# Jumonji domain protein JMJD5 functions in both the plant and human circadian systems

**Matthew A. Jones**[a], **Michael F. Covington**[a,b], **Luciano DiTacchio**[c], **Christopher Vollmers**[c], **Satchidananda Panda**[c], **and Stacey L. Harmer**[a,1]

[a]Department of Plant Biology, College of Biological Sciences, University of California, Davis, CA 95616; [b]Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251; and [c]Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037

Edited* by Steve A. Kay, University of California at San Diego, La Jolla, CA, and approved November 9, 2010 (received for review September 21, 2010)

**Circadian clocks are near-ubiquitous molecular oscillators that coordinate biochemical, physiological, and behavioral processes with environmental cues, such as dawn and dusk. Circadian timing mechanisms are thought to have arisen multiple times throughout the evolution of eukaryotes but share a similar overall structure consisting of interlocking transcriptional and posttranslational feedback loops. Recent work in both plants and animals has also linked modification of histones to circadian clock function. Now, using data from published microarray experiments, we have identified a histone demethylase, jumonji domain containing 5 (JMJD5), as a previously undescribed participant in both the human and *Arabidopsis* circadian systems. *Arabidopsis JMJD5* is coregulated with evening-phased clock components and positively affects expression of clock genes expressed at dawn. We found that both *Arabidopsis jmjd5* mutant seedlings and mammalian cell cultures deficient for the human ortholog of this gene have similar fast-running circadian oscillations compared with WT. Remarkably, both the *Arabidopsis* and human *JMJD5* orthologs retain sufficient commonality to rescue the circadian phenotype of the reciprocal system. Thus, JMJD5 plays an interchangeable role in the timing mechanisms of plants and animals despite their highly divergent evolutionary paths.**

KDM8 | JMJ31 | TOC1 | coexpression

Circadian rhythms are endogenous oscillations that attune the behavior and physiology of organisms to regular changes in their environment, such as dusk and dawn. It is estimated that 1/10th of mammalian genes and one-third of *Arabidopsis* genes are regulated in a circadian fashion (1, 2). Not surprisingly, the circadian clock modulates a broad range of processes, ranging from the regulation of growth and flowering time in plants to the control of body temperature and the sleep-wake cycle in mammals (3, 4). The coordination of these molecular and physiological processes with the external environment has been shown to provide an adaptive advantage in organisms as diverse as cyanobacteria, higher plants, and insects (5–7).

Although it is thought that circadian clocks have arisen multiple times during eukaryotic evolution (8), molecular oscillators in a diverse range of lineages share a broad overall structure consisting of interlocking transcriptional and posttranslational feedback loops (3, 4). In plants, the morning-phased Myb-like transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) repress the expression of *TIMING OF CAB1 EXPRESSION 1* (*TOC1*) by binding to a conserved motif within its promoter known as the evening element (9–11). *TOC1*, in turn, promotes expression of *CCA1* and *LHY* via an indirect mechanism, forming a negative feedback loop (12). This central *CCA1*/*LHY*/*TOC1* loop interlocks with additional morning- and evening-phased circuits to provide more robustness and flexibility to the circadian mechanism (reviewed in 3, 13).

Because genes that act together are often coregulated (14), we have characterized a gene that is coexpressed with *TOC1* on both developmental and daily time scales. This gene, *JMJD5*, contains a jumonji-C (jmjC) domain that is often found in proteins with histone demethylase activity (15); indeed, its human ortholog (also known as *KDM8*) has recently been shown to have this function (16). Although originally described as a permanent modification, histone methylation marks are now acknowledged to be dynamic alterations that can fine-tune transcription in a broad range of eukaryotic phyla (15). Components of histone methyltransferase complexes have been associated with mammalian circadian clock function (17, 18), and changes in histone methylation have been correlated with modifications in circadian gene expression in plants (19).

Here, we report that *jmjd5* mutant plants have a short-period circadian phenotype and demonstrate that JMJD5 works in concert with TOC1 to promote *CCA1* and *LHY* expression. Remarkably, we also found that mammalian cells deficient for the human JMJD5 ortholog have a short-period phenotype very similar to that of *jmjd5* plants and that both *JMJD5* orthologs retain sufficient commonality to rescue the circadian phenotype of the reciprocal system. These data suggest that *JMJD5* may play a similar role in both the *Arabidopsis* and human circadian systems despite the wide evolutionary distance separating plants and mammals.

## Results

***TOC1* and *JMJD5* Are Coexpressed.** Despite recent advances (12), the mode of action of the central plant clock gene *TOC1* is currently enigmatic. Coexpressed genes frequently share biological function (14), prompting us to identify genes that are developmentally coregulated with *TOC1*. We therefore obtained microarray expression data generated from 79 different *Arabidopsis* samples (representing a wide variety of developmental stages and diverse organs) (20) and calculated the Pearson correlation coefficients between *TOC1* and each gene on the ATH1 array. The gene most highly correlated with *TOC1* ($r = 0.77$) was *EARLY FLOWERING 3* (*ELF3*; Table S1), a gene involved both in light input to the clock and in central clock function (21–23), thereby validating our approach. Because genes involved in the circadian clock are themselves frequently expressed with a circadian oscillation (3, 13), we examined the daily patterns of expression of our candidate genes using previously published microarray data (2). Of the 10 genes most highly correlated with *TOC1*, only *ELF3* and a previously uncharacterized gene, *JMJD5* (At3g20810), were expressed with a circadian rhythm (Fig. 1*A*). The expression patterns of *TOC1* and *JMJD5* were the third most highly correlated across all genes on the microarray ($r = 0.75$; Fig. 1*B* and Table S1). Empirical resampling of randomly per-

---

Author contributions: M.A.J., M.F.C., L.D., C.V., S.P., and S.L.H. designed research; M.A.J., L.D., and C.V. performed research; M.A.J., M.F.C., L.D., C.V., and S.L.H. contributed new reagents/analytic tools; M.A.J., M.F.C., L.D., and S.P. analyzed data; and M.A.J. and S.L.H. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: slharmer@ucdavis.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1014204108/-/DCSupplemental.

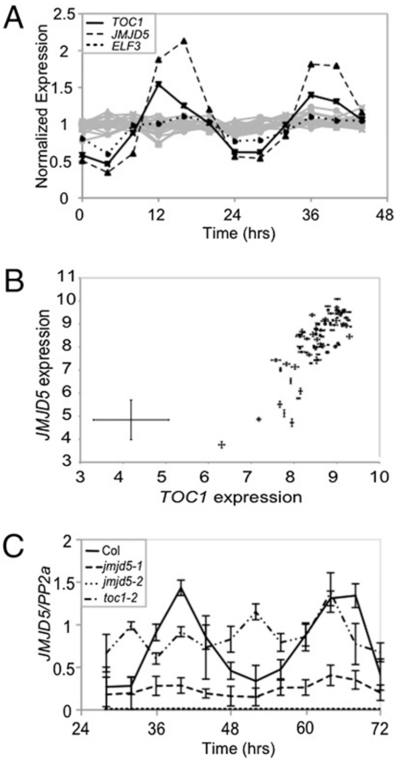


**Fig. 1.** *JMJD5* is coexpressed with *TOC1* over developmental and diurnal timescales. (*A*) Daily expression patterns of the 10 genes most highly developmentally correlated with *TOC1*. *TOC1*, *JMJD5*, and *ELF3* are shown in black, with the remaining genes shown in gray. Accession numbers for each of these genes are listed in Table S1. Expression data were obtained from the work of Covington and Harmer (51). (*B*) Scatter plot of *JMJD5* compared with *TOC1* mRNA expression levels across a range of developmental time points. Each symbol represents the mean of each different biological sample; error bars represent SEM. Data were obtained from the work of Schmid et al. (20). (*C*) qRT-PCR analysis of *JMJD5* expression under constant red light. WT Columbia (Col), *jmjd5-1*, *jmjd5-2*, and *toc1-2* seedlings were compared using oligos spanning intron 1 (Fig. S1*A* and Table S4). Plants were entrained to 12/12-h light/dark cycles for 6 d before being moved to constant conditions with 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ red light. *JMJD5* mRNA levels were normalized to *PP2a*. Data are the mean of three technical replicates; SE is shown. Presented results are representative of three independent experiments.

muted data shows that the chance for this level of correlation occurring between two randomly selected genes is only 1.3% (2).

We next investigated whether *JMJD5* expression was regulated by known clock genes. In *toc1-2* mutants, *JMJD5* expression levels gravitated toward a median level relative to the peak of expression in WT plants (Fig. 1*C*). In contrast, *JMJD5* mRNA was not detectable by quantitative RT-PCR (qRT-PCR) in seedlings overexpressing *CCA1* (Fig. S1*D*). This repression was similar to but more pronounced than the reduction of *TOC1* expression previously reported in these *CCA1-OX* plants (9) (Fig. S1*E*). Like *TOC1*, the *JMJD5* promoter contains an evening element sequence immediately upstream of the transcriptional start site (TSS) in addition to two within the 5′ UTR (Fig. S1*F*), suggesting that CCA1 might bind to this region and directly repress *JMJD5* expression. These data demonstrate that not only are *JMJD5* and *TOC1* coregulated across developmental and circadian time but they are both repressed by the morning-phased clock protein CCA1.

***jmjd5*, Like *toc1*, Is Conditionally Arrhythmic.** To determine whether *JMJD5* is simply an output of the clock or if it is involved in the clock mechanism, we introduced the *CCR2::LUC* luciferase reporter construct into two lines with T-DNA insertions within the At3g20810 locus (*jmjd5-1* and *jmjd5-2*; Fig. S1*A*). qRT-PCR using oligos spanning the first exon–exon junction revealed no detectable full-length mRNA in either mutant but a low level of a truncated form of the message in *jmjd5-1* (Fig. 1*C* and Fig. S1*B*). Because the conserved jmjC domain is located at the C terminus of the protein (Fig. S1*A*), both alleles are likely loss-of-function mutations. Consistent with this possibility, circadian rhythms in luciferase activity in both mutants were ≈0.5–1.0 h shorter than in WT (Fig. 2 *A* and *B*; $P < 0.05$, Student's *t* test). This phenotype was highly reproducible and was observed when plants were grown in a variety of light conditions (constant red light, constant blue light,

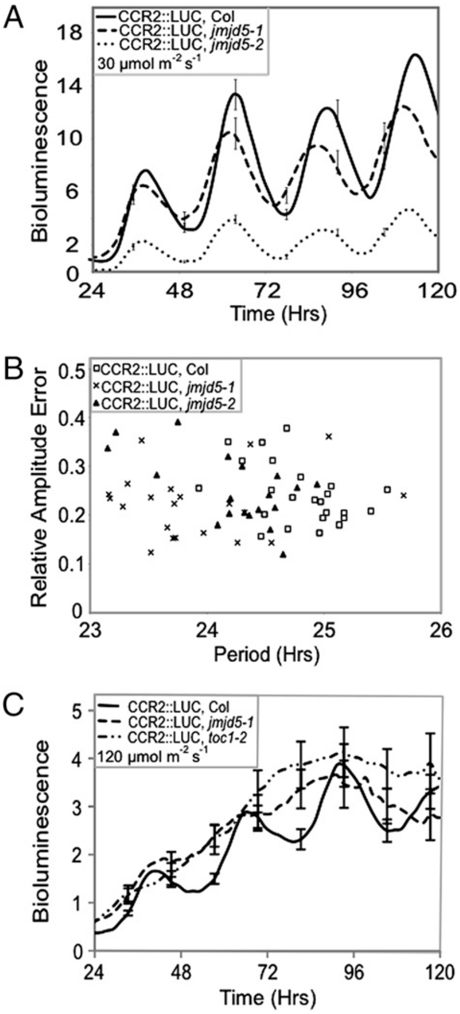


**Fig. 2.** *jmjd5* mutants have circadian phenotypes. Luciferase activity of *CCR2::LUC* plants monitored in continuous red light. (*A*) WT Columbia (Col), *jmjd5-1*, and *jmjd5-2* seedlings were entrained to 12/12-h light/dark cycles for 6 d before being moved to constant conditions with 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ monochromatic red light. The *jmjd5-2* alleles displayed reduced luciferase activity despite expressing normal levels of *CCR2* mRNA (Fig. S1*C*). Similar phenomena have previously been described in other T-DNA lines (49). Error bars indicate SEM and are displayed every 20 h for clarity ($n \geq 20$). (*B*) Scatter plot of data shown in *A* comparing circadian period with relative amplitude error, a measure of rhythmic robustness, with a perfect cosine wave having a value of 0 as calculated by Fourier fast transform-nonlinear least squares (50). Data points are presented for WT Col, *jmjd5-1*, and *jmjd5-2*. Plants were treated as described in *A*. (*C*) Bioluminescence of WT, *jmjd5-1*, and *toc1-2* seedlings maintained in higher intensity red light. Plants were entrained to 12/12-h light/dark cycles for 6 d before being moved to constant conditions with 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ red light. Data are representative of two independent experiments. Error bars are plotted every 20 h and indicate SEM ($n \geq 11$).

or constant red plus blue light; Table S2). Neither insertion allele displayed an altered rhythm under constant darkness, indicating that JMJD5 function is light-dependent (Table S2). Similar light-dependent circadian phenotypes have been reported for other clock mutations (24, 25).

Loss-of-function *toc1-2* mutants have a strong short-period phenotype in most conditions but become arrhythmic in constant red light (26). We therefore examined clock regulation of luciferase activity in *jmjd5* mutants maintained in different fluence rates of constant red light. When held under low levels of red light (~30 μmol·m$^{-2}$·s$^{-1}$), *jmjd5* mutants had a short period but preserved robust rhythms (Fig. 2 *A* and *B*). Intriguingly, at higher fluence rates of monochromatic red light (120 μmol·m$^{-2}$·s$^{-1}$), *jmjd5* seedlings became arrhythmic or had greatly dampened oscillations compared with WT plants, which had robust rhythms under these conditions (Fig. 2*C* and Fig. S2*A*). The *jmjd5* mutants continued to cycle on days 4 and 5 of free-run under a high fluence rate of white light (Fig. S2*B*), suggesting that this phenotype was specific to red light. Thus, the *jmjd5* and *toc1* mutant phenotypes are similar: Both have short free-running periods in constant blue or white light but become arrhythmic in constant red light.

**JMJD5 Is a Positive Regulator of *CCA1* and *LHY* Expression.** Although it is known that TOC1 promotes *CCA1* and *LHY* expression, the underlying mechanism is not yet fully understood (9, 12). *TOC1* and *JMJD5* are coexpressed and have comparable mutant phenotypes; thus, we speculated that JMJD5, like TOC1, might play a role in the regulation of *CCA1* and *LHY* expression. To investigate this possibility, we used qRT-PCR to examine the expression of core circadian genes in *toc1-2* and *jmjd5* mutants. Because both the *toc1* and *jmjd5* phenotypes were most severe in monochromatic red light (Fig. 2*C* and Fig. S2*A*), we chose to examine clock gene expression in plants grown in high-intensity constant red light, collecting samples over a full 2 d. Consistent with the luciferase data, qRT-PCR analysis revealed that expression of the evening-phased genes *GIGANTEA* (*GI*) and *TOC1* was disrupted, with a decrease in rhythmic amplitude (Fig. 3 *A* and *B*).

We next examined expression of *CCA1* and *LHY*, factors known to repress *GI* and *TOC1* expression (9, 27). In *jmjd5* mutants, peak levels of *CCA1* and *LHY* mRNA were reduced relative to WT (Fig. 3 *C* and *D* and Fig. S3), similar to the reduced expression seen in *toc1-2* plants (Fig. 3 *C* and *D*). These data indicate that like TOC1, JMJD5 promotes expression of the morning-phased clock genes *CCA1* and *LHY*.

***JMJD5* and *TOC1* Interact Genetically.** Given that *TOC1* and *JMJD5* are coregulated and have related mutant phenotypes, we speculated they might act at a similar step in the clock mechanism and would show genetic interactions. We therefore generated plants mutant for both genes and assessed circadian function in multiple light conditions. When monitored in constant red plus blue light, *jmjd5-1 toc1-2* double mutants had a free-running period ≈1.5 h shorter than *toc1-2* single mutants; in comparison, *jmjd5-1* single mutants had a period only 0.5 h shorter than WT controls (Fig. 4*A*). Therefore the combination of *jmjd5-1* and *toc1-2* mutations had a synergistic effect on period length. When maintained under constant red light of a moderate fluence rate (30 μmol·m$^{-2}$·s$^{-1}$), *jmjd5-1 toc1-2* mutants also showed a synergistic effect on rhythmicity. Under this condition, *jmjd5-1* single mutants were robustly rhythmic, whereas ≈70% of *toc1-2* mutants had a detectable rhythm in luciferase activity (Fig. 4*C*). In the double mutants, only 30% of plants had a significant circadian rhythm. Thus, *TOC1* and *JMJD5* showed synergistic genetic interactions for multiple clock phenotypes. In contrast, only additive effects on period and rhythmicity were observed in *jmjd5-1 lhy-100* double mutants, with the double mutants having a free-running period 0.5 h shorter than the *lhy-100* single mutants (Fig. 4 *B* and *D*).

**Human Cells Deficient for *JMJD5* also Have a Short-Period Phenotype.** Although the core circadian transcription factors are not shared across higher taxa, some proteins (such as casein kinase and cryptochrome) and protein modifications (such as histone acetylation and poly-ADP ribosylation) do play a role in the circadian clocks of diverse eukaryotes (11, 28, 29 and reviewed in 30, 31). *JMJD5* orthologs can be found in many eukaryotic genomes (Fig. S4*A* and Table S3). For example, the human JMJD5 protein (also known as KDM8) (16) is highly related to the *Arabidopsis* protein, with 39% identity and 56% similarity at the amino acid level and similar predicted secondary and tertiary protein structures (Fig. S4 *B* and *C*). Amino acid similarity rises to 68% within the conserved jmjC domain. Given the similarities between the human and *Arabidopsis* *JMJD5* genes, we investigated whether the human ortholog might also be involved in clock function. We designed an siRNA construct against the human *JMJD5* gene and transfected this into U2OS cells expressing luciferase under the control of the *BMAL1* promoter (U2OS-B6) (32). Transformation with the siRNA construct specifically knocked down endogenous levels of *JMJD5* to 20% of WT (Fig. S5 *A* and *B*). Transformed cells had a significantly shorter period than U2OS-B6 cells transfected with a scrambled siRNA

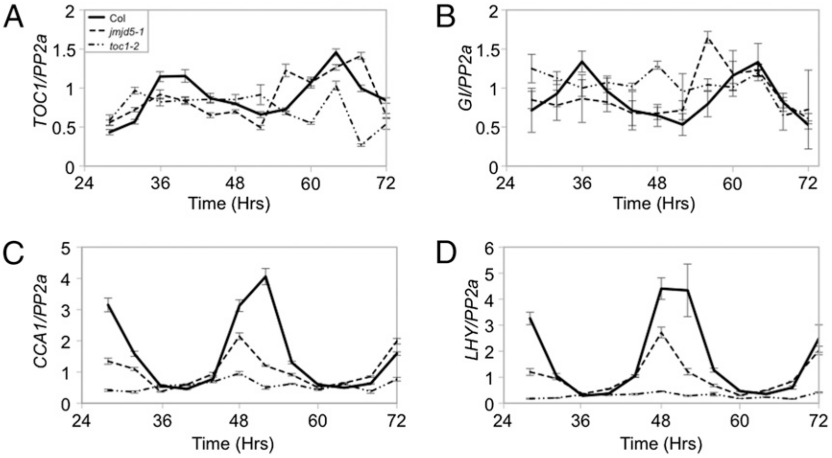


**Fig. 3.** qRT-PCR analysis of circadian clock-regulated genes. WT Columbia (Col), *jmjd5-1*, and *toc1-2* mutants were compared by qRT-PCR. Levels of *TOC1* (*A*), *GI* (*B*), *CCA1* (*C*), and *LHY* (*D*) mRNA were assessed. Plants were entrained to 12/12-h light/dark cycles for 6 d before being moved to constant conditions with 120 μmol·m$^{-2}$·s$^{-1}$ red light. mRNA levels for each gene were normalized to *PP2a*. Data are the mean of three technical replicates; SE is shown. Results are representative of three independent replicates.

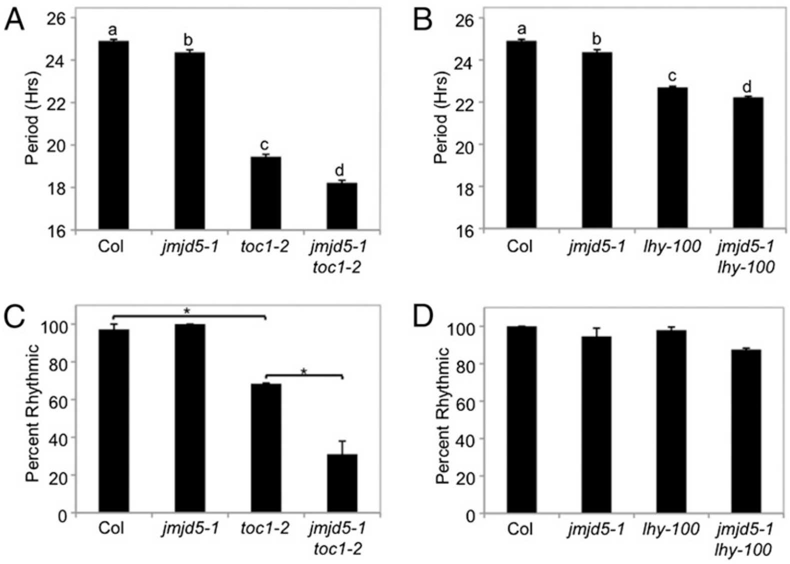


**Fig. 4.** *JMJD5* and *TOC1* interact genetically. (*A*) Period estimates of WT Columbia (Col), *jmjd5-1*, *toc1-2*, and *jmjd5-1 toc1-2* genotypes. Plants were entrained to 12/12-h light/dark cycles for 6 d before being held in continuous 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ red and blue light. Each genotype had a significantly different period length (*a–d*; $P < 0.001$, Tukey range test). Error bars represent SE ($n \geq 11$). Presented data are representative of three independent experiments. (*B*) Period estimates of WT Col, *jmjd5-1*, *lhy-100*, and *jmjd5-1 lhy-100* genotypes. Seedlings were treated and analyzed as described in *A*. Error bars represent SE ($n \geq 30$). Presented data are representative of three independent experiments. (*C*) Rhythmicity of *jmjd5-1 toc1-2* seedlings held under 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ red light. Percent rhythmicity was defined as the fraction of seedlings that returned a period estimate with a relative amplitude error <1 as determined by Fourier fast transform-nonlinear least squares (50). Seedlings and entrainment conditions were as described in *A*. Populations of *toc1-2* and *jmjd5-1 toc1-2* double-mutant seedlings had significantly worse rhythms than WT or *toc1-2* populations, respectively (*$P < 0.05$, Student's *t* test). Error bars indicate SE from three independent experiments. (*D*) Rhythmicity of *jmjd5-1 lhy-100* seedlings held under 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ red light. Seedlings and entrainment conditions were as described in *C*. Error bars indicate SE from three independent experiments.

control (Fig. 5 *A* and *B*; $P = 0.022$, Student's *t* test) but continued to show robust rhythmicity (Fig. 5*A*). This period shortening was strikingly similar to the circadian phenotype observed for *Arabidopsis jmjd5* mutants in most light conditions (Table S2).

**JMJD5 Homologs Act in Both Plant and Human Circadian Systems.** Because loss of either ortholog caused similar effects on clock function in plants and human cells, we speculated that the proteins might share a conserved cellular function. To test this possibility, we created a cell line (U2OS + AtJMJD5) that stably expressed the *Arabidopsis* gene in a U2OS-B6 background and monitored its circadian periodicity following knockdown of the endogenous human gene (Fig. 5*B*). Remarkably, U2OS + AtJMJD5 cells transfected with a human *JMJD5* siRNA had a significantly longer period than U2OS-B6 cells (Fig. 5*B*; $P = 0.0026$, Student's *t* test), indicating that the plant protein is able to function within the human circadian system. This rescue is highly significant but not complete, because a modest decrease in period length was observed in U2OS + AtJMJD5 cells when transfected with *JMJD5* siRNA compared with a scrambled siRNA control (Fig. 5*B*).

We also investigated whether the human ortholog was functional *in planta*. To test this hypothesis we introduced either the *Arabidopsis* or the human *JMJD5* gene, expressed under the control of the plant *JMJD5* promoter (Fig. S4*D*), into the *Arabidopsis jmjd5-1* mutant background and tested for rescue of the period defect. The circadian periods of T1 seedlings transformed with either the human or plant gene were then compared with those of WT and *jmjd5-1* plants (Fig. 5*C* and Fig. S4*E*). Intriguingly, seedlings transformed with either *JMJD5* ortholog displayed a significantly lengthened circadian period compared with the untransformed parental control ($P = 0.0013$ and $0.0210$ for the *Arabidopsis* and human genes, respectively). Use of T1 plants permitted characterization of at least 20 independent insertion events for each transgenic population but necessitated the use of hygromycin selection. Such treatment is unlikely to alter circadian period, however, because hygromycin-resistant homozygous T3 transgenics demonstrated no difference in period length in the presence or absence of hygromycin (Fig. S4*F*). In combination, these cross-kingdom rescue experiments demonstrate that JMJD5 functions in both the *Arabidopsis* and human circadian clocks via a conserved molecular mechanism.

## Discussion

**Characterization and Placement of *JMJD5* Within the *Arabidopsis* Circadian System.** We have successfully used a data-mining strategy to identify *JMJD5*, an evening-phased putative histone demethylase that acts within the *Arabidopsis* central clock. Similar to *TOC1*, *JMJD5* expression is strongly repressed by CCA1 (9) (Fig. S1 *D* and *E*). Indeed, it appears that the effect of *CCA1* overexpression on *JMJD5* expression levels may be more pronounced than that on *TOC1*; unlike *TOC1*, *JMJD5* mRNA was not detectable by qRT-PCR in seedlings overexpressing *CCA1* (9) (Fig. S1 *D* and *E*). Although *JMJD5* expression was altered in a *toc1-2* mutant background (Fig. 1*C*), this might be an indirect consequence of reduced *CCA1* and *LHY* mRNA levels in this genetic background (9). Similarly, mutation of the *TOC1* homologs *PSEUDORESPONSE REGULATOR 5*, *7*, and *9* (*PRR5*, *PRR7*, and *PRR9*) profoundly increased expression of *CCA1* and *LHY* and decreased expression of *JMJD5*, suggesting the PRRs may indirectly regulate *JMJD5* expression (33). *TOC1* expression was also significantly reduced in the *prr5*, *prr7*, and *prr9* mutants but to a lesser extent than that of *JMJD5* (33).

We found that *JMJD5* and *TOC1* showed a synergistic genetic interaction, whereas the interactions between *JMJD5* and *LHY* were additive (Fig. 4). Formally, synergism may arise either when two genes share overlapping functions within the same process or when they act in convergent signaling pathways. The reduced *CCA1* and *LHY* expression seen in both *jmjd5* and *toc1* single mutants (Fig. 3 *C* and *D* and Fig. S3) suggests that they may act together to promote expression of these morning-phased clock genes. Similar synergistic effects on regulation of gene expression

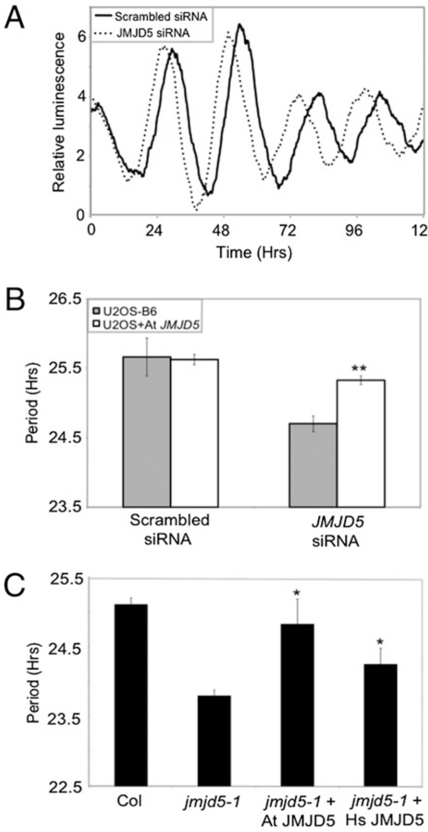


**Fig. 5.** *Arabidopsis* and human JMJD5 have conserved functions. (*A*) Circadian rhythmicity of U2OS-B6 cells stably expressing a Bmal1 promoter-driven luciferase reporter construct. Average luciferase activity of cells transfected with either scrambled siRNA or JMJD5 siRNA was assayed following synchronization with a serum shock as described by Vollmers et al. (32) ($n = 5$). Data are representative of three independent experiments. (*B*) Circadian period of human U2OS-B6 cells stably transformed with the plant *JMJD5* ortholog. U2OS-B6 cells expressing the plant JMJD5 ortholog (U2OS + AtJMJD5) had a significantly longer period than WT U2OS-B6 cells when transfected with a human JMJD5 siRNA ($^{**}P = 0.0026$, Student's *t* test). Error bars indicate SEM ($n = 5$). Cells were treated as described in *A*. Data are representative of two independent experiments. (*C*) Circadian period of *jmjd5-1* plants transformed with either the plant or human *JMJD5* gene. Plants were entrained to 12/12-h light/dark cycles for 6 d before being moved to continuous 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ red light. T1 plants transformed with either the plant or human *JMJD5* gene had a significantly longer period than *jmjd5-1* mutants ($^{*}P < 0.05$, mixed-effect linear model). Data are pooled from three independent experiments. Error bars show SEM ($n \geq 20$).

have previously been reported between transcription factors and chromatin-modifying enzymes (34–36) and between the mammalian clock genes CLOCK and BMAL1 (37).

***jmjd5*** **Phenotype Is Exacerbated by Increased Fluence Rates.** Although we observed a significant shortening of circadian rhythms in a range of light conditions (Fig. 2*A* and Table S2) and arrhythmia at higher fluence levels of monochromatic red light (Fig. 2*C*), we did not find a difference in circadian rhythms between WT and *jmjd5* mutants in constant darkness (Table S2). Similar light-dependent circadian phenotypes have been reported for *elf3* mutants (24), a gene that we similarly found to be coexpressed with TOC1 (Fig. 1*A*). The exacerbation of the *jmjd5* phenotype at increased fluence rates of monochromatic red light (Fig. 2*C*) was correlated with reduced expression of *CCA1* and *LHY* (Fig. 3 and Fig. S3). In contrast, *TOC1* expression levels were not appreciably reduced in *jmjd5* (Fig. 3*A*).

Intriguingly, JMJD5 shares these differential effects on clock gene expression with *LUX ARRHYTHMO (LUX)*, *ELF3*, and *ELF4*, which are all evening-phased genes that have yet to be ascribed specific positions within the circadian circuitry (21, 23, 38, 39). Like *JMJD5*, loss of *ELF3*, *ELF4*, or *LUX* causes decreased expression of *CCA1* and *LHY* without strongly affecting *TOC1* expression levels (38, 40–42). It is possible that these coexpressed genes act in concert in some manner to promote morning-phased clock gene expression.

**JMJD5 Functions in Both the Human and Plant Circadian Systems.** Orthologs of JMJD5 are found across eukaryotes (Fig. S4*A* and Table S3). Phylogenetic analyses suggest that these homologs originated before the separation of the animal and plant phyla, ≈1.5 billion y ago (43). Despite this ancient divergence, the human *JMJD5* gene can rescue the circadian defect of *Arabidopsis jmjd5* mutants. Given the apparent independent origins of the plant and human circadian clocks (8, 44), it would appear that JMJD5 has been recruited independently into each of these molecular oscillators.

The human JMJD5 protein has recently been shown to demethylate histone H3 dimethylated at lysine 36 (H3K36me2), both in vitro and in vivo (16). Although the enzymatic activity of the *Arabidopsis* ortholog is not yet determined, our data suggest that human and plant proteins can fulfill similar functions within the plant and human circadian systems, respectively (Fig. 5 *B* and *C* and Fig. S4*E*). All the cofactor-binding residues thought necessary for histone methylase activity (15) are found in the *Arabidopsis* JMJD5 protein (Fig. S4*B*), suggesting that it may also have this biochemical function. Despite this conservation, we cannot exclude the possibility that the human JMJD5 acts simply as a scaffold to facilitate the function of endogenous plant protein complexes rather than supplying needed enzymatic activity.

Although H3K36me2 modifications are predominantly found toward the 3′ end of actively transcribed eukaryotic genes (45–47), enrichment of this modification is also found near the TSS and within exons, where it acts as a repressive mark to suppress erroneous transcription (45, 47, 48). Both *CCA1* and *LHY* loci carry extensive H3K36me2 marks within their coding regions (48), suggesting that JMJD5 may directly demethylate the *CCA1* and *LHY* loci and thereby promote their transcription. Alternately, JMJD5 could promote gene expression less directly by modifying the combination of histone modifications present at the affected loci. Histone modifications are increasingly acknowledged to act in concert to direct transcriptional activity (15, 16, 47).

In summary, we have identified a previously undescribed component of both the *Arabidopsis* and human circadian systems that may link the clock with dynamic histone modification and thereby provide additional regulation of gene expression. Our coexpression and genetic analyses indicate that *Arabidopsis* JMJD5 acts in concert with TOC1 to positively regulate expression of *CCA1* and *LHY*. Although we do not presume that the inclusion of *JMJD5* in the molecular oscillators of both the *Arabidopsis* and human circadian systems is a retained ancestral function of this protein, it is intriguing that it functions in the clocks of these highly divergent species. At the very least, this suggests that histone methylation is an important regulatory mechanism in two very different eukaryotes. Future work will further elucidate the relationship between JMJD5 and the core clock components of both animals and plants.

## Materials and Methods

**Plant Materials and Growth Conditions.** Details on the construction of the binary vectors and the mutants used in this study are included in *SI Materials and Methods*.

**Mammalian Cell Culture.** U2OS cell lines used in this study are described in *SI Materials and Methods*.

**Luciferase Assays.** Luciferase assays were performed as described (32, 49, 50). Additional information is provided in *SI Materials and Methods*.

**PCR Techniques.** RNA isolation, cDNA preparation, and qRT-PCR techniques are described in *SI Materials and Methods*.

**Gene Expression Correlation and Phylogenetic Analyses.** Details on the statistical and phylogenetic tools used in this study are included in *SI Materials and Methods*.

**ACKNOWLEDGMENTS.** We thank Julin Maloof for statistical advice and Reetika Rawat (University of California, Davis, CA) for seed stocks. Mutants were obtained from the Arabidopsis Biological Resource Center and the European Arabidopsis Stock Centre. This study was supported by National Institutes of Health Grant GM069418 (to S.L.H.).

1. Panda S, et al. (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109:307–320.
2. Covington MF, Maloof JN, Straume M, Kay SA, Harmer SL (2008) Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome Biol*, 10.1186/gb-2008-9-8-r130.
3. Harmer SL (2009) The circadian system in higher plants. *Annu Rev Plant Biol* 60: 357–377.
4. Ko CH, Takahashi JS (2006) Molecular components of the mammalian circadian clock. *Hum Mol Genet* 15(Spec No 2):R271–R277.
5. Dodd AN, et al. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* 309:630–633.
6. Woelfle MA, Ouyang Y, Phanvijhitsiri K, Johnson CH (2004) The adaptive value of circadian clocks: An experimental assessment in cyanobacteria. *Curr Biol* 14:1481–1486.
7. Emerson KJ, Bradshaw WE, Holzapfel CM (2008) Concordance of the circadian clock with the environment is necessary to maximize fitness in natural populations. *Evolution* 62:979–983.
8. Rosbash M (2009) The implications of multiple circadian clock origins. *PLoS Biol* 7:e62.
9. Alabadí D, et al. (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science* 293:880–883.
10. Harmer SL, Kay SA (2005) Positive and negative factors confer phase-specific circadian regulation of transcription in Arabidopsis. *Plant Cell* 17:1926–1940.
11. Perales M, Más P (2007) A functional link between rhythmic changes in chromatin structure and the Arabidopsis biological clock. *Plant Cell* 19:2111–2123.
12. Pruneda-Paz JL, Breton G, Para A, Kay SA (2009) A functional genomics approach reveals CHE as a component of the Arabidopsis circadian clock. *Science* 323:1481–1485.
13. Jones MA (2009) Entrainment of the Arabidopsis circadian clock. *J Plant Biol* 52: 202–209.
14. Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95:14863–14868.
15. Mosammaparast N, Shi Y (2010) Reversal of histone methylation: Biochemical and molecular mechanisms of histone demethylases. *Annu Rev Biochem* 79:155–179.
16. Hsia DA, et al. (2010) KDM8, a H3K36me2 histone demethylase that acts in the cyclin A1 coding region to regulate cancer cell proliferation. *Proc Natl Acad Sci USA* 107: 9671–9676.
17. Brown SA, et al. (2005) PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator. *Science* 308:693–696.
18. Etchegaray JP, et al. (2006) The polycomb group protein EZH2 is required for mammalian circadian clock function. *J Biol Chem* 281:21209–21215.
19. Ni Z, et al. (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. *Nature* 457:327–331.
20. Schmid M, et al. (2005) A gene expression map of Arabidopsis thaliana development. *Nat Genet* 37:501–506.
21. Covington MF, et al. (2001) ELF3 modulates resetting of the circadian clock in Arabidopsis. *Plant Cell* 13:1305–1315.
22. McWatters HG, Bastow RM, Hall A, Millar AJ (2000) The ELF3 zeitnehmer regulates light signalling to the circadian clock. *Nature* 408:716–720.
23. Thines B, Harmon FG (2010) Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. *Proc Natl Acad Sci USA* 107:3257–3262.
24. Hicks KA, et al. (1996) Conditional circadian dysfunction of the Arabidopsis early-flowering 3 mutant. *Science* 274:790–792.
25. Eriksson ME, Hanano S, Southern MM, Hall A, Millar AJ (2003) Response regulator homologues have complementary, light-dependent functions in the Arabidopsis circadian clock. *Planta* 218:159–162.
26. Más P, Alabadí D, Yanovsky MJ, Oyama T, Kay SA (2003) Dual role of TOC1 in the control of circadian and photomorphogenic responses in Arabidopsis. *Plant Cell* 15: 223–236.
27. Mizoguchi T, et al. (2002) LHY and CCA1 are partially redundant genes required to maintain circadian rhythms in Arabidopsis. *Dev Cell* 2:629–641.
28. Panda S, Poirier GG, Kay SA (2002) tej defines a role for poly(ADP-ribosyl)ation in establishing period length of the arabidopsis circadian oscillator. *Dev Cell* 3:51–61.
29. Asher G, et al. (2010) Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. *Cell* 142:943–953.
30. Grimaldi B, Nakahata Y, Kaluzova M, Masubuchi S, Sassone-Corsi P (2009) Chromatin remodeling, metabolism and circadian clocks: The interplay of CLOCK and SIRT1. *Int J Biochem Cell Biol* 41:81–86.
31. Gallego M, Virshup DM (2007) Post-translational modifications regulate the ticking of the circadian clock. *Nat Rev Mol Cell Biol* 8:139–148.
32. Vollmers C, Panda S, DiTacchio L (2008) A high-throughput assay for siRNA-based circadian screens in human U2OS cells. *PLoS ONE* 3:e3457.
33. Nakamichi N, et al. (2009) Transcript profiling of an Arabidopsis PSEUDO RESPONSE REGULATOR arrhythmic triple mutant reveals a role for the circadian clock in cold stress response. *Plant Cell Physiol* 50:447–462.
34. Bertrand C, et al. (2005) Arabidopsis HAF2 gene encoding TATA-binding protein (TBP)-associated factor TAF1, is required to integrate light signals to regulate gene expression and growth. *J Biol Chem* 280:1465–1473.
35. Lee S, Lee B, Lee JW, Lee S-K (2009) Retinoid signaling and neurogenin2 function are coupled for the specification of spinal motor neurons through a chromatin modifier CBP. *Neuron* 62:641–654.
36. Kim J-R, et al. (2009) Enhancer of polycomb1 acts on serum response factor to regulate skeletal muscle differentiation. *J Biol Chem* 284:16308–16316.
37. Sato TK, et al. (2006) Feedback repression is required for mammalian circadian clock function. *Nat Genet* 38:312–319.
38. Hazen SP, et al. (2005) LUX ARRHYTHMO encodes a Myb domain protein essential for circadian rhythms. *Proc Natl Acad Sci USA* 102:10387–10392.
39. McWatters HG, et al. (2007) ELF4 is required for oscillatory properties of the circadian clock. *Plant Physiol* 144:391–401.
40. Schaffer R, et al. (1998) The late elongated hypocotyl mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* 93: 1219–1229.
41. Doyle MR, et al. (2002) The ELF4 gene controls circadian rhythms and flowering time in Arabidopsis thaliana. *Nature* 419:74–77.
42. Kikis EA, Khanna R, Quail PH (2005) ELF4 is a phytochrome-regulated component of a negative-feedback loop involving the central oscillator components CCA1 and LHY. *Plant J* 44:300–313.
43. Bhattacharya D, Yoonb H, Hedgesc S, Hackett J (2009) Eukaryotes (Eukaryota). *The Timetree of Life* (Oxford University Press, Oxford), p 116.
44. Young MW, Kay SA (2001) Time zones: A comparative genetics of circadian clocks. *Nat Rev Genet* 2:702–715.
45. Carrozza MJ, et al. (2005) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* 123: 581–592.
46. Bannister AJ, et al. (2005) Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes. *J Biol Chem* 280:17732–17736.
47. Luo C, Lam E (2010) ANCORP: A high-resolution approach that generates distinct chromatin state models from multiple genome-wide datasets. *Plant J* 63:339–351.
48. Oh S, Park S, van Nocker S (2008) Genic and global functions for Paf1C in chromatin modification and gene expression in Arabidopsis. *PLoS Genet* 4:e1000077.
49. Martin-Tryon EL, Kreps JA, Harmer SL (2007) GIGANTEA acts in blue light signaling and has biochemically separable roles in circadian clock and flowering time regulation. *Plant Physiol* 143:473–486.
50. Plautz JD, et al. (1997) Quantitative analysis of Drosophila period gene transcription in living animals. *J Biol Rhythms* 12:204–217.
51. Covington MF, Harmer SL (2007) The circadian clock regulates auxin signaling and responses in Arabidopsis. *PLoS Biol* 5:e222.

# Supporting Information

## Jones et al. 10.1073/pnas.1014204108

### SI Materials and Methods

**Plant Materials and Growth Conditions.** *jmjd5-1* (SAIL-811_H12) and *jmjd5-2* (GK-454C10) were obtained from the SAIL and Gabi-Kat T-DNA insertion collections, respectively (1, 2). Homozygous T-DNA insertion lines were identified using oligo sets 1 and 2 as shown in Table S4. *jmjd5-1 toc1-2* and *jmjd5-1 lhy-100* plants were generated by crossing and then genotyping for the requisite mutations as previously described (3). *CCA1-OX* lines have been described previously (4).

Binary vectors for the generation of phenotypic rescue plants were generated as follows. A genomic fragment containing *JMJD5* and a 1.5-kb upstream region was PCR-amplified using oligo set 3 (Table S4) and introduced into pENTR/D-TOPO (Invitrogen) via the TOPO cloning method to create pENTR At JMJD5. This construct was then used in conjunction with pGWB4 (5) to create pGWB4 At JMJD5 by attL × attR (LR) recombination as described in the manufacturer's protocol (Invitrogen). To generate pENTR Hs JMJD5, the coding sequence of *At JMJD5* was cut out of pENTR At JMJD5 using MslI and AscI (New England Biolabs). Hs JMJD5 cDNA was PCR-amplified from pcDNA3.2 JMJD5 using oligo set 4 (Table S4) and digested with EcoRV and AscI (New England Biolabs) before being ligated into the compatible ends within the cut pENTR vector. The pENTR Hs JMJD5 vector was then sequenced and used in conjunction with pGWB4 (5) to create pGWB4 At JMJD5 by LR recombination. Hygromycin-resistant control plasmids (Col + pMDC32) were generated by introducing At4g00050 cDNA into pMDC32 (6) by LR recombination.

All clones were moved into *Agrobacterium* strain GV3101 and transformed into WT *CCR2::LUC* or jmjd5-*1 CCR2::LUC* plants by *Agrobacterium*-mediated transformation (7). Transformants were selected on Murashige–Skoog media supplemented with 3% (wt/vol) sucrose (MSS) (3) containing 50 μg/mL hygromycin (EMD Chemicals) for 5 d before being transplanted to fresh MSS plates lacking hygromycin.

**Mammalian Cell Culture.** U2OS-B6 cells have previously been described (8). At JMJD5 cDNA was inserted into pcDNA6.2 by LR recombination as described by the manufacturer's protocol (Invitrogen). U2OS + AtJMJD5 cells were subsequently generated by seeding U2OS-B6 cells onto six-well plates before transfection with FuGENE 6 (Roche) mixed with pcDNA6.2 At JMJD5 according to the manufacturer's instructions. Forty-eight hours posttransfection, the cells were placed on normal growth media supplemented with Blasticidin S (Invitrogen). U2OS + AtJMJD5 stable populations were maintained in the presence of 2–10 μg/mL blasticidin.

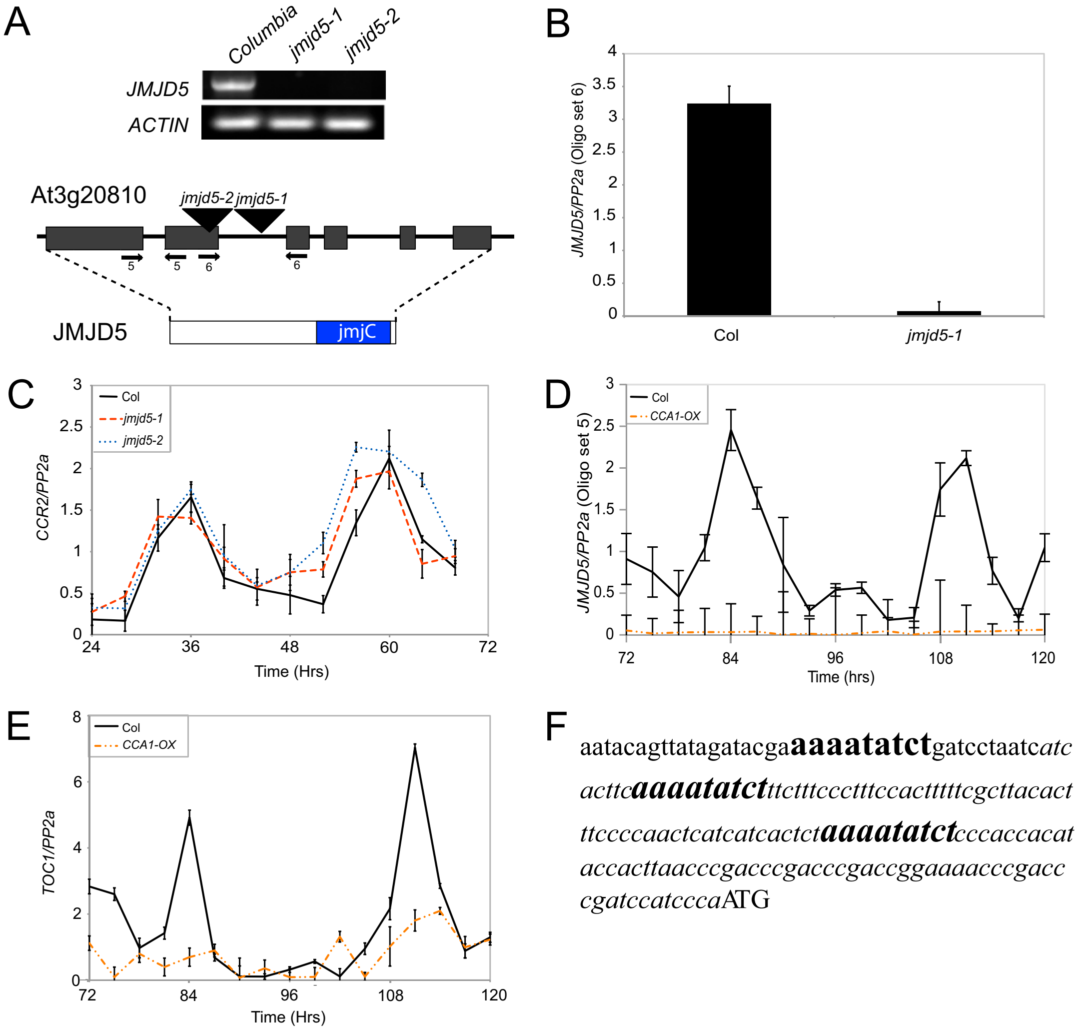
**Luciferase Assays.** Plants were entrained for 6 d in 12/12-h light/dark cycles under white light before being sprayed with 3 mM D-luciferin in 0.01% Triton X-100. Plants were then transferred to free-running conditions under either red, blue, or red plus blue light-emitting diodes as previously described (3). Imaging was completed over 5 d, and data were processed using Metamorph software (Molecular Devices). Patterns of luciferase activity were fit to cosine waves using Fourier fast transform-nonlinear least squares (9) to estimate circadian period length.

U2OS-B6 cell lines stably expressing a Bmal1 promoter-driven luciferase reporter construct were transfected with the desired siRNA in a 384-well plate, sealed, and subjected to real-time bioluminescence measurement in a TECAN M200 luminometer as described (8).

**PCR Techniques.** For seedlings, plants were grown, RNA was isolated, and qRT-PCR was performed as previously described (3). Samples were run in triplicate, and starting quantity was estimated from critical thresholds using the standard curve of amplification. Data for each sample were normalized to the respective *PP2a* expression level. For U2OS-B6 analysis, four wells of a 384-well plate were transfected per siRNA; 36–48 h posttransfection, RNA extraction was performed with an RNeasy kit (Qiagen). cDNA levels were measured via SYBR Green-based qRT-PCR (Roche) and normalized against *GADPH* expression. Primer sets used are described in Table S4.

**Gene Expression Correlation and Phylogenetic Analyses.** Genes with expression similar to that of *TOC1* were identified using AtGenExpress (10). Genes orthologous to *Arabidopsis JMJD5* were identified using BLAST (11, 12). Protein sequences were aligned using MUSCLE (13) and manually curated to remove clearly erroneous regions of alignment. A maximum likelihood phylogenetic tree was constructed using CIPRES PORTAL (14), utilizing RAxML (15) and an "LG + G + F" model of protein evolution as prescribed by ProtTest (16). Data were manipulated in JALVIEW (17).

1. Li Y, Rosso MG, Viehoever P, Weisshaar B (2007) GABI-Kat SimpleSearch: An Arabidopsis thaliana T-DNA mutant database with detailed information for confirmed insertions. *Nucleic Acids Res* 35(Database issue):D874–D878.
2. Sessions A, et al. (2002) A high-throughput Arabidopsis reverse genetics system. *Plant Cell* 14:2985–2994.
3. Martin-Tryon EL, Kreps JA, Harmer SL (2007) GIGANTEA acts in blue light signaling and has biochemically separable roles in circadian clock and flowering time regulation. *Plant Physiol* 143:473–486.
4. Wang ZY, Tobin EM (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. *Cell* 93:1207–1217.
5. Nakagawa T, et al. (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104:34–41.
6. Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* 133:462–469.
7. Clough SJ, Bent AF (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* 16:735–743.
8. Vollmers C, Panda S, DiTacchio L (2008) A high-throughput assay for siRNA-based circadian screens in human U2OS cells. *PLoS ONE* 3:e3457.
9. Plautz JD, et al. (1997) Quantitative analysis of Drosophila period gene transcription in living animals. *J Biol Rhythms* 12:204–217.
10. Schmid M, et al. (2005) A gene expression map of Arabidopsis thaliana development. *Nat Genet* 37:501–506.
11. Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402.
12. Altschul SF, et al. (2005) Protein database searches using compositionally adjusted substitution matrices. *FEBS J* 272:5101–5109.
13. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797.
14. Miller MA, et al. *The CIPRES Portals.* CIPRES. Available at http://www.phylo.org/sub_sections/portal. Accessed August 4, 2009.
15. Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
16. Abascal F, Zardoya R, Posada D (2005) ProtTest: Selection of best-fit models of protein evolution. *Bioinformatics* 21:2104–2105.
17. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ (2009) Jalview Version 2—A multiple sequence alignment editor and analysis workbench. *Bioinformatics* 25: 1189–1191.



aatacagttatagatacga**aaaatatct**gatcctaatc*atc*
*acttc****aaaatatct****ttctttccctttccactttttcgcttacact*
*ttccccaactcatcatcactct****aaaatatct****cccaccacat*
*accacttaacccgacccgacccgaccggaaaacccgacc*
*cgatccatccca*ATG

**Fig. S1.** Characterization of *Arabidopsis jmjd5* alleles. (*A*) Semiquantitative PCR analysis of *JMJD5* mRNA expression in WT Columbia (Col) and *jmjd5* mutant plants using primers spanning the full-length cDNA (oligo set 8; Table S4). Actin mRNA was amplified as a loading control. The positions of the T-DNA insertions within the *JMJD5* locus (At3g20810) are indicated below. Gray squares indicate exons, whereas black triangles demonstrate the approximate position of the T-DNA insertion for each allele. A cartoon of the translated protein is also shown, with the conserved jmjC domain indicated in blue. Numbered arrows indicate oligo sets used for qRT-PCR (Table S4). (*B*) qRT-PCR analysis of *JMJD5* message levels in *jmjd5-1* mutants. *JMJD5* message was assayed in WT Col and *jmjd5-1* mutants using oligos that amplified cDNA on either side of the T-DNA insertion point as in *A* (oligo set 6; Table S4). Plants were entrained to 12/12-h light/dark cycles for 10 d before tissue was harvested at dusk. Data are the mean of three technical replicates; SE is shown. Presented results are representative of two independent experiments. (*C*) qRT-PCR analysis of *CCR2* expression in WT Col, *jmjd5-1*, and *jmjd5-2* seedlings. Plants were treated and analyzed as described in Fig. 1*C*. *JMJD5* (*D*) and *TOC1* (*E*) expression analysis in WT Col and seedlings overexpressing *CCA1* (*CCA1-OX*). *JMJD5* and *TOC1* mRNA levels were normalized to *PP2a*. Plants were treated as described in Fig. 1*C*. Data are the mean of three technical replicates; SE is shown. Presented results are representative of two independent experiments. (*F*) Promoter analysis of *JMJD5*. The region directly upstream of the *JMJD5* start codon contains three evening elements (AAAATATCT). The 5′ UTR and TSS are indicated in italics and with capital letters, respectively.

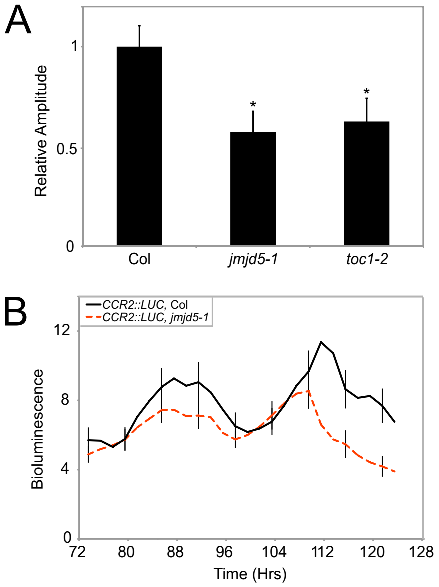


**Fig. S2.** *jmjd5-1* mutants have reduced rhythmicity under monochromatic red light. (*A*) Rhythmic amplitude of luciferase activity in *jmjd5-1* and *toc1-2* seedlings held under 120 μmol·m$^{-2}$·s$^{-1}$ red light. Mean amplitude of seedlings that returned any rhythm in the circadian range (15 < τ < 30 h; rhythmicity determined by Fourier fast transform-nonlinear least squares) (9) was normalized against the WT Columbia (Col) sample mean. Seedlings and entrainment conditions were as described in Fig. 2*C*. Error bars indicate SE ($n \geq 8$). Presented data are representative of two independent experiments. (*B*) Bioluminescence of WT Col and *jmjd5-1 CCR2::LUC* seedlings under high-intensity white light. Plants were entrained to 12/12-h light/dark cycles for 6 d before being moved to constant conditions with 130 μmol·m$^{-2}$·s$^{-1}$ white light. Seedlings were imaged for 2 d after 3 d in free-run. Error bars are plotted every 6 h and indicate SE ($n \geq$ 7). Presented data are representative of two independent experiments.

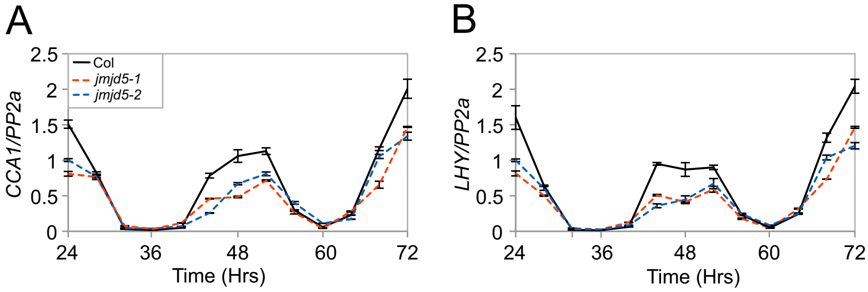


**Fig. S3.** qRT-PCR analysis of circadian clock-regulated genes in *jmjd5-2* plants. WT Columbia (Col), *jmjd5-1*, and *jmjd5-2* were compared by qRT-PCR. Levels of *CCA1* (*A*) and *LHY* (*B*) mRNA were assessed. Plants were entrained to 12/12-h light/dark cycles for 6 d before being moved to constant conditions with 120 μmol·m$^{-2}$·s$^{-1}$ red light. mRNA levels for each gene were normalized to *PP2a*. Data are the mean of three technical replicates; SE is shown. Presented results are representative of two independent experiments.

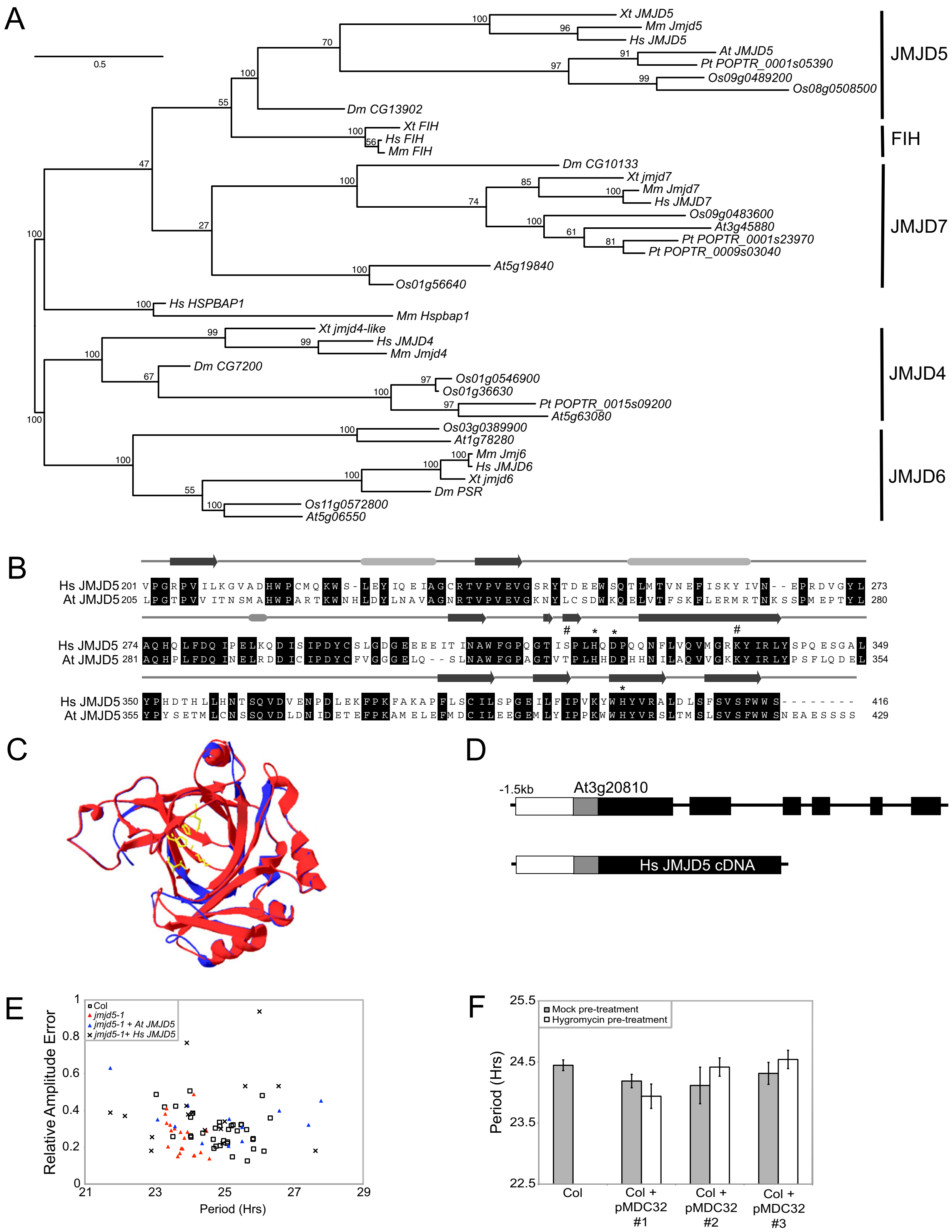


**Fig. S4.** Comparison of plant and human JMJD5 orthologs. (*A*) Maximum likelihood phylogenetic tree showing "jmjC-only" proteins in model species; note that JMJD5 orthologs form a distinct clade compared with other proteins containing an orphan jmjC domain. *Arabidopsis* JMJD5 was utilized as a template to identify candidate orthologs using BLAST (1, 2). Additional jmjC-only proteins were selected according to previous analyses (3, 4). Sequences were aligned using MUSCLE (5), and a maximum likelihood phylogenetic tree was then constructed using CIPRES PORTAL (6). Species abbreviations are as follows: *At*, *Arabidopsis thaliana*; *Dm*, *Drosophila melanogaster*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Os*, *Oryza sativa*; *Pt*, *Populus trichocarpa*; *Xt*, *Xenopus tropicalis.* Gene locus designations are given on each tip. Percentage bootstrap values of 1,000 replications are shown on an internal node. (*B*) Conservation of primary and sec-

Legend continued on following page

ondary protein structures between the jmjC domains of *Arabidopsis* and human *JMJD5* homologs. Identical amino acids are shown in black. The predicted secondary structure is shown above. The predicted Fe (II) (*) and α-keto-glutarate (#) binding residues, respectively, are shown (7). Although At JMJD5 T323 and Hs JMJD5 S318 are not conserved, jmjC histone demethylase activity has been shown in proteins with either serine or threonine in this position (7, 8) (*C*) Protein structure of the jmjC domains of *Arabidopsis* JMJD5 (blue) and human JMJD5 (red) obtained by homology modeling with the program Swiss Model using the protein structure of FIH1 (PDB ID code 1MZF) as a template (9). Conserved residues thought necessary for cofactor binding are shown in yellow. (*D*) DNA constructs transformed into *jmjd5-1* mutants. Exons are shown in black, the *Arabidopsis* 5′ UTR is shown in gray, and the *Arabidopsis JMJD5* promoter is shown in white. The *Arabidopsis* coding region was replaced with human *JMJD5* cDNA to create the *At*/*Hs JMJD5* fusion construct. (*E*) Scatter plot comparing circadian period with relative amplitude error as calculated by Fourier fast transform-nonlinear least squares (10). Circadian rhythms were monitored using a *CCR2::LUC* reporter construct. Data points are presented for WT Columbia (Col), *jmjd5-1*, *jmjd5-1* + *At JMJD5*, and *jmjd5-1* + *Hs JMJD5*. Plants were assayed as described in Fig. 5*C*. (*F*) Circadian period of transgenic plants under hygromycin selection. Plants were entrained to 12/12-h light/dark cycles for 6 d in the presence or absence of hygromycin before being transplanted to fresh plates without hygromycin. The period lengths of three independent T3 lines treated with hygromycin (Col + pMDC32) were statistically indistinguishable from those of untreated controls ($P = 0.47$, mixed-effect linear model). Data are representative of two independent experiments. Error bars show SEM ($n \geq 14$).

1. Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402.
2. Altschul SF, et al. (2005) Protein database searches using compositionally adjusted substitution matrices. *FEBS J* 272:5101–5109.
3. Takeuchi T, Watanabe Y, Takano-Shimizu T, Kondo S (2006) Roles of jumonji and jumonji family genes in chromatin regulation and development. *Dev Dyn* 235:2449–2459.
4. Lu F, et al. (2008) Comparative analysis of JmjC domain-containing proteins reveals the potential histone demethylases in Arabidopsis and rice. *J Integr Plant Biol* 50:886–896.
5. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797.
6. Miller MA, et al. *The CIPRES Portals.* CIPRES. Available at http://www.phylo.org/sub_sections/portal. Accessed August 4, 2009.
7. Trewick SC, McLaughlin PJ, Allshire RC (2005) Methylation: Lost in hydroxylation? *EMBO Rep* 6:315–320.
8. Hsia DA, et al. (2010) KDM8, a H3K36me2 histone demethylase that acts in the cyclin A1 coding region to regulate cancer cell proliferation. *Proc Natl Acad Sci USA* 107:9671–9676.
9. Dann CE, 3rd, Bruick RK, Deisenhofer J (2002) Structure of factor-inhibiting hypoxia-inducible factor 1: An asparaginyl hydroxylase involved in the hypoxic response pathway. *Proc Natl Acad Sci USA* 99:15351–15356.
10. Plautz JD, et al. (1997) Quantitative analysis of Drosophila period gene transcription in living animals. *J Biol Rhythms* 12:204–217.

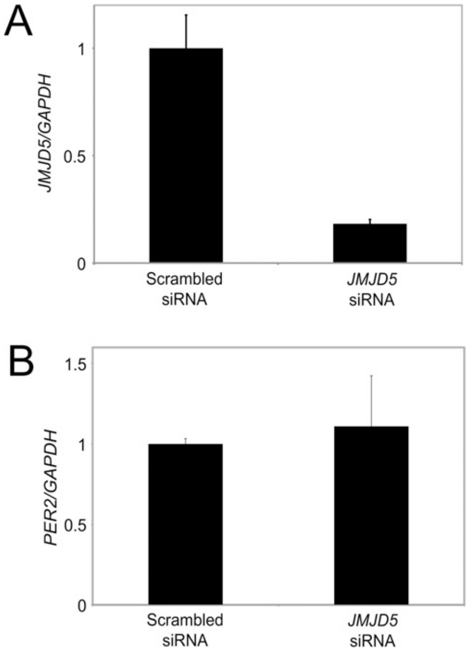


**Fig. S5.** Effect of *JMJD5* siRNA treatment of human cells. (*A*) qRT-PCR analysis of *JMJD5* message levels in U2OS-B6 cells transfected with either JMJD5 siRNA or a scrambled siRNA control (Table S4). Data are the mean of three technical replicates; SE is shown. Results are representative of three independent experiments. (*B*) *PER2* message levels were unaffected by *JMJD5* siRNA knockdown. Samples were processed and analyzed as described in *A*. Data are the mean of three technical replicates; SE is shown. Results are representative of three independent experiments.

## Table S1. Genes correlated with *TOC1* expression over developmental time

| AGI | Annotation | Pearson correlation coefficient |
|---|---|---|
| AT5G61380 | TOC1 | 1 |
| AT2G25930 | ELF3 | 0.768912673 |
| AT4G35230 | Putative protein kinase | 0.765872777 |
| AT3G20810 | JMJD5 | 0.750030994 |
| AT4G26610 | Putative protein kinase | 0.736636937 |
| AT2G11520 | CRCK3 | 0.729635179 |
| AT4G31170 | Putative protein kinase | 0.726003408 |
| AT5G41260 | Putative protein kinase | 0.725784361 |
| AT1G01800 | Short-chain dehydrogenase/reductase (SDR) family protein | 0.724870086 |
| AT1G28280 | VQ motif-containing protein | 0.712580025 |
| AT1G48210 | Putative protein kinase | 0.708676577 |

Microarray expression data generated from 79 different *Arabidopsis* samples [representing a wide variety of developmental stages and diverse organs ($n$ = 20)] were examined to identify genes coexpressed with *TOC1*. Pearson correlation coefficients between *TOC1* and each gene are presented.

## Table S2. *jmjd5* clock phenotypes in a variety of light conditions

| | cR (60 μE) | cB (20 μE) | cR + B (60 μE) | cD |
|---|---|---|---|---|
| *CCR2::LUC*, Col | 24.85 ± 0.07 | 24.12 ± 0.08 | 24.66 ± 0.10 | 25.75 ± 0.14 |
| *CCR2::LUC*, *jmjd5-1* | 23.89* ± 0.12 | 23.57* ± 0.15 | 24.10* ± 0.09 | 26.05 ± 0.29 |
| *CCR2::LUC*, *jmjd5-2* | 24.36* ± 0.09 | 23.09* ± 0.25 | 23.60* ± 0.08 | 25.19 ± 0.19 |

Circadian period estimates were obtained from plants entrained in 12/12-h light/dark cycles for 6 d and then moved to constant darkness (cD), constant red (cR) light, constant blue (cB) light, or the combination of constant red and blue (cR + B) light. Intensities of different light conditions are presented in μE ($\mu$mol·m$^{-2}$·s$^{-1}$). SEM is shown. *Significant difference between the WT period and the indicated mutant ($P < 0.01$, Student's $t$ test).

## Table S3. Percent identity between JMJD5 proteins in different eukaryotes

| | Species | Annotation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Arabidopsis thaliana* | At3g20810 | | | | | | | | | | | |
| 2 | *Populus trichocarpa* | POPTR_0001s05390 | 69 | | | | | | | | | | |
| 3 | *Oryza sativa* | Os09g0489200 | 55 | 56 | | | | | | | | | |
| 4 | *Drosophila melanogaster* | CG13902 | 35 | 33 | 33 | | | | | | | | |
| 5 | *Caenorhabditis elegans* | C06H2.3 | 28 | 28 | 30 | 30 | | | | | | | |
| 6 | *Gallus gallus* | JMJD5 | 42 | 41 | 41 | 39 | 35 | | | | | | |
| 7 | *Xenopus tropicalis* | JMJD5 | 36 | 38 | 36 | 36 | 33 | 63 | | | | | |
| 8 | *Canis familiaris* | JMJD5 | 39 | 39 | 39 | 36 | 33 | 62 | 57 | | | | |
| 9 | *Bos taurus* | JMJD5 | 40 | 39 | 40 | 39 | 34 | 63 | 60 | 83 | | | |
| 10 | *Homo sapiens* | JMJD5/KDM8 | 39 | 38 | 38 | 37 | 34 | 64 | 57 | 81 | 83 | | |
| 11 | *Mus musculus* | JMJD5 | 39 | 38 | 38 | 35 | 36 | 62 | 55 | 75 | 77 | 78 | |
| 12 | *Dictyostelium discoideum* | XM_641084.1 | 32 | 32 | 31 | 32 | 28 | 34 | 30 | 32 | 33 | 32 | 31 |

JMJD5 orthologs were identified and aligned using BLAST and MUSCLE (11–13). Percent amino acid identity is presented. National Center for Biotechnology Information sequence references and alternate annotations are provided where applicable.

**Table S4. Oligonucleotides used for this study**

| Oligo set | Description | 5′ oligo | 3′ oligo |
|---|---|---|---|
| 1 | T-DNA insertion check (WT) | 5′-GTGCGCTTGATATGGGTCTT-3′ | 5′-CTGCCCATTCGTGTTAAACC-3′ |
| 2 | T-DNA insertion check (mutant) | 5′-TAGCATCTGAATTTCATAACCAATCTCGATACAC-3′ | 5′-CTGCCCATTCGTGTTAAACC-3′ |
| 3 | At JMJD5 + promoter | 5′-CACCGGCCAAGCATG-3′ | 5′-CGAGCTAGAAGATTCTGCTTCAT-3′ |
| 4 | Hs JMJD5 cDNA | 5′-GGCGCGCCACGACCACCAGAAGC-3′ | 5′-GATATCCAATGGCTGGAGACAC-3′ |
| 5 | At JMJD5 qPCR (exon 1) | 5′-GACGTCAATGAGGTCCTTAAGA-3′ | 5′-CTCCACGGGAACGGTG-3′ |
| 6 | At JMJD5 qPCR (exon 2) | 5′-GTGGAGGTGGGGAAAAAC-3′ | 5′-CTGATCAAACAAAGGATGCTG-3′ |
| 7 | CCR2 qPCR | (1) | (1) |
| 8 | At JMJD5 (full-length cDNA) | 5′-ATGAATTCATGTCAGGAGCTACCACC-3′ | 5′-AGCGGCCGCCTACGAGCTAGAAGATTC-3′ |
| 9 | Actin | (2) | (2) |
| 10 | CCA1 qPCR | (3) | (3) |
| 11 | LHY qPCR | (3) | (3) |
| 12 | TOC1 qPCR | (3) | (3) |
| 13 | GI qPCR | (3) | (3) |
| 14 | PP2a qPCR | (4) | (4) |
| 15 | Hs JMJD5 qPCR | 5′-CCTGCCGCACAGTAAAGAAGA-3′ | 5′-CAGACACTCGTCCCTCCTG-3′ |
| 16 | PER2 qPCR | 5′-TTCCCAGCAAACGTCCCAG-3′ | 5′-GGTGCGTACCTACTCCCGT-3′ |
| 17 | GAPDH qPCR | 5′-ATGGGGAAGGTGAAGGTCG-3′ | 5′-GGGGTCATTGATGGCAACAAT-3′ |
| 18 | Scrambled siRNA | 5′-GCCUUCACAAGACUACCAGTT-3′ | |
| 19 | Hs JMJD5 siRNA | 5′-CAGCAAATACATCGTGAATGA-3′ | |

1. Martin-Tryon EL, Kreps JA, Harmer SL (2007) GIGANTEA acts in blue light signaling and has biochemically separable roles in circadian clock and flowering time regulation. *Plant Physiol* 143:473–486.
2. Brown BA, Jenkins GI (2008) UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature Arabidopsis leaf tissue by requirement for UVR8, HY5, and HYH. *Plant Physiol* 146:576–588.
3. Mockler TC, et al. (2004) Regulation of flowering time in Arabidopsis by K homology domain proteins. *Proc Natl Acad Sci USA* 101:12759–12764.
4. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible W-R (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. *Plant Physiol* 139:5–17.