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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.


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 tepod (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 tepod 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, sg3, 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 (Baurle & 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
MicroRNAs, trans-acting small interfering RNAs (ta-siRNAs) and their target genes involved in the control of the juvenile–adult phase transition. A REVIEW 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., 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.

In Arabidopsis, 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 TPI 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 Ipomoea 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 balsamina, 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., 2007).
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 day, 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 REGULATORATOR (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 fkh1 mutants are late flowering and have reduced levels of CO mRNA, as do CDF1 over-expressing...
lines (Suárez-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 (Suárez-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 PHOTOTRICHROME 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 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 is being very different from that in the expression profile of CO, these other factors may 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 (Suárez-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 euakaryotic 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 CCAAT 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 EAT1 (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 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 juvenileity 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 miRNAs 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 (Chailikyan 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; Świeczewski *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 *ArMSH*, 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 (TaGl 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 HOMEOBIN 1 (POTH1) to form a heterodimer which binds to the promoter of the GA biosynthetic gene GA20 OXIDASE 1 (GA20ox1) 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 GA20ox1 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 aspen (Populus trichocarpa) FT orthologue, PtFT1, is an inhibitor of this process and its expression decreases if plants are shifted from LDs to SDs (Böhli 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 (Böhli 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 (Böhli 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.

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