FLOWERING NEWSLETTER REVIEW

At the end of the day: a common molecular mechanism for photoperiod responses in plants?

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Abstract

Photoperiod or daylength affects a diverse set of traits in plants, including flowering and tuberization in annuals, as well as growth cessation and bud set in perennials. During the last 10–15 years, great progress has been made in the understanding of molecular mechanisms controlling photoperiodic induction of flowering, in particular in the model species *Arabidopsis thaliana*. An obvious question is to what extent the molecular mechanisms revealed in *A. thaliana* are also shared by other species and other traits controlled by photoperiod. The purpose of this review is to summarize data on the molecular mechanisms of photoperiod control in plants with a focus of annual growth rhythm in perennial plants.

Key words: Annual growth rhythm, bud set, flowering time, photoperiod.

Introduction

Adaptation to changing environments is critical to all life. The ever-changing seasons clearly are drastic changes particularly in temperate regions, but they occur in a predictable way every year. The length of the day or photoperiod enables living organisms to respond to the seasonal changes in their environment (Fig. 1). As photoperiod changes in a foreseeable way during the year, it is a more reliable indicator of season than, for example, temperature. In temperate areas, short days precede winter with low temperatures and deficit of water. In a similar way, long days precede dry periods in some desert areas. Using daylength as a cue, species may change their growth, physiology, and development in accordance with anticipated future changes in climate.

Accordingly, photoperiodic responses are known from all kingdoms of life. Among animals, the number of examples of photoperiodism in Arthropods is huge, including signals leading to diapause, migration, and reproduction (Bradshaw and Holzapfel, 2007, and references therein). Likewise, in Vertebrates, photoperiod has proven to be important for timing of reproduction in many species including teleostean fish, birds, and mammals (Dawson *et al.*, 2001). As an example, development of reproductive structures in birds is influenced by the lengthening of the day through changes in

key hormones. This results in raising of the offspring during the favourable spring and early summer periods.

In plants, timing of reproduction, including flowering, tuberization, and bulbing, is often controlled by photoperiod (Garner and Allard, 1920; Thomas and Vince-Prue, 1997). Equally important for survival and fitness is the photoperiodic control of the annual growth cycle in perennials, which in particular in temperate areas is mainly controlled by photoperiod (Wareing, 1956; Dormling, 1973; Vince-Prue, 1975; Eriksson *et al.*, 1978). Several decades of physiological studies on various photoperiodic responses in plants have revealed striking similarities in these responses (Thomas and Vince-Prue, 1997). During the last 10–15 years, great progress has also been made in the understanding of molecular mechanisms controlling photoperiodic induction of flowering, in particular in the model species *Arabidopsis thaliana* (Searle and Coupland, 2004; Imaizumi and Kay, 2006; Turk *et al.*, 2008). The purpose of this review is to summarize data on the molecular mechanisms of photoperiod control in plants with a focus of annual growth rhythm in perennial plants. Particular attention will be paid to two major questions: (i) to which extent are these molecular mechanisms shared among species in the case of
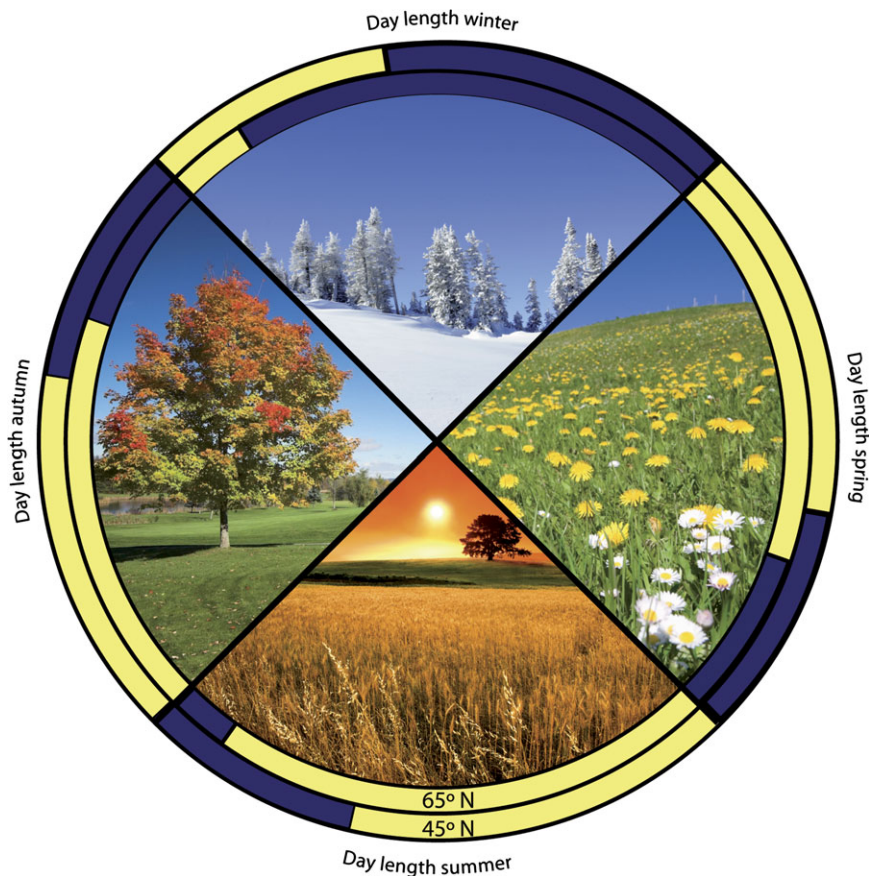
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Fig. 1. Daylength varies dramatically over season and latitudes. The figure depicts daylength at latitude 65°N and 45°N in winter (1 January), spring (1 May), summer (20 June), and autumn (10 August).

flower induction; and (ii) are some of these mechanisms shared by other traits controlled by photoperiod, such as annual growth rhythm and tuberization?

Photoperiodic control of flowering time

A number of excellent reviews have covered the recent rapid progress in our understanding of the mechanisms controlling the photoperiodic induction of flowering (e.g. Searle and Coupland, 2004; Imaizumi and Kay, 2006; Turck *et al.*, 2008). Although most research has focused on the model species *A. thaliana*, other studies have revealed that parts of the basic mechanisms are conserved in other angiosperm species with varying responses to photoperiod (Yano *et al.*, 2001; Kojima *et al.*, 2002; Hayama and Coupland *et al.*, 2004; Hayama *et al.*, 2007; Jackson, 2009). To aid discussion of photoperiodic responses controlling traits other than induction of flowering, a summary is given here of the current views of the mechanisms controlling photoperiodic induction of flowering in angiosperms.

Already at the beginning of the 20th century Julien Tournois and Hans Klebs suggested that daylength was more important than light quantity for the induction of flowering (Tournois, 1912; Klebs, 1913). A few years later, Garner and Allard (1920, 1923) clearly demonstrated that flowering and other responses were induced by long days in some species and by short days in others (Fig. 2). They
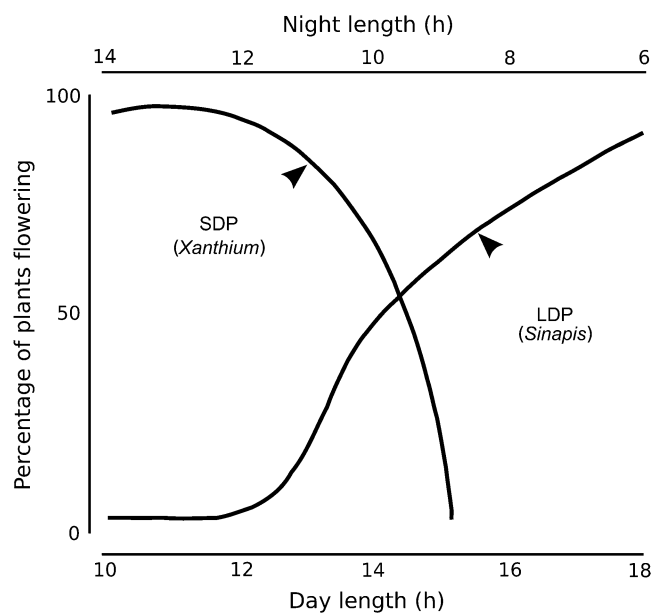

Fig. 2. Photoperiodic time measurement. Flowering response of a short day plant (SDP) and a long day plant (LDP) on light–dark cycles of different lengths. The total cycle is always 24 h. Modified from Thomas and Vince-Prue (1997).

coined the term photoperiodism for this phenomenon. Garner and Allard classified plants into three main categories based on photoperiodic response, short-day plants

(SDPs), long-day plants (LDPs), and day-neutral plants (DNPs). It soon became clear that the daylength signal was perceived in the leaves (Knott, 1934). In several species, leaves exposed to inductive daylength induced flowering when they were grafted onto plants growing in non-inductive light regimes (Lang, 1965). This finding implied that the vector of the received photoperiod signal is a trans-missible signal—a floral hormone or florigen (Chailakhyan, 1937).

What is measured: daylength or nightlength?

Even though daylength and nightlength are perfectly correlated in the 24 h cycle, plants could in principle measure the length of either the night or the day. One way to distinguish these alternatives would be to vary them independently. In the SDP *Xanthium strumarium*, Hamner and Bonner (1938) showed that long nights induced flower-ing even if these were coupled with a long day, but short days did not induce flowering if the night was also short (Fig. 3). Further studies also showed that flowering could be prevented if the long night was interrupted with a light pulse (a night break), supporting the importance of the dark period.

The situation in LDPs is, however, different. Generally they are less responsive to night break treatment (Thomas and Vince-Prue, 1997). In contrast to SDPs, longer night interruptions are needed, and the flowering response is often semi-quantitative in nature. Furthermore, in contrast to SDPs, most LDPs require far-red light at the end of the light period to interpret the light period as a long day (Thomas, 1998; Thomas and Vince-Prue, 1997). If instead far-red light is followed by red light in the second part of the day, promotion of flowering is poor or absent (Fig. 3). In SDPs the light quality given at different parts of the light period has little effect on flowering. These differences in responses to night or day are not perfectly correlated with SDPs or LDPs, and some SDPs also show a weak response to light quality during the day. For this reason, plants can be classified as either dark dominant or light dominant. SDPs are predominantly dark dominant, while LDPs are predominantly light dominant.

How is time measured?

Three basic models have been suggested for the measurement of the length of the day or night. Most favoured is one based on external coincidence, where an internal rhythm coincides with an external signal (light) under certain conditions and then induces flowering (Bünning, 1936; Pittendrigh, 1966). A more complex model is based on the coincidence of two internal rhythms, the internal coincidence model (Pittendrigh, 1972). This model has been difficult to test, and most data are compatible with the simpler external coincidence model. Both these models rely on a circadian clock controlling the internal rhythms. A third model does not require an internal clock but relies on an hourglass-type timer (Lees, 1973). Although such a model could explain data in some species, a circadian rhythm in the sensitivity to night breaks is evident in many cases (Fig. 4). A rapid dampening of a circadian timer in darkness is also compatible with an apparent hourglass behaviour.

In the external coincidence model, the phase of the rhythm in sensitivity to light, or the photoperiodic response rhythm (Lumsden, 1998), is set by light. In most SDPs the phase of the photoperiodic response rhythm is constant from the end of the light period, which is required to enable measurement of the length of the night. One hypothesis arising from the constant phase relationship with the end of the light period would be that the rhythm is suspended in light if the light period is long enough, and then released at the onset of darkness (Lumsden, 1998). Based on evidence for persistence of rhythmicity also during light in SDPs, Lumsden (1996) suggested an extended model in which the circadian clock is not completely arrested in the light but continues to oscillate around a so-called light limit cycle. In this model, the cycle still has a period of ~24 h, but will only occupy a limited part of possible phase states. The end
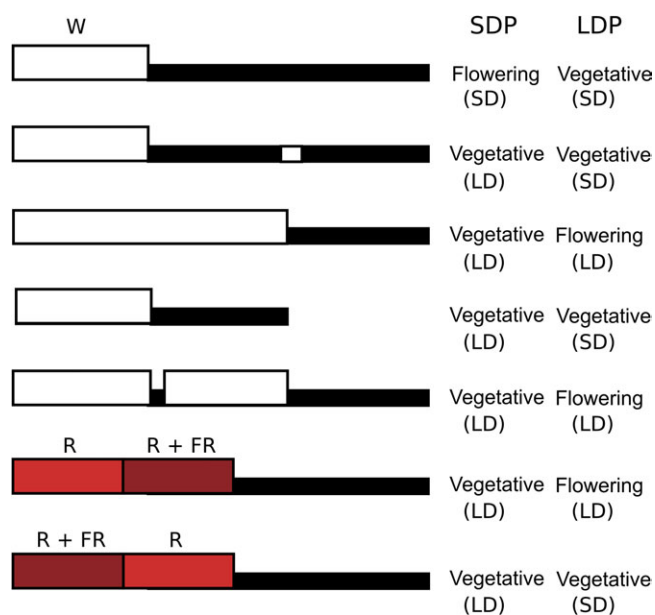

Fig. 3. Schematic illustration of typical responses of SDPs and LDPs to various combinations of light and dark. W, white light; R, red light; FR, far-red light; SDP, short day (dark-dominant) plant; LDP long day (light-dominant) plant. (SD) and (LD) indicated the photoperiod perceived by SDPs and LDPs (after Thomas, 1998).
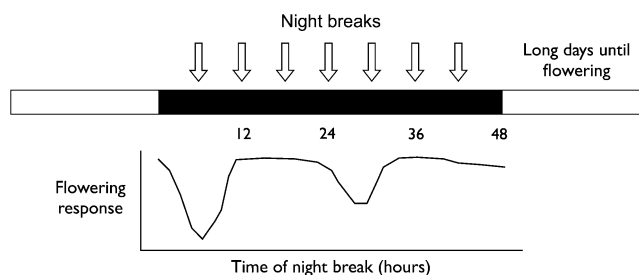

Fig. 4. Rhythymic response to the time of night break during an extended dark period in an SDP.

result will be similar but the rhythm will be released at slightly different phases depending on the phase in the light limit cycle at dusk.

As described above, LDPs are generally not responsive to short night breaks, but show a rhythm in the sensitivity to far-red light during the day. One hypothesis explaining the response of LDPs states that the photoperiodic response rhythm is reset at dawn but, in contrast to SDPs, the rhythm is not suspended but continues to run in light (Thomas and Vince-Prue, 1997; Lumsden, 1998). This rhythm establishes a phase of sensitivity to far-red light. This model fits well with molecular data from the LDP *Arabidopsis* (see below).

Molecular mechanism controlling daylength-induced flowering in long day plants

Extensive and elegant work with the model species *A. thaliana* has revealed important components of the molecular mechanisms involved in daylength-induced flowering in an LDP. *Arabidopsis thaliana* is a facultative LDP, and the induction of flowering in long days may now be explained by a molecular version of the external coincidence model.

CONSTANS is a key protein in photoperiod sensing in Arabidopsis: A key component of this model is the *CON-STANS* gene (*CO*; Putterill *et al.*, 1995). The expression of *CO* is controlled by the circadian clock, with a diurnal peak of expression during the night in short days (Suarez-Lopez *et al.*, 2001). However, the CO protein is degraded in darkness, so CO function can only be obtained if *CO* mRNA is also expressed before darkness (Fig. 5). This is achieved in long days when light is present closer to the peak of *CO* mRNA expression but also because *CO* mRNA displays a broader peak of expression in long days. The broader peak of expression results from the activity of FLAVIN-BINDING, KELCH REPEAT, F-BOX (FKF1), which degrades a repressor of *CO* (Imaizumi *et al.*, 2005). The end result is that CO protein accumulates only in long days, and then activates transcription of *FLOWERING LOCUS T* (*FT*) in vascular tissue. A number of recent studies strongly support that the FT protein then moves from the leaves to the shoot apical meristem (SAM), where it activates transcription factors that induce flowering (see below).

Degradation of CO is thought to involve SUPPRESSOR OF PHYA-1 (SPA1) and two of it homologues (SPA3 and SPA4). SPA1 is known to act in protein degradation jointly with CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) during photomorphogenesis (Laubinger *et al.*, 2006). SPA proteins interact with CO *in vitro*, and CO protein levels are elevated in *spa1 spa3 spa4* triple mutants. A number of photoreceptors are important for the control of CO stability in light (Valverde *et al.*, 2004). Phytochrome A and cryptochrome 2 that are responsive to far-red and blue light, respectively, promote the stability of CO at the end of the day by repression of degradation. In contrast, phytochrome B reduces the abundance of CO in red light, specifically in the morning. These effects are consistent with
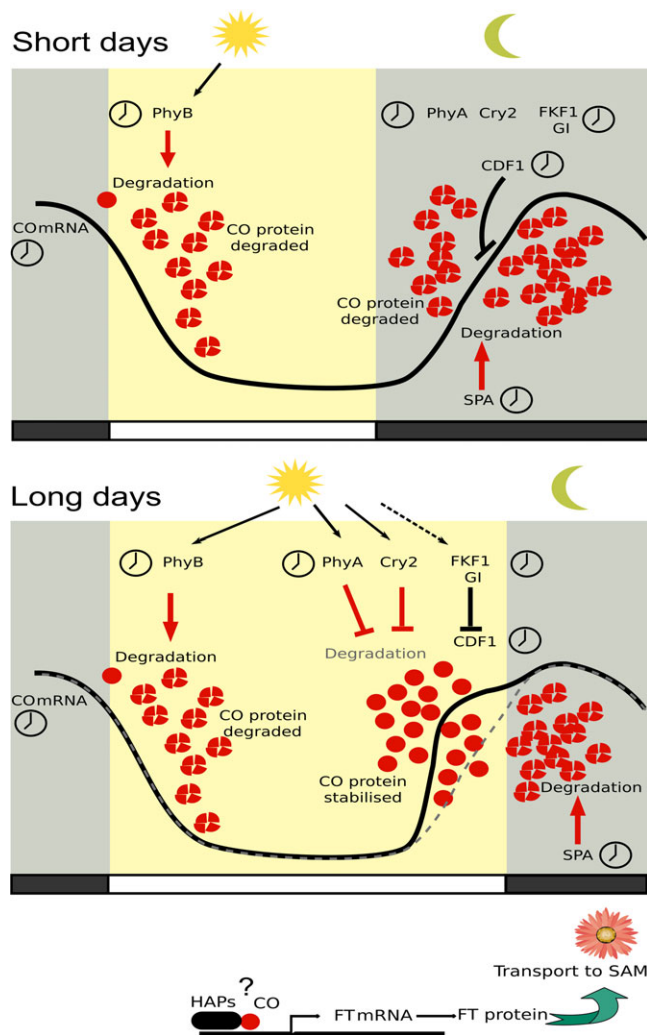

Fig. 5. Regulation of the *CONSTANS* gene at both the mRNA and protein levels provides a molecular explanation of the external coincidence model. The clock-controlled variation in *CO* mRNA levels is depicted with black curves, and CO protein is represented by red spheres (intact protein), or red split spheres (degraded protein). In short days *CO* mRNA is mainly expressed in darkness, and the resulting protein is degraded partly through the action of SPA1, 3, and 4. The protein produced in the morning is also degraded, a process that is dependent of active PhyB. In long days, the repression of *CO* mRNA by CDF1 is released through the action of FKF1 and GI, resulting in an elevated *CO* expression in the afternoon. The translated CO protein is stabilized in light through the action of PhyA and Cry2. It has been hypothesized that the stable CO protein forms a complex with HAP (haem activator protein) that binds to the *FT* promoter (Wenkel *et al.*, 2006). The FT protein is transported through the phloem to the SAM to induce flowering. Genes controlled by the circadian clock are indicated by a clock symbol.

the effects of these photoreceptors of flowering: *phyB* mutants are early flowering, while *phyA* and *cry2* mutants are late flowering.

Regulation of CO *mRNA by the circadian clock*: Recent models of the circadian clock of *A. thaliana* include at least

three interlocked negative feedback loops (Fig. 6; McClung, 2006; Locke *et al.*, 2006; Zeilinger *et al.*, 2006). According to these models, CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), two closely related MYB domain transcription factors, play a key role as they participate in two different loops. In one loop they down-regulate the expression of one pseudo-response regulator (*PRR*), *TIMING OF CAB EXPRESSION1* (*TOC1*), that acts as a positive regulator of *CCA1* and *LHY*. In a second loop, CCA1/LHY promote the transcription of two other *PRR* genes, *PRR7* and *PRR9*, both of which are negative regulators of *CCA1/LHY*. The third loop in the model, consists of GIGANTEA (GI) and TOC1, where GI up-regulates *TOC1*, which in turn represses *GI*. A number of additional components important for clock function have also been identified although their exact mode of action is still unclear. In addition to *TOC1*, genes such as *EARLY FLOWERING 4* (*ELF4*) and *LUX ARRHYTHMO* (*LUX*) have been shown to be important for proper regulation of *CCA1* and *LHY* (Hazen *et al.*, 2005; McWatters *et al.*, 2007). The large number of genes and their complex interaction at the transcriptional, post-transcriptional, and post-translational levels have stimulated the use of mathematical modelling in circadian research (Locke *et al.*, 2006; Zeilinger *et al.*, 2006). Testing the resulting models with experimental data will undoubtedly advance the understanding on clock function in *Arabidopsis* and other plant species.

One output of the circadian clock controls the transcription of *CO* through several genes including *GI*, *FKF1*, and *CYCLING DOF FACTOR1* (*CDF1*). CDF1 exhibits its peak of expression early in the day, and acts as a repressor of *CO* transcription (Imaizumi *et al.*, 2005). GI and FKF1, on the other hand, peak in the afternoon, and collectively promote the expression of *CO*. Current data suggest that GI and FKF1 form a complex on the *CO* promoter that promotes the degradation of CDF1 (Sawa *et al.*, 2007). The interaction between GI and FKF1 is promoted by blue light, adding an additional instance of external coincidence in the control of photoperiodic flowering (besides the control of CO protein stability by light). Interestingly, a majority of the available photoreceptors are involved in photoperiod sensing through the control of CO. Besides the

control of protein stability by PHYA, PHYB, CRY1, and CRY2, FKF1 acts as a blue light receptor in the control of *CO* transcription (Valverde *et al.*, 2004; Sawa *et al.*, 2007).

CO is a member of a gene family of some 17 genes in *Arabidopsis* (Griffiths *et al.*, 2003). The two most closely related members *COL1* and *COL2* showed little effect on flowering time when the expression of those genes was altered in transgenic *Arabidopsis* (Ledger *et al.*, 2001). On the other hand, more distantly related genes such as *COL3* and *COL9* may play a role in the control of flowering time. A loss-of-function mutation in *COL3* and a gain-of-function mutation in *COL9* resulted in early flowering and late flowering phenotypes, respectively, indicating that *COL3* and *COL9* function as floral repressors (Cheng and Wang, 2005; Datta *et al.*, 2006).

CO *activates a mobile florigen signal*: Classical studies have demonstrated that the site of photoperiod perception is predominantly in the leaves, which implies that a mobile signal must be transported from the leaves to the SAM. Although *CO* mRNA can be found in apical regions, it is strongly expressed in phloem companion cells. Furthermore, studies of protein stability suggested that CO protein is restricted to the phloem (An *et al.*, 2004). These results, in combination with the fact that expression of CO in the SAM does not induce flowering, showed that the mobile signal must be downstream of *CO* (An *et al.*, 2004).

Induction of *CO* expression either by dexamethasone in 35S::CO:GR transgenics or by shifting wild-type and *co* plants from short days to long days identified *FT* as the main (and perhaps only) target of CO in the leaves (Samach *et al.*, 2000; Wigge *et al.*, 2005). *FT* expression seems to be restricted to the phloem companion cells preferentially to those of the distal minor veins of source leaves (Takada and Goto, 2003; An *et al.*, 2004). Even so, expression of *FT* in the SAM results in early flowering, suggesting that FT could be the mobile signal (An *et al.*, 2004). A combination of grafting experiments using FT:GFP (green fluorescent protein) fusion proteins and artificial miRNAs (microRNAs) targeting *FT* mRNA in different tissues collectively clearly support FT protein as a strong candidate for a mobile 'florigen' signal (Lifschitz *et al.*, 2006; Corbesier *et al.*, 2007; Jaeger *et al.*, 2007; Lin *et al.*, 2007; Mathieu *et al.*, 2007; Tamaki *et al.*, 2007).

When the FT protein is present in the SAM it is thought to form a complex with FLOWERING LOCUS D (FD), a bZIP transcription factor, and activate a number of MADS-box transcription factors including the floral meristem identity genes *APETALA1* and *FRUITFULL* probably through direct binding to their promoters (Abe *et al.*, 2005; Wigge *et al.*, 2005). The earliest known marker of floral induction in the SAM, *SOC1*, is probably also a target of the presumed FT/FD complex, as *SOC1* induction is delayed in both *ft* and *fd* mutants (Borner *et al.*, 2000; Yoo *et al.*, 2005). SOC1 is a floral integrator that regulates *LEAFY* expression by binding to its promoter in a complex with AGL24 (Lee *et al.*, 2008).

CO is not the only gene that affects *FT* transcription. Within the photoperiod pathway, an miRNA, *miR172*, has
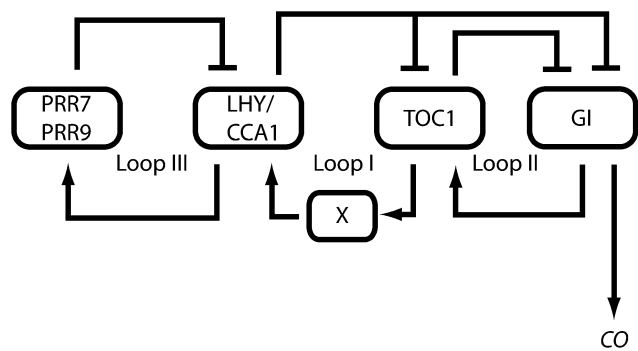
Fig. 6. A three-loop model of the circadian clock in *A. thaliana*. X represents a hypothetical protein.

been reported to control the expression of a number of *AP2*-like genes, including *TOE1*, which repress the expression of *FT* (Jung *et al.*, 2007). It was also suggested that the higher levels of *miR172* seen in long days than in short days were controlled through *GI* but were independent of *CO* (Jung *et al.*, 2007).

Besides photoperiod, long periods of low temperature (vernalization) are an important environmental determinant of flowering time. This response is mediated through the MADS-box transcription factor FLOWERING LOCUS C (FLC), which binds directly to the *FT* promoter as a repressor (Helliwell *et al.*, 2006). Exposure to low temperature represses *FLC* transcription, enabling induction of *FT* through *CO* (Henderson *et al.*, 2003). Ambient temperatures also affect flowering, most probably through the regulation of *FT* expression. High temperature induces flowering in *Arabidopsis*, a response that acts through the floral repressor *FLOWERING LOCUS M* and *FT*, but does not involve the photoperiodic pathway (Balasubramanian *et al.*, 2006). Flowering in *Arabidopsis* is also delayed at 16 °C, a response that is dependent on *FCA* and *FVE*, two genes in the autonomous pathway (Blázquez *et al.*, 2003). This temperature response also seems to require *FT*, as late flowering at low temperature was coupled to reduced FT expression, and overexpression of *FT* resulted in loss of response to low temperature (Blázquez *et al.*, 2003). SHORT VEGETATIVE PHASE (SVP), another MADS-box transcription factor that binds to the *FT* promoter and negatively regulates its expression, was recently suggested to act in the thermosensory pathway downstream of *FCA* and *FVE* (Lee *et al.*, 2007). Adding to the complexity, a recent report suggested that SVP was also partly regulated by the clock proteins CCA1 and LHY, in a pathway distinct from the one including GI and CO (Fujiwara *et al.*, 2008).

A further aspect of *FT* regulation involves chromatin regulation to establish long-term repression. Mutations in both *TERMINAL FLOWER 2* (*TFL2*) and *EARLY BOLTING IN SHORT DAYS* (*EBS*), which are implicated in chromatin remodelling, result in early flowering and elevated *FT* mRNA levels (Kotake *et al.*, 2003; Pineiro *et al.*, 2003).

Even though *FT* integrates signals from several pathways, it is not required for flowering. Silencing of *FT* and its close homologue *TWIN SISTER OF FT* (*TSF*) does not prevent flowering in *Arabidopsis* (Yamaguchi *et al.*, 2005). This suggests that another pathway promotes flowering in the absence of *FT* and *TSF* in long days as well as in the wild type in short days. Mutations in the gibberellin (GA) biosynthesis gene *GA1* prevents flowering in short days (Wilson *et al.*, 1992), and it also severely delays flowering in *co* mutants in long days (Reeves *et al.*, 2001). A GA-dependent pathway acting on genes in the apex, downstream of *FT* (*SOC1* and *LFY*), may thus function in parallel with the ones converging on *FT*. GA has been suggested to be a systemic signal for flowering in *Lolium*, and might also serve a similar function in *Arabidopsis* (Mutasa-Gottgens and Hedden, 2009).

Molecular mechanism controlling daylength-induced flowering in short day plants

The progress in understanding photoperiodic induction of flowering in the LDP *Arabidopsis* has inspired similar studies also in SDPs. Most of this work has so far focused on the monocotyledonous plant rice. It seems as if the function of the proposed florigen FT might be highly conserved in a wide range of plant species, but that the regulation of its expression determines the various responses to photoperiod evident in LDPs, SDPs, and DNPs. Very early flowering is seen when *FT* homologues are overexpressed in rice (Kojima *et al.*, 2002) and Morning Glory (*Ipomoea nil*) (Hayama *et al.*, 2007), as well as in tobacco, tomato (Lifschitz and Eshed, 2006), and poplar (Bohlenius *et al.*, 2006; Hsu *et al.*, 2006). In rice, the induction of *Hd3* (an *FT* homologue) is mediated through a *CO* homologue *Hd1*, but a different function of the rice *Hd1* homologue explains the short day response. In contrast to CO, Hd1 represses *Hd3* in long days, but promotes *Hd3* expression and subsequent flowering in short days (Yano *et al.*, 2000). The reason for this divergent function of Hd1 and CO is still unclear. The expression pattern of *Hd1* shows a similar diurnal pattern to *CO*, and Hd1 function is probably controlled by phytochrome, as a mutant lacking functional phytochromes (*photoperiod sensitivity 5*, *se5*) is early flowering under all photoperiods, although it retains the diurnal expression pattern of *Hd1* (Izawa *et al.*, 2002). These observations suggest that light activates Hd1 to function as a repressor of *Hd3*, while in darkness Hd1 instead acts as an activator of *Hd3*.

The similar diurnal expression patterns of *CO* and *Hd1* suggest a similar regulation of their expression by a circadian clock. This hypothesis is supported by the presence of rice homologues to several genes in the *Arabidopsis* clock, such as *GI*, *CCA1/LHY*, *TOC1*, *ZTL*, and *ELF3* (Murakami *et al.*, 2007). The rice *GI* homologue *OsGI* has also been shown to regulate *Hd1* expression similarly to how *GI* regulates *CO* in *Arabidopsis* (Hayama *et al.*, 2003).

Characteristic for most SDPs is that they measure the length of the night, and show a clear response to night breaks. As stated above, one hypothesis is that the photoperiod response rhythm is reset at dusk, so that time measurement always starts from the beginning of the dark period in SDPs. The expression pattern of *Hd1* does not fit easily into this model. The diurnal expression pattern of *Hd1* in long and short days shows a similar phase to that of *CO* in the LDP *Arabidopsis*. However, additional components of the photoperiod pathway, not present in *Arabidopsis*, have been identified in rice. *Early heading date1* (*Ehd1*) is one such component, which codes for a B-type response regulator with no obvious homologue in *Arabidopsis* (Doi *et al.*, 2004). *Ehd1* promotes floral transition preferentially under short day conditions, even in the absence of functional *Hd1*. Expression analysis revealed that *Ehd1* functions upstream of *Hd3a*, *RFT1*, and some MADS-box genes (Doi *et al.*, 2004), and that, in contrast to *Hd1*, *Ehd1* mRNA is induced strongly only under short day conditions, with a peak of

expression before dawn (Wu *et al.*, 2008). Furthermore, night breaks suppress accumulation of *Hd3a* mRNA and subsequent flowering. This response requires PhyB, and also functions in an *hd1* mutant. Therefore, it is possible that *Ehd1*-dependent activation of *Hd3a* expression is suppressed by a night break (Ishikawa *et al.*, 2005).

More direct evidence that flowering is controlled by a circadian rhythm set at dusk that induces an *FT* gene comes from recent work on a classical SDP model plant *Pharbitis nil* (*Ipomoea nil*). *Pharbitis nil FT* genes (*PnFT1* and *PnFT2*) are induced only if night is long enough irrespective of the length of the preceding light period (Hayama *et al.*, 2007); in other words, time is measured from the light to dark transition. As in rice, exposure to night breaks suppresses *PnFT1* and *PnFT2* mRNA levels, and this correlates with a strong reduction in flowering. In contrast to rice, *CO* genes may not be important for induction of *PnFT* in darkness. In experiments where the preceding light period before transfer to darkness was extended, *PnCO* expression was decoupled from that of *PnFT* expression so that the peaks no longer coincide. Even though additional *PnCO* genes might exist, the results suggest an additional pathway controlling *PnFT* expression in darkness as proposed for rice.

In conclusion, molecular data on the photoperiodic induction of flowering in both LDPs and SDPs suggest that the control of FT expression by coincidence between light and a rhythm generated by a circadian clock can explain flower induction, but the exact nature of how this control differs between plants with divergent responses to photoperiod is still unclear.

Photoperiod control of tuberization

Photoperiod is also an important determinant of tuberization in potato, and there is now also some evidence of a similar molecular control of this short day-induced trait. The short day response, present mainly in non-cultivated species, is inhibited by night breaks, which can be reversed by far-red light (Batutis and Ewing, 1982). In accordance with this effect, it has been shown that *PhyB* is involved and acts to repress tuberization similarly to its effect on flowering (Jackson *et al.*, 1996). Grafting experiments have also shown that the site of photoperiod perception is the leaves, suggesting that a transmissible signal is produced (Jackson *et al.*, 1998). Interestingly, grafting experiments using short day, long day, and day neutral tobacco species showed that conditions that induced flowering in the respective tobacco scions resulted in tuberization in *andigena* potato, but not when conditions that did not induce flowering were used (Chailakhyan *et al.*, 1981). These studies indicate that the mobile signals inducing flowering and tuberization could be the same. Two genes closely related to FT have been identified in potato, and one of these was also strongly induced in short days but not long days (Rodriguez-Falcon *et al.*, 2006). The expression levels of this gene were also elevated in *phyB* antisense lines, and repressed in lines overexpressing *Arabidopsis CO*, in which tuberization

was delayed. Grafting experiments could locate the interfering function of *AtCO* (perhaps with the endogenous CO) to the leaves.

Photoperiodic control of growth cessation and bud set in trees

Trees growing in temperate and frigid regions endure the often cold winters by entering a state of dormancy. This implies that growth is arrested, buds with protective scales are formed, and the meristems in buds and cambium are at rest. In parallel, the plants also start to build up frost tolerance, a process that takes several weeks and is most efficient at mild temperatures (Weiser, 1970). This means that the process must start well in advance of the onset of cold temperatures. Perhaps for this reason, but also because of unpredictable fluctuations in temperature, most plants use shortening daylength (photoperiod) as a cue to induce the process. However, in reality, photoperiod can interact with other environmental cues, including temperature and various forms of stress. Temperature can thus be important for dormancy induction, but the effect is most often seen if the temperature is outside a normal range (Heide, 1974). Still, some species such as apple and pear are insensitive to photoperiod, and recent data indicate that low temperature is the main cue for induction of growth cessation in these species (Heide and Prestrud, 2005).

During the initial stages of growth cessation and bud set, trees attain a moderate level of frost tolerance; however, low temperature has the main effect on the build up of hardiness during subsequent stages (Weiser, 1970; Dormling, 1979). At the end of this process the trees enter an endodormant stage which means that they cannot restart growth until after a longer cold period that satisfies a so-called chilling requirement. This chilling requirement could be considered analogous to the vernalization requirement for flowering in winter annual and biennial plant species, although there appear to be important differences between these two processes. Current data suggest that only dividing cells are capable of becoming vernalized, while chilling is thought to occur after cell division has ceased (Wellensiek, 1964; Burn *et al.*, 1993). Furthermore, the vernalized state is mitotically stable and is only reset in meiosis (Mylne *et al.*, 2006). In contrast, the resetting in vegetative meristems occurs every year without passing through meiosis. After the chilling requirement has been met, bud burst is induced by high temperature in the spring, so photoperiod seems to play no or a limited role in this part of the annual growth cycle.

Several vegetative processes in woody plants are controlled by daylength, including duration of extension growth, internode extension, leaf growth in conifers, leaf senescence in angiosperms, and dormancy. Dormancy in woody plants was actually one of the first traits that Garner and Allard showed to be under photoperiodic control (Garner and Allard, 1923). This observation was later confirmed in a wide range of species of both angiosperms and gymnosperms (Wareing, 1956). Like flowering, the

photoperiodic control of growth cessation is an inductive phenomenon. For example, a few short days before transfer to constant light is enough to stop extension growth in *Picea abies*.

For most native tree species from high latitudes, the main external signal for induction of growth cessation is a shortening of the photoperiod. Seedlings of the conifer Norway spruce (*P. abies*) have been extensively used to study the effects of photoperiod on growth cessation and bud set, partly because they are easy to handle experimentally. Also, for *P. abies* and other conifers, the photoperiodic response is most pronounced at the seedling stage. The extension growth of first-year seedlings consists of the expansion of stem units formed in the current season (Fig. 7). This free growth is in the following years successively replaced by predetermined growth (expansion of stem units initiated in the preceding growth period) and results in shortening of the period of extension growth. In mature Norway spruce trees, terminal bud set takes place already in June or July, and is unlikely to be under strong photoperiodic control. Still, the initiation of frost tolerance and dormancy begins in the autumn and is probably under photoperiodic control (see Clapham *et al.*, 2001*a*).

For *P. abies* seedlings growing under natural conditions, shoot extension stops and terminal buds are set in late summer in response to a shortening photoperiod, after which the cambium ceases growth, needle primordia are initiated within the buds, and frost tolerance begins to increase. Subsequently, rest dormancy (endodormancy) develops in the meristems during autumn and, with exposure to chilling temperatures (2–10 °C), changes into quiescence dormancy (ectodormancy) by midwinter, when frost tolerance is maximal. After a period of low winter temperature, bud burst is induced by high temperature in the spring, so that opening of the bud scales occurs in spring after a temperature sum (TS) has been attained.

In the autumn, bud set is induced by one or a few long nights, even if the seedlings are transferred back to continuous light after the long night treatment (Dormling *et al.*, 1968). Interestingly, the response to photoperiod is strikingly different between populations from different latitudinal origin. High latitude populations (~67°N) are induced by a single 16 h night, while seedlings of central European origin (~45°N) require four such long nights. The critical nightlength (CNL) that induces 50% bud set also differs considerably between plants of these origins. Northern populations have a CNL of 2–3 h while the CNL of southern populations is 7–10 h. Further experiments adding populations from intermediate latitudes have shown that the variation displays a strong latitudinal cline (Clapham *et al.*, 1998*a*), supporting that timing of bud set is important for adaptation to the local climate. Similar clines in CNL are also present in many other tree species, in particular those originating from high latitudes (Hurme *et al.*, 1997; Howe *et al.*, 2003; Ingvarsson *et al.*, 2006).

As stated above, two different processes have been proposed to operate in the photoperiod control of flowering (Thomas and Vince-Prue, 1997), one measuring mainly the
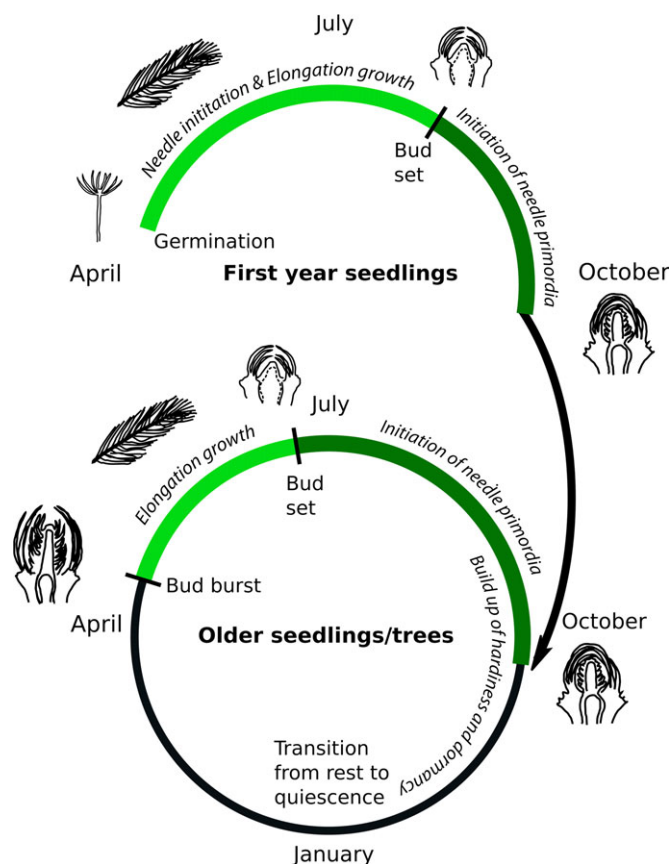

Fig. 7. Summary of the annual growth cycle of Norway spruce. In first-year seedlings, shoot extension stops and terminal buds are set in late summer in response to a shortening photoperiod, after which the cambium ceases growth, needle primordia are initiated within the buds, and frost tolerance begins to increase. Rest dormancy (endodormancy) develops in the meristems during autumn after bud set and, with exposure to chilling temperatures (2–10 °C), changes into quiescence dormancy (ectodormancy) by midwinter, when frost tolerance is maximal; and opening of the bud scales (bud burst) occurs in spring after a temperature sum (TS) has been attained. The extension growth of first-year seedlings consists of the expansion of stem units formed in the current season. This free growth is successively replaced in the following years by predetermined growth (expansion of stem units initiated in the preceding growth period) and results in shortening of the period of extension growth. In older seedlings and trees, growth cessation and terminal bud set occur in early summer, presumably under endogenous rather than photoperiodic control. The build-up of frost tolerance in late summer is, however, initiated mainly under photoperiodic control.

duration of darkness (mainly in SDPs), and the other where events occurring during the day are more important (mainly acting in LDPs). Intriguingly, both these processes seem to operate in Norway spruce, with the dark-dominant response being more important in trees from low latitudes, while the light-dominant response dominates in high-latitude populations.

Is the generally accepted external coincidence model for floral induction also at work in the photoperiod control of

bud set, i.e. is there a circadian rhythm of light sensitivity generated by an internal oscillator? It was known for a long time that night breaks could effectively repress bud set in several tree species including Norway spruce (Thomas and Vince-Prue, 1997). Clapham *et al.* (2001*b*) were able to demonstrate a circadian sensitivity to light in the photoperiodic control of bud set using a night break technique with an extended dark period. During the extended 40 h night, two peaks of higher sensitivity to a night break with ~24 h spacing were observed. The time from lights off to maximum efficiency of the night break corresponded fairly well with the CNL for populations from latitudes 46°N up to 61°N, but for a population from 66°N, no clear night break response was evident, indicating that dark time keeping might not operate. These results suggested that northern populations might behave more like light-dominant plants.

Light-dominant plants are characterized by a requirement for far-red light at certain times during the photoperiod (Vince-Prue, 1994; Thomas and Vince-Prue, 1995). Specifically, far-red light is more efficient if given after a period of white light than if it precedes it. Northern populations of Norway spruce, in contrast to southern ones, require light rich in the far-red spectrum during 16 h day extensions to prevent bud set. In a regime with a main light period of 8 h with light rich in far-red, followed by a 16 h extension with light deficient in far-red, seedlings from northern populations set a bud despite continuous illumination while Romanian seedlings continue to grow and do not set a bud under these conditions (Clapham *et al.*, 1998*b*). Furthermore, the requirement for far-red light shows a clinal variation for populations at intermediate latitudes (Clapham *et al.*, 1998*b*; Molmann *et al.*, 2006).

Flowering in SDPs is supposed to be controlled by a circadian rhythm in the sensitivity to light, the so-called photoperiodic response rhythm. From available data it seems that a similar mechanism is also at work in the control of bud set in Norway spruce seedlings adapted to lower latitudes. At dusk the photoperiodic response rhythm is released, and the time it takes to reach a light-sensitive phase is the CNL. If this phase is reached in darkness (a long night), bud set is induced. Light received at the light-sensitive phase would then constitute an external coincidence.

Molecular control of growth cessation and bud set in trees

Molecular mechanisms in angiosperms trees: The similarities of physiological responses to photoperiod in the control of annual growth rhythm and induction of flowering are striking. The first evidence that the underlying molecular mechanisms might also share conserved components came from studies of *CO* and *FT* homologues in poplar (Bohlenius *et al.*, 2006). Overexpression of the poplar *FT* homologue *PtFT1* resulted in very early flowering. Accordingly, the expression levels of *PtFT1* increased with age in wild-type plants until they flowered at 5–6 years of age. Overexpression of *PtFT1* did not only affect flowering, but

also resulted in an attenuated response to short-day-induced growth cessation and bud set. Again, the expression pattern of the endogenous *PtFT1* supported a role for the gene also in the control of growth cessation. Shifting wild-type plants from long days to short days resulted in a down-regulation of the diurnal pattern seen in long days. Furthermore, down-regulation of *PtFT1* using RNA interference (RNAi) resulted in enhanced bud set response to short days. These results collectively support a role for *FT*-like genes in the control of growth cessation. The authors also report that the diurnal expression pattern of a poplar *CO* gene, *PtCO2*, might be important in the control of *PtFT1* and subsequent bud set. The suggested model states that, like *CO* in *Arabidopsis*, *PtCO2* induces the expression of *PtFT1* if *PtCO2* is expressed in light before dusk. A differing phase of expression of *PtCO2* in genotypes adapted to different latitudes might thus explain varying daylength requirements of these genotypes. It was further suggested that the difference in phase of expression of *PtCO2* between genotypes from different latitudes was controlled by a poplar *GI* homologue *PtGI*, that showed an earlier phase of expression for more southern genotypes (Bohlenius, 2007). Transgenic *PtGI* RNAi plants also initiated bud set in long days. These data suggest a connection between bud set, *PtFT1* expression, and the circadian clock.

Earlier experiments have further shown that phytochromes are also important in the daylength control of growth cessation and bud set. Howe *et al.* (1996) used night break treatment with red and far-red light to show that short night breaks of 2 min could prevent short day-induced bud set in two *Populus* clones, and that this effect was reversed by a subsequent pulse of far-red light. Furthermore, overexpression of the oat phytochrome A gene in the *P. tremula×tremuloides* hybrid significantly changed the critical daylength and prevented cold acclimation (Olsen *et al.*, 1997). Bohlenius *et al.* (2006) also reported that such overexpression prevented FT repression in short days.

A number of quantitative trait loci (QTLs) experiments in poplars have also located one phytochrome B gene (*phyB2*) to a linkage group containing a QTL for bud set (Frewen *et al.*, 2000; Chen *et al.*, 2002). The implication of *phyB2* in the control of natural variation in bud set was further strengthened in studies of sequence variation at this locus (Ingvarsson *et al.*, 2006, 2008). Ingvarsson *et al.* (2006) identified four single nucleotide polymorphisms (SNPs) distributed over a 4 kb region of *phyB2* that displayed a significant clinal variation across latitude of origin. Three of those SNPs confer conservative amino acid substitutions, two of which also showed a significant association with bud set (Ingvarsson *et al.*, 2008). These studies exemplify the importance of studies of natural variation to identify the genetic mechanisms controlling complex traits, and to provide a link to studies of adaptation and evolution.

As mentioned above, some species of deciduous trees in the Rosaceae family do not stop growth in response to a shortened photoperiod. Rather, low temperature has been shown to induce growth cessation and dormancy, at least in apple and pear (Heide and Prestrud, 2005). The molecular

mechanisms controlling this temperature response are currently unknown, but analyses of homologues to genes in the thermosensory pathway in *Arabidopsis* (see above) might provide some insight into the temperature regulation of growth rhythm.

Molecular mechanisms in gymnosperm trees: The control of growth cessation and bud set by short days and the strong latitudinal gradient in the CNL seen in temperate poplars seems to be a dominant phenomenon in a wide variety of species of trees and shrubs of the temperate and arctic zones (Wareing, 1956; Vince-Prue, 1975; Howe *et al.*, 2003). The phenomenon is not restricted to angiosperms but also occurs in distantly related gymnosperms such as several conifer species. A long series of physiological studies on the conifer *P. abies* has yielded a comparatively detailed description of the responses to photoperiod, light quality, and the variation in these responses among genotypes from different latitudes. As these responses show striking similarities to those in angiosperms, one might ask if some of the molecular mechanisms controlling photoperiodic responses are conserved even though gymnosperms diverged from angiosperms some 300 million years ago.

Homologues to several of the genes involved in photoperiodic responses in *Arabidopsis* are present in the genome of *P. abies*. These include homologues to phytochrome and cryptochrome photoreceptors, circadian clock genes, and genes related to *CO* and *FT* (Heuertz *et al.*, 2006; Gyllenstrand *et al.*, 2007; N Gyllenstrand, unpublished data). Based on expression data, it was suggested that the seemingly ubiquitous use of an *FT*-like gene as a mobile signal in photoperiodic responses might also be true for the conifer, *P. abies* (Gyllenstrand *et al.*, 2007). One of four identified *P. abies* genes with similarity to *FT*-like genes displayed an expression pattern with a striking correlation to bud set response under varying photoperiod and light conditions. In a population from low latitudes that is characterized by a dark-dominant response, *PaFT4* was induced in long nights but not if the night was shorter than the CNL. The expression was significantly reduced if a night break was given close to the CNL (resulting in reduced bud set), but not if the night break was given later in the night at a non-responsive phase. Furthermore, *PaFT4* expression remained at background levels if the night was replaced with far-red-deficient cool-white illumination in line with the resulting suppression of bud set under these conditions. If the same experimental conditions were used on seedlings from high latitude populations with a more light-dominant response, and short CNL (1–2 h) *PaFT4* was induced under all conditions. Neither replacement of the night with far-red-deficient cool-white illumination, nor night breaks reduced *PaFT4* expression or bud set. All these expression data were collected from leaf tissue, where the expression ceased if plants that were induced to set bud were returned to constant light. It is not yet known whether PaFT4 protein moves from the leaves as a potential systemic signal.

The assumed effects of PaFT4 and poplar PtFT1 on growth cessation seem contradictory in that reduced *PtFT1*

expression is associated with growth cessation while the opposite effect is seen for *PaFT4*. In *P. abies* and other conifers, four genes have so far been identified in the family of phosphatidylethanolamine-binding protein (PEBP) genes to which *FT* belongs (Gyllenstrand *et al.*, 2007). PEBP genes in angiosperms fall into three main clades, *FT-like*, *MFT-like*, and *TFL1-like* (Chardon and Damerval, 2005; Hecht *et al.*, 2005). *MFT* (*MOTHER OF FT AND TFL1*) does not seem to have a major role in controlling flowering time, although overexpression caused a slight advance in flowering time. Two of the *P. abies* genes clearly fall in the *MFT* clade, while the two others (including *PaFT4*) cluster together at the base of the node that separates the *FT* and *TFL1* clades. Amino acids that have been shown to be important for FT function as opposed to TFL1 function are conserved in the two spruce genes, suggesting that their function might be more FT like (Gyllenstrand *et al.*, 2007). However, overexpression of *PaFT4* in *Arabidopsis* does not result in early flowering (A Karlgren *et al.*, unpublished data). No genes with amino acids crucial for TFL1 function have so far been found in bryophytes, lycopodes, or gymnosperms (N Gyllenstrand *et al.*, unpublished data), indicating that the *TFL1* genes might be a novel innovation in angiosperms. If this assumption is correct, it is difficult to predict if the *P. abies FT*-like gene should possess a function more similar to FT or TFL1.

In angiosperms, *TFL1*-like genes act antagonistically to *FT*. For example, *Arabidopsis tfl1* mutants flower early and *35S::TFL1* overexpressors flower late (Shannon and Meeks-Wagner, 1991; Ratcliffe *et al.*, 1998). Surprisingly, a large part of these opposite functions is due to the exchange of a single amino acid in the *Arabidopsis* proteins (Hanzawa *et al.*, 2005). Overexpression of a poplar *TFL1* homologue (*PtCENL-1*) has been reported to delay bud flush (Mohamed, 2006). The gene, furthermore, displayed low expression in inflorescence tissues and inflorescence buds, but strong expression in both terminal and lateral vegetative post-dormant buds (Mohamed, 2006). Expression levels dropped in new shoot tips in spring and increased buds formed in autumn. Expression was again strongly reduced in dormant buds later in autumn. These data suggest that *FT*-like and *TFL1*-like genes might also act antagonistically in the control of vegetative growth rhythm in angiosperms.

Can a function for *FT/TFL1*-like genes in growth cessation be reconciled with the well established function in induction of flowering in angiosperms, where interaction with FD induces expression of floral meristem identity genes? Lifschitz and Eshed (2006) suggested that the primary targets for both *FT*- and *TFL*-like genes in angiosperms may actually be induction and termination of growth, and that induction of flowering could be seen as a pleiotropic effect. Introduction in tomato (*Solanum lycopersicum*) of *Arabidopsis FT* under the filamentous flower promoter resulted in *FT* expression mainly in leaf primordia. Such leaf primordia-specific *FT* expression resulted in reduced stem and leaf growth, and in frequent meristem arrest in addition to early flowering (Lifschitz *et al.*, 2006). These results indicate that *FT* genes can affect

growth independently of flower formation. Lifschitz *et al.* (2006) suggested that 'floral transition and growth attenuation, instead of being the consequence of one another, are two facets of the same cellular responses'.

In woody perennials, development of reproductive structures is often intimately connected to bud set. The 'flowering' process often extends to two consecutive seasons—during the first season buds are formed and, in those latent buds, reproductive development is initiated. Development of the reproductive structures can then either continue in the same season within the buds or be partly or completely postponed until the next growing season. In several *Picea* species studied, the time of bud differentiation and initiation of leaf or reproductive primordia coincides with the completion of shoot elongation (Owens and Molder, 1977*a*, *b*). It is not inconceivable that the ancestors of *FT/TFL1*-like genes possessed a more general function in the determination of growth of primordia in developing buds.

Two *CO*-like genes were also identified in *P. abies*, but none of them was a strong candidate as a determinant of *PaFT4* expression (Gyllenstrand *et al.*, 2007). However, the expression patterns of a *GI* homologue and a gene most similar to *PRR7*, that in *Arabidopsis* functions in close connection with the circadian clock, were correlated with the expression of *PaFT4*. For example, night breaks that reduce *PaFT4* expression and bud set in low latitude populations also resulted in rapid induction of *PaPRR7* and *PaGI* in that population. In contrast, no effects of night breaks were seen on these genes in high latitude populations, in line with the lack of repression of *PaFT4* and bud set (N Gyllenstrand *et al.*, unpublished data). These data suggest that plants from low and high latitude populations are in a different phase of the photoperiodic response rhythm because if both types of plants were kept in darkness for an extended period of several days, the genes responded equally well to a night break.

An interesting observation that could provide clues to the shift from dark-dominant in low latitude populations to a more light-dominant response in high latitude populations was that in long days, putative circadian clock genes displayed a decrease in amplitude in plants from a low latitude population, compared with high latitude plants (unpublished data). This could be interpreted to suggest the dark-dominant plants went into a light limit cycle with restricted phase states. Clearly, more research is needed to elucidate the photoperiodic pathway controlling growth cessation in conifers, but current data indicate that parts of the basic mechanisms might be shared with the corresponding pathways in angiosperms.

Conclusions

Comparative studies of photoperiodic responses of flowering and annual growth rhythm in plants suggest some common molecular mechanisms for these responses. Current data support that the mobile protein FT and its homologues are universal signalling molecules transferring the result of photoperiod induction from the leaves to the meristems in the control of flowering. The limited data on growth rhythm in trees also suggest that *FT/TFL1* homologues might have a similar role in the control of growth cessation. More detailed studies on the regulation of *FT* in different species suggest a more diverse set of mechanisms and genes in regulation of *FT*-mediated control of flowering time. Common elements have been identified such as the circadian clock and several photoreceptors, but how these interact and affect *FT* expression differ both between and within LDPs and SDPs. Photoreceptors and circadian clock genes are also implicated in the control of growth cessation and bud set in poplars, and preliminary data suggest that such genes might also affect timing of bud set in gymnosperms. Studies of homologues to genes from model species provide an entry point to disentangle the mechanisms controlling timing of growth cessation and bud set in trees. However, to understand those mechanisms fully, these studies must be combined with more unbiased approaches. These could include association mapping and studies of DNA sequence variation in natural populations to identify the genes controlling divergent photoperiodic responses in different genotypes. The strong clinal pattern of response to photoperiod in many tree species makes such attempts attractive.

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