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Original Paper

CO-EXPRESSED WITH CLOCK GENES LHY AND CCA1 1 (*CEC1*) is regulated by LHY and CCA1 and plays a key role in phase setting of *GI* in *Arabidopsis thaliana*

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Abstract Many biological processes, including the control of flowering time, are regulated by the circadian clock. Although a number of clock-associated genes have been characterized in *Arabidopsis thaliana* (Arabidopsis), the complete molecular mechanisms of the circadian clock remain unclear. Here, we report that CO-EXPRESSED WITH CLOCK GENES LHY AND CCA1 1 (CEC1) plays an important role in circadian clock function in Arabidopsis. Three genes, *CEC1*, *CEC2*, and *CEC3*, are co-expressed with the clock genes *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*). The mutants, *cec1* and *cec2*, exhibited an early flowering phenotype under long-day (LD) and continuous-light (LL) conditions, possibly through an increase in *FLOWERING LOCUS T* (*FT*) mRNA. In addition, rhythmic peaks of *GIGANTEA* (*GI*) expression were delayed in the *cec1* mutant plants, but the period length and amplitude of *GI* expression were not affected under LD and LL. These results suggest that CEC1 might contribute to the modulation of circadian phases.

Key words: Arabidopsis, circadian rhythms, photoperiodic flowering.

Circadian clock mechanisms generate circadian rhythms in a wide variety of organisms from cyanobacteria to humans. In *Arabidopsis thaliana* (Arabidopsis), the internal clock regulates a number of biological activities such as leaf movement, petal opening (Bunning 1964; Engelmann and Johnson 1978), hormone biosynthesis (Thain et al. 2004), hypocotyl elongation (Dowson-Day and Millar 1999), stomatal opening (Penfield and Hall 2009), and photoperiodic flowering (Fowler et al. 1999; Park et al. 1999; Schaffer et al. 1998; Wang and Tobin 1998).

Circadian clock genes have been isolated from *Drosophila melanogaster* (Jackson et al. 1986; Konopka and Benzer 1971), *Neurospora crassa* (McClung et al. 1989), and *Mus musculus* (Sehgal et al. 1994; Sun et al. 1997). In each of these organisms, the central oscillator that generates circadian rhythms has at least two interlocked feedback loops. These feedback loops include both positive and negative feedback (Dunlap 1999; Stanewsky 2003). Cyanobacteria are unlikely to have interlocked feedback loops for the circadian oscillator but have a protein-based oscillator (Nakajima et al. 2005).

A large number of circadian clock-associated genes have been identified through genetic studies in Arabidopsis, and a gene regulatory circuit model has been proposed to generate 24-h cycles (Helfer et al. 2011; Pokhilko et al. 2012). CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) activate *PSEUDO-RESPONSE REGULATOR 9* (*PRR9*) and *PRR7* and repress the expression of *TIMING OF CAB EXPRESSION 1*(*TOC1*)/*PRR1*, *EARLY FLOWERING 4* (*ELF4*), and *LUX ARRHYTHMO* (*LUX*). *PRR9* and *PRR7* expression is repressed by ELF3, ELF4, and LUX. PRR9, PRR7, and PRR5 repress *CCA1* and *LHY*, whereas TOC1 activates *CCA1* (Dixon et al. 2011; Koimos et al. 2009; Onai and Ishiura 2005). A number of recent studies have revealed the molecular functions of these clock-associated proteins. However it is still unclear whether the model is sufficient to explain how 24-h rhythms are driven.

To explore the possibility of additional clock components, we used the co-expression database ATTED II (http://atted.jp/) to identify genes co-expressed with *LHY* and *CCA1*. Most of the genes identified were

Abbreviations: Arabidopsis, *Arabidopsis thaliana*; CCA1, CIRCADIAN CLOCK ASSOCIATED 1; CDF1, CYCLING DOF FACTOR 1; CO, CONSTANS; COL, CONSTANS-LIKE 1; ELF4, EARLY FLOWERING 4; LCL5, LHY-CCA1 LIKE5; LHY, LATE ELONGATED HYPOCOTYL; LNK1, NIGHT LIGHT INDUCIBLE AND CLOCK-REGULATED GENES 1; LUX, LUX ARRHYTHMO; OOP1, OUT OF PHASE 1; PHYB, PHYTOCHROME B; PRR, PSEDO-RESPONSE REGULATOR; RVE8, REVEILLE 8; TOC1, TIMING OF CAB EXPRESSION 1.

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circadian-clock-associated or circadian-regulated genes. For example, CYCLING DOF FACTOR 1 (CDF1) binds to the *CONSTANS* (*CO)* promoter and represses its transcription (Imaizumi et al. 2005), *CONSTANS-LIKE 1* (*COL1*) encodes a zinc finger protein that might regulate flowering (Ledger et al. 2001; Mikkelsen and Thomashow 2009; Putterill et al. 1995), and *REVEILLE 8/LHY-CCA1 LIKE5* (*RVE8/LCL5*) encodes a MYB-like transcription factor similar to *CCA1* and *LHY* that regulates the expression of the *TOC1* gene (Farinas and Mas 2011). We found three uncharacterized genes, *At3g54500*, *At3g12320*, and *At5g06980* in the LHY/CCA1 co-expression networks. *At3g54500*, *At3g12320*, and *At5g06980* were named CO-EXPRESSED WITH CLOCK GENES LHY AND CCA1 1 (*CEC1*), *CEC2*, and *CEC3*, respectively. CEC1, CEC2, and CEC3 proteins share some motifs in their sequences.

In this study, we describe the phenotypes of *cec1* and *cec2* single loss-of-function mutants. Analysis of flowering time and the mRNA levels of the floral activators CO and FLOWERING LOCUS T (FT) in these mutants suggested that CEC1 and CEC2 might play a key role in the control of photoperiodic flowering. Rhythmic expression patterns of *CEC1* and *CEC2* were similar to those of *LHY* and *CCA1* under both long-day (LD) and continuous light (LL) conditions. A double loss-of-function mutant of *LHY* and *CCA1* (*lhy*;*cca1*) reduced the amplitude and shortened the period of *CEC1* and *CEC2* expression under LL. The *cec1* plants showed a slightly delayed phase of *GI* expression under LD but the period and amplitude of *GI* expression under LL was not affected suggesting an important role of CEC1 in the circadian clock system of Arabidopsis.

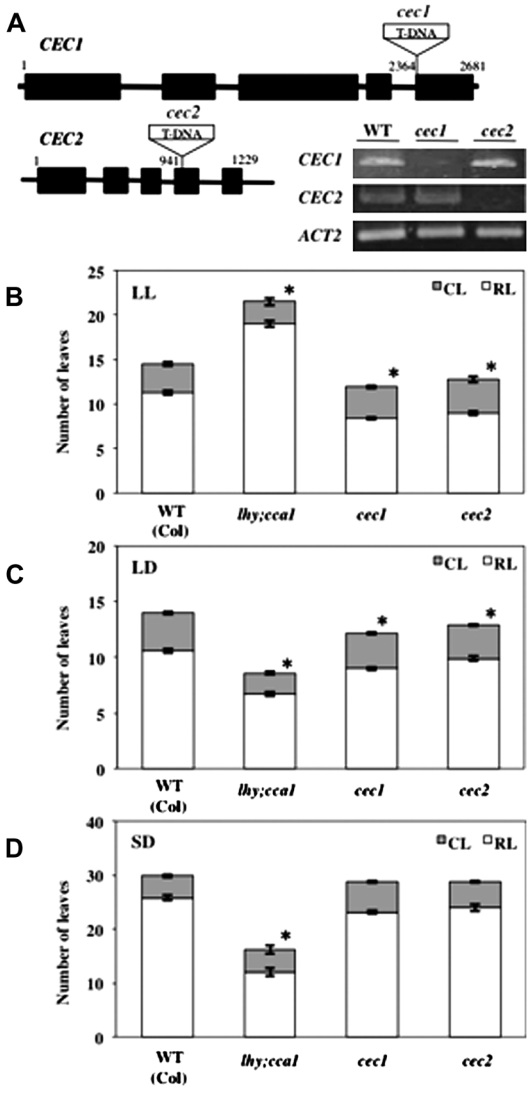

Figure 1. Accelerated flowering time in *cec1* and *cec2* mutants under LD and LL. (A) Schematic representation of the *CEC1* and *CEC2* locus in the *cec1* and *cec2* mutants. Black boxes indicate exons. (B-D) Flowering phenotype of WT, *lhy*;*cca1*, *cec1*, and *cec2* mutants in LL (B), LD (C), and SD (D). Plants were grown under continuous light (LL), 16-h light/8-h dark (LD), and 8h-light/16-h dark (SD). Numbers of cauline (CL) and rosette (RL) leaves as scored at flowering. Data are presented as means±S.E. (n≧10). Asterisks (*) represent statistical significance compared to WT values (Student's t-test, $p<0.05$). Experiments were performed twice with similar results.

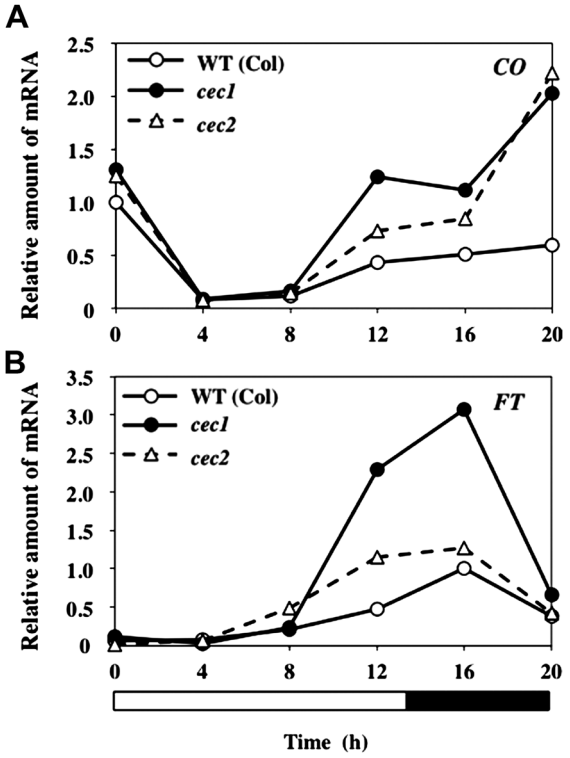

Figure 2. Increased *CO* and *FT* mRNA levels in *cec1* and *cec2* mutants under LD. WT, *cec1*, and *cec2* plants were grown under 16-h light/8-h dark cycles (LD) for 3 weeks. *CO* and *FT* mRNA levels were measured by real-time PCR and normalized to *ACT2* mRNA levels. (A) Expression of *CO* in WT, *cec1*, and *cec2* plants. (B) Expression of *FT* in WT, *cec1*, and *cec2* plants. White and black boxes represent light and dark periods, respectively. Experiments were performed twice using two independent RNA samples with similar results.

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Materials and methods

Plant material and growth conditions

The double mutant *lhy-11*;*cca1-1* [Columbia (Col)] was described previously (Niwa et al. 2007). The *cec1* (SALK_116103) and *cec2* (SALK_085551) mutants were in the Col background and were obtained from the Arabidopsis Biological Resource Center (https://abrc.osu.edu/). The mutants were genotyped using the following primers: CEC1-F, 5′-TCC AAG GGC TAA CTG CAA TGC-3′, CEC1-R, 5′-TCA CAA TTT TCT TTT GTT TCC TTG GG-3′ CEC2-F, 5′-TGT CTT CTG AAG AAT TCG TGT TGC-3′, CEC2-R, 5′-TCA GAT TCT ATC TCT TCC TCT C-3′.

Seeds were imbibed and cold treated at 4°C for 3 days in the dark before germination under light. Plants were grown in controlled environment rooms at 22°C. Light conditions were LD (16-h light/8-h dark), SD (8-h light/16-h dark), or LL (continuous light) with a photon flux density of about 40-μmol $m^{-2}\,s^{-1}$.

Measurement of flowering time

Plants were grown as described above. Flowering time was scored by growing plants on soil under LD, SD, or LL conditions and counting the number of rosette and cauline leaves on the main stem after bolting. Data are presented as the means±S.E. ($n \geqq 10$). Measurement of flowering time was performed at least twice, with similar results.

Gene expression analysis

Seeds were sown as described above and grown on soil for 3 weeks. Aerial parts were used for RNA preparation. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, http://www.qiagen.com/). Complementary DNA was synthesized using the PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time, TaKaRa, http://www.takara-bio.co.jp/). Quantitative RT-PCR analysis was performed using a Thermal Cycler Dice Real Time System TP800 (TaKaRa) using the SYBR Premix Ex Taq II (Perfect Real Time, TaKaRa), cDNA (equivalent to 10 ng of total RNA) was amplified using gene-specific primers in a 25 μl reaction volume according to the manufacturer's instructions. qRT-PCR was performed twice using two independent RNA samples, with similar results. The gene-specific primers used were: LHY-F, 5′-GAT GCA AAA CTT GTT TCA TCG GCC-3′, LHY-R, 5′-TGT TCA CAG TAG AAA CAC CCG AGC-3′, CEC1-F, 5′-CGC AGT TCT TTA TCG GCT TC-3′, CEC1-R, 5′-AGT TCT GTC TGT GGG GTT GG-3′, CEC2-F, 5′-CAT TTA CAA TCT CGG ATC TGT C-3′, CEC2-R, 5′-TTT GCG TGT CTC ATC AGT CAA-3′, GI-F, 5′-CTG TCT TTC TCC CGT TGT TTC ACT GT-3′, GI-R, 5′-TAC GAC ATT GCA TAG CGC ATC AAC A-3′, CO-F, 5′-CTC ACT ACA ACG ACA ATG GTT CCA-3′, CO-R, 5′-TCA TCT GGC TTG CAG GGT CAG-3′, FT-F, 5′-ACA ACT GGA ACA

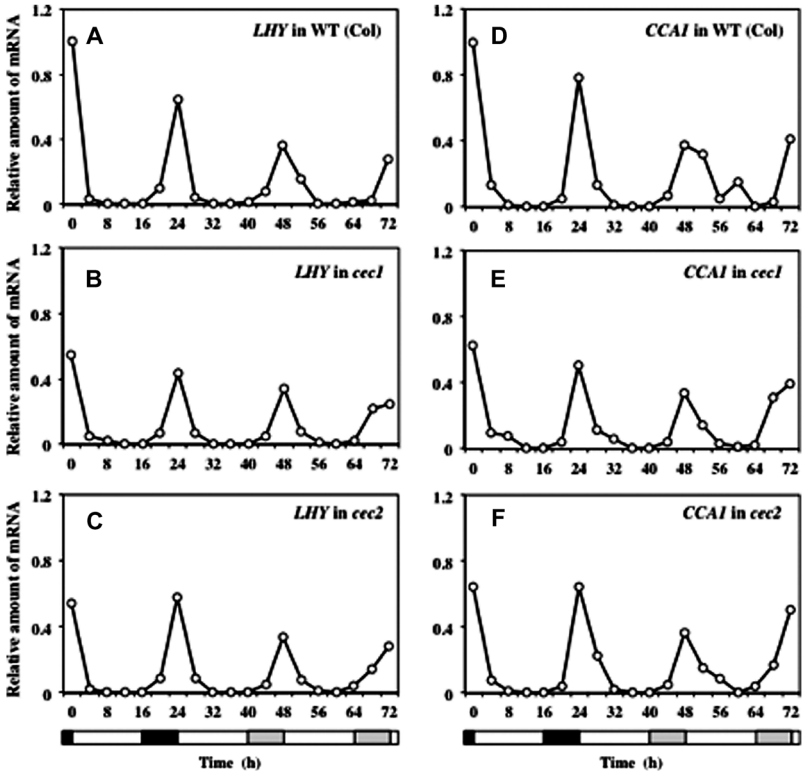

Figure 3. *LHY* and *CCA1* expression in *cec1* and *cec2* mutants. Wild-type, *cec1*, and *cec2* plants were grown in 16-h light/8-h dark cycles (LD) for 3 weeks, and then transferred to LL. *LHY* and *CCA1* mRNA levels were measured by real time PCR and normalized to *ACT2* mRNA levels. (A) Expression of *LHY* in WT. (B) Expression of *LHY* in *cec1*. (C) Expression of *LHY* in *cec2*. (D) Expression of *CCA1* in WT. (E) Expression of *CCA1* in *cec1*. (F) Expression of *CCA1* in *cec2*. White, black, and gray boxes represent light, dark, and subjective dark periods, respectively. Experiments were performed twice using two independent RNA samples with similar results.

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ACC TTT GGC AAT G-3′, FT-R, 5′-AGC CAC TCT CCC TCT GAC AA-3′. Primer set for *ACT2 and CCA1* were described by Miura et al. (2009) and Nakamichi et al. (2010), respectively.

Results

Accelerated flowering time in cec1 and cec2 mutants under LD and LL

We characterized *cec1* and *cec2* mutant T-DNA insertion lines to investigate the functional implications of the mutations for the Arabidopsis circadian clock. Homozygous mutants were verified by diagnostic PCR using T-DNA border primers and independent specific primers (Figure 1A). *CEC1* and *CEC2* transcripts were not detected in *cec1* and *cec2* mutants, respectively.

Photoperiodic flowering is tightly linked to the circadian clock, which measures day and night lengths (Mizoguchi et al. 2002; Suarez-Lopez et al. 2001; Yanovsky and Kay 2002). CCA1 and LHY regulate *CO* expression by regulating the peak of *GI* expression (Mizoguchi et al. 2005). Thus, accurate regulation of *CCA1* and *LHY* expression is important for setting the phase of clock-controlled genes to regulate flowering time (Mizoguchi et al. 2002). We therefore investigated whether the knockout mutants for *CEC1* and *CEC2* affected the photoperiodic flowering pathway. The *cec1* and *cec2* mutants exhibited an early flowering phenotype under LD (Figure 1B) and LL (Figure 1C), but not under short-day (SD, Figure 1D) conditions.

Increased CO and FT mRNA levels in cec1 and cec2 mutants under LD

We measured the expression levels of the flowering time genes *CO* and *FT* in *cec1* and *cec2* mutants under LD (Figure 2, Supplemental Figure 1). *CO* mRNA abundance increased slightly during the daytime to evening periods in the *cec1* and *cec2* mutants (Figure 2A, Supplemental Figure 1A). The *FT* mRNA level increased significantly in the *cec1* and *cec2* mutants (Figure 2B, Supplemental Figure 1B) consistent with the early flowering phenotype under LD (Figure 1C).

Phase shift of GI expression peaks in cec1 under LD and LL

To investigate whether CEC1 and CEC2 are important clock mechanism components similar to *LHY* and *CCA1*, we assessed circadian clock gene expression patterns in the *cec1* and *cec2* mutants under free-running conditions. Wild-type, *cec1*, and *cec2* plants were grown for 3 weeks under 16-h light/8-h dark cycles and then transferred to LL conditions. In the wild-type plants, free-running rhythmic expression of *LHY* and *CCA1* was seen with a peak at subjective dawn (Figures 3A, D, Supplemental Figures 2A, D), as reported previously (Mizoguchi et al. 2002). The expression patterns of both *LHY* and *CCA1* in the mutants were similar to those in wild-type plants (Figures 3B, C, E, F, Supplemental Figures 2B, C, E, F).

We determined the *GI* expression patterns in the *cec1* and *cec2* mutants. We used *GI* for our experiments because *LHY* and *CCA1* are morning-phased clock genes but *GI* is an evening-phased clock gene (Fowler et al. 1999; Park et al. 1999; Schaffer et al. 1998; Wang and Tobin 1998). In wild-type plants, *GI* mRNA showed the expected pattern of expression with a peak at Time 8 under LD (Figure 4A, Supplemental Figure 3A, Mizoguchi et al. 2002). In the *cec1* mutant, the phase of peak *GI* expression was delayed by 4 h and occurred at Time 12 (Figure 4B, Supplemental Figure 3B). A similar *GI* expression pattern with delayed phase was seen after transfer to LL with no effect on period length

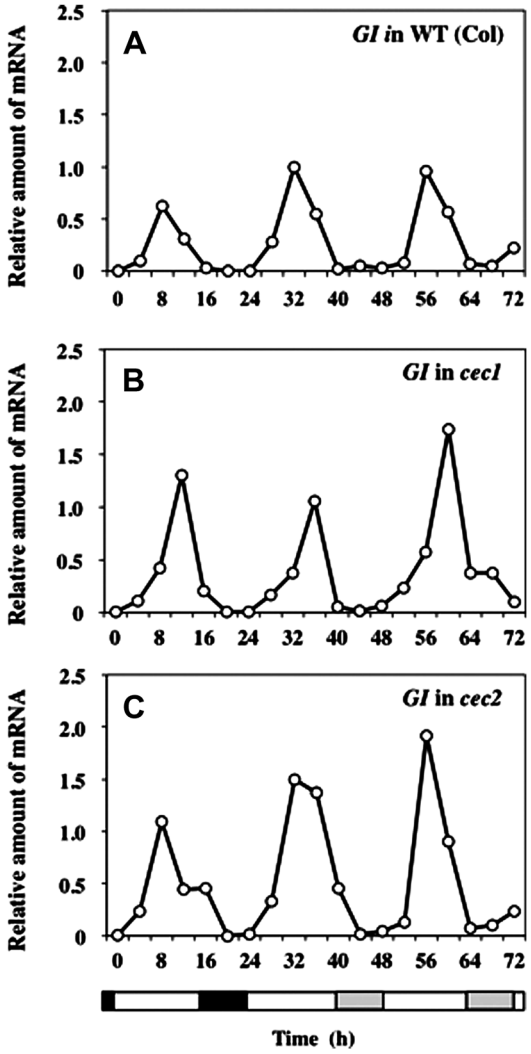

Figure 4. Phase shift in *GI* expression peaks in *cec1* under LD and LL. WT, *cec1*, and *cec2* plants were grown under 16-h light/8-h dark cycles (LD) for 3 weeks and then transferred to LL. *GI* mRNA levels were measured by real time PCR and normalized to *ACT2* mRNA levels. (A) Expression of *GI* in WT. (B) Expression of *GI* in *cec1*. (C) Expression of *GI* in *cec2*. White, black, and gray boxes represent light, dark, and subjective dark periods, respectively. Experiments were performed twice using two independent RNA samples with similar results.

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or amplitude (Figure 4B, Supplemental Figure 3B). The expression pattern of *GI* was not significantly altered in the *cec2* mutation under the same condition (Figure 4C, Supplemental Figure 3C).

Shorter period and lower amplitude of CEC1 and CEC2 expression in lhy;cca1 under free-running conditions

The abundance of *CEC1* and *CEC2* mRNAs was measured to examine their diurnal and circadian expression patterns under LD and LL conditions (Figure 5, Supplemental Figure 4). Wild-type and *lhy*;*cca1* plants were grown for 3 weeks under 16-h light/8-h dark cycles and then transferred to LL conditions. In wild-type plants, free-running rhythmic expression of both *CEC1* and *CEC2* was seen with peaks at subjective dawn in a pattern similar to *LHY* and *CCA1* expression (Figures 2A, 2C, 5A, 5C, Supplemental Figures 1A, 1C, 4A, 4C). The rhythmic expression was rapidly damped when the *lhy*;*cca1* mutants were transferred to LL (Figures 5B, D, Supplemental Figures 4B, D). Intervals between the second and third peaks of *CEC1* and *CEC2* expression in wild-type plants were 24 and 28 h, respectively (Figures 5A, C, Supplemental Figures 4A, C). In contrast, the intervals between peaks were 20 h in *lhy*;*cca1* (Figures 5B, D, Supplemental Figures 4B, D), indicating that the *lhy*;*cca1* double mutation shortened the period length and reduced the amplitude of *CEC1* and *CEC2* expression under the free-running condition.

Discussion

Possible roles of CEC1 and CEC2 in flowering time regulation in Arabidopsis

The circadian clock is an important system for maintaining proper regulation of photoperiodic flowering in light/dark cycles. In particular, LHY and CCA1 regulate a flowering pathway that includes *GI*, *CO*, and *FT* under LD or SD conditions (Mizoguchi et al. 2005). The *lhy*;*cca1* double mutant delayed flowering under LL through the canonical *GI-CO* independent pathway, although flowering was accelerated under LD or SD (Fujiwara et al. 2008). The *cec1* and *cec2* mutants exhibited an early flowering phenotype under LD, possibly through the activation of *FT* expression (Figures 1C, 2B, Supplemental Figure 1B). Our results suggest that CEC1 and CEC2 may act as repressors of *FT* expression.

Rugnone et al. identified a family of night light-inducible and clock-regulated genes (*LNK1–4*). LNK2, LNK3, and LNK4 are identical to CEC1, CEC2, and CEC3, respectively, in this study. LNK1 (At5g64170) was not present in the LHY/CCA1 co-expression networks. LNK1 and LNK2 might regulate the expression of clock genes such as *PRR5* and *ELF4* (Rugnone et al. 2013). While *lnk2* single mutants showed an early flowering phenotype under LD as did *cec1* in this work, the *lnk1*;*lnk2* double mutant showed late flowering under LD (Rugnone et al. 2013). In general, phenotypes of double mutants are thought to be much more severe than those of the corresponding single mutants. For example, the *lhy* and *cca1* single mutants showed early flowering relative to WT under SD and the *lhy*;*cca1* double loss-

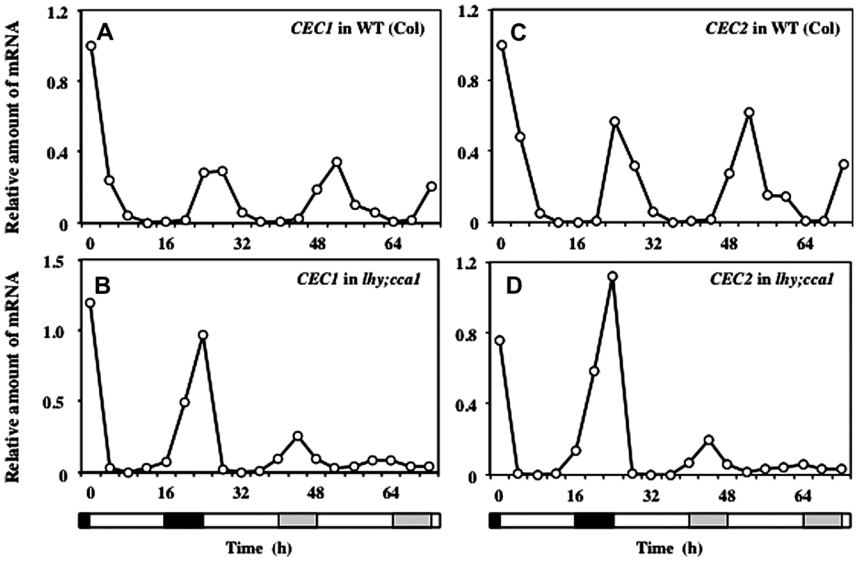

Figure 5. Reduced period and amplitude of *CEC1* and *CEC2* expression in *lhy*;*cca1* under free-running conditions. WT and *lhy*;*cca1* plants were grown under 16-h light/8-h dark cycles (LD) for 3 weeks and then transferred to LL. *CEC1* and *CEC2* mRNA levels were measured by real time PCR and normalized by *ACT2* mRNA levels. (A) Expression of *CEC1* in WT. (B) Expression of *CEC1* in *lhy*;*cca1*. (C) Expression of *CEC2* in WT. (D) Expression of *CEC2* in *lhy*;*cca1*. White, black, and gray boxes represent light, dark, and subjective dark periods, respectively. Experiments were performed twice using two independent RNA samples with similar results.

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of-function mutant flowered much earlier than WT or the single mutants (Mizoguchi et al. 2002). The late flowering phenotypes of *prr5* or *prr9* were apparent, but rather subtle. A synergistic effect was seen when *prr7* was combined with either *prr5* or *prr9* under LD (Nakamichi et al. 2005).

The reason why the *lnk1*;*lnk2* double mutant showed a phenotype opposite to the single mutants has not been elucidated (Rugnone et al. 2013). Therefore, further genetic and biochemical analyses including the examination of double, triple, and quadruple mutants of the *LNK1*, *LNK2*/*CEC1*, *LNK3*/*CEC2*, and *LNK4*/*CEC3* genes are required. *LNK1* is co-expressed with *PRR7* or *GI* according to the ATTED II database. *LNK1* and *LNK2*/*CEC1* are expressed rhythmically with peak expression occurring at noon and in the morning, respectively (Rugnone et al. 2013). Analyses combining many clock factors active in different phases will provide an effective approach to further research with the aim of identifying new clock-related genes and to investigate the PRR family.

Possible roles of CEC1 as a circadian clock component in Arabidopsis

While *LNK2*/*CEC1* and *LNK3*/*CEC2* were expressed rhythmically with expression peaks occurring in the morning, this rhythmic profile was rapidly damped when *lhy*;*cca1* was shifted to free-running conditions (Figure 5, Supplemental Figure 4). This result indicates that LHY and CCA1 regulate *CEC1* and *CEC2* gene expression. This is consistent with the fact that LHY and CCA1 are components of the central oscillator (Mizoguchi et al. 2002).

The expression of morning genes, such as *LHY* or *CCA1*, did not change in phase, but *GI* gene expression that peaks in the evening was affected in the *cec1* mutant under LD cycles (Figures 3B, 3C, 4B, Supplemental Figures 2B, 2C, 3B). The phase of *GI* expression was delayed by 4 h in the *cec1* mutant under LD and LL, but neither the period nor amplitude of *GI* expression was affected by *cec1* under LL. The *out of phase 1* (*oop1*) mutation is a *phytochrome B* (*phyB)* mutant allele (Salomé et al. 2002). The *oop1* mutant exhibited an early phase in the timing of the peaks of multiple circadian rhythms, but retained a normal period length (Salomé et al. 2002). In Arabidopsis, *PHY* genes participate in light input to the circadian clock system (Quail 2002). Furthermore, these photoreceptors regulate the entrainment of the circadian oscillator to light/dark cycles and modulate circadian clock function (Millar et al. 1995). Because the period length and amplitude of the evening gene *GI* expression was unaffected in the *cec1*-mutant plants, CEC1 might contribute to the determination of circadian phase by regulating evening genes rather than being a component of the clock itself. Again, further analyses of the double, triple, and quadruple mutants of the *LNK1*, *LNK2*/*CEC1*, *LNK3*/*CEC2*, and *LNK4*/*CEC3* genes is required to investigate the roles of the LNK/CEC family members in the control of circadian rhythms in Arabidopsis.

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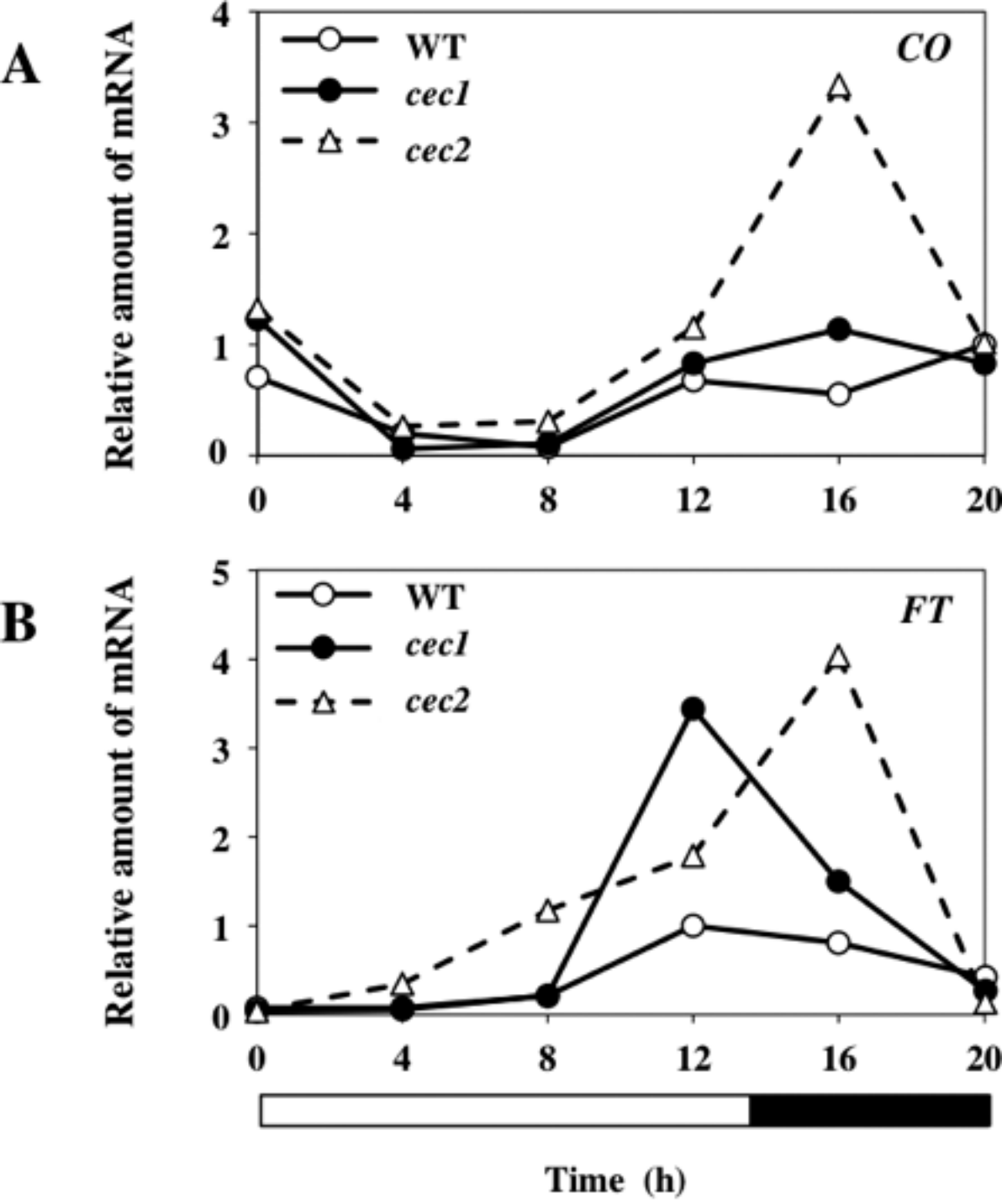
Supplemental figure legends

Supplemental Figure 1. Increased *CO* and *FT* mRNA levels in *cec1* and *cec2* mutants under LD. WT, *cec1*, and *cec2* plants were grown under 16-h light/ 8-h dark cycles (LD) for 3 weeks. *CO* and *FT* mRNA levels were measured by real-time PCR and normalized to *ACT2* mRNA levels. (A) Expression of *CO* in WT, *cec1*, and *cec2* plants. (B) Expression of *FT* in WT, *cec1*, and *cec2* plants. White and black boxes represent light and dark periods, respectively. Two experiments in Figure 2 and this figure were performed independently.

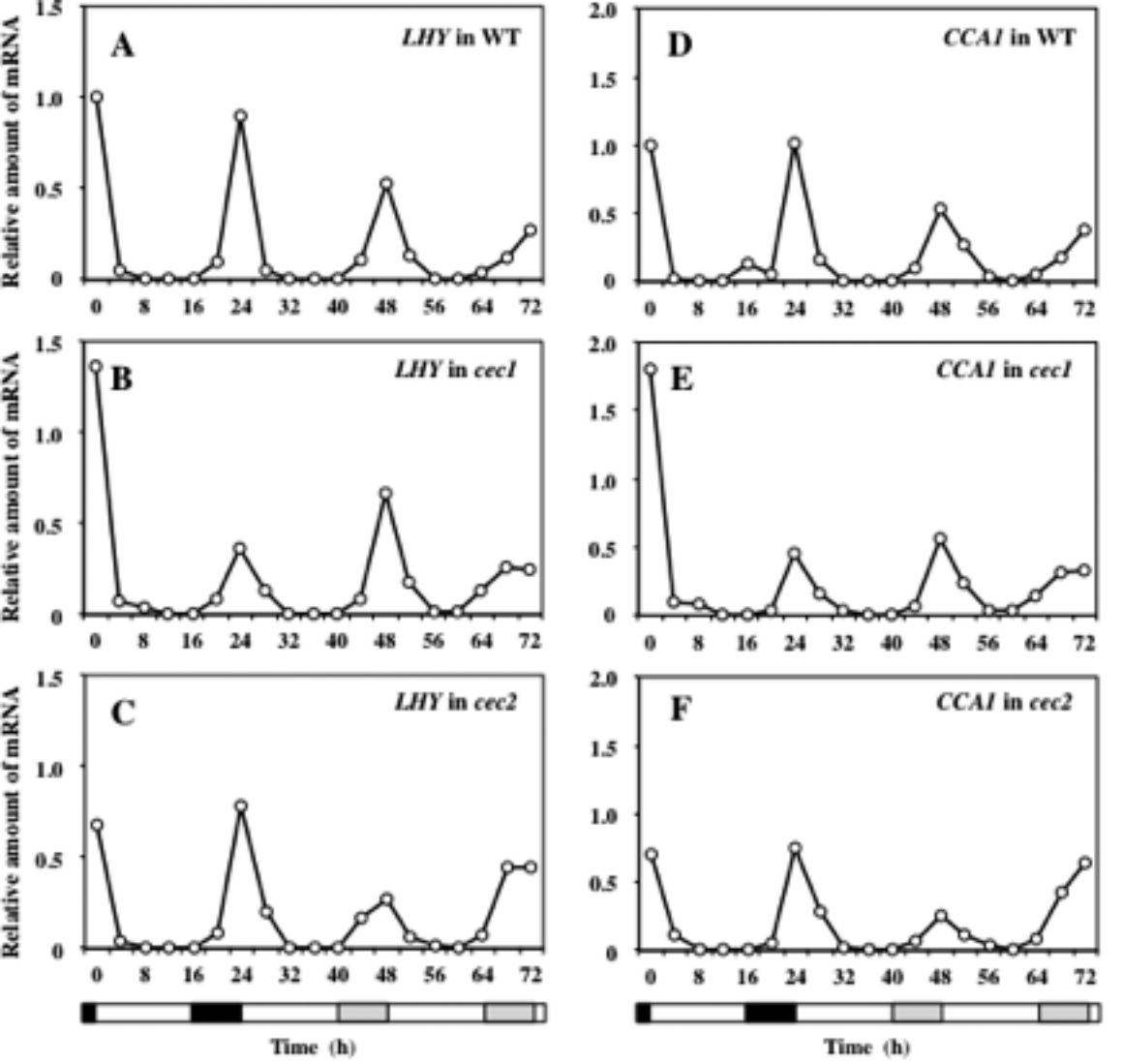
Supplemental Figure 2. *LHY* and *CCA1* expression in *cec1* and *cec2* mutants. Wild-type, *cec1*, and *cec2* plants were grown in 16-h light/ 8-h dark cycles (LD) for 3 weeks, and then transferred to LL. *LHY* and *CCA1* mRNA levels were measured by real time PCR and normalized to *ACT2* mRNA levels. (A) Expression of *LHY* in WT. (B) Expression of *LHY* in *cec1*. (C) Expression of *LHY* in *cec2*. (D) Expression of *CCA1* in WT. (E) Expression of *CCA1* in *cec1*. (F) Expression of *CCA1* in *cec2*. White, black, and gray boxes represent light, dark, and subjective dark periods, respectively. Two experiments in Figure 3 and this figure were performed independently.

Supplemental Figure 3. Phase shift in *GI* expression peaks in *cec1* under LD and LL. WT, *cec1*, and *cec2* plants were grown under 16-h light/ 8-h dark cycles (LD) for 3 weeks and then transferred to LL. *GI* mRNA levels were measured by real time PCR and normalized to *ACT2* mRNA levels. (A) Expression of *GI* in WT. (B) Expression of *GI* in *cec1*. (C) Expression of *GI* in *cec2*. White, black, and gray boxes represent light, dark, and subjective dark periods, respectively. Two experiments in Figure 4 and this figure were performed independently.

Supplemental Figure 4. Reduced period and amplitude of *CEC1* and *CEC2* expression in *lhy;cca1* under free-running conditions. WT and *lhy;cca1* plants were grown under 16-h light/ 8-h dark cycles (LD) for 3 weeks and then transferred to LL. *CEC1* and *CEC2* mRNA levels were measured by real time PCR and normalized by *ACT2* mRNA levels. (A) Expression of *CEC1* in WT. (B) Expression of *CEC1* in *lhy;cca1*. (C) Expression of *CEC2* in WT. (D) Expression of *CEC2* in *lhy;cca1*. White, black, and gray boxes represent light, dark, and subjective dark periods, respectively. Two experiments in Figure 5 and this figure were performed independently.



Supplemental Figure 1 (Hara et al.)



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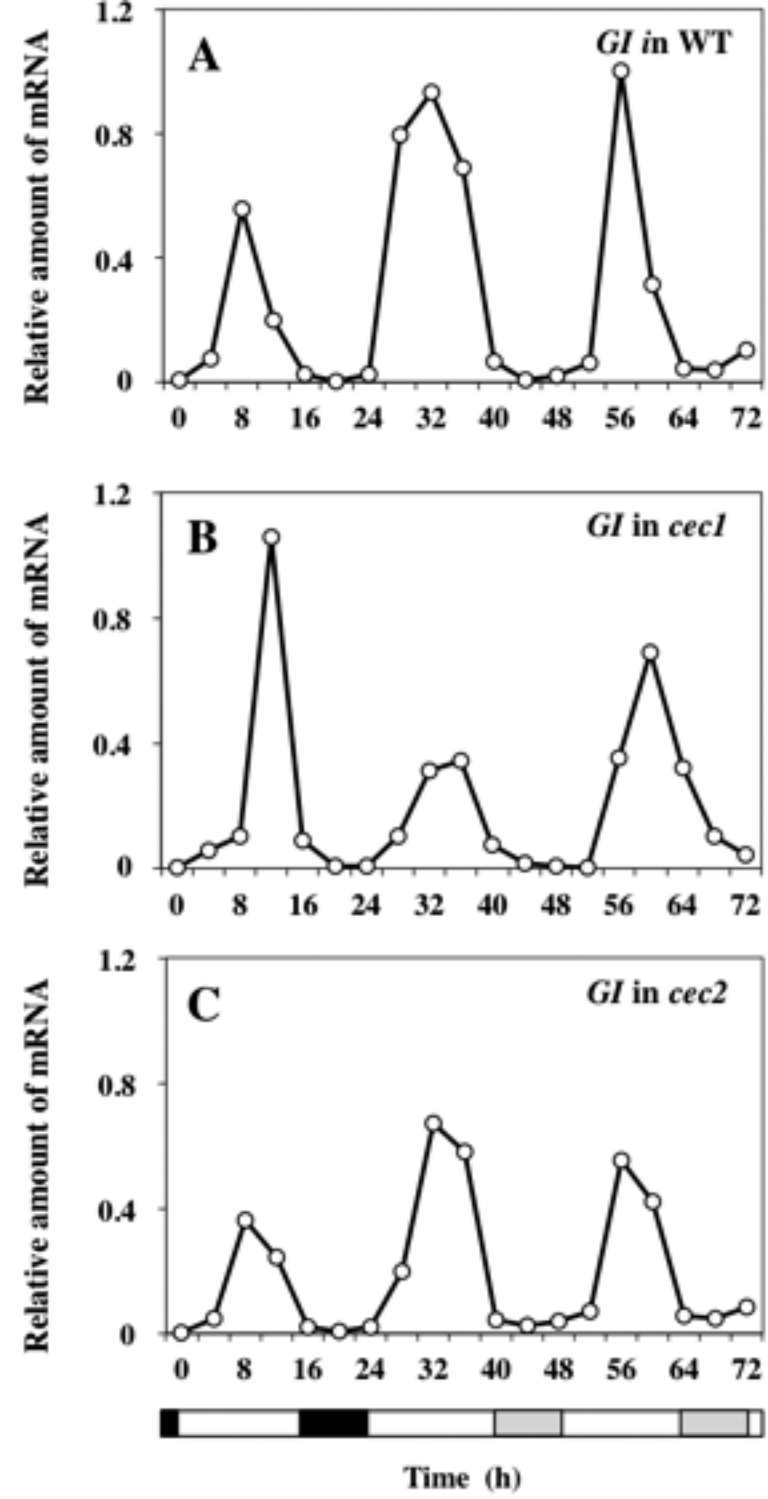
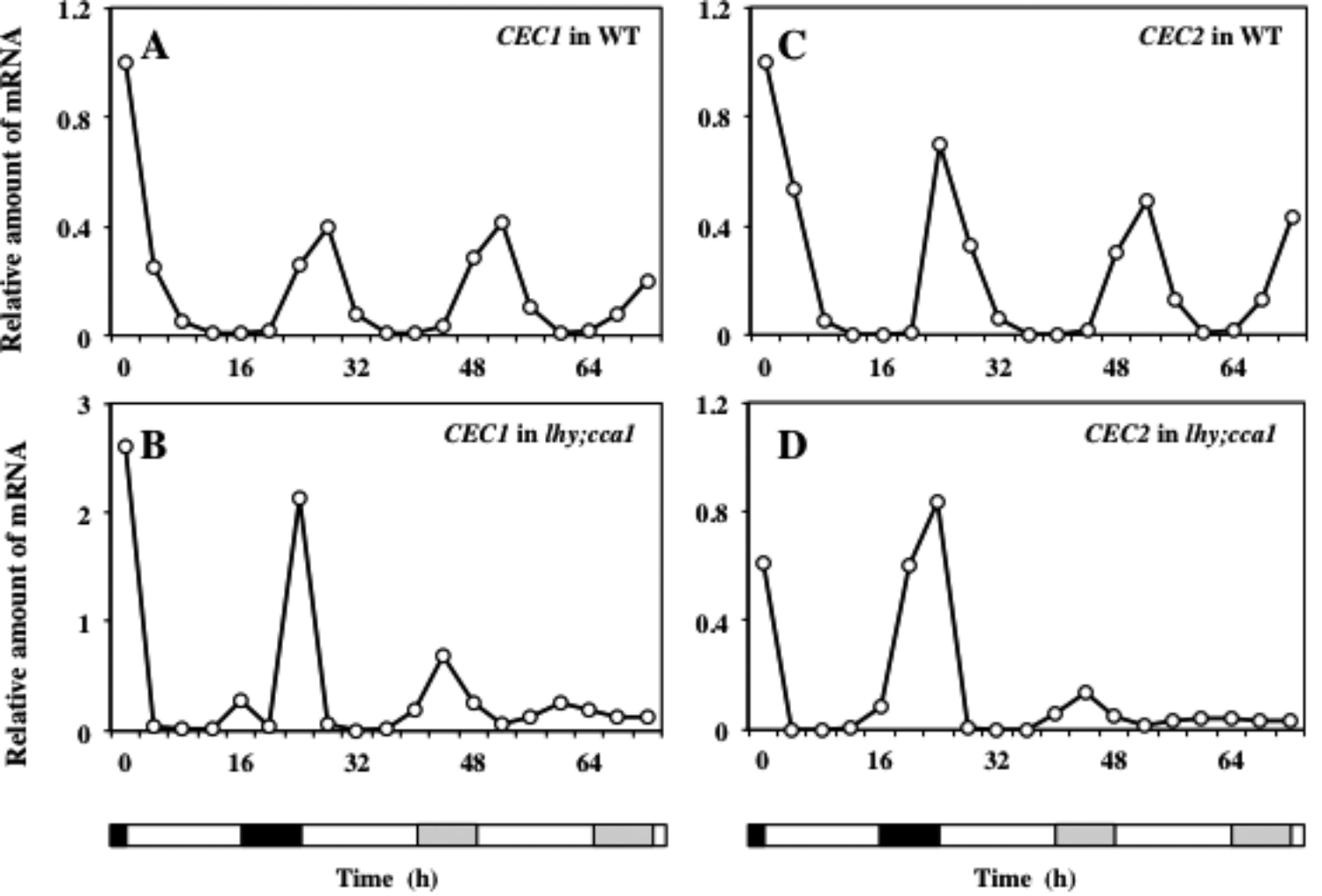


Supplemental Figure 3 (Hara et al.)



Supplemental Figure 4 (Hara et al.)