# Time for Plants. Progress in Plant Chronobiology

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## THE SEEDS OF CIRCADIAN BIOLOGY

More than 270 years ago plants played the pivotal role in a remarkable discovery: Organisms possess within themselves a surprisingly accurate timing device that synchronizes physiology with the daily environmental cycle. The field of circadian biology arose from the curious observation of de Mairan in 1729 that the daily leaf movements of plants (*Mimosa pudica*) persisted for several days after he placed them in his basement in constant darkness (24). Plant circadian rhythms continued to intrigue scientists from Linnaeus to Darwin, but little progress ensued until 2 centuries later, contemporaneous with the first years of *Plant Physiology*, when Erwin Bünning revived the field, again using plants as the subject of study (24). Plant studies are now poised to deliver novel insights of the inner workings of a unique 24-h biological clock, largely through the development of molecular genetic tools that allow the automation of rhythm analysis.

## REGULATION IN THE FOURTH DIMENSION

The pervasive influence of the circadian clock in plants is reflected in the variety of processes employed as circadian markers by researchers. Over the years, overt rhythms have been measured in processes such as stem elongation, root pressure, stomatal aperture, cell membrane potential, plastid migration, and gas exchange (24). Photoperiodism was first recognized in the 1920s by Garner and Allard (who coined the term) while studying the induction of flowering in tobacco and soybean, and plant researchers first established the involvement of the circadian clock in controlling these timed events (a relationship proposed by Bünning; 25).

Control points of the clock have been identified at all levels of gene expression: transcription, translation, and protein activity through posttranslational modification. For example, circadian regulation of $CO_2$ exchange in the leaves of crassulacean acid metabolism plants is accomplished by circadian activity of the $CO_2$ assimilatory phospho*enol*pyruvate carboxylase (PEPc) enzyme. The oscillation in activity derives from cyclic changes in enzyme phosphorylation state, which in turn is dependent on temporal regulation of the abundance of PEPc-specific kinase mRNA. Indirect evidence suggests that a cytological layer of regulation contributes as well, via circadian control of partitioning of malate, a feedback inhibitor of PEPc, between the cytoplasm and tonoplast (14).

## THE PLANT CLOCK GETS A DIGITAL DISPLAY

Despite progress in characterizing clock-regulated functions, the lack of a suitable assay that would allow identification of mutants affected in circadian timing presented a major obstacle for identifying components of the plant clock. Circadian phenomena can be detected only by repeated measurement of a physiological process, around the clock for several days, to reveal peaks and troughs of daily activity. Genetic investigations introduce the additional requirement of determining phenotypes from large numbers of progeny, preferably by a noninvasive assay. Strategies that proved useful in recent decades for screening animals, such as automated collection of locomotor activity data, were not adaptable to sessile plants. Apparatus for automated recording of circadian leaf movements were developed over a century ago (Fig. 1A; 24), but real progress in genetic and molecular elucidation of the plant clock awaited a more facile circadian assay.

Identification of timing mutants, and thus clock-associated genes, hinged on developing technologies to exploit the circadian oscillation of transcription of specific genes; their promoters could drive reporter genes whose expression is amenable to automated quantitation (12). Luciferases, as reporters, have sufficiently short-lived activity to allow detection of circadian troughs, and they generate a product that can be detected sensitively and by automated assay: light (Fig. 1B). Paired with ever-improving imaging technologies and methods for tagging, mapping, and cloning genes in Arabidopsis, bioluminescence reporting has provided the means to discover circadian clock-associated genes of plants, and to explore the connection between the circadian timing circuit and the signal transduction pathways of photomorphogenesis and floral development.

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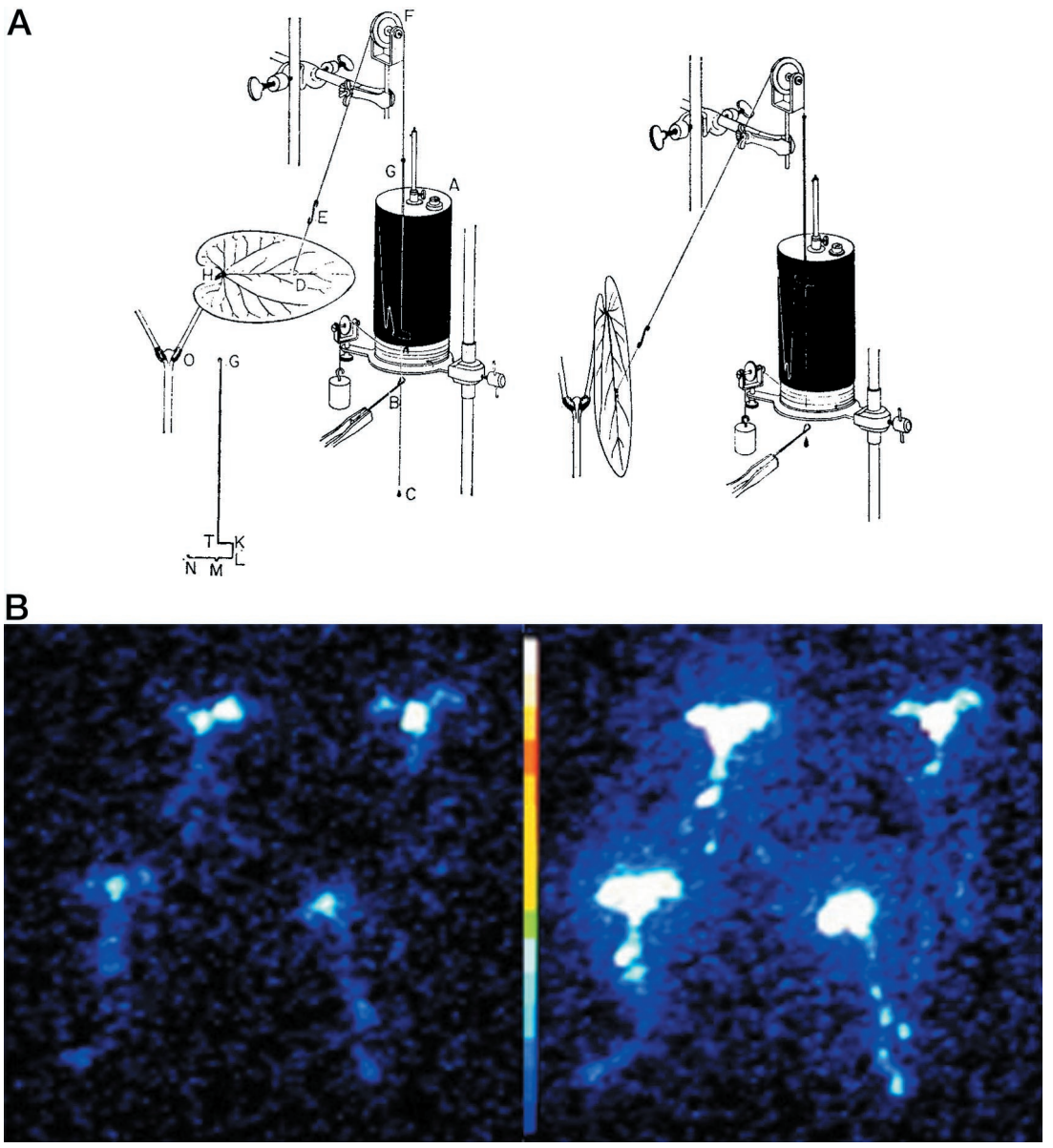


**Figure 1.** Automated monitoring systems for plant circadian rhythms. A, Leaf movement recording device designed by W. Pfeffer in the late 1800s (reprinted with permission from reference 24). The day position is shown at left, and the night position at right. B, Bioluminescence monitoring of transgenic Arabidopsis seedlings. Light emission reports expression of a *luc* (firefly luciferase) fusion to the *TOC1* promoter with a trough near dawn (left) and a peak near dusk.

## CLOCK GENETICS IN PLANTS

The first plant mutant identified in a screen specifically for circadian timing mutants was *toc1* (*timing of CAB*) of Arabidopsis (11), and this locus remains the best candidate to date for encoding a component of the plant circadian oscillator. Two point mutant alleles have been cloned, both of which cause shortening of the circadian period of all rhythms tested (23). The data are consistent with a role for *TOC1* that is central and unique to the circadian timing circuit, although its necessity for rhythmicity has not been established. TOC1 is not homologous to clock components of animals, fungi, or cyanobacteria (6); likewise, there are no homologs of most of the clock genes of other organisms in the Arabidopsis genome. Thus, it appears likely that the plant circadian mechanism will be quite different from those being revealed from other phylogenetic groups. TOC1 does not have a PAS domain, which is found in many other eukaryotic clock components (6). Rather, it exhibits motifs not described previously in circadian systems: a receiver domain common among the response regulator proteins of bacterial two-component sensory transduction systems, and a basic motif found in the

CONSTANS family of plant proteins (10, 23). The sequence features of TOC1 and its nuclear localization suggest a role in transcriptional control.

Other clock-associated genes have been described in recent years, such as those that encode the Myb-type transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (26) and LATE ELONGATED HYPOCOTYL (18), and the novel protein GIGANTEA (7, 16). Most of these were found by recognition of a circadian phenotype in mutants that were originally defined by defects in photomorphogenesis or flowering time. These mutants underscore a convergence between the circadian timing system and that of photoperiodic regulation of flowering, and the reliance of both of these processes on the perception of light.

## PHOTORECEPTORS PROVIDE ENVIRONMENTAL INPUT TO THE CLOCK

One canonical property of circadian systems is the ability to be reset, or to have the timing of peaks and troughs synchronized to the sidereal day. In plants, photoreceptors that are important for photomorphogenesis, the cryptochromes (1) and phytochromes (17), are also used to entrain the circadian clock to the day/night cycle, and they influence the endogenous period of the clock (20). Cryptochromes serve light input roles to the clock in both Arabidopsis and *Drosophila melanogaster*, but in mammals they are more closely involved in the oscillator mechanism in a light-independent capacity (1). The sharing of this class of proteins among the circadian systems of diverse organisms is not strong evidence for homology of clock mechanisms because cryptochromes likely diverged from DNA photolyases, which are universally distributed and have had ample opportunity for convergence of function throughout evolution (1).

The phytochromes as clock input photoreceptors have parallels in the circadian systems of another group, the cyanobacteria. A bacteriophytochrome, CikA (circadian input kinase), is important for resetting the clock in *Synechococcus elongatus* (19). CikA has similarity to the lyase domain of phytochromes, and to the His protein kinase domain of bacterial two component sensors. It is intriguing that the protein also carries an unusual receiver motif that, like the motif in TOC1, lacks the expected aspartyl residue needed for phosphoryl transfer in response regulator proteins (22). Whether this similarity between TOC1 and CikA is coincidental, or indicative of an important biochemical function that cyanobacterial and plant mechanisms share, has yet to be determined. As is true for cryptochromes, bacteriophytochromes and phytochromes have widely diverged family members (8), and their presence in diverse circadian input systems may owe more to the ease of sculpting a handy cofactor-binding domain for a variety of functions than to lineage.

The affected gene in the *toc7* period mutant *zeitlupe* (*ZTL*) suggests the role of another class of signal transduction proteins in providing light information to the circadian clock (20, 21). *ZTL* period phenotypes are evident in a variety of circadian-controlled processes, and are strongly fluence dependent. However, acute responses to light and photomorphogenesis are not affected by *ZTL* mutation. Thus, ZTL may define a signal input mechanism that is more specifically allied with the clock than are the previously described photoreceptor pathways. The protein sequence reveals similarity to the PAS domains of the blue-light sensitive NPH1 (3) and white-collar proteins (5) of plants and fungi, respectively, as well as kelch motifs. The latter predict a conserved tertiary structure with unknown activity, called a beta propeller, which is found in a variety of proteins of diverse cellular function. *ZTL* defines a small gene family that includes one paralog identified as the affected gene in a flowering time mutant, *fkf1* (13).

None of the known light signal transduction genes appears to be individually essential for circadian timing or entrainment to a diurnal world. Rather, each has a role in a providing a subset of light property information, fine-tuning the clock in an environment of changing fluence and light quality.

## FUTURE PROSPECTS

Insights into the molecular workings of the plant clock are unfolding at an increasing pace, and will be accelerated dramatically by various genome projects. Components of the plant circadian clock will likely have partners that interact with constituents of other systems as well, connecting the circadian timing mechanism with plant development and light-regulated processes. Additional signatures of the bacterial contributors to the genome may be evident in plants that are not seen in animals, like the receiver domain of TOC1. Homology of plant oscillators with those of other groups of organisms is unlikely, but conservation of some mechanisms is probable. Extensive posttranscriptional and especially post-translational control is expected. Shuttling of clock proteins among subcellular compartments occurs in animal systems (2, 6), and is a feature of phytochromes (27). Phosphorylation of the FRQ protein of *Neurospora crassa* and the PER protein in *D. melanogaster* influences the accumulation and turnover of these central clock proteins (6); the cyanobacterial clock protein KaiC is also phosphorylated, although the function of this modification has not been demonstrated (15). The wealth of information accessible from the genome will allow deeper investigation of protein turnover in circadian timing by assessing the contributions of identifiable players in the ubiquitination and proteasome pathways (4).

Molecular technologies will reveal a more complete view of the extent to which the clock controls plant physiology. Approaches such as differential display can expand the search for circadian regulated genes

without prejudice (9). The most comprehensive global pictures will come soon from analysis of arrays of genes identified from genome projects in Arabidopsis and other species, which will allow the recognition of patterns of circadian coregulation among groups of genes, at least some of which can be assigned to known physiological pathways.

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