Supporting Information

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SI Materials and Methods

Strains and Strain Construction. ***ZG192.*** For ZG192, a kan^r *mcherry-parB* clone was introduced into the *xylX* locus in CB15N. A tet^r *GFP-ParA* was then subsequently introduced into the *xylX* locus. Details about the construction of the *mcherry-parB* plasmid are described in ref. 1. To create the tet^r *pxyl-GFP-parA* construct, a Gateway (Invitrogen) compatible vector was created by cloning the Gateway cassette into the MCS of pXGFP4-C1. A subsequent Gateway LR reaction was performed using a *parA* entry vector (1) to create the *pxyl-GFP-parA* fusion. The resistance of this plasmid was then change from kanamycin to tetracycline using the EZ-Tn5 <TET-1> insertion kit (Epicenter Biotechnologies #EZI921T).

ZG194. For ZG194, an *mCherry-dnaN* fusion was introduced into the *xylX* locus in CB15N. Details of the *mCherry-dnaN* construct are described in ref. 1. This strain was modeled on the work from Collier and Shapiro (2).

ZG195. For ZG195, first wild-type *parA* was amplified and cloned into pET28. The $parA_{K20R}$ mutation was created using the QuikChange Site-Directed Mutagenesis Kit (Stratagene #200518), with the following primers: F-5′ CAAAAGGGTGGGGTGGGGAGGACCACGACCGCGATCAAT and R-5′ ATTGATCGCGGTCGTGGTCCTCCCCACCCCACCCTTTTG. The $parA_{K20R}$ ORF was then PCR amplified with Gateway compatible primers and Gateway cloned into gXRC (1). This mutant was based on a previously characterized mutant (3).

1. Werner JN, et al. (2009) Quantitative genome-scale analysis of protein localization in an asymmetric bacterium. *Proc Natl Acad Sci USA* 106:7858–7863.
2. Collier J, Shapiro L (2009) Feedback control of DnaA-mediated replication initiation by replisome-associated HdaA protein in *Caulobacter*. *J Bacteriol* 191:5706–5716.
3. Toro E, Hong SH, McAdams HH, Shapiro L (2008) *Caulobacter* requires a dedicated mechanism to initiate chromosome segregation. *Proc Natl Acad Sci USA* 105:15435–15440.

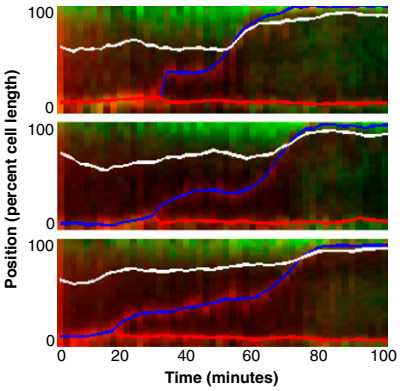


Fig. S1. ParA retracts upon ParB association and appears to pull ParB to the distal pole. Kymographs of three individual cells coexpressing GFP-ParA and mCherry-ParB. The kymographs are a composite of the cell's GFP-ParA (green) and mCherry-ParB (red) fluorescence intensities at 2-min intervals over a 100-min time-course. The red and blue curves display the positions of the proximal (red) and distal (blue) mCherry-ParB foci. The white line indicates a normalized threshold of GFP-ParA intensity (see *Materials and Methods* for details).

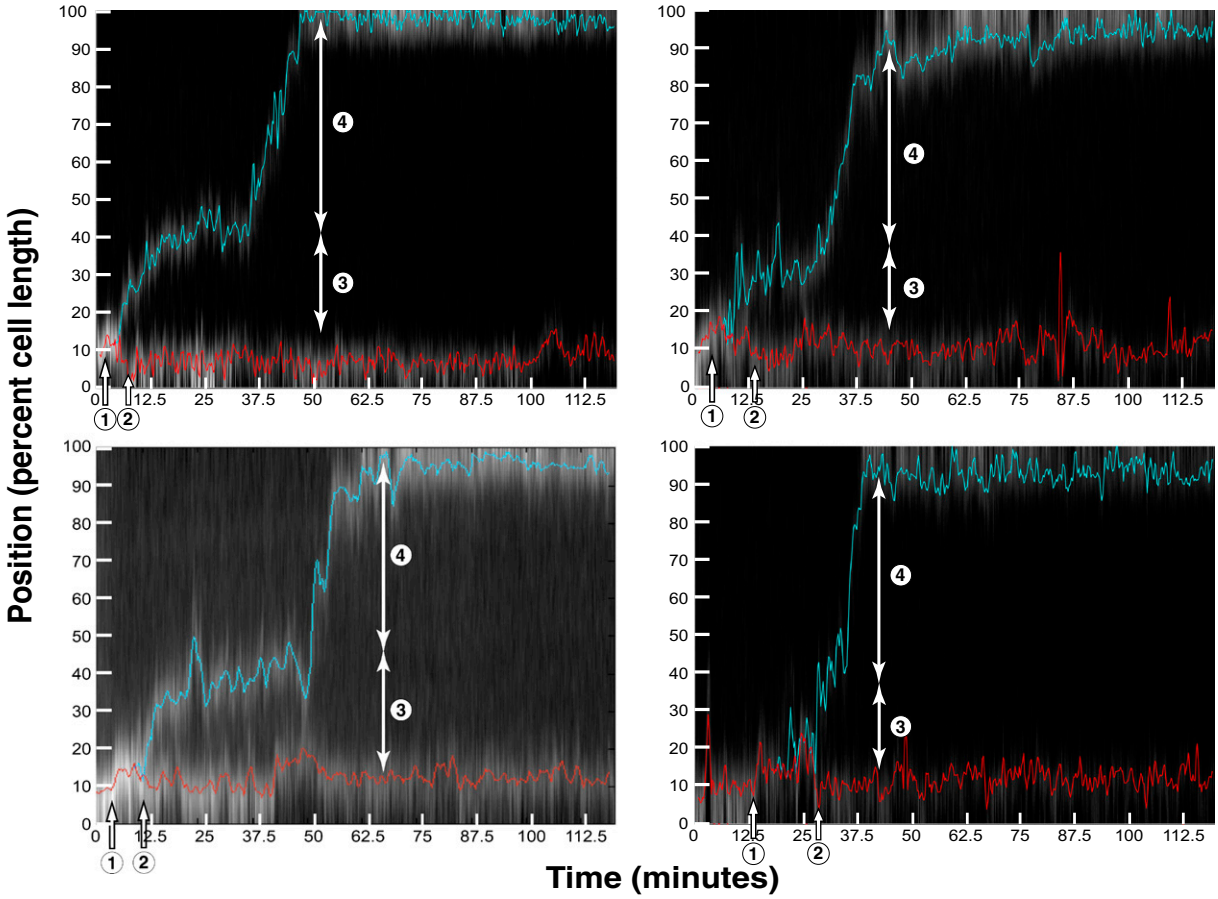


Fig. S2. Four distinct classes of motion can be observed in most segregating *Caulobacter* origins of replication (*oris*). Kymographs of four individual cells tracking the GFP-ParB-labeled *ori*. The kymographs are a composite of the cell's fluorescent intensity at each 15-s time-point over the 2-h time-course. PSICIC image analysis was used to detect and track the proximal (red) and distal (blue) GFP-ParB foci. The steps of *ori* translocation are labeled: 1, polar release; 2, polar retraction; 3, early translocation; and 4, late translocation.

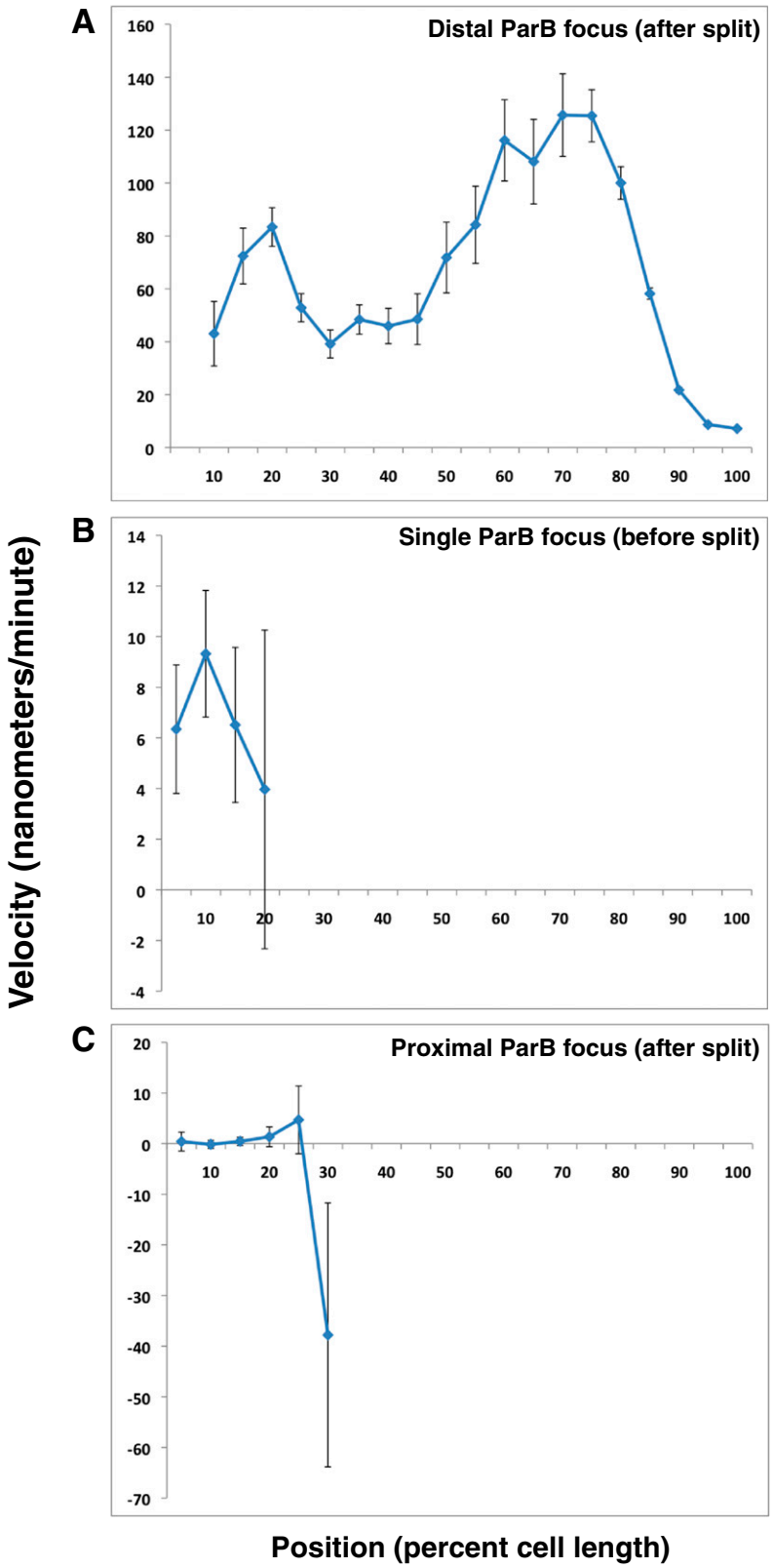


Fig. S3. Population-scale visualization of the four classes of motion. Shown are plots of average GFP-ParB instantaneous velocities as a function of relative cell position in bins of 5% of cell length (error bars = SEM). Velocities are based on mean squared displacement (values are positive when oriented toward the distal pole and negative when oriented toward the proximal pole). For each cell, GFP-ParB was separately examined both before and after two foci could be resolved (the point of ParB splitting). (*A*) Velocities of only the distal-most focus after ParB splitting, showing two peaks that represent early and late translocation. (*B*) Velocities of only the ParB focus before splitting, showing the positive velocity of polar release. (*C*) Velocities of only the proximal-most focus after ParB splitting, showing the negative velocity of polar retraction.

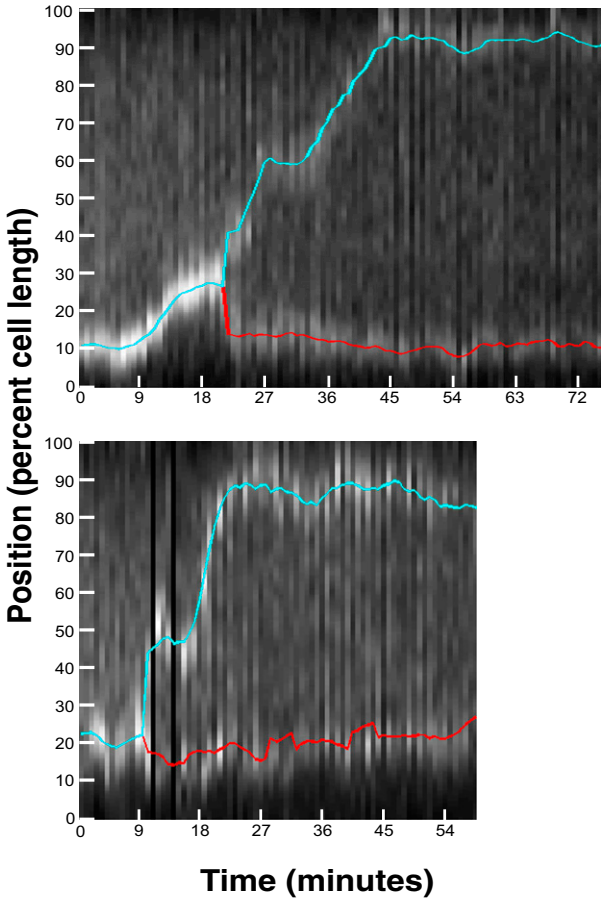


Fig. S4. A different *ori*-labeling method showed similar segregation stages. Kymographs of two individual cells tracking the LacO/LacI-CFP labeled *ori*. The kymographs are a composite of the cell's fluorescent intensity at each 45-s time-point. PSICIC image analysis was used to detect and track the proximal (red) and distal (blue) LacI-CFP foci.

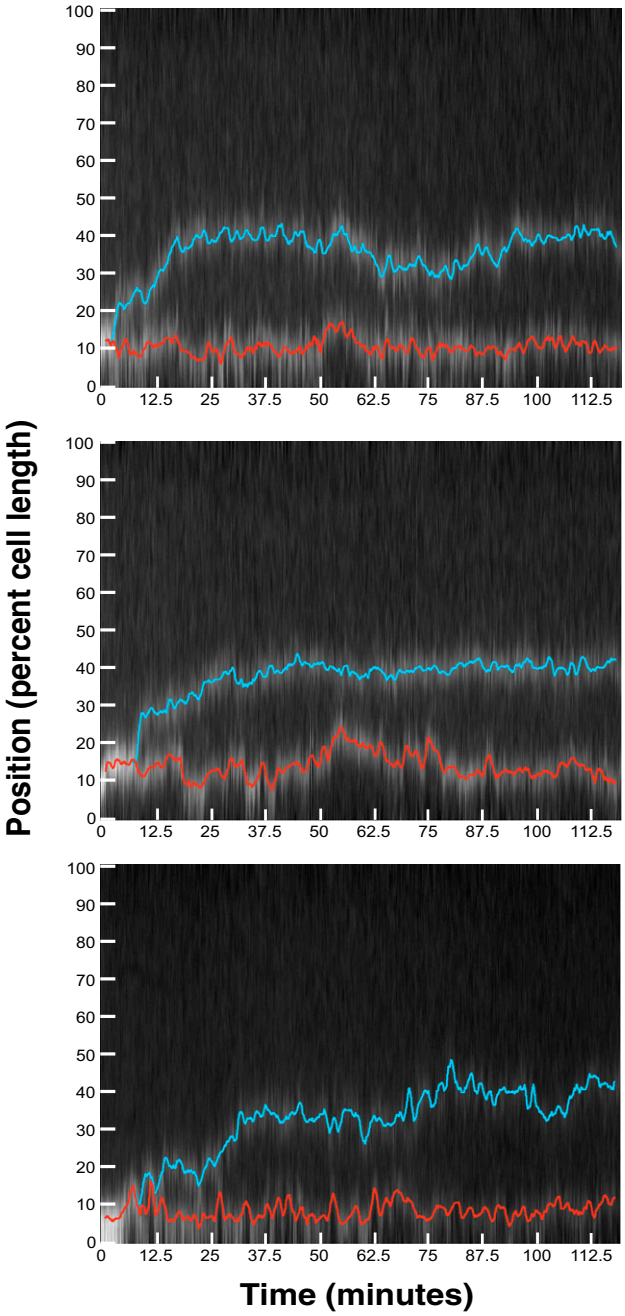


Fig. S5. Perturbing ParA specifically disrupts the late translocation step of *ori* motion. Shown are kymographs tracking the GFP-ParB-labeled *ori* in three individual cells expressing $ParA_{K20R}$-mCherry. The kymographs are a composite of the cell's fluorescent intensity at each 15-s time-point over the 2-h time-course. PSICIC image analysis was used to detect and track the proximal (red) and distal (blue) GFP-ParB foci. Note that in the cells expressing $ParA_{K20R}$-mCherry, GFP-ParB is translocated to the point where late translocation normally begins and then stably remains near midcell.

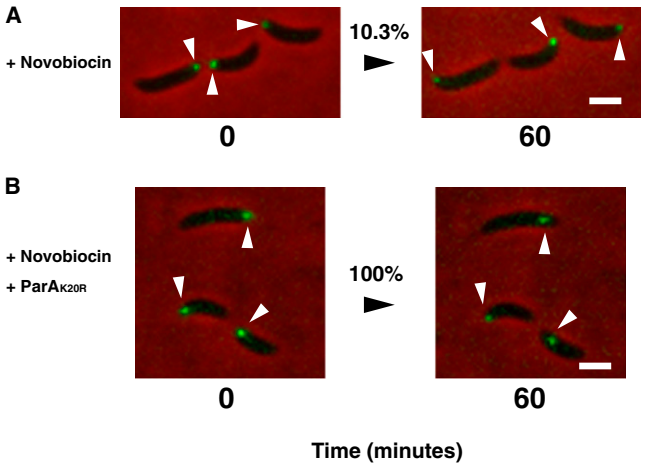


Fig. S6. Single GFP-ParB foci (denoted by arrowheads) can translocate from the distal to the proximal cell pole. (*A*) Images of cells with GFP-ParB labeled *ori* grown in the presence of novobiocin at the beginning of the cell cycle (*Left*) and after 60 min (*Right*). Of the cells imaged, 10.3 ± 2.3% exhibited this aberrant translocation. (*B*) Images of cells with GFP-ParB labeled *ori* expressing ParA$_{K20R}$-mCherry and grown in the presence of novobiocin at the beginning of the cell cycle (*Left*) and after 60 min (*Right*). No cells exhibited aberrant translocation. (Scale bar, 1 μm.)

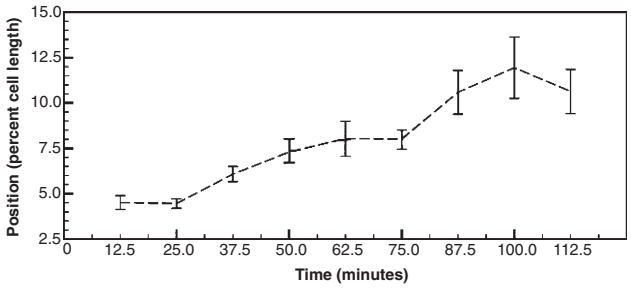
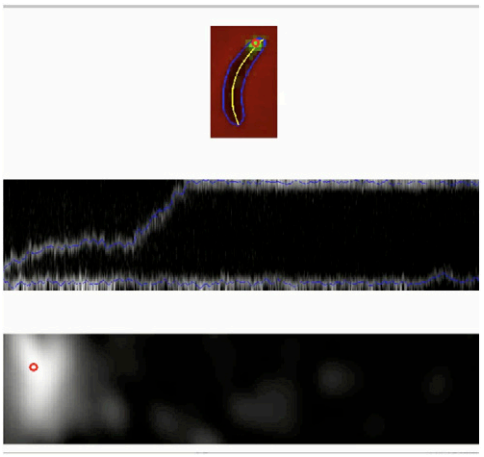


Fig. S7. Polar release occurs in the presence of the DNA replication inhibitor novobiocin. Shown is a plot of the average distance (mean ± SEM) of GFP-ParB labeled *oris* from the proximal cell pole as a function of time in cells grown in the presence of 5 μg/mL novobiocin.

Table S1. Strains

Strain	Genotype	Reference
MT174	parB::gfp-parB	(1)
ZG192	xylX::gfp-parA, mCherry-parB	Present study
ZG194	xylX::mCherry-dnaN	Present study
ZG195	parB::gfp-parB, xylX::parA(k20r)-mCherry	Present study
LS3833	CC0006::LacO, xylX::lacI-cfp	(2)

1. Thanbichler M, Shapiro L (2006) MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. *Cell* 126:147–162.
2. 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.



Movie S1. A movie illustrating the analysis pipeline with a representative cell including the raw image (*Top*), 1D PSICIC tracking (*Middle*), and 2D representation (*Bottom*).

Movie S1