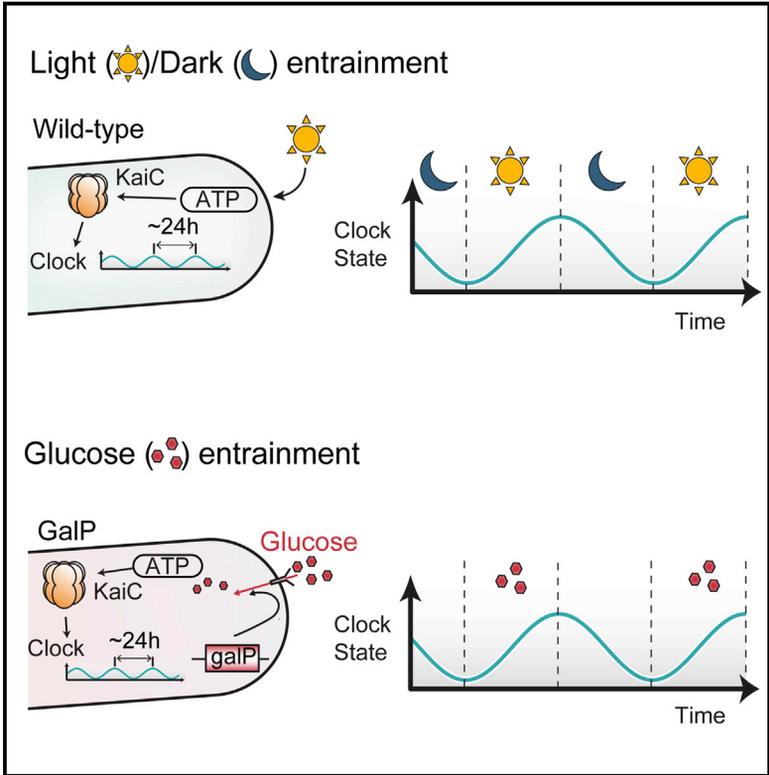


Controlling the Cyanobacterial Clock by Synthetically Rewiring Metabolism

Graphical Abstract



Authors

Gopal K. Pattanayak, Guillaume Lambert, Kevin Bernat, Michael J. Rust

Correspondence

mrust@uchicago.edu

In Brief

A fundamental problem in circadian biology is to understand the molecular mechanisms that allow cells to synchronize to their environment. By uncoupling growth from light and dark, Pattanayak et al. show that the fundamental metabolite-sensing properties of the Kai proteins track daily cycles without any dedicated light sensor.

Highlights

- Expressing a transgenic sugar transporter in cyanobacteria allows growth in the dark
- Glucose feeding overrides the effect of darkness on the circadian clock
- In complete darkness, the KaiABC clock senses rhythmic feeding
- Metabolism is the fundamental synchronizer for the cyanobacterial clock



Controlling the Cyanobacterial Clock by Synthetically Rewiring Metabolism

Gopal K. Pattanayak,¹ Guillaume Lambert,¹ Kevin Bernat,¹ and Michael J. Rust^{1,*}

¹Department of Molecular Genetics and Cell Biology, Institute for Genomics and Systems Biology, 900 E. 57th Street, KCBD 10124, Chicago, IL 60637, USA

*Correspondence: mrust@uchicago.edu

<http://dx.doi.org/10.1016/j.celrep.2015.11.031>

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

SUMMARY

Circadian clocks are oscillatory systems that allow organisms to anticipate rhythmic changes in the environment. Several studies have shown that circadian clocks are connected to metabolism, but it is not generally clear whether metabolic signaling is one voice among many that influence the clock or whether metabolic cycling is the major clock synchronizer. To address this question in cyanobacteria, we used a synthetic biology approach to make normally autotrophic cells capable of growth on exogenous sugar. This allowed us to manipulate metabolism independently from light and dark. We found that feeding sugar to cultures blocked the clock-resetting effect of a dark pulse. Furthermore, in the absence of light, the clock efficiently synchronizes to metabolic cycles driven by rhythmic feeding. We conclude that metabolic activity, independent of its source, is the primary clock driver in cyanobacteria.

INTRODUCTION

Circadian clocks are oscillatory systems found in all kingdoms of life that allow organisms to synchronize their behavior with cycles in the external environment caused by the rotation of Earth. To maintain proper alignment with the environment, clocks must respond to appropriate synchronizing cues while ignoring irrelevant fluctuations. Therefore, understanding the molecular mechanisms that transduce environmental signals into the clock is fundamental.

The simplest example of a circadian oscillator is the biochemically tractable KaiABC system from *Synechococcus elongatus* PCC7942 (hereafter *S. elongatus*), a photoautotrophic cyanobacterium, in which the three Kai proteins work together to produce ~24 hr cycles in KaiC phosphorylation (Nakajima et al., 2005). KaiC then signals through an output transcription factor to generate rhythms in gene expression and physiology (Markson et al., 2013). Exposing cells to cycles of light and dark efficiently entrains the oscillations of the Kai system, suggesting the existence of an input signaling pathway that sends information about light exposure to the clock (Kondo et al.,

1993). However, initial genetic screens for input components failed to identify clock-specific light-sensitive factors (Schmitz et al., 2000; Katayama et al., 2003).

Subsequent biochemical studies revealed that the Kai proteins are directly sensitive to key energy and redox metabolites (Wood et al., 2010). In particular, KaiC senses the ATP/ADP ratio that switches the balance between the enzyme's kinase and its phosphatase activities (Rust et al., 2011; Phong et al., 2013). These biochemical findings suggest that the role of the bacterial circadian system is to sense metabolic changes that are driven by photosynthesis, perhaps without the need for direct perception of light or dark.

Mutants with a defective clock response to a dark pulse also exhibit metabolic phenotypes with altered ATP levels in the dark (Pattanayak et al., 2014). However, these data are largely correlative and do not address whether the metabolic cycles that occur under these conditions are necessary for clock synchronization. To directly test the hypothesis that metabolic activity, not the light-dark cycle per se, is the primary timing cue, we took a synthetic biology approach to uncouple cellular metabolism from darkness. We show that supplying glucose to an engineered cyanobacterial strain capable of growth on sugar supports high levels of ATP in the dark and suppresses the normal clock-resetting effect of a dark pulse. We further show that rhythmic sugar feeding efficiently drives clock synchronization without the need for light-dark cycling. Our results show that metabolic activity is the primary clock synchronizer in cyanobacteria.

RESULTS AND DISCUSSION

Cyanobacteria Expressing a Transgenic Sugar Transporter Becomes Metabolically Active in the Dark

Although *S. elongatus* is a natural autotroph, using photosynthesis to fix inorganic carbon, it can be modified to metabolize sugar supplied in the culture medium by transgenically expressing the *galP* sugar symporter gene from *Escherichia coli* (Figure 1A; McEwen et al., 2013).

We constructed strains that carry fluorescent reporters of clock-driven transcription (Chabot et al., 2007), along with the *galP* transgene, and used live cell microscopy to ask whether this strain could grow on exogenously supplied sugars under typical clock-resetting conditions. While there was no measurable increase in biomass in the wild-type (WT) strain in the

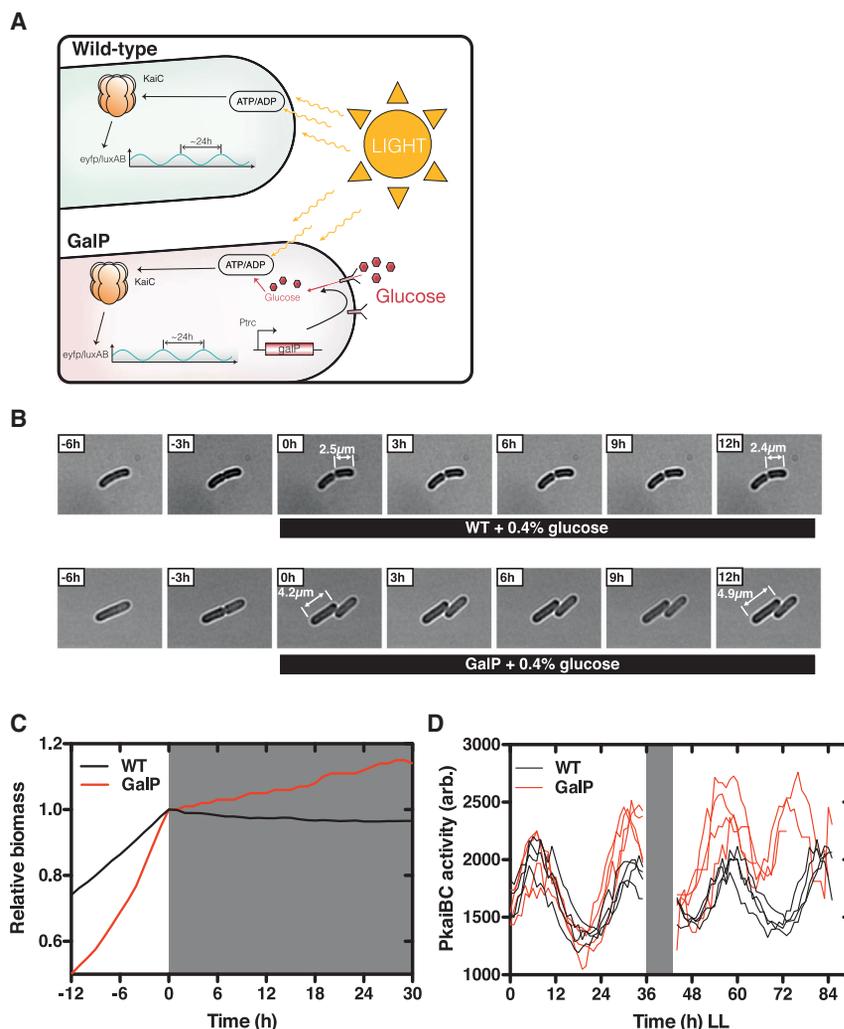


Figure 1. Transgenic *S. elongatus* Strain Expressing GalP Transporter

(A) Schematic of study design. WT cells are photoautotrophic and rely on light for both growth and clock signaling. Transgenic cells expressing the GalP sugar transporter can use glucose as an alternative energy source.

(B) Growth of GalP-expressing cells in the dark. A series of micrographs demonstrates the length increase of GalP cells under dark conditions. WT cells contract slightly under prolonged dark conditions. (C) Increase in total biomass, defined as the normalized sum of all cell lengths, before and after a light-dark transition for WT cells (black) and GalP-expressing cells (red) in the presence of 0.4% glucose.

(D) Clock response to a dark pulse reported by fluorescence in single cells. *PkaiBC::eyfp-ssrA* fluorescence traces before and after a dark pulse (shaded bar) for selected WT cells (black) and GalP-expressing cells grown in the presence of glucose (red). LL, constant light.

See also Figure S1 and Movie S1.

dark, the GalP-expressing cells continued to elongate when glucose was provided, indicating that this engineered strain could grow heterotrophically in the dark (Figures 1B and 1C; Movie S1).

We then asked whether supporting metabolism in the dark with exogenous sugar would suppress the normal clock-resetting effect of a dark pulse. Using time-lapse microscopy experiments, we monitored clock-driven gene expression before and after a dark pulse perturbation. Visualizing rhythms in single cells suggested that supplying cells with sugar altered the response of the clock to a dark pulse delivered near subjective dusk (Figures 1D and S1).

Glucose Feeding Supports Dark Metabolism and Blocks the Normal Clock-Resetting Effect of Darkness

To systematically probe the responsiveness of the circadian clock, we used a light-emitting diode (LED) array device to perturb cells with dark pulses throughout the clock cycle. We then monitored clock time following the perturbation using a bioluminescent reporter of gene expression (Mackey et al., 2007; Pattanayak et al., 2014).

The resulting phase response curve shows that dark-induced phase shifts are nearly completely suppressed in the engineered strain when it is actively growing on sugar (Figures 2A–2C and S2), presumably because sugar uptake can compensate for the loss of photosynthetic metabolism in the dark. The presence of glucose in the culture medium did not substantially alter the free-running period of the circadian clock (25.5 ± 0.2 hr with glucose versus 25.2 ± 0.2 hr without glucose).

The ATP/ADP ratio is known to modulate phosphorylation of the central clock enzyme KaiC, and experimentally manipulating this nucleotide ratio causes phase shifts in the purified protein oscillator (Rust et al., 2011). We therefore asked whether the suppression of phase resetting in the dark caused by sugar uptake is accompanied by a reduced change in the ATP/ADP ratio. We found that sugar feeding elevates the ATP/ADP ratio in the dark, resulting in diminished metabolic contrast during the dark pulse (Figure 2D). Glucose-driven support of a high ATP/ADP ratio in the dark requires the presence of the *galP* transgene (Figure 2D). These results suggest that changes in energy charge are required for strong clock resetting and that maintaining metabolic activity can override the effect of darkness.

Rhythmic Feeding of Glucose Synchronizes the Clock in the Absence of Light-Dark Cues

To test this metabolic hypothesis directly, we asked whether metabolic cycling, driven by rhythms in sugar uptake, could efficiently entrain the circadian clock in the absence of light-dark cues. We designed an experiment in which cultures are initially entrained by a light-dark cycle and then kept in the dark for 48 hr while glucose concentrations are cycled either in phase

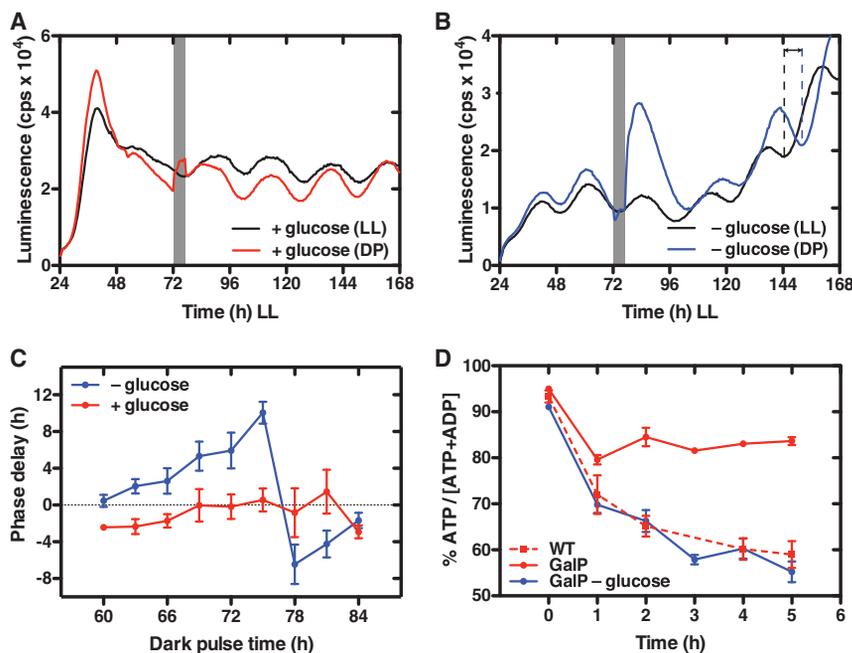


Figure 2. Glucose Feeding Supports Cellular Energy and Blocks Clock Resetting in the Dark

(A and B) Bioluminescence rhythms (*PpsbA1::luxABCDE*) in the GalP-expressing strain in the presence or absence of glucose. After entrainment with one light-dark cycle (12 hr:12 hr), control samples (black) were kept in constant light (LL) while experimental samples (blue or red) were subjected to a 5 hr dark pulse (DP; shaded bar). Phase shift between the conditions is called out with dashed lines.

(C) Phase delay of the bioluminescence rhythm of GalP-expressing cells in the absence (blue) or presence (red) of glucose caused by a 5 hr dark pulse at the indicated time. Phase delays relative to an untreated control (LL) were estimated by fitting the log-transformed time series to a sinusoid. Bars represent standard deviations (n = 4).

(D) Drop in the %ATP/[ATP+ADP] energy charge during a 5 hr dark in GalP-expressing cells or in WT cells (dashed line) in the presence (red) or absence (blue) of glucose. Bars represent SE (n = 4–6). See also Figure S2.

(condition 1) or out of phase (condition 2) with the starting clock state (Figure 3A). We carried out this experiment with oppositely phased glucose cycles and sampled cultures in the dark to monitor KaiC phosphorylation, an indicator of internal clock state. Repeated cycles of sugar feeding are able to effectively reprogram the clock phase in GalP-expressing transgenic cells (Figure 3B) but not in the WT (Figure S3). When these cells are then released into constant light, there is a persistent phase shift between the oppositely cycled cultures, measured by a transcriptional reporter (Figure 3C). Consistent with the shifted rhythms in KaiC phosphorylation, we found that rhythmic feeding of glucose in the dark drives rhythms in the ATP/ADP ratio (Figure 3D). We conclude that light-dark cycles per se are not an essential part of clock entrainment and that metabolism is the fundamental synchronizer for the cyanobacterial clock. However, there could be cryptic light-sensitive clock input mechanisms that might be important under other growth conditions.

Higher organisms can use multiple cues from the environment to reset circadian clocks to the appropriate time. In mammals, light signals are transduced to molecular clock circuitry through neural connections from the retina. The same clock circuitry can respond to feeding cues in peripheral tissues (Dibner et al., 2010). The problem of sensing timing cues from the environment appears to be solved simply in cyanobacteria, where changing light availability during the day-night cycle can be perceived through its impact on metabolic activity, without the need for a dedicated light-sensing apparatus. This architecture supports a scenario in which circadian oscillations arose out of 24 hr metabolic cycles imposed on microbes by the environment (Edgar et al., 2012). The clock machinery in many organisms may have ancient evolutionary roots in the fundamental tasks of metabolic sensing and regulation, a scenario hinted at by the conserved involvement of the peroxiredoxin system in circadian

rhythms (Edgar et al., 2012), and perhaps by the similarities between cyanobacterial KaiC and the F₁ ATP synthase (Egli et al., 2012; Nishiwaki and Kondo, 2012).

Many microbes, including *Legionella* spp., are not photosynthetic but maintain the *kaiBC* genes (Dvornyk et al., 2003). The function of the *kai* genes outside of cyanobacteria is not known, but they can play important roles in regulating growth in the context of virulence (Loza-Correa et al., 2014). Many other microbes, such as the members of the mammalian gut microbiome, must contend with a rhythmic environment without perceiving light directly (Thaiss et al., 2014). Our results here show that the *kai* system can function as a generalized metabolic timing system in the absence of light-dark cues. This finding suggests a framework for studying the broader family of *kai* genes outside of cyanobacteria and points to the possibility of using this system in a synthetic biology context to sense and respond to metabolic fluctuations.

EXPERIMENTAL PROCEDURES

Cyanobacterial Strains

The bioluminescent reporter strains used in this study are based on a bacterial (*Vibrio harveyi*) luciferase system and are derivatives of *S. elongatus* PCC 7942 (Chen et al., 2009). The WT luminescence reporter strain WT/pAM2195 (MRC1005) carries *PpsbA1::luxABCDE*, so the cultures produce the enzyme luciferase and the long-chain aldehyde substrate for luciferase simultaneously (Mackey et al., 2007). In plasmid pAM2195, *PpsbA1::luxABCDE* is integrated at neutral site (NS) 2.1 with a chloramphenicol resistance cassette (Mackey et al., 2007). The *yfp-ssrA* reporter strain WT/JRCS35 (MRC1006) carries *PkaiBC::yfp-ssrA*, so the cultures express yellow fluorescent protein (YFP) rhythmically. In plasmid JRCS35, *PkaiBC::yfp-ssrA* is integrated at NS2 with a kanamycin cassette (Chabot et al., 2007). The other strains used in this study, i.e., the GalP-expressing strains in the MRC1005 background (MRC1007) and in the MRC1006 background (MRC1008), respectively, are described later.

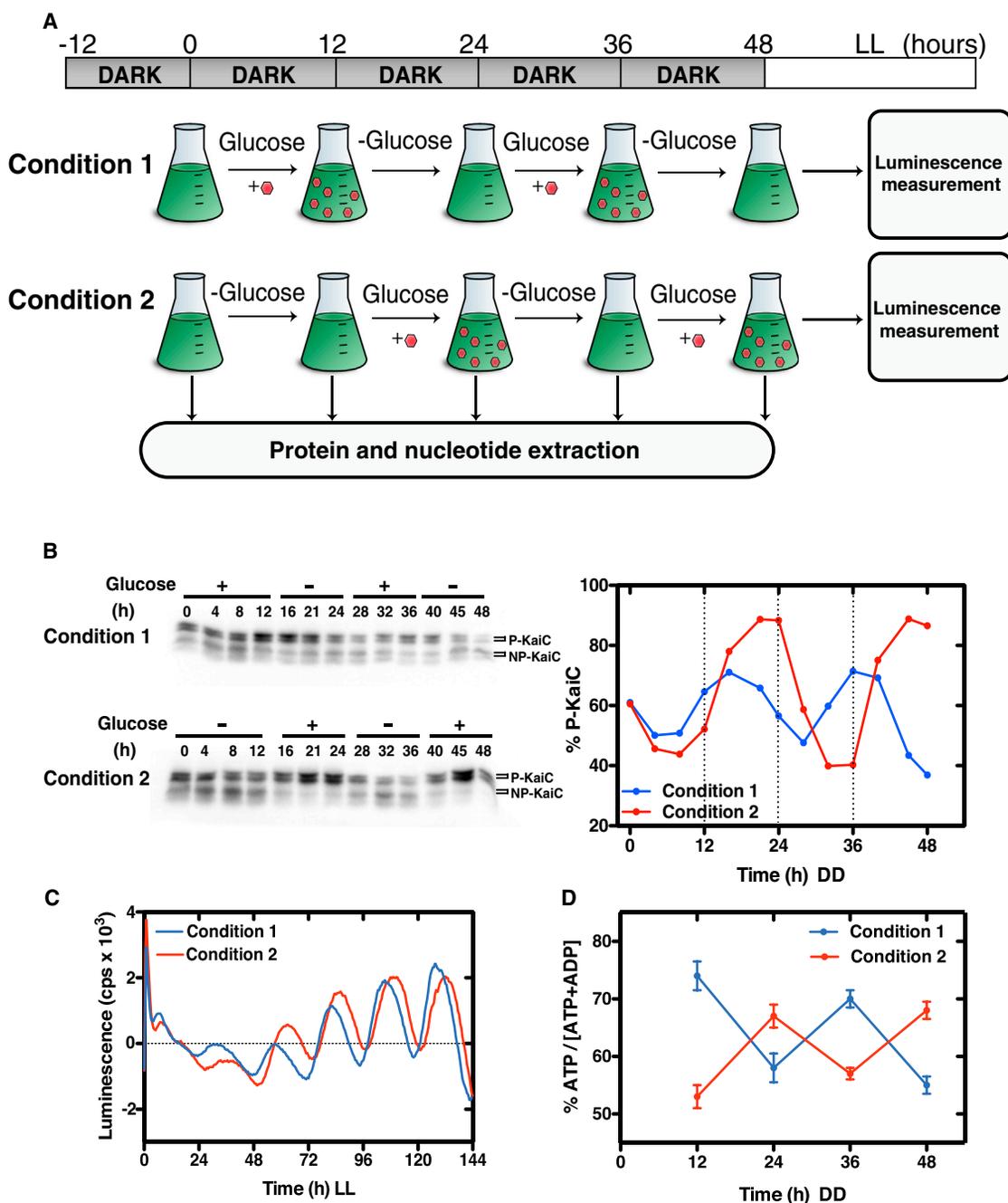


Figure 3. Rhythmic Glucose Feeding Entrains the Clock in the Absence of Light-Dark Cues

(A) Schematic of glucose feeding experiment in the dark. GalP-expressing cells were transferred into continuous dark. During this dark period, cells were fed rhythmically, 12 hr with glucose and 12 hr no glucose, and harvested for protein and nucleotide analysis. After 48 hr of rhythmic glucose feeding, cells were washed to remove glucose from the media and transferred to constant light (LL) without glucose to monitor bioluminescence rhythms. All experiments were done at least twice.

(B) Western blot (α KaiC) time series during glucose cycling (left). Densitometric estimate of oscillations in the fraction of P-KaiC (right). DD, constant dark.

(C) Resulting phase of bioluminescence oscillations (*PpsbAI::luxABCDE*) after transfer to continuous light without glucose.

(D) Rhythms in %ATP/[ATP+ADP] in the dark during glucose cycling. Bars represent SE (n = 4–6).

See also Figure S3.

Molecular Cloning and Construction of the GalP Expression Plasmid

The *galP* gene was amplified from *Escherichia coli* genomic DNA using two pairs of oligonucleotide primers, 5'-CTA ACA ATT GAT GCC TGA CGC TAA

AAA ACA GGG GCG-3' (*galP* F) and 5'-CTA TAG ATC TTT AAT CGT GAG CGC CTA TTT CGC GCA GTT-3' (*galP* R). The DNA fragment (1,395 bp) amplified by *galP* F/*galP* R primers was digested with MfeI and BglII (underlined) and

inserted into pAM2991 plasmid (spectinomycin resistance) digested with EcoRI and BamHI restriction enzyme sites, respectively, to create the pMR0094 plasmid. In pMR0094, *galP* expression is under the control of *P_{trc}* promoter (isopropyl β -D-1-thiogalactopyranoside [IPTG] inducible); pAM2991 is an NS1 plasmid.

Transformation of *S. elongatus*

S. elongatus transformations were performed as previously described (Golden et al., 1986). The pAM2195 plasmid was used to transform WT *S. elongatus* cells to generate the WT/pAM2195 (MRC1005) reporter strain. Similarly, the JRC35 (Chabot et al., 2007) plasmid was used to transform WT *S. elongatus* cells to generate the MRC1006 reporter strain. The pMR0094 plasmid carrying the *galP* gene under the control of the *trc* promoter was used to transform the MRC1005 luminescence reporter strain and the MRC1006 YFP reporter strain independently to generate the GalP-expressing strains MRC1007 and MRC1008, respectively. The transformed colonies carrying the *galP* gene were grown repeatedly in BG-11 liquid medium supplemented with appropriate antibiotics, followed by genomic DNA extraction and confirmation of the presence of the *galP* gene by PCR analysis using two pairs of oligonucleotide primers, 5'-TTG ACA ATT AAT CAT CCG GCT CGT ATA-3' (*P_{trc}* F) and *galP* R (5'-CTA TAG ATC TTT AAT CGT GAG CGC CTA TTT CGC GCA GTT-3'), respectively.

Culture Conditions

For all experiments, cyanobacterial strains were grown in BG-11 liquid medium without the addition of sodium bicarbonate and were supplemented with 20 mM HEPES (pH 7.0; henceforth referred as BG-11-A), with appropriate combinations of antibiotics at 30°C under continuous illumination ($\sim 75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) by cool white fluorescent light (Phillips) and with shaking at 170 rpm, except as noted. Cell densities were monitored by measuring the optical density at 750 nm (OD_{750}).

Nucleotide Extraction and Analysis

We extracted nucleotides from different cyanobacterial cultures as described (Pattanayak et al., 2014). Luciferase assay for ATP and ADP was done using a luminometer (Glomax, Promega). The nucleotide extraction and luciferase assay procedures are detailed in Supplemental Experimental Procedures.

Microscopy and Single-Cell Analysis

Cells were grown in the presence of 4 g/l glucose. Each strain (WT+glucose and GalP+glucose) was first entrained by two 12 hr:12 hr light-dark cycles under red LED illumination in a black 96-well plate. Cultures were then released into constant light ($\sim 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 48 hr and then transferred to the microscope. On the microscope, the cells were subjected to 12 hr of light followed by continuous dark (L12DD) and imaged automatically using $\mu\text{Manager}$ (Edelstein et al., 2010). In Figure 1C, the sum of the lengths of all cells detected in the WT+glucose and GalP+glucose experiments under L12DD conditions were plotted as a function of time. The total length of the population was rescaled with respect to its length immediately before lights off. In Figure 1D, selected cells with a similar phase were selected among the WT+glucose and GalP+glucose experiments (so that the dark pulse occurred approximately 7 hr after the peak reporter signal), and their YFP fluorescence signals were plotted as a function of time. Methods for sample preparation, imaging, and image processing are described in Supplemental Experimental Procedures.

Circadian Bioluminescence Measurements

Bioluminescence rhythms were assayed from luciferase reporter strains illuminated by a custom-built LED (Super Bright LEDs) array on a TopCount luminometer (PerkinElmer) as detailed in Supplemental Experimental Procedures.

Rhythmic Feeding of Glucose to Cultures in the Dark

Two flasks of MRC1007 cultures (125 ml each) were grown in BG-11-A liquid medium under continuous illumination until the OD_{750} reached ~ 0.6 . Cultures were exposed to one light-dark cycle (12 hr:12 hr) to synchronize the circadian clock. Five hours before the onset of the 12 hr dark pulse, IPTG (0.1 mM) was added to induce the cells to express GalP. Immediately after the end of the

12 hr dark pulse, glucose (4 g/l) was added to one flask (culture condition 1), and the other flask (culture condition 2) remained without glucose. Then, both flasks were kept in the dark for 12 hr with shaking at 40 rpm. After 12 hr of dark, cultures were centrifuged (4,000 rpm for 10 min) at room temperature to remove glucose and IPTG from the growth medium. The supernatants were discarded, and the cell pellets were washed with 100 ml of BG-11-A liquid medium. This process was repeated, and finally the cell pellets were resuspended in 100 ml of BG-11-A medium. Now, glucose (4 g/l) and IPTG (0.1 mM) were added to culture condition 2, but only IPTG (0.1 mM, no glucose) was added to culture condition 1; the cultures were kept in the dark for a further 12 hr. We repeated the washing process, removing glucose from culture condition 2 and adding glucose to culture condition 1. By doing this, we altered the glucose content rhythmically in both flasks. We rhythmically altered the glucose content every 12 hr for 48 hr in complete darkness. The negative control in Figure S3 uses the same protocol.

Cultures grown for 48 hr in the dark under this rhythmic feeding regimen were then washed twice to remove glucose from the culture media and inoculated (30 μl) onto 250 μl BG-11 agar pads with no glucose in a 96-well black plastic plate. The plate was placed under red LEDs (light intensity was adjusted to $\sim 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), and the bioluminescence rhythms were assayed on a PerkinElmer TopCount luminometer as described (Pattanayak et al., 2014).

Total Protein Extraction and Western Blot Analysis

Cultures were harvested in the dark, and the pellets were frozen in liquid nitrogen and stored at -80°C . For total protein extraction, each frozen pellet was thawed in 150 μl of lysis buffer (8 M urea + 20 mM HEPES [pH 8.0]). Glass beads (0.1 mm, acid washed) were added to the cell suspension, and cells were broken by vigorously vortexing the tube for 5 min in cycles of mixing for 30 s and cooling on ice for 1 min. The cell suspension was centrifuged at low speed (1,000 rpm for 3 min) in a microcentrifuge, and the supernatant fraction was collected. A sample was taken from each supernatant fraction to measure the protein concentration by Bradford assay. Equal amounts of total protein were added to 3x DTT-containing SDS-PAGE sample buffer. The samples were heated at 70°C for 10 min and were loaded onto SDS-PAGE gels (10%). Electrophoresis conditions were: 4.5 hr at a 35 mA constant current at 12°C .

Total protein samples (10 μg) were separated by SDS-PAGE and transferred onto polyvinylidene fluoride membranes. The membrane filters were blocked for 2 hr at room temperature by incubating in 2% nonfat dry milk/Tris-buffered saline with 0.1% Tween 20 (TBST). Membranes were then incubated overnight at 4°C with a polyclonal anti-KaiC antibody (1:10,000 dilutions in 2% nonfat dry milk/TBST). After washing three to four times (15 min each), membranes were incubated for 2 hr at room temperature with 1:10,000 dilutions of secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit). The blots were developed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) according to the manufacturer's directions. The blots were photographed with a charge-coupled device camera using the Chemi-Doc MP Imaging System (Bio-Rad). The percentage of KaiC phosphorylation was estimated by dividing the image into a region that corresponds to the phosphorylated bands in the in vitro clock reaction phosphorylated KaiC (P-KaiC) and a region that corresponds to unphosphorylated KaiC (NP-KaiC) protein (Rust et al., 2007), though NP-KaiC can correspond to multiple bands of unknown identity in the in vivo samples. The percentage of P-KaiC was calculated by densitometry on the blot image.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and one movie and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.11.031>.

AUTHOR CONTRIBUTIONS

G.K.P. and K.B. carried out biochemistry experiments. G.L. carried out microscopy experiments. G.K.P., G.L., and M.J.R. designed the study and wrote the paper.

ACKNOWLEDGMENTS

We thank Susan S. Golden (University of California, San Diego) and Alexander van Oudenaarden (Hubrecht Institute, Utrecht) for the generous gifts of plasmids. We thank Shota Atsumi (University of California, Davis) for useful discussions about the GalP-expressing strain. We thank members of the M.J.R. lab for comments on the manuscript. The work was supported by a Burroughs-Wellcome Career Award at the Scientific Interface (to M.J.R.) and by the NIH (GM107369-01).

Received: August 10, 2015

Revised: October 26, 2015

Accepted: November 7, 2015

Published: December 10, 2015

REFERENCES

- Chabot, J.R., Pedraza, J.M., Luitel, P., and van Oudenaarden, A. (2007). Stochastic gene expression out-of-steady-state in the cyanobacterial circadian clock. *Nature* 450, 1249–1252.
- Chen, Y., Kim, Y.I., Mackey, S.R., Holtman, C.K., Liwang, A., and Golden, S.S. (2009). A novel allele of kaiA shortens the circadian period and strengthens interaction of oscillator components in the cyanobacterium *Synechococcus elongatus* PCC 7942. *J. Bacteriol.* 191, 4392–4400.
- Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* 72, 517–549.
- Dvornyk, V., Vinogradova, O., and Nevo, E. (2003). Origin and evolution of circadian clock genes in prokaryotes. *Proc. Natl. Acad. Sci. USA* 100, 2495–2500.
- Edelstein, A., Amodaj, N., Hoover, K., Vale, R., and Stuurman, N. (2010). Computer control of microscopes using μ Manager. *Curr. Protoc. Mol. Biol.* 92, 1–17.
- Edgar, R.S., Green, E.W., Zhao, Y., van Ooijen, G., Olmedo, M., Qin, X., Xu, Y., Pan, M., Valekunja, U.K., Feeney, K.A., et al. (2012). Peroxiredoxins are conserved markers of circadian rhythms. *Nature* 485, 459–464.
- Egli, M., Mori, T., Pattanayek, R., Xu, Y., Qin, X., and Johnson, C.H. (2012). Dephosphorylation of the core clock protein KaiC in the cyanobacterial KaiABC circadian oscillator proceeds via an ATP synthase mechanism. *Biochemistry* 51, 1547–1558.
- Golden, S.S., Brusslan, J., and Haselkorn, R. (1986). Expression of a family of psbA genes encoding a photosystem II polypeptide in the cyanobacterium *Anacystis nidulans* R2. *EMBO J.* 5, 2789–2798.
- Katayama, M., Kondo, T., Xiong, J., and Golden, S.S. (2003). IdpA encodes an iron-sulfur protein involved in light-dependent modulation of the circadian period in the cyanobacterium *Synechococcus elongatus* PCC 7942. *J. Bacteriol.* 185, 1415–1422.
- Kondo, T., Strayer, C.A., Kulkarni, R.D., Taylor, W., Ishiura, M., Golden, S.S., and Johnson, C.H. (1993). Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. *Proc. Natl. Acad. Sci. USA* 90, 5672–5676.
- Loza-Correa, M., Sahr, T., Rolando, M., Daniels, C., Petit, P., Skarina, T., Gomez Valero, L., Dervins-Ravault, D., Honoré, N., Savchenko, A., and Buchrieser, C. (2014). The *Legionella pneumophila* kai operon is implicated in stress response and confers fitness in competitive environments. *Environ. Microbiol.* 16, 359–381.
- Mackey, S.R., Ditty, J.L., Clerico, E.M., and Golden, S.S. (2007). Detection of rhythmic bioluminescence from luciferase reporters in cyanobacteria. *Methods Mol. Biol.* 362, 115–129.
- Markson, J.S., Piechura, J.R., Puszynska, A.M., and O’Shea, E.K. (2013). Circadian control of global gene expression by the cyanobacterial master regulator RpaA. *Cell* 155, 1396–1408.
- McEwen, J.T., Machado, I.M.P., Connor, M.R., and Atsumi, S. (2013). Engineering *Synechococcus elongatus* PCC 7942 for continuous growth under diurnal conditions. *Appl. Environ. Microbiol.* 79, 1668–1675.
- Nakajima, M., Imai, K., Ito, H., Nishiwaki, T., Murayama, Y., Iwasaki, H., Oyama, T., and Kondo, T. (2005). Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* 308, 414–415.
- Nishiwaki, T., and Kondo, T. (2012). Circadian autodephosphorylation of cyanobacterial clock protein KaiC occurs via formation of ATP as intermediate. *J. Biol. Chem.* 287, 18030–18035.
- Pattanayak, G.K., Phong, C., and Rust, M.J. (2014). Rhythms in energy storage control the ability of the cyanobacterial circadian clock to reset. *Curr. Biol.* 24, 1934–1938.
- Phong, C., Markson, J.S., Wilhoite, C.M., and Rust, M.J. (2013). Robust and tunable circadian rhythms from differentially sensitive catalytic domains. *Proc. Natl. Acad. Sci. USA* 110, 1124–1129.
- Rust, M.J., Markson, J.S., Lane, W.S., Fisher, D.S., and O’Shea, E.K. (2007). Ordered phosphorylation governs oscillation of a three-protein circadian clock. *Science* 318, 809–812.
- Rust, M.J., Golden, S.S., and O’Shea, E.K. (2011). Light-driven changes in energy metabolism directly entrain the cyanobacterial circadian oscillator. *Science* 331, 220–223.
- Schmitz, O., Katayama, M., Williams, S.B., Kondo, T., and Golden, S.S. (2000). CikA, a bacteriophytochrome that resets the cyanobacterial circadian clock. *Science* 289, 765–768.
- Thaiss, C.A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, A.C., Abramson, L., Katz, M.N., Korem, T., Zmora, N., et al. (2014). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 159, 514–529.
- Wood, T.L., Bridwell-Rabb, J., Kim, Y.I., Gao, T., Chang, Y.G., LiWang, A., Barondeau, D.P., and Golden, S.S. (2010). The KaiA protein of the cyanobacterial circadian oscillator is modulated by a redox-active cofactor. *Proc. Natl. Acad. Sci. USA* 107, 5804–5809.