Circadian and solar clocks interact in seasonal flowering

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The plant maintains a 24-h circadian cycle that controls the sequential activation of many physiological and developmental functions. There is empirical evidence suggesting that two types of circadian rhythms exist. Some plant rhythms appear to be set by the light transition at dawn, and are calibrated to circadian (zeitgeber) time, which is measured from sunrise. Other rhythms are set by both dawn and dusk, and are calibrated to solar time that is measured from mid-day. Rhythms on circadian timing shift seasonally in tandem with the timing of dawn that occurs earlier in summer and later in winter. On the other hand, rhythms set to solar time are maintained independently of the season, the timing of noon being constant year-round. Various rhythms that run in-phase and out-of-phase with one another seasonally may provide a means to time and induce seasonal events such as flowering.

Keywords: circadian rhythm; circadian time; dawn; dusk; mid-day; photoperiod; seasonal flowering; solar time; zeitgeber time

Introduction

The plant's circadian rhythm provides an interface in the signalling network between the environment and its internal programmes.[1] In this regard, the circadian clock of plants maintains a period of 24 h in keeping with the earth's rotation. One or more core oscillators are believed to regulate this cycle, the effects of which pervade to the cellular level to control the timing of many physiological and developmental functions. Certain plant behaviour patterns, such as petal movement and various intercellular signalling processes, are diurnal adjustments repeated from one day to the next. Other functions may occur only once during a specific and limited phase of plant growth and development, hypocotyl elongation being an example. Still other physiological episodes may be recurrent annual events that require interaction of the circadian rhythm with the environment. Photoperiod changes interrelate with the circadian rhythms of some plant species to induce seasonal synchronous flowering.

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Innate circadian rhythmicity in plants can be maintained for a period in free run even when the plants are transferred to constant environmental conditions, such as continuous light or darkness. In the longer term, nevertheless, the plant has to refer periodically to an external reference to maintain precision. The timing marker for a circadian rhythm must bear some differentiating characteristics, *e.g.* some form of transient discontinuity in the light, in order that the plant has an awareness of its arrival to set its rhythm by. Hence, the two light transitions of sunrise and sunset are natural timing references ('zeitgebers') that the plant can recognise and make use of to maintain its circadian rhythms. From current molecular biology research, especially that based on the *Arabidopsis* model, there is broad acceptance of the existence of circadian clock regulation through transcriptional, translational and post-translational processes forming a negative feedback loop that involves a complementation of genes. In this connection, the rhythmic genes *LATE ELONGATED HYPOCOTYL* (*LHY*), *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) and *TIMING OF CAB EXPRESSION 1* (*TOC1*) are among the key clock oscillator components that the plant employs to regulate its 24-h period.[2–4]

Arabidopsis shows many outputs from its circadian clock in the form of the rhythms of various cyclic genes that regulate plant function.[5,6] With the clock oscillator tracking the passage of time, the plant assigns to these individual gene rhythms their proper phases so that each gene is expressed at the prescribed time. While variables such as temperature may influence the circadian cycle,[7,8] light is well recognised as a major factor in circadian rhythm entrainment with dawn and dusk seen as important entrainment references.[1,5]

This article is concerned with gene rhythms that are the output of the circadian clock. Here, I examine various implications of the widely held contentions that the circadian rhythms of plant genes are principally referenced to light cues that occur at dawn, or at dusk, or at dawn and dusk.

Setting circadian rhythms by the light transitions at dawn and dusk

At which points on the circadian clock are the various circadian rhythms of the plant re-set daily? Are sunrise and

sunset equally important for the entrainment of circadian rhythms? An experimental approach commonly used to determine which light cue is involved in setting the plant's circadian cycle is to see how its rhythm is altered when the photoperiod is changed. Several studies on a number of selected genes have been carried out in this manner in the past although it remains unclear whether these individual examples are representative of rhythmic plant genes as a whole. The recent study of Michael et al.[9] on the nuclear transcriptome of *Arabidopsis thaliana* provides a good opportunity to evaluate the circadian behaviour of a very large number of rhythmic genes. Their investigation includes a study of phase shifts in rhythmic genes maintained under a short photoperiod cycle of 8 h light:16 h darkness and a long photoperiod cycle of 16 h light:8 h darkness. Under these lighting regimes, the timing of rhythmic genes that are referenced to dawn ('lights-on') or to dusk ('lights-off') can be expected to be affected in the following way:

1. A circadian rhythm set exclusively by the light transition at dawn would be re-set when the lights are turned on. Thereafter, the duration of the photoperiod has no bearing on the phase of the genes. Hence, genes set by the dawn are expected to show no phase difference between short and long photoperiods when timed from 'lights-on'.
2. A circadian rhythm set exclusively by the light transition at dusk would be re-set when the lights are turned off. Since the lights for the long photocycle are turned off 8 h after 'lights-off' for the short photocycle, the plant's rhythm under the former conditions should show a phase delay of 8 h timed from 'lights-on'. Hayama et al.[10] provide an example in *Pharbitis* of such a phase delay arising from a circadian rhythm that is set by dusk.

In their findings, Michael et al.[9] observe a cluster of rhythmic genes that show minimal (0–1 h) phase shift when the photoperiod is changed from 8 to 16 h. These are the expected genes that have their rhythms set by the light transition at dawn; their rhythms are unaffected by any subsequent change in the photoperiod as mentioned above. Contrary to what might be expected of genes set by the light transition at dusk, there is a paucity of genes showing an 8-h phase shift when the photoperiod is lengthened from 8 to 16 h. Therefore, unlike the darkness-to-light transition at sunrise, there do not appear to be many genes that employ the light-to-darkness transition at sunset to generate an independent cycle in *Arabidopsis*, separate from the cycle set at dawn.

Besides the cluster of genes that are unaffected by the photoperiod and show no phase shift, it is particularly noteworthy that the study of Michael et al.[9] also reveals another two large clusters of rhythmic genes positioned 12 h apart, each displaying a phase delay of 4 h when the photoperiod is lengthened from 8 to 16 h. What is the significance of a 4-h phase delay and how does such a phase shift come about? And why are there two such daily clusters of rhythmic genes that peak 12 h apart? To elucidate this matter further, I revisit past research to examine results on changes to the phases of rhythmic genes when the plant is maintained under short or long photoperiods.

Empirical evidence for an alternate circadian rhythm running on solar time

In the study of Roden et al.,[11] the authors present the 24-h cycles of *LHY* and *COLD CIRCADIAN RHYTHM RNA BINDING 2* (*CCR2*) subjected to 8-h (short day) or 16-h (long day) light periods. The authors write: '. . . under inhibitory, short-day conditions (8L16D), the onset of *lhy*::*luc* expression occurred 6 hr before dawn. Under inductive, long-day conditions (16L8D), expression of *lhy*::*luc* was delayed and only anticipated dawn by 2 hr at most. In contrast, the timing of *CCR2* expression relative to dawn was unchanged'. Some salient points can be gathered from the authors' statement. The time lapse from sunrise to initiation of *CCR2* expression is fixed at about 6 h, irrespective of the day length (Fig. 1). It is for this reason that gene expression peaks in the late afternoon under long-day lighting, but peaks after sunset under short-day lighting. Although *CCR2* is a dusk-expressing gene, it takes its circadian rhythm cue at dawn. This is therefore a clear case of the circadian cycle being timed by sunrise.

Roden et al. do not say how the *LHY* cycle might be entrained, but it is clearly not by sunrise since *LHY* gene expression is initiated 4 h *later* during long days as compared with short days. This discrepancy of 4 h is significant. If the *LHY* cycle were an independent rhythm calibrated by sunset, the longer photocycle should be running late by 8 h, and not 4 h, since illumination for the longer photoperiod is turned off 8 h after 'lights-off' for the shorter photoperiod.

The 8-h light phase in the short-day treatment has its mid-point ('mid-day') 4 h after dawn, whereas 'mid-day' for the long-day treatment occurs 8 h after dawn. The difference is 4 h, matching the phase difference between the long and the short photoperiod rhythms. Accordingly, the time lapse from 'mid-day' (or midnight) to initiation of *LHY* gene expression is unchanged for both short days and long days (Fig. 1). These observations are consistent with the circadian rhythm not being calibrated to the night/day light transition (the *beginning* of the light period or 'dawn'), but to the *mid-point* ('mid-day') of the light period. Essentially, the *LHY* rhythm is running on solar time, which is referenced to mid-day, and not on circadian (zeitgeber) time, which is referenced to sunrise.

In another study, McWatters et al.[12] confirm the circadian characteristic of the *CCR2* rhythm in wild-type *Arabidopsis* when they observe the initiation of gene activity 6–7 h after dawn, irrespective of the photoperiod. Just as Roden et al.

observe, this is consistent with rhythm entrainment at daybreak. McWatters et al. also present the rhythmic cycles of another two genes, *CCA1* and *CHLOROPHYLL A/B-BINDING PROTEIN 2* (*CAB2*), which are pointedly different from that of *CCR2*. When timed from dawn, the phases of *CCA1* and *CAB2* are delayed 4 h upon changing the photoperiod from 8 to 16 h. However, there is no phase difference when the phases are timed from noon or midnight. This is again consistent with the gene rhythms being referenced to solar time.

Solar time is based on the 24-h cycle that uses the position of the sun as the reference. The clock starts at noon (ST 0), when the sun traverses the meridian ('overhead'), and so ST 12 is midnight. Slight discrepancies in the length of the solar day occur over the year due to the tilt of the earth and its elliptic orbit around the sun. Chronometer time averages out these discrepancies. In essence, chronometer time is 'average solar time' and it retains slight discrepancies with true solar time. Chronometer time starts at midnight so as to be more amenable for civilian use. For that reason, it is also known as 'civil time'. Circadian time, also called zeitgeber time (zeitgeber = German for time giver), is the 24-h cycle that uses sunrise (ZT 0) as the reference. As sunrise occurs earlier when the days are long in summer and later when the days are short in winter, circadian time displays a seasonal drift relative to solar time or chronometer time.

The existence of two plant circadian rhythms operating in *Arabidopsis* has earlier been noted by Michael et al.[8] The authors report that the circadian cycles of two genes, *CAB2* and *CATALASE 3* (*CAT3*), are controlled by separate clocks. They observe that *CAT3* has a consistent phase when timed from daybreak, irrespective of the photoperiod. The authors do not say when light entrainment takes place for *CAB2*, but state only that the two clocks are distinguishable on the basis of their sensitivity to temperature. Nevertheless, it can be seen from the results they present that, at constant temperature, *CAB2* activity initiates and peaks at a consistent phase when timed from mid-day or midnight, irrespective of the photoperiod, confirming McWatters et al.[12] Michael et al. report that 'the phase of *CAB2* is sensitive but that of *CAT3* is insensitive to photoperiod'. That statement is valid to the extent that the authors only adopt sunrise as the entrainment reference for both the genes on the assumption that both rhythms run on circadian time. If the authors had used mid-day or midnight as the reference, the situation would have been reversed. *CAT3* would then be seen to be sensitive to the photoperiod whereas *CAB2* would not be. Essentially, both *CAB2* and *CAT3* are insensitive to the photoperiod (showing consistent phase) when viewed within the respective time-frame to which each gene belongs: the *CAB2* rhythm running on solar time and thus calibrated against mid-day/midnight, and the *CAT3* rhythm running on circadian time and thus calibrated against dawn.

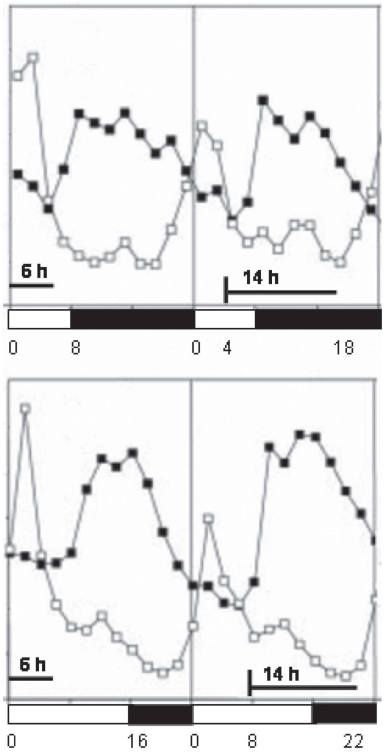

Figure 1. Depiction of circadian cycles of *CCR2* and *LHY* expression under 24-h short-day (upper panels) and long-day (lower panels) lighting. *CCR2* expression (filled squares) is entrained at 'dawn' (beginning of the light phase). In contrast, *LHY* expression (unfilled squares) is referenced to 'mid-day' (middle of the light phase). Thus, *CCR2* expression initiates 6 h from sunrise ('lights-on'), regardless of the photoperiod, and *LHY* expression initiates 14 h from the mid-point of the light phase (or 2 h from the mid-point of the dark phase), regardless of the photoperiod. Light and dark phases are indicated by unshaded and shaded bars, with hours from the beginning of the light phase indicated below (adapted from Roden *et al.*[11] Copyright (2002) National Academy of Sciences, U.S.A.).

At this juncture, the question arises whether the 4-h phase difference seen between the 8-h short-day and the 16-h long-day treatments (that feature in all of the abovementioned examples) may be no more than a quirk of coincidence peculiar to these two photoperiods only. Would a similar relationship be observed if other photoperiods were compared? The results of McWatters et al.[12] indicate that *CCA1* circadian rhythms are referenced to solar time when maintained under 8, 12 and 16 h of light. In a study encompassing a wider range of photoperiods, Millar and Kay[13] investigate the circadian behaviour of *CAB* in plants subjected to light periods of 1, 3, 6, 12, 18 and 21 h. (The *CAB*

family of genes, members of which are generally synchronous in their circadian rhythms,[14,15] includes the aforementioned *CAB2*.) They find that *CAB* expression generally peaks close to the middle of the subjective light period, suggesting conformation to solar time.

In later referring to this work, Millar[1] describes gene expression 'peaking at a phase about 40% of the way through the predicted light interval'. The figure 40% refers to all the photoperiod treatments investigated in that particular study. *CAB* rhythms under 6- and 12-h photoperiods in the study actually peak at their mid-points, just as McWatters et al.[12] and Michael et al.[8] find for their *CAB* genes in *Arabidopsis* under 8- and 16-h photoperiods. To maintain a constant phase relative to mid-day or midnight (and thereby comply with solar time), peak activity is delayed by approximately 1 h for every 2 h added to the photoperiod. Therefore, the phase of the rhythm running on solar time shifts according to the photoperiod when timed from sunrise. Millar and Kay[13] consider photoperiod-dependent entrainment of the circadian cycle to be unique to *CAB*, but this is clearly not the case as the abovementioned examples show.

Unlike *CCA1* or *LHY*, which are referenced to mid-day/midnight, *CAB* expression is not only referenced to mid-day/midnight, but is also programmed to peak around mid-day. This has an important bearing on the light-gathering function of *CAB* and other photosynthesis-related genes.[16] If a cycle that is set at mid-day also peaks at noon, it would mean that the gene would always be expressed when sunshine is most intense for the day, regardless of the season. This is advantageous in the higher latitudes where the daily window of sunlight can be very narrow in winter.

The fact that the rhythmic genes calibrated to solar time include *CCA1* and *LHY* is worthy of special note. As mentioned earlier, the interactive regulation between *CCA1* and its partial homologue *LHY* on the one part, and *TOC1* on the other, forms a feedback loop that perpetuates clock function in *Arabidopsis*.[2–4] The observation that *CCA1* and *LHY* circadian rhythms are regulated to solar time[11,12] would predict the *TOC1* rhythm behaving likewise. Indeed, the findings of Perales and Más[17] and Para et al.[18] confirm this. They show that the phases of *TOC1* expression under short and long photoperiods remain constant when measured from mid-day, in accordance with the expectations of solar timing. Hence, the solar time frame does not only apply to the setting of many individual circadian rhythms that are the output of the circadian clock oscillator, but it is also relevant to the calibration of the clock oscillator itself.

Coming back to the earlier mentioned findings of Michael et al.,[9] the clusters of gene activities displaying a phase delay of 4 h when the photoperiod is changed from 8 to 16 h are what might be expected if the genes concerned are referenced to solar time. Judging by the large number of cyclic genes that show this trait,[9] it would appear that circadian rhythms running on solar time are not at all rare in *Arabidopsis*; they are at least as common, if not more so, than gene rhythms referenced to circadian time.

Whether a rhythm is referenced to sunrise or mid-day/midnight can generally be determined by observing which calibrating reference is being followed under short and long photoperiods. Regardless of the photoperiod, the phase (*e.g.* a peak or trough) of the rhythm does not change in position relative to sunrise when the rhythm is running on circadian time. The peak or trough of the rhythm does not change in position relative to mid-day/midnight when the rhythm is running on solar time. Sometimes, as in the case of *LHY* and *CCA1*, the trough (*i.e.* initiation of gene activity) occurring during the night gives a more accurate assessment of circadian timing whereas the peak activity may be forced by light.[3,19]

How does the plant know when it is noon?

The growth chamber, with its 'lights-on'/'lights-off'-controlled photoperiods of uniform lighting, does not provide the plant with any mid-day or midnight cue in the course of the light or dark phases. If the circadian rhythms of many plant genes run on solar time, and solar time is referenced to mid-day, how does the plant know when it is mid-day? A rhythm that utilises a cue occurring at any time of the solar clock – and not just noon – is still referenced to mid-day and remains on solar time. Even in the absence of such light cues during the light phase in an experimental growth chamber, the plant can still set its circadian rhythm to solar time based on the timings of dawn and dusk alone.

An example of how the light transitions at 'lights-on' and 'lights-off' in the experimental growth chamber can provide cues to calibrate a circadian rhythm to solar time is shown in Fig. 2. According to this model, the light transition at dawn triggers the initiation of a signal at the cellular level (perhaps a gene action) that rises to a peak and then declines to base level. A complementary signal is triggered by the light transition at dusk, and the signals from dawn and dusk interact where these signals overlap. It can be seen from Fig. 2 that the points of maximum signal overlap (interaction) occur at a consistent phase from mid-day and midnight regardless of the photoperiod, and can thus serve as cues for solar timing. The interaction of the dawn and dusk signals in this model provides a working proposition for Millar's surmise that 'at least two signals must participate in entrainment, from a selection comprising the sharp transitions at dawn and dusk and the intervals of continuous light and darkness'.[1]

The model in Fig. 2 does not predict a single solar timing cue either, but predicts two solar timing cues over each 24-h period, and these are spaced 12 h apart. If these timing cues induced the simultaneous initiation of a large number of genes

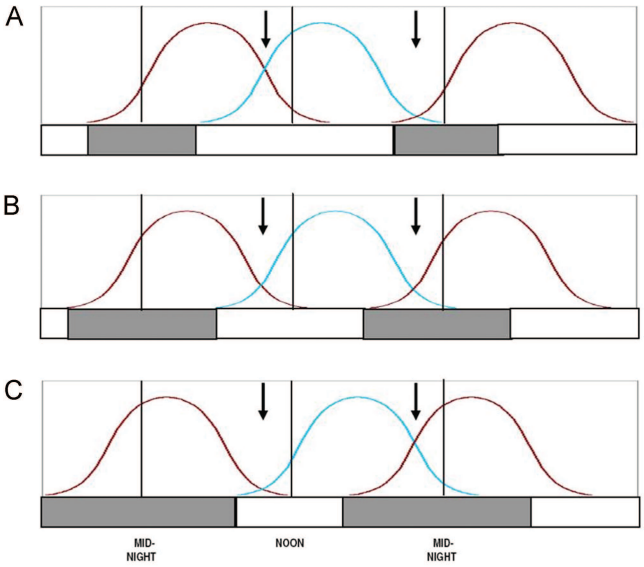

Figure 2. How the plant circadian rhythm is set on solar time based on the timing of 'lights-on' and 'lights-off' in the experimental growth chamber – an example. The shaded and unshaded bars represent the dark and light phases, respectively. At the transition between darkness and light (dawn or 'lights-on'), a photoinductive signal (the dawn signal) is activated. Its activity (light blue line) rises to a peak and then tapers to baseline. At the light transition at dusk ('lights-off'), a complementary photoinductive signal (the dusk signal, dark brown line) is activated. The overlap of the two signals generates the reference cue for solar time. It can be seen that that the points of maximum overlap of the dawn and dusk signals (arrows) are always constant relative to mid-day or midnight (*i.e.* mid-day plus or minus a constant, midnight plus or minus a constant), regardless of whether the photoperiod is 16 h (**A**), 12 h (**B**) or 8 h (**C**). Thus, the model provides for a repeating 12-h timing reference set by the light transitions at dawn and dusk. It enables the plant to calibrate its rhythm to solar time without the necessity for any light cue occurring at mid-day or at any other point during the light phase. Re-setting the light transition at either dawn or dusk, which changes the photoperiod, would re-set the solar time reference and the phases of genes that are calibrated to this reference. The above depicts a generic example where the durations of the dawn and dusk signals (from initiation to peak and back to baseline) are arbitrarily 20 h. If the duration of the signals were exactly 24 h, then the intersection peaks of the dawn and dusk signals would occur at midnight and mid-day. This relationship holds true even where the ratio of the light-to-dark periods, or the dark-to-light periods were extreme, *e.g.* 2 h:22 h in the 24-h cycle.

with generally similar initiation-to-peak lag times, heightened gene activities 12 h apart can then be expected to follow each cue. That is, in fact, what Michael et al.[9] observe in their oligonucloeotide microarray studies of gene activities under day lengths of 8 and 16 h. From these findings, they conclude that many *Arabidopsis* gene phases are referenced against two daily set points that are separated by 12 h. Together with the earlier results of Edwards et al.[20] and Harmer et al.,[16] who set the day length at 12 h, activities of a large number of genes are found to peak twice a day at these circadian times: ZT 6 and ZT 18 under an 8-h photoperiod,[9] ZT 8 and ZT 20 under a 12-h photoperiod[16,20] and ZT 10 and ZT 22 under a 16-h photoperiod.[9]

While the circadian timings of these peaks may seem unconnected when considered in isolation, a common thread emerges when these timings are read together on the solar clock. ZT 6, ZT 8 and ZT 10 all correspond to the solar time ST 2 (or 2 p.m. chronometer time) under their respective photoperiods, while ZT 18, ZT 20 and ZT 22 all correspond to ST 14 (2 a.m.). Essentially, therefore, large clusters of genes regulated to solar time peak around 2 p.m. and 2 a.m., irrespective of the photoperiod. Wave forms of several genes tracked individually suggest that they are in the main unimodal over the 24-h period, and that the two observed daily clusters are from different sets of genes peaking.[16,20]

There has been the suggestion that the two daily gene activity peaks are examples of genes 'anticipating the dawn or dusk' to prepare the plant ahead of various cellular activities that take place either in light or in darkness.[9,16] However, this is not the case. If, on the one hand, the solar timings of the gene peaks are fixed at 2 p.m. and 2 a.m. and, on the other hand, the solar timings of dawn and dusk are fixed for any given day length (*e.g.* dawn is 4 a.m for a 16-h photoperiod, 8 a.m. for an 8-h photoperiod, etc.), the proximity of the two peaks to dawn or dusk is variable and is essentially determined by the day length. Thus, the 2 p.m. peak emerges 2,[9] 4[16,20] or 6 h[9] prior to dusk when the photoperiod is 8, 12 and 16 h, respectively. Barring extreme photoperiods for which data are lacking, the 2 p.m. peak advances towards the dusk by 1 h, whereas the 2 a.m. peak retreats from the dawn by 1 h for every 2 h decrease in day length, and vice versa as the day length increases.

When rhythms coincide: the induction of seasonal flowering

Plant rhythms set on circadian time and solar time may provide a mechanism to measure seasonal changes in day length and to time, and induce various events that occur at specific times of the year. In this respect, the Internal Coincidence Model[21] postulates that two endogenous rhythms coincide seasonally to trigger such episodes. Among the best studied seasonal activities in plants growing in temperate regions is seasonal flowering. Nevertheless, the Internal Coincidence Model has not gained wide acceptance in this regard, at least partly because there have not been any substantial suggestions as to what these endogenous rhythms might be that control seasonal flowering.

Before considering how two circadian rhythms might coincide seasonally, it is useful to first consider how coincidence of two rhythms *cannot* be achieved. If two 24-h circadian cycles were set to the same reference (*e.g.* noon) that does not undergo

seasonal adjustment, the two cycles would end up moving in lock-step synchrony with one another year-round, and would never shift between the 'in-phase' and 'out-of-phase' states. The same happens if both rhythms track the season, but are tied to the same timing reference (*e.g.* sunrise). On the other hand, seasonal coincidence of two rhythms can be achieved where one rhythm shifts seasonally in its timing relative to noon and the other remains constant in its timing relative to noon. Plant rhythms running differentially on circadian time and solar time are therefore suitable candidates for the endogenous rhythms that are integral to the Internal Coincidence Model.

The foregoing notwithstanding, the currently prevailing hypothesis to explain seasonal flowering is based on the External Coincidence Model,[22] which postulates that flowering in long-day plants (those that require long days and short nights for flowering) is induced when proteins associated with flowering are synthesised in the presence of light.[11,23] Because of the increased day length in late spring or summer, genes controlling flowering that are expressed just after sunset in winter would be expressed when it is still light in summer. A major concern with this model stems from the fact that short-day plants (that require short days and long nights for flowering) have genes connected to flowering that are very closely related to their counterparts in long-day plants. Whereas such broad similarities suggest that the control of seasonal flowering would be identical in these two groups, their light requirements in this respect are diametrically opposite. One resolution to this paradox is to regard light or darkness as having no direct bearing on the flowering

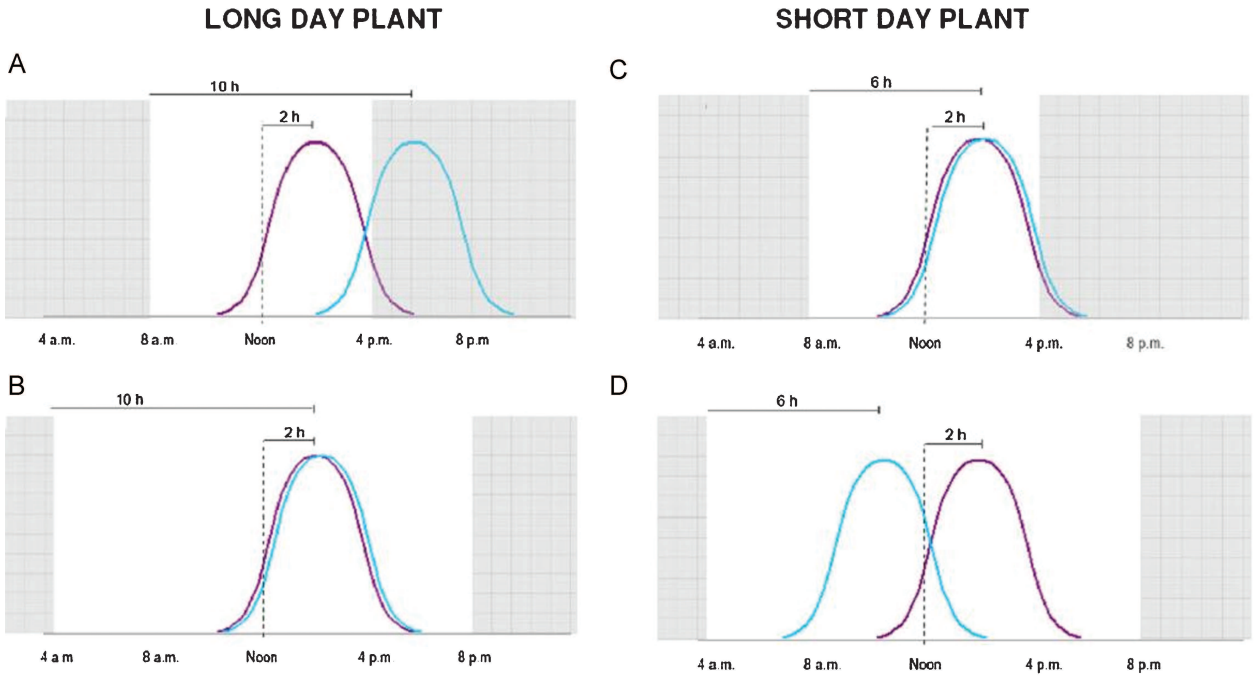

Figure 3. Induction of seasonal flowering in long-day (**A**,**B**) and short-day plants (**C**,**D**) according to the Internal Coincidence Model. A hypothetical example is shown of two concurrent endogenous circadian rhythms operating during short days of 8-h photoperiods (A,C) and long days of 16-h photoperiods (B,D). Light and dark phases are indicated by unshaded and shaded areas. The phase (peak activity or some other reference point) of the gene associated with flowering induction ('flowering gene') that is calibrated to solar time (dark brown line) is unchanged regardless of the photoperiod when measured from mid-day (dashed vertical line). On the other hand, the phase of the flowering gene that is calibrated to circadian time (light blue line) is unchanged regardless of the photoperiod when measured from sunrise. In the example of the long-day plant, the flowering gene on solar time has its phase 2 h after mid-day (ST 2), while its complementary flowering gene on circadian time has its phase 10 h after sunrise (ZT 10). In the short-day plant, the flowering gene on solar time also has its phase 2 h after mid-day (ST 2), whereas the flowering gene on circadian time has its phase 6 h after sunrise (ZT 6). Flowering is induced when the phases of the two genes coincide. For long-day plants, there is no coincidence of gene expression during short days (A). However, expression of the flowering gene on circadian time is advanced relative to noon when the sun rises earlier as day length increases. This facilitates coincidence of the flowering gene calibrated on solar time with that calibrated on circadian time during long days, with the latter still maintaining its phase of 10 h from sunrise (B). The converse is true for short-day plants (C,D). In the model depicted in this figure, sensitivity to day length is due to the difference in the phases of the flowering genes referenced to circadian time for short- and long-day plants (whereas the phases of genes running on solar time are similar in both groups of plants). There is also the alternative model that has the phases of flowering genes on circadian time being similar, and sensitivity to day length being due to the discrepancy in the phases of the flowering genes running on solar time. It can be seen from in this scheme that even a slight change (*e.g.* due to a single mutation) in the phase of the flowering gene on either rhythm can alter the timing of flowering quite considerably.

stimulus and changes in seasonal day length serve essentially to re-set one or more of the plant's endogenous rhythms in accordance with the Internal Coincidence Model.

An example of how seasonal flowering might be induced according to the Internal Coincidence Model is given in Fig. 3. This explanation, which is based on the seasonal coincidence of the circadian rhythm set on solar time with that set on circadian time, applies equally well to both long- and short-day plants.

Conclusions and next steps

Some characteristics of light entrainment of *Arabidopsis* circadian rhythms inferred from the published studies discussed above are as follows:

1. The light transition at dawn sets various circadian gene rhythms that run on circadian (zeitgeber) time. The phases of gene rhythms on circadian time remain generally constant regardless of the photoperiod, if they are timed from sunrise.
2. The light transition at dusk sets few (if any) circadian gene rhythms that are timed from sunset. However, this light transition plays a more important role, in concert with the light transition at dawn, to set various circadian gene rhythms that run on solar time. The phases of gene rhythms on solar time remain generally constant regardless of the photoperiod, if they are timed from mid-day or midnight. If timed from sunrise, rhythms on solar time show a phase delay of about 1 h for every 2 h increase in the photoperiod.
3. *Arabidopsis* circadian rhythms running on solar time are at least as common, if not more so, than rhythms referenced to circadian time. Among the genes with rhythms on solar time are three key components of the circadian clock oscillator: *CCA1*, *LHY* and *TOC1*. It would appear, therefore, that not only do many circadian rhythms follow solar time, but the solar timeframe is fundamental to the clock oscillator itself.
4. Cyclic gene expression occurs throughout the day, but a large number of genes regulated to solar time peak around ST 2 and ST 14 (2 p.m. and 2 a.m. chronometer time), irrespective of the photoperiod. This is consistent with the presence of two daily set points separated by 12 h.[9] The proximity of the two peaks to dawn and dusk under certain day lengths gives the appearance of the genes 'anticipating the dawn or dusk'.[9,16] In fact, the proximity of these clusters of heightened activities to dawn or dusk is variable; it is dependent on, and predictable from the photoperiod.
5. Since the timing of dawn changes with the season (the sun rises earlier in summer and later in winter) whereas that of mid-day or midnight does not, genes timed by the rhythms running on circadian time and on solar time are activated in-phase and out-of phase with one another according to the season. The two rhythms are therefore suitable candidates for the endogenous rhythms integral to the Internal Coincidence Model for seasonal flowering that is compatible with both long- and short-day plants.
6. Rhythmic genes that express at dawn or at dusk do not necessarily receive their circadian light cues only at dawn or dusk, respectively. If they did, then the gene events would either coincide with the cue or follow it, but never precede it. For example, *CCR2* expression is in fact timed from the dawn even though the gene is expressed in the late evening or night.[11] This explains how *CCR2* is expressed before sunset under long photoperiods and after sunset under short photoperiods. Similarly, *LHY* is an example of a morning-expressing gene that is timed from the preceding midnight or the previous day's noon. This explains how *LHY* gene expression can be triggered even before sunrise.

There is considerable scope to elucidate further the hypothetical standpoints discussed above. The concept of circadian rhythms entrained to the solar clock is supported by a body of empirical evidence that shows rhythmic gene phases conforming to solar time under different experimental day lengths, commonly 8, 12 and 16 h. Other photoperiod comparisons can be attempted if there is felt the need for further confirmation of the phenomenon. The hypothesised solar cues depicted in Fig. 2 can be tested using a Nanda–Hamner modification[24] of Michael et al.'s[9] microarray analysis by shortening the period from 24 to 20 h while maintaining a day length of 8 h. The mechanics underlying Fig. 2 predict that two major gene activity peaks would continue to be observed in the shortened cycle. Compared with the 24-h cycle, the phase (timed from 'lights-on') of one peak in the 20-h cycle would remain unchanged while the phase of the other would be altered so that the two peaks would be separated by one half of the cycle period (10 h).

Another investigation to pursue is the identification of the hypothesised rhythmic genes that enable the Internal Coincidence Model of flowering. Among the well-researched *Arabidopsis* genes thought to be closely associated with the promotion of day length-dependent flowering are GIGANTEA (GI) and CONSTANS (CO),[23,25,26] which are entrained to solar time[27] and circadian time,[23] respectively, thus allowing for seasonal coincidence of their rhythms. These and other associated flowering-related genes need to be examined further. Here again, high-throughput data generated from microarrays could provide useful leads in identifying genes that conform to the scheme in Fig. 3. Prospective flowering genes (or orthologues) identified from long- and short-day plants (*Lemna* or *Nicotiana*, which have both long- and short-

day species, might be especially useful) can then be intertransposed to observe their effects on flowering of the resulting transgenics.

There is always the concern of whether artificial lighting in growth chambers represents a reasonable emulation of the natural environment. In nature, the ambient light changes not only in intensity, but also in its spectral properties throughout the day (*e.g.* the blue-to-red wavelength ratio and the red-to-far-red ratio in sunlight peak at noon).[28] While plant rhythms are sensitive to specific light wavelengths,[29,30] the uniform artificial lighting regimes of current experimental growth chambers do not indicate whether light spectral quality may provide alternate or additional (perhaps reinforcing) markers for circadian rhythm entrainment in nature. With light-emitting diode (LED) technology deployed to replicate the sequence of ambient light at different times of the day, it would be especially interesting to see how the plant's circadian rhythm might be affected by setting the peak in light intensity or in blue-to-red light ratio away from the mid-point of the light phase.

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