A circadian rhythm in the accumulation of the core clock protein KaiC has been proposed to be important for proper circadian timing in the cyanobacterium Synechococcus elongatus PCC 7942 under continuous light conditions. Cycling in the abundance of the KaiC protein is delayed to the rhythm of its mRNA by ~8 h, consistent with the proposed function of KaiC as a negative feedback regulator of kaiBC transcription. Here, we present temporal profiles of the synthesis and degradation of KaiC protein that determine the rhythm of its accumulation. The rate of KaiC synthesis shows a robust circadian oscillation, which is delayed to the mRNA rhythm slightly and advances the rhythm of KaiC accumulation by ~6 h. The stability of KaiC protein also shows circadian fluctuations, such that KaiC degradation is suppressed during the mid-subjective night. These results suggest that transcriptional, translational, and posttranslational processes are important for the proper circadian changes in KaiC accumulation. Moreover, the turnovers of the phosphorylated and nonphosphorylated forms of KaiC show robust circadian rhythms with an anti-phase relationship to each other. Interestingly, when translation was inhibited, KaiC degradation and phosphorylation proceeded within at least 4 h in a circadian phase-dependent manner. Thus, the circadian timing seems flexible even when any perturbation in protein synthesis occurs.

Circadian clocks drive endogenous oscillations with a period of ~24 h in the molecular, physiological, behavioral, and ecological activities of most organisms (1). Cyanobacteria are the simplest organisms known to exhibit circadian rhythms and provide both genetic and physiological model systems for circadian biology (2–4). In Neurospora, Arabidopsis, Drosophila, and mammals, transcription/translation-based negative feedback regulation of the clock genes has been suggested to be a key process in the generation of basic oscillations (5). In the unicellular cyanobacterium Synechococcus elongatus PCC 7942, the kaiA, kaiB, and kaiC genes have been characterized as essential clock regulators. Of these three genes, kaiC forms an operon with the kaiB gene and is expressed in a robust circadian fashion. KaiC is an ATP-binding autokinase protein (6) that negatively regulates its own expression (7) in a KaiA-dependent manner (8). Interestingly, the overexpression of kaiC represses not only the kaiBC promoter but also most gene promoters (9). Therefore, KaiC is thought to be a promoter-nonspecific, genome-wide transcriptional modifier, possibly acting via its effect on the basic transcription machinery or on the state of chromosome compaction (9–11).

Although the biochemical activities of the Kai proteins remain unclear, they show dynamic circadian patterns in their accumulation, protein complex formation, and posttranslational modification. The KaiB and KaiC proteins accumulate in a circadian fashion, peaking at circadian time (CT)1 15–18.2 under continuous light conditions, which delays the rhythm of kaiBC mRNA accumulation by ~8 h (12). This type of time lag between mRNA and protein rhythms has also been observed for some negative regulators in eukaryotic clock systems and is thought to be important in causing feedback loops to oscillate (5, 13).

KaiC forms a hexamer in an ATP-dependent manner in vitro (10, 14). The KaiC hexamer forms larger protein complexes with KaiA, KaiB, and a sensory histidine kinase, SasA, during the subjective night (15–18). KaiC undergoes phosphorylation at Ser and Thr residues, most probably through its own autokinase activity. Phosphorylation of KaiC shows a robust circadian rhythm peaking at CT16. KaiA enhances the (auto)phosphorylation of KaiC both in vitro and in vivo (8), and this effect is inhibited by KaiB, which possibly enhances the auto-dephosphorylation activity of KaiC (11, 18, 19). The kaiA2 mutation in the KaiA protein lengthens the circadian period by ~8 h (7) and reduces KaiC phosphorylation (8). The kaiC15 mutation, which was mapped to one of two KaiA-binding domains of KaiC, suppresses the effects of the kaiA2 mutation, restoring the wild-type magnitude of KaiC phosphorylation and circadian period length (8). Thus, KaiA-mediated KaiC phosphorylation seems important for circadian pacemaking.

Importantly, a temporal increase in the amount of KaiC shifts the phase of the Synechococcus clock (7, 12). Therefore, the rhythmic accumulation of KaiC is thought to be important for circadian timing. However, no quantitative information on KaiC protein turnover that determines its accumulation rhythm has been available. Here, we present temporal profiles of KaiC protein synthesis and degradation. Our results suggest that rhythmic protein synthesis and degradation are both important for the proper circadian accumulation of KaiC protein.

This paper is available on line at http://www.jbc.org

1 The abbreviations used are: CT, circadian time; LL, continuous light; Chm, chloramphenicol.
2 CT indicates the subjective time of day in h under constant environmental conditions where CT0 corresponds to the subjective dawn.

Received for publication, May 26, 2004, and in revised form, June 29, 2004
Published, JBC Papers in Press, June 30, 2004, DOI 10.1074/jbc.M405861200

Keiko Imai, Taeko Nishiwaki, Takao Kondo, and Hideo Iwasaki‡
From the Division of Biological Science, Graduate School of Science, Nagoya University, and CREST, Japan Science and Technology Corporation (JST), Furo-cho, Chikusa, Nagoya 464-8602, Japan

‡ To whom correspondence should be addressed: Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602, Japan. Tel.: 81-52-789-2507; Fax: 81-52-789-2963; E-mail: iwasaki@bio.nagoya-u.ac.jp.
Circadian Rhythms in KaiC Protein Synthesis and Stability

EXPERIMENTAL PROCEDURES

Bacterial Strains and Culture—NUC42 (20) and NUC301 2 were used as wild-type reporter strains. NUC301 carries a Pm::luxAB reporter cassette with a kanamycin-resistance gene at a specific targeting site designated NS1. kaiC13 (also named CLab) (21) was used as an arrhythmic mutant strain. The PpsbaA::luxAB reporter unit of this strain was replaced with a Pm::luxAB cassette (with a selectable marker gene at the specific target site, NS1) to evaluate kaiBC promoter activity. Synechococcus cells were grown in modified BG-11 medium (22) under continuous light (LL) conditions (50 \( \mu \)M \( \cdot \)m \(^{-2}\) \( \cdot \)s \(^{-1}\) from white fluorescent lamps) at 30 °C.

Methionine Uptake Analysis—Methionine uptake was analyzed as described by Chen et al. (23) with some modifications. Synechococcus cells were grown in a continuous culture system to an optical density of 0.25 at 730 nm (\( A_{730} \)), exposed to two cycles of alternating 12-h light/12-h dark, and then placed under LL conditions. Cells were collected at the appropriate times, resuspended in 800 \( \mu \)l of fresh BG-11 medium containing 1.48 MBq of \(^{[35}\)S\)methionine (1000 Ci/mmol; Amersham Biosciences), and incubated at 30 °C under LL for 30 min. After centrifugation (20,000 \( \times \) g, 2 min), cell pellets were washed with 1 ml of 1% casamino acid and then filtered through prewashed 0.45-\( \mu \)m filters (Millipore). The filters were rinsed twice with 5 ml of cold fresh medium, and the radioactivity was measured with a liquid scintillation counter (Aloka LSC-5100). To correct for background counts, 800 \( \mu \)l of cell suspension was denatured at 90 °C for 20 min, cooled on ice, and then mixed with 1.48 MBq of \(^{[35}\)S\)methionine. The cells were immediately processed as described above.

KaiC Protein Synthesis Rate Analysis—Cells were collected from the continuous culture system at the appropriate times, resuspended in 600 \( \mu \)l of fresh BG-11 medium containing 1.11 MBq of \(^{[35}\)S\)methionine (1000 Ci/mmol), and incubated at 30 °C under LL for 30 min. After centrifugation, cell pellets were washed once with 1 ml of 1% casamino acid and then twice with 1 ml of fresh BG-11 medium, immediately frozen, and stored at −80 °C. Cells were resuspended in 500 \( \mu \)l of lysis buffer (20 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 1% SDS, 1 \( \mu \)l/ml leupeptin, 1 \( \mu \)g/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride, pH 8.0) and disrupted using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) with zirconium beads (0.2 mm diameter). The cell suspension was denatured at 90 °C for 2 min, cell pellets were washed with 1 ml of 1% casamino acid and then filtered through prewashed 0.45-\( \mu \)m filters (Millipore). The filters were rinsed twice with 5 ml of cold fresh medium, and the radioactivity was measured with a liquid scintillation counter (Aloka LSC-5100). To correct for background counts, 800 \( \mu \)l of cell suspension was denatured at 90 °C for 20 min, cooled on ice, and then mixed with 1.48 MBq of \(^{[35}\)S\)methionine. The cells were immediately centrifuged, cell pellets were washed with 1 ml of 1% casamino acid and then filtered through prewashed 0.45-\( \mu \)m filters (Millipore). The filters were rinsed twice with 5 ml of cold fresh medium, and the radioactivity was measured with a liquid scintillation counter (Aloka LSC-5100). To correct for background counts, 800 \( \mu \)l of cell suspension was denatured at 90 °C for 20 min, cooled on ice, and then mixed with 1.48 MBq of \(^{[35}\)S\)methionine. The cells were immediately processed as described above.

KaiC Stability Analysis—Cells were collected from the continuous culture system at the appropriate times, resuspended in 600 \( \mu \)l of fresh BG-11 medium containing 1.11 MBq of \(^{[35}\)S\)methionine (1000 Ci/mmol), and incubated at 30 °C under LL for 30 min. After centrifugation, cell pellets were washed once with 1 ml of 1% casamino acid and then twice with 1 ml of fresh BG-11 medium, immediately frozen, and stored at −80 °C. Cells were resuspended in 500 \( \mu \)l of lysis buffer (20 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 1% SDS, 1 \( \mu \)l/ml leupeptin, 1 \( \mu \)g/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride, pH 8.0) and disrupted using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) with zirconium beads (0.2 mm diameter). The cell suspension was denatured at 90 °C for 2 min, cell pellets were washed with 1 ml of 1% casamino acid and then filtered through prewashed 0.45-\( \mu \)m filters (Millipore). The filters were rinsed twice with 5 ml of cold fresh medium, and the radioactivity was measured with a liquid scintillation counter (Aloka LSC-5100). To correct for background counts, 800 \( \mu \)l of cell suspension was denatured at 90 °C for 20 min, cooled on ice, and then mixed with 1.48 MBq of \(^{[35}\)S\)methionine. The cells were immediately processed as described above.

RESULTS

Circadian Oscillations in the Rate of KaiC Synthesis—To analyze the temporal dynamics of KaiC protein synthesis, we used a combinatorial approach with a pulse-chase labeling method with \(^{[35}\)S\)methionine and immunoprecipitation. Chen et al. (23) demonstrate the circadian rhythms of Synechococcus sp. RF-1 using the uptake of amino acids. Therefore, we initially examined a temporal profile of \(^{[35}\)S\)methionine uptake activity in S. elongatus. Cells carrying a bacterial luciferase reporter gene were used to monitor kaiBC expression (7) were grown in a continuous culture system under standard LL conditions after a 12-h dark treatment to reset the clock. Every 6 h, the cells were removed and incubated with liquid medium containing \(^{[35}\)S\)methionine for 30 min under LL. The radioactivity of the incorporated \(^{[35}\)S\)methionine was then measured to estimate the temporal profile of methionine uptake (Fig. 1B). Methionine uptake activity remained constant throughout the circadian cycle, whereas kaiBC promoter activity showed robust circadian fluctuations (Fig. 1A) (7).

For the protein synthesis assay, cells were incubated with \(^{[35}\)S\)methionine for 30 min at 2- or 4-h intervals under LL. Proteins were extracted and subjected to immunoprecipitation with anti-KaiC antisera. The immunoprecipitated materials were subjected to SDS-PAGE, immunoblotting, and autoradiography. As shown in Fig. 1C (middle panel), a robust circadian oscillation in the rate of KaiC protein synthesis was demonstrated, which peaked at CT12. From the same continuous culture, we simultaneously collected other cell samples and processed them for Northern and Western blot analyses to compare kaiBC mRNA and KaiC accumulation profiles. As reported previously, the levels of kaiBC mRNA and KaiC protein show circadian rhythms peaking at around CT10 and CT18, respectively (Figs. 1, C and D, upper and lower panels) (7, 12, 16). Thus, the rate of KaiC synthesis changes in a circadian manner in parallel with the accumulation of kaiBC mRNA with a slight delay, whereas it advances to the rhythm of KaiC protein accumulation by about 6 h. To our knowledge, this is the first demonstration of a circadian protein synthesis profile of clock-related proteins in any organisms. Under LL, KaiC undergoes robust circadian phosphorylation, which peaks at CT16 (8). KaiC protein was detected as double bands on immunoblots after SDS-PAGE on 10% gels as shown in Fig. 1C. The upper band with lower mobility corresponds to the phosphorylated form of KaiC, and the lower band corresponds to the non-phosphorylated form (8). Our autoradiography results show that the \(^{[35}\)S\)-labeled KaiC protein also appeared as double bands, indicating that the newly synthesized KaiC was phosphorylated rapidly within 30 min.

Circadian Rhythms in the Rate of KaiC Degradation—The accumulation profile of KaiC protein is determined by the balance between its synthesis and degradation. Xu et al. (11) recently demonstrated the half-lives of the non-phosphorylated and phosphorylated forms of KaiC to be 8.8 and 2.0 h, respectively. This is based on the stability of transiently overexpressed KaiC in a kaiC-null background strain under the control of the Escherichia coli trc promoter that was controlled by pulsed administration of the inducer isopropyl-\( \beta \)-thiogalactopyranoside. However, that experiment did not examine the temporal profile of endogenously expressed KaiC. To estimate a temporal profile of the rate of KaiC degradation, we used a different approach and monitored the stability of KaiC in the presence of the protein synthesis inhibitor Chm during the course of a circadian cycle. Fig. 2A shows that total protein synthesis was inhibited by Chm at 400 and 800 \( \mu \)g/ml. Cells were removed from a continuous culture grown under LL after a 12-h dark treatment. They were incubated with or without Chm (400 \( \mu \)g/ml) for 4 h at 4-h intervals under LL, processed, fractionated by SDS-PAGE on 12% gels, and then subjected to immunoblot analysis with anti-KaiC antisera (Fig. 2B). As shown in Fig. 2, B–D, from late subjective night to early subjective day (CT20, CT24, and CT4), KaiC was reduced to around 70% within 4 h in the presence of Chm (indicating that a half-life was about 6 h), whereas around the mid-subjective night (CT16),

\(^{3}\) Y. Kitayama, unpublished result.

\(^{4}\) We used \(^{[35}\)S\)methionine incorporation to measure the protein synthesis rate (12).
KaiC was more stable (~15% reduction, indicating an estimated half-life of ~20 h). In our experiment, the average half-life of KaiC protein was ~10 h. The fact that KaiC is most stable when its accumulation rhythm peaks suggests that a circadian change in the stability of KaiC contributes to the proper phasing of the rhythm of its amplitude or accumulation (see "Discussion").

We also examined the stability of KaiC in an arrhythmic mutant strain, kaiC13 (7, 9). This mutant encodes the amino acid substitution G460E in KaiC. The level of KaiC accumulation in this mutant was continuously low, and the phosphorylated form of KaiC was more abundant than the non-phosphorylated form throughout the circadian cycle (Fig. 3B). No difference in the rate of degradation of KaiC was found at 8 and
16 h under LL in the mutant as shown in Fig. 3, C and D, confirming that the stability of KaiC is modified in a circadian fashion in wild-type cells. Moreover, the stability of KaiC protein is lower in the kaiC13 mutant than in the wild-type strain. The lower level of KaiC accumulation in the mutant strain is due to both a reduction in the level of kaiBC expression and a decrease in the stability of KaiC. Moreover, the phosphorylated form of KaiC in the kaiC13 mutant appeared more stable than the non-phosphorylated form (Fig. 3C).

Differential Turnover of Phosphorylated and Non-phosphorylated Forms of KaiC—Finally, we analyzed the temporal profiles of the phosphorylated and non-phosphorylated forms of KaiC in the presence of Chm. Fig. 4A presents a schematic diagram of phosphorylated and non-phosphorylated KaiC protein turnover. We analyzed the same protein samples as were used to collect the data shown in Fig. 2 by separating both forms of KaiC on 10% gels (Fig. 4B). We calculated the relative changes in phosphorylated KaiC (upper band) and non-phosphorylated KaiC (lower band) during a 4-h treatment with Chm. When the translation of KaiC was inhibited by Chm at CT4 or CT8, the level of phosphorylated KaiC increased, whereas the level of non-phosphorylated KaiC dramatically decreased because of both degradation and phosphorylation (Fig. 4, A and D). These results are consistent with the obser-
viation that both total KaiC and phosphorylated KaiC increased from CT4 to CT12 in the absence of Chm, whereas non-phosphorylated KaiC decreased (Fig. 4C) (8). These results also indicate that KaiC phosphorylation does not require de novo protein synthesis during the subjective day. In the presence of Chm during the late subjective night (CT20–24), the level of phosphorylated KaiC was reduced dramatically because of both degradation and dephosphorylation (Fig. 4A), whereas the level of non-phosphorylated KaiC was less affected. Thus, the turnovers of the phosphorylated and non-phosphorylated forms of KaiC show robust circadian rhythms and the non-phosphorylated and phosphorylated forms have an anti-phase relationship to each other, even in the presence of Chm.

**DISCUSSION**

The levels of kaiBC mRNA and KaiC protein oscillate under LL, peaking at −CT8 and −CT16, respectively (Fig. 1D) (7, 8, 12). In this study, we revealed temporal changes in the rates of synthesis and degradation of KaiC. Rhythmic KaiC synthesis peaked at CT12 with a slight delay in the rhythm of mRNA accumulation (Fig. 1, C and D). Thus, the rate of KaiC protein synthesis seems primarily dependent on the level of kaiBC mRNA accumulation. Consistent with this finding, Xu et al. (11) have demonstrated that transiently induced kaiBC mRNA is degraded rapidly. We found that the rate of KaiC protein degradation is modified in a circadian phase-dependent manner (Fig. 2D). KaiC was most stable at CT16 (the calculated half-life at this time point was ~20 h), at which time its accumulation and phosphorylation levels were maximal (8, 12). From the late subjective night to early subjective day, the stability of KaiC protein decreased (with a half-life of ~6 h) and the level of its accumulation decreased.

We previously estimated the circadian profile of the absolute level of cellular KaiC (18). The levels of KaiC protein oscillate with an average content of ~10,000 molecules/cell. Based on previous results, we estimated a quantitative profile of KaiC protein turnover (synthesis and degradation) per single cell (Fig. 5). The amount of KaiC protein degraded within 4 h (Fig. 5B) was calculated on the basis of the amount accumulated (Fig. 5A) and the mean value of its degradation rate at each time point (Fig. 2D). Furthermore, to address the relevance of circadian changes in KaiC synthesis and stability to the accumulation rhythm profile, we simulated KaiC accumulation profiles with constant rates of KaiC synthesis or degradation (Fig. 5C). We confirmed that the accumulation profile (Fig. 5C, black solid line), which was generated from the calculated amounts of synthesized and degraded KaiC protein shown in Fig. 5A, correlates well with the experimental data (Fig. 5C, gray solid line). If the KaiC synthesis rate is constant at the average level, the resulting KaiC accumulation rhythm is largely dampened with a slight phase delay. On the other hand, if the degradation rate is constant, the amplitude of the rhythm of KaiC accumulation is also reduced with a slight phase advance. These results suggest that oscillations in both KaiC protein synthesis and degradation are important in generating a KaiC accumulation profile with proper amplitude and phasing and thereby determine the ~8-h delay between the mRNA and protein rhythms.

When Chm was added during the subjective day (CT4–12), the level of phosphorylated KaiC increased and the level of non-phosphorylated KaiC decreased. The level of non-phosphorylated KaiC decreased greatly when Chm was added compared with the levels in the absence of Chm (Fig. 4B). This might occur, because the degradation rate of non-phosphorylated KaiC is rapid during this time or because the non-phosphorylated form of KaiC is phosphorylated by (auto)phosphorylation activity. The increase in the phosphorylated form of KaiC, regardless of the presence of Chm (Fig. 4, B and D), supports the latter possibility. During the subjective night, the rate of KaiC protein synthesis becomes lower, probably because
of a reduced kaiBC mRNA pool. At −CT16, KaiC protein becomes relatively stable. Previous gel-filtration and co-immunoprecipitation studies have demonstrated that the KaiC hexamer forms a tight complex with KaiA, KaiB, and SasA during mid-subjective night (16–18). Therefore, it is plausible that such posttranslational modification or complex formation transiently changes the stability of KaiC. The increased KaiC stability would further promote the accumulation of KaiC while its synthesis rate decreased. The levels of the non-phosphorylated and phosphorylated forms of KaiC are also stable in the presence of Chm from CT16 to CT20 (Fig. 4). From the late subjective night to early subjective day (CT20 to CT4), the rate of KaiC synthesis is maintained at a low level and the rate of KaiC degradation increases. Therefore, the net amount of KaiC decreases. Moreover, the level of phosphorylated KaiC is also reduced during the subjective night (regardless of the presence of Chm) because of degradation or dephosphorylation. This dramatic change seems to be attributed to the formation of a KaiC-containing complex with KaiB, which accelerates KaiC auto-dephosphorylation activity at around CT20 (11, 17–19). Moreover, these results indicate that, even when translation is inhibited, KaiC degradation and phosphorylation proceed within at least 4 h in a circadian phase-dependent manner, suggesting a flexibility in the circadian timing that accommodates any perturbation in protein synthesis.

In Neurospora and Drosophila, the stability of the clock proteins, Period and Frequency, is modulated in a circadian fashion because of their rhythmic phosphorylation (5). In S. elongatus, KaiC is also rhythmically phosphorylated, most probably through autophosphorylation (Fig. 4B) (8). However, the effect of KaiC phosphorylation on its stability remains unclear. Using a transient KaiC overinducer in a kaiC-depleted strain, Xu et al. (11) suggest that the stability of phosphorylated KaiC is lower than that of non-phosphorylated KaiC. However, in this study, we demonstrated that KaiC is most stable when its phosphorylation peaks at around CT16 (Fig. 4D). Thus, there appears no simple correlation between the phosphorylation status and the stability of KaiC.

REFERENCES

1. Pittendrigh, C. S. (1993) Annu. Rev. Physiol. 55, 17–54
2. Golden, S. S., Ishiura, M., Johnson, C. H., and Kondo, T. (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 327–354
3. Iwasaki, H., and Kondo, T. (2000) Plant Cell Physiol. 41, 1013–1020
4. Ditty, J. L., Williams, S. B., and Golden, S. S. (2003) Annu. Rev. Genet. 37, 513–543
5. Young, M. W., and Kay, S. A. (2001) Nat. Rev. Genet. 2, 702–715
6. Nishiwaki, T., Iwasaki, H., Ishiura, M., and Kondo, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 495–499
7. Ishiura, M., Kutsuna, S., Aoki, S., Iwasaki, H., Anderson, C. B., Tanabe, A., Golden, S. S., Johnson, C. H., and Kondo, T. (1998) Science 281, 1519–1523
8. Iwasaki, H., Nishiwaki, T., Kitayama, Y., Nakajima, M., and Kondo, T. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15978–15983
9. Nakahira, Y., Katayama, M., Miyashita, H., Kutsuna, S., Iwasaki, H., Oyama, T., and Kondo, T. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 881–885
10. Mori, T., Saveliev, S. V., Xu, Y., Stafford, W. F., Cox, M. M., Inman, R. B., and Johnson, C. H. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 17203–17208
11. Xu, Y., Mori, T., and Johnson, C. H. (2003) EMBO J. 22, 2117–2126
12. Xu, Y., Mori, T., and Johnson, C. H. (2000) EMBO J. 19, 3349–3357
13. Dunlap, J. C. (1999) Cell 96, 271–290
14. Hayashi, F., Suzuki, H., Iwase, R., Uzumaki, T., Miyake, A., Shen, J. R., Imada, K., Furukawa, Y., Yonekura, K., Namba, K., and Ishiura, M. (2003) Genes Cells 8, 287–298
15. Iwasaki, H., Taniguchi, Y., Ishiura, M., and Kondo, T. (1999) EMBO J. 18, 1137–1145
16. Iwasaki, H., Williams, S. B., Kitayama, Y., Ishiura, M., Golden, S. S., and Kondo, T. (2000) Cell 101, 223–233
17. Kageyama, H., Kondo, T., and Iwasaki, H. (2003) J. Biol. Chem. 278, 2388–2395
18. Kitayama, Y., Iwasaki, H., Nishiwaki, T., and Kondo, T. (2003) EMBO J. 22, 2127–2134
19. Williams, S. B., Valonakis, I., Golden, S. S., and LiWang, A. C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15357–15362
20. Nishimura, H., Nakahira, Y., Imai, K., Tsuruhara, A., Kondo, H., Hayashii, H., Hirai, M., Saito, H., and Kondo, T. (2002) Microbiology 148, 2963–2990
21. Kondo, T., Tsinoremas, N. F., Golden, S. S., Johnson, C. H., Kutsuna, S., and Ishiura, M. (1994) Science 266, 1233–1236
22. Kondo, T., Strayer, C. A., Kulikarni, R. D., Taylor, W., Ishiura, M., Golden, S. S., and Johnson, C. H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5672–5676
23. Chen, T. H., Chen, T. L., Hung, L. M., and Huang, T. C. (1991) Plant Physiol. 97, 55–59
Circadian Rhythms in the Synthesis and Degradation of a Master Clock Protein 
KaiC in Cyanobacteria

Keiko Imai, Taeko Nishiwaki, Takao Kondo and Hideo Iwasaki

J. Biol. Chem. 2004, 279:36534-36539. 
doi: 10.1074/jbc.M405861200 originally published online June 30, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M405861200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 23 references, 14 of which can be accessed free at http://www.jbc.org/content/279/35/36534.full.html#ref-list-1