDGMENTS
We thank Anjana Badrinarayanan, Matthew Bush, Mark Buttner, Michael Laub, and Susan Schlimpert for discussion and comments on the manuscript. We thank Christine Jacob-Wagner and Martin Thanbichler for materials. This study was supported by a Royal Society University Research Fellowship (UF140053), a Royal Society Research Grant (RG150448) to T.B.K.L. and a BBSRC grant-in-add (BBS/E/J/000C0683) to the John Innes Centre.
REFERENCES

- Attaiech, L., Minnen, A., Kjos, M., Gruber, S., and Veening, J.-W. (2015). The ParB-parS Chromosome Segregation System Modulates Competence Development in Streptococcus pneumoniae. MBio 6, e00662-15.
- Badrinarayanan, A., Le, T.B.K., and Laub, M.T. (2015a). Bacterial Chromosome Organization and Segregation. Annu. Rev. Cell Dev. Biol. 31, 171–199.
- Badrinarayanan, A., Le, T.B.K., and Laub, M.T. (2015b). Rapid pairing and resegregation of distant homologous loci enables double-strand break repair in bacteria. J. Cell Biol. 210, 385–400.
- Belitsky, B.R., and Sonenshein, A.L. (2013). Genome-wide identification of Bacillus subtilis CodY-binding sites at single-nucleotide resolution. Proc. Natl. Acad. Sci. U. S. A. 110, 7026–7031.
- Bergé, M., Campagne, S., Mignonet, J., Holden, S., Théraulaz, L., Manley, S., Allain, F.H.-T., and Viollier, P.H. (2016). Modularity and determinants of a (bi-)polarization control system from free-living and obligate intracellular bacteria. ELife 5.
- Bowman, G.R., Comolli, L.R., Zhu, J., Eckart, M., Koenig, M., Downing, K.H., Moerner, W.E., Earnest, T., and Shapiro, L. (2008). A Polymeric Protein Anchors the Chromosomal Origin/ParB Complex at a Bacterial Cell Pole. Cell 134, 945–955.
- Breier, A.M., and Grossman, A.D. (2007). Whole-genome analysis of the chromosome partitioning and sporulation protein Spo0J (ParB) reveals spreading and origin-distal sites on the Bacillus subtilis chromosome. Mol. Microbiol. 64, 703–718.
- Easter Jr., J., and Gober, J.W. (2002). ParB-Stimulated Nucleotide Exchange Regulates a Switch in Functionally Distinct ParA Activities. Mol. Cell 10, 427–434.
- Ebersbach, G., Briegel, A., Jensen, G.J., and Jacobs-Wagner, C. (2008). A self-associating protein critical for chromosome attachment, division, and polar organization in caulobacter. Cell 134, 956–968.
- Figge, R.M., Easter, J., and Gober, J.W. (2003). Productive interaction between the chromosome partitioning proteins, ParA and ParB, is required for the progression of the cell cycle in Caulobacter crescentus. Mol. Microbiol. 47, 1225–1237.
- Fogel, M.A., and Waldor, M.K. (2006). A dynamic, mitotic-like mechanism for bacterial chromosome segregation. Genes Dev. 20, 3269–3282.
- Graham, T.G.W., Wang, X., Song, D., Etson, C.M., Oijen, A.M. van, Rudner, D.Z., and Loparo, J.J. (2014). ParB spreading requires DNA bridging. Genes Dev. 28, 1228–1238.
- Gruber, S., and Errington, J. (2009). Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in B. subtilis. Cell 137, 685–696.
- Ireton, K., Gunther, N.W., and Grossman, A.D. (1994). spo0J is required for normal chromosome segregation as well as the initiation of sporulation in Bacillus subtilis. J. Bacteriol. 176, 5320–5329.
- Jakimowicz, D., Chater, K., and Zakrzewska-Czerwińska, J. (2002). The ParB protein of Streptomyces coelicolor A3(2) recognizes a cluster of parS sequences within the origin-proximal region of the linear chromosome. Mol. Microbiol. 45, 1365–1377.
- Jecz, P., Bartosik, A.A., Glabski, K., and Jagura-Burczy, G. (2015). A single parS sequence from the cluster of four sites closest to oriC is necessary and sufficient for proper chromosome segregation in Pseudomonas aeruginosa. PloS One 10, e0120867.
- Lagage, V., Boccard, F., and Vallet-Gely, I. (2016). Regional Control of Chromosome Segregation in Pseudomonas aeruginosa. PLOS Genet. 12, e1006428.
- Le, T.B., Imakakev, M.V., Mirny, L.A., and Laub, M.T. (2013). High-resolution mapping of the spatial organization of a bacterial chromosome. Science 342, 731–734.
Leonard, T.A., Butler, P.J., and Löwe, J. (2005). Bacterial chromosome segregation: structure and DNA binding of the Soj dimer—a conserved biological switch. EMBO J. 24, 270–282.

Lim, H.C., Surovtsev, I.V., Beltran, B.G., Huang, F., Bewersdorf, J., and Jacobs-Wagner, C. (2014). Evidence for a DNA-relay mechanism in ParABS-mediated chromosome segregation. ELife 3, e02758.

Lin, D.C.-H., and Grossman, A.D. (1998). Identification and Characterization of a Bacterial Chromosome Partitioning Site. Cell 92, 675–685.

Livny, J., Yamaichi, Y., and Waldor, M.K. (2007). Distribution of Centromere-Like parS Sites in Bacteria: Insights from Comparative Genomics. J. Bacteriol. 189, 8693–8703.

Minnen, A., Attaiech, L., Thon, M., Gruber, S., and Veening, J.-W. (2011). SMC is recruited to oriC by ParB and promotes chromosome segregation in Streptococcus pneumoniae. Mol. Microbiol. 81, 676–688.

Mohl, D.A., and Gober, J.W. (1997). Cell cycle-dependent polar localization of chromosome partitioning proteins in Caulobacter crescentus. Cell 88, 675–684.

Mohl, D.A., Easter, J., and Gober, J.W. (2001). The chromosome partitioning protein, ParB, is required for cytokinesis in Caulobacter crescentus. Mol. Microbiol. 42, 741–755.

Murray, H., Ferreira, H., and Errington, J. (2006). The bacterial chromosome segregation protein Spo0J spreads along DNA from parS nucleation sites. Mol. Microbiol. 61, 1352–1361.

Sanchez, A., Cattoni, D.I., Walter, J.-C., Rech, J., Parmeggiani, A., Nollmann, M., and Bouet, J.-Y. (2015). Stochastic Self-Assembly of ParB Proteins Builds the Bacterial DNA Segregation Apparatus. Cell Syst. 1, 163–173.

Schwartz, M.A., and Shapiro, L. (2011). An SMC ATPase mutant disrupts chromosome segregation in Caulobacter. Mol Microbiol 82, 1359–1374.

Sullivan, N.L., Marquis, K.A., and Rudner, D.Z. (2009). Recruitment of SMC by ParB-parS Organizes the Origin Region and Promotes Efficient Chromosome Segregation. Cell 137, 697–707.

Taylor, J.A., Pastrana, C.L., Butterer, A., Pernstich, C., Gwynn, E.J., Sobott, F., Moreno-Herrero, F., and Dillingham, M.S. (2015). Specific and non-specific interactions of ParB with DNA: implications for chromosome segregation. Nucleic Acids Res. 43, 719–731.

Thanbichler, M., and Shapiro, L. (2006). MipZ, a Spatial Regulator Coordinating Chromosome Segregation with Cell Division in Caulobacter. Cell 126, 147–162.

Toro, E., Hong, S.-H., McAdams, H.H., and Shapiro, L. (2008). Caulobacter requires a dedicated mechanism to initiate chromosome segregation. Proc. Natl. Acad. Sci. 105, 15435–15440.

Tran, N.T., Laub, M.T., and Le, T.B. (2017). SMC Progressively Aligns Chromosomal Arms In Caulobacter crescentus But Is Antagonized By Convergent Transcription. BioRxiv 125344. (Accepted in Cell Reports)

Vecchiarelli, A.G., Hwang, L.C., and Mizuuchi, K. (2013). Cell-free study of F plasmid partition provides evidence for cargo transport by a diffusion-ratchet mechanism. Proc. Natl. Acad. Sci. U. S. A. 110, E1390-1397.

Vecchiarelli, A.G., Neuman, K.C., and Mizuuchi, K. (2014). A propagating ATPase gradient drives transport of surface-confined cellular cargo. Proc. Natl. Acad. Sci. U. S. A. 111, 4880–4885.

Viollier, P.H., Thanbichler, M., McGrath, P.T., West, L., Meewan, M., McAdams, H.H., and Shapiro, L. (2004). Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. Proc Natl Acad Sci U A 101, 9257–9262.
Figure 1. ParB occupies 10 kb DNA region near the origin of replication

(A) The distribution of FLAG-tagged ParB on Caulobacter chromosome between +4030 kb and +4042 kb. ChIP-seq signals were reported as the number of reads at every nucleotide along the genome (RPBPM value). The whole-genome ChIP-seq profile of ParB is shown in the inset. For the whole genome profile, the ChIP-seq signals were reported as the number of reads at every kb along the genome (RPKPM value).

(B) ChIP-seq profile of FLAG-tagged YFP.

(C) ChIP-seq profile of FLAG-tagged ParB (G101S) mutant.

(D) IDAP-seq profile of ParB-(His)6 with sonication-fragmented genomic DNA from Caulobacter. IDAP-seq reads were sorted to either the upper strand (red) or to the lower strand (blue) of the reference genome to enable identification of parS sites (see also Fig. 2 and Fig. S3). Putative parS sites (1 to 7) are noted with asterisks (see also Fig. 2).

(E) IDAP-seq profile of a negative control in which ParB-(His)6 was omitted.
Figure 2. Identification of parS sequences by in vitro DNA purification with deep sequencing (IDAP-seq)

Sequencing reads were sorted to either the upper DNA strand (red) or to the lower strand (blue) of the Caulobacter reference genome, as suggested in the original IDAP-seq publication (Belitsky and Sonenshein, 2013, Fig. S3). The sequence in between the summit of the upper strand profile and that of the lower strand profile defines the parS sequence required for binding to ParB in vitro (See also Fig. S3). (A) IDAP-seq profile of ParB-(His)_6 in the genomic region between +4031 kb and +4039 kb. (B-G) IDAP-seq profile of ParB-(His)_6 surrounding each individual parS site. Palindromic nucleotides within the identified parS site are shaded in orange and green.
Surface Plasmon Resonance (SPR) was used to measure binding affinity of ParB (50 nM, 200 nM and 500 nM) to 24-bp double-stranded DNA that contains individual putative parS site. The level of ParB binding to DNA was expressed as a percentage of the theoretical maximum response, Rmax, assuming a single ParB dimer binding to one immobilized double-stranded DNA oligomer. This normalization process enabled the various responses to be readily compared, irrespective of the quantity length of the DNA tethered on an SPR chip surface. A wider range of ParB concentration (6.25 nM, 12.5 nM, 25 nM, 50 nM, 100 nM, 200 nM, 400 nM, 600 nM and 800 nM) was used to estimate the binding constant (Kd) of ParB to individual parS site (Fig. S4). The sequences of parS are shown with palindromic nucleotides shaded in orange and green. Convergent arrows on top of parS sequence indicate that parS sites are palindromic. Thicker arrow signifies that the second half of parS sequences (GTGAAA, in green) is conserved among Caulobacter parS sites.

Figure 3. ParB-parS in vitro binding affinities correlate to their in vivo ChIP-seq enrichment

Surface Plasmon Resonance (SPR) was used to measure binding affinity of ParB (50 nM, 200 nM and 500 nM) to 24-bp double-stranded DNA that contains individual putative parS site. The level of ParB binding to DNA was expressed as a percentage of the theoretical maximum response, Rmax, assuming a single ParB dimer binding to one immobilized double-stranded DNA oligomer. This normalization process enabled the various responses to be readily compared, irrespective of the quantity length of the DNA tethered on an SPR chip surface. A wider range of ParB concentration (6.25 nM, 12.5 nM, 25 nM, 50 nM, 100 nM, 200 nM, 400 nM, 600 nM and 800 nM) was used to estimate the binding constant (Kd) of ParB to individual parS site (Fig. S4). The sequences of parS are shown with palindromic nucleotides shaded in orange and green. Convergent arrows on top of parS sequence indicate that parS sites are palindromic. Thicker arrow signifies that the second half of parS sequences (GTGAAA, in green) is conserved among Caulobacter parS sites.
Figure 4. *Caulobacter* ParB binds to *parS* and spreads to flanking DNA in a heterologous *E. coli* host

A cassette composed of individual *parS* (red line) site and an apramycin resistance marker *aac(3)IV* was inserted at the *yybD* locus on an *E. coli* chromosome. T18-ParB (WT) (black) or T18-ParB (G101S) (blue) were expressed from an IPTG-inducible promoter, and their distribution on the *E. coli* chromosome were determined by α-T18 ChIP-seq. ChIP-seq signals were reported as the number of reads at every nucleotide along the genome (RPBPM value). A cassette composed of a scrambled *parS* site 3 and an apramycin resistance marker was also inserted at the *yybD* locus and serves as a negative control.
Figure 5. Plasmid-borne parS reduces the fitness of Caulobacter
Low-copy number plasmid harbouring individual parS site was conjugated from E. coli S17-1 to wild-type Caulobacter. The same number of E. coli and Caulobacter cells were used for each conjugation. A ten-fold serial dilution was performed and spotted on PYE plates supplemented with both nalidixic acid and kanamycin or just with nalidixic acid. Addition of kanamycin enforces the retention of parS plasmid, while omitting kanamycin allows plasmid loss. All cells were spotted on the same +kanamycin or -kanamycin plates, and pictures were taken after 3 day incubation at 30°C.
Figure 6. The position of an ectopic parS on the chromosome is critical for the fitness of Caulobacter

Micrograph of parB::cfp-parB Caulobacter cells (A) without an extra ectopic parS^{3+4}, (B) with an extra ectopic parS^{3+4} at +200 kb, (C) at +1000 kb, or (D) at +1800 kb. Cell length of an exponentially-growing cells were quantified and presented as histograms. Vertical dotted red lines indicate the mean cell length. The number of CFP-ParB foci (green) per cell was also quantified and plotted as histograms. Note that we could not observe foci corresponding to an extra ectopic parS^{3+4} perhaps due to the limited numbers of ParB bound to this shorter cluster. Most observable foci are likely due to the original parS^{1-7} cluster that reside ~8 kb near ori.
Figure 7. Tn5-seq reveals the positional bias of the centromeric parS site on Caulobacter chromosome

(A) Wild-type Caulobacter cells were mutagenized with the parS⁺ or parS⁻ transposon, and the number of insertions was binned to 10-kb segments along the genome. The ratio between insertion frequency for the parS⁺ transposon and that of the parS⁻ transposon was calculated and plotted as a log10 scale against genomic position. Two hotspots for insertion of the parS⁺ transposon are marked with asterisks (*). The vertical dotted line (black) shows the position of the native parS cluster. The horizontal bar (orange) indicates the permissive zone for extra parS insertions.

(B-C) Comparison between parS⁺ (blue) and parS⁻ (black) transposon insertions for the genomic segment between +4025 kb and +4043 kb, and between +158 kb and +175 kb.

(D) parS⁺/parS⁻ Tn5-seq profiles for Flip1-5 (blue) and Flip 2-5 (orange) strains. The horizontal axis represents genome position in kilobases for each strain. A schematic genomic map of Caulobacter showing the position of parS and ori are presented in the inset. The inverted DNA segment (green arrow) is indicated together with the end points of the inversion (1, 2, and 5).
Figure S1. Genomic distributions of wild-type Caulobacter ParB and spreading-defective ParB (G101S) and ParB (R104A) variants. (A) Caulobacter strains parB::P_{xyl}-parB\_van::P_{van}-flag-parB WT, G101S, or R104A were restruck on PYE + xylose to induce the expression of the wild-type untagged ParB, or on PYE + glucose + vanillate to repress the expression of the wild-type untagged ParB while expressing the FLAG-tagged ParB WT, G101S, or R104A. The FLAG-tagged version of wild-type ParB is functional and can complement the depletion of wild-type untagged ParB while the spreading mutant ParB (G101S) or ParB (R1014A) cannot. (B) ChIP-seq profiles of FLAG-ParB (WT), FLAG-ParB (G101S), and (R104A) (using α-FLAG antibody), of CFP-ParB (using α-GFP antibody), and of ParB (using polyclonal α-ParB antibody). Note: the red dagger (†) symbol on the CFP-ParB ChIP-seq profile indicates the genomic region where sequencing reads were missing. This is because CFP-ParB ChIP-seq reads were mapped to the wild-type Caulobacter reference genome instead of to the genome of parB::cfp-parB strain.
Figure S2. Identification of potential spreading-defective ParB mutants in *Caulobacter crescentus*. (A) A sequence alignment of *Caulobacter* ParB and homologs to highlight the conservation of the Box II motif (square bracket). Identical amino acids residues are shown in red. Vertical arrows indicate the position of G101, R103, R104, and R106 residues. (B) Surface Plasmon Resonance (SPR) was used to measure binding affinity of ParB WT, ParB (G101S) and ParB (R104A) at 200 nM to a 24-bp double-stranded DNA that contains parS site 4. The level of ParB variants binding to DNA was expressed as a percentage of the theoretical maximum response, R_max, assuming a single ParB dimer binding to one immobilized double-stranded DNA oligonucleotides. This normalization process enabled the various responses to be readily compared, irrespective of the quantity and length of the DNA tethered on an SPR chip surface. (C) Immunoblot analysis of FLAG-tagged ParB WT vs. G101S and R104A. Cells were depleted of wild-type untagged ParB for 5 hours, then vanillate was added for an additional hour to allow for expression of FLAG-tagged ParB. Equal amount of total protein was loaded on each well of the SDS-PAGE.
Figure S3. Methodology for the analysis of in vitro affinity purification with deep sequencing (IDAP-seq) data. Sequencing reads were sorted to either the upper DNA strand (A) or to the lower strand (B) of the Caulobacter reference genome (Belitsky and Sonenshein, 2013). The 5’ nucleotides are shown as circles. Solid circles are for DNA fragments with an intact ParB binding site (black rectangle), and open circles are for DNA fragments with a partial or no ParB binding site. Only DNA fragments with an intact ParB binding site will be pulled down during affinity purification and contribute to the sequencing coverage. (C) A schematic strand-specific coverage map of IDAP-seq. A footprint of ParB can be identified in between the two edges of the upper-strand peak (red) and the lower-strand peak (blue). The schematic picture was adapted from Belitsky and Sonenshein (2013) with permission from A. Sonenshein.
Figure S4. Determination of binding affinity constants ($K_d$) of ParB-$parS$ interactions.

24-bp duplex DNA containing each individual $parS$ was tethered on an SPR chip surface. Increasing concentrations of ParB (6.25 nM, 12.5 nM, 25 nM, 50 nM, 100 nM, 200 nM, 400 nM, 600 nM and 800 nM) were flown through the SPR chip surface. Binding of ParB to $parS$ site was recorded and expressed as response unit (RU). Response units were plotted against ParB concentration and curve fitted to estimate $K_d$ value ± standard deviation. $K_d$ for $parS$ site 1 and site 6 were not determined due to very little specific binding of ParB to the tested DNA.

$K_d = 30 ± 3$ nM
$K_d = 62 ± 4$ nM
$K_d = 133 ± 17$ nM
$K_d = 144 ± 28$ nM
$K_d = 170 ± 28$ nM
$K_d$ (ND)
Figure S5. T18-ParB is functional in Escherichia coli. (A) A ParB protein was expressed from an IPTG-inducible promoter as a C-terminal fusion to the T18 fragment of Bordetella pertussis adenylate cyclase. Known interacting partners of ParB were expressed as fusion proteins to the T25 fragment of B. pertussis: T25-ParB, ParA-T25 and MipZ-T25. Interactions between ParB and partners were assessed on a solid MacConkey agar or by β-galactosidase assay. Three biological replicates were performed for each pair of interacting partners. A negative control (T25 fragment alone) and a positive control: T25-ZIP and T18-ZIP were also included. (B) ChIP-seq profiles of T18-ParB at parS site 4, site 6 and site 1 in an E. coli heterologous host. T18-ParB protein was expressed by addition of 500 µM IPTG for an hour before fixing with formaldehyde for ChIP-seq. DNA bound to T18-ParB was immunoprecipitated using α-T18 conjugated sepharose beads. A scrambled parS site 3 was also inserted at the ybbD locus to serve as a negative control. ChIP-seq signals were reported as the number of reads at every nucleotide along the genome (RPBPM value).
Figure S6. Tn5-seq reveals the positional bias of the centromeric parS site on Caulobacter chromosome. (A) A comparison of parS+ (blue) and parS- (black) transposon insertions for the genomic segment between +0 kb and +168 kb (ori proximal). (B) A comparison of parS+ (blue) and parS- (black) transposon insertions for the genomic segment between +9912 kb and +1008 kb (mid-arm). (C) A comparison of parS+ (blue) and parS- (black) transposon insertions for the genomic segment between +1991 kb and +2008 kb (ter proximal). Axes labeled in purple color highlights different scaling. (D) Wild-type (black) or Δsmc (red) Caulobacter cells were mutagenized with parS+ or parS- transposon, and the number of insertions was binned to 10-kb segments along the Caulobacter chromosome. The ratio between insertion frequency for parS+ transposon and that of parS- transposon was calculated and plotted as a log10 scale against genomic position.