Tansley review

Plant responses to photoperiod

Author for correspondence:
Stephen D. Jackson
Tel: +44 (0)24 76575091
Fax: +44 (0)24 76574500
Email: Stephen.jackson@warwick.ac.uk

Received: *5 August 2008*
Accepted: *29 September 2008*

Stephen D. Jackson

Warwick HRI, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, UK

Contents

	Summary	1
I.	The importance of photoperiod	1
II.	The competence to respond to photoperiod	3
III.	The photoperiodic response pathway	5
IV.	Systemic signals	7
V.	Moderating factors	8
VI.	The flowering response to photoperiod in other species	9
VII.	Tuberization in potato	10
VIII.	Bud set and growth cessation in trees	11
	References	12

Key words: bud set, flowering, juvenility, photoperiod, tuberization.

Summary

Photoperiod controls many developmental responses in animals, plants and even fungi. The response to photoperiod has evolved because daylength is a reliable indicator of the time of year, enabling developmental events to be scheduled to coincide with particular environmental conditions. Much progress has been made towards understanding the molecular mechanisms involved in the response to photoperiod in plants. These mechanisms include the detection of the light signal in the leaves, the entrainment of circadian rhythms, and the production of a mobile signal which is transmitted throughout the plant. Flowering, tuberization and bud set are just a few of the many different responses in plants that are under photoperiodic control. Comparison of what is known of the molecular mechanisms controlling these responses shows that, whilst common components exist, significant differences in the regulatory mechanisms have evolved between these responses.

New Phytologist (2008) **doi**: 10.1111/j.1469-8137.2008.02681.x

I. The importance of photoperiod

The ability to co-ordinate certain developmental processes to particular times of the year when environmental conditions are likely to be more favourable confers distinct advantages. Timing reproduction to springtime so that vulnerable young offspring have the maximum possible time to develop before experiencing the harsh conditions of winter, for example,

would result in a greater survival rate of the offspring. There is thus a selective advantage for plants and animals that have acquired mechanisms enabling them to sense seasonal differences through the detection and response to changes in photoperiod. The photoperiod is the amount of light and darkness in a daily cycle of 24 h. At the equator (zero latitude) the photoperiod is a constant 12 h light and 12 h dark but, because of the tilt of the earth's axis towards the sun, as you move from the equator towards either of the earth's poles the lengths of the light and dark periods change to become unequal divisions of the 24-h cycle. The differences in daylength and nightlength become more extreme the closer you get to the poles, where photoperiods of 24 h light or 24 h darkness are experienced at certain times of the year. The annual rotation of the earth around the sun causes the photoperiod at a particular latitude to change throughout the year (except at the equator), with daylengths becoming longer in summer and shorter in winter, the summer solstice being the time when the length of the day has reached its annual maximum for a particular latitude, and the winter solstice being the time when the daylength is shortest. The annual cycle of variation in photoperiod is consistent from year to year (in the Northern Hemisphere the summer solstice is always around 20/21 June and the winter solstice around 21/22 December) and it is thus a reliable indicator of the time of year, much more reliable than temperature which also shows seasonal variations but is far less predictable.

In animals and birds the secretion of the hormone melatonin from the pineal gland is strongly inhibited by light; thus it is secreted during the dark period and for longer periods during the long nights and short days of winter than during the short nights and long days of summer (Goldman, 2001). The photoperiodic signal is thus translated into the duration of melatonin secretion. The melatonin signal activates specific receptors in discrete regions of the brain and pituitary gland and regulates annual rhythms in reproduction, moulting, body weight, hibernation and migration (Duncan, 2007). This mechanism enables precise timing of behavioural, or developmental, events such as the springtime arrival of swallow migrations from the Southern Hemisphere around the same day each year. Co-ordinated responses as a result of photoperiodic control are also observed in the plant kingdom, where at particular times of the year synchronous flowering of plants of the same species occurs to maximize cross-fertilization.

In addition to enabling an organism to co-ordinate various responses to particular times of the year, the ability to respond to photoperiod also enables an organism to anticipate variations in environmental conditions that can be predicted to occur around the same time each year. In northern latitudes, for example, shortening daylength in autumn is used as a cue by many trees and perennial plant species for the induction of cold hardiness and bud dormancy in anticipation of the freezing winter temperatures yet to come. Furthermore, the ability to respond to photoperiod can also help an organism occupy a niche in either space or time; some species such as the liverwort can survive in the desert by using long days as a signal to go into a dormant state during the arid summer period, whereas ground-level woodland plants may use short days to induce flowering in early spring, enabling them to complete seed production before the leaf canopy fully forms and limits the available light (Thomas & Vince-Prue, 1997).

There are three main photoperiod response types: short-day plants (SDP) in which the response is induced when the photoperiod is shorter than the critical daylength (CDL); long-day plants (LDP) in which the response is induced when the photoperiod exceeds the CDL; and day-neutral plants (DNP) which do not respond to photoperiod. The CDL is thus the point at which the photoperiod switches from being noninductive to inductive, and the value of the CDL varies considerably among species and among plants within the same species. A common misconception is that SDPs only flower in short photoperiods and LDPs only in long photoperiods; in fact, some SDPs such as *Xanthium strumarium* have a long CDL (15.5 h) and will thus be able to flower in long days (LDs) of 15 h light, whereas there are LDPs such as certain cultivars of *Lolium perenne* and *Lolium temulentum* that have low CDLs and are able to flower in short days (SDs) of 9 h (Thomas & Vince-Prue, 1997). Plants in which flowering can only occur in the inducing photoperiod have what is termed an obligate response, whereas plants in which flowering is promoted by LDs or SDs, but which can still flower in the other photoperiod, have a facultative response. In some tropical species the difference between an inducing and noninducing photoperiod can be as little as 30 min, implying that plants are able to measure time very accurately (Borchert *et al.*, 2005). This is important as small errors in measurement of the photoperiod can result in premature, or delayed, induction of the response of up to several weeks. Plants are able to measure time by means of an endogenous time-keeping mechanism called the circadian clock which is described in more detail later. The CDL is not fixed and is known to vary with environmental conditions and plant age; for example, in *Hyoscyamus niger* the CDL gets shorter with lower night temperatures, and seedlings of *Pharbitis nil* have a shorter CDL than adult plants (Thomas & Vince-Prue, 1997).

Sometimes the response to photoperiod is used in conjunction with other responses to other environmental stimuli to create a particular developmental life-cycle. Biennial plants such as henbane (*Hyoscyamus niger*) will not flower in the first year of growth despite encountering inductive photoperiods because they require a long period of cold over the winter months to satisfy a vernalization requirement, following which they will be able to flower and set seed. This results in a life-cycle spanning 2 yr, with flowering timed to coincide with spring or early summer in the second year. There are also several known cases where other environmental factors such as vernalization, high temperature, high irradiance, or low nitrogen can substitute for photoperiodic induction of flowering, or where the

response to photoperiod may be modulated or even repressed by other environmental factors (Bernier & Perilleux, 2005). The molecular basis of the interactions between the photoperiodic pathway and other pathways that affect flowering is still unclear; however, it appears that genes that act primarily in one pathway can sometimes be subject to regulation by other pathways. Thus one should always consider the photoperiodic pathway as a component of an interacting network of pathways regulating flowering rather than in isolation.

Whilst flowering is only one of many responses that plants have to photoperiod, it is the one that has been the most intensely studied and most of the molecular mechanisms described here relate to the control of flowering. Other responses such as tuberization, bud break and the onset of cold hardiness or dormancy also rely on the basic photoperiodic detection mechanism and common features with the control of flowering will be discussed.

II. The competence to respond to photoperiod

The competence to respond to florally promotive conditions, such as inducing photoperiods, changes during plant development. Most plants have a juvenile phase which prevents floral induction until a certain developmental stage has been reached, thus ensuring that the plant has sufficient resources to be able to sustain flower and subsequent fruit production. Juvenile plants are unable to respond to an inductive stimulus that would be sufficient to induce flowering in an adult plant. The juvenile phase can be as short as a few days in herbaceous species such as Arabidopsis, or can extend to several years in woody tree species (Hackett, 1985). Light integral, temperature, photoperiod and gibberellic acid (GA) have all been shown to affect the length of the juvenile phase and thus the point at which the plant can respond to photoperiod (Hackett, 1985; Chien & Sussex, 1996; Telfer *et al.*, 1997; Adams *et al.*, 1999, 2001). In many plants the juvenile to adult phase change is associated not only with the onset of the competence to flower but also with phenotypical changes such as alterations in leaf shape in ivy (*Hedera helix*) and maize (*Zea mays*), and the development of abaxial trichomes in adult Arabidopsis plants (Poethig, 1990; Bongard-Pierce *et al.*, 1996; Telfer *et al.*, 1997). Numerous mutants with altered juvenile phase lengths have been identified through the use of these phenotypical markers, including the *teopod* (*tp*) and *early phase change* (*epc*) mutants of maize, which have extended and shortened juvenile phases, respectively (Poethig, 1988; Dudley & Poethig, 1993; Vega *et al.*, 2002). Interestingly, studies on the *teopod* mutants, where sectors of wild-type tissue were created in *tp1* and *tp2* mutants, indicate that the *TP1* and *TP2* genes affect juvenility non-cell-autonomously (Dudley & Poethig, 1993). Whilst most mutants have been found to have an altered length of the juvenile phase, the rice *mori1* mutant is unable to undergo the juvenile to adult phase transition at all, and as a result will not flower even if grown in inducing SD photoperiods (Asai *et al.*, 2002).

Studies in Arabidopsis have identified genes involved in determining the length of the juvenile phase, including *HASTY* (*HST*), *ZIPPY* (*ZIP*), *SERRATE* (*SE*) and *SQUINT* (*SQN*) (Clarke *et al.*, 1999; Berardini *et al.*, 2001; Bollman *et al.*, 2003; Hunter *et al.*, 2003). Mutations in all of these genes result in a shortened juvenile phase, indicating that the function of these genes is to maintain the length of the juvenile phase. The involvement of microRNAs (miRNAs) and trans-acting small interfering RNAs (ta-siRNAs) in controlling the length of the juvenile phase has been established following the findings that *SE* is known to act in an miRNA gene silencing pathway (Grigg *et al.*, 2005), *ZIP* encodes an ARGONAUTE protein which is required for the production and/or stability of ta-siRNAs (Fahlgren *et al.*, 2006; Hunter *et al.*, 2006), and *HST* is involved in the synthesis or stability of some miRNAs (Park *et al.*, 2005). Furthermore, plants mutated in other genes known to play a role in gene silencing, *SUPPRESSOR OF GENE SILENCING 3* (*SGS3*), *RNA-DEPENDENT POLYMERASE 6* (*RDR6*) and *DICER-LIKE 4* (*DCL4*), were also found to have a shortened juvenile phase (Peragine *et al.*, 2004; Xie *et al.*, 2005). Like *ZIP*, these three genes are involved in the biosynthesis of ta-siRNAs and, as with *ZIP*, their mutant phenotypes are mainly related to the juvenile–adult phase change, rather than the highly pleiotropic phenotypes of miRNA biosynthesis mutants such as *hst* (Bollman *et al.*, 2003). This has led to the suggestion that ta-siRNAs are likely to have a more restricted role in plant development than miRNAs (Willmann & Poethig, 2005). A model of how ta-siRNAs affect the juvenile to adult phase change has been proposed where the target of the ta-siRNA is a gene that promotes the adult state (or represses the juvenile state). Disruption of the biosynthesis of ta-siRNAs as in the *zip*, *sgs3*, *rdr6* and *dcl4* mutants would mean that the transcript of the target gene is not degraded, thus resulting in a shortening of the juvenile phase and a more rapid transition to the adult state (Bäurle & Dean, 2006).

The identities of the miRNAs involved in the production of the ta-siRNAs, and the identities of the ta-siRNAs and their target genes involved in the control of juvenility are currently the subject of much research. MiR390 is involved in the production of the TAS3 family of ta-siRNAs which target the mRNAs of several *AUXIN RESPONSE FACTOR* (*ARF*) genes, including *ARF3*, for degradation (Fahlgren *et al.*, 2006). It was shown that regulation of transcript levels of the *ARF3* gene by TAS3 ta-siRNAs affects juvenile phase length, demonstrating that *ARF3* is one target gene involved in the control of juvenility (Fig. 1).

Over-expression of miR156 has been shown to extend the juvenile phase, in the most part through its down-regulation of the SBP-box gene *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* (*SPL3*) (Wu & Poethig, 2006). In addition to regulation of its transcript levels, *SPL3* is also regulated at

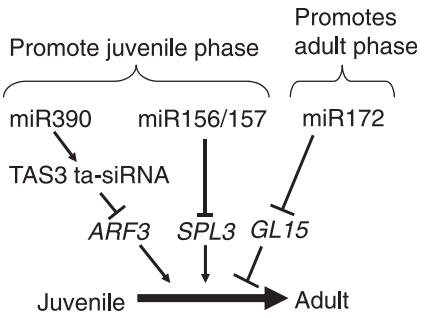

Fig. 1 MicroRNAs, trans-acting small interfering RNAs (ta-siRNAs) and their target genes involved in the control of the juvenile–adult phase transition. *AUXIN RESPONSE FACTOR 3* (*ARF3*) and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* (*SPL3*) promote the transition to the adult phase in Arabidopsis, whilst *GLOSSY15* (*GL15*) in maize (*Zea mays*) inhibits it.

the translational level by an miRNA-responsive element, complementary to miR156 and miRNA157, in the 3′ untranslated region of the *SPL3* mRNA (Gandikota *et al.*, 2007). *SPL3* and other miR156-regulated SBP-box genes, *SPL4*, *SPL5*, *SPL9* and *SPL15*, have been shown to be target genes involved in promoting the adult state and the end of the juvenile phase, as well as flowering (Wu & Poethig, 2006; Schwarz *et al.*, 2008). In the *hst-6* mutant the levels of miR156 are reduced and *SPL3* mRNA levels are increased (Park *et al.*, 2005; Wu & Poethig, 2006), observations consistent with the reduced juvenile phase length of this mutant.

The level of miR156 was shown to be higher in juvenile tissue than adult tissue (Wu & Poethig, 2006); this is opposite to the pattern of expression of another miRNA, miR172, that also affects juvenile phase length in maize (Lauter *et al.*, 2005). miR172 targets an *APETALA2* (*AP2*)-like gene, *GLOSSY15* (*GL15*), in maize which is expressed in juvenile leaves and which promotes the juvenile phase. In Arabidopsis, miR172 targets other *AP2*-like genes which are involved in repressing the floral transition (Aukerman & Sakai, 2003; Schmid *et al.*, 2003; Jung *et al.*, 2007), and this is described in more detail in section III (4). The reciprocal expression pattern of miR156 and miR172, and consequently their target genes *SPL3* and *GL15*, which are known to inhibit and promote the juvenile phase, respectively, suggests that these miRNA genes might be controlled by the same regulatory pathway (Wu & Poethig, 2006).

Thus post-transcriptional regulation by both ta-siRNAs and miRNAs is involved in the regulation of the length of the juvenile phase. It will be interesting to see if intercellular movement of these small RNA molecules accounts for the non-cell-autonomous action of the *TP1* and *TP2* genes reported by Dudley & Poethig (1993). It will also be interesting to establish whether environmental conditions that affect juvenile phase length do so through affecting the production or action of miRNAs and ta-siRNAs. It has already been shown that photoperiod and light quality affect miR172 levels in Arabidopsis, with levels being higher in LDs and in blue light (Jung *et al.*, 2007).

There is evidence that the juvenile state exists both in leaves and at the apex. Juvenile shoots of *Bryophyllum* were able to flower after grafting onto florally induced mature plants (Zeevaart, 1962), as were juvenile seedlings of *Ipomaea batatas* after grafting onto induced *P. nil* stocks (Takeno, 1991), indicating that in these cases the properties of the leaves on the stock plants were the determining factor. Conversely, grafting of juvenile buds of Japanese larch (*Larix kaempferi*) onto mature trees did not cause them to flower, whereas when mature buds were grafted flowering ensued, suggesting that the state of the apex was the determining factor in this case (Robinson & Wareing, 1969). Thus, changes in leaves and/or apices may be involved in the transition from the juvenile phase to the adult, florally competent phase, depending upon the species. Experiments in maize have shown that this transition is not rapid, with leaves that are being formed during the transitional period exhibiting both juvenile traits at the tip and adult traits at the base, and that these changes occur in response to factors that originate outside of the shoot apical meristem (SAM) (Orkwiszewski & Poethig, 2000).

In addition to the juvenile to adult phase change, the competence of the apex of adult plants to respond to inducing signals also changes with time. As plants get older the SAM responds more readily to inducing signals; this has been shown by grafting tobacco (*Nicotiana tabacum*) apices of differing ages onto stock plants (Singer *et al.*, 1992). In Arabidopsis this phenomenon may in part be attributed to changes in expression of the meristem identity gene *LEAFY* (*LFY*) in the apex, which gradually increases during vegetative growth in noninducing conditions (Blazquez *et al.*, 1997). It has also been suggested that the apex changes in its competence to respond to LFY activity, as photoperiod was shown to modulate the effect of constitutive *LFY* over-expression on flowering time (Weigel & Nilsson, 1995); indeed, analysis of *LFY* over-expression in late-flowering mutants demonstrated that some flowering time genes affected *LFY* transcription whereas others affected the response to LFY (Nilsson *et al.*, 1998), and the molecular basis for this is now starting to be understood (Chae *et al.*, 2008; Lee *et al.*, 2008). Alteration of the competence of the apex to respond to LFY activity is likely to be a mechanism to control flowering in tobacco, petunia (*Petunia hybrida*) and *Impatiens balsimina*, as *LFY* homologues in these species have been shown to be expressed both in noninduced vegetative apices and in florally induced apices (Kelly *et al.*, 1995; Pouteau *et al.*, 1997; Souer *et al.*, 1998). Other genes shown to be involved in controlling the competence of the apex to respond to floral inducing signals are *PENNYWISE* (*PNY*) and *POUND-FOOLISH* (*PNF*). Mutations in these genes prevent the vegetative to floral transition in inducing conditions despite the induced state of the plant, as indicated by the induction of the floral integrator gene *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) in the apex (Smith

et al., 2004). It has recently been shown that *pny pnf* double mutants prevent the activation of *LFY* by FLOWERING LOCUS T (FT) (Kanrar *et al.*, 2008).

In plants that require a vernalization response, the competence of the apex to respond to inducing signals, such as inducing photoperiods, is affected by vernalization through the regulation of a repressor of flowering. In Arabidopsis this repressor is FLOWERING LOCUS C (FLC). High levels of FLC repress *FT* expression and also prevent induction at the apex (Searle *et al.*, 2006). *FLC* expression is itself regulated by the autonomous and vernalization flowering pathways, as well as by the *FRIGIDA* (*FRI*) gene. The vernalization and autonomous pathways act to reduce the levels of *FLC* expression, thereby relieving the repression at the apex and increasing its competence to be induced by other pathways such as the photoperiodic pathway (Mouradov *et al.*, 2002; Henderson *et al.*, 2003; Bäurle & Dean, 2006).

III. The photoperiodic response pathway

The ability to respond to photoperiod requires a mechanism to detect daylength. In Arabidopsis, which is a facultative LDP that is induced to flower earlier in LDs than in SDs, this mechanism has been shown to involve the interaction of light signals which are perceived by photoreceptors such as phytochromes, cryptochromes, and the blue light receptor F-box proteins ZEITLUPE (ZTL) and FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1), with components of the circadian clock, and the *CONSTANS* (*CO*) gene and protein whose rhythmic expression is driven by the circadian clock. The CO protein is a major regulator of photoperiodic flowering and it directly induces the expression of the floral integrator gene *FT* and the closely related *TWIN SISTER OF FT* (*TSF*) (Samach *et al.*, 2000; Wigge *et al.*, 2005; Yamaguchi *et al.*, 2005). The CO protein is expressed at very low levels and its abundance is the limiting factor in the induction of flowering by photoperiod, as demonstrated by the fact that reducing CO levels by half in heterozygous plants delays flowering (Robson *et al.*, 2001). The photoperiodic pathway precisely regulates levels of the CO protein during the course of the day, with levels of the CO protein increasing from *c.* 10 h after dawn onwards, reaching high levels by 16 h after dawn or later (i.e. towards the end of a LD) (Valverde *et al.*, 2004). The coincidence of high levels of *CO* expression with light is necessary for floral induction, as was demonstrated by experiments where flowering could be induced by altering the light/dark regime, or *CO* expression, such that high levels of *CO* expression occurred in the light period in SDs (Roden *et al.*, 2002; Yanovsky & Kay, 2002).

1. The circadian clock

The circadian clock is an endogenous timekeeping mechanism based upon several interconnected negative feedback loops. These feedback loops enable the clock to continue to cycle in constant conditions, that is, without entrainment by zeitgeber (German for 'time-giver') signals such as changes in light or temperature conditions which act to synchronize the circadian clock with the external environment. The clock controls many responses that need to be co-ordinated to particular times of the daily cycle, and several reviews have been published on this topic recently (Gardner *et al.*, 2006; McClung, 2006; Hotta *et al.*, 2007), so the clock will not be described in great detail here. The basic negative feedback loop consists of the *TIMING OF CAB EXPRESSION 1* (*TOC1*) gene whose product positively regulates two partially redundant Myb transcription factors, *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*). LHY and CCA1 proteins then feed back to negatively regulate the expression of *TOC1* through binding to an evening element in its promoter (Alabadi *et al.*, 2001). The stability of the TOC1 protein is also regulated, in this case by ZTL, which targets TOC1 for degradation by the 26S proteasome (Más *et al.*, 2003). Other feedback loops involve the *PSEUDO RESPONSE REGULATOR* (*PRR*), *GIGANTEA* (*GI*) and *LUX ARRHYTHMO* (*LUX*) genes (Gardner *et al.*, 2006; McClung, 2006).

2. The role of light

The light signal has three principal functions in the photoperiodic response mechanism.

(i) It entrains the clock to a 24-h cycle (the clock has a free-running period of between 22 and 29 h (Michael *et al.*, 2003), so without entrainment would get out of phase with the normal day/night cycle within a few days). Both red light acting through the phytochromes phyA, phyB, phyD and phyE (the role of phyC not having been established) and blue light acting through ZTL and the cryptochromes cry1 and cry2 are involved in entrainment of the clock (Somers *et al.*, 1998; Devlin & Kay, 2000; Kim *et al.*, 2007b). Light signals entrain the clock by inducing the expression of genes that are key components of the clock, such as *LHY*, *CCA1* and *PRR9* (Wang & Tobin, 1998; Kim *et al.*, 2003; Farré *et al.*, 2005). Light also affects clock components at the post-transcriptional level, as blue light enhances the stability of ZTL by promoting its interaction with another clock component, GI; this confers a rhythm on ZTL protein levels which results in an amplified and sharper peak in TOC1 protein levels (Kim *et al.*, 2007b). Correct entrainment of the clock is important as it sets the phase of expression of clock-regulated genes such as *CO*, which are outputs from the clock, in relation to the daily light/dark cycle.

(ii) It promotes the blue-light-dependent interaction between FKF1 and GI which is necessary for the degradation of a transcriptional repressor of *CO* called CYCLING DOF FACTOR 1 (CDF1) (Sawa *et al.*, 2007), and which thus promotes *CO* expression. Both *gi* and *fkf1* mutants are late flowering and have reduced levels of *CO* mRNA, as do *CDF1* over-expressing

lines (Suàrez-López *et al.*, 2001; Imaizumi *et al.*, 2003, 2005). *FKF1*, *GI* and *CDF1* are all under circadian control but, whereas *FKF1* and *GI* have similar phases of expression, peaking 8–10 h after dawn, *CDF1* expression peaks earlier in the morning (Fowler *et al.*, 1999; Imaizumi *et al.*, 2003, 2005). It is proposed that CDF1 is bound to the *CO* promoter and inhibits *CO* transcription in the first part of the day. GI and FKF1, which are produced later in the day, form a complex in a blue-light-dependent manner, which binds to CDF1, enabling FKF1 to target CDF1 for degradation by the 26S proteasome, thus relieving the repression of *CO* and allowing its expression towards the end of a LD (Sawa *et al.*, 2007).

(iii) It regulates CO protein stability. Red light acting through phyB promotes the degradation of CO by the proteasome, whereas far-red and blue light acting through phyA and the cryptochromes, respectively, increase the stability of CO. The phyB-mediated degradation predominates during the morning, whereas this is antagonized towards the end of the day by the action of phyA and the cryptochromes, resulting in the stabilization of CO at the end of a LD (Valverde *et al.*, 2004). In the dark, the CO protein is targeted for degradation by the proteasome by the CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) protein, a RING finger ubiquitin ligase that regulates the stability of transcription factors involved in the plant's response to light (Osterlund *et al.*, 2000; Holm *et al.*, 2001; Seo *et al.*, 2003). COP1 activity is higher in the dark than in the light as a result of exclusion of COP1 from the nucleus in the light (von Arnim & Deng, 1994) and also through direct repression by cryptochromes in the light (Wang *et al.*, 2001). Although *CO* is expressed at high levels during the dark period in both SDs and LDs (Suàrez-López *et al.*, 2001), the action of COP1 prevents accumulation of the CO protein during the dark period (Jang *et al.*, 2008). The degradation of CO by COP1 may involve members of the SUPPRESSOR OF PHYTOCHROME A-105 (SPA) family of proteins which have been shown to bind to both CO and COP1, and to regulate the ubiquitin ligase activity of COP1 (Saijo *et al.*, 2003; Laubinger *et al.*, 2006).

3. The role of CO

The light-dependent regulation of CO at both the transcriptional and post-transcriptional levels modifies the circadian oscillation of *CO* expression to allow higher levels of expression of *CO* towards the end of a LD (between 10–16 h after dawn), and also promotes the stability of the CO protein at this time of the day. This allows high levels of CO protein to accumulate during the light period, causing a strong induction of the *FT* gene which ultimately results in flowering (Fig. 2). In SDs, however, *CO* expression does not rise to high levels during the daytime. There is no GI/FKF1-induced daytime peak of *CO* expression because the circadian rhythm of expression of *GI* and *FKF1* is such that the proteins are not present in sufficient amounts to relieve the CDF1-mediated repression of *CO*. *FT*

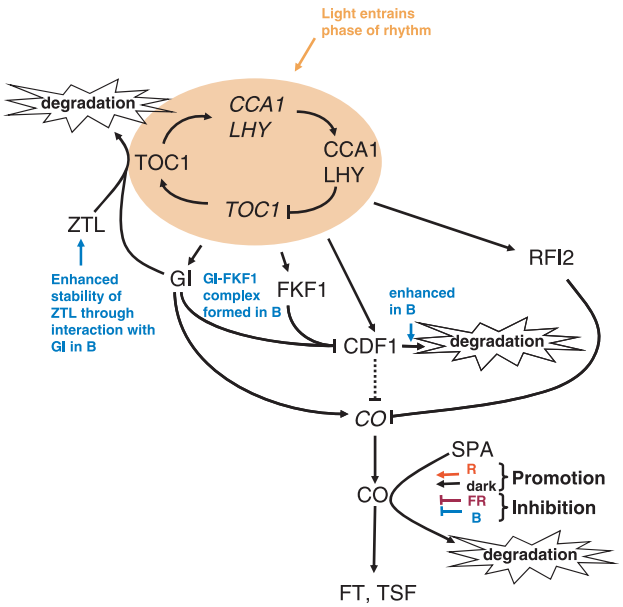

Fig. 2 Light effects on the CONSTANS (CO)-dependent photoperiodic pathway. Not all of the components of the clock are shown. Arrows indicate induction, and bars at the end of lines indicate inhibition. Proteins are designated by normal type, and gene transcripts by italics. R, red light; FR, far-red light; B, blue light. CCA1, CIRCADIAN CLOCK ASSOCIATED 1; CDF1, CYCLING DOF FACTOR 1; FKF1, FLAVIN-BINDING KELCH REPEAT F-BOX 1; FT, FLOWERING LOCUS T; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYL; RFI2, RED AND FAR RED INSENSITIVE 2; SPA, SUPPRESSOR OF PHYTOCHROME A-105; TOC2, TIMING OF CAB EXPRESSION 2; TSF, TWIN SISTER OF FT; ZTL, ZEITLUPE.

expression is not induced and neither is flowering. It has to be remembered that in most experimental designs a LD consists of 16 h light and a SD of 8 or 10 h light, whereas natural daylengths vary in a continuum between (and beyond) these values. In LDPs such as Arabidopsis, the CDL is the point at which the photoperiod has become long enough to be florally inductive, that is, has reached the threshold point where CO protein levels have risen high enough to cause the induction of *FT* and flowering. As mentioned above, the CDL varies among plants of different species, and also within species, which must be as a result of slightly altered expression patterns of *CO* and genes affecting CO protein accumulation.

In addition to GI, FKF1 and CDF1 there are other factors that also affect *CO* expression. Reduced levels of the CDF1 repressor in *CDF1* RNAi lines do not result in de-repression of *CO* expression as might be expected, indicating that other repressors of *CO* transcription exist. Furthermore, the late-flowering phenotype of the *fkf1* mutant cannot be completely restored by reduced levels of *CDF1* expression in these *CDF1* RNAi lines, indicating that FKF1 is not exerting its effect through the degradation of CDF1 alone, but may also act upon these other unknown repressors of *CO* (Imaizumi *et al.*, 2005). Similarly, over-expression of *GI* in the *fkf1* mutant background causes early flowering, indicating that *GI* affects

flowering via factors other than *FKF1* (Sawa *et al.*, 2007). As the GI protein is present in SDs as well as LDs (David *et al.*, 2006), its abundance being very different from that in the expression profile of *CO*, these other factors must act to prevent activation of *CO* by GI at inappropriate times of the day. One such factor could be the RING finger protein RED AND FAR RED INSENSITIVE 2 (RFI2) which is reported to repress *CO* expression primarily in LDs and is thought to act together with *GI* (Chen & Ni, 2006).

CO promotes flowering by inducing the expression of the floral integrator genes *FT*, *TSF* and *SOC1* (Onouchi *et al.*, 2000; Samach *et al.*, 2000; Yamaguchi *et al.*, 2005). *FT* and *TSF* are induced directly by *CO*, and they have a peak of expression at the end of a LD caused by the high CO protein abundance at this time of day (Suàrez-López *et al.*, 2001; Yanovsky & Kay, 2002; Yamaguchi *et al.*, 2005). *SOC1* is then induced in turn by *FT* (Yoo *et al.*, 2005). CO does not possess a typical DNA-binding domain and therefore it is likely to need to bind to another protein partner(s) in order to bind to sequences in the *FT* promoter. It has recently been shown that CO can interact *in planta* with the Arabidopsis orthologues of the mammalian HEME ACTIVATOR PROTEIN 3 (HAP3) and HAP5 (Wenkel *et al.*, 2006), and also that a tomato (*Solanum lycopersicum*) CO homologue (Tomato CO-LIKE 1 (TCOL1)) can interact with the tomato HAP5 protein (Ben-Naim *et al.*, 2006). It is proposed that CO is able to replace HAP2 in the HAP2/HAP3/HAP5 trimeric HAP complex (also called the CCAAT box factor (CBF), or nuclear factor Y (NF-Y)) which binds to CCAAT boxes in eukaryotic promoters (Wenkel *et al.*, 2006). In tomato it was shown that the TCOL1-HAP complex binds to CCAAT motifs of the yeast *CYC1* and *HEM1* promoters, demonstrating that CO-like proteins are able to bind DNA through interacting with the HAP complex (Ben-Naim *et al.*, 2006). The HAP complex has been shown to also bind CAAT motifs in tobacco (Kusnetsov *et al.*, 1999), and there are several of these motifs in the *FT* promoter region. It has yet to be shown, however, that the CO/HAP3/HAP5 complex can bind the *FT* promoter directly.

Perception of daylength occurs in the leaf. *CO* is expressed in the vascular tissues of hypocotyls, cotyledons and leaves, and also in the apex (Takada & Goto, 2003; An *et al.*, 2004); however, whilst its expression from the phloem companion cell-specific sucrose transporter (*SUC2*) promoter was sufficient to complement the *co-2* mutation, its expression from meristem-, epidermis- or root-specific promoters was not (An *et al.*, 2004). These results indicate that CO acts specifically in the phloem to induce flowering. CO is a direct activator of *FT*, and *FT* expression is also observed in the vascular tissue of cotyledons and in the apical part of the leaves (but not the basal parts, or in the primary veins). Expression of *FT* in phloem companion cells is required for the induction of flowering, as flowering is prevented if its expression in these cells is reduced by artificial miRNAs (Mathieu *et al.*, 2007). *FT* gene expression was not observed in the SAM (An *et al.*, 2004); however, unlike *CO* it can induce flowering if expressed from a meristem-specific promoter or indeed an epidermis-specific promoter (An *et al.*, 2004). Thus, whilst the functionality of the CO protein appears to be restricted to the phloem, where its role is to induce *FT*, FT can exert its influence if it is present in other tissues in the plant.

4. CO-independent pathways

Photoperiod can also regulate flowering time via a separate pathway that does not involve *CO*. *GI* regulates an miRNA called miR172, the target genes of which are the *AP2*-like genes *TARGET OF EAT 1* (*TOE1*), *TOE2*, *TOE3*, *SCHLAFMUTZE* (*SMZ*) and *SCHNARCHZAPFEN* (*SNZ*), which it down-regulates post-transcriptionally. Over-expression of all of these genes apart from *TOE3* causes late flowering, indicating that these are floral repressors (Aukerman & Sakai, 2003; Jung *et al.*, 2007). Over-expression of miR172, however, causes extremely early flowering even in a *co* mutant background, and, whilst *CO* expression is unaltered by miR172 over-expression in wild-type plants, *FT* expression is up-regulated (Jung *et al.*, 2007). TOE1 is a repressor of *FT* expression, and so miR172 induces flowering through the alleviation of the repression of *FT* by TOE1. The expression of miR172 increases with plant age until flowering, and the transcript levels of its target genes *TOE1*, *TOE2*, *SMZ* and *SNZ* (but not *TOE3*) all decrease with age in a complementary fashion (Jung *et al.*, 2007). The fact that miR172 levels are in part regulated by genes involved in the autonomous pathway, such as *FCA*, *FLK* and *FVE*, may account for this age-related regulation. It is possible that the repression of *FT* by high levels of TOE1 in very young plants helps to prevent the meristem responding to inducing signals during the juvenile phase; however, this remains to be determined (as mentioned previously miR172 is already known to regulate *GL15*, which affects juvenility in maize). The levels of miR172 are increased in blue light but decreased in red light, and are much higher in plants grown in LDs than in those grown in SDs, thus implicating miR172 in the promotion of flowering in inducing LDs. GI regulates miR172 abundance in a clock-independent manner, as miR172 levels do not have any rhythmic oscillations. *GI* therefore has a dual role in the photoperiodic control of flowering, regulating both the *CO*- and miR172-mediated induction of *FT* expression (Jung *et al.*, 2007), and both pathways are required for the promotion of flowering in LDs as disruption of either results in late flowering in LDs.

IV. Systemic signals

Classical grafting experiments clearly demonstrated the existence of a graft-transmissible flower-inducing signal that moved from induced leaves through the phloem to the apex (reviewed in Thomas & Vince-Prue, 1997). The FT protein has been detected by mass spectroscopy in the phloem of *Brassica napus*

and *Cucurbita maxima* (Giavalisco *et al.*, 2006; Lin *et al.*, 2007). This is not so surprising considering that *FT* is expressed in phloem companion cells and that it has been shown that there is nonselective loading of proteins of up to 67 kDa from companion cells into the phloem sieve elements (Stadler *et al.*, 2005); thus there would be no restriction to the entry of the small 20-kDa FT protein into the phloem. However, a raft of recent publications have shown that in several species, including rice (*Oryza sativa*) and Arabidopsis, the FT protein is also able to move intracellularly from the end of the vasculature into the SAM (Corbesier *et al.*, 2007; Jaeger & Wigge, 2007; Mathieu *et al.*, 2007; Tamaki *et al.*, 2007), and even across graft unions (Corbesier *et al.*, 2007; Lin *et al.*, 2007). Elegant experiments where the effects of the FT protein were uncoupled from those of *FT* mRNA demonstrated that movement of the FT protein alone from the phloem into the SAM was sufficient to induce flowering (Jaeger & Wigge, 2007; Mathieu *et al.*, 2007), providing a convincing argument that the FT protein (and its paralogues such as TSF; Mathieu *et al.*, 2007) is a component of the mobile flower-inducing signal.

It is well established that mRNAs can also move through the phloem throughout the plant to control developmental processes such as tuberization and leaf development (Kim *et al.*, 2001; Haywood *et al.*, 2005; Banerjee *et al.*, 2006). In many of the above experiments, movement of the *FT* mRNA across a graft union or into the SAM was not detected; however, Tamaki *et al.* (2007) did report the detection of low levels of mRNA of the rice *FT* orthologue *Heading date 3a* (*Hd3a*) in rice shoot apices although it is not expressed there. So the question of whether *FT* mRNA does move, and what its function may be, may still be open to debate. Similarly, the role of small RNA molecules in the spread of the induced state throughout the plant remains to be established. Several miRNAs have been detected in phloem sap, including miR156 (Yoo *et al.*, 2004), which has been shown to affect the floral transition through its regulation of SBP-box genes (Wu & Poethig, 2006; Schwarz *et al.*, 2008), although transport of gene-silencing RNAs into the apex may be prevented by the RNA surveillance system present at the SAM (Foster *et al.*, 2002).

When the FT protein arrives in the apex it interacts with the bZIP transcription factor FLOWERING LOCUS D (FD) to form a transcriptional complex that activates the meristem identity gene *AP1* (Abe *et al.*, 2005; Wigge *et al.*, 2005). Mutations in *FD* do not completely suppress the early flowering phenotype of *FT* over-expressing plants, indicating that FT does not act through FD alone (Abe *et al.*, 2005; Wigge *et al.*, 2005). FT is also known to up-regulate *SOC1* expression in the SAM (Yoo *et al.*, 2005). SOC1 forms a complex with another MADS box protein, AGAMOUS-LIKE 24 (AGL24), which translocates it to the nucleus where it binds the *LFY* promoter to induce *LFY* expression (Lee *et al.*, 2008). LFY induces the expression of *AP1*, and vice versa.

Other compounds that affect flowering are also transported from leaves to the apex. These compounds range from hormones such as gibberellins and cytokinins to metabolites such as sucrose, nitrate and glutamine, some of which may act by altering the rate of cell division at the SAM (Bernier & Perilleux, 2005). Photoperiodic induction in *Sinapis* and *Xanthium* leads to increased export of sucrose and cytokinin from the leaf, and it is proposed that this results in an increase in hexoses at the SAM, which triggers the observed increase in cell division at the SAM in *Sinapis* following photoperiodic induction (Gonthier *et al.*, 1987; Bernier & Perilleux, 2005). Increasing cell division in the SAM can cause early flowering, as was shown by over-expressing the Arabidopsis *CYCLIN D2* gene in tobacco (Cockcroft *et al.*, 2000); however, whether these compounds are part of the inducing signal *per se*, or whether their transport to the apex is an early event following induction, is a question that has been difficult to answer. The observation that tobacco callus derived from induced plants could form flowers if grown on media supplemented with glucose, but that callus derived from noninduced plants did not (Chailkhyan *et al.*, 1975), suggests that the latter may be the case. However, in Arabidopsis, mutations in the sucrose transporter gene *AtSUC9* resulted in early flowering only in SDs and not in LDs (Sivitz *et al.*, 2007), and down-regulation of the sucrose transporter gene *SUT4* in potato (*Solanum tuberosum*) resulted in increased sucrose export from source leaves and enabled *S. tuberosum* ssp. *andigena*, which normally only tuberizes in SDs, to tuberize in LDs, an effect that was graft-transmissible (Chincinska *et al.*, 2008). It has been suggested that, in potato, sucrose may link light quality perception by photoreceptors to GA signals regulating tuberization.

V. Moderating factors

As mentioned previously, the photoperiodic pathway is a component of an interacting network that regulates flowering, and the influence of photoperiod on flowering is moderated by other factors such as temperature and the developmental age of the plant. In Arabidopsis, FLC is a general repressor of flowering; it acts in both the phloem to inhibit *FT* and *SOC1* expression, and in the SAM where it inhibits *FD* and *SOC1* expression, thereby affecting the competence of the SAM to respond to the FT protein (Searle *et al.*, 2006). The levels of *FLC* expression are tightly controlled and different flowering pathways, such as the vernalization and autonomous pathways, act to reduce *FLC* expression, thereby relieving the repression of flowering and allowing flowering to be induced by other pathways such as the photoperiodic pathway (Mouradov *et al.*, 2002; Henderson *et al.*, 2003; Bäurle & Dean, 2006). It is through the interaction between the autonomous and vernalization pathways that control FLC levels, and the floral inductive pathways such as the photoperiodic pathway, that the Arabidopsis plant regulates both the ability to produce the inducing FT signal and the competence of the SAM to respond to this signal, and is thus able to control the seasonal/developmental timing of the floral transition. FLC orthologues have been identified in *Brassica* and sugar beet (*Beta vulgaris*) (Reeves *et al.*, 2007), but in other

species such as wheat (*Triticum aestivum*) the vernalization response is conferred by a different repressor which performs the same function (Yan *et al.*, 2004).

In Arabidopsis, the repression of *FLC* by the autonomous and vernalization pathways is mediated through chromatin modifications, and this may involve siRNAs (He & Amasino, 2005; Bäurle & Dean, 2006; Swiezewski *et al.*, 2007). Similarly, chromatin remodelling factors appear to regulate chromatin structure around the *FT* locus, as mutations in the *EARLY BOLTING IN SHORT DAYS* (*EBS*) and *TERMINAL FLOWER 2* (*TFL2*) genes result in elevated *FT* expression levels and early flowering in both LDs and SDs (Kotake *et al.*, 2003; Pineiro *et al.*, 2003); levels of *TSF* are also up-regulated in the *ebs* mutant (Yamaguchi *et al.*, 2005). Furthermore, chromatin modifications, mediated independently of *FLC* through *At MSI1*, are also involved in the regulation of *SOC1* levels (Bouveret *et al.*, 2006). In fact, it has been shown that there is widespread decondensation of gene-rich chromatin in leaves of Arabidopsis plants undergoing the floral transition, and this chromatin decondensation requires the blue light receptor cry2, indicating that it is under light regulation (Tessadori *et al.*, 2007).

In addition to the photoperiodic pathway, other pathways also induce flowering. Apart from its effects on the clock, *CO* expression and protein stability, light quality acts to promote flowering independently of *CO* and the photoperiodic pathway through the action of *PHYTOCHROME AND FLOWERING TIME 1* (*PFT1*), which acts downstream of phyB to regulate *FT* (Cerdán & Chory, 2003). The delayed flowering of the *pft1* mutant in LDs indicates that PFT1 is able to affect the induction of FT by the photoperiodic pathway, and thus may be able to modulate the extent of photoperiodic induction under different light qualities, for example in vegetative shade. Changes in ambient temperature affect the flowering response quite dramatically; growing Arabidopsis plants in SDs at 27°C rather than at 23°C will induce plants to flower as efficiently as transferring them to inductive LD conditions. This induction does not involve the photoperiodic or autonomous pathways, but does require an active GA response pathway, and acts through *FT* (Balasubramanian *et al.*, 2006). By contrast, growing Arabidopsis plants at a cooler temperature of 16°C delays flowering except in the *fca* and *fve* mutants of the autonomous pathway suggesting that these genes play a role in the flowering response to cooler temperatures (Blázquez *et al.*, 2003). The *SHORT VEGETATIVE PHASE* (*SVP*) gene was shown to function downstream of these genes in the thermosensory pathway, and it acts to repress flowering by binding to the *FT* promoter (Lee *et al.*, 2007). Temperature also affects the role of phytochromes in regulating the flowering response, as the repression of flowering mediated by phyB and phyD observed at 22°C is abolished at 16°C; at the lower temperature, flowering is repressed by the action of phyE instead (Halliday *et al.*, 2003).

The hormone GA induces flowering in Arabidopsis; however, its effect is predominantly in SDs when the photoperiodic pathway is not active. GA acts to directly induce *LFY* expression via a domain in the *LFY* promoter that is different to the one required for photoperiodic induction of *LFY* (Blázquez & Weigel, 2000). This domain binds the GAMYB transcription factor which in turn is regulated by a miRNA, miR159 (Achard *et al.*, 2004). GA also induces the expression of *SOC1* (Moon *et al.*, 2003), and antagonizes the repression of floral homeotic genes by the DELLA protein REPRESSOR OF GA1–3 (RGA) (Yu *et al.*, 2004). It should be noted that in some species, for example roses, GA is inhibitory for flowering (Roberts *et al.*, 1999). Abscisic acid (ABA) delays flowering through binding to the FCA protein and preventing its complex formation with FY, which is necessary to repress *FLC* expression (Razem *et al.*, 2006). Stress conditions also affect flowering time; the stress hormone salicylic acid induces *FT* expression and promotes flowering (Martínez *et al.*, 2004), whilst conversely nitric oxide, which is produced under biotic and abiotic stresses, represses *CO* and *GI* and elevates *FLC* expression to inhibit flowering (He *et al.*, 2004). Thus there are many factors that are able to influence the induction of flowering by photoperiod, and relatively little is known about the molecular interactions that are involved.

VI. The flowering response to photoperiod in other species

The molecular mechanisms outlined above primarily describe the photoperiodic flowering response in the LDP Arabidopsis. This has greatly helped our understanding of the variation in photoperiodic responses in crop plants such as barley (*Hordeum vulgare*) and wheat, which are also both LDPs. A major gene controlling this response to LDs in barley, the *Photoperiod-H1* (*Ppd-H1*) gene, is a pseudo-response regulator (*PRR*) gene which has highest similarity to the Arabidopsis *PRR7* gene (Turner *et al.*, 2005). *PRR7* in Arabidopsis is involved in the re-setting of the clock in response to light signals, and mutants have altered expression of clock genes and are late flowering in LDs (Farré *et al.*, 2005). The mutant *ppd-H1* allele, which confers late flowering in LDs, was shown to cause a delay in the induction of the barley *CO* genes *HvCO1* and *HvCO2* (Turner *et al.*, 2005). The *ppd-H1* allele also prevented the induction of the barley *FT* gene (*HvFT*) in LDs and this is thought to be attributable to the reduced levels of *HvCO1* and *HvCO2* gene expression in LDs, although it is also possible that the *ppd-H1* mutation affects another pathway that regulates *FT* expression independently of *HvCO1* and *HvCO2*.

The barley *Ppd-H1* gene is collinear with the wheat *Ppd-D1* locus, which affects the sensitivity to photoperiod. The allele conferring reduced photoperiod sensitivity and early flowering, *Ppd-D1a*, was widely used in the 'green revolution' to breed varieties that were adapted to a broader range of environments. This *Ppd-D1a* allele has recently been cloned and has been shown to contain a 2-kb deletion in the upstream region of a *PRR* gene which leads to mis-expression of this gene (Beales *et al.*, 2007). The expression of the wheat *FT* gene

(*TaFT*) was up-regulated in both the light and the dark in a substitution line carrying the *Ppd-D1a* allele, which explains the early-flowering phenotype. The timing of expression of the wheat *GI* and *CO* genes (*TaGI* and *TaCO1*) was unchanged, although *TaCO1* expression in the dark was reduced. This shows that the up-regulation of *TaFT* is independent of *TaCO1*, although it could be caused by altered expression of another *CO*-like gene in wheat, or indeed it could be mediated through a *CO*-independent pathway. Thus, whilst the barley *ppd-H1* and wheat *Ppd-D1a* alleles are both mutations in *PRR* genes, the mutations are of a different nature and result in distinct phenotypes (late flowering in LDs, and early flowering in both LDs and SDs, respectively). It does suggest, however, that *PRR* genes are able to provide great adaptive flexibility as they can cause early or late flowering and thus they appear to be good targets for selection.

A lot of research has been carried out to investigate whether similar mechanisms to those described in Arabidopsis also operate in controlling flowering in SDPs such as rice. Orthologues of *GI*, *CO* and *FT* have been identified in rice; these are *OsGI*, *HEADING DATE 1* (*Hd1*) and *Hd3a*, respectively. These genes function differently in rice. *OsGI* promotes the expression of the rice *CO* orthologue *Hd1*, as is the case in Arabidopsis. Unlike the situation in Arabidopsis, however, *Hd1* inhibits the rice *FT* orthologue *Hd3a* in LDs but promotes its expression in SDs, and induction of *Hd3a* then promotes flowering in SDs (Izawa *et al.*, 2002; Kojima *et al.*, 2002; Hayama *et al.*, 2003). *Hd3a* is a member of a small *FT*-like gene family in rice. Other members include *RICE FLOWERING LOCUS T1* (*RFT1*) and *FT-LIKE* (*FTL*), whose expression is also up-regulated in SDs and over-expression of which also causes early flowering as with *Hd3a* (Izawa *et al.*, 2002). The expression of these three *FT*-like genes is elevated in a chromophore-biosynthetic mutant of rice, *photoperiodic sensitivity 5* (*se5*), which is deficient in active phytochrome. The phytochrome-mediated repression of these genes does not occur through the circadian clock, or reduced *Hd1* expression, as these are both unaltered in the *se5* mutant. This suggests that phytochromes are not involved in the entrainment of the circadian clock in rice as they are in Arabidopsis and that there are two pathways controlling flowering in rice – a clock/*Hd1*/*Hd3a* pathway and a phytochrome/*Hd3a* pathway (Izawa *et al.*, 2002). *EARLY HEADING DATE 1* (*Ehd1*) is a B-type response regulator that induces the expression of *Hd3a* and *RFT1* in SDs in an *Hd1*-independent manner (Doi *et al.*, 2004), and thus may be involved in the second pathway. *OsMADS51* is a promoter of flowering that acts upstream of *EHD1* and which in turn appears to be regulated by *OsGI* (Kim *et al.*, 2007a). *OsGI* therefore seems to be involved in both the *Hd1* and the *Ehd1* pathways, just as *GI* is involved in the *CO*-dependent and *CO*-independent flowering pathways in Arabidopsis. As in Arabidopsis and several other species, the FT orthologue in rice has been shown to be a mobile flowering signal following demonstration that the Hd3a protein is able to move from the vascular tissue into the SAM to induce flowering (Tamaki *et al.*, 2007). There is also recent evidence that, as with the Arabidopsis *FT* gene, chromatin modifications may be involved in the regulation of *RFT1* (Komiya *et al.*, 2008). Whether the other rice *FT*-like genes are also subject to this type of regulation is as yet unknown.

As rice is a monocot and Arabidopsis is a dicot, it is perhaps unsurprising that these distantly related species respond differently to photoperiod. However, different photoperiodic responses are also found between members of the same family in both dicots and monocots, indicating that SD and LD responses have evolved independently several times rather than as a single evolutionary event. This is proposed to be the case for Pharbitis (*Ipomoea nil*), which is an SDP like rice and, as is the case in rice, the Pharbitis *FT* homologues *PnFT1* and *PnFT2* are expressed in SDs but not LDs (Hayama *et al.*, 2007). However, the regulation of *PnFT1* and *PnFT2* expression appears to be through a different mechanism to the one that has been described above for rice. In Pharbitis, *PnFT1* and *PnFT2* expression appears to be regulated by the circadian clock in a more direct manner than that of *FT* or *Hd1*, with peaks of expression of *PnFT1* and *PnFT2* occurring during the dark period following a SD always at a set time after dusk. The regulation of *PnFT1* and *PnFT2* does not appear to be so dependent upon the Pharbitis *CO* homologue *PnCO* as *FT* and *Hd3a* are on *CO* and *Hd1* in Arabidopsis and rice, respectively (Hayama *et al.*, 2007).

VII. Tuberization in potato

Tuberization in potato is another photoperiodic response that has been studied at the molecular level. SDs promote tuberization and LDs inhibit it. Whilst this response to photoperiod has been bred out of most commercially grown potatoes, some potato species cultivated in South America, such certain lines of *Solanum demissum* and *S. tuberosum* ssp. *andigena*, will only tuberize in SDs and will not tuberize in LDs. These potato species exhibit a typical SD response in that tuberization in SDs can be inhibited by a night break, and the effect of an inhibitory night break of red light can be reversed by a far-red light treatment (Batutis & Ewing, 1982). Like flowering, tuberization represents a major developmental switch from vegetative growth to reproductive growth, and involves many physiological changes in addition to the formation of tubers at the stolon tips. Stems become shorter and thicker, leaves become larger and broader, and there is increased flower bud abortion and accelerated senescence (Steward *et al.*, 1981).

There is some evidence that some of the molecular components involved in the photoperiodic control of flowering may also play a role in the photoperiodic control of tuberization. It has been shown that phyB is involved in the photoperiodic control of tuberization and, as in the control of flowering, it acts to repress the response. *PHYB*-antisense plants are able to tuberize as well in noninducing LDs as they do in inducing SDs (Jackson *et al.*, 1996). It has also been shown that phyB regulates the

production of a graft-transmissible signal, as grafting a *PHYB*-antisense plant onto wild-type plants grown in noninducing LDs enabled the wild-type plant to tuberize (Jackson *et al.*, 1998). PhyA also plays a role in daylength perception in potato (Heyer *et al.*, 1995), and both phyA and cryptochromes are involved in entraining the circadian clock in potato (Yanovsky *et al.*, 2000).

A possible role for *CO* in the tuberization response was suggested by over-expressing the Arabidopsis *CO* gene in potato, which resulted in delayed tuberization (Martinez-Garcia *et al.*, 2002). A *GI* homologue and several *CO*-like genes are reported to have been isolated from potato, as have homologues of *FT* – one of which is reported to be up-regulated in SDs but not LDs (Rodriguez-Falcon *et al.*, 2006). It would thus appear that potato has all the basic photoperiod pathway components; it will be interesting to see whether the endogenous *GI*, *CO* and *FT* genes do actually play a role in the photoperiodic control of tuberization. Interestingly, in some lines of the potato *S. tuberosum* ssp. *andigena*, tuberization is under photoperiodic control but flowering is not. This raises interesting questions about how a conserved photoperiodic response mechanism would be able to regulate different plant responses – perhaps different mobile signals are involved? One mobile signal that has been implicated in the regulation of tuberization is the mRNA of the potato *StBEL5* transcription factor (Banerjee *et al.*, 2006). SDs, which induce tuberization, promote both the expression of the gene and movement of the RNA to the stolon tips, resulting in enhanced tuberization. In addition to its expression in the phloem tissue of leaves, petioles and roots, *StBEL5* is expressed in stolons in both SDs and LDs. The expression of *StBEL5* in both photoperiods, and in tissues growing in the dark underground, makes it unlikely that its expression is regulated by CO (*CO* requires light for its expression, and to prevent the protein being degraded). CO may, however, play a role in the increased transport of *StBEL5* mRNA into the stolons in SDs, which may raise the transcript levels over a certain threshold to induce tuberization. StBEL5 interacts with a KNOX transcription factor called POTATO HOMEOBOX 1 (POTH1) to form a heterodimer which binds to the promoter of the GA biosynthetic gene *GA20 OXIDASE 1* (*GA20 ox1*) and inhibits GA biosynthesis (Chen *et al.*, 2004). This would reduce the levels of GA, which is inhibitory for tuberization (Jackson & Prat, 1996) in the stolon. It is possible that the effect of phyB on tuberization may also be mediated through gibberellins, as *GA20 ox1* expression levels were altered in *PHYB*-antisense plants (Jackson *et al.*, 2000), although in this case its expression was up-regulated in leaves.

VIII. Bud set and growth cessation in trees

The onset of bud set and growth cessation which precedes dormancy in trees is a photoperiodic response that is induced by the SDs of autumn. The apsen (*Populus trichocarpa*) *FT* orthologue, *PtFT1*, is an inhibitor of this process and its expression decreases if plants are shifted from LDs to SDs (Böhlenius *et al.*, 2006). Plants over-expressing *PtFT1* do not exhibit growth cessation and bud set even after an extended period in SDs, whereas RNAi lines with decreased levels of *PtFT1* show an enhanced response. The levels of *PtFT1* were shown to be dependent upon levels of the *P. trichocarpa CO* orthologue, *PtCO*, which induces *PtFT1* when it peaks in the light at the end of a LD. The rhythm of *PtCO* expression was found to be different in trees originating from different latitudes and which exhibit different CDLs for the onset of bud set and growth cessation; trees from higher latitudes stop growing earlier in the autumn and have longer CDLs than trees from warmer southern latitudes. This was shown to be a result of *PtCO* expression peaking earlier in the day in the trees from southern latitudes, meaning that the days have to be shorter before this peak of *PtCO* expression occurs in the night, resulting in no induction of *PtFT1* expression and allowing growth cessation and bud set to occur (Böhlenius *et al.*, 2006). Aspen is thus using the same mechanism to control bud set and growth cessation as Arabidopsis is using to control flowering, except in aspen the presence of PtFT1 in LDs acts as an inhibitor of the SD response.

It has been suggested that *PtFT1* may also have a role in determining the length of the juvenile phase in trees, as expression levels increase in young trees as they get older (Böhlenius *et al.*, 2006). Higher levels of expression of *FT2* in *Populus deltoids* were observed in mature trees compared with juvenile trees, and over-expression of *FT2* caused a severe shortening of the juvenile phase, enabling trees to flower in their first year of growth when normally they would flower only after 7–10 yr once the juvenile phase had been completed (Hsu *et al.*, 2006). If this is true then *FT* and its orthologous genes will join an expanding list of genes, including *HST*, miR172, and the SBP-box genes, that are known to play a role in both the juvenile to adult transition and the transition from vegetative to reproductive growth. For a complete understanding of the response to photoperiod it is necessary to consider these two processes together as one continuum, going from the photoperiod-insensitive phase to the photoperiod-sensitive phase, rather than as two separate developmental processes.

To conclude, for plants to respond to photoperiod they need the basic mechanisms for light detection, for timekeeping, and for integrating these external and endogenous signals. Genes homologous to many of the Arabidopsis genes that are known to play a role in the photoperiodic control of flowering have been isolated from an increasingly large number of plant species, and many of these genes have been shown to be true orthologues as they share the same function. In some cases, such as the regulation of growth cessation and bud set in aspen, the control mechanism appears to be very similar to that controlling flowering in Arabidopsis, with PtFT1 acting as a repressor rather than an inducer. In other species, for example Pharbitis and rice, the response to SDs appears to involve different mechanisms to the one that has been defined in

Arabidopsis. In most cases, however, *FT* (or its orthologue) appears to be the end target gene for all these pathways and clearly plays an essential role; this may be because of the ability of the protein to move as a signal molecule through the plant. This may not be the case for all photoperiodic responses in all species though, as there is evidence that RNA can also act as a signal, as is the case for tuberization in potato. The role of miRNAs in the control of flowering has also been established, and some of these, such as miR156, have been shown to be present (and presumably mobile) in the phloem. There is therefore still much research to be carried out to elucidate the photoperiodic control mechanisms in species other than Arabidopsis, as clearly different species have evolved different mechanisms to respond to photoperiod.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309**: 1052–1056.

Achard P, Herr A, Baulcombe DC, Harberd NP. 2004. Modulation of floral development by a gibberellin-regulated microRNA. *Development* **131**: 3357–3365.

Adams SR, Pearson S, Hadley P. 2001. Improving quantitative flowering models through a better understanding of the phases of photoperiod sensitivity. *Journal of Experimental Botany* **52**: 655–662.

Adams SR, Pearson S, Hadley P, Patefield WM. 1999. The effects of temperature and light integral on the phases of photoperiod sensitivity in *Petunia* × *hybrida*. *Annals of Botany* **83**: 263–269.

Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Más P, Kay SA. 2001. Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science* **293**: 880–883.

An H, Roussot C, Suárez-López P, Corbesier L, Vincent C, Piñeiro M, Hepworth S, Mouradov A, Justin S, Turnbull C *et al.* 2004. CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development* **131**: 3615–3626.

von Arnim AG, Deng XW. 1994. Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* **79**: 1035–1045.

Asai K, Satoh N, Sasaki H, Satoh H, Nagato Y. 2002. A rice heterochronic mutant, mori1, is defective in the juvenile-adult phase change. *Development* **129**: 265–273.

Aukerman MJ, Sakai H. 2003. Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. *Plant Cell* **15**: 2730–2741.

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D. 2006. Potent induction of *Arabidopsis thaliana* flowering by elevated growth temperature. *PLoS Genetics* **2**: 0980–0989.

Banerjee AK, Chatterjee M, Yu Y, Suh SG, Miller WA, Hannapel DJ. 2006. Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. *Plant Cell* **18**: 3443–3457.

Batutis EJ, Ewing EE. 1982. Far-red reversal of red light effect during long-night induction of potato (*Solanum tuberosum* L.) tuberization. *Plant Physiology* **69**: 672–674.

Bäurle I, Dean C. 2006. The timing of developmental transitions in plants. *Cell* **125**: 655–664.

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA. 2007. A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **115**: 721–733.

Ben-Naim O, Eshed R, Parnis A, Teper-Bamnolker P, Shalit A, Coupland G, Samach A, Lifschitz E. 2006. The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and DNA. *Plant Journal* **46**: 462–476.

Berardini TZ, Bollman K, Sun H, Poethig RS. 2001. Regulation of vegetative phase change in *Arabidopsis thaliana* by cyclophilin 40. *Science* **291**: 2405–2407.

Bernier G, Perilleux C. 2005. A physiological overview of the genetics of flowering time control. *Plant Biotechnology Journal* **3**: 3–16.

Blázquez MA, Ahn JH, Weigel D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nature Genetics* **33**: 168–171.

Blazquez MA, Soowal LN, Lee I, Weigel D. 1997. LEAFY expression and flower initiation in Arabidopsis. *Development* **124**: 3835–3844.

Blázquez MA, Weigel D. 2000. Integration of floral inductive signals in Arabidopsis. *Nature* **404**: 889–892.

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O. 2006. CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* **312**: 1040–1043.

Bollman KM, Aukerman MJ, Park MY, Hunter C, Berardini TZ, Poethig RS. 2003. HASTY, the Arabidopsis ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. *Development* **130**: 1493–1504.

Bongard-Pierce DK, Evans MMS, Poethig RS. 1996. Heteroblastic features of leaf anatomy in maize and their genetic regulation. *International Journal of Plant Sciences* **157**: 331–340.

Borchert R, Renner SS, Calle Z, Navarrete D, Tye A, Gautier L, Spichiger R, von Hildebrand P. 2005. Photoperiodic induction of synchronous flowering near the Equator. *Nature* **433**: 627–629.

Bouveret R, Schönrock N, Gruissem W, Hennig L. 2006. Regulation of flowering time by Arabidopsis MSI1. *Development* **133**: 1693–1702.

Cerdán PD, Chory J. 2003. Regulation of flowering time by light quality. *Nature* **423**: 881–885.

Chae E, Tan QK, Hill TA, Irish VF. 2008. An Arabidopsis F-box protein acts as a transcriptional co-factor to regulate floral development. *Development* **135**: 1235–1245.

Chailkhyan MK, Aksdenova NP, Konstantinova TN, Bavrina TL. 1975. The callus model of plant flowering. *Proceedings of the Royal Society of London, Series B* **190**: 333–340.

Chen H, Banerjee AK, Hannapel DJ. 2004. The tandem complex of BEL and KNOX partners is required for transcriptional repression of ga20ox1. *Plant Journal* **38**: 276–284.

Chen M, Ni M. 2006. RFI2, a RING-domain zinc finger protein, negatively regulates CONSTANS expression and photoperiodic flowering. *Plant Journal* **46**: 823–833.

Chien JC, Sussex IM. 1996. Differential regulation of trichome formation on the adaxial and abaxial leaf surfaces by gibberellins and photoperiod in *Arabidopsis thaliana* (L.) Heynh. *Plant Physiology* **111**: 1321–1328.

Chincinska IA, Liesche J, Krugel U, Michalska J, Geigenberger P, Grimm B, Kuhn C. 2008. Sucrose transporter StSUT4 from potato affects flowering, tuberization, and shade avoidance response. *Plant Physiology* **146**: 515–528.

Clarke JH, Tack D, Findlay K, Van Montagu M, Van Lijsebettens M. 1999. The SERRATE locus controls the formation of the early juvenile leaves and phase length in Arabidopsis. *Plant Journal* **20**: 493–501.

Cockcroft CE, den Boer BG, Healy JM, Murray JA. 2000. Cyclin D control of growth rate in plants. *Nature* **405**: 575–579.

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C. *et al.* 2007. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316**: 1030–1033.

David KM, Armbruster U, Tama N, Putterill J. 2006. Arabidopsis GIGANTEA protein is post-transcriptionally regulated by light and dark. *FEBS Letters* **580**: 1193–1197.

Devlin PF, Kay SA. 2000. Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. *Plant Cell* **12**: 2499–2509.

Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A. 2004. Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes & Development* **18**: 926–936.

Dudley M, Poethig RS. 1993. The heterochronic teopod1 and teopod2 mutations of maize are expressed non-cell-autonomously. *Genetics* **133**: 389–399.

Duncan MJ. 2007. Circannual prolactin rhythms: calendar-like timer revealed in the pituitary gland. *Trends in Endocrinology and Metabolism* **18**: 259–260.

Fahlgren N, Montgomery TA, Howell MD, Allen E, Dvorak SK, Alexander AL, Carrington JC. 2006. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. *Current Biology* **16**: 939–944.

Farré EM, Harmer SL, Harmon FG, Yanovsky MJ, Kay SA. 2005. Overlapping and distinct roles of PRR7 and PRR9 in the Arabidopsis circadian clock. *Current Biology* **15**: 47–54.

Foster TM, Lough TJ, Emerson SJ, Lee RH, Bowman JL, Forster RL, Lucas WJ. 2002. A surveillance system regulates selective entry of RNA into the shoot apex. *Plant Cell* **14**: 1497–1508.

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J. 1999. GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. *EMBO Journal* **18**: 4679–4688.

Gandikota M, Birkenbihl RP, Hohmann S, Cardon GH, Saedler H, Huijser P. 2007. The miRNA156/157 recognition element in the 3′ UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *Plant Journal* **49**: 683–693.

Gardner MJ, Hubbard KE, Hotta CT, Dodd AN, Webb AAR. 2006. How plants tell the time. *Biochemical Journal* **397**: 15–24.

Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Kehr J. 2006. Towards the proteome of *Brassica napus* phloem sap. *Proteomics* **6**: 896–909.

Goldman BD. 2001. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *Journal of Biological Rhythms* **16**: 283–301.

Gonthier R, Jacqmard A, Bernier G. 1987. Changes in cell-cycle duration and growth fraction in the shoot meristem of *Sinapis* during floral transition. *Planta* **170**: 55–59.

Grigg SP, Canales C, Hay A, Tsiantis M. 2005. SERRATE coordinates shoot meristem function and leaf axial patterning in Arabidopsis. *Nature* **437**: 1022–1026.

Hackett WP. 1985. Juvenility, maturation and rejuvenation in woody plants. *Horticultural Reviews* **7**: 109–155.

Halliday KJ, Salter MG, Thingnaes E, Whitelam GC. 2003. Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator *FT*. *Plant Journal* **33**: 875–885.

Hayama R, Agashe B, Luley E, King R, Coupland G. 2007. A circadian rhythm set by dusk determines the expression of FT homologs and the short-day photoperiodic flowering response in Pharbitis. *Plant Cell* **19**: 2988–3000.

Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K. 2003. Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* **422**: 719–722.

Haywood V, Yu TS, Huang NC, Lucas WJ. 2005. Phloem long-distance trafficking of GIBBERELLIC ACID-INSENSITIVE RNA regulates leaf development. *Plant Jounal* **42**: 49–68.

He Y, Tang RH, Hao Y, Stevens RD, Cook CW, Ahn SM, Jing L, Yang Z, Chen L, Guo F, *et al.* 2004. Nitric oxide represses the Arabidopsis floral transition. *Science* **305**: 1968–1971.

He YH, Amasino RM. 2005. Role of chromatin modification in flowering-time control. *Trends in Plant Science* **10**: 30–35.

Henderson IR, Shindo C, Dean C. 2003. The need for winter in the switch to flowering. *Annual Review of Genetics* **37**: 371–392.

Heyer AG, Mozley D, Landschutze V, Thomas B, Gatz C. 1995. Function of phytochrome A in potato plants as revealed through the study of transgenic plants. *Plant Physiology* **109**: 53–61.

Holm M, Hardtke CS, Gaudet R, Deng XW. 2001. Identification of a structural motif that confers specific interaction with the WD40 repeat domain of Arabidopsis COP1. *EMBO Journal* **20**: 118–127.

Hotta CT, Gardner MJ, Hubbard KE, Baek SJ, Dalchau N, Suhita D, Dodd AN, Webb AAR. 2007. Modulation of environmental responses of plants by circadian clocks. *Plant, Cell & Environment* **30**: 333–349.

Hsu CY, Liu Y, Luthe DS, Yuceer C. 2006. Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. *Plant Cell* **18**: 1846–1861.

Hunter C, Sun H, Poethig RS. 2003. The Arabidopsis heterochronic gene ZIPPY is an ARGONAUTE family member. *Current Biology* **13**: 1734–1739.

Hunter C, Willmann MR, Wu G, Yoshikawa M, de la Luz Gutierrez-Nava M, Poethig SR. 2006. Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. *Development* **133**: 2973–2981.

Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA. 2005. FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. *Science* **309**: 293–297.

Imaizumi T, Tran HG, Swartz TE, Briggs WR, Kay SA. 2003. FKF1 is essential for photoperiodic-specific light signalling in Arabidopsis. *Nature* **426**: 302–306.

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K. 2002. Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. *Genes & Development* **16**: 2006–2020.

Jackson SD, Heyer A, Dietze J, Prat S. 1996. Phytochrome B mediates the photoperiodic control of tuber formation in potato. *Plant Journal* **9**: 159–166.

Jackson SD, James P, Prat S, Thomas B. 1998. Phytochrome B affects the levels of a graft-transmissible signal involved in tuberisation. *Plant Physiology* **124**: 423–430.

Jackson SD, James PE, Carrera E, Prat S, Thomas B. 2000. Regulation of transcript levels of a potato gibberellin 20-oxidase gene by light and phytochrome B. *Plant Physiology* **124**: 423–430.

Jackson SD, Prat S. 1996. Control of tuberisation in potato by gibberellins and phytochrome B. *Physiologia Plantarum* **98**: 29–32.

Jaeger KE, Wigge PA. 2007. FT protein acts as a long-range signal in Arabidopsis. *Current Biology* **17**: 1050–1054.

Jang S, Marchal V, Panigrahi KC, Wenkel S, Soppe W, Deng XW, Valverde F, Coupland G. 2008. Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. *EMBO Journal* **27**: 1277–1288.

Jung JH, Seo YH, Seo PJ, Reyes JL, Yun J, Chua NH, Park CM. 2007. The GIGANTEA-regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in Arabidopsis. *Plant Cell* **19**: 2736–2748.

Kanrar S, Bhattacharya M, Arthur B, Courtier J, Smith HM. 2008. Regulatory networks that function to specify flower meristems require the function of homeobox genes PENNYWISE and POUND-FOOLISH in Arabidopsis. *Plant Journal* **54**: 924–937.

Kelly AJ, Bonnlander MB, Meeks-Wagner DR. 1995. NFL, the tobacco homolog of FLORICAULA and LEAFY, is transcriptionally expressed in both vegetative and floral meristems. *Plant Cell* **7**: 225–234.

Kim JY, Song HR, Taylor BL, Carre IA. 2003. Light-regulated translation mediates gated induction of the Arabidopsis clock protein LHY. *EMBO Journal* **22**: 935–944.

Kim M, Canio W, Kessler S, Sinha N. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. *Science* **293**: 287–289.

Kim SL, Lee S, Kim HJ, Nam HG, An G. 2007a. OsMADS51 is a short-

day flowering promoter that functions upstream of Ehd1, OsMADS14, and Hd3a. *Plant Physiology* **145**: 1484–1494.

Kim WY, Fujiwara S, Suh SS, Kim J, Kim Y, Han L, David K, Putterill J, Nam HG, Somers DE. 2007b. ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. *Nature* **449**: 356–360.

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M. 2002. Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. *Plant & Cell Physiology* **43**: 1096–1105.

Komiya R, Ikegami A, Tamaki S, Yokoi S, Shimamoto K. 2008. Hd3a and RFT1 are essential for flowering in rice. *Development* **135**: 767–774.

Kotake T, Takada S, Nakahigashi K, Ohto M, Goto K. 2003. Arabidopsis TERMINAL FLOWER 2 gene encodes a heterochromatin protein 1 homolog and represses both FLOWERING LOCUS T to regulate flowering time and several floral homeotic genes. *Plant & Cell Physiology* **44**: 555–564.

Kusnetsov V, Landsberger M, Meurer J, Oelmuller R. 1999. The assembly of the CAAT-box binding complex at a photosynthesis gene promoter is regulated by light, cytokinin, and the stage of the plastids. *The Journal of Biological Chemistry* **274**: 36009–36014.

Laubinger S, Marchal V, Gentilhomme J, Wenkel S, Adrian J, Jang S, Kulajta C, Braun H, Coupland G, Hoecker U. 2006. Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* **133**: 3213–3222.

Lauter N, Kampani A, Carlson S, Goebel M, Moose SP. 2005. microRNA172 down-regulates glossy15 to promote vegetative phase change in maize. *Proceedings of the National Academy of Sciences, USA* **102**: 9412–9417.

Lee J, Oh M, Park H, Lee I. 2008. SOC1 translocated to the nucleus by interaction with AGL24 directly regulates leafy. *Plant Journal* **55**: 832–843.

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH. 2007. Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. *Genes & Development* **21**: 397–402.

Lin M, Belanger H, Lee Y, VarkonyiGasic E, Taoka K-C, Miura E, XoconostleCazares B, Gendler K, Jorgensen R, Phinney B *et al.* 2007. FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the Cucurbits. *Plant Cell* **19**: 1488–1506.

Martínez C, Pons E, Prats G, León J. 2004. Salicylic acid regulates flowering time and links defence responses and reproductive development. *Plant Journal* **37**: 209–217.

Martinez-Garcia JF, Virgos-Soler A, Prat S. 2002. Control of photoperiod-regulated tuberization in potato by the Arabidopsis flowering-time gene CONSTANS. *Proceedings of the National Academy of Sciences, USA* **99**: 15211–15216.

Más P, Kim WY, Somers DE, Kay SA. 2003. Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. *Nature* **426**: 567–570.

Mathieu J, Warthmann N, Kuttner F, Schmid M. 2007. Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. *Current Biology* **17**: 1055–1060.

McClung CR. 2006. Plant circadian rhythms. *Plant Cell* **18**: 792–803.

Michael TP, Salome PA, Yu HJ, Spencer TR, Sharp EL, McPeek MA, Alonso JM, Ecker JR, McClung CR. 2003. Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* **302**: 1049–1053.

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I. 2003. The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. *Plant Journal* **35**: 613–623.

Mouradov A, Cremer F, Coupland G. 2002. Control of flowering time: interacting pathways as a basis for diversity. *Plant Cell* **14** (Suppl): S111–130.

Nilsson O, Lee I, Blazquez MA, Weigel D. 1998. Flowering-time genes modulate the response to LEAFY activity. *Genetics* **150**: 403–410.

Onouchi H, Igeño MI, Périlleux C, Graves K, Coupland G. 2000. Mutagenesis of plants overexpressing CONSTANS demonstrates novel interactions among Arabidopsis flowering-time genes. *Plant Cell* **12**: 885–900.

Orkwiszewski JA, Poethig RS. 2000. Phase identity of the maize leaf is determined after leaf initiation. *Proceedings of the National Academy of Sciences, USA* **97**: 10631–10636.

Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature* **405**: 462–466.

Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS. 2005. Nuclear processing and export of microRNAs in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **102**: 3691–3696.

Peragine A, Yoshikawa M, Wu G, Albrecht HL, Poethig RS. 2004. SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. *Genes & Development* **18**: 2368–2379.

Pineiro M, Gomez-Mena C, Schaffer R, Martinez-Zapater JM, Coupland G. 2003. EARLY BOLTING IN SHORT DAYS is related to chromatin remodeling factors and regulates flowering in Arabidopsis by repressing FT. *Plant Cell* **15**: 1552–1562.

Poethig RS. 1988. Heterochronic mutations affecting shoot development in maize. *Genetics* **119**: 959–973.

Poethig RS. 1990. Phase change and the regulation of shoot morphogenesis in plants. *Science* **250**: 923–930.

Pouteau S, Nicholls D, Tooke F, Coen E, Battey N. 1997. The induction and maintenance of flowering in *Impatiens*. *Development* **124**: 3343–3351.

Razem FA, El-Kereamy A, Abrams SR, Hill RD. 2006. The RNA-binding protein FCA is an abscisic acid receptor. *Nature* **439**: 290–294.

Reeves PA, He Y, Schmitz RJ, Amasino RM, Panella LW, Richards CM. 2007. Evolutionary conservation of the FLOWERING LOCUS C-mediated vernalization response: evidence from the sugar beet (*Beta vulgaris*). *Genetics* **176**: 295–307.

Roberts A, Blake P, Lewis R, Taylor J, Dunstan D. 1999. The effect of gibberellins on flowering in roses. *Journal of Plant Growth Regulation* **18**: 113–119.

Robinson LW, Wareing PF. 1969. Experiments on the juvenile-adult phase change in some woody species. *New Phytologist* **68**: 67–78.

Robson F, Costa MMR, Hepworth SR, Vizir I, Piñeiro M, Reeves PH, Putterill J, Coupland G. 2001. Functional importance of conserved domains in the flowering-time gene CONSTANS demonstrated by analysis of mutant alleles and transgenic plants. *Plant Journal* **28**: 619–631.

Roden LC, Song HR, Jackson S, Morris K, Carre IA. 2002. Floral responses to photoperiod are correlated with the timing of rhythmic expression relative to dawn and dusk in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **99**: 13313–13318.

Rodriguez-Falcon M, Bou J, Prat S. 2006. Seasonal control of tuberization in potato: conserved elements with the flowering response. *Annual Review of Plant Biology* **57**: 151–180.

Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoecker U, Deng XW. 2003. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes & Development* **17**: 2642–2647.

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G. 2000. Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. *Science* **288**: 1613–1616.

Sawa M, Nusinow DA, Kay SA, Imaizumi T. 2007. FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. *Science* **318**: 261–265.

Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU. 2003. Dissection of floral induction pathways using global expression analysis. *Development* **130**: 6001–6012.

Schwarz S, Grande AV, Bujdoso N, Saedler H, Huijser P. 2008. The microRNA regulated SBP-box genes SPL9 and SPL15 control shoot maturation in Arabidopsis. *Plant Molecular Biology* **67**: 183–195.

Searle I, He YH, Turck F, Vincent C, Fornara F, Kröber S, Amasino RA, Coupland G. 2006. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes & Development* **20**: 898–912.

Seo HS, Yang JY, Ishikawa M, Bolle C, Ballesteros ML, Chua NH. 2003. LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* **423**: 995–999.

Singer SR, Hannon CH, Huber SC. 1992. Aquisition of competence for floral development in *Nicotiana* buds. *Planta* **188**: 546–550.

Sivitz AB, Reinders A, Johnson ME, Krentz AD, Grof CP, Perroux JM, Ward JM. 2007. Arabidopsis sucrose transporter AtSUC9. High-affinity transport activity, intragenic control of expression, and early flowering mutant phenotype. *Plant Physiology* **143**: 188–198.

Smith HM, Campbell BC, Hake S. 2004. Competence to respond to floral inductive signals requires the homeobox genes PENNYWISE and POUND-FOOLISH. *Current Biology***14**: 812–817.

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

Souer E, van der Krol A, Kloos D, Spelt C, Bliek M, Mol J, Koes R. 1998. Genetic control of branching pattern and floral identity during *Petunia* inflorescence development. *Development* **125**: 733–742.

Stadler R, Wright KM, Lauterbach C, Amon G, Gahrtz M, Feuerstein A, Oparka KJ, Sauer N. 2005. Expression of GFP-fusions in Arabidopsis companion cells reveals nonspecific protein trafficking into sieve elements and identifies a novel post-phloem domain in roots. *Plant Journal* **41**: 319–331.

Steward F, Moreno V, Roca W. 1981. Growth form and composition of potato plants as affected by environment. *Annals of Botany* **48** (Suppl. 2): 45.

Suàrez-López P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G. 2001. CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. *Nature* **410**: 1116–1120.

Swiezewski S, Crevillen P, Liu F, Ecker JR, Jerzmanowski A, Dean C. 2007. Small RNA-mediated chromatin silencing directed to the 3′ region of the Arabidopsis gene encoding the developmental regulator, FLC. *Proceedings of the National Academy of Sciences, USA* **104**: 3633–3638.

Takada S, Goto K. 2003. TERMINAL FLOWER 2, an Arabidopsis homolog of HETEROCHROMATIN PROTEIN 1, counteracts the activation of FLOWERING LOCUS T by CONSTANS in the vascular tissues of leaves to regulate flowering time. *Plant Cell* **15**: 2856–2865.

Takeno K. 1991. Flowering response of *Ipomaea batatas* scions grafted onto *Pharbitis nil* stocks. *Physiologia Plantarum* **63**: 682–686.

Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K. 2007. Hd3a protein is a mobile flowering signal in rice. *Science* **316**: 1033–1036.

Telfer A, Bollman KM, Poethig RS. 1997. Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*. *Development* **124**: 645–654.

Tessadori F, Schulkes RK, van Driel R, Fransz P. 2007. Light-regulated large-scale reorganization of chromatin during the floral transition in Arabidopsis. *Plant Journal* **50**: 848–857.

Thomas B, Vince-Prue D. 1997. *Photoperiodism in plants*. London, UK: Academic Press.

Turner A, Beales J, Faure S, Dunford RP, Laurie DA. 2005. The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. *Science* **310**: 1031–1034.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* **303**: 1003–1006.

Vega SH, Sauer M, Orkwiszewski JAJ, Poethig RS. 2002. The early phase change gene in maize. *Plant Cell* **14**: 133–147.

Wang H, Ma LG, Li JM, Zhao HY, Deng XW. 2001. Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. *Science* **294**: 154–158.

Wang ZY, Tobin EM. 1998. Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. *Cell* **93**: 1207–1217.

Weigel D, Nilsson O. 1995. A developmental switch sufficient for flower initiation in diverse plants. *Nature* **377**: 495–500.

Wenkel S, Turck F, Singer K, Gissot L, Le Gourrierec J, Samach A, Coupland G. 2006. CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of Arabidopsis. *Plant Cell* **18**: 2971–2984.

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. 2005. Integration of spatial and temporal information during floral induction in Arabidopsis. *Science* **309**: 1056–1059.

Willmann MR, Poethig RS. 2005. Time to grow up: the temporal role of smallRNAs in plants. *Current Opinion in Plant Biology* **8**: 548–552.

Wu G, Poethig RS. 2006. Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. *Development* **133**: 3539–3547.

Xie ZX, Allen E, Wilken A, Carrington JC. 2005. DICER-LIKE 4 functions in trans-acting small interfering RNA biogenesis and vegetative phase change in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **102**: 12984–12989.

Yamaguchi A, Kobayashi Y, Goto K, Abe M, Araki T. 2005. TWIN SISTER of FT (TSF) acts as a floral pathway integrator redundantly with FT. *Plant and Cell Physiology* **46**: 1175–1189.

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J. 2004. The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. *Science* **303**: 1640–1644.

Yanovsky MJ, Izaguirre M, Wagmaister JA, Gatz C, Jackson SD, Thomas B, Casal JJ. 2000. Phytochrome A resets the circadian clock and delays tuber formation under long days in potato. *Plant Journal* **23**: 223–232.

Yanovsky MJ, Kay SA. 2002. Molecular basis of seasonal time measurement in Arabidopsis. *Nature* **419**: 308–312.

Yoo BC, Kragler F, Varkonyi-Gasic E, Haywood V, Archer-Evans S, Lee YM, Lough TJ, Lucas WJ. 2004. A systemic small RNA signaling system in plants. *Plant Cell* **16**: 1979–2000.

Yoo SK, Chung KS, Kim JK, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH. 2005. CONSTANS activates SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 through FLOWERING LOCUS T to promote flowering in Arabidopsis. *Plant Physiology* **139**: 770–778.

Yu H, Ito T, Zhao Y, Peng J, Kumar P, Meyerowitz EM. 2004. Floral homeotic genes are targets of gibberellin signaling in flower development. *Proceedings of the National Academy of Sciences, USA* **101**: 7827–7832.

Zeevaart JAD. 1962. The juvenile phase in *Bryophyllum daigremontianum*. *Planta* **58**: 531–542.

