which were spare, and the remainder containing one each of elevated winter temperature, controlled summer drought, supplemented summer rainfall, elevated winter temperature and summer drought, elevated winter temperature and supplemented summer rainfall, control, and cable control (ambient conditions with unconnected heating cables). At the end of the growing season, all plots were cut to a height of 4 to 5 cm to maintain a short turf. At Buxton, point quadrat surveys were conducted on four occasions per year, with the first in April and the last in late September, from 1994 to 1997. During the same period, vegetation surveys took place every 6 weeks at Wytham. More recently (1998 onwards) point quadrat sampling has been conducted only once (Buxton) or twice (Wytham) per year. Analysis was restricted here to data collected each year in late June/early July, the time of maximum plant growth and immediately before the imposition of the drought treatment. Thus, the data collected in any year do not reflect the immediate effects of the drought treatment applied in that year.

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REPORTS

CikA, a Bacteriophytochrome That Resets the Cyanobacterial Circadian Clock

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The circadian oscillator of the cyanobacterium *Synechococcus elongatus*, like those in eukaryotes, is entrained by environmental cues. Inactivation of the gene *cikA* (circadian input kinase) shortens the circadian period of gene expression rhythms in *S. elongatus* by approximately 2 hours, changes the phasing of a subset of rhythms, and nearly abolishes resetting of phase by a pulse of darkness. The CikA protein sequence reveals that it is a divergent bacteriophytochrome with characteristic histidine protein kinase motifs and a cryptic response regulator motif. CikA is likely a key component of a pathway that provides environmental input to the circadian oscillator in *S. elongatus*.

The cyanobacterium *S. elongatus* PCC 7942 (*1*) exhibits circadian rhythms of gene expression that can be monitored using luciferase reporter genes (*2*). These bioluminescence rhythms persist with a period of approximately 24 hours, are temperature compensated, and their phase can be reset by light/dark transitions or by temperature cues (*3*). The cyanobacterial clock exhibits these characteristics of eukaryotic circadian clocks despite a lack of apparent homology between its protein components and those identified in other groups of organisms (*4*). For example, the complete genome sequence of *Synechocystis* sp. strain PCC 6803 is devoid of sequences similar to clock genes of *Drosophila*, such as *period*, *timeless*, *Clock*, and *cycle*, or the *frequency* gene of *Neurospora* (*4*, *5*). Likewise, no homologs of the cyanobacterial *kaiA*, *kaiB*, or *kaiC* genes, essential for circadian rhythmicity (*6*), have been detected thus far in eukaryotes. Other cyanobacterial genes that, when mutated, affect relay of temporal information from the clock to downstream genes include a sigma factor (*7*) and a putative carboxylase (*8*). A histidine protein kinase, SasA, interacts with the KaiC protein and works with the oscillator either at a point of environmental input or of output transduction to all downstream genes (*9*). We describe here a new clock-associated gene, *cikA*, that lies on an input pathway that supplies phase-setting information to the *S. elongatus* clock.

The *cikA* gene was identified from a Tn5 transposon insertion mutant (*2*) that showed subtle alteration in light-responsive regulation of a photosystem II gene, *psbAII* (*10*). Expression of a *psbAII*::*luxAB* (bacterial luciferase) fusion in the mutant was 50 to 80% of wild type under low light conditions and showed exaggerated induction on exposure to higher light intensity (*11*). However, a more striking circadian (*2*, *12*) phenotype was noted: the period of bioluminescence oscillation was shortened by approximately 2 hours (22.80 ± 0.45 versus 24.71 ± 0.25, $n = 12$), and the relative timing of peaks (phase angle) was offset by approximately 6 hours (Fig. 1A).

Reduction of both period and amplitude was observed with all reporters (Fig. 1, A to D) (e.g., periods for *kaiB*::*luxAB*, 22.36 ± 0.47 hours versus 25.24 ± 0.35 hours, $n = 12$; for *purF*::*luxAB*, 22.75 ± 0.24 hours versus 24.86 ± 0.33 hours, $n = 12$). Nonetheless, expression from the *kaiB* promoter, indicative of clock gene expression, remained robustly rhythmic with no notable alteration in phase angle (Fig. 1B). The bioluminescence rhythm from a *purF*::*luc* reporter (firefly luciferase) was also affected in both amplitude and period (Fig. 1C), indicating that the phenotype is not related to the substrates of bacterial luciferase and that it extends to class 2 genes [*purF* peaks at subjective dawn and is defined as class 2; the majority of gene expression patterns in the organism peak near subjective dusk and are defined as class 1 (*13*)]. A gentamycin resistance cassette inserted in both orientations with respect to the *cikA* open reading frame (ORF) caused phenotypes identical to those of the original Tn5 insertion mutant (Fig. 1D). Note that the *kaiA*::*luxAB* reporter showed an altered phase-angle phenotype; thus, in the *cikA* genetic background, the relative phasing of *kaiA* and *kaiBC* expression is uncoupled without dramatically affecting circadian timing (Fig. 1, B and D), as was previously demonstrated for mutation of the *cpmA* gene (*8*).

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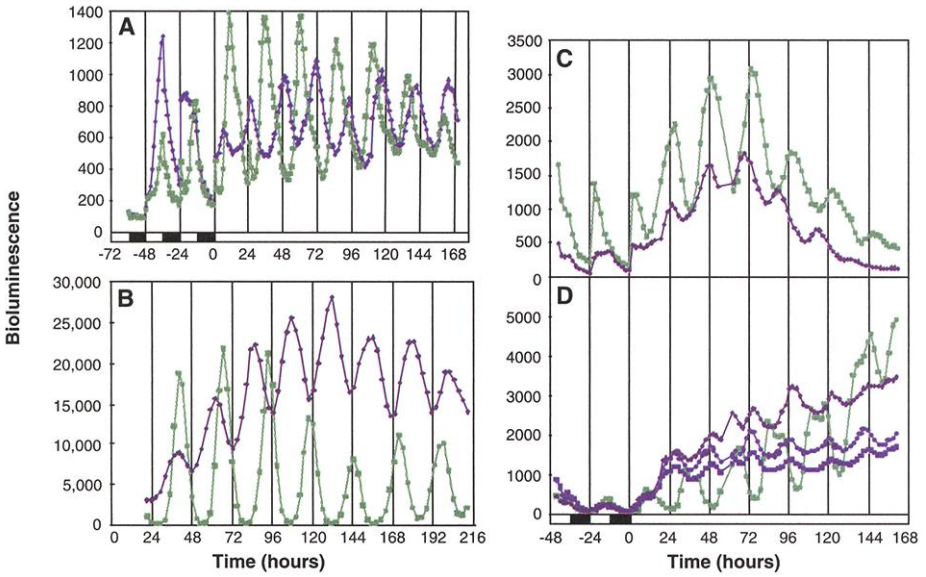

Fig. 1. Circadian phenotypes of reporter strains in which *cikA* is inactivated by transposon Tn5 insertion. Bioluminescence (counts per second) is shown from **(A)** translational *luxAB* fusion to *psbAII* (*10*); **(B)** transcriptional *luxAB* fusion to the promoter of *kaiB* (*6*); **(C)** translational fusion of a firefly luciferase gene (*luc*) to *purF* (*33*); and **(D)** translational *luxAB* fusion to *kaiA* (*6*). In (A), (C), and (D), black bars on the abscissa indicate dark incubation periods; otherwise, samples were kept in continuous light (onset = time 0). In (B), samples were subjected to one 12-hour dark incubation before release into continuous light. Green, wild type; blue, *cikA* insertion mutant. (D) Blue diamonds, Tn5 insertion mutant; blue circles and squares, two orientations of inactivating gentamycin resistance cassettes.

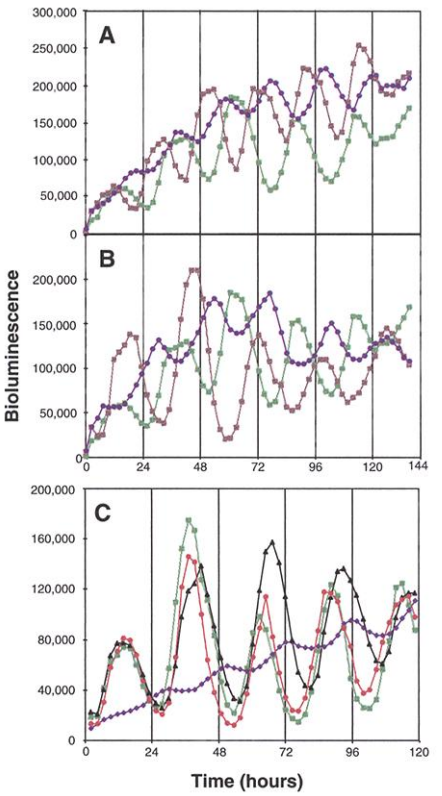

Fig. 2. **(A** and **B)** *cikA* disruption in period mutants of *Synechococcus*. Wild-type (green; period 25.11 ± 0.46 hours, n = 12) and period mutant *kai* backgrounds (rose) carry a *psbAI::luxAB* fusion (*6*, *34*). (A) Rose, period mutant B22a (21.34 ± 0.10 hours, n = 5); blue, B22a/*cikA* (20.92 ± 0.34 hours, n = 5). (B) Rose, period mutant C28a, (26.36 ± 0.83 hours, n = 5); blue, C28a/*cikA* (24.34 ± 0.13, n = 5). **(C)** Genetic complementation of the *cikA* phenotype. Ectopic insertion of a copy of *cikA* (red) restored the period, amplitude, and phasing phenotypes of a *cikA* disruption mutant (blue) to match those of wild type (green), as measured by a *psbAI::luxAB* reporter. Ectopic insertion of the extra copy of *cikA* into a wild-type background (black). Axes labeled as in Fig. 1.

Mutant *kai* alleles in a *psbAI::luxAB* reporter background that affect period (*6*) allowed us to examine the effect of *cikA* inactivation in both short- and long-period mutants of *S. elongatus*. The *cikA* mutation caused a very small, but reproducible, additional period shortening of the *psbAI::luxAB* reporter rhythm in the *kaiB* missense mutant B22a, and a dramatic phase-angle change (Fig. 2A). The phase-angle alteration is particularly marked in the *kaiC* long-period mutant C28a background (Fig. 2B), in which the C28a/*cikA* double mutant and wild type have a stable period length relationship throughout the run, but their bioluminescence peaks are offset by approximately 9 hours. The additive effect of the combined mutations suggests that CikA and Kai proteins perform independent, nonoverlapping functions.

Genetic complementation confirmed that inactivation of the *cikA* gene is responsible for the mutant phenotypes, rather than possible polar effects of the transposon insertion on nearby genes. Wild-type amplitude, period, and phase-angle properties were all restored to *psbAI::luxAB* bioluminescence when an ectopic copy of *cikA* was provided to a *cikA* mutant strain (Fig. 2C).

Persistence of robust circadian rhythms in the *cikA* genetic background indicates that the product of this gene is not essential for circadian oscillator function. The global effect on period of more than eight tested genes (*14*), including representatives of classes that were assigned to distinct output pathways by mutational analyses, suggests that the *cikA* product is not part of one of these pathways, unless it functions as does SasA in close association with the clock (*9*). To determine whether CikA provides environmental input to the oscillator, we tested the ability of *cikA*-inactivated reporter strains to reset the phase of the clock in response to a 5-hour dark pulse (*15*). During portions of the circadian cycle, wild-type *S. elongatus* responds to this stimulus by changing the phase of subsequent peaks by 10 to 12 hours after cells are returned to continuous light (Fig. 3). In contrast, *cikA* mutant strains show little phase resetting in this assay. These data are consistent with CikA functioning in an input pathway to the circadian oscillator.

We named the gene *cikA*, for circadian input kinase, on the basis of mutant phenotypes and inference from sequence analysis. The most striking features in the deduced protein sequence (*16*) are histidine protein kinase motifs that conform to all conserved blocks for that family (Fig. 4A, blocks H, N, D/F, and G) (*17*). The carboxyl terminus is similar to the receiver domains of response regulators, most notably PhoB (Fig. 4C) (*18*). Although other key residues of this motif are present, the invariant Asp in this family, which is the residue phosphorylated by a cognate histidine protein kinase in each case, is absent from the sequence (Fig. 4C) (*16*). Thus, if the CikA histidine protein kinase domain transfers a phosphoryl group to its receiver domain, another residue must become phosphorylated. Alternatively, phosphotransfer may not be the role of this segment of the protein; perhaps it interacts with other regulatory partners, and this contact is modulated by autophosphorylation within the H box.

The amino-terminal sequence reveals that the protein belongs to the expanding family of bacteriophytochromes (*19*), similar to *Synechocystis* sp. strain 6803 Cph1 (*20*), *Fremyella diplosiphon* RcaE (*21*), *Deinococcus radiodurans* BphP (*22*), and *Arabidopsis thaliana* PhyE (*23*) (Fig. 4B). This raises the possibility that CikA is a photoreceptor. However, unlike other known phytochromes and bacteriophytochromes, CikA lacks the conserved Cys residue expected as a bilin ligand for phytochromes (*24*, *25*). It also lacks the His residue reported to be the bilin ligand for *D. radiodurans* BphP, which corresponds to His 323 in the PhyE sequence (Fig. 4B) (*22*). This suggests several possi-

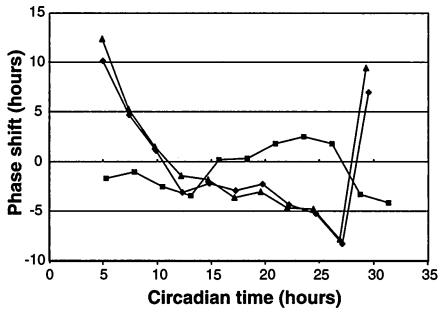

Fig. 3. Phase-resetting of the *psbAI::luxAB* bioluminescence rhythm in wild-type (diamonds), *cikA* (squares), and complemented *cikA* (triangles) genetic backgrounds in response to a 5-hour dark pulse. At the indicated circadian time on the abscissa, samples received 5 hours of dark incubation, then were returned to continuous light for monitoring of the circadian rhythm (*15*). The ordinate for each data point indicates the offset of the phase of peaks after the treatment, relative to a control not pulsed with darkness: phase advance (positive values) or phase delay (negative values). To accommodate differences in circadian period between strains, actual time was converted to circadian time (one circadian hour = free running period × 24^{-1}).

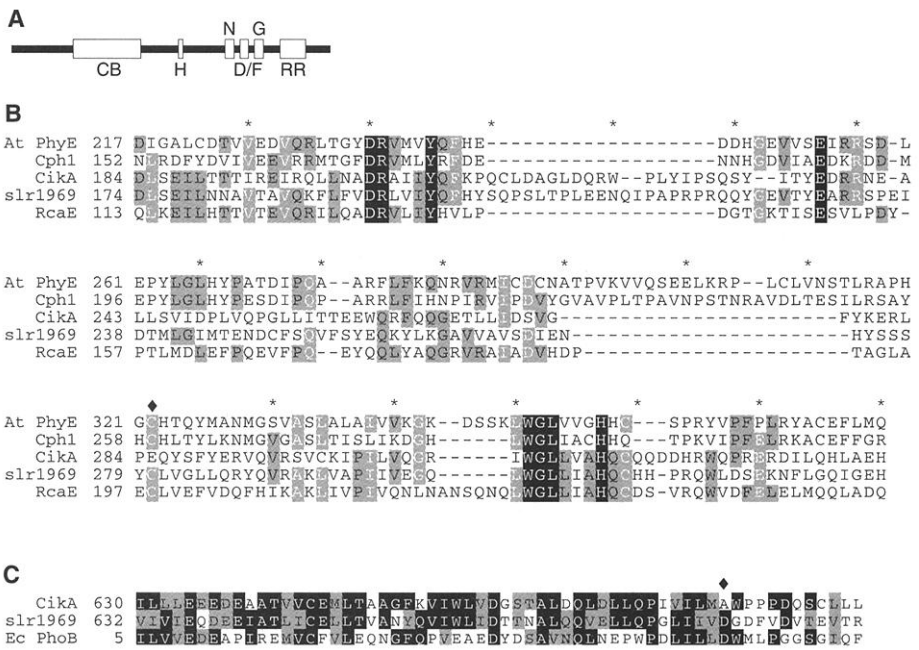

Fig. 4. (**A**) Graphic representation of the 754–amino acid CikA protein, indicating relative size and distribution of identifiable motifs: chromophore binding domain of phytochromes (CB); H, N, D/F, and G boxes of histidine protein kinases; and a receiver domain of response regulators (RR). (**B**) Comparison of chromophore binding domains of CikA, PhyE from *Arabidopsis thaliana* (GenBank accession no. X76610), Cph1 (Kazusa DNA Institute CyanoBase ORF slr0473), and slr1969 (Kazusa DNA Institute CyanoBase ORF slr1969) from *Synechocystis* sp. strain PCC 6803, and RcaE from *Fremyella diplosiphon* (GenBank accession no. U59741). Black diamond, PhyE residue 322 bilin chromophore ligand. Residues conserved: in all sequences, white letters on black; in four out of five, white on gray; in three out of five, black on gray. Numbers at the beginning of each line indicate position in the respective protein sequence. Asterisks mark each tenth residue in alignment. (**C**) Comparison of receiver domains of CikA, slr1969 from *Synechocystis* sp. strain PCC 6803, and PhoB from *Escherichia coli* (GenBank accession no. P08402). Black diamond, residue expected to be Asp in response regulator receiver domains. Black background, identical residues; gray background, chemically similar residues. For alignments (B and C), we used a ClustalW 1.8 alignment tool accessed through the BCM Search Launcher (*35*). Alignment in (B) was modified by hand on the basis of information from 54 phytochrome-like sequences with assistance from C. Lagarias (*36*).

bilities for CikA structure and function: it does not bind a bilin chromophore, it binds a chromophore (bilin or another cofactor) noncovalently, or it binds a chromophore by a novel attachment.

The similarity of CikA to phytochromes provides the first potential evolutionary parallel between cyanobacterial and eukaryotic circadian systems. Phytochromes play several distinct roles in relaying light information to the circadian clocks of plants (*26*). Although the white collar proteins of *Neurospora*, important for light-dependent processes and for circadian clock function (*27*), bear similarity to phytochromes, the correspondence is through shared PAS domains. No direct link can be drawn between the white collar proteins and CikA, which lacks a PAS domain and resembles a different part of the phytochrome sequence—the chromophore binding domain.

A subsequent direct screen for transposon mutants that affect phase resetting has identified five independent *cikA* mutants, and no other loci, as causing clear resetting phenotypes (*28*). This further supports a key role for CikA in providing environmental input to reset the cyanobacterial circadian clock.

References and Notes

1. This strain has been reported without a specific name as *Synechococcus* sp. strain PCC 7942 (Pasteur Culture Collection accession no. 7942). The strain PCC 6301 has been proposed as the living neotype of *S. elongatus* (*29*, *30*); a pending update to *Bergey's Manual of Determinative Bacteriology* will reflect this nomenclature. PCC 7942 is very closely related to PCC 6301 (*31*, *32*) and, thus, can also be assigned to *S. elongatus*.
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15. The experimental design for Fig. 3 was modified from phase-response curve protocols developed by K. Okamoto, C. Inoue, and T. Kondo (unpublished data). Supplemental information is available to *Science* Online subscribers www.sciencemag.org/feature/data/1051545.shl.
16. Nucleotide and deduced amino acid sequences for *cikA* are entered in the GenBank database (accession no. AF258464). Absence of the Cys residue corresponding to PhyE 322 was confirmed by direct sequencing from a polymerase chain reaction amplification product of PCC 7942 chromosomal DNA in the chromophore binding domain. Absence of the conserved Asp in the receiver domain is supported by independent database entry of sequence flanking the *S. elongatus* PCC 6301 *gsa* gene, the locus immediately downstream of *cikA* (GenBank accession no. X53695).
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37. We thank C. Strayer, M. Straume, and V. Cassone for assistance with statistical analysis, C. Johnson and Y. Ouyang for the *purF::luc* reporter construct, D. Hodgson for the gentamycin resistance cassette, C. Inoue and K. Okamoto for information that influenced the design of phase-resetting experiments, and C. Lagarias for help in aligning phytochrome chromophore binding domains. This work was supported in part by grants from the NIH (GM37040) and NSF (MCB9513367) to S.S.G. and the Human Frontier Science Program grant to S.S.G. and T.K. (with co–principal investigators C. Johnson and M. Ishiura). M.K. and S.B.W. were supported by postdoctoral fellowships from the Japan Society for the Promotion of Science and NIH National Research Service Award, respectively.

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Cloning of the *Arabidopsis* Clock Gene *TOC1*, an Autoregulatory Response Regulator Homolog

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The *toc1* mutation causes shortened circadian rhythms in light-grown *Arabidopsis* plants. Here, we report the same *toc1* effect in the absence of light input to the clock. We also show that *TOC1* controls photoperiodic flowering response through clock function. The *TOC1* gene was isolated and found to encode a nuclear protein containing an atypical response regulator receiver domain and two motifs that suggest a role in transcriptional regulation: a basic motif conserved within the *CONSTANS* family of transcription factors and an acidic domain. *TOC1* is itself circadianly regulated and participates in a feedback loop to control its own expression.

The endogenous circadian clock enables organisms to anticipate and adapt to daily variations in the environment and to temporally coordinate internal processes. In animals, fungi, and bacteria, genetic screens for altered circadian rhythms have revealed molecular clock components. The generally conserved core mechanism consists of autoregulatory transcriptional loops in which positive factors act on genes encoding negative factors that in turn feed back to block their own expression (*1*). Although plant models have proven valuable for understanding circadian input and output pathways, our understanding of processes at the core of the plant circadian system is lacking.

We therefore executed a screen for rhythm mutants in *Arabidopsis*, from which we identified the *toc1* (*timing of CAB expression*) mutant (*2*). The defining phenotype is a shortened period of luciferase-reported *CAB* gene expression (~21 hours, versus ~24.5 hours in the wild type) under constant light conditions (LL). All clock phenotypes tested are similarly affected by the *toc1-1* mutation, which is semidominant, as are mutant alleles of diverse clock genes (*1–3*). Moreover, the effects of the *toc1-1* mutation are specific to the clock system, with no defects seen, for instance, in clock-independent light responses (*3*). This is noteworthy because disruption of photoreceptors and phototransduction components that participate in clock entrainment can alter period in LL (*4, 5*). However, perturbations of these components produce specific, differential effects depending on the quality and quantity of light, whereas the *toc1-1* effect is essentially the same in all light conditions (*3*). To further address this issue, we assayed the bioluminescence rhythm of *toc1-1* and wild-type seedlings during extended dark incubation (DD) using a new reporter, *ccr2::luc*. *CCR2* (*COLD–CIRCADIAN RHYTHM–RNA-BINDING 2*) is a clock-controlled gene whose LL expression rhythm is shortened by the *toc1-1* mutation (*6*). The *ccr2::luc* reporter (including a luciferase gene fusion) reveals that *toc1-1* has a similar effect on the period of gene expression in DD (Fig. 1), consistent with a role for *TOC1* outside of light input pathway(s) to the clock.

Mutation of *TOC1* also affects photoperiodic regulation of floral induction. Wild-type *Arabidopsis* flowers earlier in long days [16 hours light, 8 hours dark (16:8 LD)] than in short days (8:16 LD), but this differential response is greatly reduced in *toc1-1* (*3*). This phenotype is likely the result of clock-based misinterpretation of photoperiodic information in *toc1-1* rather than direct effects of *toc1-1* on floral induction pathways. To test this possibility, we measured the transition to flowering of *toc1-1* lines grown in LD cycles of 21 hours total duration, where the environmental period (T) more closely matched the period of the endogenous clock (τ) (Fig. 2). Correct photoperiodic response was restored in *toc1-1* plants grown in this regime, where *toc1-1* plants flowered much later in short days (7:14 LD) than in long days (14:7 LD). The *toc1-1* flowering defect therefore can be fully explained by its circadian defect. The cause is not simply incorrect measurement of light or dark intervals: Mutant plants given 7 hours light in a 24-hour period (7:17 LD) also flowered early (Fig. 2). These data, combined with results of experiments measuring gene expression in *toc1-1* and wild-type plants entrained to altered T cycles (*3*), also suggest a possible mechanism underlying this defect: incorrect modulation of phase and/or waveform of clock-controlled regulatory factors when τ varies greatly from T.

To further investigate its role in the *Arabidopsis* circadian system, we isolated the *TOC1* gene. Genetic mapping delimited

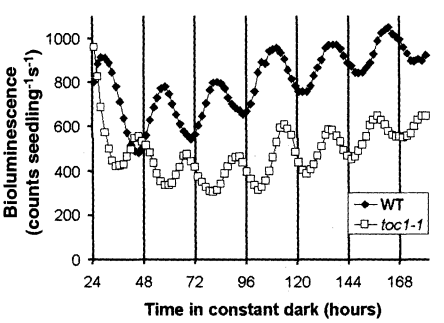

Fig. 1. Bioluminescence rhythms from *toc1-1* and wild-type seedlings in constant darkness (DD). *ccr2::luc* transgenic seedlings (*7*) were grown in 12:12 LD for 8 days before transfer to DD. Bioluminescence was recorded at the indicated times (*7*). Traces represent averages of 21 to 23 seedlings from each line. Period estimates (variance-weighted means ± variance-weighted SD) for each line were calculated as described (*26, 27*): wild type = 27.5 ± 1.16 hours, *toc1-1* = 22.3 ± 0.39 hours.

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