# Supporting Information

## Boyd et al. 10.1073/pnas.1312793110

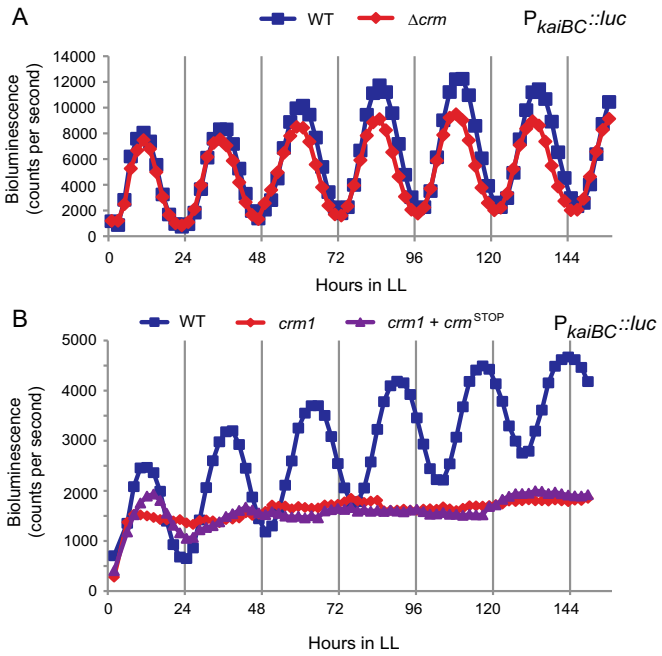


**Fig. S1.** (*A*) Average bioluminescence traces of wild type (WT) (blue trace) and *crm* deletion mutant (red trace) measured from *kaiBC::luc* reporter. Segregation of the mutant was confirmed via PCR. No substantial differences in gene expression rhythms were detected. (*B*) Start codon of *crm* ORF is required for complementation of arrhythmia of the *crm1* insertion strain. Representative bioluminescence traces of WT (blue), *crm1* (red), and *crm1* transformed with a construct wherein the start codon of *crm* is substituted with two consecutive TAG stop codons (violet) are shown. None of the 12 independent *crm* start→stop strains tested demonstrated complementation of arrhythmia. LL, constant light.

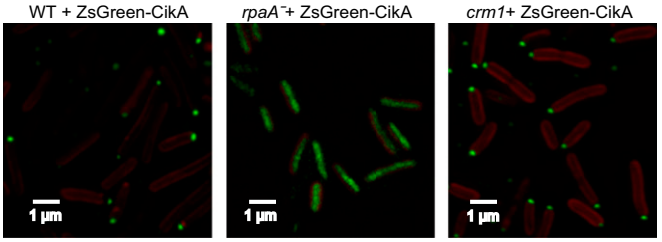


**Fig. S2.** Combined fluorescence micrographs of WT, *rpaA*$^-$, and *crm1* strains expressing ZsGreen-tagged CikA (green). Autofluorescence from photosynthetic pigments is in red. Disruption of *rpaA* abolishes the polar localization of CikA, whereas CikA localization is not disrupted in *crm1* cells.

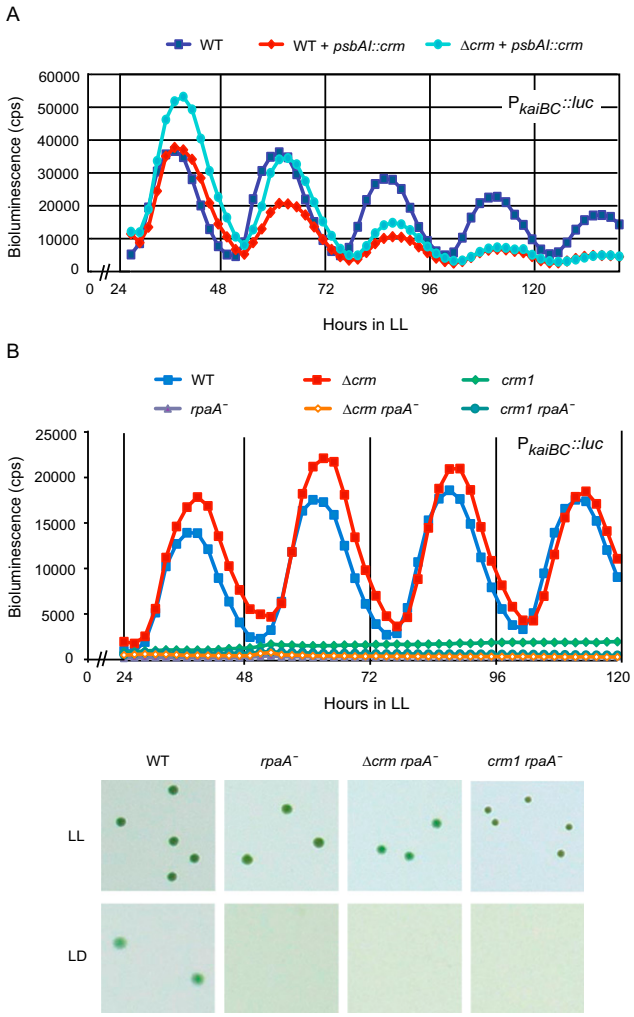


**Fig. S3.** (*A*) Expression of native *crm* from the strong *psbAI* promoter results in significant damping of amplitude of rhythms in both WT and *crm* deletion backgrounds after several days in free run after 2 d of entrainment to a 12-h light:dark (LD) cycle. Average bioluminescence traces are shown. (*B*) Genetic disruption of *rpaA* in either a *crm1* or a *crm* deletion background reflects phenotypes of an *rpaA* null. (*Upper*) Representative bioluminescence traces of *kaiBC::luc* promoter activity. (*Lower*) Colony growth in LL vs. LD.

**Table S1. RNA transcript expression in the *crm* genomic region**

| Oligonucleotide primer set | Position relative to Tn*5* insertion | Average cycle threshold | |
|---|---|---|---|
| | | WT | *crm1* |
| F1/R3 | Downstream | | |
| Forward: GCTATCTCAACAGCAGTTC | | 19 | 19 |
| Reverse: CGTCATAGCCAGACATCTC | | | |
| F4/R4 | Upstream | | |
| Forward: CCTCAAGGCAGCCTAGA | | 20 | 19 |
| Reverse: CTGAGCCTTGATCAACTCA | | | |
| Control | *rpoA* | 16 | 16 |

**Table S2. Cyanobacterial strains used in this study**

| Strain | Genetic characteristics* | Antibiotic resistance | Source or reference |
|---|---|---|---|
| AMC06 | WT | none | Laboratory collection |
| AMC541 | WT $P_{kaiBC}$::*luc* | Cm | Ditty et al. 2003[1] |
| AMC601 | WT $P_{purF}$::*luc* | Km | Ditty et al. 2005[2] |
| AMC603 | WT $P_{psbAI}$::*luc* | Km | Ditty et al. 2005[2] |
| AMC704 | *kaiC* in-frame deletion | Cm | Ditty et al. 2005[2] |
| AMC1722 | Tn*5* 8S15-E11 in AMC541 | Km, Cm | Dong et al. 2010[3] |
| AMC2051 | uni-gene set (UGS) 18-B-10 (Tn*5* 8S15-E11 in AMC06) | Km | This study |
| AMC1782 | pAM4420 in AMC06 | Km | Takai et al. 2006[4] |
| AMC2082 | pAM4226 in AMC1782 | Km, Sp, Sm | This study |
| AMC2034 | pAM4664 in AMC541 | Km, Cm, Sp, Sm | This study |
| AMC2039 | pAM4420 in AMC541 | Km, Cm | This study |
| AMC2087 | pAM2225 in AMC2051 | Km, Sp, Sm | This study |
| AMC2086 | pAM2224 in AMC2051 | Km, Sp, Sm | This study |
| AMC2140 | Tn*5* 8S15-E11 in AMC704 | Km, Cm | This study |

*pAM plasmids were used to transform cyanobacterial strains (AMC) by homologous recombination.
Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin.


1. Ditty JL, Williams SB, Golden SS (2003) A cyanobacterial circadian timing mechanism. *Annu Rev Genet* 37:513–543.
2. Ditty JL, et al. (2005) Stability of the *Synechococcus elongatus* PCC 7942 circadian clock under directed anti-phase expression of the *kai* genes. *Microbiol* 151:2605–2613.
3. Dong G, et al. (2010) Elevated ATPase activity of KaiC applies a circadian checkpoint on cell division in *Synechococcus elongatus*. *Cell* 140(4):529–539.
4. Takai N, et al. (2006) A KaiC-associating SasA-RpaA two-component regulatory system as a major circadian timing mediator in cyanobacteria. *Proc Natl Acad Sci USA* 103(32): 12109–12114.


**Table S3. Plasmids used in this study**

| Plasmid | Characteristics | Antibiotic resistance | Source or reference |
|---|---|---|---|
| 8S15-I10 | Tn*5* transposon insertion in *crm* | Km | Holtman et al. 2005[1] |
| pAM1303 | Promoter-less NSI vector | Sp, Sm | Laboratory collection |
| pAM2224 | *psbAI::luc* promoter fusion | Sp, Sm | Laboratory collection |
| pAM2225 | *purF::luc* promoter fusion | Sp, Sm | Laboratory collection |
| pAM4420 | $Km^R$ cassette insertion in *rpaA* | Km | This study |
| pAM4226 | WT *rpaA* | Sp, Sm | Laboratory collection |
| pAM4664 | pSyn1_D/TOPO vector | Sp, Sm | Life Technologies |
| pSyn2.0 | pSyn2_D/TOPO vector; *psbAI* promoter | Sp, Sm | Life Technologies |
| pAM4666 | Genomic region containing antisense RNA in pSyn1_D/TOPO vector | Sp, Sm | This study |


1. Holtman CK, et al. (2005) High-throughput functional analysis of the *Synechococcus elongatus* PCC 7942 genome. *DNA Res* 12(2):103–115.