LIVING BY THE CALENDAR: HOW PLANTS KNOW WHEN TO FLOWER

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Reproductive processes in plants and animals are usually synchronized with favourable seasons of the year. It has been known for 80 years that organisms anticipate seasonal changes by adjusting developmental programmes in response to daylength. Recent studies indicate that plants perceive daylength through the degree of coincidence of light with the expression of *CONSTANS*, which encodes a clock-regulated transcription factor that controls the expression of floral-inductive genes in a light-dependent manner.

DAYLENGTH
(photoperiod). The duration of the illuminated phase of a daily light/dark cycle.

PHOTOPERIODIC RESPONSE
The biological response to changes in daylength, or photoperiod, that are associated with seasonal adaptations.

CIRCADIAN RHYTHM
A rhythm with an approximate 24-h period.

ENTRAINMENT
The synchronization or adjustment of a rhythm to another cycle of similar periodicity. In the case of circadian rhythms, it refers to their synchronization to the 24-h solar cycle in response to changes in environmental cues such as light and temperature that normally occur at dawn and dusk.

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Seasonal changes in light, temperature and rainfall have strongly influenced the evolution of life on earth. Most organisms adjust the timing of crucial developmental processes so that they occur at times of the year that maximize their chance of survival and reproductive success[1]. In 1920, Garner and Allard discovered that the onset of flowering in many species is triggered by changes in DAYLENGTH, or photoperiod, which is an environmental cue associated with seasonal progression[2]. They found that some plants flower faster (or only) when the photoperiod is shorter than some critical value, others when its longer, and a third group flower independently of daylength; these are known as short-day plants (SDPs), long-day plants (LDPs) and day-neutral plants, respectively.

Here we review the significant advances that have been made recently towards the elucidation of the molecular mechanisms that underlie daylength measurement in the LDP *Arabidopsis* and the SDP rice. We also discuss briefly how *Arabidopsis* plants integrate daylength measurement with temperature perception to ensure an appropriate seasonal regulation of flowering time.

Models of daylength measurement

Several models have been developed during the past century to explain PHOTOPERIODIC RESPONSES[3]. The hourglass model proposes that responses to daylength result simply from the direct effects of light on some reaction (or reactions), such that certain processes are induced or repressed when the duration of light or darkness allows key regulatory products (the sand of the hourglass) to reach some threshold level (FIG. 1a). If this is true, increasing the hours of darkness in the light/dark cycle should either promote (SDPs) or inhibit (LDPs) flowering until a threshold duration is reached, after which further increments should have no other effect. In contrast to this prediction, the floral response of many plant species to cycles of 8 h of light and increasing hours of darkness varies rhythmically, with a maximum response every time the total length of the cycle is 24 h or a multiple of it, and a minimum response at intermediate cycle lengths[4]. CIRCADIAN RHYTHMS in floral responses are also observed when plants that are grown under cycles of 8 h of light and 64 or more hours of darkness are exposed to short pulses of light at different times during the dark interval[4].

These observations provided strong support for the hypothesis that was proposed by Erwin Bünning, who postulated as early as 1936 that time measurement in seasonal responses relies on a circadian oscillator that is similar to that used by organisms to time multiple processes throughout the day[5] (BOX 1). What Bünning stated specifically was that a circadian clock drives a rhythm in a light-sensitive process, and that photoperiodic responses are promoted (LDPs), or inhibited (SDPs), when the illuminated part of the day overlaps with the most sensitive phase of this endogenous rhythm. A refinement of Bünning's hypothesis, which includes the role of light in synchronizing, or ENTRAINING, the clock to the solar cycle (BOX 1), is known as the external coincidence model[6] (FIG. 1b).

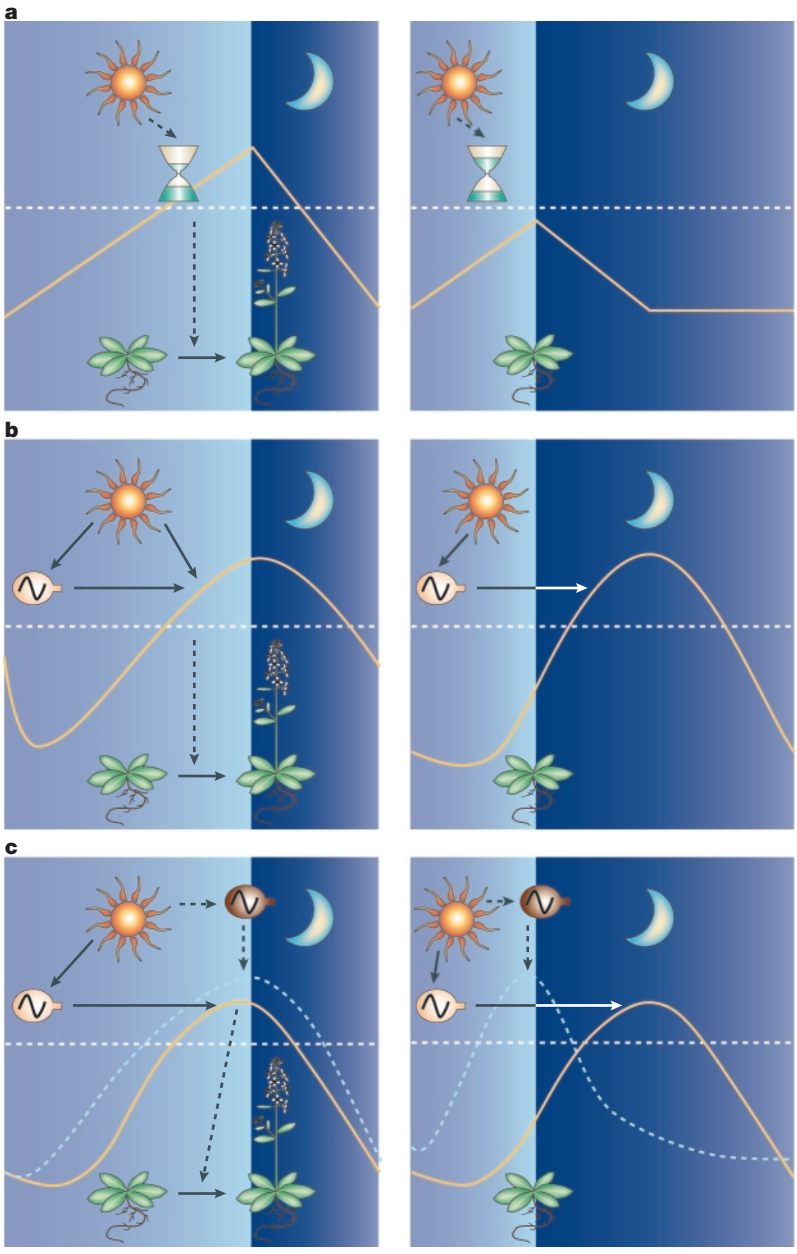


Figure 1 | **Physiological models of photoperiodic time measurement. a** | The hourglass model states that daylength is measured through some regulatory product (yellow line), the accumulation of which is light dependent. Photoperiodic responses are triggered when this product accumulates above a certain threshold level (white dotted line). **b** | The external coincidence model proposes that daylength measurement relies on a circadian oscillator that controls the levels of some regulatory molecule (yellow line), the activity of which is modulated by light. Photoperiodic responses are triggered when the illuminated part of the day overlaps with a phase of the cycle during which the levels of the regulatory molecule are above a certain threshold (white dotted line). In this model, light functions in two ways: first, by promoting (long-day plants) or inhibiting (short-day plants) flowering when it is present at a particular phase of the circadian cycle; and second, by setting the phase of the circadian oscillator that controls the levels of the key regulator. **c** | The internal coincidence model implies that photoperiodic responses are induced by the coincidence of two or more rhythms that overlap only under certain photoperiods. This would be the case if some rhythms are timed at a fixed interval from dawn (yellow line) and others from dusk (light-blue dotted line), because they are driven by distinct circadian oscillators (beige and brown, respectively)[105] or by similar or identical clocks in separate tissues containing cell-specific regulatory factors[106,107], which respond differently to light/dark transitions.

In the 1960s, Pittendrigh proposed that photoperiodic responses could be triggered by the coincidence of two or more endogenous rhythms[7]. This model, which is known as internal coincidence, proposes that light functions exclusively through its effect on clock entrainment, by ensuring that the phases of two or more rhythms overlap only under inductive photoperiods. This could happen, for example, if some rhythms are timed at a fixed interval from dawn and others from dusk (FIG. 1c).

In agreement with Bünning's hypothesis (that circadian clocks constitute the timing mechanism of seasonal responses), several mutants that were identified in *Arabidopsis* on the basis of their defective photoperiodic regulation of flowering — such as *early flowering 3* (*elf3*)[8], *late elongated hypocotyl* (*lhy*)[9], *gigantea* (*gi*)[10,11] and *early flowering 4* (*elf4*)[12] — also show aberrant circadian rhythms. Conversely, mutants that were originally isolated for their circadian defects, such as *timing of cab expression 1* (*toc1*)[13–15] and *zeitlupe* (*ztl*)[16], also show a reduced daylength sensitivity.

A caveat to this correlation is that most of the clock mutants mentioned above also show defective PHOTOMORPHOGENIC RESPONSES, which makes it unclear whether their photoperiodic defects are due to alterations in light or circadian signalling. An exception to this is the clock mutant *toc1-1*, which is a unique allele of *TOC1* that shows 21-h rhythms irrespective of light conditions[13–15] and lacks obvious light-dependent morphological phenotypes[14,17]. Furthermore, the daylength-insensitive, early-flowering phenotype of *toc1-1* is observed under environmental cycles of 24 h (but not under cycles of 21 h), which match the endogenous period of this mutant[15]. This indicates that the photoperiodic insensitivity of *toc1-1* is entirely due to its clock defect, and shows that the circadian clock, which is responsible for timing processes throughout the day, is a key component of the molecular calendar, which times developmental processes throughout the year.

The plant circadian clock

The identification of mutants that are defective in both flowering time and circadian rhythmicity not only provided genetic evidence for a role of circadian clocks in daylength measurement, but cloning of the genes is allowing us to understand the molecular basis of the plant circadian oscillator (FIG. 2). In all organisms analysed so far, circadian rhythms are based on transcriptional/translational feedback loops in which some proteins negatively control their own expression by antagonizing the action of positively regulating transcription factors[18].

We recently proposed that a feedback loop that is crucial for robust circadian rhythmicity in *Arabidopsis* relies on the interaction of *TOC1*, *LHY* and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*)[19]. CCA1 and LHY are two MYB-related transcription factors, the mRNA and protein levels of which cycle with a peak at dawn[9,20]. The overexpression of *CCA1* or *LHY* from a constitutive promoter leads to the downregulation of

TEMPERATURE COMPENSATION
The ability of circadian clocks to maintain a relatively constant pace over a wide temperature range.

PHOTOMORPHOGENIC RESPONSE
The morphological and physiological adaptation of plants to changes in the quality and quantity of their light environment.

PSEUDO-RESPONSE REGULATOR
A protein that shares strong sequence similarity to response regulators of bacterial two-component signalling systems, but that lacks the conserved residues that are phosphorylated by a sensor kinase, which modulates its activity.

Box 1 | General properties of circadian clocks

Circadian clocks regulate many biological activities in eukaryotes and prokaryotes, by synchronizing them with the environmental day/night cycles[18]. Examples of processes in plants that are under circadian control include leaf movement, the opening and closing of stomatal pores and the expression of genes that are involved in the photosynthetic process, cell elongation and flowering-time regulation. The control of these processes by circadian clocks allows plants to anticipate periodic changes that occur in their environment. Under constant environmental conditions, however, circadian clocks free-run with periods close to, but not exactly, 24 h. To keep pace with the solar cycle and maintain their anticipatory function throughout the year, circadian clocks are adjusted by environmental signals such as the light–dark transitions at dawn and dusk, a phenomenon that is known as entrainment. In addition, the free-running period of circadian rhythms is largely unaffected by temperature. This property of the circadian system, which is known as TEMPERATURE COMPENSATION, allows circadian clocks to measure time precisely throughout the year.

each other's expression and a generalized arrhythmia[9,20], which is a phenotype that is also observed when their functions are simultaneously reduced[21,22]. This implies that CCA1 and LHY are partially redundant components of a negative-feedback loop that is essential for generating and/or sustaining circadian rhythms.

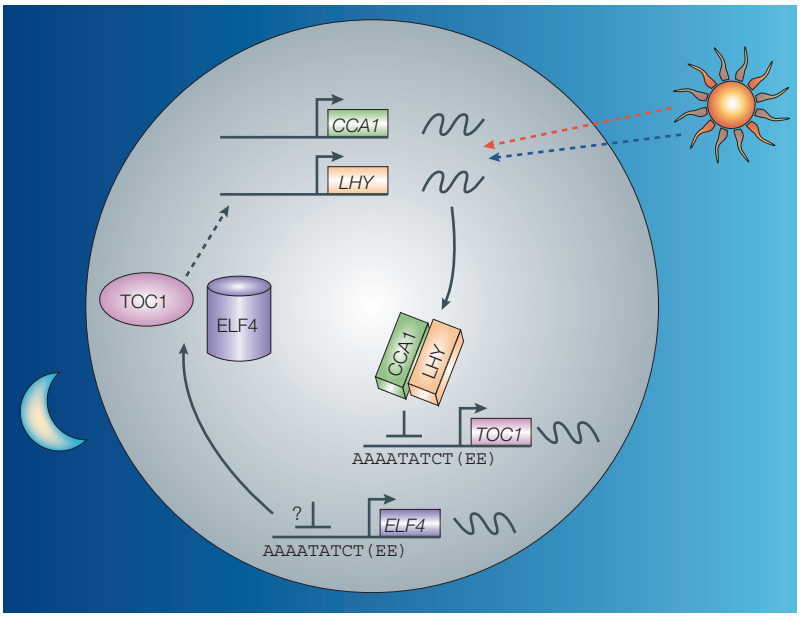


Figure 2 | **Molecular interactions that shape the plant circadian oscillator.** Circadian rhythms in *Arabidopsis* are thought to depend on a transcriptional/translational negative-feedback loop. During the late evening, TOC1 (timing of cab expression 1) and ELF4 (early flowering 4) activate expression of the transcription factor genes *CCA1* (*CIRCADIAN CLOCK ASSOCIATED 1*) and *LHY* (*LATE ELONGATED HYPOCOTYL*) through an as-yet-unidentified mechanism. CCA1 and LHY levels peak at dawn, presumably when phytochromes that are activated by light move to the nucleus to upregulate the expression of these transcription factors. In addition, high levels of CCA1 and LHY repress the expression of *TOC1* through their binding to the evening element (EE; AAATATCT) that is present in the *TOC1* promoter. EEs are also present in the promoter of *ELF4*, which indicates that its expression might also be repressed by CCA1 and LHY. Owing to repression of their positive regulator (or regulators) — TOC1 and, presumably, ELF4 — CCA1 and LHY levels decrease during the day. This, in turn, releases the inhibition on *TOC1* (and possibly *ELF4*) expression, which finally peaks at dusk when CCA1 and LHY levels are at their trough. Closing the cycle, TOC1 and ELF4 accumulate during the evening, promoting again the expression of *CCA1* and *LHY*. This model is an oversimplification of the molecular interactions that form the circadian oscillator in *Arabidopsis*. Post-transcriptional regulations, such as phosphorylation of CCA1 and LHY by casein kinase 2, and regulation of the stability of as-yet-unidentified clock components by the putative photoreceptors zeitlupe (ZTL), flavin-binding kelch repeat F-box 1 (FKF1) and LOV kelch protein 2 (LKP2), also have crucial but less well-understood roles in regulating clock progression. In addition, there are four homologues of *TOC1* in the *Arabidopsis* genome that cycle and peak at different times of the day, which might also have an important role in the circadian system[15,23,24,108,109].

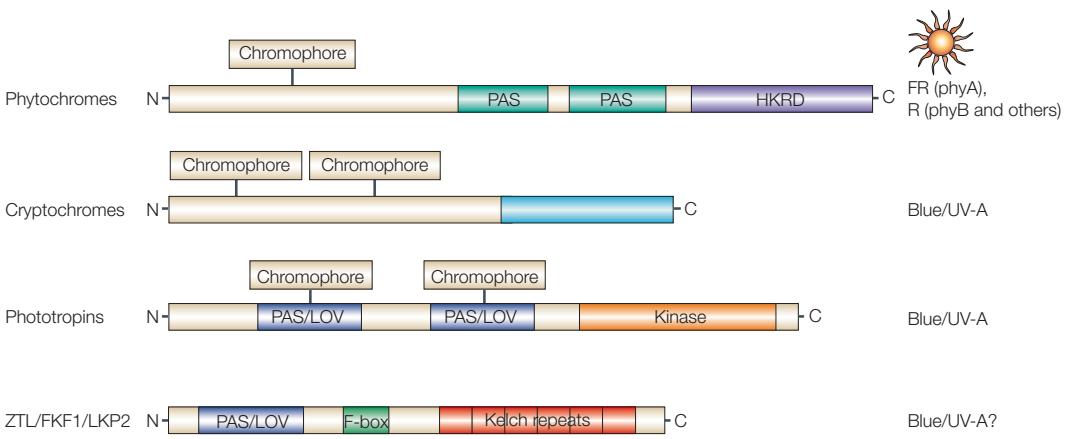
TOC1 encodes a PSEUDO-RESPONSE REGULATOR, the expression of which cycles with a peak at dusk[15,23,24]. Consistent with a central role for TOC1 in the circadian oscillator, mutations in *TOC1* shorten the period of all circadian rhythms tested so far[13–15,19]. In addition, increments in the dosage of *TOC1* lengthen the period of circadian oscillations[17], and overexpression of *TOC1* from a constitutive promoter causes arrhythmicity[17,25]. TOC1 has several motifs that indicate that it has a role in transcriptional regulation[15] and, indeed, *CCA1* and *LHY* mRNA levels are reduced in the *toc1-2* mutant[19] (which is probably a null allele[15]). *CCA1* expression is also markedly reduced in *elf4*, a mutant that is arrhythmic in both continuous light and continuous darkness[12]. ELF4 lacks homology to proteins of known function, but its expression cycles with a phase that is similar to that of *TOC1*, which indicates that these two proteins could function together to promote *CCA1* and *LHY* expression[12]. Closing the feedback loop, CCA1 and LHY downregulate their own expression by repressing that of *TOC1*. This occurs through their binding to a 9-nucleotide-element in the *TOC1* promoter (AAATATCT) that is crucial for its circadian regulation[19].

Interestingly, the element that CCA1 and LHY recognize in the promoter of *TOC1* is over-represented in a cluster of evening-phased genes and is therefore known as the evening element (EE)[26]. Furthermore, the EE is almost identical to the sequence that CCA1 recognizes in the promoter of *LIGHT HARVESTING CHLOROPHYLL A/B BINDING PROTEIN 1* (AAAAATCT), a clock-regulated gene, the expression of which peaks in the morning[27]. So, in addition to being part of a feedback loop that is crucial for circadian rhythmicity, CCA1 and LHY might function to repress the expression of several evening-phased genes and promote that of morning-phased genes, providing a direct mechanism that links the circadian oscillator to numerous biochemical and physiological processes. Indeed, circadian clocks from plants to mammals regulate metabolic and developmental activities by controlling directly or indirectly the expression of key regulatory genes[26,28], a mechanism that has a crucial function in the photoperiodic regulation of flowering time in *Arabidopsis* and rice (see below).

The photoperiodic photoreceptors

An important step towards understanding how plants measure daylength is the identification of the pigments

Box 2 | **The plant photoreceptors**



Phytochromes are red-light/far-red-light (R/FR) photoreceptors that perceive light through a tetrapyrrole chromophore that is bound covalently to their amino-terminal photosensory domain[30]. The carboxy-terminal domain contains two PAS (for period circadian protein, Ah receptor nuclear translocator protein and single-minded protein) repeats, which initiate a signalling cascade by mediating direct interactions with molecules such as the basic-helix-loop-helix transcription factor PIF3, and a histidine-kinase-related domain (HKRD), which might phosphorylate direct targets such as phytochrome kinase substrate 1 (a protein that negatively regulates phytochrome signalling)[30]. The light-labile phytochrome (phy)A is more active in FR, whereas phyB and other light-stable phytochromes are more active in R. This difference is due in part to their differential light-stability, but also to other properties that are specific to the phyA domain[103].

Cryptochromes are blue/UV-A photoreceptors that bind pterin and flavin chromophores at their amino-terminal domain[104]. Blue-light activation of cryptochromes initiates a signalling cascade through their carboxy-terminal domain[104]. This signalling cascade operates in part through the direct inactivation of constitutive photomorphogenic 1 (COP1), which is a general repressor of photomorphogenic responses[104].

Phototropins have two PAS/LOV domains that bind a flavin mononucleotide (FMN) chromophore[38]. The absorption of blue light triggers the formation of covalent adducts between FMN and cysteine residues in the PAS/LOV domains, which induce a conformational change that is thought to initiate a signalling cascade through activation of the serine/threonine kinase activity at the carboxy-terminal domain[38].

Zeitlupe (ZTL), flavin-binding kelch repeat F-box 1 (FKF1) and LOV kelch protein 2 (LKP2) share a unique combination of motifs, which includes an amino-terminal PAS/LOV domain, an F-box domain that probably recruits proteins for ubiquitylation and subsequent degradation, and six kelch repeats that mediate protein–protein interactions[16,39–42]. The PAS/LOV domain of this family of proteins might bind FMN, allowing these molecules to target specific proteins for degradation in a light-dependent manner.

that discriminate days from nights. This has been accomplished by using both physiological and genetic approaches. In SDPs, red light is the most effective wavelength to inhibit flowering when given as a short pulse of light in the middle of a long night, which functions as a night-break treatment that mimics the effect of long days[4]. A short pulse of red light given in the middle of a long night is also very effective at accelerating flowering in some LDPs[4], but in *Arabidopsis* and other LDPs far-red light and blue light are much more effective than red light[4,29].

Plants monitor changes in light by using at least three families of photoreceptors (BOX 2). The red-light and far-red-light region of the spectrum is perceived by phytochromes, a small family of CHROMOPROTEINS that is encoded by five genes in *Arabidopsis*, *PHYTOCHROME* (*PHY*) *A*, *B*, *C*, *D* and *E*[30]. The characterization of mutants that are defective for one or several phytochromes indicates that the light-stable phyB, phyD and phyE mediate responses to red light, whereas the light-labile phyA is the main photoreceptor that discriminates far-red light from darkness[30]. Consistent with this, *Arabidopsis* plants that lack phyA flower much later than wild-type seedlings when grown under SHORT-DAY CONDITIONS that are extended for several hours with incandescent light, which is rich in far-red light[31]. phyA also has an important role in the perception of long days in the pea, another LDP[32,33]. By contrast, phyB, phyD and phyE only have secondary roles in the photoperiodic regulation of flowering time in *Arabidopsis*, because even wild-type plants do not discriminate short days from long days when these days are provided as monochromatic red light[34]. Nonetheless, phyB contributes partially to daylength discrimination in *Arabidopsis* through its interactions with other photoreceptors[34] (see below). On the other hand, rice *phyA* mutants flower simultaneously with wild-type plants[35], whereas a rice mutant that is defective in the biosynthesis of the phytochrome CHROMOPHORE (which is common to all phytochromes) is insensitive to photoperiod, and flowers as early on long

CHROMOPROTEIN
A protein that is linked to a chromophore, which allows the holoprotein (protein plus chromophore) to work as a photoreceptor.

SHORT-/LONG-DAY CONDITIONS
In *Arabidopsis*, short-day conditions usually consist of 8–10-h photoperiods, and long-day conditions of 14–16-h photoperiods. The length of the day that, when exceeded, promotes or inhibits flowering varies for each species.

CHROMOPHORE
A molecule that selectively absorbs certain wavelengths.

days as it does on short days[36]. So, stable phytochromes seem to be the main photoperiodic photoreceptors in this SDP.

The effects of blue light on plant growth and development have been known since the nineteenth century, but the corresponding photoreceptors — cryptochromes and phototropins — were identified only recently[37,38]. Phototropins 1 and 2 have a crucial function in blue-light-dependent phototropic responses and are also known to control chloroplast movement and stomatal opening[38]. So far, no effect on flowering time has been reported for the phototropins, but their chromophore-binding site (which is a PAS/LOV domain) is highly similar to the PAS (for period circadian protein, Ah receptor nuclear translocator protein and single-minded protein) signal-sensor domain that is present in ZTL, flavin-binding kelch repeat F-box 1 (FKF1) and LOV kelch protein 2 (LKP2), which are part of an novel family of proteins that regulate flowering time and circadian rhythms in *Arabidopsis*[16,39–42]. The similarity between the PAS/LOV domains of phototropins, ZTL, FKF1 and LKP2 indicates that the latter proteins could function as new photoperiodic and/or circadian photoreceptors.

Finally, cryptochromes are soluble flavoproteins that share strong similarity to bacterial DNA photolyases[37]. They were identified originally for their role in the blue-light-dependent inhibition of stem growth in *Arabidopsis*, in which they are encoded by two genes, *CRYPTOCHROME* (*CRY*) *1* and *CRY2* (REFS 43,44). More recently, cryptochromes have also been found in flies and mammals, in which they work as blue-light circadian photoreceptors and/or as crucial components of the circadian clock[45]. Interestingly, *cry2* mutants in *Arabidopsis* flower much later than wild-type plants during long but not short days, which indicates that cry2 has an important role in the photoperiodic regulation of flowering time[46]. The effect of cry2 under white-light conditions requires the presence of active phyB[47], with which it associates physically in the nucleus[48]. In blue light, cry2 regulates flowering time redundantly with cry1 and phyA[34]. So, cry2 and phyA seem to be the principal photoperiodic photoreceptors, discriminating day from night in *Arabidopsis*, and their interaction with other photoreceptors might ensure that they flower appropriately under the wide range of light environments that plants experience throughout their life cycle[34,47–49].

Daylength measurement in *Arabidopsis*

The ability of plants to tell the time and discriminate day from night is essential for daylength measurement. But how are these two processes integrated to differentiate long days from short days? Is it exclusively through the effect of light on clock entrainment (as predicted by the internal coincidence model), or does it also involve the clock regulation of a direct effect of light on photoperiodic responses (as proposed by the external coincidence model)?

Light regulation of clock progression. If daylength measurement operates exclusively through an internal coincidence mechanism, the reduced photoperiodic sensitivity of photoreceptors and light-signalling mutants should be explained completely by defects in the entrainment of the circadian system to the solar cycle.

The role of individual photoreceptors in clock entrainment has recently been analysed in *Arabidopsis*. These studies indicate that phyA is required for clock adjustment under very low-intensity red light and high-intensity far-red light, whereas phyB, phyD and phyE mediate clock entrainment in response to high-intensity red light[50–52]. On the other hand, cry1 is important for clock resetting by high-intensity blue light, whereas cry1, cry2 and phyA function redundantly at low and intermediate intensities of this wavelength[50,51,53]. ZTL, FKF1 and/or LKP2 could also function as extra circadian photoreceptors. This is predicted, not only on the basis of their similarity to phototropins, but also on the light-dependent circadian phenotypes of *ztl* mutants[16,42]. Consistent with this hypothesis, *phyAphyBcry1cry2* quadruple mutants still respond to light signals that reset the clock[54], which indicates that other photoreceptors function redundantly with them in the control of these processes. Interestingly, ZTL interacts *in vitro* with phyB and cry1 (REF. 42). So, alternatively, or in addition to their role as circadian photoreceptors, ZTL family members could be functioning as components of phytochrome and cryptochrome signalling pathways that regulate clock progression.

Although there is no direct evidence for how circadian photoreceptors and light-signalling components ultimately reset the clock, two possibilities can be envisioned. First, ZTL family members share, in addition to the PAS/LOV domain, an F-box region that might target proteins for degradation[55], as well as six terminal kelch repeats, which most probably mediate specific protein–protein interactions[56]. So, ZTL, FKF1 and/or LKP2 could reset the circadian oscillator by targeting clock components for degradation in a light-dependent manner.

Second, resetting of the clock by light could result from changes in the expression of a clock component. In dark-grown seedlings, a short pulse of light induces (or upregulates) *CCA1* and *LHY* expression through the interaction of the phytochromes with phytochrome-interacting factor 3 (PIF3), a basic-helix-loop-helix (bHLH) transcription factor that binds to a CACGTG element in the promoter of these genes[57]. Interestingly, TOC1 and PIF3 interact in yeast and *in vitro*, which indicates that this interaction could modulate the effect of phytochrome (or phytochromes) on the circadian oscillator[25]. Consistent with this possibility, plants with severely reduced *TOC1* mRNA levels (such as *toc1-2* and *TOC1* RNA-interference mutant plants) have morphological and circadian alterations when exposed to red light, as well as a decreased response to red-light pulses that induce *CCA1* and *LHY* expression[17]. These phenotypes contrast strongly with the light-independent circadian defects of the *toc1-1* allele (see above), which is caused by a missense mutation[15]. So, in addition to having a light-independent role in the circadian system[15],

PAS/LOV
PAS is a signalling domain that was identified initially in period circadian protein, Ah receptor nuclear translocator protein and single-minded protein. It mediates protein–protein interactions and/or binds small ligands. LOV domains are a subset of PAS domains that are found in signalling proteins that are activated by light, oxygen or voltage.

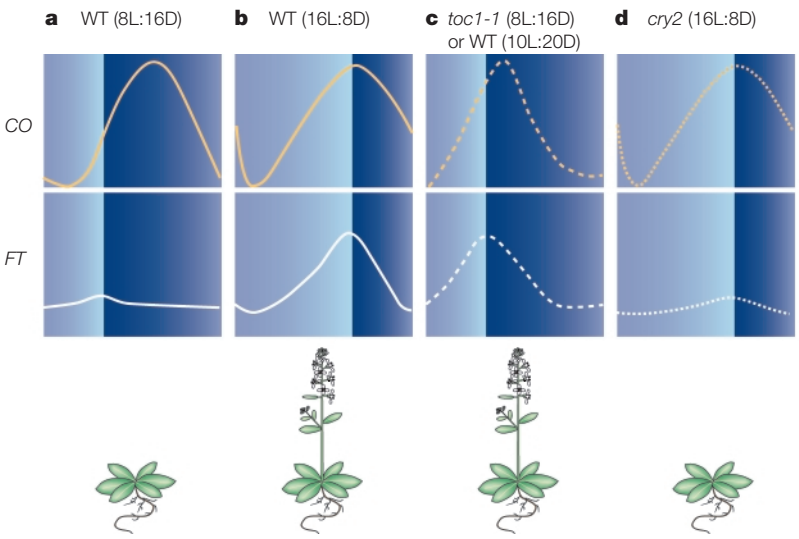


Figure 3 | **Photoperiodic regulation of *CO* and *FT* expression. a** | In wild-type (WT) *Arabidopsis* seedlings that are grown on short days (8 h light: 16 h dark), *CONSTANS* (*CO*) expression peaks during the night-time and is mostly confined to the dark period (upper panel). Under this condition, *FLOWERING LOCUS T* (*FT*) expression is very low but shows a small increase at dusk (lower panel). **b** | The peak of *CO* expression is broader on long days (16 h light: 8 h dark) and, although *CO* mRNA levels are still highest at night, there is a significant overlap between high *CO* levels and the illuminated part of the day at dusk (upper panel). Consistent with CO induction of *FT* expression being light dependent, *FT* mRNA levels are highest at dusk on long days (lower panel). **c** | *CO* expression can be shifted towards daytime in plants grown under short-day conditions by accelerating the clock, as in *toc1-1* mutants, or by increasing the total length of the external light/dark cycle to 30 h (10 h light: 20 h dark; upper panel). This causes an upregulation of *FT* mRNA levels that lead ultimately to an acceleration of flowering time (lower panel). **d** | The pattern of *CO* expression in cryptochrome *cry2* mutants grown on long days (a condition in which they are late flowering) is similar to that observed in wild-type plants (compare part **d** and **b**, upper panels). On the other hand, *FT* mRNA levels are reduced markedly in *cry2* mutants compared with wild-type seedlings under this environmental condition (compare part **d** and **b**, lower panels).

TOC1 might modulate phytochrome signalling to the clock and other developmental processes through its interaction with PIF3 and/or other related bHLH transcription factors.

Finally, ELF3 and GI are two other proteins that function in phytochrome regulation of clock progression. The circadian phenotypes of *gi* and *elf3* mutants are light dependent and are also associated with reduced *CCA1* and *LHY* mRNA levels[8,10,11,58]. Furthermore, both the *elf3* and *gi* mutants show defects in their photomorphogenic responses, in addition to their alterations in flowering time and circadian rhythms, which indicates that they are involved in the early steps of phytochrome signalling[59–62]. Although the molecular and biochemical nature of GI action is largely unknown, ELF3 seems to antagonize light responses through a direct interaction with phyB[58,62,63].

It is important to note, however, that in most of the cases analysed so far, the reduced photoperiodic sensitivity of photoreceptor and light-signalling mutants is not clearly attributable to defects in clock entrainment. For example, the *cry2* mutant is severely impaired in the photoperiodic regulation of flowering under high-intensity white light[44] but only shows minor alterations in clock entrainment under this condition[48,50]. In addition, the opposite is observed in the phyA signalling mutant *fhy1*, which is impaired in a subset of physiological responses to far-red light[65,66], including clock entrainment[53], but flowers like wild-type seedlings when grown under short-day conditions that are extended with light rich in far-red light[31]. This indicates strongly that phyA regulates clock progression and flowering time by at least two partially independent signalling pathways.

Clock regulation of light signalling. The lack of a strong correlation between flowering time and circadian defects in most light-signalling mutants indicates that light has a more direct role in the regulation of flowering time and, as proposed by the external coincidence model, clock regulation of light signalling would ensure that flowering is promoted only under long-day conditions. So, how does the clock regulate the sensitivity to light signals that induce photoperiodic responses? There is good evidence that the clock regulates several early steps of the phytochrome and cryptochrome signalling cascades.

To begin with, the circadian clock controls the expression of all of the phytochromes and cryptochromes[26,67–69]. Indeed, robust diurnal oscillations in proteins levels are detected for phyA[34,70] and cry2 (REFS 34,71) particularly under short-day conditions, although these oscillations are mainly the result of direct effects of light on phyA and cry2 stability[34,70]. Evidence that changes in cry2 levels during the course of the day are important for daylength discrimination is provided by a newly discovered *cry2* allele, which increases the stability of the protein and causes an early-flowering, daylength-insensitive phenotype[71].

In addition, the subcellular localization and/or activity of phyA and other phytochromes might change rhythmically, as indicated by diurnal cycles in the ability of these proteins to localize to nuclear speckles[72]. The biological role of these speckles is uncertain, but they might represent active transcriptional complexes, as phytochrome molecules operate in part by interacting with PIF3 and other nuclear-localized transcription factors to modulate the expression of several genes[57,73].

Finally, several positive and negative regulators of phytochrome signalling, such as ELF3, GI and SUPPRESSOR OF PHYA1 (SPA1), are also controlled by the clock at the transcriptional level[10,26,58,74] and, at least for ELF3, clock regulation of its function has also been shown[58,63]. So, diurnal variations in the level or activity of any of these proteins might contribute to ensuring that direct effects of light on flowering can only take place at particular times of the day on long, but not short, days.

Measuring daylength through CONSTANS. In addition to the possibilities discussed above, recent studies indicate strongly that *Arabidopsis* plants discriminate short days from long days by integrating circadian and light signalling pathways at the level of *CONSTANS* (*CO*). Mutations in *CO* delay flowering on long but not short days[75] and, in contrast to the flowering-time mutants

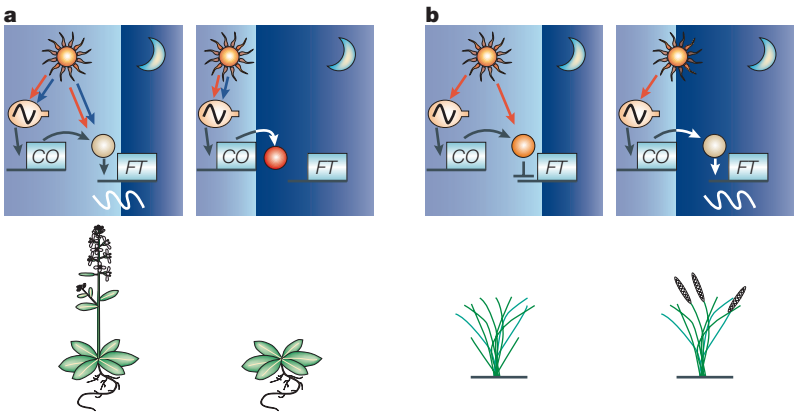


Figure 4 | **The regulation of flowering time by photoperiod in *Arabidopsis* and rice.** The expression of *CONSTANS* (*CO*) orthologues in the short-day plant rice and the long-day plant *Arabidopsis* is regulated in similar ways, by ensuring that there is a much longer overlap of *CO* expression with the illuminated part of the day on long days than on short days. **a** | In *Arabidopsis*, light perceived by photoperiodic photoreceptors phyA and cry2 (red and blue arrows, respectively) activates *FLOWERING LOCUS T* (*FT*) expression through CO (beige circle), and this promotes flowering on long days. On short days, *FT* expression remains low, as there is minimal overlap between light and CO (red circle), and this delays the floral transition. **b** | In rice, light perceived by stable phytochromes (red arrow) represses the expression of *FT-like* genes through CO (orange circle), whereas CO activates their expression in the dark (beige circle). As *FT-like* genes promote flowering, rice plants flower more rapidly on short days than long days. The beige circle represents CO functioning as an activator of *FT* expression, the orange circle represents CO acting as a repressor of *FT-like* genes and the red circle represents an inactive form of CO.

described previously, do not cause pleiotropic alterations in circadian rhythms or light-regulated responses. *CO* encodes a transcriptional regulator that accelerates flowering time on long days through the direct upregulation of *FLOWERING LOCUS T* (*FT*)[75–79]. The biochemical mechanism of FT action is unknown, but increments in *FT* mRNA levels above a certain threshold promote the expression of MERISTEM-IDENTITY GENES[77], which in turn trigger the transition from vegetative to reproductive development at the shoot apical MERISTEM.

Interestingly, *CO* expression is regulated by the circadian clock in such a way that it is confined mostly to the dark period on short days[80] (FIG. 3a), whereas high levels of *CO* mRNA overlap with the illuminated part of the day at dawn and dusk on long days (FIG. 3b). As the protein is unstable, CO abundance most probably follows that of its mRNA[80]. In addition, *FT* mRNA levels (which are a direct readout of CO activity) are highest on long days at times when the coincidence of light with high levels of *CO* expression is maximal[80] (FIG. 3b). These observations led to the proposal that clock control of *CO* expression, combined with light regulation of CO function, could be an important mechanism that mediates the photoperiodic regulation of flowering time in *Arabidopsis*[80].

The most compelling evidence that the precise timing of *CO* expression is important for daylength discrimination comes from studies of *CO* mRNA levels in the clock mutant *toc1-1* (REF. 81; FIG. 3c). As mentioned above, this 21-h-clock mutant flowers earlier than wild-type seedlings on short days of 24 h in total. Under these environmental conditions, the phase of *CO* expression is shifted towards daytime in *toc1-1* compared with wild-type plants; as a result, higher levels of *CO* mRNA occur at dusk[81,82] (FIG. 3c). On the other hand, *CO* expression in the mutant is confined to the dark part of the day when they are grown under short-day conditions of 21 h, when *toc1-1* mutants flower as late as wild-type plants. Furthermore, *co* mutants do not affect flowering time on short days of 24 h, but strongly delay flowering in a *toc1-1* background under these conditions. Taken together, these results indicate that the early-flowering phenotype of *toc1-1* on short days is caused by the shift in *CO* expression towards daytime.

Further evidence that the precise timing of *CO* expression relative to the light/dark cycle is important for appropriate interpretation of daylength comes from studies in which wild-type plants are grown in non-24-h light/dark cycles[81,83]. In general, the phase of circadian rhythms is advanced relative to light/dark transitions when the total duration of the external cycle is longer than the period of circadian oscillations[6,83,84], as observed for the 21-h-clock mutant *toc1-1* on 24-h short days[81,82]. Consistent with these observations, *CO* expression is advanced towards daytime in wild-type plants that are grown during short days of 28 h (9.3 h light: 18.7 h dark)[83] or 30 h (10 h light: 20 h dark)[81] (FIG. 3c). This shift in *CO* expression towards daytime correlates with a strong acceleration of flowering time in wild-type plants[81,83] but not in *co* mutants (M.J.K. and S.A.K., unpublished data).

Evidence that CO function is light dependent comes from studies of *FT* expression. First, *FT* expression is low in wild-type plants that are grown on short days (that is, when *CO* expression is restricted to the dark part of the day)[81] (FIG. 3a). Second, high levels of *FT* mRNA can be detected on short days at dusk if *CO* expression is shifted towards daytime, as occurs in *toc1-1* mutants that are grown in cycles of 24 h[81,82], or in wild-type seedlings that are grown in cycles of 30 h[81] (FIG. 3c). Third, overexpression of CO causes elevated *FT* mRNA levels only in the presence of light[80,81]. Fourth, *FT* expression is reduced markedly in *phyA* and *cry2* photoreceptor mutants[81], whereas *CO* expression is largely unaffected[80,81] (FIG. 3d). Finally, a single exposure to a few hours of light acutely upregulates *FT* mRNA levels in wild-type plants that are grown on short days, and this effect is severely impaired in *co*, *phyA* and *cry2* mutants[81].

In summary, the above results fit an external coincidence mechanism in which light that is perceived by phyA and cry2 directly promotes flowering by activating *FT* expression. This effect of light requires functional CO, and clock regulation of *CO* expression ensures that high CO levels and light overlap only under long-day conditions. So, *FT* mRNA levels accumulate to levels that are sufficient to promote flowering only under this condition (FIG. 4a).

Daylength measurement in rice

A problem that has intrigued many scientists for decades is how a given developmental response, such

MERISTEM-IDENTITY GENE
A gene, such as *LEAFY* and *APETALA1*, that triggers the initiation of flowers, instead of leaves, from the shoot apical meristem.

MERISTEM
A small group of undifferentiated cells from which plant organs are formed.

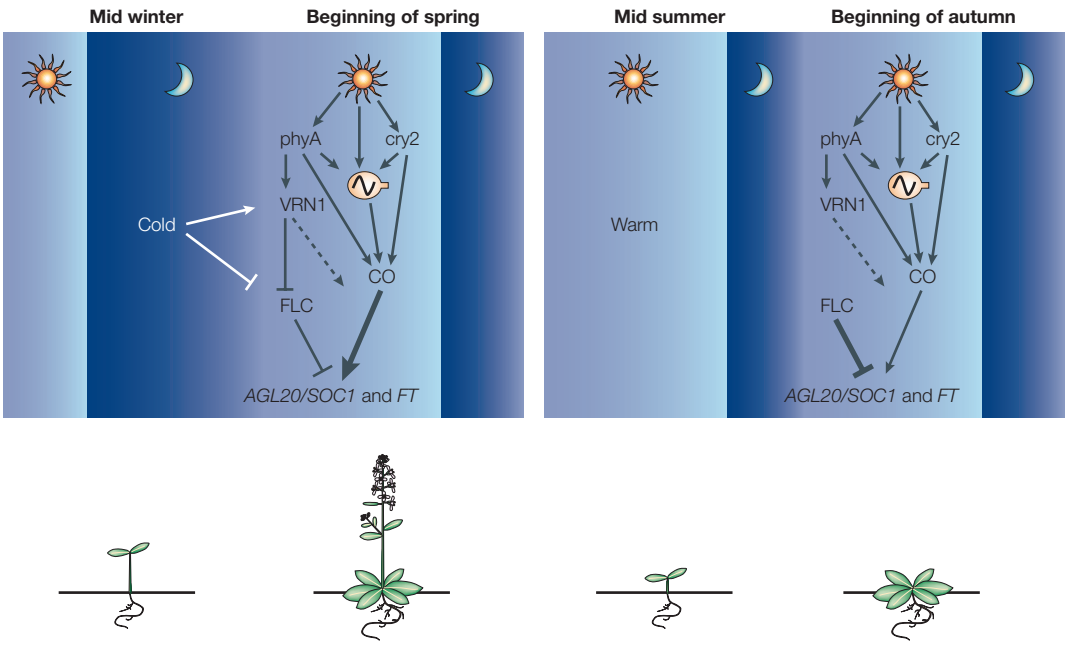


Figure 5 | **The *Arabidopsis* calendar.** Some ecotypes of *Arabidopsis*, as well as some cereals such as wheat, discriminate between the beginning of spring and that of autumn by flowering under long-day conditions only when these have been preceded by the cold temperatures of the winter. This results from the integration of temperature and photoperiodic signalling pathways in the control of the expression of two genes, *AGAMOUS-LIKE 20* (*AGL20*)/*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*), that promote the transition from vegetative to reproductive development. These genes are antagonistically regulated by FLOWERING LOCUS C (FLC) — a MADS-box transcription factor that is downregulated in the cold and that delays the floral transition — and CONSTANS (CO). Plants that germinated during the summer do not flower in the early autumn or late summer despite favourable photoperiodic conditions, because high levels of FLC impair the ability of CO to activate the expression of *AGL20*/*SOC1* and *FT*. On the other hand, plants that start growing in the winter are exposed to low temperatures that reduce *FLC* levels, and this allows CO to activate the expression of *AGL20*/*SOC1* and *FT* when the plants start perceiving the long days of spring through the photoreceptors cryptochrome 2 (cry2) and phytochrome A (phyA). In addition, the ability of plants to 'remember' the low temperatures of the winter when they are exposed to the warmer conditions of the spring requires the activity of vernalization (VRN)1 and VRN2 (only VRN1 is shown), two proteins that mediate an epigenetic downregulation of *FLC*. Interestingly, VRN1 is also partially required for the activation of *FT* expression by light perceived through phyA, and therefore constitutes another point of crosstalk between the temperature and photoperiodic signalling pathways that regulate flowering time.

as flowering, can be triggered by long days in some species and by short days in others. Do SDPs and LDPs measure daylength by similar mechanisms? If so, where lies the difference in their response to a given photoperiod? Several genes that affect the photoperiodic regulation of flowering time have recently been identified in rice, allowing the mechanisms that underlie photoperiodic responses in SDPs and LDPs to be compared.

The first flowering-time gene to be cloned in rice was *PHOTOPERIODIC SENSITIVITY 5* (*SE5*), a haem oxygenase that functions in the biosynthesis of the phytochrome chromophore[36]. *se5* mutants flower as early on long days as on short days, which indicates that phytochromes are photoperiodic photoreceptors in rice as well as in *Arabidopsis*[36]. Interestingly, the period and phase of circadian rhythms are largely unaffected in *se5* mutants, which indicates that phytochromes contribute to the photoperiodic control of flowering time in rice by a mechanism that is, at least in part, different from clock regulation[85].

Other genes that affect the photoperiodic regulation of flowering time in rice are *Heading date* (*Hd*) *1*, *Hd3a* and *Hd6*, all of which were originally identified as QUANTITATIVE TRAIT LOCI (QTL) that are responsible for natural variation in photoperiodic sensitivity[86]. Remarkably, they have all been cloned and shown to encode proteins already known for their role in clock or flowering-time regulation in *Arabidopsis*. *Hd6* encodes the α-subunit of protein kinase 2 (CK2)[87]. Interestingly, CK2 has been shown to have an important role in clock regulation in plants[88,89], fungi[90] and flies[91]. This indicates that, despite differences in the molecular nature of clock components among organisms from different kingdoms, there might be a common evolutionary origin for circadian clocks. In *Arabidopsis*, the β-subunit of CK2 interacts with and phosphorylates CCA1 and LHY *in vitro*[88], whereas its overexpression causes shortening of circadian rhythms and early flowering irrespective of the photoperiod[89]. In rice, the expression of a functional *CK2* α-allele delays flowering time, presumably through its effect on clock progression[87].

QUANTITATIVE TRAIT LOCUS (QTL). A genetic locus that is identified through the statistical analysis of complex traits (such as plant height or body weight). These traits are typically influenced by more than one gene and also by the environment.

The above findings indicate strongly that circadian clocks have a similar role in daylength measurement in SDPs and LDPs. But how is the activity of phytochromes integrated with the circadian system to distinguish short from long days in rice? An answer to this question is emerging from studies of *Hd1* and *Hd3a*, the two other flowering-time genes that were identified by QTL analysis[85,92,93]. Notably, *Hd1* encodes a protein with strong similarity to CO[92]. Consistent with daylength being measured in a similar way by SDPs and LDPs, the expression of this rice *CO* homologue is controlled by the clock in a similar way to its *Arabidopsis* counterpart[85,93]. Furthermore, the rice CO also affects flowering time by regulating the expression of *FT*-like genes, one of which is encoded by *Hd3a*, a gene that (as with *FT* in *Arabidopsis*) functions to promote flowering when expressed above a threshold level[85,93].

Why then do long days promote flowering in *Arabidopsis* and repress it in rice? The answer seems to lie in the fact that *FT* expression in *Arabidopsis* is activated by the coincidence of CO with light perceived by phyA and cry2 (FIG. 4a). On the other hand, the expression of *FT*-like genes in rice is activated by the *CO* homologue in the dark and is repressed by the coincidence of this protein with light perceived by phytochromes[85] (FIG. 4b). The molecular nature of this contrasting effect of light on *FT* expression in SDPs and LDPs remains to be determined, but it is unlikely to rely on differences in the CO proteins themselves, as a *CO* homologue from a SDP can rescue the flowering-time defects of an *Arabidopsis co* mutant[94].

A role for temperature in the plant calendar

Changes in daylength are a reliable indicator of seasonal progression, but daylength *per se* is not completely informative of the time of year. Some plants discriminate between equivalent photoperiods in the beginning of autumn and spring by flowering under long-day conditions only when these are preceded by a prolonged exposure to cold, a phenomenon that is known as vernalization[95]. The effect of cold on flowering time is mediated in part through a reduction in the levels of *FLOWERING LOCUS C* (*FLC*), which encodes a MADS-BOX transcription factor that delays the floral transition[96,97]. Recent evidence indicates that FLC functions directly by repressing the expression of *AGAMOUS-LIKE 20* (*AGL20*)/*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), which encodes another MADS-box transcription factor that accelerates flowering[98]. Interestingly, *AGL20/SOC1* was previously shown to be a direct target of CO transcriptional activity[76]. So, the convergence of CO and FLC activity at the promoter of *AGL20/SOC1* could be one mechanism by which cold and photoperiodic signalling pathways are integrated to ensure an appropriate seasonal control of flowering time (FIG. 5).

Two other proteins that are implicated in the control of flowering time by vernalization are VERNALIZATION (VRN) 1 and VRN2 (REFS 99,100). Both proteins are required for the maintenance of low levels of *FLC* mRNAs that are established by cold treatments once the plants are exposed to warmer conditions[99,100]. VRN1 and VRN2 seem to be involved in chromatin remodelling, which fits with the hypothesis that vernalization downregulates *FLC* expression by EPIGENETIC mechanisms. Consistent with a role of VRN1 in the photoperiodic regulation of flowering, *vrn1* mutants also affect the flowering response to day-length extensions that are perceived by phyA[99], and overexpression of VRN1 accelerates flowering irrespective of cold exposure by upregulating *FT* expression[99]. So, VRN1 is probably another point of crosstalk between the photoperiodic and vernalization pathways in the regulation of flowering time (FIG. 5).

Conclusions and future directions

The emerging picture of the molecular organization of the plant calendar is in agreement with the external coincidence model of photoperiodic time measurement that was proposed almost 70 years ago by Erwin Bünning. The photoperiodic regulation of flowering time in plants arises in part from direct effects of light on *FT* mRNA levels, coupled with a circadian rhythm of sensitivity to this signal exerted through clock regulation of *CO* expression. One question that would be interesting to address is how *CO* expression is modulated by the clock and photoperiod to ensure that there is a coincidence of CO with light in long but not short days. Interestingly, *CO* expression is upregulated by light in etiolated seedlings[73], and its expression in light-grown plants is severely altered in *gi* and *elf3* mutants that are defective in both circadian and light-regulated responses[80]. This indicates that the photoperiodic regulation of *CO* expression might result from a complex interplay between light and clock signalling pathways.

Beyond this issue, one of the most fundamental problems that needs to be addressed in the near future is the molecular and biochemical basis of the modulation of CO function by light, and the contrasting effect of light on CO function in SDPs and LDPs. One possibility is that the light-dependent regulation of CO function could result from the effects of cry2 and phyA on CO levels, activity and/or subcellular localization. In addition, as CO probably functions as a transcriptional regulator without binding DNA directly[98], light could also affect its function by regulating the levels or activity of DNA-binding proteins that are required for CO to modulate gene expression. In turn, the opposite effect of photoperiod on flowering in SDPs and LDPs could be due to differences between these two groups in the light signalling pathways that regulate CO function. The signalling pathways by which phytochromes and cryptochromes ultimately affect the expression of flowering-time genes are largely unknown but, at least for phytochromes in *Arabidopsis*, it might involve the precise coupling of kinase and phosphatase activities[101].

Interestingly, CO seems to mediate the photoperiodic regulation of developmental processes in species other than rice and *Arabidopsis*. In *Pharbitis nil*, which is the model SDP for the study of photoperiodic control of floral initiation, a homologue of the *Arabidopsis CO*

MADS BOX
A conserved DNA-binding domain that is found in a family of transcriptional regulators that are present in animals, fungi and plants.

EPIGENETIC
A heritable change in gene expression that is controlled by modifications in DNA methylation and/or chromatin structure.

is also controlled by the circadian clock and rescues the flowering-time defect of *Arabidopsis co* mutants[94]. In addition, the overexpression of *Arabidopsis CO* in potato disrupts the short-day induction of tuberization[102]. So, it is tempting to speculate that CO is the central player that mediates the daylength measurement in all plants, and whether a particular response is induced or repressed by short days or long days might be determined by the effect of light on the cofactors with which CO interacts to regulate different genes and responses.

Finally, it is interesting to note that the vernalization requirement of many cereals can be replaced by exposures to short days[4]. Does the effect of short days in these LDPs operate through *CO*-like genes? Does it also involve the downregulation of *FLC*-like genes mediated by *VRN* genes? Finding answers for all of these questions will lead to a more complete understanding of the plant calendar, which should enhance our ability to increase crop performance at different latitudes.

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