Supporting Information

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SI Results

Transposon-Mediated Conditional Mutagenesis Screen for Activators of CckA. Given the relationship between CckA localization and CckA activity, we carried out a genetic screen using automated fluorescence microscopy to identify the genes that control the localization of CckA. We then assayed candidate mislocalization mutants for CckA activity. The host strain used for this screen had its *cckA* locus replaced by a *cckA-cfp* allele and had a genetic background compatible with that of the CckA/FixL P_{fixK}-*lacZ* reporter system for assessing the CckA activity of candidate mutants. We took advantage of a recently developed transposon mutagenesis methodology designed to disrupt both nonessential and essential genes that incorporates an outward-facing vanillate-inducible promoter P_{van}, allowing for the conditional expression of flanking genes otherwise disrupted by transposon insertion (1). We used this method to generate a library of 6,144 P_{van}*Tn5* insertion mutants of the fluorescence microscopy host strain. Our prescreening of this library for vanillate-conditional or small swarm size on large-format swarmer agar plates (1), indicating the growth and/or morphological defects anticipated for mutants with impaired CckA activation, identified 101 mutants for further investigation by automated fluorescence microscopy. One of these mutants that exhibited an aberrant CckA localization pattern had a transposon insertion that mapped to *divL* (28 amino acids from the C terminus) encoding a cell cycle–dependent protein kinase (Fig. 1 *A* and *B*).

SI Experimental Procedures

Materials. Quick T4 DNA ligase, shrimp alkaline phosphatase (SAP), exonuclease I (ExoI), and endonucleases were purchased from Fermentas and New England Biolabs. DNA oligos were purchased from the Stanford Protein and Nucleic Acid Biotechnology Facility. One Shot TOP10 chemically competent *Escherichia coli* was purchased from Invitrogen and used for cloning purposes. The 0.1-cm electroporation cuvettetes were purchased from Bio-Rad. DNA sequencing was performed by Sequetech. KOD Hot Start DNA polymerase was purchased from Novagen. BioMix Red DNA polymerase was purchased from Bioline. DNA miniprep and gel extraction kits were purchased from Qiagen. The CP-7200 colony picker was acquired from Norgren Systems. The Orbit 300 shaker was purchased from Labnet. The 240 × 240-mm vented QTray polystyrene culture plates were purchased from Genetix. Tolnaftate was purchased from MP Biomedicals.

Growth Conditions and Buffers. When appropriate, media were supplemented with antibiotics at the following concentrations (liquid/solid media for *Caulobacter*, liquid/solid media for *E. coli*, in μg/mL): kanamycin, 5/25, 30/50; gentamycin, 0.6/5, 15/20; oxytetracycline, 1/2, 12/12; chloramphenicol, 1/1, 20/30; and rifampicin, 2.5/5, 25/50. The SDS lysis buffer contained 4% SDS, 100 mM EDTA, and 50 mM Tris-HCl (pH 7). The K2 low-salt buffer contained 50 mM Tris-HCl (pH 7), 100 mM NaCl, 50 mM EDTA, and 2% Triton X-100. The K2 high-salt buffer contained 50 mM Tris-HCl (pH 7), 500 mM NaCl, and 50 mM EDTA. The SDS-loading buffer contained 125 mM Tris-base, 20% glycerol, 2% SDS, and 0.01 mg/mL bromophenol blue, adjusted to pH 6.8. CoIP buffer contained 20 mM Hepes (pH 7.5), 100 mM NaCl, and 20% glycerol. The wash buffer contained 50 mM Tris-HCl (pH 7.4) and 150 mM NaCl.

Transposon-Mediated Conditional Mutagenesis and Swarmer Plate Prescreen. Strain NJH530 was mated (2) with strain BC1429 (1) in the presence of 0.5 mM vanillate to achieve conjugal transfer of plasmid pVMCS-2_TnP_{van}1_linker and subsequent Tn5 transposon-mediated conditional mutagenesis (1). The resulting mutant *Caulobacter* colonies were picked with a CP-7200 colony picker into 96-well plates containing PYE media supplemented with 0.5 mM vanillate, and grown at 28 °C and 60 rpm on an Orbit 300 shaker. Cultures were replica-plated, as described previously (1), onto PYE 0.25% agar swarmer plates supplemented with 2.5 μg/mL of tolnaftate with or without 0.5 mM vanillate, and grown at 28 °C. The resulting plates were imaged at 300 dpi on a Hewlett-Packard HP Scanjet 7400c flat-bed scanner using Adobe Photoshop CS2. Images of corresponding vanillate and no vanillate replica plates were overlaid and visually inspected for mutant strains with small or conditional swarm size.

Fluorescence Microscopy Screen. Transposon-mutated NJH530 strains with small or conditional swarm size were inoculated in replicate into 24-well plates containing PYE media supplemented with or without 0.5 mM vanillate, grown at 28 °C and 60 rpm, and immobilized onto 1.0% agar in M2G before imaging with phase and epifluorescent microscopy. On visual inspection, mutant strains differing from WT NJH530 in terms of morphology, CckA or CpdR localization pattern, or cellular concentration were reinoculated in replicate into 24-well plates containing M2G media with or without 0.5 mM vanillate, grown at 28 °C and 60 rpm, and immobilized onto 1.0% agar in M2G before begin reimaged with phase and epifluorescent microscopy. Transposon insertion sites were mapped with two rounds of arbitrary PCR using Biomix Red polymerase and cell culture as a template, followed by SAP and ExoI cleanup, PCR product primer extension sequencing, and blastn (3) sequence alignment against the *Caulobacter* CB15 genome (4), as described previously (1).

In Vivo Phosphorylation. Cells were initially grown in PYE and then in M2G in log phase, and were finally diluted in M5GG media overnight, avoiding overgrowth above 0.3 OD_{660nm}. Then 1 mL of cells grown in M5GG at 0.3 OD_{660nm} were labeled with 30 μCi of Easytides adenosine 5′-triphosphate [gamma-^{32}P] (PerkinElmer) for 5 min. The sample was pelleted by centrifugation at 16,000 × *g* for 1 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in SDS lysis buffer with 10 units/μL of Ready-Lyse lysozyme *s*olution (Epicentre Biotechnologies) and 0.5% n-dodecyl-β-D-maltoside (Pierce), pipetting up and down until the pellet was viscous. The lysate was resuspended in 600 μL of K2 low-salt buffer ice-cold, and then 20 μL of Protein A Agarose (Roche) (previously washed in K2 low-salt buffer) and another 600 μL of K2 low-salt buffer ice-cold were added. The sample was centrifuged at 16,000 × *g* for 4 min at 4 °C. The supernatant was collected and placed in a tube containing 20 μL of Protein A Agarose (Roche) [previously washed in K2 low-salt buffer and incubated with 2 μL of anti-CckA antibodies at 4 °C (5)], and mixed by rocking for 1 h at 4 °C. After that incubation, the sample was spun at 800 × *g* for 1 min at 4 °C. The supernatant was discarded, and the agarose beads were washed in 1 mL of K2 low-salt buffer ice-cold and spun at 800 × *g* for 1 min at 4 °C. Then the supernatant was discarded, and the agarose beads were washed in 1 mL of K2 high-salt buffer ice-cold and spun at 800 × *g* for 1 min at 4 °C. This operation was repeated twice. After all supernatant was removed, the agarose beads were resuspended with 30 μL of

SDS loading buffer and incubated for 5 min at 37 °C and for 2 min at 60 °C. The sample was spun at 800 × *g* for 1 min at 4 °C, loaded in a prechilled 8% SDS-polyacrylamide gel, run at 200 V at 4 °C until the front disappeared, and visualized on a Molecular Dynamics PhosphorImager. Using the software ImageQuant version 5.2 from Molecular Dynamics, we quantified the area of interest from each lane in Fig. 2*A*, WT p*xylX-ctrA*$_{D51E}$, Δ*divL* p*xylX-ctrA*$_{D51E}$, and Δ*cckA* p*xylX- ctrA*$_{D51E}$, respectively. Compared with the intensity of the lane WT p*xylX-ctrA*$_{D51E}$, the quantitative analysis showed approximately 80% less intensity for both lanes Δ*divL* p*xylX- ctrA*$_{D51E}$ and Δ*cckA* p*xylX- ctrA*$_{D51E}$ (which completely lacks CckA and is therefore a "background control"). Subtracting the intensity of the lane Δ*cckA* p*xylX- ctrA*$_{D51E}$ from the lane Δ*divL* p*xylX- ctrA*$_{D51E}$, we conclude that there is no CckA phosphorylated in the strain Δ*divL* p*xylX- ctrA*$_{D51E}$.

Coimmunoprecipitation Assay. Cells were grown in 500 mL of PYE until an OD_{660nm} of 0.3 was reached. Cultures were spun at 11,300 × *g* for 20 min at 4 °C, after which the pellet was washed with CoIP buffer and spun again similarly. Cells were resuspended in 10 mL of CoIP buffer with 0.5% n-dodecyl-β-D-maltoside (Pierce), 1× Ready-Lyse lysozyme solution (Epicentre Technologies), and 50 units of DNase I (Fermentas) and rocked during 15 min at room temperature. Then the samples were passed through a French press at 16,000 psi and spun at 11,300 × *g* for 20 min at 4 °C, after which the supernatant was precleared with 50 μL of Protein A Agarose beads (Roche). After the preclearing, an aliquot from this lysate was resuspended in SDS loading buffer and kept at −20 °C until immunoblot analysis. The remainder of the whole sample was incubated with 50 μL of anti-FLAG M2-agarose affinity gel (FLAGIPT-1; Sigma-Aldrich), with rocking at 4 °C overnight. On the next day, samples were pelleted by centrifugation at 800 × *g* for 2 min at 4 °C, washed three times with CoIP buffer supplemented with 0.5% n-dodecyl-β-D-maltoside and three times with wash buffer, and then resuspended in 50 μL of SDS loading buffer and kept at −20 °C until immunoblot analysis. Before immunoblot analysis, CoIP samples were boiled for 5 min and spun at 800 × *g* for 2 min at room temperature, and the supernatant was loaded on 10% SDS-polyacrylamide. The lysate samples that had been kept at −20 °C were also boiled for 5 min and loaded on 10% SDS polyacrylamide for immunoblot analysis.

Immunoblot Analysis. *Caulobacter* cultures were grown in M2G until an OD_{660nm} of 0.3 was reached. Then a 1- mL sample was pelleted by centrifugation at 16,000 × *g*, resuspended in 100 μL of SDS loading buffer, and boiled for 5 min. The proteins were separated on 10% SDS polyacrylamide gels and transferred to a PVDF membrane (Immobilon; Millipore). Immunodetection was performed with anti-CckA antibodies at a dilution of 1:10,000 (5) following standard procedures. Donkey anti-rabbit IgG conjugated to HRP (Jackson ImmunoResearch) was used as a secondary antibody. Immunocomplexes were visualized using Western Lightning chemiluminescence reagent (PerkinElmer) and Kodak Bio-Max MR film.

Construction of Plasmids and Strains. Strain AA725 was generated by transducing *divL::Tn5* into a clean NJH530 background with ΦCr30 lysate derived from the original *divL::Tn5* mutant identified on our fluorescence microscopy screen. The *divL::Tn5* mutant contains an insertion in the *divL* locus (bp 3726490 in the *Caulobacter crescentus* CB15 genome) of the sequence derived from plasmid pVMCS-2_TnP_{van}1_linker.

Strain AA889. A fragment containing *divL* was amplified by PCR from plasmid pNJH275 using oligos NJH408 and NJH415 with an extension time of 2 min, 30 s. This *divL* fragment was digested with *Nde*I and *Afl*II and then ligated into a similarly digested pVCHYC-4 vector backbone. The sequence of the resulting plasmid, pNJH281, was confirmed by primer extension sequencing using the oligos Pvan F and mCherry Nterm R. Plasmid pNJH281 was transformed into strain NJH429, resulting in strain AA889.

Strain AA972. *vanA::divL*$_{Y550F}$ was transduced with ΦCr30 lysate from strain NJH599 into strain NJH564.

Strain AA980. Plasmid pLB07 (L. Britos, unpublished), a pNPTS138-based plasmid to generate a chromosomal deletion of the locus CC3476, was introduced into the strain NJH564 in the presence of 0.5 mM vanillate. The Δ*divL* allele and integrated LB07 plasmid were contransduced with ΦCr30 lysate from the resulting strain into strain LS2715 (6) in the presence of 0.3% xylose. To eliminate the integrated LB07 plasmid, we performed the two-step *sacB* counterselection procedure described above in the presence of 0.3% xylose and searched for colonies with undeleted CC3476 locus by PCR using 72F and 63R oligos (L. Britos, unpublished). The resulting strain was named AA980.

Strain AA987 was obtained after the sequential introduction of plasmids p*fixK* and pNJH143 into strain AA972.

Strain AA1001. *cckA::cckA-cfp* was transduced with ΦCr30 lysate from a CB15N strain harboring plasmid pGB024 into strain LS2715 (6).

Strain AA1003. *cckA::cckA-cfp* was transduced with ΦCr30 lysate from a CB15N strain harboring plasmid pGB024 into strain AA980. Strain AA1015 was obtained by integrating the sequence *divL-m2* at the *divL* locus after transformation of CB15N with the plasmid p*divL-m2*. To create p*divL-m2*, a *divL* sequence fragment of ∼2.3 kb was obtained after digestion of the plasmid pNJH281 by *Nde*I and *Afl*II, and was cloned into the vector pFLGC-2, which had been previously cut with the same enzymes.

Strain NJH206. Because WT *Caulobacter* CB15N is not the optimal genetic background for CckA/FixL reporter system performance, we made several appropriate modifications. To diminish background β-galactosidase activity, we began with strain JOE2321, in which *lacA* has been deleted in-frame (7). We deleted *fixL* to eliminate the endogenous anaerobic stress–signaling pathway (8), which can induce expression from the P_{fixK} promoter in a CckA/FixL-independent manner. We then deleted *fixT*, encoding a negative regulator of FixLJ-mediated signaling, to eliminate the negative-feedback loop contained within the endogenous anaerobic stress–signaling pathway (8), which would reduce the maximal level of CckA/FixL activity–induced expression from the P_{fixK} promoter. The pNPTS138-*fixL* deletion vector (8) was transformed into JOE2321 (Table S1) by electroporation as described previously (2), and in-frame gene deletion of *fixL* was done with a two-step *sacB* counterselection procedure (9). Colony PCR, with Biomix Red polymerase and oligos NJH151 and NJH152 (Table S2), was used to screen for the presence of the *fixL* deletion. The 25-μL PCR mixture, containing 5% DMSO, was made following the manufacturer's protocol. PCR was initiated by melting at 94 °C for 1 min, 45 s, followed by 32 cycles of melting at 94 °C for 15 s, annealing at 62 °C for 30 s, and a 1-min, 45-s extension at 72 °C. The pNPTS138-*fixT* deletion vector (8) was transformed to the resulting Δ*lacA* Δ*fixL* strain, and the in-frame gene deletion of *fixT* (CC0753) was accomplished as described above. Colony PCR, with oligos NJH162 and NJH163 and a 1-min, 15-s extension time, was used to screen for the presence of the *fixT* deletion. The p*fixK* FixL/J system LacZ reporter plasmid (8) was then transformed into the resulting Δ*lacA* Δ*fixL* Δ*fixT* strain, resulting in strain NJH206.

Strain NJH227. The genomic region containing *cckA* (CC1078) was amplified from *Caulobacter* CB15N genomic DNA (strain CB15N) with KOD Hot Start DNA polymerase and oligos NJH164 and NJH165. The 50-μL PCR mixture, containing 5% DMSO, was made in accordance with the manufacturer's protocol. The PCR was initiated by melting at 94 °C for 1 min, 45 s, followed by 32 cycles of melting at 94 °C for 15 s, annealing at 62 °C for 30 s, and a 2-min, 20-s extension at 68 °C. The resulting PCR product was then purified by electrophoresis through 1.2%

agarose, followed by gel extraction. The genomic region containing *fixL* (CC0759) was similarly amplified with oligos NJH151 and NJH152 and a 1-min, 40-s extension time. The four *cckA/fixL* constructs were then generated using the splicing by overlap extension (SOE) method (10).

The *cckA* genomic region template (see above) was amplified with oligos NJH140 and NJH109 (5′ $CckA_{K298}$ SOE PCR) and a 1-min extension time. The *fixL* genomic region template (see above) was similarly amplified with oligos NJH108 and NJH137 (3′ $CckA_{K298}$ SOE PCR) and a 45-s extension time. The 5′ $CckA_{K298}$ and 3′ $CckA_{K298}$ first-round SOE PCR products was mixed 1:1 as a template for second-round SOE PCR reactions, then amplified using oligos NJH140 and NJH137 with a 1-min, 45-s extension time. These four second-round SOE PCR products were then digested with NdeI and EcoRI, ligated with Quick T4 DNA ligase with similarly digested pMT383 vector (11) backbone, and then transformed into One Shot TOP10 chemically competent *E. coli* cells (all in accordance with the manufacturer's protocol). The sequence of the resulting plasmid, pNJH92, was confirmed by primer extension sequencing using oligos NJH128, NJH129, and NJH178.

To make plasmid pX31, a fragment was amplified by PCR using oligos XylHindIII.for and XylBamHI.rev and plasmid pXGFP4 (M. Alley, unpublished) as a template. This fragment contains ~500 bp of the upstream sequence of *xylX* (CC0823) together with part of the multiple cloning site, including the BamHI site. The PCR product was digested with HindIII and BamHI and ligated into similarly digested pMR31 (R. Roberts, unpublished) vector backbone, generating the pX31. Plasmid pNJH92 was digested with NdeI and EcoRI, and the insert (containing *cckA/fixL*) was ligated with similarly digested pX31 vector backbone and then transformed into One Shot TOP10 chemically competent *E. coli* cells, resulting in plasmid pNJH143. Plasmid pNJH143 was transformed into strain NJH206, resulting in strain NJH227.

Strains NJH278-9. The PCR product of the amplification of the genomic region containing *fixL* (see above) was reamplified with KOD polymerase and oligos NJH136 and NJH190 and an extension time of 1 min, 45 s. Plasmid pNJH92 was similarly reamplified with oligos NJH140 and NJH190. These two PCR products were then digested with NdeI and EcoRI, ligated with similarly digested pMT383 vector backbone, and transformed into One Shot TOP10 chemically competent *E. coli* cells. The sequences of the resulting plasmids, pNJH151 and pNJH152, were confirmed by primer extension sequencing using oligos NJH128, NJH129, and (for pNJH152) NJH178. Plasmids pNJH151-2 were digested with NdeI and EcoRI, and the inserts (containing *fixL* and *cckA/fixL*, respectively) were ligated with similarly digested pNJH156 vector backbone (12), then transformed into One Shot TOP10 chemically competent *E. coli* cells, resulting in plasmids pNJH164-5, respectively. Plasmids pNJH164-5, each containing *fixL-eyfp* or *cckA/fixL-eyfp* under the control of the xylose-inducible promoter P_{xyl}, were transformed into strain CB15N, followed by transformation with plasmid pGB024 (G. Bowman, unpublished) resulting in strains NJH278-9.

Strain NJH429. A fragment containing the 3′ terminus of *cckA* was amplified from plasmid pGB024 by PCR using oligos JLB5 and NJH292 with a 45-s extension time. This *cckA* fragment was then digested with *Hind*III and *Age*I and ligated into similarly digested pYFPC-3 vector backbone (13). The sequence of the resulting plasmid, pNJH221, was confirmed by primer extension sequencing using oligos JLB5 and NJH292. Plasmid pNJH221 was transformed into strain CB15N, resulting in strain NJH429.

Strain NJH530. Plasmid pGB024, containing *cckA-cfp*, was amplified with KOD polymerase and oligos NJH383 and NJH384 and a 1-min, 45-s extension time, and the resulting PCR product was digested with EcoRI and BamHI. The genomic region downstream of *cckA* was similarly amplified from *Caulobacter* CB15N genomic DNA with oligos Down.cckA.for and Down.cckA.rev and a 45-s extension time, and the resulting PCR product was digested with BamHI and HindIII. These two digested PCR products were ligated with EcoRI- and HindIII-digested pNPTS138 (M. Alley, unpublished) vector backbone (triple ligation), and then transformed into One Shot TOP10 chemically competent *E. coli* cells. The sequence of the resulting plasmid, pNJH250, was confirmed by primer extension sequencing using oligos NJH208 and NJH209.

A fragment containing 193 bp upstream through the entire *cpdR-yfp* coding sequence was amplified by PCR using oligos UpSpeI.cpdR.for and UpHindIII.yfp.rev2, and plasmid p*cpdR-yfp* (14) as a template. A second fragment containing 594 bp immediately downstream of the *cpdR* coding sequence was amplified by PCR using oligos DownHindIII.cpdR.for and DownEcoRI. cpdR.rev, and CB15N genomic DNA as a template. The first PCR product was digested with SpeI and HindIII, and the second PCR product was digested with HindIII and EcoRI. These two digested PCR products were then ligated with SpeI- and EcoRI-digested pNPTS138 vector backbone (triple ligation), resulting in plasmid pAA665. To increase the length of the upstream sequence, ~2 kb upstream through the entire *cpdR* coding sequence was amplified using oligos 2KB_Up_CpdR_SpeI_for and RR7Xyl.rev, and CB15N genomic DNA as a template. This PCR product was then digested with SpeI and BstBI and ligated into similarly digested pAA665 vector backbone, resulting in plasmid pNJH266, (*cpdR* contains a BstBI restriction site 75 bp into the coding sequence.)

The pJC343 deletion vector (7) was transformed into MT231, and the in-frame gene deletion of *lacA* was done through the two-step *sacB* counterselection procedure described above. Colony PCR, with oligos NJH269 and NJH270 and a 2-min, 45-s extension time, was used to screen for the presence of the *lacA* deletion. The pNPTS138-*fixL* and pNPTS138-*fixT* deletion vectors were then sequentially transformed into the resulting Δ*vanA* (CC2393) Δ*lacA* strain and used to achieve the in-frame gene deletions of *fixL* and *fixT*, as described above. Plasmid pNJH250 was transformed into the resulting Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT* strain, and the replacement of *cckA* with *cckA-cfp* was done through the two-step *sacB* counterselection procedure described above. Colony PCR, with oligos NJH383 and NJH384 and a 1-min, 45-s extension time, was used to screen for the presence of the *cckA::cckA-cfp* replacement. Plasmid pNJH266 was transformed into the resulting Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT cckA::cckA-cfp* strain, and the replacement of *cpdR* with *cpdR-eyfp* was done as described above. Colony PCR, with oligos NJH385 and NJH386 and a 1-min extension time, was used to screen for the presence of the *cpdR::cpdR-eyfp* replacement, resulting in strain NJH530.

Strain NJH551. Strain NJH530 was sequentially transformed with plasmids p*fixK* and pNJH143, resulting in strain NJH551.

Strain NJH558. Strain AA725 was sequentially transformed with plasmids p*fixK* and pNJH143, resulting in strain NJH558.

Strain NJH564. A fragment containing *divL* was amplified from *Caulobacter* CB15N genomic DNA by PCR using oligos NJH408 and NJH409 with a 2-min, 30-s extension time. This *divL* fragment was digested with *Nde*I and *EcoR*I, and then ligated into similarly digested pVCHYC-4 (13) vector backbone. The sequence of the resulting plasmid, pNJH275, was confirmed by primer extension sequencing using oligos Pvan F and mCherry Nterm R. Fragments containing the upstream or downstream genomic regions of *divL* were amplified from *Caulobacter* CB15N genomic DNA by PCR using oligos NJH410 and NJH411 or NJH412 and NJH413, respectively, with a 1-min extension time. These *divL* upstream and downstream fragments were digested with HindIII and BamHI or with BamHI and EcoRI, respectively, and triply ligated into HindIII- and EcoRI-digested pNPTS138 vector backbone. The sequence of the resulting plasmid, pNJH277, was confirmed by primer extension sequencing using oligos NJH410 and NJH413. Strain NJH530 was sequentially transformed with plasmids pNJH275 and then

pNJH277, and the in-frame deletion of *divL* was accomplished in the presence of 0.5 mM vanillate with the two-step *sacB* counterselection procedure described above, resulting in strain NJH564. Colony PCR, with oligos NJH410 and NJH413 and a 2-min, 30-s extension time, was used to screen for the presence of the *divL* deletion.

Strain NJH576. A fragment containing *divL*Δ28 was amplified by PCR using plasmid pNJH275 as a template and oligos NJH408 and NJH420, with an extension time of 2 min, 30 s. This *divL*Δ28 fragment was digested with NdeI and AflII, and then ligated into similarly digested pVCHYC-4 vector backbone. The sequence of the resulting plasmid, pNJH283, was confirmed by primer extension sequencing using oligos Pvan F and mCherry Nterm R. Strain AA725 was transformed with plasmid pNJH283, resulting in strain NJH576.

Strain NJH599. The 5′ terminus of *divL* was amplified by PCR using plasmid pNJH275 as a template and oligos NJH408 and NJH422 with a 1-min, 45-s extension time (5′ SOE PCR). The 3′ terminus of *divL* was similiarly amplified with oligos NJH423 and NJH409 with a 45-s extension time (3′ SOE PCR). These two first-round SOE PCR products were mixed 1:1 as a template for the second-round SOE PCR, then amplified using oligos NJH408 and NJH409 with a 2-min, 30-s extension time.

The second-round SOE PCR product was digested with NdeI and EcoRI and ligated with similarly digested pVCHYC-2 vector backbone. The sequence of the resulting plasmid, pNJH288, was confirmed by primer extension sequencing using oligo NJH409. Plasmid pNJH288 was then transformed into strain NJH530, resulting in strain NJH599.

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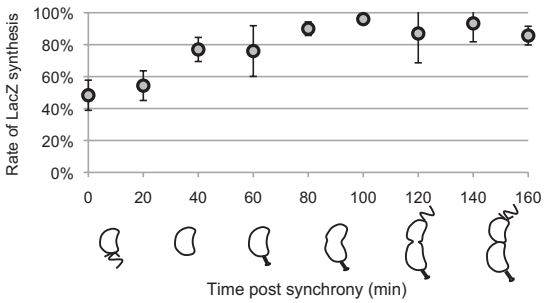

Fig. S1. Cell cycle lacZ reporter activity of the CckA/FixL chimera. Relative rates of de novo LacZ synthesis as a function of the cell cycle for the CckA/FixL strain (NJH227), as assayed by [35S]-methionine pulse experiments. Data points represent the average, and error bars show the SD, of three independent experiments, with the exceptions of t = 80 and 120 min (n = 2) and t = 100 min (n = 1).

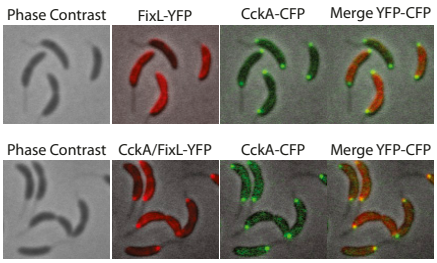

Fig. S2. Subcellular localization of the CckA/FixL chimera. Phase contrast and fluorescence images of FixL-YFP, CckA-CFP, and YFP-CFP merge from strain NJH278, and CckA/FixL-YFP, CckA-CFP, and YFP-CFP merge from strain NJH279. Cells were grown in M2G media supplemented with 0.003% xylose for 1 h to induce expression of *fixL-eyfp* or *cckA/fixL-eyfp*, respectively.

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Table S1. Plasmids and strains used in this study

Plasmid	Description	Reference
p*divL-m2*	pFLGC-2 *divL-m2*	This study
pFixK	FixL/J system $P_{fixK(CC0752)}$-LacZ reporter plasmid	(8)
pFLGC-2	C-terminal Flag tag integration vector	(13)
pGB024	*CckA-cfp* integration disruption vector	
pJC343	pNPTS138-Δ*lacA*	(7)
pLB07	pNPTS138-based vector for the markerless in-frame deletion of CC3476	L. Britos, unpublished
pMR31	High-copy expression vector	R. Roberts, unpublished
pMT383	pP_{van}-*ftsZ-eyfp* expression integration vector	(11)
pNJH92	pMT383-$cckA_{K298}fixL_{Q258}$	This study
pNJH143	pX31-P_{xyl} $cckA_{K298}fixL_{Q258}$	This study
pNJH151	pMT383-*fixL-eyfp*	This study
pNJH152	pMT383-$cckA_{K298}fixL_{Q258}$-*eyfp*	This study
pNJH156	pX31-P_{xyl} *gfpuv* (-*eyfp*) expression vector	(12)
pNJH164	pX31-P_{xyl} *fixL-eyfp*	This study
pNJH165	pX31-P_{xyl} $cckA_{K298}fixL_{Q258}$-*eyfp*	This study
pNJH221	pYFPC-3 *cckA-eyfp*	This study
pNJH250	pNPTS138-*cckA-cfp*	This study
pNJH266	pNPTS138-*cpdR-eyfp*	This study
pNJH275	pVCHYC-4 *divL*	This study
pNJH277	pNPTS138-ΔdivL	This study
pNJH281	pVCHYC-4 *divL-mCherry*	This study
pNJH283	pVCHYC-4 *divLΔ28-mCherry*	This study
pNJH288	pVCHYC-2 $divL_{Y550F}$	This study
pNPTS138	Suicide vector containing *sacB* for gene deletions	M. Alley, unpublished
pNPTS138-*fixL*	Vector for the markerless in-frame deletion of *fixL*	(8)
pNPTS138-*fixT*	Vector for the markerless in-frame deletion of *fixT*	(8)
pVCHYC-2	pP_{van}-*mCherry* expression integration vector	(13)
pVCHYC-4	pP_{van}-*mCherry* expression integration vector	(13)
pX31	High-copy xylose-inducible expression vector	This study
pXGFP4	Xylose-inducible expression vector	M. Alley, unpublished
pYFPC-3	C-terminal -*eyfp* fusion integration vector	(13)
Strain		
AA725	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT divL::Tn5 cckA::cckA-cfp cpdR::cpdR-eyfp*	This study
AA889	*Caulobacter* CB15N *cckA::cckA-yfp vanA::divL-mCherry*	This study
AA972	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT* Δ*divL cckA::cckA-cfp cpdR::cpdR-eyfp vanA::*$divL_{Y550F}$	This study
AA980	*Caulobacter* CB15N Δ*divL* pIDC42	This study
AA987	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT* Δ*divL cckA::cckA-cfp cpdR::cpdR-eyfp vanA::*$divL_{Y550F}$ p*fixK* pNJH143	This study
AA1001	*Caulobacter* CB15N *cckA::cckA-cfp* pIDC42	This study
AA1003	*Caulobacter* CB15N Δ*divL cckA::cckA-cfp* pIDC42	This study
AA1015	*Caulobacter* CB15N *divL::divL-m2*	This study
BC1429	*E. coli* S17-1 pVMCS-2_TnP_{van}1_linker	(1)
CB15N	*Caulobacter* NA1000	(15)
JOE2321	*Caulobacter* CB15N Δ*lacA*	(7)
LS2715	*Caulobacter* CB15N pIDC42	(6)
LS3360	*Caulobacter* CB15N Δ*cckA* pIDC42	(16)
LS4379	*Caulobacter* CB15N *hfq::hfq-m2*	S. Landt, unpublished
MT231	*Caulobacter* CB15N Δ*vanA*	(13)
NJH206	*Caulobacter* CB15N Δ*lacA* Δ*fixL* Δ*fixT* p*fixK*	This study
NJH227	*Caulobacter* CB15N Δ*lacA* Δ*fixL* Δ*fixT* p*fixK* pNJH143	This study
NJH278	*Caulobacter* CB15N *cckA::cckA-cfp* pNJH164	This study
NJH279	*Caulobacter* CB15N *cckA::cckA-cfp* pNJH165	This study
NJH429	*Caulobacter* CB15N *cckA::cckA-eyfp*	This study
NJH530	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT cckA::cckA-cfp cpdR::cpdR-eyfp*	This study
NJH551	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT cckA::cckA-cfp cpdR::cpdR-eyfp* p*fixK* pNJH143	This study
NJH558	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT divL::Tn5 cckA::cckA-cfp cpdR::cpdR-eyfp* p*fixK* pNJH143	This study
NJH564	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT* Δ*divL cckA::cckA-cfp cpdR::cpdR-eyfp vanA::divL*	This study
NJH576	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT divL::Tn5 cckA::cckA-cfp cpdR::cpdR-eyfp vanA::divLΔ28-mCherry*	This study
NJH599	*Caulobacter* CB15N Δ*vanA* Δ*lacA* Δ*fixL* Δ*fixT cckA::cckA-cfp cpdR::cpdR-eyfp vanA::*$divL_{Y550F}$	This study

Table S2. DNA oligos used in this study

Name	Site	Sequence 5′-3′ (endonuclease site underlined)
2KB_Up_CpdR_SpeI_for	SpeI	ctccccactagttccacccccgaccgtcaggaacact
Down.cckA.for	BamHI	GCGGATCCagcagctgcaggcggcgtagaag
Down.cckA.rev	HindIII	GATAAGCTTtcatccacggcctgacgtctggc
DownEcoRI.cpdR.rev	EcoRI	ccgccgaattcgtggtgccgatgttcatgctgct
DownHindIII.cpdR.for	HindIII	ccgccaagcttcacaaaaagtttcgacaggcggc
JLB5	HindIII	tatAataagcttcgagacggcggtcatgaacct
mCherry Nterm R		gatgatggccatgttatcctcctcgccc (13)
NJH108		gtgtccgagcagaagcagaacgagctggtccacgtc
NJH109		gacgtggaccagctcgttctgcttctgctcggacac
NJH128		Atccaatcttgacgtccgtttgattacg
NJH129		Gtgaacagctcctcgcccttgctcacca
NJH136	NdeI	ctataacCATAtgacattcaggaagccgccac
NJH137	EcoRI	TgAATTCtcagtcatcgatggtctcctt
NJH140	NdeI	aacctcgcATatggccgacttgcagctccaggacaagg
NJH151		Cagcaggaacgccaacgact
NJH152		Aagctggccgaaggtcgaag
NJH162		Tcgcgcaggatccgataggt
NJH163		Ctgcgcatcatcgtctccac
NJH164		tcgctggctcgccgtaagaa
NJH165		gctggtgcgtaatccgaagt
NJH178		gcgccaaggccggtgtgctgttcg
NJH190	EcoRI	gactgcagaattctgtcatcgatggtctccttctc
NJH208		ttcacacaggaaacagctatgaccatga
NJH209		ggccagtccgtaatacgactcacttaa
NJH269		acagatggccgaactggttg
NJH270		aagtagccgaccgtgacgaa
NJH292	AgeI	cgccatACCGGTccgcctgTagctgctgcttgacg
NJH383	*EcoR*I	gcttcgagaAggAATtcatgaacctggccgtcaacgc
NJH384	*BamH*I	Actgcaggatcctacttgtacagctcgtccatgcc
NJH385		gagaagcgcgatcacatggt
NJH386		agacgctgaaggcgatgaac
NJH408	NdeI	agccacgcATATgatgacttcgtacgacctgatcctc
NJH409	EcoRI	gccgcgAgaATTcctagaagccgagttcg
NJH410	HindIII	gAccctcaagcTTcgAcagaaccgccggctgcagg
NJH411	BamHI	cgaggatGGATCcgtacgaagtcatcaggcgcgtggctac
NJH412	BamHI	ggcAaAgGGATccgaactcggcttctaggcggtcg
NJH413	EcoRI	cagcacgGaAttcagcgAcaccacgttataggccagcgg
NJH415	AflII	cgcgtaacgttcACTtAAGtagaagccgagttcg
NJH420	AflII	ttcgacACTTAAGttgaccagggccaggc
NJH422		gtcggcaatgtctcctTcgagctgcgcacgc
NJH423		gcgtgcgcagctcgAaggagacattgccgac
Pvan F		gccgaccgactgagacgctcacaagc (13)
RR7Xyl.rev	BamHI	cgggatccggatcaggccgccatcatc
UpHindIII.yfp.rev2	HindIII	gcaggaagcttttacttgtacagctcgtccatgcc
UpSpeI.cpdR.for	SpeI	ctccagcactagtgacgaaagctcttcgctcgaaag
XylBamHI.rev	BamHI	ccggtggatcccgggcccgcggtaccgtcg
XylHindIII.for	HindIII	cccaagcttcaccagccacaggcccgtgcc