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Review

Dance of plants with circadian clock

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Abstract Endogenous oscillator called circadian clock controls many physiological aspects. Since the identification of *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) genes as the 1st candidates for clock components in plants, many clock-associated genes have been identified in *Arabidopsis* and other plant species. The 1st negative feedback model composed of LHY, CCA1 and TIMING OF CAB EXPRESSION 1 (TOC1) has recently been modified based on possible functions of new players. Photoperiodic flowering is controlled by clock and our knowledge on molecular mechanisms underlying the clock-controlled process has much advanced in this decade. Recently, we have started to understand how the clock regulates organ movements and elongations in a model plant, *Arabidopsis*. In this short review, i) a history of construction of models on circadian rhythms in *Arabidopsis*, ii) modified models on circadian system in *Arabidopsis*, iii) recent progress on understanding molecular mechanisms underlying organ movements controlled by a circadian clock and iv) advantages of using tomato as a model system for chronobiology are summarized and discussed.

Key words: Circadian clock, Circumnutation, LATE ELONGATED HYPOCOTYL (LHY), Micro-Tom, PSEUDO RESPONSE-REGULATOR (PRR).

Circadian clock is an endogenous oscillator with an approximate period of 24 h that can be entrained to the exact period of daily oscillations in light and temperature (Dunlap 1999). This process enables an organism to phase its biological activities to the correct time of day. The circadian rhythm has been reported in many processes in various organisms (Dunlap 1999) from cyanobacteria, fungi and plants to humans. The first report about a circadian rhythm is in plant leaf movement. In 1729, De Mairan, a French astronomer, found that leaf movement in *Mimosa pudica* which had been already known to open (horizontal) during day and close (vertical) during night persisted even after transfer to continuous dark (DD) condition. This suggests that the leaf movements were not simple response to the light and were controlled by the endogenous circadian clock.

Since the 1st report on the clock-controlled phenomena, our knowledge on molecular mechanisms of the circadian clock has much advanced. In this review, a short history of models explaining the circadian rhythms in plants is shown in the 1st and 2nd sections. Recent progress on characterization of organ movements, classical but still mysterious and uncovered outputs controlled by the circadian clock in plants are shown in the 3rd section. Finally, advantages to use tomatoes for studies on circadian rhythms are discussed in more detail in the 4th section.

Negative feedback models at the early stage of the *Arabidopsis* clock studies

Two Myb-related genes, *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), have been shown to be closely associated with clock function in *Arabidopsis* (Schaffer et al. 1998; Wang and Tobin 1998). Expression of *LHY* and *CCA1* oscillates with a circadian rhythm. Over-expression of either *LHY* or *CCA1* causes arrhythmic expression of clock-controlled genes (*CCGs*) and reduction of their own expression. Lack of *CCA1* in a T-DNA insertion mutant line (*cca1-1*) shortened the period of the rhythm in the expression of *LHY* and other *CCGs* (Green and Tobin 1999).

TOC1 gene is also a part of central oscillator function in *Arabidopsis* (Millar et al. 1995). The *toc1* mutant was initially identified in a screen for mutations that disrupt the circadian-clock-controlled pattern of expression of the gene encoding the chlorophyll *a*/*b* binding protein (CAB) by using a *CAB* promoter fusion to *luciferase* (*luc*). In *toc1* mutants, circadian clock regulation still occurs, but the circadian-clock-controlled peaks in gene expression occur approximately every 21 hours instead of every 24 hours. Leaf movement and stomatal conductance rhythms were similarly ~3 hours shorter than wild type (Somers et al. 1998). These results

Abbreviations: CCA1, CIRCADIAN CLOCK ASSOCIATED 1; CO, CONSTANS; FT, FLOWERING LOCUS T; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYL; LL, continuous light; LD, long day; SD, short day; TOC1, TIMING OF CAB EXPRESSION 1.

indicate that TOC1 plays pivotal roles in a variety of clock-controlled processes throughout development in *Arabidopsis*. *TOC1* gene has been identified by a map-based cloning and encodes a protein with a response regulator domain and a CCT motif that is conserved in CONSTANS (CO), CONSTANS LIKE (COL) and TOC1 (Putterill et al. 1995; Strayer et al. 2000; Ledger et al. 2001).

Histidine-to-aspartate (His-Asp) phosphorelay (or two-component) systems are very common signal transduction mechanisms that are implicated in a wide variety of cellular responses to environmental stimuli (Appleby et al. 1996). A His-to-Asp phosphorelay system consists of two or more common signal transducers, a sensor exhibiting His-kinase activity, a response regulator containing a phospho-accepting Asp in its receiver domain and a His-containing phosphotransmitter (HPt) (Parkinson and Kofoid 1992, Mizuno 1998). The *TOC1* gene was also identified as *PSEUDO-RESPONSE REGULATOR 1* (*PRR1*) gene during the course of systematic studies of the His-to-Asp phosphorelay system in *Arabidopsis* (Matsushika et al. 2000).

The *TOC1*/*PRR1* mRNA oscillates with high hhamplitude in wild-type plants in constant light (LL) (Matsushika et al. 2000, Strayer et al. 2000). The *TOC1*/*PRR1* mRNA level is constant in both *LHY-1* (*LHY*-OX) and *CCA1-OX* plants under the same light condition (Alabadí et al. 2001, Figure 1B). High and constant expression levels of either *LHY* or *CCA1* result in low and constant levels of *TOC1*/*PRR1* transcript in LL. This suggests that LHY and CCA1 are negative regulators of *TOC1*/*PRR1*. This idea is supported by the fact that *TOC1*/*PRR1* transcript oscillates 12 hours out of phase with both *LHY* and *CCA1* transcripts in wild-type plants (Strayer et al. 2000). Moreover, both LHY and CCA1 bind to a region in the *TOC1*/*PRR1* promoter that is critical for its clock regulation, suggesting that both LHY and CCA1 may act directly to negatively regulate *TOC1*/*PRR1* expression (Alabadí et al. 2001) (Figure 1B).

We have isolated three loss-of-function alleles of *LHY* (*lhy-11*, *lhy-12* and *lhy-13*), and demonstrate that these mutations shorten the period of the *CCG* expressions and leaf movements under continuous conditions (Mizoguchi et al. 2002). A similar short period phenotype was previously shown for *cca1-1* null mutant (Green and Tobin 1999). We constructed *lhy cca1* double mutants and these plants lost free-running rhythms in *CCG* expressions after a few cycles in LL (Mizoguchi et al. 2002). Therefore LHY and CCA1 have partially redundant functions that are essential for the maintenance of circadian rhythmicity in LL. In addition, under light/dark cycles the *lhy cca1* double mutants show dramatic shifts earlier in the phase of expression of *CCGs* that normally peak in the evening. These *CCGs* include *GIGANTEA* (*GI*) and *TOC1/PRR1* that are associated with the generation of circadian rhythms, and are required for peak expression levels of *LHY* and *CCA1* (Mizoguchi et al. 2002).

Plants lacking *CCA1* and with *LHY* function strongly reduced (*cca1-1 lhy-R*) were generated using double-stranded RNA interference (RNAi) technology (Alabadí et al. 2002). These plants were unable to maintain sustained oscillations of *CCG* expressions and leaf movements in LL. These phenotypes are quite similar to those seen in *lhy cca1* double knockout mutants (Mizoguchi et al. 2002). The *cca1-1 lhy-R* disrupted circadian rhythms not only in LL but in DD using bioluminescence analysis of the *COLD*, *CIRCADIAN RHYTHM 2* (*CCR2*): *luc* reporter (Alabadí et al. 2002). Results obtained by two independent groups clearly show that both LHY and CCA1 are required for maintenance of circadian rhythms under continuous conditions.

Regulations between *LHY* and *CCA1* that peak at dawn, and *TOC1*/*PRR1* and *GI* that peak in the evening appear to be reciprocal. Loss-of-function mutation of *toc1* (*toc1-2*) reduced peak levels of *LHY* and *CCA1* expressions (Alabadí et al. 2001) (Figure 1B). The *gi* mutation had similar effect on the high amplitude of oscillation for both *LHY* and *CCA1* expressions (Mizoguchi et al. 2002). These results suggest that *TOC1*/*PRR1* and *GI* appear to function to control gene expression of *LHY* and *CCA1* in a positive regulatory loop (Figure 1C).

Over-expression of *TOC1/PRR1* dampened the free-running robust rhythms of *LHY* and *CCA1* (Matsushika et al. 2002). In particular, rhythmic expression of *GI* gene was completely suppressed in TOC1/PRR1-OX plants under LL. Based on results obtained from both gain-of-function mutants (lhy-1, *CCA1-OX* and *TOC1*/*PRR1-OX*) and loss-of-function mutants (*lhy*, *cca1*, *lhy cca1*, *toc1* and *gi*), a negative feedback model composed of these clock components in *Arabidopsis* was proposed (Figure 1C). In this model, *LHY* and *CCA1* which peak at dawn negatively regulate their own expression and also expressions of *TOC1/PRR1* and *GI*. On the other hand, *TOC1/PRR1* and *GI* genes peak in the evening and appear to function as positive regulators of *LHY* and *CCA1*.

Circadian clock genes and modified models on circadian system in *Arabidopsis*

More than 10 *Arabidopsis* genes that are highly associated with circadian clock functions have been isolated. Recently, a triple mutant of *prr9*, *prr7* and *prr5*, has been characterized and phenotypes of the triple mutant plants have been compared to those of double

mutants, *prr7 prr5*, *prr9 prr7* and *prr9 prr5* (Nakamichi et al. 2005) (Figure 1E). Also novel classes of proteins such as EARLY FLOWERING 4 (ELF4) (Doyle et al. 2002, Figure 1D) and PHYTOCLOCK1(PCL1)/LUX ARRHYTHMO (LUX) (Hazen et al. 2005; Onai and Ishiura 2005) (Figure 1F) as candidates for clock genes have been isolated in *Arabidopsis*. *GI* has been re-considered as a clock associated gene (Locke et al. 2005; Mizoguchi et al. 2005) (Figure 1D, G). A current view of a modified clock model composed of PRRs, PCL/LUX and GI as well as LHY, CCA1 and TOC1 appears to be quite complex (Figure 1H). We should modify it again when we will identify new players for the circadian clock in *Arabidopsis*.

Organ movement as one of the clock-controlled outputs in plants

Photoperiodic flowering, mRNA stability (Lidder et al. 2005), starch degradation and maltose metabolism (Lu et al. 2005), photosynthesis (Dodd et al. 2005a), Ca^{2+} level (Dodd et al. 2005b reviewed) and organ movements (Niinuma et al. 2005; Someya et al. 2006) have been shown to be controlled by the circadian clock. Among these, the regulation of flowering time has been well characterized and summarized in other recent reviews (Hayama and Coupland, 2003; Salome and McClung, 2004; Calvino et al. 2005; Mizoguchi et al. 2006). In this section, recent progress on the characterization of the organ movements such as leaf movements, stem elongation and circumnutation are summarized (Figure 2A).

Leaf movements in plants with or without pulvini

At least two types of leaf movements have been reported. One is the leaf movement caused with pulvinus, the organ on the bottom part of the leaf. For example, legumes have pulvini and the leaf movement system with pulvinus has been investigated in *Phaseolus coccineus*, *Phaseolus vulgaris*, *Samenea saman* and *Albizza lophanta* (Engelmann and Johnsson 1998). Changes of turgor volume in the upper and lower parts of pulvinus result in the leaf movements and the upper and lower parts are called flexor and extensor, respectively (Satter et al. 1974). The extensor and flexor cells swell and shrink, respectively, during closure, whereas the extensor and flexor cells shrink and swell, respectively, during opening (Coté 1995). Circadian rhythm of leaf movement, which usually opens during day and closes during night, is mediated by circadian volume changes in the extensor and flexor. Satter and Morse (1990) detached pulvini from the plants and removed their leaves. The pulvini still maintained to bend rhythmically under DD with a period of approximate 24 hours. Moreover, the circadian rhythm of volume change was also observed in the protoplasts isolated from the extensor and flexor cells, similar to in those of pulvinar cells *in situ* (Mayer and Fisher 1994). These results suggest that the cells in extensor and flexor have own endogenous circadian clocks.

Ion efflux such as K^+, H^+, Cl^-, malate, and other small organic anions affects the turgor changes (Satter et al. 1974; Kiyosawa et al. 1979). K^+ and main counter-ion to K^+, Cl^-, increase in the swelling cells and simultaneously decrease in the shrinking cells (Satter and Galston 1981; Satter et al. 1988). The K^+ permeability is controlled by light (Lowen and Satter 1989, Kim et al. 1992; Suh et al. 2000) and the circadian clock (Kim et al. 1993). The extensor and flexor cells in *S. samae* have four genes encoding K^+ channels (Moshelion et al. 2002a). Expression of the four K^+ channel genes show diurnal rhythms under light and dark cycles and three of them are controlled by circadian clock.

Second class of leaf movements is observed in plants such as *Arabidopsis* and these plants lack the pulvinus. Although most of plants do not have the special motor organs pulvini, they show leaf movements that are under the control of circadian rhythms (Engelmann et al. 1992). This change of leaf position is caused by differential growth in its petiole between upper and lower sides (Engelmann and Johnsson 1998) and is also reported to be related with cell division and elongation (Poethig and Sussex 1985). Aquaporin, a water channel, is suggested to be involved in the rhythmic leaf movement with puilvini (Moshelion et al. 2002b). Expression of *Samanea saman Aquaporin 2* (*SsAQP2*) is restricted to the movement-associated parts of the leaf and is cotrolled by circadian clock.

Leaf movements have been used as rhythmic markers in plants for chronobiology. Leaf movements are usually recorded from the side to obtain three circadian parameters, period, phase and amplitude. The amplitude is the vertical distance between the highest position and the lowest position of leaf during one cycle. The period is the duration for one up-and-down movement of a leaf. The phase is the time when the leaves are in the highest position. Plants with short and thick petioles, for example *Arabidopsis* plants with *erecta* (*er*) mutation, showed normal period and phase in the leaf movements, but lower amplitude compared to that of wild-type plants (Swarup et al. 1999). We have to interpret experimental results carefully, when we adapt the leaf movement as a rhythmic marker, because leaf movement is affected by morphology of plants used for the experiments.

Hypocotyl and inflorescence stem elongations

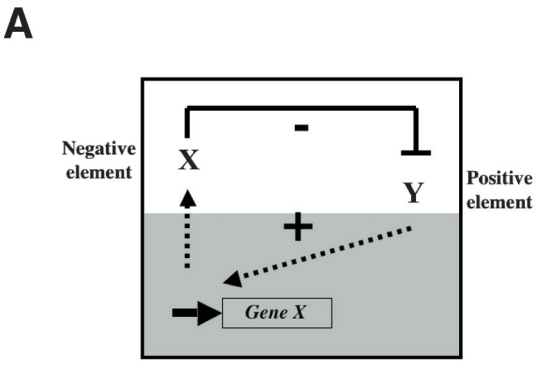
Elongation rates of hypocotyl and infloresence stem oscillate with a circadian rhythm (Dowson-Day et al. 1999; Jouve et al. 1998). Hypocotyl is usually composed of approximately 22 cells at longitude axis and its

elongation depends on cell elongation (Gendreau et al. 1997). By contrast, stem elongation is associated with both cell division in the shoot apical meristem and cell elongation.

Elongation is influenced by plant hormones auxin, gibberellins, brassinosteroids, cytokinins, abscisic acid and ethylene, positively and negatively (Chory et al. 1944, Creelman and Mullet 1997; Kende and Zeevaart 1997; McGrath and Ecker 1998). For example, Indole-3-acetic acid (IAA) plays an important role in circadian rhythm of stem elongation. Endogenous levels of IAA and IAA-aspartate that is biologically inactive until conjugate is cleaved off fluctuated with circadian period (Jouve et al. 1999). Decapitation suppressed elongation and circadian rhthmicity of the inflorescence node of *Arabidopsis*. Application of IAA but not IAA-aspartate after decapitation re-established the elongation and its circadian rhythmicity in the first inflorescence node of *Arabidopsis* (Jouve et al. 1999). Circadian rhythm of free IAA level may be resulted from the inter-conversion between IAA and its conjugates (Hangarter and Good 1981; Kleczkowski and Schell 1995). IAA metabolism is thought to be essential for circadian rhythmicity of elongation of the stem and hypocotyl in *Arabidopsis* (Jouve et al. 1999). Ethylene is also known to influence cell elongation. However, rhythmic elongation does not require the rhythmic production of ethylene, because hypocotyl elongation in ethylene mutants such as *ethylene-overproducer 2* (*eto2*), *ethylene-insensitive 4* (*ein4*) and *ethylene-resistant 1* (*etr1*), showed circadian rhythms (Thain et al. 2004).

In *Arabidopsis*, several mutants lacking circadian clock-associated genes have altered hypocotyl lengths (Schaffer et al. 1998; Wang and Tobin 1998; Somers et al. 2000; Nozue and Maloof 2006), raising a possibility that these hypocotyl phenotypes are caused by altered circadian rhythms. EARLY FLOWERING 3 (ELF3) plays a key role in gating light signals to the circadian clock (Hicks et al. 1996; McWatters et al. 2000; Covington et al. 2001; Liu et al. 2001). The *elf3* mutants are conditionally arrhythmic in various circadian outputs under LL (Zagotta et al. 1992; Hicks et al. 1996). The *elf3* mutant plants also show arrhythmic hypocotyl elongation (Dowson-Day et al. 1999). The arrhythmicity in *elf3* appears to be caused by abolishing the growth arrest during subjective dawn. Therefore, loss-of-function of *ELF3* makes longer hypocotyls than wild-type plants (Dowson-Day et al. 1999).

Figure 1. A brief history of the hypothetical models of circadian clock. (A) The initial model showing the core feedback loop central to circadian oscillators (modified from Dunlap 1999). This model provides a view of what some of the common elements might be in the logic underlying the assembly of circadian oscillators. In this model, the positive element Y acts as a transcriptional activator to induce clock gene (X) expression. The protein products (negative element) of the clock gene X, in turn, block the action of the positive element, thus indirectly repressing their own expression. (B) The initial negative feedback model of *Arabidopsis* clock composed of LHY, CCA1 and TOC1/PRR1 (modified from Alabadi et al. 2001). This model is mainly based on both LHY and CCA1 proteins bind to a region in the TOC1 promotor and genes expression patterns as following: Light activates *LHY* and *CCA1* expression at dawn. LHY and CCA1 activate *CAB* expression and possibly other genes with a phase similar to *CAB*. LHY and CCA1 simultaneously repress *TOC1* and other evening genes. The reduction of *LHY* and *CCA1* expression levels during the day allows *TOC1* transcript levels to rise and reach maximum levels toward the end of the day, when *LHY* and *CCA1* expression levels are lowest. TOC1 appears to participate in the positive regulation of *LHY* and *CCA1* expression, which reach maximum levels at dawn. (C) A model showing relations among LHY, CCA1, TOC1, GI and other evening phased genes to control the *Arabidopsis* clock (modified from Mizoguchi et al. 2002). *LHY* and *CCA1* transcription rises early in the day, which may reflect a direct response to light as well as circadian regulation. The model proposes that *LHY* and *CCA1* genes (red) act as negative elements that repress *GI* and *TOC1* (blue) expression and feedback to repress their own expression. As the expression of *LHY* and *CCA1* subsides, the levels of *GI* and *TOC1* mRNA rise and eventually peak in the evening. The *GI* and *TOC1* genes promote expression of *LHY* and *CCA1*. (D) The modified clock model showing the dual roles of GI in acting within the circadian clock to regulate period length and circadian phase, while also more directly promoting expression of a circadian clock output pathway that includes *CO* and *FT* and promotes flowering (modified from Mizoguchi et al. 2005). GI, TOC1, and ELF4 all promote *LHY* and *CCA1* expression. The flowering pathway is one of many output pathways controlled by the circadian clock, and three other pathways expressed at different times of the day are illustrated. Main loop is shown in pink. (E) The modified clock model based on multi-loops composed of not only LHY, CCA1 and TOC1/PRR1 but also PRR9, PRR7 and PRR5 (modified from Mizuno and Nakamichi 2005). This model supposes that the PRR9/PRR7/PRR5 circuitry interlocks with the main loop (pink line) in such a way that the PRR9/PRR7/PRR5 circuitry forms two other positive/negative loops (green and blue lines). The analysis of the *prr9 prr7* and the *prr7 prr5* double mutants under temperature cycle suggested that the multi-loop clock can also entrained by temperature cycle. TOC1 interacts with certain bHLH factors including PIF3/4 (PHYTOCHROME INTERACTING FACTOR 3 and 4) and four other homologous bHLH factors, PIL1/2/5/6 (PIF3-LIKE 1/2/5/6). Two PIFs in turn interact with phyB. (F) The modified multi-loop model including new players PCL/LUX and ELF4 (modified from Onai and Ishiura 2005). *pcl1* and *elf4* mutations caused arrhythmia in multiple circadian outputs (Onai and Ishiura 2005). *elf4* mutation disrupted rhythmic CCA1 expression. In this model, PCL1 is proposed to down-regulate mRNA levels of *GI*, *TOC1*, *ELF4* and *PCL1* itself. Unidentified factors X is proposed to activate *LHY* and *CCA1*, and Y and Z repress *TOC1* and *ELF4*, respectively, in this model. (G) The two-loops model based on computer-simulation (modified from Locke et al. 2005). This model proposes that each loop may receive input signals from light, and that each loop may include a hypothetical component (X and Y) that had not been explicitly identified (Locke et al. 2005). Analysis of the model predicted the properties of these components, including an acute light induction at dawn that is rapidly repressed by LHY and CCA1. TOC1 is activated by light indirectly via hypothetical gene Y. Y activates TOC1 transcription and both LHY and TOC1 repress Y transcription, forming a second feedback loop. Recently, GI has been proposed to be a strong candidate for X (Locke et al. 2006) and also the three-loops models with PRR9 and PRR7 in the 3rd loop have been reported (Locke et al. 2006, Zeilinger et al. 2006). (H) One of the current views of the *Arabidopsis* clock composed of multi-loops. LHY, CCA1, TOC1/PRR1, PRR9, PRR7, PRR5, PCL/LUX, GI and ELF4 are included in this model. Pink lines represent a core-loop. Asterisks (*) indicate that LHY/CCA1 proteins directly bind promoters of *TOC1* (Alabadi et al. 2001) and *LUX/PCL1* (Hazen et al. 2005).



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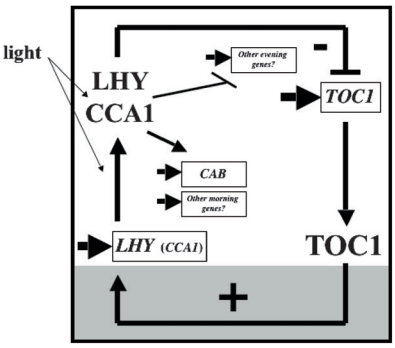

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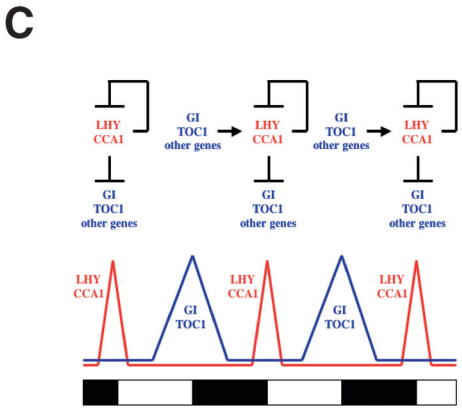

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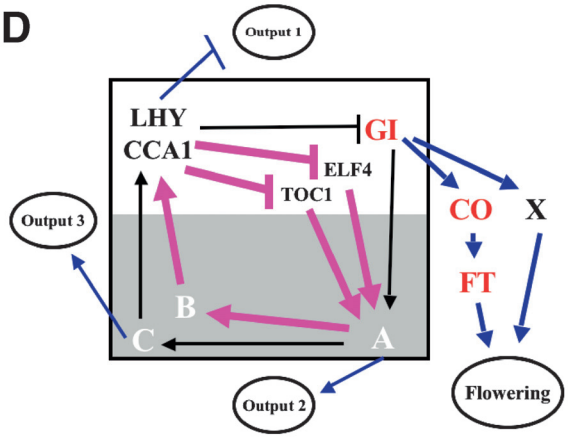

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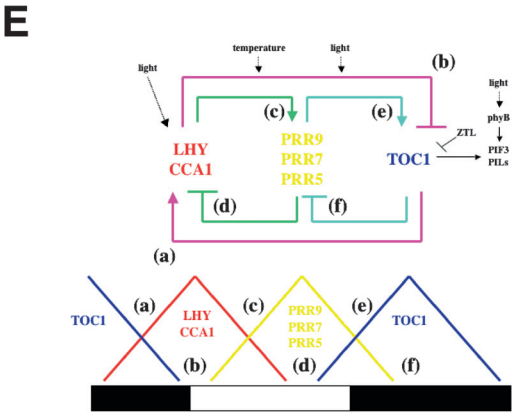

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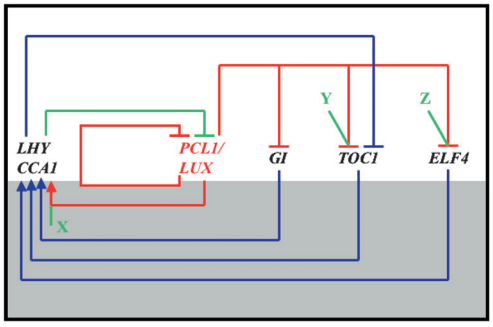

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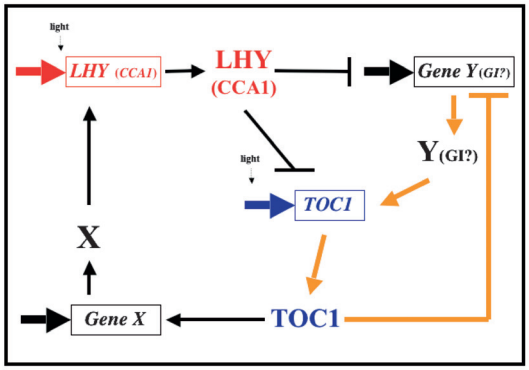

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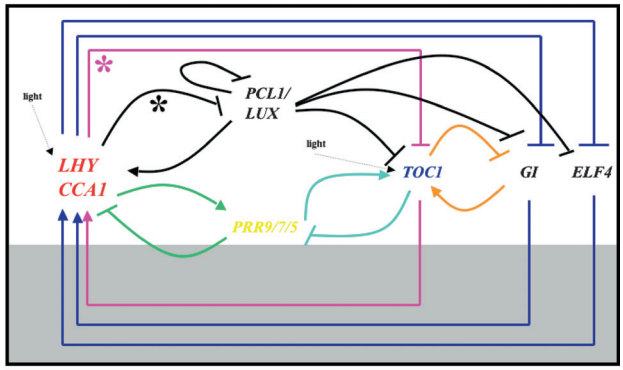

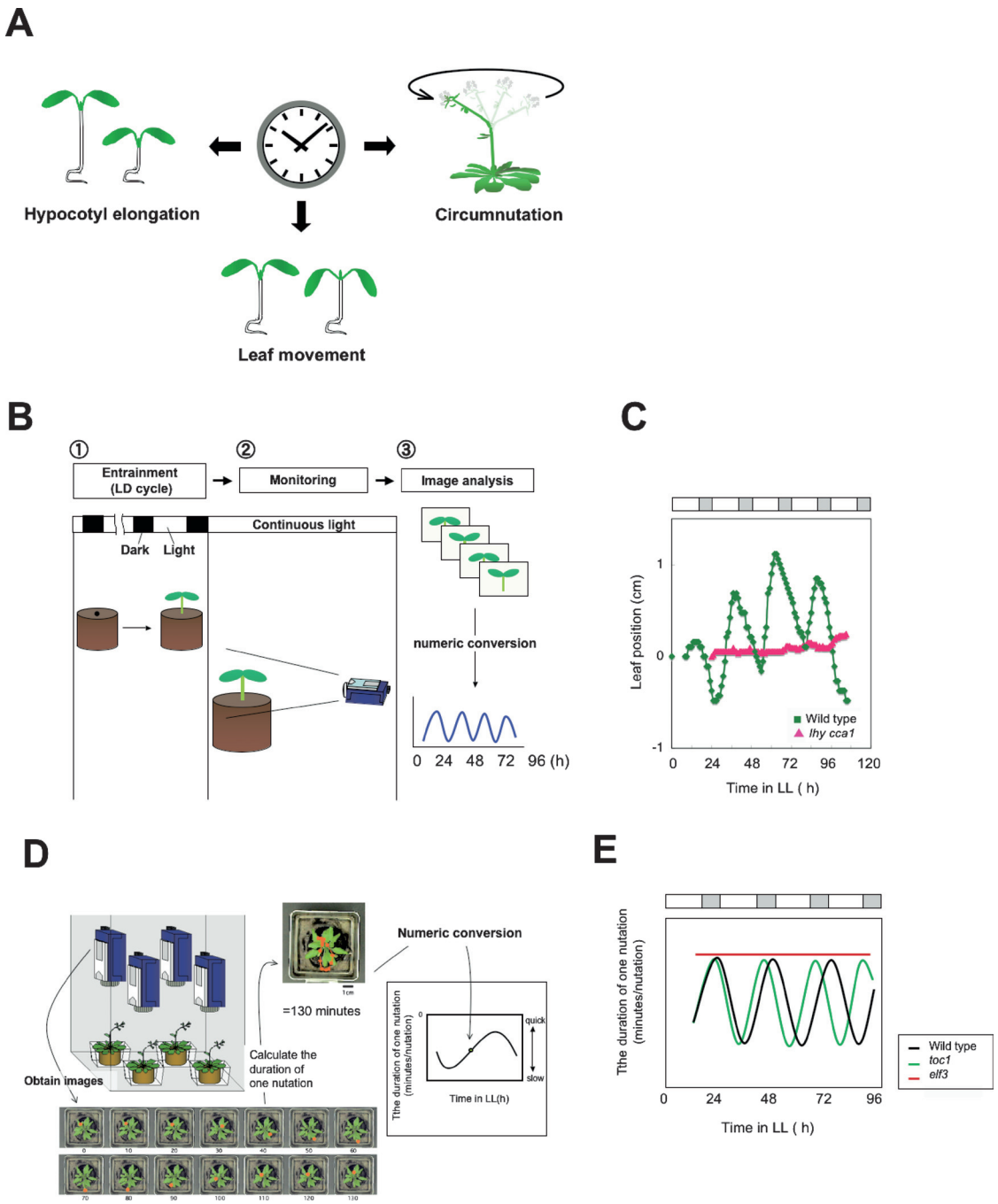

Figure 2. Organ movements controlled by circadian clock in plants. (A) Circadian clock controls various organ movements including hypocotyl elongation, leaf movement and circumnutation. (B) A procedure of monitoring circadian rhythms of leaf movements. At first, plants were grown under LD conditions and monitored their leaf movements after transfer to continuous condition. The movement of the leaf tips was monitored under LL, from above with a video camera (DCR-TRV30, Sony Corporation, Tokyo, Japan). Data were recorded at 5 min. intervals for several days. The data was obtained by numeric conversion with the movies. (C) Circadian rhythms of leaf movements in *Arabidopsis* wild-type (*Ler*) and a clock mutant, *lhy cca1*, under LL. The rhythms were monitored as described in (B). Open and gray bars along the horizontal axis represent subjective day and subjective night periods, respectively. These are measured in hours after the start of the LL treatment (Time in LL). (D) Acquisition of circumnutation data of *Arabidopsis*. The images were obtained at 5 min. intervals, and data at 10 min. intervals are shown. The coordinates of the inflorescence stem tip (●) were analyzed by the NIH image program (National Institutes of Health, Bethesda, MD, USA) (ref). The duration of one nutation was estimated at 130 min. in this case. (E) A schematic representation of arrhythmic and short period of circumnutation of influorescence stems in *elf3* and *toc1*, respectively. The modulation of circumnutation speed in *elf3* (red), *toc1* (green) and wild-type (black) plants were monitored in LL as shown in (D). Time in LL shows hours after transfer of the plants to LL. Open and dark boxes represent subjective day and subjective night, respectively.

Circumnutation

Circumnutations are revolving movements of plant elongating organs, which result from helical growth (Brown 1993) and partly reversible length variations occurring in the cells of the moving part of the organ (Carré et al. 1998). The circumnutating organ tip described pendulum-like, elliptical, or circular pattern (Melin 1975a; Melin 1975b). It usually takes 1 to 5 h to make one nutation (Lubkin 1994). Although it had been predicted, it was not proven clearly for a long time that the rotation movement showed circadian rhythm or not (Niinuma et al. 2005). This is mainly because the circumnutation is a complicated integration of physiological phenomena. In the cells of the bending zone of circumnutating organ, partly reversible length variations (Carré et al. 1998) are associated with differences of turgor and ion concentration (Lubkin 1994). The circumnutating organ has areas with rhythmically changing cell volumes that have been shown to move around the organ (Millet et al. 1988). Moreover, circumnutation is variable in character according to the plant species, the organ, or even on certain individual traits such as the age of the plant (Baillaud 1962; Darwin and Darwin 1880; Vanden Dressche 2000). Circumnutation is strongly affected by external stimuli such as temperature, light intensity, and mechanical stress (Anderson-Bernadas et al. 1997; Someya et al. 2006).

Characterization of clock mutants, *elf3* and *toc1* demonstrated that circumnutation is one of the outputs controlled by circadian clock (Niinuma et al. 2005). The modulation of circumnutation speed in inflorescence stem of wild-type is rhythmic with the phases of highest and lowest speed at subjective dawn and dusk, respectively (Figure 2D, E). The *toc1* mutation shortened the period of the circadian rhythm of the downstream events (Somers et al. 1998). In circumnutation, the *toc1-1* mutant has a shorter period length compared to that of wild-type and elf3 mutation seems to show constant nutation speed and abolish the rhythm of modulation of circumnutation speed, consistent with previous reports (Figure 2E, Niinuma et al. 2005).

Circumnutation is observed in elongating organ. Elongation rate of stem and hypocotyls is under the control of circadian rhythms (Jouve et al. 1998; Dowson-Day et al. 1999), as described in section "Hypocotyl and inflorescence stem elongations". Highest speed of circumnutation and growth arrest occurred at subjective dusk and lowest speed of circumnutation and maximal elongation rate at subjective dawn, respectively (Niinuma et al. 2005). These results suggest that there may be some relationships between speed of circumnutation and elongation rate. Alternatively, speed of circumnutation and elongation rate may be two independent outputs controlled by the circadian clock. In *elf3* mutant, constant and relatively high speed of cicumnutation and elongation rate persisted in LL (Niinuma et al. 2005). These results support the latter idea that circumnutation and elongation are controlled independently by the clock.

Tomato as a model system for chronobiology

Advantages to use the *Solanum lycopersicum* cv. Micro-Tom are often discussed in this issue. We think that the Micro-Tom is a good model plant for comparative genetics on connection between circadian clock and the photoperiodic flowering (Mizoguchi et al. 2007, this issue). Photoperiodic flowering responses of the long day (LD) (*Arabidopsis*), short day (SD) (rice) and day-neutral (DN) plants (tomato) are shown in Figure 3E. Tight connection between circadian clock and the photoperiodic flowering is shown in a facultative LD plant, *Arabidopsis* (Mizoguchi et al. 2006; Mizoguchi et al. 2007, this issue). Several processes, however, have been reported to be controlled by circadian clock in tomatoes (Samach et al. 2007, this issue). For examples, circadian clock controls leaf movement of plants. Although *Arabidopsis* shows a robust oscillation of leaf movement (Figure 2C), *Arabidopsis* is a small plant and therefore to follow the rhythm of this plant species is rather difficult. Seedlings of the Micro-Tom are much larger than those of *Arabidopsis* and to follow and monitor the rhythm of Micro-Tom are easier than those of *Arabidopsis* (Figure 3A).

We observed and analyzed a circadian rhythm of leaf movement of the Micro-Tom by using digital video (Figure 3A, B). LHY and GI play key roles in the control of the photoperiodic flowering and circadian rhythms in *Arabidopsis* (Mizoguchi et al. 2002; Mizoguchi et al. 2005; Locke et al. 2006; Mizoguchi et al. 2006). The Micro-Tom has genes related to the *Arabidopsis LHY* and *GI* and the expression of them showed similar daily and circadian rhythms to those of *Arabidopsis* (Figure 3C, D) This suggests that these genes may also be involved in the regulation of circadian rhythms in tomato. Although genes related to the key factors of the photoperiodic flowering in *Arabidopsis* such as *LHY*, *CCA1*, *GI*, *CO* and *FT* (*FLOWERING LOCUS T*) have been isolated in some of LD and SD plants, molecular mechanisms underlying the DN-type of the photoperiodic flowering in tomatoes have been remained unknown (Mizoguchi et al. 2007, this issue). Recently, it has been shown that a *FT* homolog of tomato has a similar important role in flowering to that of *Arabidopsis FT* (Lifschitz and Eshed 2006). Do the *GI* and *CO* homologs affect the expression of *FT* homolog in tomato (Figure 3F)? If not, what is a main difference in the *GI-CO-FT* pathway between the DN plant, tomato, and the LD plant, *Arabidopsis*? How is expression of the *FT* homolog gene regulated? To

isolate Micro-Tom mutants with delayed or accelerated flowering phenotypes and to identify genes responsible for these phenotypes will be crucial steps for the comparison of mechanisms underlying three classes of the photoperiodic flowering responses in plants. The Micro-Tom mutant populations generated by EMS-mutagenesis (Watanabe et al. 2007, this issue) and γ-ray irradiation (Matsukura et al. 2007, this issue) will be useful to screen mutants with altered flowering time, photoperiodic flowering response, and circadian rhythms.

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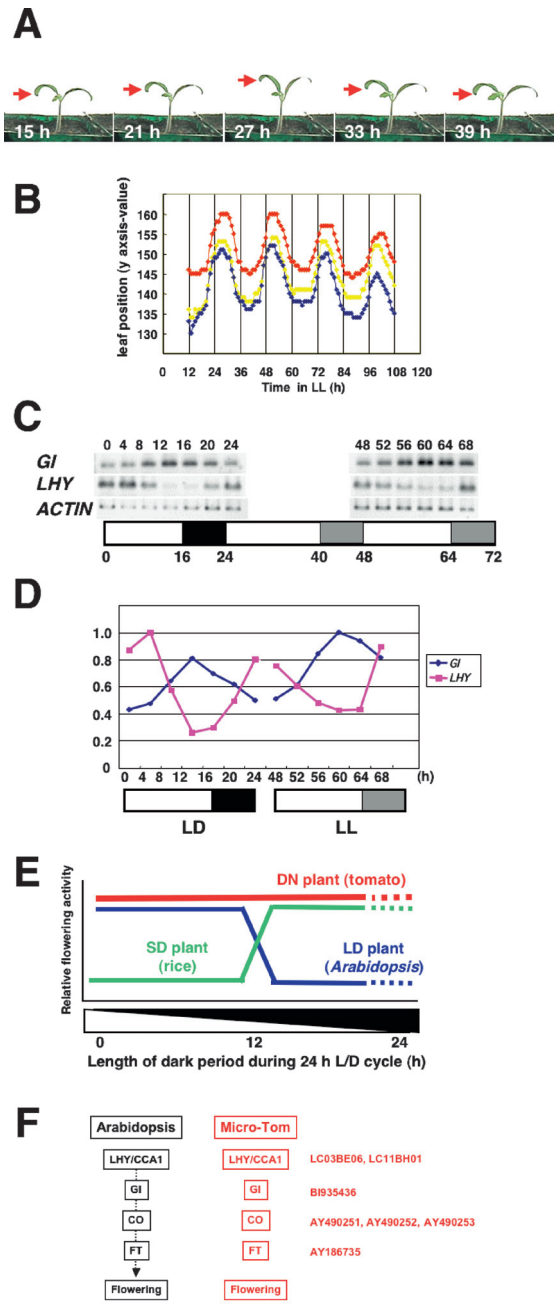

Figure 3. Circadian rhythms of Micro-Tom. (A) Circadian rhythms of leaf angle in sequence of images of a wild-type seedling (Micro-Tom) under LL. Time in LL is shown. Positions of the leaf tip (red arrows) were monitored every 6 hours as shown in Figure 2(B). (B) Circadian rhythms of leaf angles of three tomato seedlings under LL. The position of the leaf tip and hypocotyls apex was derived as described in Figure 2. Leaf movements were calculated as the vertical distance between the hypocotyl apex and the leaf tips. (C) The expression of the Micro-Tom *LHY* (LC03BE06/LC11BH01) and *GI* (B1935436) homologs and actin (*ACT*) genes was analyzed by RT-PCR in Micro-Tom plants grown in LD (16 h light/8 h dark) and LL. (D) Results are presented as a proportion of the highest value after standardization with respect to *ACT* levels. Open and filled bars along the horizontal axis under LD represent light and dark periods, respectively. These are measured in hours from dawn (zeitgeber time; ZT). Open and gray bars along the horizontal axis under LL represent subjective day and subjective night periods, respectively. (E) A schematic model showing the day-length responses of *Arabidopsis* (LD plant), rice (SD plant) and tomato (Micro-Tom; DN plant) are represented in blue, green and red, respectively. (F) Tomato homologs of *LHY/CCA1*, *GI*, *CO* and *FT* that play key roles in the photoperiodic flowering in *Arabidopsis* (ref). Accession numbers of the tomato genes are shown.

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