The Circadian Clock. A Plant's Best Friend in a Spinning World[1]

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The circadian clock is an intricate, even delicate, regulator of plant physiology, yet at least one of the selective pressures that drove its evolution is brutally simple. Plants must be exposed to sunlight for photosynthesis, and sunlight is not available continuously. Therefore, plants are stuck with a day/night cycle of light and temperature, with the possible exceptions of buried, germinating seedlings and polar inhabitants. Each day's solar energy propels their metabolism into a spate of carbon fixation, which must end at nightfall. Locomotion would not alleviate the problem. Plants, like other eukaryotes and some prokaryotes, have adapted to the day/night cycle by evolving the circadian system, which drives matching rhythms in very many aspects of metabolism, physiology, and behavior (Harmer et al., 2001; Young and Kay, 2001).

The hallmarks of circadian regulation are very similar in all organisms, most obviously the persistence of biological rhythms even under constant environmental conditions. The rhythms are all reset by light and/or temperature signals in a characteristic fashion that synchronizes the clock with the environment. This process of "entrainment" is crucial to ensure that rhythmic processes occur at an appropriate time of day (circadian phase), particularly because the period of circadian clocks in the absence of entraining signals often differs from 24 h. Plant circadian rhythms in nature are always entrained to 24 h by the day/night cycle; the non-24 h period is expressed only in exceptional circumstances (or in the laboratory). Therefore, the circadian clock contributes to plant physiology by regulating the phase of entrained rhythms, and natural selection acts primarily on phase, not on period.

The period of the clock that we measure in constant conditions will nonetheless affect the phase of entrainment, all else being equal, so a rhythm with a longer period under constant conditions will have a later phase under entrainment. This relationship can be used experimentally to alter the phase of entrainment (see below in the discussion of photoperiodic regulation; Yanovsky and Kay, 2002). The converse relationship does not necessarily hold: A rhythm with an early phase can arise without a change in period (for example, in the phyB mutant; Hall et al., 2002).

THE RHYTHM SECTION. WHICH PLANT PROCESSES ARE CLOCK-REGULATED?

Microarray experiments indicate that at least 6% of Arabidopsis genes are rhythmically expressed, with expression peaks at all phases throughout the day and night (Harmer et al., 2000; Schaffer et al., 2001). This circadian gene expression produces the rhythms that pervade plant physiology, some of which are obvious (such as the "sleep movements" of legume leaves, noted since classical times), others less so. In several cases, genes that affect a common pathway or process are expressed at the same phase, suggesting that the phase might be important in the function of that process. Many genes encoding enzymes of phenylpropanoid biosynthesis have peak RNA levels before dawn, perhaps because it is advantageous to accumulate photoprotective flavonoids before the sun rises (Harmer et al., 2000). A large proportion (68%) of the rhythmically regulated genes also directly respond to environmental stress (Kreps et al., 2002), so rhythmic expression of these genes in anticipation of predictable environmental changes might prepare the plant to withstand a stress (or make best use of a resource). Thus, circadian regulation would complement the plant's subsequent response to the stress. Recent experimental evidence shows a fitness detriment in some Arabidopsis clock mutants (Green et al., 2002), and more is likely to follow from natural variants and further physiological studies. Photoperiodism is a special case in which a circadian rhythm is combined with light signaling. The photoperiod sensor allows plants to respond to the annual cycle of day length by making flowers, tubers, or frost-tolerant buds at appropriate seasons. The selective advantages of correct seasonality are very clear; recent reports have significantly enhanced our understanding of this mechanism (see below).

The circadian clock is itself a target of regulation by environmental response pathways, so it stands at the interface between external and endogenous regula-

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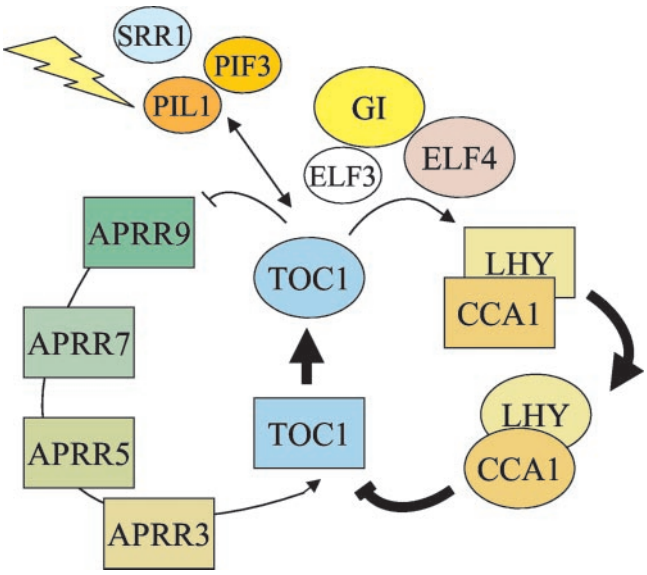

Figure 1. A current view of the circadian system in Arabidopsis. Proteins are encircled, proteins that are unlikely to function in the core timing loop have smaller symbols, and genes are shown in rectangles. *CCA1* and *LHY* are expressed in the early day; their respective proteins repress *TOC1* transcription (bold line). CCA1/LHY proteins decay at the end of the day, derepressing *TOC1* transcription. The accumulation of TOC1, ELF3, ELF4, and GI proteins during the night activates the transcription of *CCA1/LHY* the next morning by an unknown mechanism (light arrow). Interactions between TOC1 and PIL1/PIF3 proteins may occur at the *CCA1* and *LHY* promoters, enhancing their expression in a light-dependent fashion. *APRR9* transcription is light activated (flash); it is the first gene to be expressed in the *APRR/TOC1* quintet, followed by *APRR7*, *APRR5*, *APRR3*, and *TOC1*. *TOC1* can repress *APRR9* RNA expression; otherwise, it is not very clear how the protein quintet interact. Components that have not been located relative to the loops shown here are omitted (e.g. TEJ and the ZTL family).

tors. Light-signaling pathways from both phytochromes (phys) and cryptochromes (crys) regulate clock components to achieve entrainment, for example (for review, see Fankhauser and Staiger, 2002). There are also reports of hormonal effects upon circadian timing from several plant species. Perhaps surprisingly, circadian timing is not coordinated among cells, so it is possible to set circadian rhythms of gene expression to several different phases in different parts of a single plant or even of a single leaf (Thain et al., 2000). Moreover, the circadian system appears to differ slightly among cell types. The evidence for this is that circadian rhythms in different cell types can have different circadian periods in wild-type plants tested under identical conditions. A recent example was provided by the expression rhythm of a *LUC* reporter that is expressed in the leaf epidermis compared with one that is expressed in the mesophyll (Hall et al., 2002, and refs. therein). A possible advantage is that the rhythms controlled by one clock can alter their phase relative to the rhythms controlled by another clock. The biochemical difference in the clocks between cell types is unknown. It might be relatively minor because all the circadian rhythms tested in Arabidopsis depend upon a common set of genes. These "clock genes" or "clock-associated genes" function within, or close to, each cell's circadian clock: Their products produce and maintain the oscillation that drives all other circadian rhythms.

PORTRAIT OF THE ARABIDOPSIS CIRCADIAN CLOCK

The known clock mechanisms in all organisms include a gene circuit with negative feedback, involving 24-h rhythms in the levels of positively and negatively acting transcriptional regulators, in all organisms (Harmer et al., 2001; Young and Kay, 2001). These rhythmic feedback loops probably have arisen by convergent evolution because the sequences of the proteins involved share little or no homology across taxa, despite the overt similarities among the circadian rhythms that they control. The genes that we now review represent the plant kingdom's solution to the problems posed by the rhythmic environment (Staiger, 2002; Hayama and Coupland, 2003); we restrict our discussion to work on Arabidopsis, the species in which these genes were first identified (Fig. 1). The first consistent model of the Arabidopsis circadian oscillator (Alabadi et al., 2001) suggested that it is comprised of three main players, the genes encoding Myb-related transcription factors *CCA1* (*CIRCADIAN CLOCK ASSOCIATED 1*) and *LHY* (*LATE ELONGATED HYPOCOTYL*) and a pseudoresponse regulator *TOC1*

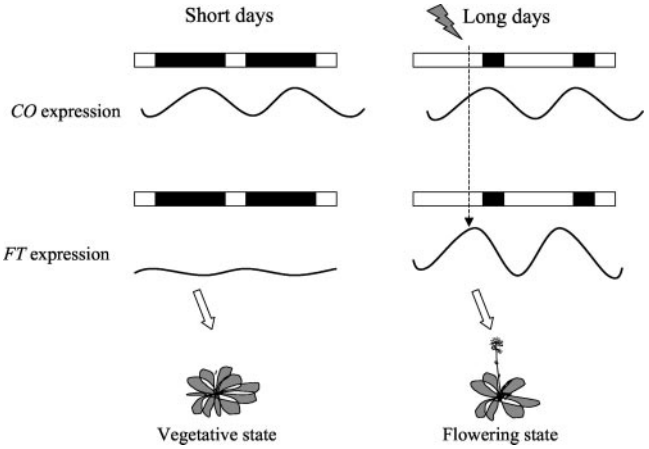

Figure 2. Photoperiodic control of flowering. The detection of day length through the circadian system is dependent on light signals perceived through the photoreceptors cry2 and phyA, integrated at the level of *CO* expression. When and only when *CO* is expressed at high levels in the light phase, expression of the *FT* gene is induced. *FT* activates downstream flowering genes, committing the plant to the flowering state. Dark bars indicate the dark hours and yellow bars indicate the light hours of the light/dark cycle. The flash indicates light perceived, and the dotted arrow indicates the light-activated *CO* signal that activates *FT* expression.

(*TIMING OF CAB EXPRESSION 1*; also known as Arabidopsis *PRR1* [*PSEUDORESPONSE REGULATOR 1*]). Strong loss-of-function alleles of *TOC1*, a double mutant of *LHY* and *CCA1*, or constitutive overexpression of any of these genes cause arrhythmia under prolonged constant light and darkness (Schaffer et al., 1998; Wang and Tobin, 1998; Alabadi et al., 2002; Makino et al., 2002; Mizoguchi et al., 2002; Mas et al., 2003). It is too early to speculate on the origin of the rhythms that remain in these plants under light/dark cycles and in some cases persist transiently in constant conditions.

There is good evidence that CCA1/LHY proteins act redundantly in the late night and early day, binding to an evening element (EE; AAATATCT) in the promoter of *TOC1* and repressing its expression (Harmer et al., 2000; Alabadi et al., 2001, 2002; Mizoguchi et al., 2002). When CCA1/LHY levels fall late in the day, the TOC1 protein is proposed to activate the transcription of *CCA1/LHY*, thus forming the outline of a transcriptional feedback loop (Alabadi et al., 2001). The accumulation pattern of TOC1 protein in plants has not been reported yet. This activation is probably indirect because it requires at least three other genes that are co-expressed with *TOC1* in the evening: *ELF3* (*EARLY FLOWERING 3*; Schaffer et al., 1998), *GI* (*GIGANTEA*; Fowler et al., 1999), and *ELF4* (*EARLY-FLOWERING 4*; see below, Doyle et al., 2002). The biochemical functions of the cognate proteins are unclear. Paradoxically, *CCA1* expression is somewhat reduced rather than activated by overexpression of *TOC1* (Makino et al., 2002; Mas et al., 2003). Thus, the current portrait of the Arabidopsis clock amounts to a silhouette; we discern the familiar negative feedback loop but, as in other species, evolution has endowed the Arabidopsis clock with more than the minimum mathematical requirement for an oscillation.

LIGHT INPUT PATHWAYS IN CHIAROSCURO

The light input to the oscillator is provided by the photoreceptor families, crys and phys, which mediate signaling by high-intensity blue light and low-intensity blue light or red light, respectively. This area of the plant circadian system has been reviewed recently (Devlin, 2002; Fankhauser and Staiger, 2002). One aspect of light input deserves further mention, namely the gating mechanism or "zeitnehmer" (time bringer), by which the clock rhythmically regulates its input signals. In the *elf3* mutant, unregulated light input stops the clock 8 to 10 h after dawn; therefore, timing breaks down in long days (McWatters et al., 2000; Covington et al., 2001). *ELF3* is, therefore, an essential part of the plant circadian system, though it may not be central to rhythm generation. ELF3 protein can bind to PHYB; thus, it may inhibit light signaling in the evening (Covington et al., 2001). This zeitnehmer mechanism is analogous to the rhythm of light responsiveness that creates the photoperiod sensor (see below).

In contrast to the photoreceptors, their signaling pathway(s) to the clock components are not yet well defined. In etiolated seedlings, PIF3 (PHYTOCHROME INTERACTING FACTOR 3) binding to a G-box sequence (CACGTG) in the *CCA1* promoter might mediate *CCA1* activation by light, when activated phyA and/or phyB bind to PIF3 at the promoter (for review, see Quail, 2002). In green tissues, Carré and coworkers recently showed that light regulates LHY protein levels rather than RNA abundance, which can account for driven rhythms of *LHY* overexpressors in light/dark cycles (Kim et al., 2003). However, the weak rhythmicity that remains in *lhy; cca1* double mutant plants is still entrained by light/dark cycles, so it is unclear whether these genes are essential for entrainment of the clock (Alabadi et al., 2002; Mizoguchi et al., 2002).

Other potential mechanisms of light input have been suggested. Previous studies of the *toc1-1* allele revealed no light-dependent effects of *TOC1*; however, *toc1-1* is now classed as a weak allele because it maintains circadian rhythms under all lighting conditions, albeit with a 21-h period. The more recent work used RNAi transgenes and a stronger allele, *toc1-2*, that severely reduce *TOC1* RNA levels and result in arrhythmia specifically under red light (Mas et al., 2003). The strong alleles also reduce the responsiveness of hypocotyl elongation and *CCA1* gene expression to red light, suggesting that *TOC1* might be involved in phy signaling to targets other than the clock. The only hint of a biochemical function for TOC1 protein is its potential to bind to PIF3 and a related protein, PIL1 (PIF3-LIKE 1; Makino et al., 2002), but this at least provides a potential link to photoreceptor signaling. Under blue or white light, the severe *toc1* mutants remain rhythmic with a short period, so signaling from phy requires more *TOC1* than cry signaling, though both are affected in the mutants (Mas et al., 2003).

An intriguing gene family is made up of *ZTL* (*ZEITLUPE*), *FKF* (*FLAVIN-BINDING KELCH REPEAT F-BOX*), and *LKP2* (*LOV DOMAIN KELCH PROTEIN 2*). Mutations or misexpression of each gene can affect circadian rhythms, but most interestingly, the *ztl* mutant's effect on circadian period varies depending on the fluence rate of ambient light (Devlin, 2002). Their protein sequences share a PER-ARNT-SIM domain, multiple kelch domains, and an F box. Similar PER-ARNT-SIM domains of other proteins bind a flavin chromophore, as in the plant phototropin photoreceptors (Briggs and Christie, 2002), so a similar cofactor might confer light dependence on ZTL function. Kelch domains are typically involved in protein interactions, and other F-box proteins recruit target proteins to E3 ubiquitination complexes, marking the target proteins for degradation. This suggests a function in the light-dependent ubiquitination of a clock

component(s), which might provide light input independently of *LHY/CCA1* (for review, see Fankhauser and Staiger, 2002).

NEWCOMERS AMONG THE CLOCK-ASSOCIATED GENES

System identification (finding all the relevant components) is an important step in understanding biological regulation. However, finding the primary function of a gene starting from a mutant phenotype is never trivial. The intimate connections between light signaling and circadian regulation often result in overlapping phenotypes: The *srr1* mutant (sensitivity to red light reduced) is a recent example that shows defects both in phyB signaling and in circadian rhythms (Staiger et al., 2003). The challenge is exacerbated by gene duplications: Additional members of the CCA1/LHY protein family have been identified, for example (for review, see Carre and Kim, 2002), so we eagerly await their functional characterization.

ELF4 IS NECESSARY FOR CIRCADIAN RHYTHMS AND PHOTOPERIODISM

The *elf4* mutant was identified by its early flowering in short photoperiods (Doyle et al., 2002). It carries a T-DNA insertion in the *ELF4* gene, which encodes a predicted protein of 111 amino acids without identifiable protein signatures. Its small size alone might lead to speculation that it functions as a post-translational protein modifier or a secreted signal. The *ELF4* transcript shows robust, circadian expression in wild-type plants, with a peak in the evening. The *elf4* mutation affects the rhythms of the circadian-regulated genes *CAB* (*CHLOROPHYLL A/B BINDING PROTEIN*, also known as *LHCB*) and *CCR2* (*COLD-CIRCADIAN RHYTHM-RNA BINDING 2*). Their expression in a population of seedlings (studied using *LUC* [luciferase] reporter gene fusions) rapidly became arrhythmic under constant conditions, but individual seedlings were transiently rhythmic with widely varying periods. Circadian leaf movements were similarly affected, suggesting a role for ELF4 in the accuracy and persistence of circadian rhythms. Overall, *elf4*'s rhythmic defects resembled those of *lhy;cca1* double mutant plants; consistent with this interpretation, the *elf4* mutation drastically reduces expression of *CCA1* (Doyle et al., 2002). A striking feature of *elf4* plants is their very early flowering in short photoperiods, whereas under long-day conditions, they flowered at about the same time as wild type. A high expression of *CO* (*CONSTANS*) in the mutant during short days is the likely cause of the early flowering seen under these conditions. *ELF4* appears to be closely linked with the circadian oscillator; whether its circadian defect is the sole cause of its early flowering (see below) remains to be investigated.

TEJ IMPLICATES PROTEIN POLY(ADP-RIBOSYL)ATION IN MAINTAINING PERIOD LENGTH

The *tej* mutant (Panda et al., 2002) was identified in the screen for mutants with altered rhythms of *CAB:LUC* expression, which also identified the first *toc1* and *ztl* mutants. *tej* mutant plants showed a light-independent period lengthening of 2 h in *CAB*, *CCA1*, and *CCR2* expression; leaf movement rhythms were similarly affected. Flowering time was slightly earlier than wild type under both long and short days. The recessive mutation results from an amino acid substitution in the TEJ protein, which functions as a poly (ADP-Rib) glycohydrolase. Poly(ADP-ribosyl)ation is a posttranslational protein modification, which is conferred by poly (ADP-Rib) polymerase and removed by poly (ADP-Rib) glycohydrolase; in other species, it has been implicated in DNA repair, DNA damage signaling, and the regulation of transcription and proteasome function (refs. in Panda et al., 2002). Applying an inhibitor of poly (ADP-Rib) polymerase, 3-aminobenzamide, rescued the phenotype of *tej*. This strongly suggests that the mutant phenotypes were due to excessive poly(ADP-ribosyl)ation of a clock-related protein, the identity of which is unknown (Panda et al., 2002).

THE *APRR/TOC1* QUINTET. IS THEIR DAILY ROUND IMPORTANT FOR CIRCADIAN TIMING?

TOC1 shares sequence homology with a set of pseudoresponse regulator genes, *APRR9*, *7*, *5*, and *3*, which have been termed the "APRR quintet" (Matsushika et al., 2000; Strayer et al., 2000; Suzuki et al., 2001). *APRR9*, *APRR7*, *APRR5*, and *APRR3* and *TOC1* (*APRR1*) are expressed sequentially every 2 to 3 h, starting soon after dawn with the expression of *APRR9*, until the evening when *TOC1* is expressed. *APRR9* expression is also phy activated (Matsushika et al., 2000). The interactions among these proteins are unknown, but recent studies of transgenic plants overexpressing *APRR9*, *APRR5*, and *TOC1* show that their expression is interrelated. *TOC1* overexpression, for example, abolishes *APRR9* expression under constant light and damps rhythmic *APRR7-APRR3* expression to low levels (Makino et al., 2002). Plants that overexpress *APRR9* or *APRR5* affect flowering time and show a red light-dependent short-hypocotyl phenotype (also found for *TOC1* overexpressors). *APRR9* overexpression confers an early phase and/or a short period on many rhythmic genes under constant white light (Matsushika et al., 2002). In *APRR5* overexpressors, *APRR9* and *APRR7* gene expression was reduced toward the trough level, whereas expression of *APRR3* and *TOC1* was in-

creased toward the peak level (Sato et al., 2002). This is consistent with a cascade mechanism, in which regulation proceeds along the quintet from *APRR9* to *TOC1*. Studies of null mutants in these genes are now required to understand their function in the circadian system. The quintet might provide a flexible output mechanism that can regulate a gene at any desired phase from dawn to dusk. They might thus participate in *TOC1* activation toward the end of the day, counteracting its repression by CCA1/LHY.

PHOTOPERIODIC REGULATION OF FLOWERING TIME

Principle

The photoperiodic regulation of seasonal events such as flowering requires a measurement of the duration of daylight (or nighttime darkness because long summer days are necessarily followed by short nights). Most Arabidopsis strains are "long-day" plants, which respond to the photoperiod sensor by flowering quickly under long days (after producing six to eight leaves under 16-h-light/8-h-dark cycles) and much more slowly under short days (producing approximately 30 leaves under 8-h-light/16-h-dark cycles). The sensor must involve at least a timing function to measure duration and one or more light sensors to determine when the day or night begins and ends. However, the way in which these elements are combined cannot be determined a priori. A series of elegant physiological studies have shown that the sensor is located in the leaves, the timer involves a circadian clock, but the relevant photoreceptors vary among species (for review, see Lumsden and Millar, 1998). Red-/far-red-sensitive phys are often involved (they were first discovered in studies of photoperiodism), but blue light, perceived by crys, is important in Arabidopsis and its relatives (for example, see Mockler et al., 1999). Even this information does not uniquely identify the mechanism because it does not specify what the photoreceptors control.

Current evidence strongly favors the "external coincidence" model, in which Erwin Bünning proposed that the photoreceptors generate a flowering signal, possibly the same signal that was later shown to move to the shoot apex to initiate floral development. He proposed that the photoreceptor function was rhythmic; in other words, the signal could be generated only at a specific circadian phase, so light at other phases would have no effect on flowering (see Fig. 2; Bünning, 1936). The logic of this mechanism is relatively simple. If the light-signaling phase occurs at the end of the day, for example, then short-day responses are triggered if the daylight has already ended such that this phase passes in darkness; long-day responses are triggered if it passes in light. The circadian rhythm must regulate the signaling pathway from photoreceptors to flowering signal, restricting its function to the correct phase relative to the day/night cycle. The photoreceptors have two functions because they are required to entrain the circadian clock (which both phys and crys do in Arabidopsis) and to generate the flowering signal. In the alternative "internal coincidence" model, Colin Pittendrigh pointed out that the photoreceptors might not generate a flowering signal directly, but rather that they might entrain two different circadian clocks to different phases, depending on the photoperiod (Pittendrigh, 1972). In some photoperiods, overlap between the two clocks would generate the flowering signal. The differences between the models have been reviewed elsewhere (Samach and Coupland, 2000). We note that since Pittendrigh's work, the circadian rhythms of rodents have been discussed in terms of two circadian clocks with different entrainment: a "morning" clock that entrains to dawn and an "evening" clock that entrains to dusk (for recent update, see Daan et al., 2001).

Practice

Recent publications strongly support an external coincidence mechanism in Arabidopsis (Suarez-Lopez et al., 2001; Blazquez et al., 2002; Roden et al., 2002; Yanovsky and Kay, 2002), which has also been reviewed elsewhere (Davis, 2002; Hayama and Coupland, 2003; Yanovsky and Kay, 2003). The key gene in this model is *CO* (*CONSTANS*). Studies of the *co* mutant and *CO* overexpression lines had already shown that *CO* was necessary and sufficient for rapid flowering in long days, so it was clearly an important component of the mechanism (Suarez-Lopez et al., 2001). The recent work first showed that the circadian clock generates a rhythm in the level of *CO* RNA (see Fig. 2), which starts to rise from about 8 h after dawn to reach a broad peak from about 14 to 20 h, before falling back in the late night to its minimum in the early day (Suarez-Lopez et al., 2001). This pattern alone is reminiscent of the "light sensitivity" rhythm described above. Furthermore, the CO protein is likely to be unstable, such that its abundance could follow the pattern of *CO* RNA (Suarez-Lopez et al., 2001). Second, the levels of *CO* expression consistently predict the flowering time of a range of mutants that alter *CO* regulation (Suarez-Lopez et al., 2001; Doyle et al., 2002). Third, the genes *FT* (*FLOWERING LOCUS T*) and *AGL20* (*AGAMOUS-LIKE 20*; also known as *SOC1* [*SUPRESSOR OF CONSTANS 1*]) are activated by *CO* and in turn activate the developmental regulators *LEAFY* and *APETALA1* at the shoot apex (for review, see Hayama and Coupland, 2003). Fourth, a signaling pathway or pathways from the photoreceptors cry2 and phyA requires *CO* to activate *FT* and *AGL20*, and phyA is most effective at the end of the day when *CO* is expressed (Johnson et al., 1994; Mockler et al., 1999; Yanovsky and Kay, 2002). However, the photoreceptors have little effect on the phase or level of *CO* RNA expression, so they

presumably affect CO protein accumulation or function (Yanovsky and Kay, 2002). Last, correct entrainment of a circadian clock is essential because altering the phase of circadian rhythms relative to the day/night cycle alters flowering time. This is true when phase is altered either by a short-period clock mutation (*toc1-1*) grown in normal, 24-h light/dark cycles or by growing the wild type in light/dark cycles longer or shorter than 24 h (Roden et al., 2002; Yanovsky and Kay, 2002). For both treatments, the effect on flowering time can be predicted from the observed coincidence of *CO* RNA expression with light, but both treatments would affect many circadian rhythms in addition to *CO* expression.

This tale can be told as a prime example of hypothesis-driven research, though 65 years of technical development were required to identify the components of the external coincidence model. The simplest interpretation of the data is as follows: The rhythm of *CO* expression would create a light-sensitive phase starting from about 8 h after dawn. Little or no *CO* RNA accumulates at any time during a short, 8-h day (and no light is present when *CO* is later expressed); therefore, phyA and cry2 do not activate *FT* and *AGL20*. In contrast, *CO* RNA reaches high levels by the end of a long, 16-h day, and phyA and cry2 activate *FT* expression in a pattern that strikingly matches the coincidence between light and *CO* RNA. Thus, *FT* and *AGL20* expression are the first steps of the pathway that are known to be regulated by photoperiod. Several important elements remain to be discovered, notably the mechanism that allows the photoreceptors to alter CO function. All the genes involved are known or predicted transcriptional regulators, with the possible exception of *FT*, so the long-distance photoperiod signal also remains a mystery. It is expected to function downstream of *CO*, and grafting studies in transgenic potato indicate that overexpressing Arabidopsis *CO* in the leaf is sufficient to initiate the signal (Martinez-Garcia et al., 2002).

Bünning proposed a minimal model so that we might find ancillary functions that reinforce or stabilize the photoperiod switch. The *CO* RNA rhythm alters its phase by 2 to 3 h relative to dawn, for example, and changes waveform and peak level under short- versus long-day conditions (Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002). Most strikingly, the level of CRY2 protein may be regulated by photoperiod, just as *FT* and *AGL20* are. CRY2 instability in blue light has been reported (Shalitin et al., 2002), but one report showed that this was specific for plants in short days, whereas in long days CRY2 was stable at a high level (El-Din El-Assal et al., 2001). The simplest model (above) suggests that CRY2 levels should have little effect in short days because *CO* RNA levels are low throughout the day, but if anything, cry2 degradation might further delay flowering. Consistent with this suggestion, a *cry2* allele (*CRY2-Cvi*) that partially suppressed degradation also had an early flowering phenotype (El-Din El-Assal et al., 2001). The external coincidence model requires only one rhythmic component to gate the signaling pathway from photoreceptors to flowering. In fact, several rhythms might affect the pathway at different stages because photoreceptors are also regulated by the circadian clock via nuclear translocation (Kircher et al., 2002) and interaction with ELF3 (see above). Ultimately, a quantitative analysis of the physiological and molecular data by computational modeling will help to understand the contributions of these and other effects.

FUTURE PROSPECTS

The identification of the molecular components of the plant circadian system and its associated photoperiod sensor will accelerate with the wider application of genome-wide data collection, quantitative biochemical studies of individual components, and mathematical modeling. Methods of regulating gene expression in specific cell types will be applied to manipulate the clock, matching the reporter gene methods for monitoring rhythms in specific tissues. Some classic questions will be re-visited using these new tools: Circadian timing can differ among cells, for example, so which leaf cells are responsible for the *CO* expression rhythm (Salisbury and Denney, 1971)? We look forward to understanding how the Arabidopsis model is altered in plant species with other adaptations of timing. To name but a few, the phase jump from diurnal to nocturnal gas exchange, when *Mesembryanthemum crystallinum* switches from C3 to CAM metabolism; the photoperiodic responses of some crop species, which contribute to determining harvest times; and the short-day response of many trees, which must trigger the formation of dormant buds before temperatures drop below freezing in the winter.

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