Cell

## SUPPLEMENTAL REFERENCES

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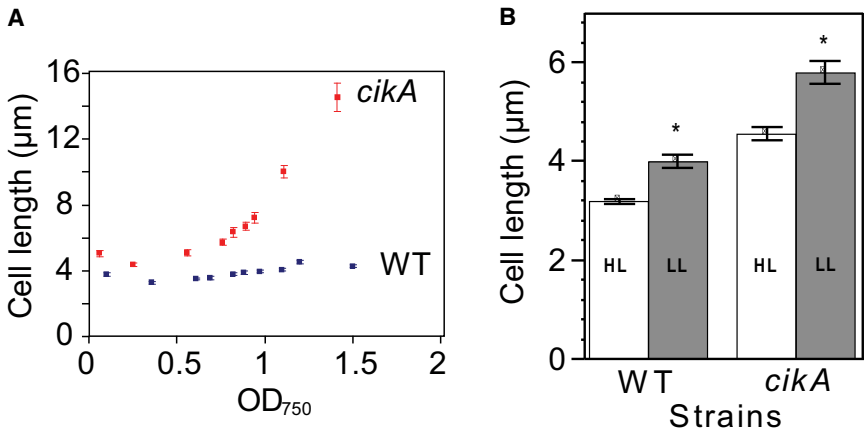


**Figure S1. Cell Length Is Affected by Cell Density and Light Intensity, Related to Figure 1**

It is known that bacterial cell size can be significantly affected by metabolism and physiological state. In order to standardize the conditions under which cell length of *Synechococcus elongatus* PCC 7942 is measured and compared, we investigated the effect of cell density and light intensity on cell length.

(A) Cell length increases in both the WT and *cikA* mutant strains as the cultures grow denser. Cultures were started and grown as described in the Experimental Procedures of the main text. WT cells lengthened by ~1 μm from 3.3 ± 0.1 μm (mean ± SEM) at $OD_{750}$ = 0.36 to 4.3 ± 0.1 μm at $OD_{750}$ = 1.5. Cell length is more severely affected in the *cikA* mutant, increasing from 4.4 ± 0.1 μm at $OD_{750}$ = 0.25 to 14.6 ± 0.9 μm at $OD_{750}$ = 1.4. Notice that cell length from both strains decreases slightly from the first OD reading to the second reading before it steadily increases thereafter; this observation is likely because cells from the first measurement are from a denser culture that is subsequently diluted. Thus, cell density has a significant effect on cell elongation in *S. elongatus* cells. However, as cells grow denser, self-shading causes an effective reduction of light intensity. Thus, we also investigated the effect of light intensity on cell length.

(B) Cells of WT and *cikA* mutant under low light (LL) (10 $\mu E\, m^{-2}\, s^{-1}$) and high light (HL) (100 $\mu E\, m^{-2}\, s^{-1}$) conditions were grown to the same OD and, in both strains, cells grown at low light are significantly longer than those at high light conditions.

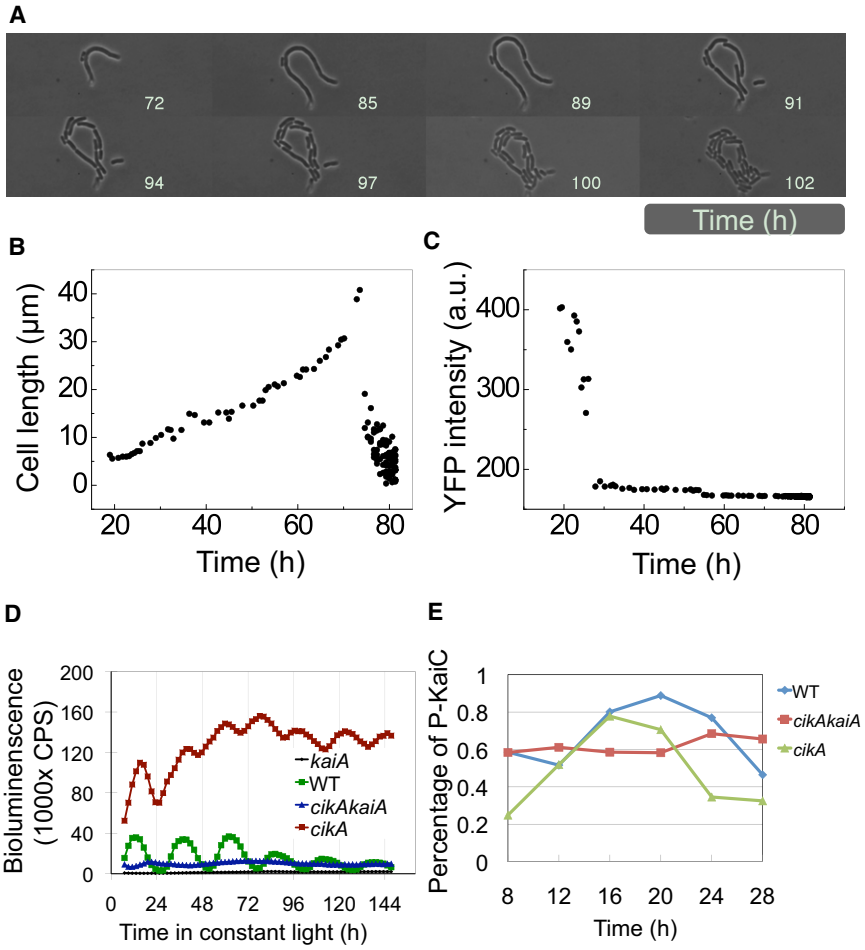


**Figure S2. Cell Elongation Caused by KaiA-YFP Overexpression Is Reversible; No Rhythms of Gene Expression or Phosphorylation Are Present in the Δ*cikA*Δ*kaiA* Mutant, Related to Figure 2**

If constitutive phosphorylation of KaiC is the cause of cell elongation, overexpression of KaiA would block cell division and the return of KaiA to preinduction levels would then unlock KaiC from its hyperphosphorylated state and initiate new rounds of cell division. To test this hypothesis, we fused KaiA to YFP genetically and expressed it from the *trc* promoter in the WT background. The KaiA-YFP fusion protein is fully functional, complements a *kaiA* null mutant when expressed without IPTG, and stimulates constitutive KaiC phosphorylation with the addition of IPTG (data not shown). This fusion allows us to monitor the relative expression level of KaiA-YFP and cell length at the same time using timelapse microscopy. We induced expression with 1 mM IPTG for 7 days, washed off IPTG, and started monitoring a group of cells continuously.

(A) Snapshots of cells at 8 different time points after IPTG washoff. Cells kept growing without dividing for about 3 days after IPTG was washed off, after which many rounds of cell division quickly occurred within a 30 hr period to bring cell length to WT.

(B) Quantification of cell length over time after IPTG washoff of one representative cell and its daughter cells.

(C) Quantification of KaiA-YFP intensity over time of the cells from (B). It took more than 60 hr for KaiA-YFP to return to the basal level after IPTG washoff, and cells kept growing without dividing for an additional 10 hr after basal levels of KaiA-YFP were achieved, after which many rounds of cell division quickly occurred to bring cell length close to normal. The 10 hr lag between basal KaiA-YFP level and cell division is likely due to the slow process of KaiC de-phosphorylation (Rust et al., 2007).

(D) Promoter activity of *kaiBC* is arrhythmic in the Δ*cikA*Δ*kaiA* mutant. All strains have P*kaiBC*::*luc* reporters except the *cikA* mutant, which has a P*kaiBC*::*luxAB* reporter. Cultures are synchronized by two cycles of light:dark (12 hr:12 hr) before released in constant light.

(E) Quantification of KaiC phosphorylation percentages in vivo. Cultures are synchronized by two light:dark cycles before being released into constant light and sampled every 4 hr. Immunoblot analysis was performed and quantified using ImageJ. KaiC in the Δ*cikA*Δ*kaiA* mutant remains 60% phosphorylated during our sampling period.

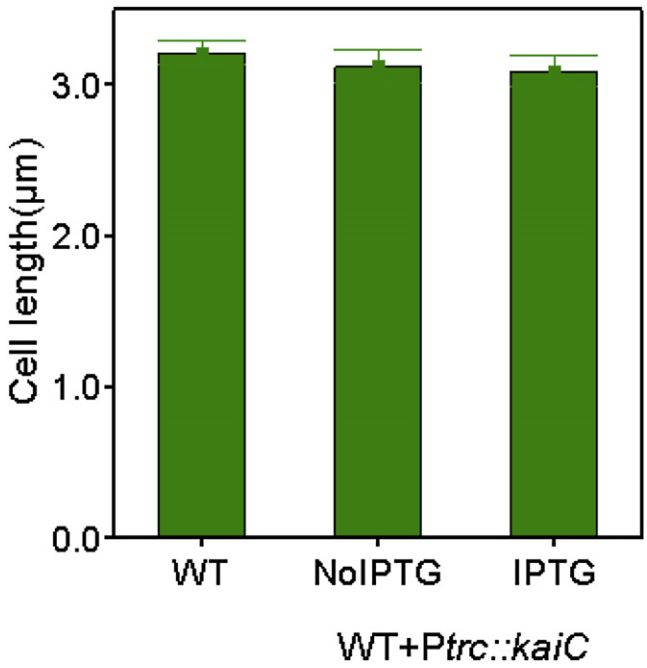


**Figure S3. Overexpression of KaiC Has No Effect on Cell Division, Related to Figure 4**
KaiC was placed under the control of the *trc* promoter and induced with 1 mM IPTG for 3 days before cell length was measured.

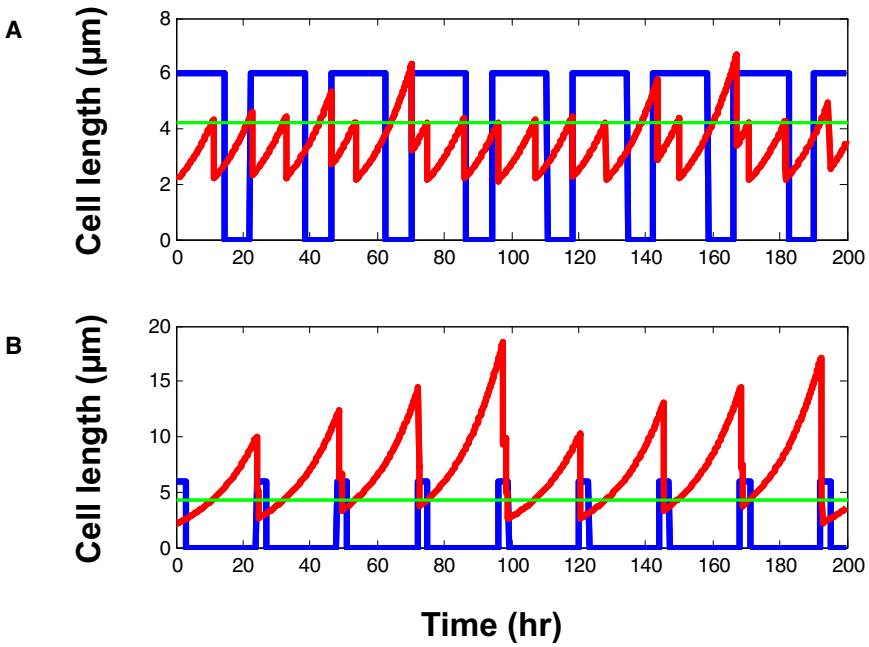


**Figure S4. Simulation of Cell Length Based on the Gating Model, Related to Figure 6C**

Starting with a single cell with certain cell length, the cell length (red line) grows exponentially over time until it hits to a length threshold (green line) and the cell commits to a cell division event. However, the probability of cell division is modulated by gating; i.e., if a cell is inside the "gate" (blue line) where cell division is inhibited, a cell keeps growing without division even above the length threshold. (A) is for WT and (B) is for the Δ*cikA* mutant.

**Table S1. Plasmids Used in This Study, Related to Experimental Procedures**

| Plasmid | Characteristics | Antibiotic resistance | Source or reference |
|---|---|---|---|
| **8S15-E11** | Tn*5* transposon inserted after codon #11 of *rpaA* | Km | (Holtman et al., 2005) |
| **pAM1303** | Cloning vector with NS I integration sequence | Sp, Sm | (Andersson et al., 2000) |
| **pAM1579** | Cloning vector with NS II integration sequence | Km | Lab collection |
| **pAM2152** | $Gm^R$ Ω-cassette inserted in partially deleted *cikA* | Gm, Ap | (Mutsuda et al., 2003) |
| **pAM2176** | $Gm^R$ Ω-cassette inserted in SmaI site of *sasA* | Gm, Ap | Lab collection |
| **pAM2255** | *E. coli* cloning vector with *trc* promoter | Ap | (Mutsuda et al., 2003) |
| **pAM2302** | P*kaiBC::kaiC* in NS I vector | Sp, Sm | (Ditty et al., 2005) |
| **pAM3685** | P*trc::kaiA-yfp* in pAM1303 | Sp, Sm | This study |
| **pAM3842** | P*trc::kaiA* based on pAM3685 | Sp, Sm | This study |
| **pAM3868** | P*kaiBC::kaiC487* based on pAM2302 | Sp, Sm | (Kim et al., 2008) |
| **pAM3870** | P*kaiBC::kaiC-AA* based on pAM2302 | Sp, Sm | (Kim et al., 2008) |
| **pAM3875** | Gateway cloning vector based on pAM1579 | Km, Cm | This study |
| **pAM3876** | P*kaiBC::kaiC* in pAM3875 | Km | This study |

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|---|---|---|---|
| **pAM3910** | P*kaiBC::kaiC497* based on pAM2302 | Sp, Sm | (Kim et al., 2008) |
| **pAM4039** | P*kaiBC::kaiC-DE* based on pAM3876 | Km | This study |
| **pAM4040** | P*kaiBC::kaiC-DT* based on pAM3876 | Km | This study |
| **pAM4041** | P*kaiBC::kaiC-AE* based on pAM3876 | Km | This study |
| **pAM4042** | P*kaiBC::kaiC-SE* based on pAM3876 | Km | This study |
| **pAM4043** | P*kaiBC::kaiC-DA* based on pAM3876 | Km | This study |
| **pAM4131** | P*trc::ftsZ* cloned into pAM1303 | Sp, Sm | This study |
| **pAM4223** | P*trc::ftsZ* in pAM3875 | Km | This study |
| **pAM4238** | P*kaiBC::kaiCR393C* from pAM2302 | Sp, Sm | This study |
| **pAM4239** | P*kaiBC::kaiC-A251V* from pAM2302 | Sp, Sm | This study |
| **pAM4245** | P*kaiBC::kaiC-AA-K52H* based on pAM3870 | Sp, Sm | This study |
| **pAM4246** | P*kaiBC::kaiC-AA-K294H* based on pAM3870 | Sp, Sm | This study |
| **pAM4247** | P*kaiBC::kaiC-AA-E77,78Q* based on pAM3870 | Sp, Sm | This study |
| **pAM4248** | P*kaiBC::kaiC-AA-E318,319Q* based on pAM3870 | Sp, Sm | This study |
| **pAM4249** | P*kaiBC::kaiC-AA-E77,78,318,319Q* based on pAM3870 | Sp, Sm | This study |

**Table S2. Cyanobacterial Strains Used in This Study, Related to Experimental Procedures**

| Strains | Genetic characteristics | Antibiotic resistance | Source or reference |
|---|---|---|---|
| **AMC541** | WT | Cm | (Ditty et al., 2003) |
| **AMC702** | *kaiA* in-frame deletion | Cm | (Ditty et al., 2005) |
| **AMC704** | *kaiC* in-frame deletion | Cm | (Ditty et al., 2005) |
| **AMC705** | *kaiBC* in-frame deletion | Cm | (Ditty et al., 2005) |
| **AMC1005** | *cikA* null by $Gm^R$ Ω-cassette insertion | Gm, Km, Cm | (Mutsuda et al., 2003) |
| **AMC1161** | *kaiA* null by $Km^R$ Ω-cassette insertion | Km, Cm | (Ditty et al., 2005) |
| **AMC1275** | *sasA* in-frame deletion | Cm | Lab collection |
| **AMC1548** | pAM3685 in AMC1161 | Km, Sp, Sm | This study |
| **AMC1619** | pAM3870 in AMC704 | Cm, Sp, Sm | (Kim et al., 2008) |
| **AMC1621** | pAM3868 in AMC704 | Cm, Sp, Sm | (Kim et al., 2008) |
| **AMC1623** | pAM3910 in AMC704 | Cm, Sp, Sm | (Kim et al., 2008) |
| **AMC1624** | pAM2302 in AMC705 | Sp, Sm, Cm | This study |
| **AMC1627** | pAM2152 in AMC704 | Gm, Sp, Sm | This study |
| **AMC1628** | pAM3842 in AMC704 | Cm, Sp, Sm | This study |
| **AMC1670** | pAM4039 in AMC704 | Km | This study |
| **AMC1671** | pAM4040 in AMC704 | Km | This study |
| **AMC1672** | pAM4041 in AMC704 | Km | This study |
| **AMC1673** | pAM4042 in AMC704 | Km | This study |
| **AMC1674** | pAM4043 in AMC704 | Km | This study |
| **AMC1675** | pAM3842 in AMC541 | Km | This study |

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|---|---|---|---|
| **AMC1679** | pAM2152 in AMC702 | Gm, Cm | This study |
| **AMC1700** | kaiCR393C in AMC704 | Cm, Sp, Sm | This study |
| **AMC1701** | KaiCA251V in AMC704 | Cm, Sp, Sm | This study |
| **AMC1708** | pAM2176 in AMC1624 | Sp, Sm, Gm, Cm | This study |
| **AMC1709** | 8S15-E11 in AMC1624 | Sp, Sm, Km, Cm | This study |
| **AMC1710** | pAM2595 in AMC1708 | Sp, Sm, Gm, Km | This study |
| **AMC1713** | pAM2152 in AMC1275 | Gm, Cm | This study |
| **AMC1714** | pAM2152 in AMC1161 | Km, Gm, Cm | This study |
| **AMC1722** | 8S15-E11 in AMC541 | Km, Cm | This study |
| **AMC1735** | pAM4245 in AMC704 | Sp, Sm, Cm | This study |
| **AMC1736** | pAM4246 in AMC704 | Sp, Sm, Cm | This study |
| **AMC1737** | pAM4247 in AMC704 | Sp, Sm, Cm | This study |
| **AMC1738** | pAM4248 in AMC704 | Sp, Sm, Cm | This study |
| **AMC1739** | pAM4249 in AMC704 | Sp, Sm, Cm | This study |