Plant Physiology, September 1999, Vol. 121, pp. 9–19, www.plantphysiol.org © 1999 American Society of Plant Physiologists

Update on Development

The Physiology and Molecular Bases of the Plant Circadian Clock

David E. Somers*

Department of Cell Biology, The Scripps Research Institute, La Jolla, California 92037

Periodicity in biology comes in many shapes and sizes. Among the very rapid are ultradian rhythms in the courtship song of the *Drosophila* male, which recur every 50 to 60 s (Edmunds, 1988). Running at a slightly faster pace is the glycolytic oscillator in yeast, which exhibits self-sustained oscillations in NADH fluorescence. These are typically between 2 and 70 min long, though periods of up to 6 h have been obtained by manipulating metabolite concentrations (Edmunds, 1988). Hypocotyl circumnutations with period lengths ranging from 25 min to 8 h are part of the process of stem elongation in Arabidopsis and *Sinapis* (Engelmann and Johnsson, 1998).

Infradian rhythms (>24 h) on the order of 4 to 5 d constitute the estrus cycles of some rodents, whereas the menstrual cycle in higher primates is generally between 25 and 35 d (Moore-Ede et al., 1982). Finally, some of the longest cycles occur as circannual rhythms that manifest as certain activities in animals (e.g. mating or hibernation) or changes in plant development (e.g. flowering or dormancy) that occur at a specific time just once a year (Sweeney, 1987; Edmunds, 1988).

Lying between these temporal extremes are processes that oscillate with a nearly 24-h (circadian) periodicity. This type of rhythm, with a period so closely matched to the rotation rate of the earth, occurs ubiquitously in both prokaryotes (e.g. cyanobacteria) and many eukaryotes, and regulates a wide range of physiological and developmental processes. The recent molecular cloning of a number of novel components of the circadian clock system has changed the landscape of the field and greatly improved our view of this timing mechanism. In this *Update* I will focus on how these and other findings have advanced our understanding of the molecular basis of the circadian clock in plants.

WHAT ARE CIRCADIAN RHYTHMS?

Two hundred and seventy years ago, the French astronomer Jean Jacques d'Ortous de Mairan initiated, with a single page report, the experimental approach to the study of endogenous biological rhythms (de Mairan, 1729; Sweeney, 1987). He used a "sensitive" plant (mimosa) that was already known to fold its leaves and leaflets closed at night and reopen them during the day. When he placed the plants in constant darkness, he found that leaf opening and closing persisted just as if the plants were seeing the day and night. With this came the first experimental evidence for the persistence of an endogenous rhythmicity in the absence of environmental cues.

Prior to de Mairan's report, and for many years after, rhythmic movements in plants were assumed to be caused by the daily cycles of light and dark. Indeed, in 1751 Linneaus designed a garden consisting of flowers that opened and closed their petals at specific but different times of day (Fig. 1). In this scheme, by simply looking out the window and noting which species were open or closed one might tell the hour of the day (Moore-Ede et al., 1982).

Skeptics of the idea of an endogenous source of the rhythmicity suggested that subtle, unknown environmental signals were being detected by the plants and were responsible for the maintenance of rhythmicity in the dark. However, 100 years after de Mairan, a second Frenchman, Augustin de Candolle, again working with mimosa, observed that in continuous dark the period length of the leaf opening and closing rhythm was not exactly 24 h, but closer to 22 to 23 h (Moore-Ede et al., 1982). Although he did not note it at the time, this result argued against an environmental signal based on the 24-h rotation of the earth as the source for maintaining rhythmicity. It also anticipated by another 100 years the results of Erwin Bünning, who identified two variants of common bean that differed in their endogenous period length by 3 h. When crossed, the progeny exhibited period lengths ranging between the extremes of the two parents, suggesting that this property of circadian rhythms is a genetically based polygenic trait (Bünning, 1935).

From the results of numerous studies from these initial observations to the present, a number of characteristics have come to define a circadian rhythm (Fig. 2) (Edmunds, 1988). First and foremost is an endogenous period that is approximately 24 h long (circadian). Only when a 24-h environmental cycle is imposed on the system does the period become exactly 24 h. Indeed, within limits, a non-24-h environmental cycle (e.g. 10 h of light/10 h of dark) will constrain the clock to oscillate with just that period length (i.e. 20 h). This adjustment arises from a second primary feature of circadian rhythms: entrainability. This is the process by which the clock is synchronized to the outside world. In all organisms studied to date, the pri-

* E-mail dsomers@scripps.edu; fax 858–784–9840.

Figure 1. Depiction of the flower clock (eine Blumen-Uhr) designed by Linneaus. The left half of the figure (6 AM–12 PM) shows when the petals of different species are opening; the right half (12 PM–6 PM) shows times of petal closing (except evening primrose, which starts to open its flowers after 5 PM). Note that some species can act to time both morning and afternoon events. (From Moore-Ede et al., 1982.)

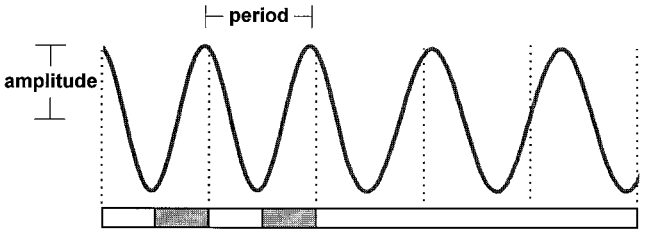

Figure 2. Four characteristics of circadian rhythms (see text). An idealized rhythm is shown under entraining conditions (light/dark cycles) and during free-run in constant conditions (continuous light). In this example the endogenous free-running period is longer than the cycle of the entrainment schedule. As long as light/dark cycles continue, the period of the oscillator is identical to the period length of the entrainment schedule cycle, resulting in a consistent difference in time (i.e. phase relationship) between any chosen point on the curve (e.g. peak expression) and a given phase marker (e.g. the dark-to-light transition; dotted line). During free-run, this constant relationship breaks down as the oscillator reverts to its longer, endogenous period and the peak of expression drifts further from "subjective" dawn. Dark (shaded bar) and light (white bar) are indicated below the trace. (Adapted from Edmunds, 1988.)

mary, though not exclusive, entraining stimuli are temperature and light. Diurnal oscillations in temperature (high/low) or light (light/dark) are the cues that adjust the circadian system with each cycle. This occurs because a step up or step down in light or temperature can alter the position, or phase, of the oscillation. In some organisms (e.g. *Drosophila*) a short 15-min light pulse is sufficient to fully entrain the clock, whereas plants often require 3 to 4 h of illumination.

A third characteristic of circadian rhythms that is not shared with other biological or biochemical periodicities is temperature compensation. Although a temperature shift can reset the phase of the clock, when stabilized, this new temperature regime has very little effect on endogenous period length. Most biochemical processes are sensitive to temperature, with reaction rates doubling or tripling with each 10°C change in temperature (Q_{10} = 2–3) (Johnson et al., 1998). In contrast, the Q_{10} values of circadian rhythms lie between 0.8 and 1.4. For example, we have tested Arabidopsis over a 20°C temperature range and found no more than a 2.5-h change in period length (Q_{10} = 1.0–1.1) (Somers et al., 1998b).

The fourth feature, the persistence of rhythmicity in the absence of periodic input, is perhaps the most intriguing aspect of circadian biology. The primary focus of most approaches to understanding the mechanism of the circadian clock has been to identify the components and their interactions that allow the maintenance of a self-sustained oscillation in a non-periodic environment.

THE CIRCADIAN CLOCK IS A SIGNALING SYSTEM LINKING THE ENVIRONMENT TO PHYSIOLOGY AND DEVELOPMENT

An oscillator alone is not a clock. To be meaningful to the organism a clock must be linked to the outside world (Fig. 3). The daily occurrence of light/dark transitions provides the time-setting information by which clock-controlled processes are appropriately phased. For example, processes required for photosynthesis are phased early in the day, whereas other genes, such as catalase, show peak expression at night (Fejes and Nagy, 1998). In the absence of external input to the clock, oscillations from a pacemaker can control various processes, but there is no synchronization with the environment and no temporal information is relayed. A familiar example is the periodic beating of the heart, which continues to pump blood at a fairly even pace day and night.

A clock must also control the rhythmicity of events in the organism. An oscillator that is not coupled to other processes cannot act as a meaningful timer. In plants a wide variety of processes are controlled by the clock. Gene transcription, Ca^{2+} levels, and some enzyme activities are examples of intracellular processes under circadian clock regulation (Sweeney, 1987; Johnson et al., 1998). At a higher level of organization, rhythms in stomatal opening, leaf movement, and hypocotyl expansion occur through coordination of cell and tissue level events by the circadian oscillator (Engelmann and Johnsson, 1998; Webb, 1998; Dowson-Day and Millar, 1999). Finally, the circadian pacemaker can mediate fundamental changes in plant development. The photoperiodic control of flowering time and the onset of bud dormancy are two examples of how a 24-h clock can underlie processes that occur on a non-circadian time scale (Thomas and Vince-Prue, 1996).

Both the input pathway(s) to the oscillator and the output pathway(s) leading to the control of the overt rhythms are signal transduction pathways, and are linked by the central oscillator (Fig. 3). Current research on the circadian clock is concerned with identifying candidate components and positioning them within one of the three realms of the system. As noted below, the borders between these are becoming fuzzy as new information emerges, but the model has provided the conceptual framework from which most clock research has proceeded (Johnson et al., 1998).

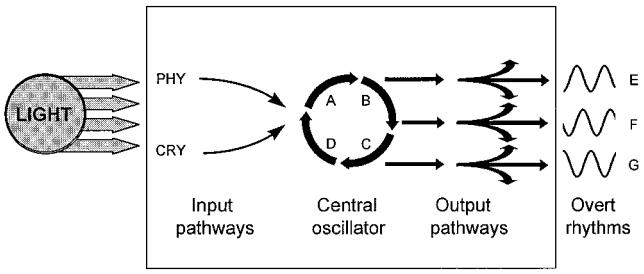

Figure 3. Model of a simple circadian system. The three primary components include: an input (entrainment) pathway(s), the central oscillator, and an output pathway(s). The phytochromes (PHY) and the cryptochromes (CRY) are two classes of photoreceptors known to mediate the first step of the light entrainment pathway (Somers et al., 1998a). Interactions among the components (A–D) of the central oscillator create the autoregulatory negative-feedback loop that generates the approximately 24-h oscillations. Three different hypothetical couplings of the central oscillator to possible output pathways are shown to indicate that differently phased overt rhythms with the same period (E–G) can arise from a single pacemaker.

THE "HANDS" OF THE CLOCK: THE OUTPUT PATHWAY LEADS TO MANY AND VARIED CLOCK-REGULATED PROCESSES IN PLANTS

Most model circadian systems have been developed around a limited number of clock-regulated outputs. For example, until recently, all of the progress in understanding the *Drosophila* circadian clock has come through the observation of eclosion (adult emergence) and locomotor activity. Work in rodents has relied largely on wheel-running activity, and circadian-regulated sporulation is still the primary morphological assay for clock function in *Neurospora*.

In contrast, research into the circadian clock in plants has benefited from studying a large number of different output processes. These afford the opportunity to probe the nature of the clock itself, as well as to understand the effects of the clock on plant growth and development. Each "hand" of the clock becomes an additional assay of the activity of the oscillator. As noted above, there are different classes of outputs and each can tell us something different about the organization of the clock system.

Gene Regulation

The list of genes under the control of the circadian clock is constantly growing (Kreps and Kay, 1997; Fejes and Nagy, 1998). There are examples of circadian regulation of gene expression at each step: transcription (Millar and Kay, 1991; Liu et al., 1996), transcript abundance (Fujiwara et al., 1996; Zheng et al., 1998), translation (Mittag et al., 1994), and posttranslational processing (Nimmo, 1998). Since the central oscillator itself is strongly based on temporal variation in gene expression (see below), it is possible that the output pathway from the oscillator to clock-controlled gene expression will be very short compared with more complex processes such as cell expansion and flowering.

The first examples of clock-controlled gene regulation in plants were mRNA levels of light-induced genes involved in photosynthesis (Kreps and Kay, 1997). Among these are the chlorophyll *a/b*-binding proteins, part of the light-harvesting complex in chloroplasts that are coded for by a small gene family (*CAB* and *LHCb*). In most species the circadian peak of expression of these genes is a few hours after dawn, as would be expected for proteins required for photosynthesis.

Other genes related to photosynthesis are also expressed rhythmically. These include the small subunit of Rubisco (Pilgrim and McClung, 1993), Rubisco activase (Liu et al., 1996), and, in CAM plants, PEP carboxylase kinase (Nimmo, 1998). In contrast to the phasing of genes of the light reactions, peak expression of PEP carboxylase kinase mRNA occurs at midnight, accurately reflecting the requirement of the enzyme at that time (Nimmo, 1998).

Non-photosynthetic enzymes under the control of the circadian clock include the catalases, which are primarily involved in eliminating toxic H_2O_2 from the cell. The circadian regulation of the small catalase gene families in maize and Arabidopsis has been intensively studied (Zhong and McClung, 1996; Polidoros and Scandalios, 1998). It is particularly interesting that the peaks of expression of *CAT2* and *CAT3* in Arabidopsis are 12 h out of phase with each other (Zhong and McClung, 1996). This difference probably reflects different roles for each in plant metabolism, but also raises the question of how they are coupled to an oscillator. Although it is possible that entirely different clocks control these two genes, there is evidence (see below) that at least some differently phased outputs are under the control of the same clockwork.

The transcript levels of two closely related putative RNA-binding proteins from Arabidopsis, *CCR1* (*Atgrp8*) and *CCR2* (*Atgrp7*), cycle with a peak expression 8 to 12 h after dawn in the wild type (Kreps and Simon, 1997). Although the function of these proteins is unclear, overexpression of *CCR2* in Arabidopsis strongly depresses the cycling of the endogenous *CCR2* transcript (Heintzen et al., 1997). This finding, together with the observation that endogenous CCR2 protein levels normally oscillate with a 4-h phase delay relative to its transcript, strongly suggests that the transcript and protein act together as components of an autoregulatory negative feedback loop. However, *CCR2* overexpression has no effect on the period or levels of other unrelated circadian-regulated transcripts tested, such as *CAB3* and *CAT3*. In addition, the *toc1* (timing of cab) mutation, which shortens the period length of a wide range of clock-controlled processes (Somers et al., 1998b), also shortens the cycling of the *CCR2* transcript in constant light (Kreps and Simon, 1997). Therefore, circadian control of *CCR2* gene expression most likely lies downstream of the effects of *toc1* on the clock and may define a "slave" oscillator that is still subject to temporal control from a "master" oscillator, but may itself control the amplitude and phase of an undefined subset of clock-controlled outputs (Heintzen et al., 1997).

Ca^{2+} Signaling

Much less is known about the effect of the clock on subcellular processes unrelated to gene expression in plants. One recent insight came from the constitutive expression of the Ca^{2+}-dependent photoprotein aequorin in tobacco and Arabidopsis. In both species, this assay revealed circadian oscillations in cytosolic [Ca^{2+}] in constant light and constant dark after entrainment in light/dark cycles (Johnson et al., 1995). When the reporter protein is targeted to the chloroplast, similar rhythms are observed, but only in the dark and they damp rapidly. These results demonstrate the potential for circadian regulation of Ca^{2+}-dependent enzymes such as calmodulin and Ca^{2+}-dependent protein kinases. Such enzymes could be part of output pathways, causing or contributing to, for example, a circadian cycle of activity of key metabolic enzymes. Ca^{2+}-dependent regulation of the input pathway might also occur, since Ca^{2+} has been shown to participate in phytochrome-mediated light regulation (Neuhaus et al., 1997). This potential for Ca^{2+} oscillations in a dual role of mediator of output and input signaling has precedence in the animal field. Pharmacological and electrophysiological studies in the sea slug (*Bulla*) show Ca^{2+} as an important

component that mediates signaling to and from the central oscillator (Block et al., 1995).

Cell and Tissue Phenotypes

Circadian control of cell expansion/contraction is the basis of rhythms in leaf movements, hypocotyl elongation, and stomatal aperture size. In legumes, endogenously controlled leaf movements arise through alternate shrinking and swelling of specialized extensor and flexor cells that lie on opposite sides of the pulvinus. Circadian rhythms in the membrane potentials of protoplasts made from these flexor and extensor cells are 12 h out of phase with each other, and result directly from changes in the state of the K^+ channels in the two cell types (Kim et al., 1993). In the intact pulvinus, flexor and extensor cells are discretely localized but closely adjacent to each other. This recapitulation of their endogenous activity in cell culture is one of the best pieces of evidence to suggest that the clock can act cell autonomously in higher plants.

In contrast, circadian leaf movements (and rhythms in hypocotyl growth) in Arabidopsis and other plants probably arise from differential cell expansion in the organ, since rhythmicity ceases after leaf (or hypocotyl) growth is complete (Engelmann and Johnsson, 1998; Dowson-Day and Millar, 1999). Using simple video technology, leaf movement rhythms can be followed for more than a week in a single seedling, and are a rapid way to assess the effects of a mutation or treatment on the circadian clock (Millar et al., 1995a; Hicks et al., 1996; Schaffer et al., 1998). The cellular bases of these movements, however, are not understood.

The size of the stomatal aperture is influenced by a wide range of environmental and physiological factors, as well as by the endogenous circadian clock (Assman, 1993; Webb, 1998). Together with the accessory cells, the paired guard cells form a discrete, specialized unit imbedded in the epidermis. When the epidermis is peeled away from the blade in fava bean, rhythms in stomatal aperture persist, strongly suggesting that circadian regulation of the stomatal apparatus is cell autonomous (Gorton et al., 1989). These data also support the notion of complete circadian clock systems running independently in each cell.

A comparison of stomatal conductance rhythms and leaf movement rhythms in the same plant (common bean) raised the question of whether the same type of clock controls these two outputs. The period length of the rhythm in stomatal opening and photosynthesis, both occurring in the leaf blade, is shorter (approximately 24 h) than the pulvinus-based leaf movement rhythm (approximately 27 h) (Hennessey et al., 1992). One interpretation of these results is that separate oscillators control the rhythms in these two tissues, each with intrinsically different free-running periods and possibly comprised of distinctly different components. Alternatively, both organs may differ only in the nature of the light input pathway leading to the oscillator, since the free-running circadian period can be modulated by the intensity of the light input to the oscillator (see below). If the pulvinar region is less sensitive to illumination than the blade, identical oscillators in the two tissues might run at slightly different rates. Ways to address this question would be to observe the period length of two or more very different outputs in the same cell or tissue (Roenneberg, 1996), or to determine how extensively a period length mutation in a known oscillator component acts on various outputs throughout the plant (see below and Millar [1998] for further discussion).

Flowering and Dormancy

The processes by which the circadian clock is involved in the control of flowering and dormancy are too complex and too ambiguous to fully review here (see Vince-Prue, 1994; Thomas and Vince-Prue, 1996). Unlike most circadian phenotypes that manifestly cycle with a 24-h rhythm, the transition from vegetative to reproductive growth occurs as the endpoint of a series of processes to which the circadian clock contributes an element of timing. Nonetheless, much of the primary evidence for a role of the clock in photoperiodic timing comes from experiments in which light pulses were administered over 3- to 4-d time courses. In Arabidopsis and barley, there was a 24-h rhythm in the acceleration of flowering time caused by 6-h far-red light treatments given over a 72-h period (Deitzer et al., 1982; Deitzer, 1984; Carré, 1998). This rhythmic change in the sensitivity to light is found in both short- and long-day plants (Vince-Prue, 1994), suggesting that the circadian clock is involved in regulating flowering time in both types of reproductive strategies. However, it is also apparent that the way in which the clock is incorporated into the entire flowering process differs greatly both within and between these two flowering time systems (Vince-Prue, 1994; Thomas and Vince-Prue, 1996).

Physiological studies such as these cannot easily address another version of the same question raised earlier: is the same type of oscillator responsible for both photoperiodic timing and circadian timing of daily rhythmic processes? In Arabidopsis, the answer has been approached by examining day-neutral flowering-time mutants, lines that flower equally rapidly or slowly in both short and long days (Koornneef et al., 1991; Carré, 1998). This strategy assumes that if the circadian clock is severely disrupted (or eliminated, resulting in arrythmicity), the timing information inherent in day-length differences will not be perceived or processed by the plant and flowering time will be the same in both short and long days. *elf3* (early-flowering 3) (Hicks et al., 1996), *lhy* (long hypocotyl) (Schaffer et al., 1998), and *toc1* (in ecotype Landsberg *erecta*) (Somers et al., 1998b) are three such day-neutral lines and share the additional feature of being disrupted in other circadian phenotypes. Although these findings suggest that the same circadian clock controls photoperiodic timing and at least a subset of other circadian processes, the phenotypes of these mutants are still sufficiently ambiguous or incomplete to discourage conclusive positioning of these genes within the system (see below).

ENTRAINMENT OF THE CENTRAL OSCILLATOR OCCURS VIA THE LIGHT INPUT PATHWAY

Since light is a universal entraining stimulus for all known circadian systems, there is intense interest in tracing the signaling pathway that provides the ultimate timing cues that entrain the clock. In addition to the phase-setting signal that a light pulse provides, changing the incident light intensity can vary the period length of a free-running rhythm. As a general rule, increasing light intensity tends to lengthen the period in nocturnal organisms and shorten the period in diurnal organisms (including plants) (Aschoff, 1979; Millar et al., 1995b; Somers et al., 1998a, 1998b). However, the relationships can be more complex than that. In the dinoflagellate *Gonyalaux*, increasing the red-light intensity lengthens the period, whereas increasing blue or white light decreases period length (Roenneberg, 1996). Together these results suggest that at least two types of photoreceptors mediate light input to this alga (Roenneberg and Deng, 1997).

Similar experiments in Arabidopsis have begun to sort out the roles of the eight or more photoreceptors in higher plants that might mediate light signaling to the circadian clock. To facilitate easy monitoring of the activity of the oscillator, the *cab2*-luciferase reporter (*cab2::luc*) was used as a bioluminescent assay of clock-controlled gene transcription. When the luciferin substrate is applied, this promoter drives robust rhythms of luminescence from intact seedlings that can be monitored with a highly sensitive video camera (Millar et al., 1992). With this system it was found that the period length (τ) of *cab2::luc* expression in Arabidopsis significantly lengthens when plants are moved from continuous light (τ = 24.5 h) to continuous darkness (τ = approximately 30 h) (Millar et al., 1995b). Using this assay, previous evidence (Johnson et al., 1998) for a role of phytochromes in the entrainment of the clock has been verified using phytochrome-deficient mutants of Arabidopsis. *hy1-6*, deficient in all five of the photoreversible phytochromes (*PHYA*, *PHYB*, *PHYC*, *PHYD*, and *PHYE*), shows a slightly longer period in red and white light than the wild type, as if the plants were partially blind (Millar et al., 1995b).

Subsequent studies using type-specific phytochrome mutants show that over a nonoverlapping range of light intensities, both phyA and phyB mediate red-light signaling to the clock (Somers et al., 1998a). Under dim-red light, plants deficent in phyA show a 2-h period lengthening compared with wild type, whereas a loss of phyB lengthens period only in high-fluence red light. PhyA also acts to mediate blue light input to the clock but, again, only at low light intensities. Not surprisingly, the dedicated blue-light photoreceptors cryptochrome 1 (cry1) and cryptochrome 2 (cry2) also play roles. A deficiency in the more abundant species, cry1, causes period lengthening in both high- and low-fluency blue light, whereas the absence of cry2 alone has only a minor effect on period length (Somers et al., 1998a).

In an ecological context these results make sense. Together the phytochromes and cryptochromes sense the full spectrum of visible light. This allows a sensitive monitoring of the changes in light quality and quantity that occur at dusk and dawn. In addition, a green leaf canopy and the proximity of neighboring plants both have marked effects on the ratio of red and far-red light that reaches a plant (Ballaré et al., 1987). The above findings suggest that the plant recruits a diversity of photoreceptors to ensure that the pace of the circadian oscillator remains unaffected by this wide range of fluence rates and spectral qualities it encounters in the environment.

Photoreceptors only initiate light signaling to the clock. The next steps are to identify the components that lie downstream and determine which are specific to the circadian clock and how many are shared with other light-responsive processes. One fruitful approach has been to examine the effects of constitutive photomorphogenic mutants of Arabidopsis on period length. Mutants of this class (*det/cop/fus*) act downstream of photoreceptors and are the converse of the partially blind photoreceptor mutants, since they develop some of the features of light-grown plants in complete darkness (Fankhauser and Chory, 1997). However, the circadian phenotype of mutants at three loci, *DET1*, *DET2*, and *COP1*, differ in important respects. The free-running period of *det1-1* is much shorter than that of the wild type, both in continuous light and constant dark (Millar et al., 1995b). Surprisingly, it is even shorter in the dark (approximately 18 h) than in the light (approximately 20 h), suggesting that light may antagonize or moderate the action of this non-null-mutant form of DET1 on the clock, possibly through a parallel signaling pathway.

On the other hand, the extremely short period is consistent with the notion that this mutation mimics constitutive very high light input, although periods this short have never been observed in wild-type plants under high irradiance. A partially active allele of *COP1*, *cop1-6*, shows a similar, though less extreme phenotype, maintaining a period in continuous darkness (23.5 h) similar to wild type in continuous light (Millar et al., 1995b). Both DET1 and COP1 are nuclear-localized proteins that negatively regulate a host of light-dependent phenotypes (Fankhauser and Chory, 1997; Torii and Deng, 1997), and their circadian phenotypes in continuous darkness are consistent with these genes acting within the light input pathway to the clock at a point still common to many other light-regulated processes. In contrast, DET2 is an enzyme of the brassinosteroid biosynthetic pathway (Fankhauser and Chory, 1997) and has little to no effect on period in continuous darkness (τ = approximately 30 h) (Millar et al., 1995b), suggesting that this hormone does not play a primary role in regulating the pace of the oscillator.

The *elf3* mutant was originally identified through a screen for early-flowering mutants. This recessive mutation causes a long hypocotyl in white light and equally rapid flowering in long and short days. Subsequently, it was found that the rhythms of *cab2::luciferase* activity and leaf movement are absent in the light in this mutant. However, there are still oscillations in luminescence in dark-grown seedlings, indicating that the arrhythmicity is light dependent (Hicks et al., 1996). These data strongly suggest that ELF3 acts as part of the light input pathway to the clock. But it is also possible that separate oscillators operate in the

light and dark and that ELF3 acts only as a key component of the former. Alternatively, ELF3 may be part of the output pathway, with *elf3* masking the activity of the oscillator only in the light. This ambiguity in knowing where ELF3 acts within the circadian clock system derives in part from its arrhythmic phenotype.

ARRHYTHMICS: WHERE DO THEY BELONG?

Positioning a component within a signaling pathway first requires the appropriate assignment to a place within the circadian system. There is no single criterion that allows one to unequivocally determine if a candidate is an input, output, or oscillator component, but any decision requires that cycling be observable under at least some conditions. Therefore, a mutation that causes the loss of rhythmicity itself can confound analysis because the essential assay is lost. In addition, arrhythmicity may arise from disruptions in any of the three subsystems of the clock.

The Oscillator

A loss-of-function mutation in a non-redundant rhythmic component of the central oscillator will stop the clock. This condition, when coupled with the isolation of both long- and short-period alleles of the locus, has been one of the more reliable identifiers of a bona fide oscillator component (Dunlap, 1996). In *Drosophila* and *Neurospora*, constitutive high expression of some oscillator genes also disrupts cycling (Aronson et al., 1994; Zeng et al., 1994). In either case, it is the loss of rhythmicity in the component that brings the pacemaker to a halt.

The Output Pathway

Two alterations in the output pathway that can lead to arrhythmicity are decoupling and masking. In the former, a mutation eliminates or impairs signaling from the oscillator to the overt rhythm, resulting in constitutively high or low expression (arrhythmicity). Deletions within the promoters of clock-controlled genes that eliminate rhythms in transcription or transcript abundance are one example (Carré and Kay, 1995; Bell-Pedersen et al., 1996). In masking, the signaling system is intact, but other processes override circadian regulation. This might arise from constitutive overexpression or misexpression of a normally circadian-regulated component (Heintzen et al., 1997). In both cases, the central pacemaker may be functioning normally but may simply not be visible because the overt rhythms that normally report its activity have been disrupted.

The Input Pathway

As noted above, the conditional light-dependent arrhythmicity of the *elf3* mutation suggests that this lesion is in a component of the light input pathway. Furthermore, many organisms that show robust rhythmicity in continuous darkness become arrhythmic with increasing light, presumably due to excessive or inappropriate light input to the oscillator (Aschoff, 1979; Konopka et al., 1989). Therefore, a lesion in the input pathway that mimics high-intensity light signaling in such systems could cause arrhythmicity. Finally, formal modeling of clock systems has shown that if the level of an input component itself cycles, as influenced by feedback from the pacemaker, then the loss of this component may result in a more rapidly damped rhythm in constant conditions (Roenneberg and Merrow, 1998).

Clearly, arrhythmicity per se gives no clues as to how and where a component acts within the circadian system. Along with *elf3*, two recently described arrhythmic lines of Arabidopsis, CCA-1-ox (circadian clock associated) and *lhy*, have brought this discussion to the forefront. Although the aberrant expression of these two genes affects many clock-controlled processes, their normal roles within the circadian clock remain unclear (see below).

THE CIRCADIAN OSCILLATOR IS AN AUTOREGULATORY NEGATIVE FEEDBACK LOOP

Recently, there has been extremely rapid progress in understanding the molecular nature of the circadian oscillator in animals and cyanobacteria (Golden et al., 1997; Ishiura et al., 1998; Wilsbacher and Takahashi, 1998; Dunlap, 1999). These advances have built upon the model of the oscillator as an autoregulatory negative-feedback loop. At its simplest, this scheme requires two interacting components that rise and fall in abundance or activity and between which there is a time delay (Merrow et al., 1997). Classical and molecular genetics have helped to identify genes in *Drosophila*, *Neurospora*, and mammals that fit these criteria, and a common molecular mechanism has begun to emerge (Fig. 4). In all three systems, both the mRNA and protein products of key genes (state variables) cycle in abundance and/or activity. The 24-h delay is caused in part by the time it takes for the protein product(s) to enter the nucleus, where they contribute to the repression of their own transcription, and/or in the time required for these proteins to degrade in the nucleus (Merrow et al., 1997; Dunlap, 1999).

Other components that do not cycle but are critical to the proper functioning of the oscillator are termed parameters, and mutations in these components can alter or stop cycling altogether (Kloss et al., 1998). The five molecular components known to participate in this feedback loop in *Drosophila* include genes that code for transcription factors (*dClock*, *dbmal1*), a protein kinase (*doubletime*), and novel proteins (*period*, *timeless*) (Wilsbacher and Takahashi, 1998). Between flies and mammals there has been a remarkable conservation of function among these proteins, suggesting a common origin of the circadian oscillator in these two groups (Dunlap, 1999). Fewer components of the oscillator have been identified in *Neurospora* (*frequency*, *white collar-1*, *white collar-2*), but, apart from a motif (the PAS domain) that facilitates protein-to-protein interactions (Young, 1998; Dunlap, 1999), none of the proteins appears to be an ortholog of other known clock components. The positive components of the loop are the transcription factors and kinase activities, while the negative factors (*period*, *timeless*)

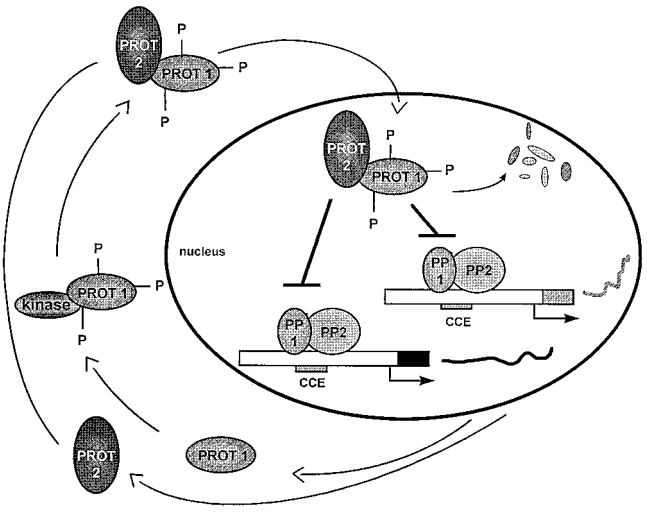

Figure 4. Current working model of a eukaryotic circadian clock. This generalized scheme is drawn largely from work with *Drosophila* and mouse, although all of the available evidence from *Neurospora* supports a similar mechanism. PROT1 and PROT2 are two nuclear-localized proteins that act to negatively regulate their own transcription by blocking the activity of two bHLH-containing DNA-binding proteins (PP1 and PP2). PP1 and PP2 share a common domain (PAS) that facilitates protein-to-protein interactions and may contribute to their heterodimerization and binding to the circadian clock element (CCE) present on the promoters of PROT1 and PROT2. In the absence of PROT1 and PROT2, PP1 and PP2 activate the transcription of both PROT genes. This results in increased protein levels of both PROT gene products in the cytoplasm, which are further regulated by light (PROT2 is light labile) and/or phosphorylation state (PROT1 is destabilized by phosphorylation). Association of PROT2 with PROT1 helps stabilize PROT1 levels and facilitates the transport of the heterodimer to the nucleus. PP1/PP2-dependent transcription is inhibited by the pair until turnover of PROT1 and PROT2 depletes levels, allowing transcription to proceed, and then the cycle begins again. Degraded proteins are indicated as small, grouped ovals. P, Phosphorylation.

facilitate the disruption of the transcription of their own genes and others (Dunlap, 1999).

In cyanobacteria (*Synechococcus*), all known period-length mutations occur within a three-gene cluster and range between 16 and 60 h (Ishiura et al., 1998). An autoregulatory feedback loop is likely the basis of this clock system too, although the requisite lag in the 24-h rhythm probably arises differently than in the eukaryotic system, since there is no cytoplasmic/nuclear partition to cross. These genes have been cloned and none shows similarity to known eukaryotic clock components (Ishiura et al., 1998).

In plants, no unequivocal oscillator components have been identified. Attempts to use known components from other organisms to probe plant genomes have generally not been successful. Two untested potential exceptions are the recently identified Arabidopsis homolog of a *Drosophila* casein kinase I (*doubletime*) (Kloss et al., 1998), and a putative Arabidopsis *timeless* gene (Zylka et al., 1998), which in flies plays a critical role in the nuclear import of the *period* protein (Young, 1998). Otherwise, the two cloned loci in Arabidopsis, *CCA-1* and *LHY*, which have been shown to disrupt clock function, are unrelated to known animal, fungal, or bacterial clock components.

CCA-1 and LHY are closely related proteins, each with a single myb-related DNA-binding motif (Wang et al., 1997; Schaffer et al., 1998; Wang and Tobin, 1998). CCA-1 was originally identified by its ability to bind a phytochrome-responsive region of the *cab1* promoter in Arabidopsis, which was later noted to fall within a 38-bp region sufficient to confer circadian regulation (Carré and Kay, 1995; Wang et al., 1997). In wild-type plants, the mRNA levels of both genes cycle with a 24-h period and with a very similar early-morning peak phase. When overexpressed at constitutively high levels, both cause arrhythmicity in all circadian outputs so far examined. The levels of endogenous *CCA-1* and *LHY* mRNAs are clamped low when *CCA-1* is overexpressed (CCA1-ox), whereas *LHY* mRNA abundance is intermediate in an *LHY*-overexpressing mutant (*lhy*).

The cycling of transcript levels of other clock-regulated genes such as *CAB*, *CAT3* (catalase), and *CCR2* (an RNA-binding protein) are also reduced or eliminated in both overexpressing lines. *CAB* expression peaks in the late morning, whereas *CAT3* and *CCR2* transcript levels peak in the late afternoon. That such differently phased rhythms are affected suggests that both CCA-1 and LHY act on, or close to, the fundamental processes of the clock, and not just on one branch of the output pathway. This conclusion is strengthened by the finding that leaf movements are arrhythmic (*lhy*), and hypocotyls are lengthened (*lhy* and CCA-1-ox) in these lines. Flowering time under long days is delayed in both overexpressors, which is consistent with their both having roles in the circadian clock system (Schaffer et al., 1998; Wang and Tobin, 1998).

These observations fulfill many of the criteria previously established to help identify a candidate as a bona fide state variable of the circadian oscillator. These criteria include: (a) the component (or process) itself oscillates with circadian periodicity, (b) the component feeds back to control its own level and rhythmicity, (c) clamping the level of the component to any constant value (null to constitutive overexpression) stops the clock and any overt rhythmicity, and (d) an induced change in the level (or activity) of the component causes a phase shift in the cycling of the component and all overt rhythms within less than one circadian cycle (Aronson et al., 1994).

The mRNA oscillations of both *LHY* and *CCA-1* satisfy criterion a, and in the case of CCA-1 the protein level has also been shown to cycle (Schaffer et al., 1998; Wang and Tobin, 1998). Overexpression of either transcript eliminates rhythmic expression of the respective endogenous gene, suggesting that each protein normally feeds back to control its own transcription (criterion b). Since all other rhythmic activity is blocked as well, overexpression of these proteins appears to stop the clock (criterion c). However, because of possible complications arising from ectopic expression and potential cross-talk between closely related family members, overexpression data must be interpreted cautiously. For example, aberrantly high levels of an output component may mask oscillator activity, as noted above, and may also affect output pathways not normally controlled by that

component. A more ideal approach would be to use an inducible/repressible promoter-gene fusion system akin to the one developed and implemented in *Neurospora* (Aronson et al., 1994) to induce a short pulse of light-independent expression of the candidate component. A phase shift in the peak of both the endogenous level of the induced gene and other outputs would fulfill criterion d.

The strong degree of sequence similarity between *LHY* and *CCA-1* raises the possibility that they belong to a gene family of proteins with redundant or partially redundant functions (Schaffer et al., 1998; Wang and Tobin, 1998). A null mutation in *CCA-1* (*cca-1*) shortens the free-running period of the circadian oscillations of three clock-controlled genes (*CAB2*, *LHY*, and *CAT2*), suggesting that LHY and CCA-1 are not fully redundant proteins (Green and Tobin, 1999). This result also shows that CCA-1 is not an essential or unique oscillator component, since the clock continues to function in its absence. However, this change in free-running period does suggest that CCA-1 is not a simple output regulator, but may instead be part of an input pathway to the clock, linking light signaling to the oscillator itself (Green and Tobin, 1999).

Most of the genes identified as coding for components of the oscillator in non-plant organisms arose from genetic screens for period length changes or as homologs of these genes (Wilsbacher and Takahashi, 1998). The strong genetics infrastructure of Arabidopsis has made it a good choice for the same approach in plants. Using the *cab2::luc* luminescence reporter system described above, Millar et al. (1995a) conducted a screen for mutants in which the peak of free-running cycling bioluminescence was shifted either later or earlier than wild type. More than 20 individuals were recovered and displayed period lengths ranging from 21 to 27 h (wild type = 24.5 h). The best characterized of these lines, *toc1-1*, runs with a period of 21 h in continuous white light. The morphology of the mutant is wild type, consistent with the normal phenotypes observed for period length mutants in other organisms. This simple observation suggests that that clock is not based on metabolic processes that are fundamental to the maintenance of the organism, but, rather, arises from interactions between processes that are at least in part specific to the clockwork itself. At the same time, close examination of the *toc1-1* line shows wide-ranging effects of this semidominant mutation on circadian-related phenotypes throughout plant growth and development.

The *toc1-1* mutant affects all clock-controlled processes so far examined. At the cellular level in green seedlings, the period of cycling in transcription (*CAB2*) and mRNA abundance (*CAB2* and *CCR2*) of two differently phased genes are both shorter than wild type by 2 to 3 h in the *toc1* background (Millar et al., 1995a; Kreps and Simon, 1997). *CAB2* expression peaks about 4 to 6 h after dawn, whereas peak CCR2 mRNA abundance occurs 5 to 6 h later. This different phasing, together with the aforementioned effects of CCR2 overexpression, suggests that different signaling pathways lead from the oscillator to control of each of these two genes, and that TOC1 acts upstream of the divergence. *toc1-1* also shortens the *cab2::luc* rhythm induced by a red-light pulse in etiolated seedlings, indicating that neither photosynthesis nor the photosynthetic apparatus is necessary for an intact circadian clock. Leaf movement and stomatal conductance rhythms in *toc1-1* are also proportionately shorter than in the wild type (Millar et al., 1995a; Somers et al., 1998b).

The severity of the effects of *toc1-1* on flowering time depends on the ecotype. In the C24 background the difference between short- and long-day-plant flowering time was reduced relative to wild-type, and this was accentuated in the Laer ecotype to the extent that the plants flowered nearly as early in short- as in long-day conditions. Clearly, *toc1-1* interacts in an allele-specific way with other loci that affect the transition to flowering. These results also demonstrate a link between quantitative changes in the pace of the clock and the processing of the day-length information that lead to the switch to reproduction (Somers et al., 1998b).

The evidence is strong, but circumstantial, that *TOC1* encodes a clock component. A shorter period length could arise through a mutation in the light input pathway, causing an increased sensitivity to light relative to the wild-type. However, over a 500-fold range of red-light intensity the period of *toc1-1* is consistently 2 to 3 h shorter than in the wild type, suggesting that *toc1-1* acts constitutively and independently of light intensity (Somers et al., 1998b). Cloning and manipulation of the protein, as described above, should resolve the question.

Together, the comprehensive effect that *toc1-1* has on cellular, tissue-level, and developmental processes strongly suggests that one type of oscillator mediates most, if not all, of the circadian responses in this plant. Alternatively, different but closely related oscillators that share one or more components (such as *TOC1*) may be present in different tissues and organs in the plant. These possibilities will become more easily addressed as more period-affecting mutations are recovered and characterized.

PERSPECTIVE

The above considerations make it clear that the model shown in Figure 3 is oversimplified. Feedback loops that act on only a subset of circadian responses (e.g. CCR2 overexpression) indicate that a clock system may be built from combinations of master/slave oscillator interactions, which may even be tissue or organ specific. Coupling of two or more master oscillators within a cell or whole organism have been inferred from physiological studies with algae and humans (see Moore-Ede et al., 1982; Roenneberg, 1996).

The levels of cryptochrome transcript and protein abundance in flies and mouse cycle with a 24-h rhythm (Emery et al., 1998; Miyamoto and Sancar, 1998). These results suggest a very close connection between photoperception and the oscillator, leading some to suggest that CRY participates directly in the animal clockwork, and blurring the distinction between input pathway and the oscillator. Although there are no published reports of clock-regulated expression of any higher-plant photoreceptors, light pulse experiments have shown a circadian rhythmicity in the rapid light induction of light-regulated *CAB* expression in

Arabidopsis (Millar and Kay, 1996). If such clock-regulated light signaling also affects the light input pathway to the oscillator itself, then a feedback loop formed by the central oscillator controlling its own input (Roenneberg and Merrow, 1998) will obscure the neat borders outlined in Figure 3. As with much of biology, the complete story will likely be complex. A combination of molecular genetics and experimental physiology currently appears to be the best approach to sorting out what may be a network of feedback loops and interconnected oscillators, large and small, that we now simply term the circadian clock.

ACKNOWLEDGMENTS

I wish to thank members of the Kay lab for critical reading of this manuscript. Thanks also to Steve Kay, under whose auspices I have been working, for his support throughout, and to Martin Moore-Ede who provided the color image of the flower clock. My apologies to the many researchers whose work could not be cited here because of space limitations.

Received January 21, 1999; accepted June 8, 1999.

LITERATURE CITED

Aronson BD, Johnson KA, Loros JJ, Dunlap JC (1994) Negative feedback defining a circadian clock: autoregulation of the clock gene *frequency*. Science **263:** 1578–1584

Aschoff J (1979) Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. Z Tierpsychol **49:** 225–249

Assman SM (1993) Signal transduction in guard cells. Annu Rev Cell Biol **9:** 345–375

Ballaré CL, Sanchez RA, Scopel AL, Casal JJ, Ghersa CM (1987) Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. Plant Cell Environ **10:** 551–557

Bell-Pedersen D, Dunlap JC, Loros JJ (1996) Distinct *cis*-acting elements mediate clock, light, and developmental regulation of the *Neurospora crassa eas* (*ccg*-2) gene. Mol Cell Biol **16:** 513–521

Block GD, Geusz ME, Khalsa SBS, Michel S (1995) A clockwork *Bulla*: cellular study of a model circadian system. Semin Neurosci **7:** 37–42

Bünning E (1935) Zur Kenntnis der erblichen Tagesperiodizität bei den Primärblattern von *Phaseolus multiflorus*. Jarhb Wiss Bot **81:** 411–418

Carré IA (1998) Genetic dissection of the photoperiod-sensing mechanism in the long-day plant *Arabidopsis thaliana. In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 257–269

Carré IA, Kay SA (1995) Multiple DNA-protein interactions at a circadian-regulated promoter element. Plant Cell **7:** 2039–2051

Deitzer GF (1984) Photoperiodic induction in long-day plants. *In* D Vince-Prue, KE Cockshull, eds, Light and the Flowering Process. Academic Press, London, pp 51–63

Deitzer GF, Hayes RG, Jabben M (1982) Phase-shift in circadian rhythm of floral promotion by far-red energy in *Hordeum vulgare.* Plant Physiol **69:** 597–601

de Mairan J (1729) Observation botanique. *In* Histoire de l'Academie Royale des Sciences, pp 35–36

Dowson-Day MJ, Millar AJ (1999) Circadian dysfunction causes aberrant hypocotyl elongation patterns in Arabidopsis. Plant J **17:** 63–71

Dunlap JC (1996) Genetic and molecular analysis of circadian rhythms. Annu Rev Genet **30:** 579–601

Dunlap JC (1999) Molecular bases for circadian clocks. Cell **96:** 271–290

Edmunds LN (1988). Cellular and Molecular Bases of Biological Clocks. Springer-Verlag, New York

Emery PT, So WV, Kaneko M, Hall JC, Rosbash M (1998) CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell **95:** 669–679

Engelmann W, Johnsson A (1998) Rhythms in organ movement. *In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 35–50

Fankhauser C, Chory J (1997) Light control of plant development. Annu Rev Cell Dev Biol **13:** 203–229

Fejes E, Nagy F (1998) Molecular analysis of circadian clock-regulated gene expression in plants: features of the 'output' pathways. *In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 99–118

Fujiwara S, Ishida N, Tsuzuki M (1996) Circadian expression of the carbonic anhydrase gene, *Cah1*, in *Chlamydomonas reinhardtii*. Plant Mol Biol **32:** 745–749

Golden SS, Ishiura M, Johnson CH, Kondo T (1997) Cyanobacterial circadian rhythms. Annu Rev Plant Physiol Plant Mol Biol **48:** 327–354

Gorton HL, Williams WE, Binns ME, Gemmell CN, Leheny EA, Shepherd AC (1989) Circadian stomatal rhythms in epidermal peels from *Vicia faba*. Plant Physiol **90:** 1329–1334

Green RM, Tobin EM (1999) Loss of the circadian clock-associated protein 1 in *Arabidopsis* results in altered clock-regulated gene expression. Proc Natl Acad Sci USA **96:** 4176–4179

Heintzen C, Nater M, Apel K, Staiger D (1997) AtGRP7, a nuclear RNA-binding protein as a component of a circadian-regulated negative feedback loop in *Arabidopsis thaliana*. Proc Natl Acad Sci USA **94:** 8515–8520

Hennessey TL, Freeden AL, Field CB (1992) Evidence of multiple circadian oscillators in bean plants. J Biol Rhythms **7:** 105–113

Hicks KA, Millar AJ, Carré IA, Somers DE, Straume M, Meeks-Wagner R, Kay SA (1996) Conditional circadian dysfunction of the *Arabidopsis* early-flowering 3 mutant. Science **274:** 790–792

Ishiura M, Kutsuna S, Aoki S, Iwasaki H, Andersson CR, Tanabe A, Golden SS, Johnson CH, Kondo T (1998) Expression of a gene cluster *kaiABC* as a circadian feedback process in cyanobacteria. Science **281:** 1519–1523

Johnson CH, Knight M, Trewavas A, Kondo T (1998) A clockwork green: circadian programs in photosynthetic organisms. *In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 1–34

Johnson CH, Knight MR, Kondo T, Masson P, Sedbrook J, Haley A, Trewavas A (1995) Circadian oscillations of cytosolic and chloroplastic free calcium in plants. Science **269:** 1863–1865

Kim HY, Cote GC, Crain RC (1993) Potassium channels in *Samanea saman* protoplasts controlled by phytochrome and the biological clock. Science **260:** 960–962

Kloss B, Price JL, Saez L, Blau J, Rothenfluh A, Wesley CS, Young MW (1998) The *Drosophila* clock gene *double-time* encodes a protein closely related to human casein kinase Iε. Cell **94:** 97–107

Konopka RJ, Pittendrigh CS, Orr D (1989) Reciprocal behavior associated with altered homeostasis and photosensitivity of *Drosophila* clock mutants. J Neurogenet **6:** 1–10

Koornneef M, Hanhart CJ, Van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet **229:** 57–66

Kreps JA, Kay SA (1997) Coordination of plant metabolism and development by the circadian clock. Plant Cell **9:** 1235–1244

Kreps JA, Simon AE (1997) Environmental and genetic effects on circadian clock-regulated gene expression in Arabidopsis. Plant Cell **9:** 297–304

Liu Z, Taub CC, McClung CR (1996) Identification of an *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase/oxygenase activase (RCA) minimal promoter regulated by light and the circadian clock. Plant Physiol **112:** 43–51

Merrow MW, Garceau NY, Dunlap JC (1997) Dissection of a circadian oscillation into discrete domains. Proc Natl Acad Sci USA **94:** 3877–3882

Millar AJ (1998) The cellular organization of circadian rhythms in plants: not one but many clocks. *In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 51–68

Millar AJ, Carré IA, Strayer CA, Chua N-H, Kay SA (1995a) Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. Science **267:** 1161–1163

Millar AJ, Kay SA (1991) Circadian control of *cab* gene transcription and mRNA accumulation in *Arabidopsis*. Plant Cell **3:** 541–550

Millar AJ, Kay SA (1996) Integration of circadian and phototransduction pathways in the network controlling *CAB* gene transcription in *Arabidopsis*. Proc Natl Acad Sci USA **93:** 15491–15496

Millar AJ, Short SR, Hiratsuka K, Chua N-H, Kay SA (1992) Firefly luciferase as a reporter of regulated gene expression in higher plants. Plant Mol Biol Rep **10:** 324–337

Millar AJ, Straume M, Chory J, Chua N-H, Kay SA (1995b) The regulation of circadian period by phototransduction pathways in *Arabidopsis*. Science **267:** 1163–1166

Mittag M, Lee D-H, Hastings W (1994) Circadian expression of the luciferin-binding protein correlates with the binding of a protein to the 3′ untranslated region of its mRNA. Proc Natl Acad Sci USA **91:** 5257–5261

Miyamoto Y, Sancar A (1998) Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. Proc Natl Acad Sci USA **95:** 6097–6102

Moore-Ede MC, Sulzman FM, Fuller CA (1982) The Clocks That Time Us. Harvard University Press, Cambridge, MA

Neuhaus G, Bowler C, Hiratsuka K, Yamagata H, Chua NH (1997) Phytochrome-regulated repression of gene expression requires calcium and cGMP. EMBO J **16:** 2554–2564

Nimmo HG (1998) Circadian regulation of a plant protein kinase. Chronobiol Int **15:** 109–118

Pilgrim ML, McClung CR (1993) Differential involvement of the circadian clock in the expression of genes required for ribulose-1,5-bisphosphate carboxylase/oxygenase synthesis, assembly and activation in *Arabidopsis thaliana*. Plant Physiol **103:** 553–564

Polidoros AN, Scandalios JG (1998) Circadian expression of the maize catalase *Cat3* gene is highly conserved among diverse maize genotypes with structurally different promoters. Genetics **149:** 405–415

Roenneberg T (1996) The complex circadian system of *Gonyaulax polyedra*. Physiol Plant **96:** 733–737

Roenneberg T, Deng TS (1997) Photobiology of the *Gonyaulax* circadian system. 1. Different phase response curves for red and blue light. Planta **202:** 494–501

Roenneberg T, Merrow M (1998) Molecular circadian oscillators: an alternative hypothesis. J Biol Rhythms **13:** 167–179

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carré IA, Coupland G (1998) The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. Cell **93:** 1219–1229

Somers DE, Devlin PF, Kay SA (1998a) Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science **282:** 1488–1490

Somers DE, Webb AAR, Pearson M, Kay S (1998b) The short-period mutant, *toc1-1*, alters circadian clock regulation of multiple outputs throughout development in *Arabidopsis thaliana*. Development **125:** 485–494

Sweeney BM (1987) Rhythmic Phenomena in Plants. Academic Press, San Diego

Thomas B, Vince-Prue D (1996) Photoperiodism in Plants. Academic Press, London

Torii KU, Deng XW (1997) The role of COP1 in light control of Arabidopsis seedling development. Plant Cell Environ **20:** 728–733

Vince-Prue D (1994) The duration of light and photoperiodic responses. *In* RE Kendrick, GHM Kronenberg, eds, Photomorphogenesis in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 447–490

Wang ZY, Kenigsbuch D, Sun L, Harel E, Ong MS, Tobin EM (1997) A Myb-related transcription factor is involved in the phytochrome regulation of an *Arabidopsis Lhcb* gene. Plant Cell **9:** 491–507

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

Webb AAR (1998) Stomatal rhythms. *In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 69–79

Wilsbacher LD, Takahashi JS (1998) Circadian rhythms: molecular basis of the clock. Curr Opin Genet Dev **8:** 595–602

Young MW (1998) The molecular control of circadian behavioral rhythms and their entrainment in *Drosophila*. Annu Rev Biochem **67:** 135–152

Zeng H, Hardin PE, Rosbash M (1994) Constitutive expression of the *Drosophila period* protein inhibits *period* mRNA cycling. EMBO J **15:** 3590–3598

Zheng CC, Porat R, Lu PZ, O'Neill SD (1998) *PNZIP* is a novel mesophyll-specific cDNA that is regulated by phytochrome and a circadian rhythm and encodes a protein with a leucine zipper motif. Plant Physiol **116:** 27–35

Zhong HH, McClung CR (1996) The circadian clock gates expression of two Arabidopsis catalase genes to distinct and opposite phases. Mol Gen Genet **251:** 196–203

Zylka MJ, Shearman LP, Levine JD, Jin X, Weaver DR, Reppert SM (1998) Molecular analysis of mammalian timeless. Neuron **21:** 1115–1122