# Supporting Information

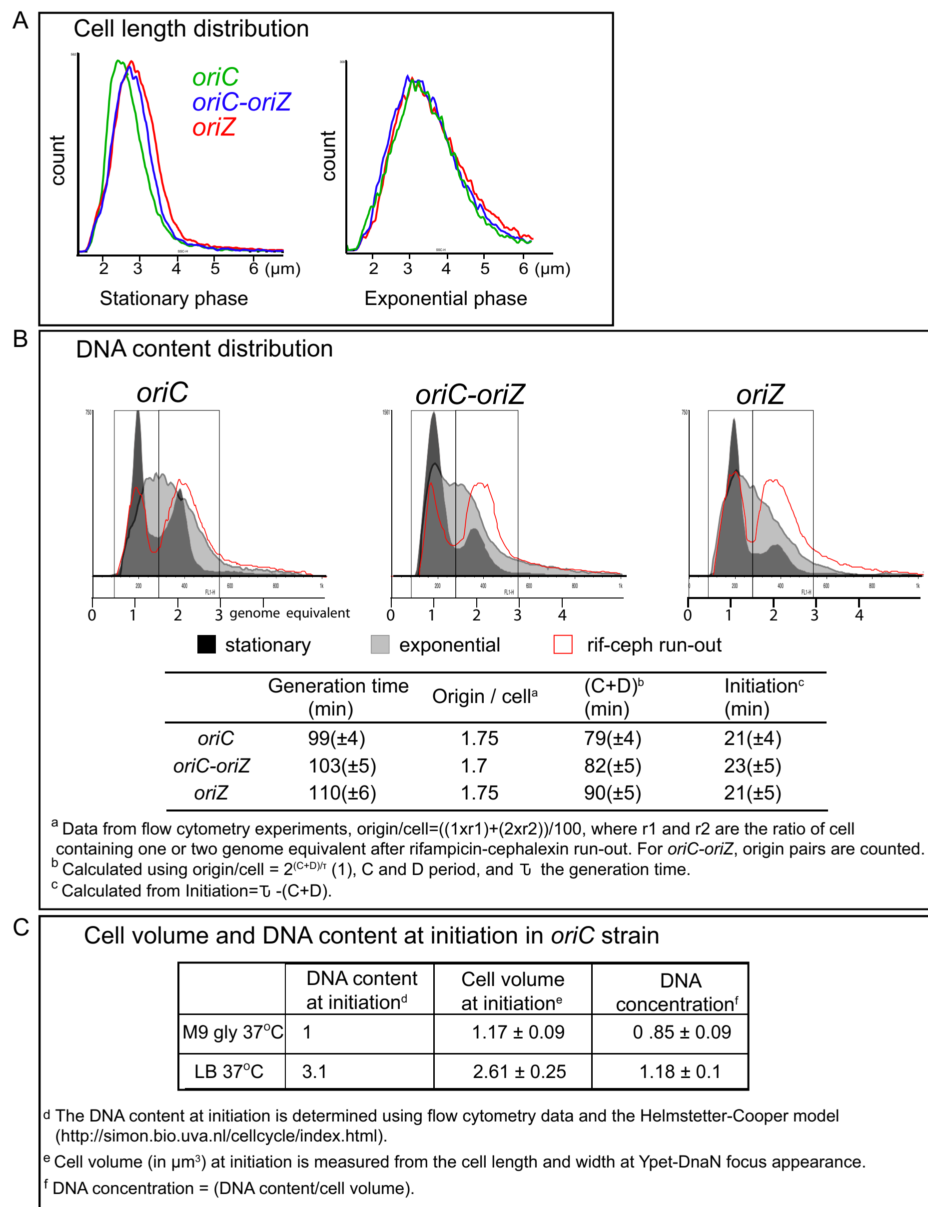
## Wang et al. 10.1073/pnas.1100874108

### SI Materials and Methods

**Fluorescent Labeling of Newly Replicated DNA in Synchronized Cells.** *dnaC2* strains carrying only WT *oriC* and *oriC-oriZ*, respectively, were grown in M9 glycerol medium at 30 °C to an OD of approximately 0.1 and synchronized at 42 °C for 3 h. EdU and dAdenosine was added to a final concentration of 20 and 100 μg/mL respectively and then cultures were transferred to 30 °C. Cells were fixed by adding cold methanol to a final concentration of 72% at 8 or 15 min after temperature shift. Labeling of new DNA was done using a modified protocol described by Ferullo et al. (1). Cells were subsequently pelleted and washed twice with filtered PBS solution, and incubated at room temperature for 30 min in Click-iT reaction mixture (Invitrogen) containing Alexa Fluor 594. Cells were washed twice in PBS solution and spotted on 1% agarose pads for microscopy.

**Introduction of the *C. crescentus* Partitioning System into *E. coli*.** To introduce the *C. crescentus* partitioning system into the *E. coli* chromosome, the 0.6-kb fragment in *gidA* promoter region containing the *parS* cluster (2) from WT *C. crescentus* CB15N was cloned into a plasmid and subsequently inserted into *E. coli yieM* locus, which is 2.3 kb from *E. coli oriC*, using λ-red recombination. PopZ-YFP was expressed from a plasmid with pBR322 origin under the control of arabinose promoter (pGB268) (3). CFP-ParB (cloned from pGB331) (2) and ParA was expressed from a p15A plasmid under the control of rhamnose promoter. L-arabinose 0.2% and/or 0.02% L-rhamnose was added for induction.


1. Ferullo DJ, Cooper DL, Moore HR, Lovett ST (2009) Cell cycle synchronization of Escherichia coli using the stringent response, with fluorescence labeling assays for DNA content and replication. *Methods* 48:8–13.
2. Thanbichler M, Shapiro L (2006) MipZ, a spatial regulator coordinating chromosome segregation with cell division in Caulobacter. *Cell* 126:147–162.
3. Bowman GR, et al. (2008) A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. *Cell* 134:945–955.




| | Generation time (min) | Origin / cell[a] | (C+D)[b] (min) | Initiation[c] (min) |
|---|---|---|---|---|
| *oriC* | 99(±4) | 1.75 | 79(±4) | 21(±4) |
| *oriC-oriZ* | 103(±5) | 1.7 | 82(±5) | 23(±5) |
| *oriZ* | 110(±6) | 1.75 | 90(±5) | 21(±5) |

[a] Data from flow cytometry experiments, origin/cell=((1xr1)+(2xr2))/100, where r1 and r2 are the ratio of cell containing one or two genome equivalent after rifampicin-cephalexin run-out. For *oriC-oriZ*, origin pairs are counted.
[b] Calculated using origin/cell = $2^{(C+D)/\tau}$ (1), C and D period, and τ the generation time.
[c] Calculated from Initiation=τ -(C+D).

| | DNA content at initiation[d] | Cell volume at initiation[e] | DNA concentration[f] |
|---|---|---|---|
| M9 gly 37°C | 1 | 1.17 ± 0.09 | 0 .85 ± 0.09 |
| LB 37°C | 3.1 | 2.61 ± 0.25 | 1.18 ± 0.1 |

[d] The DNA content at initiation is determined using flow cytometry data and the Helmstetter-Cooper model (http://simon.bio.uva.nl/cellcycle/index.html).
[e] Cell volume (in µm³) at initiation is measured from the cell length and width at Ypet-DnaN focus appearance.
[f] DNA concentration = (DNA content/cell volume).

**Fig. S1.** (*A*) Cell length distribution measured by flow cytometry of steady-state and late stationary phase cells grown in M9 minimal medium with 0.2% glycerol. *oriC*, *oriC-oriZ*, and *oriZ* strains display very similar cell length distribution in both conditions. (*B*) DNA content distribution in exponential growth and after rifampicin/cephalexin treatment (*Materials and Methods*). The stationary-phase pattern is used to calibrate the DNA content corresponding to one and two genome equivalents. The table underneath summarizes the calculations of cell cycle features. (*C*) Cell volume and DNA content at initiation in *oriC* strain calculated by using microscopy and flow cytometry data, as well as the Helmstetter–Cooper model (http://simon.bio.uva.nl/cellcycle/index.html).

1. Helmstetter CE (1996) Timing of synthetic activities in the cell cycle. *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds Neidhardt FC et al. (ASM, Washington, DC), 2nd Ed, pp 1627–1639.

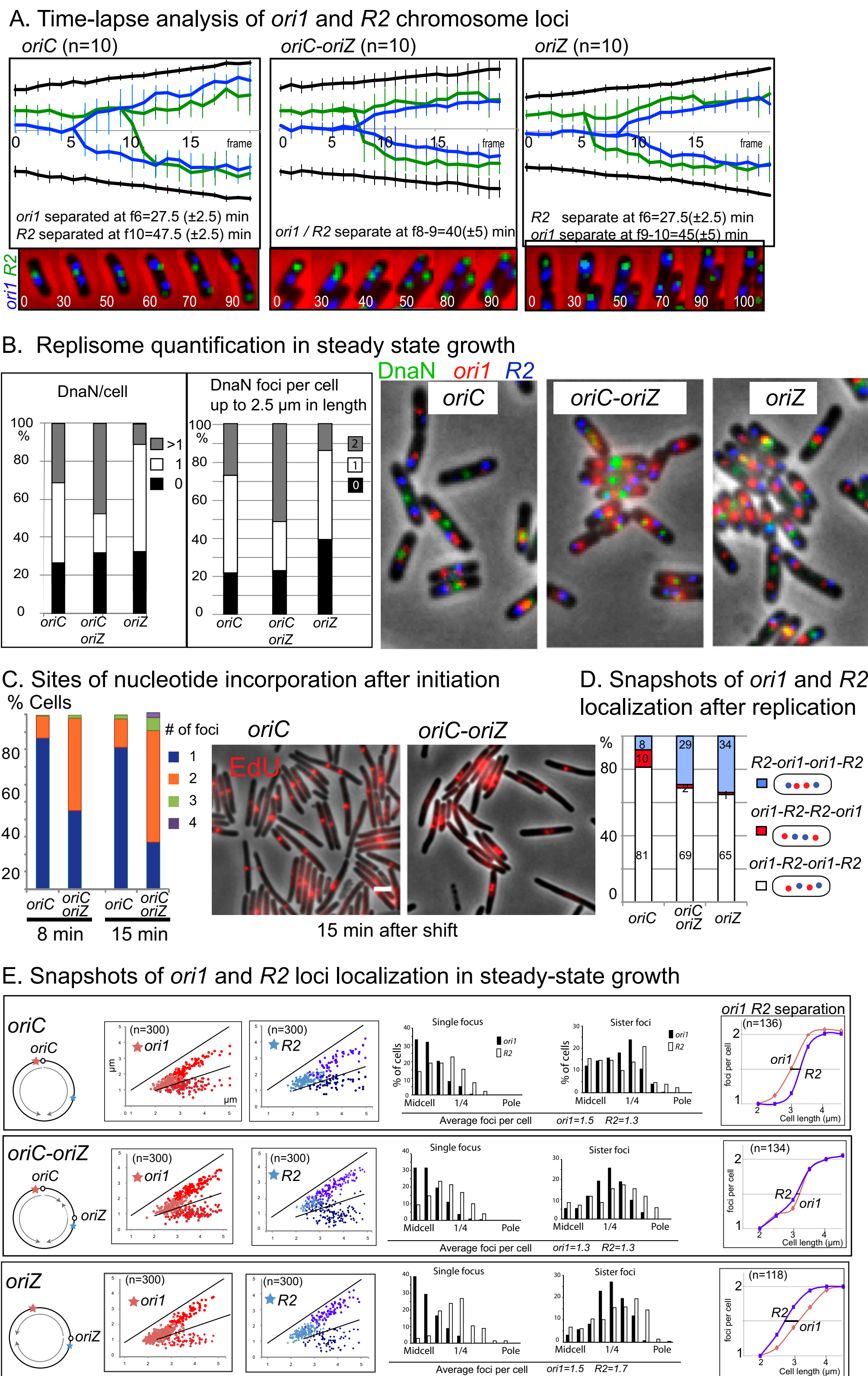



**Fig. S2.** (*A*) Time-lapse analysis of relative positioning of replisomes, *ori1* and *R2*, in *oriC*, *oriC-oriZ*, and *oriZ* strains (5 min per frame). The traces and variances were generated by using an automated particle tracking methodology (*Materials and Methods*). Cell poles (black line), *ori1* (blue line), *R2* (green line) are monitored. A representative time-lapse image set is shown below, with indicated times in minutes. If sister loci separation is observed frame X, loci separation could have occurred between X − 1 and X, leading to a 5-min uncertainty. The average timing of separation reported in Fig. 3 is then considered to be exactly between X and X − 1, with a ±2.5-min uncertainty. (*B*) Left histogram shows the number of DnaN foci per cell in steady state growth, in *oriC*, *oriC-oriZ*, and *oriZ*. Right histogram shows the number of DnaN foci per cell among cells smaller than 2.5 μm in length. Micrographs show exponentially growing cells, with simultaneous localization of DnaN-Ypet (green), *ori1* (red), and *R2* (blue). (*C*) Fluorescent labeling of newly replicated DNA. *oriC* and *oriC-oriZ* strains were

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synchronized by using *dnaC2* allele at restrictive temperature, and allowed for initiation at permissive temperature in the presence of nucleotide analogue EdU, which was later fluorescently labeled by using "click" chemistry (*SI Materials and Methods*). The proportion of cells with one to four foci is shown for *oriC* and *oriC-oriZ* strains at 8 and 15 min after release (*Left*). The data were obtained by counting more than 400 cells for each strain. Representative micrographs for both strains at 15 min after release are shown (*Right*). (Scale bar, 2 μm.) (*D*) Histogram of the relative positioning pattern of *ori1* and *R2* after replisome disappearance, observed for exponentially growing *oriC*, *oriC-oriZ*, and *oriZ* strains. (*E*) Snapshot analysis of *ori1* and *R2* loci positioning in exponential growth. The distance between cell pole and foci was measured for 300 cells and reported on the dot plots for *ori1* (red) and *R2* (blue). The graph on the right shows the average number of *ori1* and *R2* as a function of the cell size and indicates the relative separation of sister *ori1* and *R2*.

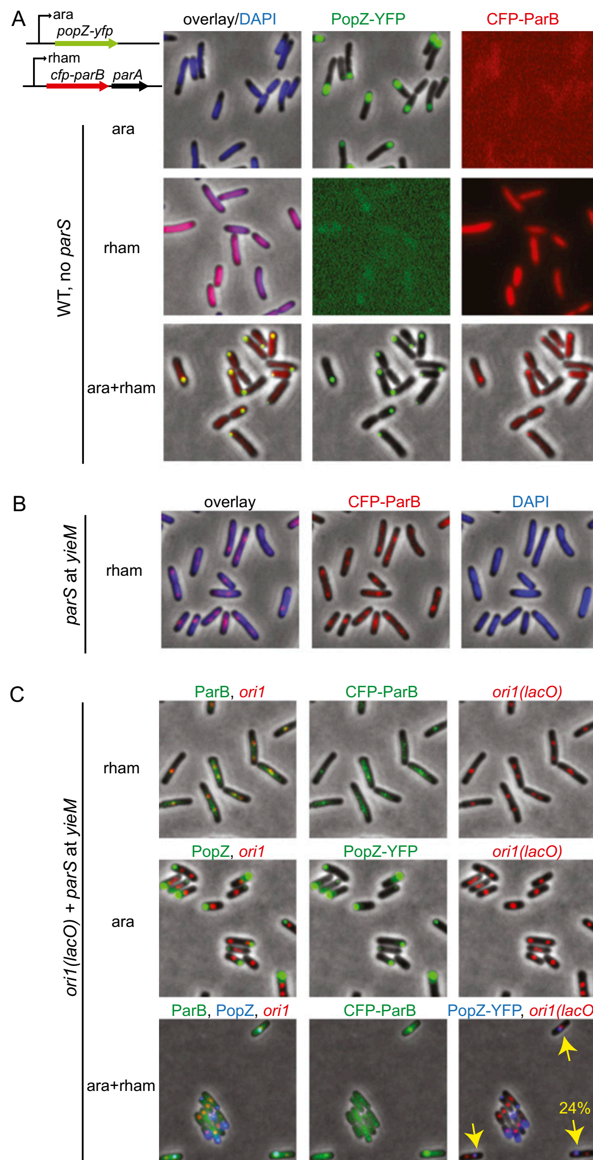


**Fig. S3.** Attempts to tether the *E. coli* origin region to a cell pole by using the *C. crescentus* PopZ-ParAB-*parS* system. (*A*) *oriC* cells were transformed with two compatible plasmids that express arabinose-controllable PopZ-YFP and rhamnose-controllable CFP-ParB and ParA (*SI Materials and Methods*). *Top*: Arabinose was added to induce PopZ-YFP (green). *Middle*: Rhamnose was added to induce CFP-ParB (red) and ParA. *Bottom*: Arabinose and rhamnose were added for induction. (*B*) AB1157 carrying *parS* sites at *yieM* (2.3 kb from *oriC*) was transformed with the two plasmids. Rhamnose was added to induce the expression of CFP-ParB (red) and ParA. DAPI stain is in blue. (*C*) AB1157 carrying *parS* sites at *yieM*, *ori1* (*lacO*), and chromosomal LacI-mCherry (red) constitutively expressed from the *dnaA* promoter was transformed with the two plasmids. *Top*: Rhamnose was added to induce the expression of CFP-ParB (green) and ParA. *Middle*: Arabinose was added to induce PopZ-YFP (green). *Bottom*: Arabinose and rhamnose were added to induce PopZ-YFP (blue) and CFP-ParB (green) and ParA. Yellow arrows show the colocalization of *ori1* (*lacO*; LacI-mCherry), PopZ-YFP, and CFP-ParB. The data show that ParB associates with PopZ at poles when *parS* sites are absent. In the presence of *parS*, 76% of cells ($n = 879$) had ParB foci bound to *parS* at the normal *oriC* position and PopZ foci at the poles bound by free ParB unassociated with *parS*. The remaining 24% of cells had additionally recruited PopZ to ParB-bound *parS* sites at the normal *oriC* position. Therefore, polar PopZ is unable to tether the *oriC* region to poles through interactions with ParB-*parS*.