Supporting Information

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SI Experimental Procedures

Bacterial Culture and Growth Conditions. *E. coli* cultures were grown in LB broth or on LB agar plates at 37 °C and *Caulobacter* cultures were grown in PYE broth or on PYE agar plates at 30 °C (1). When noted, kanamycin (Kan) was added to a final concentration of 30 μg/ml in broth and 50 μg/ml in agar (abbreviated 30:50) for *E. coli* and 25:5 for *Caulobacter*. Spectinomycin and streptomycin (Spec/Strep) were used at 50:50 for Spec and 30:30 for Strep in *E. coli*; tetracycline (Tet) at 12:12 for *E. coli* and 2:1 for *Caulobacter*; Chloramphenical (Cm) at 20:30 for *E. coli* and 1:1 for *Caulobacter*; Rifampin (Rif) at 50:50 for *E. coli*; and Nalidixic acid (Nal) at 50:50 for *Caulobacter*. Also when noted, *E. coli* cultures were induced with 1 mM IPTG and *Caulobacter* cultures were induced with 0.03% xylose.

Entry Vector Library and Gateway Destination Vector Construction. Entry vector cloning was performed by using methods and reagents from the Invitrogen Gateway cloning system. A total of 3,763 pairs of PCR primers (Operon Biotechnologies) were designed to clone the ORFs of each annotated protein in *Caulobacter crescentus* (CB15) (2). All primer sequences are available on request. Each forward and reverse primer contains a 5′ *attB* sequence for Gateway cloning and a 3′ ORF-specific sequence, enabling amplification of specific ORFs and subsequent recombination into the donor vector pDONR223 (3) *attP* sites. Each forward primer contained an ATG start codon. PCR amplification was performed in a 96-well format with KOD polymerase (Novagen) by using the manufacturer's instructions and CB15 chromosomal DNA as a template, and verified for approximate length on E-Gels (Invitrogen). The PCR products were recombined into pDONR223 by using BP Clonase II (Invitrogen) as described in the BP reaction protocol provided by Invitrogen. Following transformation of the BP reaction into *E. coli* DH5α, colony PCR was performed by using pDONR223-specific primers flanking the insert region to verify the correct size of the inserted ORF. The 5′ and 3′ ends of each ORF in its entry vector were sequenced by using M13 primers (Agencourt). BLAST analysis of the resulting quality-trimmed sequences verified that the correct and full-length ORF was present with no detectable point mutations.

Destination Vector Construction. Two destination vectors were engineered to allow xylose-inducible expression in *Caulobacter* of each ORF-encoded protein fused to mCherry at either the C terminus (gXRC) or N terminus (gXRN). gXRC was generated by digesting pXGFP4 [gift of M. R. Alley (Anacor Pharmaceuticals, Palo Alto, CA)] with NdeI and Asp-718 and ligated with a similarly digested Gateway cassette PCR product amplified from pTGW (*Drosophila* Genomics Resource Center). The GFP in the resulting construct was subsequently replaced with mCherry by digesting with NotI and Asp-718 and ligating with a similarly digested mCherry PCR product amplified from pmCherry (Clontech). gXRN was created by digesting pXGFP4-C1 (gift of M. R. Alley) with BglII and Asp-718 and ligating with a similarly digested Gateway cassette PCR product amplified from pTGW. The GFP was replaced in the resulting construct by digesting with BglII and NdeI and ligating with a similarly digested mCherry PCR product.

In Vivo Left-Right (LR) Reaction Procedure. To create the inducible N- or C-terminal fusion expression vectors containing each *Caulobacter* ORF, a high-throughput "in vivo LR" gene-transfer method was developed by building on previous studies with the *xis* and *int* genes that mediate the LR recombination reaction (4, 5). First, plasmids from the entry clone library (Spec/Strep-resistant) were isolated by using the Direct-prep 96-well miniprep kit (Qiagen) and transformed into an *E. coli* strain possessing a plasmid (pXINT129; Kan^R) containing IPTG-inducible copies of the *int* and *xis* genes that mediate the LR reaction (4). LB containing Kan, Spec, and Strep was used to select for transformants. To perform the in vivo LR, these strains were combined in 96-well format with a $CcdB^R$ *E. coli* strain containing either gXRC or gXRN, LS980 (conjugation helper strain), and a Rif^R DH5α *E. coli* strain. In this mixture, the destination vector is the only Kan^R vector that can be mobilized into the Rif^R acceptor, but because the acceptor is $CcdB^S$, this mobilization kills the acceptor unless the destination vector's *ccdB*-containing Gateway cassette has been replaced with the entry vector's ORF. The mixture was pinned onto LB plates containing IPTG by using a 48-pin frogger (Dan-Kar Corporation) and incubated at 37 °C overnight. The bacteria were then transferred, using the frogger, to LB broth containing Kan and Rif. This strategy yields a 99% success rate of generating transformants without the need for purified recombination reagents.

Conjugation into *Caulobacter*. After overnight growth at 37 °C, the expression vectors were conjugated into *Caulobacter* CB15N by triparental mating. Briefly, strains were washed once with LB and mixed with LS980 and CB15N at a ratio of 1:1:1, pinned onto PYE plates, and allowed to incubate 48 h at 30 °C. Each spot was restreaked for individual colonies on PYE plates containing Kan and Nal and incubated for 48 h at 30 °C. This purification step was repeated to ensure an isogenic population of *Caulobacter* containing the expression vector. To verify that the *Caulobacter* strains contained expression vectors with inserts in the Gateway cassette, colonies were patched onto PYE plates containing either Kan and Cm or Kan alone. Strains possessing expression vectors became Cm^S because of replacement of the Cm^R gene in the Gateway cassette with the entry vector ORF. These expression vector-containing *Caulobacter* strains were grown in PYE broth containing Kan overnight and stored at −80 °C in PYE broth containing 20% glycerol.

High-Throughput Imaging. Overnight cultures of the CB15N strains containing expression vectors were diluted 1:15 in PYE broth containing Kan, grown 2 h, induced with xylose, and subsequently grown for an additional 2 h (final OD_{660} = 0.4–0.5). Samples were placed on 48-pedestal slides (1% agarose) and fields of ≈50–200 cells were chosen for imaging. All imaging was performed with a Nikon90i epifluorescent microscope equipped with a 100 × 1.4 NA objective (Nikon), Rolera XR cooled CCD camera (QImaging), and NIS Elements software (Nikon). Phase contrast and fluorescent images were taken of each field by using a script that automated positioning of the microscope from one pedestal to the next, acquiring the images, and saving them by using a standardized file naming system.

Image Scoring and Validation. Protein localization and image quality in each fluorescent and phase contrast image were scored by 2 individuals using a MATLAB (MathWorks) program, "Localization Scorer," that is freely available on request. The results of this scoring generated an initial set of *Caulobacter* strains containing localized proteins. This set of strains was manually re-arrayed into 96-well plates, reimaged to confirm the

localization pattern, and sequenced by using primers directed against gXRC/gXRN in an analysis similar to that described for identifying the ORF identity in entry vectors. Seven different people compared the original and rearrayed images to establish a consensus localization pattern. Strains containing proteins whose localization was not reproducible, whose sequence was not correct, or that were scored as not localized by more than 2 scorers were eliminated from the localized set of proteins.

Statistical Analysis. Quantitative attributes of the localized *Caulobacter* proteins were compared to those of all *Caulobacter* proteins by using a 2-sample T test (Microsoft Excel) when appropriate. The overlaps between protein subsets were compared by using the hypergeometric distribution (MATLAB, MathWorks). Data extraction was performed by using BioPerl (bioperl.org) scripts. For ease of visualization in Fig. 2*A*, the P-values derived from these tests were converted to E-values by plotting the $\log_{10}$ of the inverse of the P value as positive for overrepresentation and negative for underrepresentation. To compare GO terms, we extracted the functional subcategory classification for each protein from NCBI and compared the fraction of localized and all proteins represented in each class. To compare cell-cycle-regulated proteins, we used the classifications of a previous report on cell-cycle-dependent transcription (6) to compare the cell-cycle-regulated fraction of localized and all proteins.

Quantitative Analysis. Cell outlines were identified, cell length was measured, and one-dimensional intensity profiles were calculated for each cell by using the PSICIC software toolkit, as previously described (7). Cells were considered localized if the maximum fluorescence was a threshold value over the mean fluorescence for that cell. The threshold used, 2×10^4, was determined by manual inspection. Because the stalked pole cannot be reliably identified in every cell, all distances measured reflect the distance to the nearest pole and are thus reported on a scale from 0–50% of cell length. The half-maximal width was calculated by identifying the minimum continuous interval of the intensity profile that contains both the maximum and the median of the intensity profile, such that each endpoint has an intensity value less than the mean of the maximum intensity. The length of this span is divided by two to give the mean distance from the peak to the half-maximal value on either side of the peak. If such a span does not exist, as in cases where the peak is close to the cell pole and is cut off in one direction before reaching its half-maximal width, then the width in that direction is ignored and only the complete value is used.

1. Ely B (1991) Genetics of *Caulobacter crescentus. Methods Enzymol* 204:372–384.
2. Nierman WC, et al. (2001) Complete genome sequence of Caulobacter crescentus. *Proc Natl Acad Sci USA* 98:4136–4141.
3. Rual JF, et al. (2004) Human ORFeome version 1.1: A platform for reverse proteomics. *Genome Res* 14:2128–2135.
4. Platt R, Drescher C, Park SK, Phillips GJ (2000) Genetic system for reversible integration of DNA constructs and lacZ gene fusions into the Escherichia coli chromosome. *Plasmid* 43:12–23.
5. Schroeder BK, et al. (2005) Development of a functional genomics platform for Sinorhizobium meliloti: Construction of an ORFeome. *Appl Environ Microbiol* 71:5858–5864.
6. Laub MT, McAdams HH, Feldblyum T, Fraser CM, Shapiro L (2000) Global analysis of the genetic network controlling a bacterial cell cycle. *Science* 290:2144–2148.
7. Guberman JM, Fay A, Dworkin J, Wingreen NS, Gitai Z (2008) PSICIC: Noise and asymmetry in bacterial division revealed by computational image analysis at sub-pixel resolution. *PLoS Comput Biol* 4:e1000233.
8. Jensen RB, Wang SC, Shapiro L (2001) A moving DNA replication factory in *Caulobacter crescentus. EMBO J* 20:4952–4963.
9. Alley MR (2001) The highly conserved domain of the *Caulobacter* McpA chemoreceptor is required for its polar localization. *Mol Microbiol* 40:1335–1343.
10. Iniesta AA, McGrath PT, Reisenauer A, McAdams HH, Shapiro L (2006) A phosphosignaling pathway controls the localization and activity of a protease complex critical for bacterial cell cycle progression. *Proc Natl Acad Sci USA* 103:10935–10940.
11. Huitema E, Pritchard S, Matteson D, Radhakrishnan SK, Viollier PH (2006) Bacterial birth scar proteins mark future flagellum assembly site. *Cell* 124:1025–1037.
12. Wheeler RT, Shapiro L (1999) Differential localization of two histidine kinases controlling bacterial cell differentiation. *Mol Cell* 4:683–694.
13. Jacobs C, Domian IJ, Maddock JR, Shapiro L (1999) Cell cycle-dependent polar localization of an essential bacterial histidine kinase that controls DNA replication and cell division. *Cell* 97:111–120.
14. Bowman GR, et al. (2008) A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. *Cell* 134:945–955.
15. Ebersbach G, Briegel A, Jensen GJ, Jacobs-Wagner C (2008) A self-associating protein critical for chromosome attachment, division, and polar organization in caulobacter. *Cell* 134:956–968.
16. Gitai Z, Dye N, Shapiro L (2004) An actin-like gene can determine cell polarity in bacteria. *Proc Natl Acad Sci USA* 101:8643–8648.
17. Dye NA, Pincus Z, Theriot JA, Shapiro L, Gitai Z (2005) Two independent spiral structures control cell shape in *Caulobacter. Proc Natl Acad Sci USA* 102:18608–18613.
18. Wang SC, Shapiro L (2004) The topoisomerase IV ParC subunit colocalizes with the *Caulobacter* replisome and is required for polar localization of replication origins. *Proc Natl Acad Sci USA* 101:9251–9256.
19. McGrath PT, Iniesta AA, Ryan KR, Shapiro L, McAdams HH (2006) A dynamically localized protease complex and a polar specificity factor control a cell cycle master regulator. *Cell* 124:535–547.
20. Obuchowski PL, Jacobs-Wagner C (2007) PfII, a protein involved in flagellar positioning in Caulobacter crescentus. *J Bacteriol* 190:1718–1729.
21. Thanbichler M, Shapiro L (2006) MipZ, a spatial regulator coordinating chromosome segregation with cell division in Caulobacter. *Cell* 126:147–162.
22. Paul R, et al. (2004) Cell cycle-dependent dynamic localization of a bacterial response regulator with a novel di-guanylate cyclase output domain. *Genes Dev* 18:715–727.
23. Jacobs C, Hung D, Shapiro L (2001) Dynamic localization of a cytoplasmic signal transduction response regulator controls morphogenesis during the Caulobacter cell cycle. *Proc Natl Acad Sci USA* 98:4095–4100.
24. Aaron M, et al. (2007) The tubulin homologue FtsZ contributes to cell elongation by guiding cell wall precursor synthesis in Caulobacter crescentus. *Mol Microbiol* 64:938–952.
25. Costa T, Priyadarshini R, Jacobs-Wagner C (2008) Localization of PBP3 in Caulobacter crescentus is highly dynamic and largely relies on its functional transpeptidase domain. *Mol Microbiol* 70:634–651.
26. Ryan KR, Huntwork S, Shapiro L (2004) Recruitment of a cytoplasmic response regulator to the cell pole is linked to its cell cycle-regulated proteolysis. *Proc Natl Acad Sci USA* 101:7415–7420.
27. Sciochetti SA, Ohta N, Newton A (2005) The role of polar localization in the function of an essential Caulobacter crescentus tyrosine kinase. *Mol Microbiol* 56:1467–1480.
28. Ausmees N, Kuhn JR, Jacobs-Wagner C (2003) The bacterial cytoskeleton: An intermediate filament-like function in cell shape. *Cell* 115:705–713.
29. 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.

Other Supporting Information Files

Table S1
Table S2