The genetic basis of flowering responses to seasonal cues

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Abstract | Plants respond to the changing seasons to initiate developmental programmes precisely at particular times of year. Flowering is the best characterized of these seasonal responses, and in temperate climates it often occurs in spring. Genetic approaches in *Arabidopsis thaliana* have shown how the underlying responses to changes in day length (photoperiod) or winter temperature (vernalization) are conferred and how these converge to create a robust seasonal response. Recent advances in plant genome analysis have demonstrated the diversity in these regulatory systems in many plant species, including several crops and perennials, such as poplar trees. Here, we report progress in defining the diverse genetic mechanisms that enable plants to recognize winter, spring and autumn to initiate flower development.

Photoperiod
The duration of light that a plant is exposed to.

Vernalization
Prolonged exposure to low winter temperatures that in many plant species is required to accelerate flowering.

Floral integrator genes
A set of genes that regulate the floral transition and are the convergence point of diverse flowering pathways.

Life histories
A variety of strategies related to key biological events in the lifetime of an organism, such as juvenile-to-adult transition, reproduction and senescence. These strategies have been adopted by different species during evolution in order to maximize the number of viable offspring they produce.

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Many plants show precisely controlled seasonal patterns in flowering. Decades of physiological analyses have dissected such behaviours into responses to discrete environmental cues, such as day length (photoperiod) and winter temperatures (vernalization)[1]. Genetic pathways that promote flowering in response to these cues were defined in the model species *Arabidopsis thaliana*, and the regulatory proteins involved were identified. Here we describe how the expression of key transcription factors in *A. thaliana* is regulated by day length or by temperature, creating plasticity in flowering behaviour so that it occurs only under certain environmental conditions. These environmentally responsive transcription factors converge on a small number of floral integrator genes that initiate the early stages of flowering, and this convergence creates a coordinated response to seasonal cues.

The strict regulation of flowering time is essential for reproductive success, enabling completion of seed development in favourable environmental conditions. This adaptive effect has been used in agriculture to ensure that plants flower synchronously and at the optimal time to maximize seed yields[2]. Recent advances in genomics of crops have allowed the isolation of several of the genes that control flowering in species such as rice, barley, maize, tomatoes, sunflowers and sugar beet. Some of these genes are orthologues of those that were previously characterized in *A. thaliana*, and their functions are conserved. However, others have proved to be homologous to *A. thaliana* flowering-time genes but to have distinct functions, and many are not found in *A. thaliana*. These recent analyses have demonstrated the surprising rapidity with which the regulatory pathways controlling flowering responses to environmental cues have evolved, even among closely related species. Such comparative approaches are also being used to study flowering control in plants that exhibit different life histories. Whereas *A. thaliana* and many of the crops mentioned above are annual plants that flower once in their life cycle and then die, perennial species that flower repeatedly and live for many years dominate many ecological niches. Recently, model species for studying perennialism have been developed, allowing for understanding the differences in the genetic control of flowering between annual and perennial species.

Here we first describe our current understanding of the mechanisms of control of flowering by day length in *A. thaliana* and contrast this with other species, including crops. We follow with a discussion of the diverse mechanisms regulating vernalization response. Then we report on the recent progress in understanding seasonal flowering patterns in perennial species, including those of perennial trees such as poplars.

Flowering in response to changing day length

Predictable differences in day length are associated with the changing seasons, particularly at high latitudes. Plants can be divided into three major groups on the basis of their responses to photoperiod: long-day plants flower when the day exceeds a critical length (normally in summer), short-day plants flower when the day is shorter than a critical length (normally in autumn) and day-neutral plants flower independently of day length[3].

The model annual plant species *A. thaliana* was initially used as a genetic system to investigate the molecular basis of day-length response. It is widely distributed in the northern hemisphere and flowers earlier in response to long days, which are typical of spring and summer[4]. By contrast, the subtropical species maize and rice are short-day plants[5]. Other short-day plants are found at high latitude, such as *Xanthium strumarium* (cocklebur), which flowers in North America in late summer as day length shortens[6]. Day-neutral species include *Lycopersicon esculentum* (tomato)[5].

Mechanisms of time measurement in the control of flowering — the long-day plant* Arabidopsis thaliana *as a model. Isolation of flowering-time mutants in *A. thaliana* initially contributed to understanding how plants detect and respond to day length. In initial studies into flowering time control, the timing of *A. thaliana* flowering was carefully measured by counting the number of leaves formed before the initiation of flowering. This developmental measure allowed the identification of mutants impaired in genes encoding regulators of flowering time rather than those involved in more general processes, such as controlling growth rate[4]. One class of mutants flowered later than the wild type under long days but at the same time as wild-type plants under short days. These mutants were proposed to be compromised in a pathway that promotes flowering specifically in response to long days[4,7]. The genes *GIGANTEA* (*GI*), *FLAVIN KELCH F BOX 1* (*FKF1*), *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) have major regulatory roles in this pathway[8–12]. These genes are expressed in the vascular tissue of leaves, and this is consistent with physiological experiments that showed that the perception of photoperiod takes place in the leaves[13], although some genes, such as *GI* and *FKF1*, are also expressed more widely[14,15].

Activation of the photoperiodic flowering pathway leads to transcriptional activation of *FT*, which occurs only under long days and is closely associated with flowering (FIG. 1). This long-day-dependent activation of *FT* requires CO, which is a zinc finger transcriptional regulator containing two B boxes and a CO, CO-LIKE and TOC1 (also known as APRR1) domain (CCT domain)[11,16]. CO probably activates *FT* transcription by directly interacting with the *FT* promoter[17] (BOX 1). Transcriptional and post-translational regulation of CO ensures that it activates *FT* transcription only under long days (FIG. 1a).

CO transcription is regulated by light and the circadian clock. Light activation of *CO* transcription is induced by the interaction between the plant-specific protein GI and the ubiquitin ligase FKF1: two proteins that are also circadian clock components[18,19]. FKF1 is able to sense light through its attached chromophore, and under long days this light-dependent interaction between FKF1 and GI releases the repression of *CO* mRNA transcription by inducing degradation of the transcriptional repressors known as CYCLING DOF FACTORs (CDFs)[20]. Thus at the end of a long day, but not in short days, GI and FKF1 interact, triggering the degradation of CDFs and a rise in *CO* mRNA (FIG. 1).

Post-translational regulation of CO is also essential for a flowering response to long days. CO protein is ubiquitylated by a ubiquitin ligase complex that includes CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SUPPRESSOR OF PHYTOCHROME A (SPA1), facilitating CO degradation by the 26S proteosome[21–23]. Activity of this complex is repressed by light so that it mainly promotes the degradation of CO protein in the dark. Thus only the peak of *CO* mRNA that occurs in the light at the end of a long day after degradation of the CDFs by GI–FKF1 leads to CO protein accumulation (FIG. 1a). This stabilization of CO at the end of a long day involves the photoreceptors PHYTOCHROME A (PHYA; a far-red-light receptor) and CRYPTOCHROME 2 (CRY2; a blue-light receptor)[24,25]. Direct interactions between activated CRY2 and SPA1 or COP1 reduce the catalytic activity of COP1–SPA1 in the light, and CO is not efficiently degraded[25] (FIG. 1a). This post-translational regulation of CO is enhanced in the afternoon under long days by FKF1. In response to blue light, FKF1 binds to CO, increasing its stability[26]. Additionally, in the morning, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1) — which is a RING-finger-containing E3 ubiquitin ligase — contributes to the instability of CO[27] (FIG. 1a). The regulatory logic of this pathway shares aspects of the classical external coincidence model that has been proposed to explain photoperiodic responses[28]. In this model, circadian patterns of *CO*, *GI* and *FKF1* transcription create diurnal rhythms of their mRNAs, and whether these rhythms coincide with exposure to an external signal, such as light, under long days determines whether the regulatory proteins are stabilized to promote flowering.

Systemic signalling from leaf to the shoot apex involves movement of FT protein. The perception of photoperiod takes place in the leaf, but floral transition occurs at the shoot apical meristem (SAM)[13]. The existence of 'florigen', which is a graft-transmissible signal produced in the leaves that induces floral initiation at the shoot apex, was proposed more than 70 years ago[29]. Recently, the following genetic experiments in *A. thaliana* strongly suggested that FT protein is at least a part of the florigen signal. FT is a member of the CETS protein family (which consists of CENTRORADIALIS (CEN), TERMINAL FLOWER 1 (TFL1) and FT), which shares homology with phosphatidylethanolamine-binding proteins or RAF kinase inhibitor proteins (RKIPs)[9,10,30] that are present in organisms as diverse as bacteria and mammals[31,32]. In plants, FT is proposed to be a component of transcriptional complexes. Misexpression of *FT* from promoters that are specific to the phloem companion cells of the vascular tissue or the SAM corrected the late flowering phenotypes of the *co* and *ft* mutants as well as *ft tsf* double mutants (in which the *FT* paralogue *TWIN SISTER OF FT* (*TSF*) is also mutated)[32–35]. Furthermore, expression of an artificial microRNA (miRNA) that targets *FT* mRNA caused late flowering when expressed in the phloem companion cells but not when expressed in the meristem[36]. These experiments were consistent with the idea that FT protein can act in the phloem and

Circadian clock
An endogenous time-keeping mechanism with a cycle time of approximately 24 hours that regulates the transcription of many plant genes.

Phytochrome
Phytochrome proteins are red- and far-red-light-absorbing photoreceptors. They covalently bind to light-absorbing linear tetrapyrrole chromophore, photochromobilin, which is able to absorb light of wavelengths between 650 nm (red) and 740 nm (far red). Activated phytochromes are imported into the nucleus and directly interact with transcription factors.

Photoreceptors
Proteins with attached chromophores that absorb light changing the conformation of the protein and initiating photoreceptor signalling.

Cryptochrome
A blue-light-absorbing photoreceptor. Cryptochromes contain two noncovalently bound chromophores (pterin and flavin). Studies in *Arabidopsis thaliana* suggest that pterin absorbs at a wavelength of 380 nm and flavin at 450 nm. Flavin photoreduction and autophosphorylation of conserved tryptophans appear to be important steps of the cryptochrome signalling pathway.

Raf-kinase inhibitor proteins
Proteins that are present in diverse organisms. In mammals, they participate in cell differentiation, the cell cycle, apoptosis and cell migration by affecting different signalling pathways.

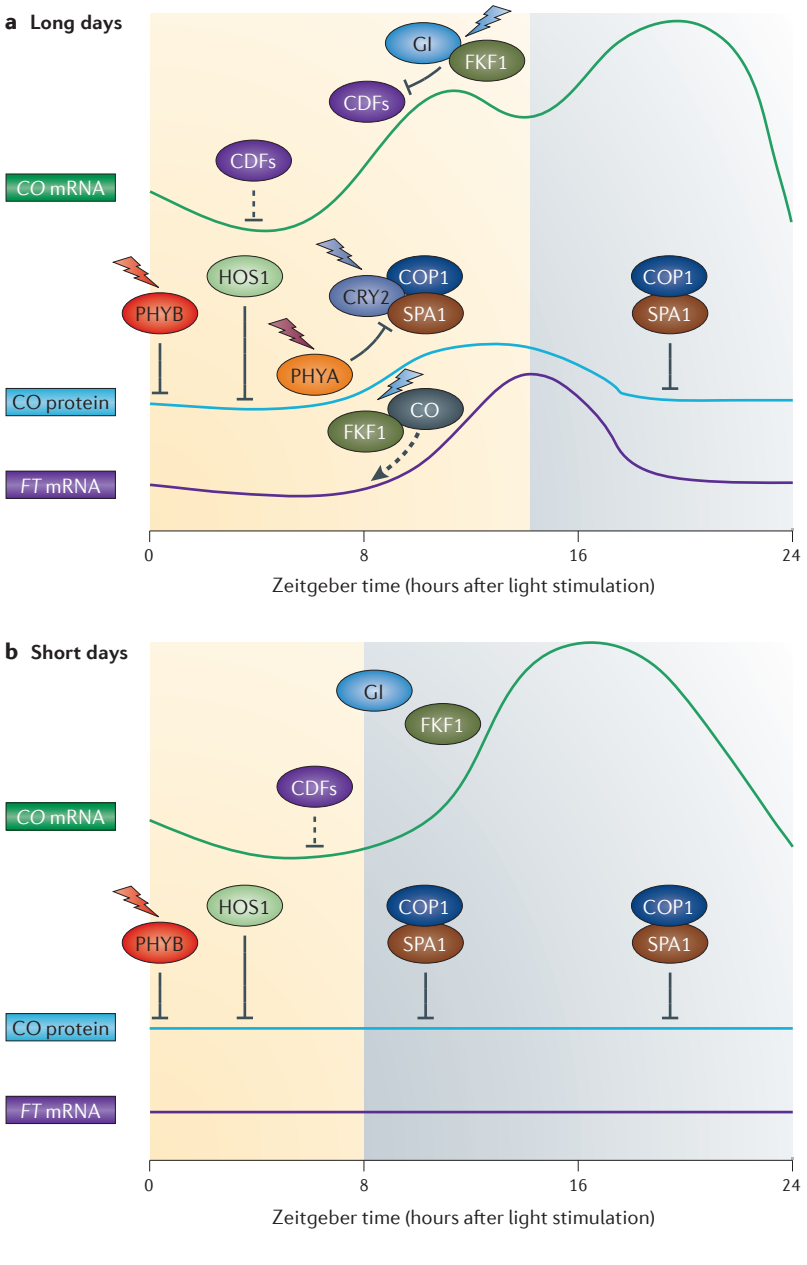
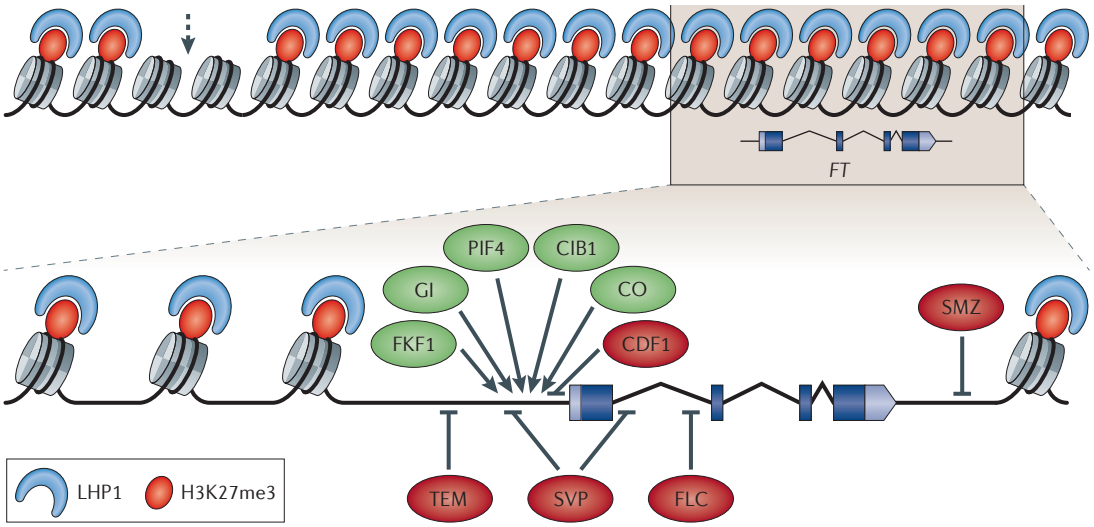


Figure 1 | **Transcriptional and post-translational regulation of *CONSTANS* controls photoperiodic flowering of *Arabidopsis thaliana*.** **a** | *CONSTANS* (*CO*) regulation under long days of spring or early summer. The peak of *CO* mRNA that occurs 12–16 hours after dawn in the light under long days is essential for the day-length-dependent promotion of flowering[12,14,20] and is regulated by the circadian clock component GIGANTEA (GI) — a plant-specific protein with no domains of known function. When plants are exposed to light under long days 10–14 hours after dawn[20], GI physically interacts with FLAVIN KELCH F BOX 1 (FKF1), which is a chromophore containing F box ubiquitin ligase[13]. This interaction stabilizes FKF1 and promotes the degradation of CYCLING DOF FACTORs (CDFs)[20,136]. The CDFs act redundantly to repress *CO* transcription[136], and at least CDF1 is a direct transcriptional repressor of *CO*[20]. CO protein expressed at the end of the long day is stabilized because the CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)–SUPPRESSOR OF PHYTOCHROME A (SPA1) ubiquitin ligase, which triggers degradation of CO in the dark, is inhibited in the light[21,137]. This inhibition occurs at least in part because activated CRYPTOCHROME 2 (CRY2) forms a complex with SPA1 (REF. 25). CRY2 also interacts directly with and inhibits COP1 (REFS 25,138,139). PHYTOCHROME A (PHYA) also inhibits the COP1–SPA1 complex, but the mechanism by which this occurs is unknown. In addition, FKF1 stabilizes CO in the afternoon[26]. In the dark, the photoreceptors are not active, and therefore the COP1–SPA1 complex triggers the degradation of CO protein. In addition, if CO protein is present in the early part of the day, it is degraded by a COP1-independent pathway that is activated by PHYB (red light receptor) but is otherwise poorly understood at the mechanistic level[21,137]. HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1) is another ubiquitin ligase that was recently shown to promote degradation of CO mainly in the morning[27]. This combination of transcriptional and post-translational regulation leads to a transient peak in CO protein at the end of the day, when CO can activate *FT* transcription. **b** | CO regulation under short days of winter or autumn. The degradation of the CO transcriptional repressors, the CDFs, does not occur under short days because the mRNAs of the light-dependent CDF degradation protein complex GI–FKF1 only rises around 10 hours after dawn. They are thus only expressed in darkness, and therefore the proteins do not form an active complex[20]. The CDF proteins are therefore present to repress CO transcription. Under these conditions, CO mRNA only peaks in the dark, and the CO protein is degraded through the activity of the COP1–SPA1 ubiquitin ligase, as described above. In the morning, CO protein is degraded by a pathway requiring PHYB.

the meristem to promote flowering but that its mRNA is only required in the phloem, where the native *FT* gene is expressed. Thus FT protein might move directly between the phloem companion cells and the shoot meristem through the sieve elements of the phloem. In support of this conclusion in *A. thaliana* and rice, FT or FT-like proteins that are fused to GFP and that are expressed in the companion cell were detected at the meristem[33,37]. The movement of FT protein was also suggested from analysis of endogenous FT-like proteins by mass spectrometry in the phloem sap of *Brassica napus*, *Cucurbita maxima* and rice[37–39]. FT protein is smaller than the size exclusion limit of the plasmodesmata, so FT might passively move into the sieve elements by diffusion. However, as the abundance of FT protein is low, an active transport mechanism is also possible[39]. Recently, an FT-interacting protein that is present in the plasmodesmata of the phloem companion cells of *A. thaliana* was found to be required for transport of FT protein into the phloem sieve elements[40].

Box 1 | **Regulation of *Arabidopsis thaliana FT* transcription**



Transcription of *FLOWERING LOCUS T* (*FT*) is a point of convergence of different seasonal cues and is tightly regulated. A 5.7 kb region 5′ of the ATG represents the *FT* promoter and contains all of the elements that are required to mediate spatial and temporal expression of *FT* in response to photoperiod[104]. CONSTANS (CO) activates *FT* transcription in response to long days and was recently demonstrated to bind *in vitro* and *in vivo* to the proximal region of the *FT* promoter[17,26]. Interestingly, although CO binds to the proximal region of the promoter close to the transcription start site, a more distal region (shown by the dotted arrow in the figure) also seems to be essential for CONSTANS (CO)-dependent *FT* activation. This upstream region might be recognized by an unknown activator complex that enhances the affinity of CO to bind to the region located in the proximal promoter or is required for CO function. The CCAAT-box-binding complex has been proposed to carry out such a function[105,106]. GIGANTEA (GI), FLAVIN KELCH F BOX 1 (FKF1) and CYCLIC DOF FACTOR 1 (CDF1), which affect expression of *FT* by regulating *CO* mRNA level, are also reported to bind directly to the *FT* locus[20,26,107]. Indeed, tagged CDF1 proteins expressed in the *fkf1* mutant strongly associate near the *FT* transcription start site, suggesting that FKF1 contributes to *FT* transcriptional activation by removing CDF repressors from its locus[26]. The basic helix–loop–helix transcription factor PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) binds directly to the proximal *FT* promoter in response to high temperature when it activates *FT* transcription independently of CO[108], and another basic helix–loop–helix transcription factor, CIB1, binds to a similar region and activates *FT* in response to blue light[109]. LIKE HETEROCHROMATIN PROTEIN1 (LHP1) is a direct repressor of *FT* transcription. In *lhp1* mutants, *FT* mRNA is highly expressed, and flowering is accelerated independently of photoperiod[110]. LHP1 is a chromodomain protein that colocalizes with histone H3 modified by trimethylation on lysine 27 (H3K27me3), which is a repressive chromatin mark formed by Polycomb repressive complex 2 (REF. 111). LHP1 is proposed to be a plant-specific component of Polycomb repressive complex 1 (REF. 111), chromatin immunoprecipitation (ChIP) experiments (such as ChIP followed by microarray (ChIP–chip) and ChIP followed by high-throughput sequencing (ChIP–seq)) showed the pattern of accumulation of these repressive marks along the locus of *FT* (upper panel of the figure). Both H3K27me3 and LHP1 widely cover the *FT* locus, conferring its transcriptional repression, whereas those regions that are free of repressive marks have been proposed to constitute a window of open chromatin that is accessible to regulatory factors[104]. Many of the other proteins that bind the *FT* locus are also transcriptional repressors. The MADS box proteins SHORT VEGETATIVE PHASE (SVP) and FLOWERING LOCUS C (FLC) confer vernalization requirement and repress *FT* by binding CArG boxes present in its promoter and first intron[72,76,112]. SVP and FLC are proposed to bind *FT* in a heteromeric complex[76,77]. The AP2-like transcription factor TEMPRANILLO (TEM) genes *TEM1* and *TEM2* also repress *FT* expression[113]. Moreover, TEM1 binds directly to the 5′ untranslated region of *FT*. A balance between *CO* and TEM genes has been described as an important mechanism in the transcriptional control of *FT* particularly in young plants[113]. Other repressors of *FT* belong to a small group of AP2-like genes, mRNAs of which are targets of miR172. This group includes *APETALA2* (*AP2*), *TARGET OF EAT 1* (*TOE1*), *TOE2*, *SCHNARCHZAPFEN* (*SNZ*) and *SCHLAFMÜTZE* (*SMZ*). However, only SMZ is known to bind directly to a region 1.5 kb downstream of the *FT* stop codon[114].

Polycomb-repressive complex 2
A protein complex that is highly conserved between plants and animals and includes methylase enzymes that cause trimethylation of lysine 27 on histone H3 leading to repression of gene transcription.

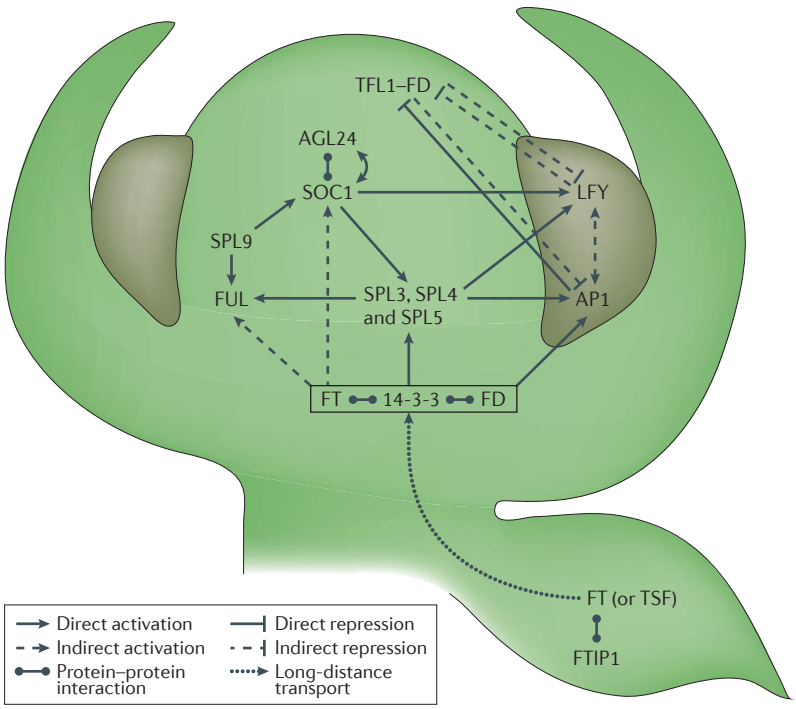
Polycomb-repressive complex 1
A protein complex found in animals that is only weakly conserved in plants and is required for repression of gene transcription by recognition of trimethylated lysine 27 on histone H3.

The contribution of other 'florigenic signals' in addition to FT protein cannot be excluded. Indeed, the mRNA of *FT* was found to spread through the plant in studies using a viral expression system[41] or by analysis of grafted *A. thaliana* plants[42]. However, although floral signals were shown to move across graft junctions in tomatoes, extensive RNA analyses failed to detect movement of mRNA of *SFT*, the tomato FT orthologue[43]. Furthermore, expression of an artificial miRNA that targets *FT* mRNA in the apex of *A. thaliana* appeared to exclude a role for *FT* mRNA in the shoot meristem of wild-type plants[35,42].

Transport of FT from the leaves to the apex is followed by a series of genetic and morphological changes that culminate in the formation of flowers (BOX 2). FLOWERING LOCUS D (FD) is a basic leucine zipper

Box 2 | **The genetic network of floral transition at the shoot apical meristem**

FLOWERING LOCUS T (FT) is made in the companion cells of the leaves and is transported from the leaves to the meristem through the phloem sieve elements. Recently, movement of FT from the companion cells to the sieve elements was shown to require the interaction between FT and a novel endoplasmic reticulum membrane protein called FT-INTERACTING PROTEIN 1 (FTIP1)[40]. TWIN SISTER OF FT (TSF) is a closely related protein and probably acts in a similar way to FT. At the shoot meristem, genetic data indicate that the FT–FLOWERING LOCUS D (FD) complex activates expression of flowering genes shown as a network in the meristem. In *ft tsf* double mutants and *fd* mutants, transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) in response to long days is delayed in comparison to wild-type plants[34,72]. *SOC1*, which encodes a MADS box transcription factor, is the earliest gene shown to be upregulated in response to long days at the meristem, and mutations in the gene cause late flowering[115,116]. Strikingly, inactivation of both *SOC1* and the related MADS box gene *FRUITFULL* (*FUL*; also known as *AGL8*) almost entirely suppresses the extreme early flowering caused by overexpression of *FT* from different heterologous promoters, suggesting that *SOC1* and *FUL* are essential for the promotion of flowering by FT[117,118]. When SOC1 is expressed in the meristem, it interacts with AGL24, another MADS box transcription factor, and promotes the activation of transcription of *LEAFY* (*LFY*), which is a meristem identity gene that is involved in the initiation of flower development[119]. *SOC*, *FUL*, *LFY* and *APETALA1* — another floral meristem identity gene — are also activated by SQUAMOSA BINDING PROTEIN LIKE (SPL) transcription factors, which are expressed in the meristem in response to FT and FD and were recently proposed to be direct targets of FD on the basis of chromatin immunoprecipitation experiments[120–122]. Interestingly, SOC1 also binds to the SPL genes, suggesting that FD might act at different layers of the hierarchy to upregulate both *SOC1* and *SPL* gene transcription[121]. Similarly, the FT–FD complex has been proposed directly to activate *AP1*, which is expressed in the cells that will give rise to the flower and confer floral identity on this primordium[45]. However, recent detailed analysis of the *AP1* promoter questioned whether FD binds directly[123], Nevertheless, in rice, FD was also proposed to bind directly to the promoter of the AP1 homologue MADS15 via a similar element[46]. The expression of both meristem identity genes *AP1* and *LFY* is antagonized by TERMINAL FLOWER1 (TFL1), which is a protein that is related to FT, preventing their ectopic expression in the centre of the shoot meristem. In young floral primordia, AP1 and LFY repress *TFL1* transcription[124,125]. Recently, TFL1 was also shown to depend on FD to trigger the transcriptional repression of its targets[126], suggesting a pivotal role of FD, depending on whether it interacts with FT or TFL1.



(bZIP) domain transcription factor that is expressed in the SAM and that interacts with FT and TSF in yeast two-hybrid assays, and genetic experiments indicate that it is partially required for FT-mediated promotion of flowering[44,45]. The interaction between FT-like proteins and bZIP domain transcription factors is also observed in other species, including rice and tomatoes[30,46]. However, recent work with rice proteins suggests that this interaction is not direct and that 14-3-3 proteins function as a bridge in the interaction between HEADING DATE 3A (HD3A), which is the rice homologue of FT, and rice FD. *In vitro*, HD3A and rice FD only interact if GF14C (a 14-3-3 protein) is added, and in the yeast two-hybrid system, a yeast 14-3-3 protein is assumed to substitute for the plant protein[46]. Nevertheless, these data provide strong support for the idea that a major function of FT is to act in protein complexes at the apex with bZIP domain transcription factors to regulate genes that promote flowering in the SAM (BOX 2).

FT-like genes contribute to diverse responses to day length. Transcriptional activation of FT-like genes occurs in response to the day length that induces flowering and can mediate the different flowering responses to short days or long days. As discussed above, FT-like genes are induced by exposure to long days in *A. thaliana*, barley and peas[9,10,47,48], but in response to short days in rice, potatoes and Japanese morning glory[46,49,50]. In sunflowers, *FT* homologues are induced by long days or short days, depending on the accession, but again transcription of *FT* homologues correlates with floral induction[51]. The large evolutionary distances between these species and their widely different environmental responses suggest that FT-like proteins represent a universal, or at least very widely conserved, floral inducing signal. However, the mechanisms controlling transcription of FT-like genes appear to differ greatly and thereby generate diverse seasonal responses to day length (FIG. 2).

Flowering response mechanisms in short-day plants. Most genetic analysis of short-day flowering responses has been carried out in rice. Initial genetic identification of genes required to confer a short-day flowering response in rice identified the *CO* and *FT* homologues *HD1* and *HD3A*, respectively, and implicated the rice homologue of *GI*, suggesting that the photoperiod pathway is likely to be highly conserved between *A. thaliana* and rice, despite their different responses to day length[52] (FIG. 2). This conservation of mechanism between rice and *A. thaliana* was complicated by the identification of rice genes that do not have counterparts in *A. thaliana* but that have important functions in photoperiodic responses. In particular, *EARLY HEADING DATE 1* (*EHD1*), which encodes a B-type response regulator, activates transcription of *HD3A* and its paralogue *RICE FT-LIKE 1* (*RFT1*) under short days[53,54], independently of HD1. Furthermore, the rice protein encoded by *GHD7* (for grain number, plant height and heading date) has a crucial role in photoperiodic response but no obvious *A. thaliana* counterpart. It contains a CCT domain, which is also present in CO[55] (FIG. 2). Such observations

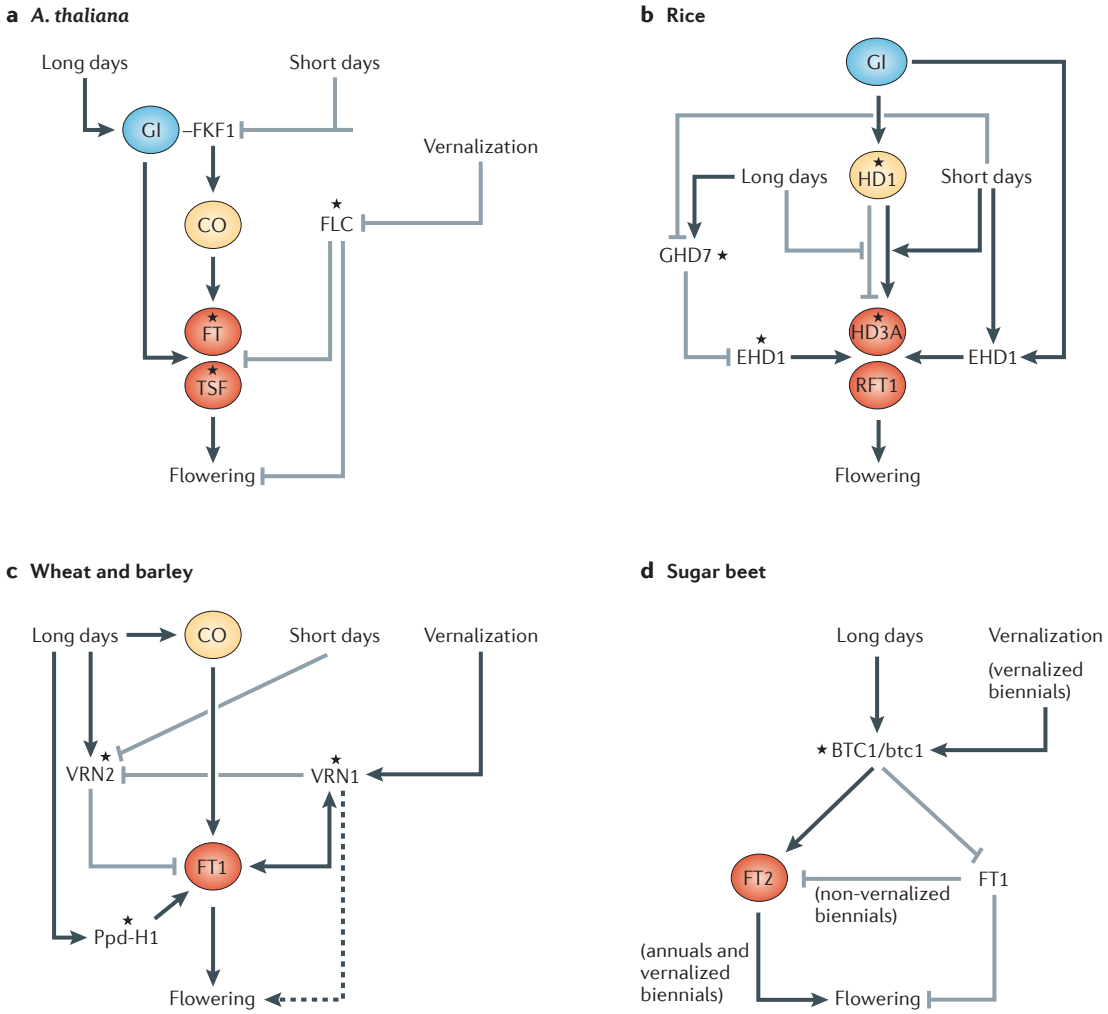


Figure 2 | **Comparison of flowering time regulation by day length and vernalization in different species.** Grey and black lines represent repression and induction, respectively. Genes marked with a star present allelic diversity associated with natural variation of flowering time. Red shapes indicate homologues of FT, yellow shapes indicate homologues of CONSTANS (CO) and blue shapes indicate homologues of GIGANTEA (GI). **a** | In *Aradbidopsis thaliana*, during vernalization FLOWERING LOCUS C (FLC) expression falls, allowing induction of FLOWERING LOCUS T (FT) and TWIN SISTER OF FT (TSF) transcription by CO under subsequent long days, as shown in FIG. 1. **b** | In contrast to *A. thaliana*, the rice CO homologue HEADING DATE 1 (HD1) represses flowering under long days by inhibiting transcription of the *HD3A* gene, but under short days it promotes the transcription of the *FT*-like genes *HD3A* and *RICE FT-LIKE 1* (*RFT1*). HD3A and RFT1 in turn promote flowering in a similar manner to FT and TSF in *A. thaliana*. GHD7 is a repressor of flowering under long days and acts by repressing *EARLY HEADING DATE 1* (*EHD1*) transcription[55]. EHD1 activates *HD3A* and *RFT1* transcription in short days. Rice GI promotes *HD1* transcription, as observed for GI and *CO* in *A. thaliana*. Current models of short-day flowering in rice suggest that coincidence of light with diurnal rhythms in transcription of *EHD1* or *GHD7* lead to either activation or repression of *HD3A* transcription, respectively, and that this can confer precise responsiveness to seasonal changes in day length[140,141]. Rice does not require vernalization to promote flowering. **c** | In the temperate cereals wheat and barley, flowering is promoted by long days. CO-like proteins are involved in the activation of FT-like genes in response to long days[87]; however, the pseudo-response regulator Ppd-H1 is also required for transcriptional activation of FT-like genes under long days[58]. Similarly to *A. thaliana*, vernalization is required to accelerate flowering in these species. During vernalization, the transcription of *VERNALIZATION 1* (*VRN1*), which encodes a MADS box transcription factor related to the *A. thaliana* proteins FRUITFULL and APETALA1 (REFS 85,86,142), is increased. VRN1 promotes inflorescence development and represses transcription of *VRN2*, which encodes a protein containing a zinc finger motif and a CCT domain[83]. VRN2 blocks expression under long days of at least one of the FT-like genes in these species (namely, *FT1*), and its expression is repressed during the winter by vernalization via VRN1. Exposure to short days also represses *VRN2*, allowing *FT1* expression, which promotes flowering in summer. **d** | In sugar beet, *BTC1* regulates both photoperiod and vernalization signals. In annuals, the dominant *BTC1* allele promotes flowering under long days by repressing *FT1* and activating *FT2*. Biennial sugar beet carries a recessive *btc1* allele, which does not block expression of the floral repressor *FT1*. *btc1* becomes active enough to repress *FT1* and to induce *FT2*, thereby promoting floral initiation but only after experiencing low temperatures of winter.

14-3-3 proteins
A family of acidic proteins present in all eukaryotes and involved in a wide range of biological processes. 14-3-3 proteins directly interact with many other proteins containing phospho-serine and phospho-threonine residues and thereby affect their activity. Interestingly, 14-3-3 proteins can mediate the nuclear-cytoplasmic shuttling of some of their targets.

B-type response regulator
Transcription factors that contain an amino-terminal receiver domain and a long carboxy-terminal extension with a MYB-like DNA-binding domain. In *Arabidopsis thaliana*, these genes participate in the two-component cytokinin signal transduction pathway.

suggest that although the role of FT-like genes appears to be highly conserved, the genes controlling their transcription in response to photoperiod vary during evolution, allowing transcription of FT-like genes in response to different day lengths.

Furthermore, recently FT-like genes were shown to control seasonal developmental responses other than flowering. In Andean varieties of potato, tuberization is induced under short days. This response is controlled by transport of the FT-like protein SP6A, which is only expressed under short days, from the leaves to the roots[51]. By contrast, the protein encoded by the paralogous gene *SP3D* promotes flowering and is expressed under all day lengths, contrasting day-neutral flowering with short-day-dependent tuberization[50].

Natural genetic variation in photoperiodic flowering. Variation for day-length responses within species has been described in *A. thaliana* and crop cultivars. *A. thaliana* accessions vary strongly in their responses to photoperiod[56]. Natural variation in photoperiod response is often associated with earlier flowering under short days, thereby reducing the difference in flowering time between long days and short days (recently reviewed in REF. 57). The importance of the variation in photoperiod response in the adaptation of *A. thaliana* to different environments remains unclear; however, related variation has been important in the domestication and optimization of crop plants. For example, in barley, the *Ppd-H1* gene promotes early flowering in response to long days and encodes a pseudo-response regulator protein containing a CCT domain[58]. Cultivation of barley originated in the Middle East, and accessions in this region tend to carry active *Ppd-H1* alleles, causing flowering to occur rapidly in spring in response to long days and before summer drought. However, barley crop varieties used in northern Europe mainly carry mutant *ppd-H1* alleles that reduce the response to long days, thereby allowing the plant to grow throughout the milder summers of this region and to generate higher seed yields. Similar reduction of photoperiodic response has been associated with the domestication of other plants originating at lower latitudes, such as rice and maize[2,59,60].

Most analyses of genetic variation for flowering time in *A. thaliana* accessions were carried out under controlled environmental conditions in growth chambers. However, plants are exposed to more complex environmental conditions in nature, such as simultaneous variation in temperature and day length, gradual changes in day length during the course of the seasons, as well as differences in light intensity and humidity. Experiments carried out with *A. thaliana* under field conditions demonstrated that the flowering time of particular mutants depended strongly on the time of germination, which in turn determined the environmental conditions to which the plants would be exposed[61,62]. More recently, *A. thaliana* accessions were used for such field experiments, and this was combined with the application of genome-wide association mapping, which allowed high-resolution linkage mapping of alleles that influence flowering time under such conditions[63]. One such experiment suggested that *TSF* contributes more strongly to natural variation in flowering time than could be predicted from experiments under controlled growth conditions[64], although this correlation detected by genome-wide association still requires confirmation by other means.

Flowering in response to winter temperatures

Seasonal flowering is also conferred by exposure to low winter temperatures, a response called vernalization. Exposure to cold for several weeks is typically required for a full vernalization response, which is in contrast to the induction of flowering by day length, in which exposure to a few days of appropriate duration is sufficient to induce flowering. Plants that require vernalization to flower encode repressors that block flowering during summer or autumn until the plant is exposed to low winter temperatures. Exposure to cold then overcomes the block on flowering often by reducing expression of the repressor and thereby allowing flowering to proceed the following spring. The genetics of this system was initially studied by comparing varieties of *A. thaliana* that differ in their requirement for vernalization (reviewed in REF. 65). This approach has identified important components and defined a regulatory framework for the vernalization response, but the mechanism by which the plant senses low temperature remains unclear.

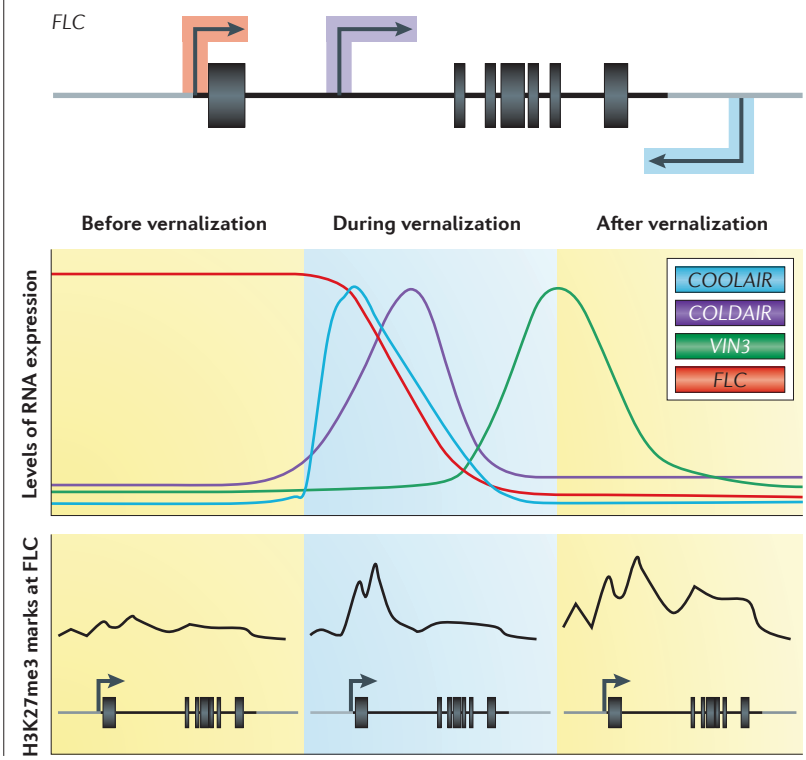
***Vernalization inhibits transcription of a flowering repressor in* A. thaliana.** *A. thaliana* accessions are usually classified into two major types, summer annuals and winter annuals, on the basis of their flowering responses under laboratory conditions and their genotypes. Summer annuals flower rapidly when grown under long days, and the whole life cycle takes only a few weeks. By contrast, winter annuals can grow for months under long days without flowering. However, if these are grown at a low temperature (4 °C) for 6 to 12 weeks, they will flower rapidly when returned to long days, as these are conditions that mimic the transition from winter to spring. Although the distinct flowering-time behaviours of summer and winter annuals under laboratory conditions clearly distinguishes them, it does not always accurately predict their behaviour under more complex natural conditions, in which accessions can show a normal rather than a bimodal distribution of flowering time[64]. Analysis of the genetic differences between summer and winter annuals demonstrated that winter annuals contain active alleles at two loci, *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*), whereas summer annuals carry mutations in one or both of these genes[66–71].

FRI and *FLC* have been isolated from winter annual accessions. *FRI* encodes a coiled-coil protein that promotes *FLC* transcription, probably by affecting its chromatin structure[68]. FLC is a MADS box transcription factor that directly binds to floral promoting genes and blocks their transcription, thereby acting as a repressor of flowering[66,67]. FLC acts both in the SAM and vascular tissue to bind directly to the flowering genes *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FT* and to repress their transcription[72]

Summer annuals and winter annuals
Summer-annual plants typically complete a full life cycle very rapidly during spring and summer because they do not require vernalization to induce flowering. Winter-annual plants usually live for longer because flowering is not induced until they experience vernalization in winter and then flower the following spring.

Box 3 | **Repression of FLC transcription by vernalization**

Repression of FLOWERING LOCUS C (*FLC*) transcription during vernalization correlates with the expression of non-coding RNAs at the *FLC* locus and the appearance of histone modifications on the gene. Around 10 days after the start of vernalization, the expression of the antisense non-coding RNA *COOLAIR* reaches a peak and then subsides to low levels[127]. This peak of *COOLAIR* correlates with the time that *FLC* mRNA abundance starts to fall. *COOLAIR* is expressed from a promoter encoded at the 3′ end of the *FLC* gene, and inserting this promoter at the 3′ end of GFP marker gene expressed from the heterologous *Cauliflower mosaic virus 35S* promoter causes a cold-dependent reduction in GFP mRNA. Although these experiments suggest that *COOLAIR* expression is instrumental in reducing *FLC* expression, other experiments suggest that it is not required for *FLC* silencing[128]. This discrepancy might be reconciled by *COOLAIR* acting redundantly with regulatory elements encoded elsewhere in the gene. Also, paradoxically, in warm temperatures increased expression of *COOLAIR* correlates with increased transcription of *FLC*[127,129]. Around 20 days after the start of vernalization, a second non-coding RNA, *COLDAIR*, reaches a peak in expression. This sense RNA is encoded in the first intron of *FLC* and appears to be expressed from a sequence — the vernalization response element — that was previously shown to be important for silencing *FLC* expression[130]. Recent experimental data suggest that this non-coding RNA is not required to reduce *FLC* expression during vernalization but is essential for silencing *FLC* after vernalization. Furthermore, proteins required for chromatin changes at *FLC* bind directly to *COLDAIR*, suggesting that this RNA has a function in recruiting such proteins to the *FLC* locus[130]. Specifically, the CURLY LEAF protein, which is a component of the Polycomb repressive complex 2 (PRC2) that is required to introduce histone H3 lysine 27 trimethylation (H3K27me3)[130,131] at target genes binds directly to *COLDAIR*. CURLY LEAF (CLF) and other components of PRC2 are required for stable silencing of FLC after vernalization[132]. In the first 4 weeks of vernalization, this chromatin mark increases around the transcriptional start site and the first exon of *FLC*, and later in vernalization it spreads further across the first intron. After vernalization, H3K27me3 remains enriched at the locus and spreads further across the whole coding sequence[133,134]. After *COLDAIR* and *FLC* mRNA fall in expression around 40 days after the start of vernalization, the transcription of the *VERNALIZATION INSENSITIVE 3* (*VIN3*) gene is induced. *VIN3* encodes a protein required for silencing of *FLC* that may interact with PRC2, creating a Polycomb complex that specifically silences gene expression during vernalization[130,135].



(BOXES 1,2). Thus FLC delays flowering by blocking the transcription of genes in the photoperiodic flowering pathway, therefore demonstrating a point of convergence of the photoperiodic and vernalization flowering pathways. Recent genome-wide analysis showed that in addition to these two genes, FLC binds to around 780 other genes involved in diverse processes, such as stress and growth regulator signalling[73].

Generally, MADS box transcription factors do not bind as monomers but rather as multimeric complexes with other MADS box proteins[74]. Genetic and biochemical data suggest that FLC acts together with the related MADS box protein SHORT VEGETATIVE PHASE (SVP). Genetic experiments demonstrated that FLC requires SVP to delay flowering strongly[75–77]. Furthermore, SVP and FLC physically interact, and SVP also binds to the *SOC1* and *FT* genes to repress their transcription[75,77] (BOX 1). The mechanism by which the FLC–SVP complex represses transcription remains unclear, but SVP has been shown to interact in several assays with LIKE HETEROCHROMATIN 1 (LHP1), a protein that is implicated in the repression of transcription by chromatin modifications[78]. Thus winter-annual accessions might show late flowering owing to direct binding of FLC–SVP to target genes, thereby recruiting chromatin modifiers that block transcription.

Exposure to low temperatures overcomes the block on flowering caused by the FLC–SVP complex by the reduction of *FLC* mRNA in response to low temperatures (BOX 3). This reduction of *FLC* mRNA level occurs progressively over several weeks, so that by the end of the vernalization period, *FLC* mRNA is at low levels[67]. The requirement for several weeks of cold exposure ensures that flowering does not occur after exposure to a few days of cold in autumn but only after the full duration of winter. The duration of low temperature exposure required for vernalization varies between accessions of *A. thaliana*, which is likely to be due to allelic variation at *FLC*[79].

After vernalization, when plants are returned to higher growth temperatures that mimic spring, *FLC* mRNA abundance remains low. This memory of having been exposed to low temperatures allows the plant to respond to the long days of spring without the block imposed by FLC. *FLC* repression during vernalization can therefore be considered in two phases: the initial repression of *FLC* transcription and the stabilization of this repression to keep *FLC* expression at low levels after the end of vernalization[67,80]. These phases are associated with the expression of non-coding RNAs at the *FLC* locus as well as the accumulation of chromatin marks on the gene (BOX 3).

Evolution of the vernalization response. The repression of FLC homologues during vernalization has also been described in other members of the Brassicaceae[81,82]. However, more distantly related species from different plant families appear to use distinct regulatory mechanisms to confer vernalization requirement, suggesting that these have arisen by convergent evolution. In temperate crops, such as the cereals wheat and barley, vernalization requirement is an important agronomic trait

used to extend the period of growth of crops, increasing the size of the plant and seed yields. In this way, varieties that require vernalization to flower can be sown late in summer and will not flower until spring or early summer the following year. Such vernalization-requiring varieties carry active alleles of genes that delay flowering until vernalization has occurred, and this is analogous to the effect of *FRI* and *FLC* in *A. thaliana*.

In vernalization-requiring varieties of temperate cereals, the promotion of flowering by long days is blocked until they have been exposed to winter temperatures (FIG. 2c). The interaction between the photoperiod responses and vernalization is mediated by VRN2, which is not found in *A. thaliana*. Under long days, VRN2 blocks the expression of at least one of the *FT* genes found in cereals[83,84]. Its transcription is repressed after exposure to vernalization by the MADS box transcription factor *VERNALIZATION 1* (*VRN1*) that is expressed in response to vernalization exposure[85]. *VRN2* expression is also repressed by a short-day-mediated repression by a mechanism that is still unclear[84,86] (FIG. 2c). Thus, at the onset of spring as day length is extended, transcription of FT-like genes (such as *FT1*) is activated through the photoperiodic flowering pathway and this promotes flowering[46,58,87]. Therefore, as in *A. thaliana*, the vernalization and photoperiod pathways are tightly interlinked in cereals.

Vernalization requirement in sugar beet is conferred by a third mechanism. Premature flowering of sugar beet before harvest reduces yield of the sugar-containing beets and complicates harvest. This is prevented by sowing biennial varieties (flowering of which is accelerated by vernalization) in the spring and harvesting them in the autumn before flowering. In contrast to these biennials, annual varieties of sugar beet flower without vernalization and carry dominant alleles at the *BTC1* locus that overcomes vernalization requirement[88]. Analysis of the FT-like gene family of sugar beet identified two members with contrasting expression profiles[89] (FIG. 2). In biennial sugar beet, expression of one of these genes, *FT2*, correlates with flowering and is only expressed in long days after vernalization, as described for *FT* in winter-annual accessions of *A. thaliana*. A second FT-like gene, *FT1* is expressed in long days before vernalization and is repressed after vernalization. Further analysis of these genes showed that *FT1* confers a block on flowering before vernalization, and its repression by vernalization allows *FT2* to be induced and flowering to proceed. Thus, in sugar beet the vernalization-sensitive repressor that blocks *FT2* transcription before vernalization is another FT-like gene *FT1*. In annual varieties, the *BTC1* gene both represses *FT1* and promotes *FT2* transcription, allowing flowering to proceed without vernalization[88] (FIG. 2d). The FT1 and FT2 proteins differ in three amino acids at the carboxy-terminal end of the protein — differences that are sufficient to explain the evolution of the repression function of FT1 (REF. 89). Interestingly, FT homologues that are able to repress flowering were also recently described in sunflowers[51], suggesting that this mechanism has evolved independently in different families of flowering plants.

Biennial
Flowering requires exposure to vernalization and the plant must reach a certain size before being sensitive to vernalization.

Polycarpic
A plant that flowers and produces fruit more than once during its lifetime.

Flowering control in perennials

The plants discussed above flower only once in their life cycle and are predominately annual plants that live for only one year or less. By contrast, many natural ecosystems are dominated by polycarpic perennials that flower multiple times during their lifespan and live for many years. In these perennials, patterns of seasonal flowering occur repeatedly, so that each year the plant flowers and then returns to vegetative growth. Thus, in contrast to annual plants, in which the transition to flowering ultimately leads to senescence of the plant and the end of the life cycle, such perennial plants cycle between periods of flowering and vegetative growth. The distinction between perennials and annuals has evolved many times in the flowering plants. Recently, several model genetic systems have been developed in which to study the control of seasonal flowering patterns in perennials.

***Repeated flowering in perennial relatives of* A. thaliana.** Some members of the *Arabidopsis* genus and of the Brassicaceae family are perennials. For example, *Arabidopsis lyrata* and *Arabis alpina* are diploid perennials, providing possibilities to study how the mechanisms conferring seasonal flowering in *A. thaliana* differ in closely related species to generate the perennial life cycle[81,90,91]. The seasonal responses of *A. alpina* differ in ways that are characteristic of the perennial life cycle. The reference accession *A. alpina* Pajares collected in northern Spain will only flower if it is exposed to vernalization[81]. However, plants younger than 4 weeks old do not respond to vernalization, indicating that these plants have not reached maturity and are unable to undergo the floral transition (FIG. 3). This age-dependent response to vernalization is not shown by annual *A. thaliana* but is a typical feature of perennials. The *A. alpina TERMINAL FLOWER 1* (*TFL1*) gene is required to prevent vernalization of young plants[92]. This gene is the orthologue of the *A. thaliana TFL1* gene, which acts to delay flowering (BOX 2), particularly under short days[93,94]. The lack of response of young plants to vernalization suggests that in nature such plants would not respond to vernalization during their first winter but would only flower after their second winter when they have reached maturity. Such responses may be important in ensuring that plants have acquired sufficient biomass before flowering to allow them to sustain the perennial life cycle. Interestingly, a related role for *TFL1* has been demonstrated in perennial poplars[95] and apples[96], in which reduction of *TFL1* mRNA levels also shortens the time to maturity.

Another striking feature of the life cycle of many perennials is the capacity to restrict flowering to a short episode and thereafter to return to vegetative growth. This may be important to ensure that the plant can carry out sufficient vegetative growth to sustain flowering in following years. Variation in flowering period also occurs between crop varieties of annual species, although how this is controlled has not been studied. After vernalization, perennial *A. alpina* Pajares flowers for only a few weeks before returning to vegetative growth. The *A. alpina* orthologue of *FLC*, which is called *PERPETUAL FLOWERING 1* (*PEP1*), has an essential

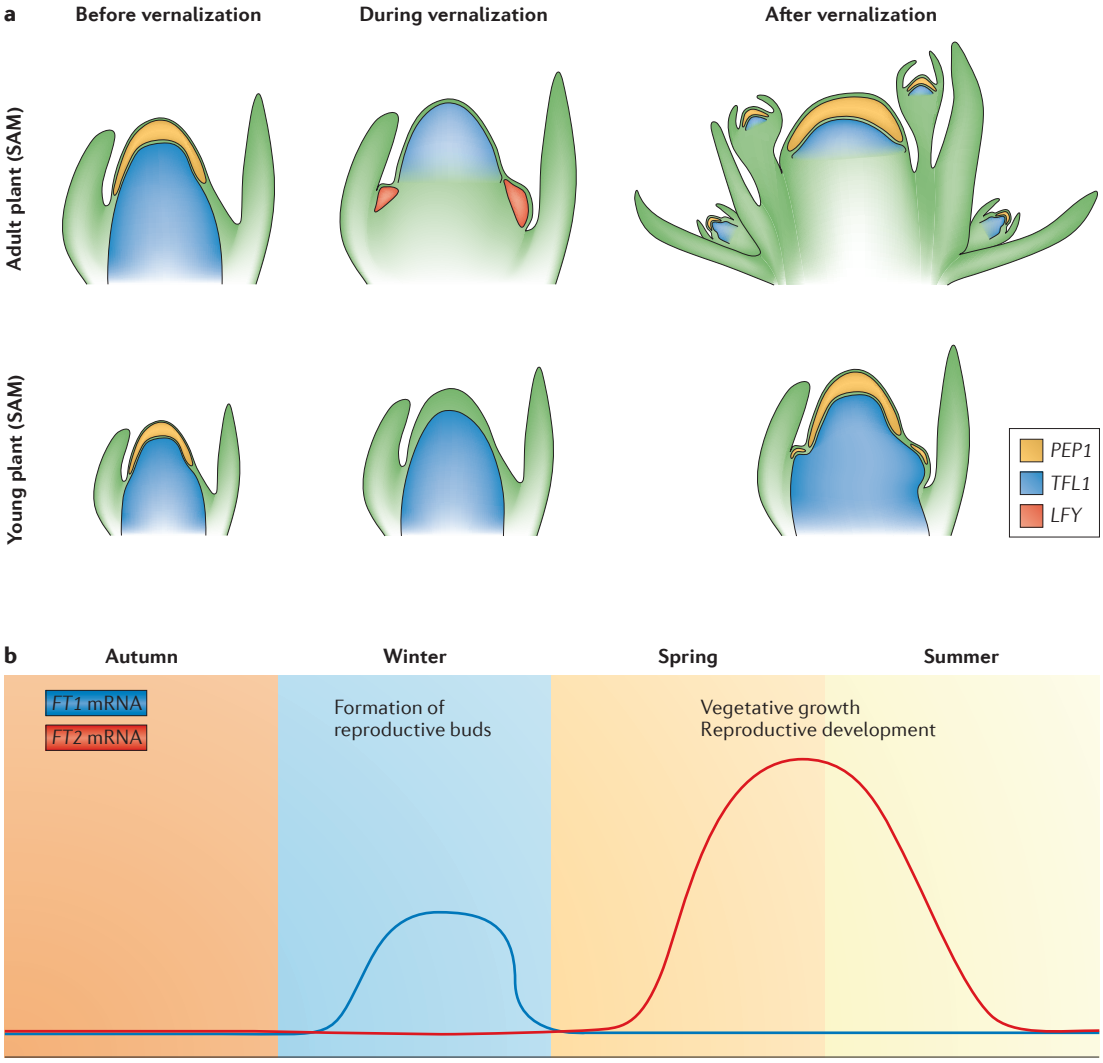


Figure 3 | **Seasonal control of flowering in perennial species. a** | Expression patterns of genes involved in the regulation of perennial flowering in *Arabis alpina*. *PERPETUAL FLOWERING 1* (*PEP1*) and *TERMINAL FLOWER 1* (*TFL1*) are expressed in the shoot apical meristem (SAM) before vernalization in adult plants that are competent to flower (upper panel) and in young plants that are not competent to flower (lower panel)[81,92]. In adult plants, the level of the *PERPETUAL FLOWERING 1* (*PEP1*) mRNA falls during vernalization. In addition, the pattern of *TFL1* mRNA changes during vernalization until it is focused only in the centre of the inflorescence meristem, and this is associated with induction of *LEAFY* (*LFY*) mRNA at the flanks of the SAM and the development of floral primordia. After vernalization, *PEP1* expression rises again in vegetative axillary meristems, blocking flowering until the following year. In young plants, vernalization also promotes reduction of *PEP1* mRNA, but in this case *TFL1* persists in expression in the meristem, blocking *LFY* activation and the floral transition. After vernalization, *PEP1* mRNA level increases again in the SAM and axillary meristems, preventing flowering of these young plants until the next year. **b** | The role of *FLOWERING LOCUS T* (*FT*) homologues in *Populus* spp. Low temperatures that are typical of winter promote *FT1* expression, which in turn triggers the formation of a limited number of reproductive buds[100]. However, warm temperatures that are typical of spring and summer suppress *FT1* transcription. In turn, warm temperature and long days induce *FT2* expression, which promotes vegetative growth.

role in restricting flowering to a short period[81]. Plants that are homozygous for *pep1* mutations flower without vernalization, as do *flc* mutants in *A. thaliana*, but they also flower continuously and do not return to vegetative growth. Therefore, *PEP1* has an additional role in the perennial life cycle by facilitating the return to vegetative growth after flowering. This correlates with a different temporal expression pattern of *PEP1* compared with that of *FLC*. The *PEP1* gene is not stably repressed by vernalization as *FLC* is (FIGS 2,3), but rather after vernalization its mRNA abundance rises again, blocking the flowering of any shoots that have not already flowered during vernalization. These shoots are then able to flower in subsequent years after exposure to vernalization conditions during following winters[81]. Similar results were obtained for the *FLC* orthologue of perennial *Arabidopsis halleri*[91]. These studies demonstrate the key role of a single locus that encodes a repressor

of flowering in conferring a perennial trait, and related observations have been made in other perennial species. For example, an inversion in *Mimulus guttatus* was associated with delayed flowering and perennialism[97], whereas in *Fragaria vesca* (wild strawberries) the *TFL1* homologue is proposed to have a similar role to *PEP1* in *A. alpina* by restricting flowering to a short seasonal episode[98].

Perennial flowering in trees. Long-lived trees are extreme and familiar examples of the perennial life cycle. Poplar species have emerged as molecular-genetic models for seasonal control of flowering in trees. In these plants, a single-shoot meristem cycles from vegetative growth to flowering and back to vegetative growth[99]. Nevertheless, this cycle is strictly seasonal. During winter, when plants are exposed to low temperatures, reproduction is initiated, whereas the floral buds are formed and open in spring. By contrast, during summer, when the plants are exposed to long days and warm temperatures, vegetative growth occurs[100]. This synchronization of flowering and vegetative growth with the changing seasons is similar to that observed in *A. alpina*[81,92]. However, *FLC* homologues do not exist in poplar species, and these periods of flowering and vegetative growth are conferred by different FT-like genes (FIG. 3b). *FT1* mRNA rises in response to low temperatures of winter and promotes reproduction. By contrast, *FT2* mRNA is induced by exposure to long days and warm temperatures of spring and early summer and promotes growth[100]. Thus, these two FT-like gene paralogues are closely related to seasonal changes in meristem function in perennial poplar species. Furthermore, the growth-promoting increase of poplar *FT* mRNA levels under long days correlates with expression of poplar *CO* mRNA in the light, suggesting conservation of the *CO*–*FT* module in this photoperiodic response[101].

Conclusions

Molecular genetic analysis of seasonal patterns of flowering in diverse annual and perennial species has demonstrated some shared features. In particular, vernalization-response pathways have evolved independently in different species as repressors of photoperiodic pathways until plants have been exposed to winter temperatures. Also, the activation of transcription of FT-like genes by day length is a feature of photoperiodic response with different regulatory mechanisms, ensuring that the day length that induces *FT* activation is reversed in long-day plants compared with short-day plants. Indeed, CETS proteins, particularly FT-like but also TFL1-like proteins, have important roles in all species examined, and in perennials the importance of the repressive function of TFL1-like genes appears to be increased. In addition, although FT-like genes are characteristically involved in floral promotion, they can control other seasonal responses, such as repressors of vernalization response or induction of tuberization and growth[102].

Despite this progress, mechanistic questions remain. How FT-like proteins promote flowering or other developmental processes remains unclear. Although their involvement in transcriptional regulation through interaction with bZIP domain transcription factors has robust support in *A. thaliana* and rice, this may not be sufficient to explain their activity. In both species, mutations in FT-like proteins confer strong flowering phenotypes, but mutations in the cognate bZIP-transcription-factor-encoding genes have only weak phenotypes. Similarly, the mechanisms by which the seasonal regulation of FT-like gene transcription is controlled can differ greatly even between closely related species, but only in *A. thaliana* and rice are the major players and the regulatory logic known. This is of particular interest in the repeated evolution of the differences between annual and perennial species or between short-day and long-day photoperiodic response types.

An unexpected recent observation is that seasonal responses measured in the field are not always those expected from experiments carried out under controlled growth environments[61,64,103]. Further genetic analysis under field conditions is likely to reveal more components in seasonal flowering and to indicate which are most important in nature, some of which are likely to affect responses to environmental cues not discussed in detail here, such as variation in photosynthetically active radiation or relative humidity[103]. Such analyses together with functional studies of alleles found in nature may allow us soon to predict novel combinations of alleles that would confer a desired and precise seasonal response in crop plants.

1. Lang, A. Physiology of flowering. *Annu. Rev. Plant Physiol.* **3**, 265–306 (1952).
2. Purugganan, M. D. & Fuller, D. Q. The nature of selection during plant domestication. *Nature* **457**, 843–848 (2009).
3. Garner, W. W. & Allard, H. A. Effect of the relative length of day and night and other factors of the environment of growth and reproduction in plants. *J. Agric. Res.* **18**, 553–603 (1920).
4. Koornneef, M., Hanhart, C. J. & van der Veen, J. H. A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **229**, 57–66 (1991).
5. Thomas, B. & Vince-Prue, B. *Photoperiodism in Plants* 2nd edn (Academic Press, 1997).
6. Ray, P. M. & Alexander, W. E. Photoperiodic adaptation to latitude in *Xanthium strumarium*. *Am. J. Bot.* **53**, 806 (1966).
7. Redei, G. P. Supervital mutants of *Arabidopsis*. *Genetics* **47**, 443–460 (1962).
8. Fowler, S. *et al.* GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J.* **18**, 4679–4688 (1999).
9. Kardailsky, I. *et al.* Activation tagging of the floral inducer FT. *Science* **286**, 1962–1965 (1999).
10. Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M. & Araki, T. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286**, 1960–1962 (1999).
11. Putterill, J., Robson, F., Lee, K., Simon, R. & Coupland, G. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* **80**, 847–857 (1995).
12. Imaizumi, T., Tran, H. G., Swartz, T. E., Briggs, W. R. & Kay, S. A. FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. *Nature* **426**, 302–306 (2003).
13. Knott, J. E. Effect of a localized photoperiod on spinach. *Proc. Am. Soc. Hortic. Sci.* **31**, 152–154 (1934).
14. Winter, D. *et al.* An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. *PLoS ONE* **2**, e718 (2007).
15. Nelson, D. C., Lasswell, J., Rogg, L. E., Cohen, M. A. & Bartel, B. FKF1, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* **101**, 331–340 (2000).
16. Suarez-Lopez, P. *et al.* CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* **410**, 1116–1120 (2001). **This study, together with references 12 and 136, provided evidence for how transcriptional and post-translational regulation of CO could combine to provide a photoperiodic flowering response.**
17. Tiwari, S. B. *et al.* The flowering time regulator CONSTANS is recruited to the FLOWERING LOCUS T promoter via a unique *cis*-element. *New Phytol.* **187**, 57–66 (2010).

18. Baudry, A. *et al.* F-box proteins FKF1 and LKP2 act in concert with ZEITLUPE to control *Arabidopsis* clock progression. *Plant Cell* **22**, 606–622 (2010).
19. de Montaigu, A., Toth, R. & Coupland, G. Plant development goes like clockwork. *Trends Genet.* **26**, 296–306 (2010).
20. Sawa, M., Nusinow, D. A., Kay, S. A. & Imaizumi, T. FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. *Science* **318**, 261–265 (2007).
The authors demonstrate how blue-light induced formation of the complex between FKF1 and GI, and along with reference 136 they show how this affects *CO* transcription. This complex was later also proposed to affect FT directly (see reference 26).
21. Jang, S. *et al. Arabidopsis* COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. *EMBO J.* **27**, 1277–1288 (2008).
22. Liu, L. J. *et al.* COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in *Arabidopsis*. *Plant Cell* **20**, 292–306 (2008).
23. Laubinger, S. *et al. Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* **133**, 3213–3222 (2006).
24. Briggs, W. R. & Olney, M. A. Photoreceptors in plant photomorphogenesis to date. Five phytochromes, two cryptochromes, one phototropin, and one superchrome. *Plant Physiol.* **125**, 85–88 (2001).
25. Zuo, Z., Liu, H., Liu, B., Liu, X. & Lin, C. Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in Arabidopsis. *Curr. Biol.* **21**, 841–847 (2011).
26. Song, Y. H., Smith, R. W., To, B. J., Millar, A. J. & Imaizumi, T. FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. *Science* **336**, 1045–1049 (2012).
27. Lazaro, A., Valverde, F., Pineiro, M. & Jarillo, J. A. The *Arabidopsis* E3 ubiquitin ligase HOS1 negatively regulates CONSTANS abundance in the photoperiodic control of flowering. *Plant Cell* **24**, 982–999 (2012).
28. Pittendrigh, C. S. The entrainment of circadian oscillations by skeleton photoperiods. *Science* **144**, 565 (1964).
29. Zeevaart, J. A. Florigen coming of age after 70 years. *Plant Cell* **18**, 1783–1789 (2006).
30. Pnueli, L. *et al.* Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. *Plant Cell* **13**, 2687–2702 (2001).
31. Zeng, L., Imamoto, A. & Rosner, M. R. Raf kinase inhibitory protein (RKIP): a physiological regulator and future therapeutic target. *Expert Opin. Ther. Targets* **12**, 1275–1287 (2008).
32. An, H. L. *et al.* CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* **131**, 3615–3626 (2004).
33. Corbesier, L. *et al.* FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* **316**, 1030–1033 (2007).
This was one of the first studies to demonstrate long-distance transport of FT protein from the leaves to the meristem and that this has a role in flowering. Related studies in rice and other plant species came to similar conclusions (see also references 35–39 and 43).
34. Jang, S., Torti, S. & Coupland, G. Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in *Arabidopsis*. *Plant J.* **60**, 614–625 (2009).
35. Mathieu, J., Warthmann, N., Kuttner, F. & Schmid, M. Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis*. *Curr. Biol.* **17**, 1055–1060 (2007).
36. Tamaki, S., Matsuo, S., Wong, H. L., Yokoi, S. & Shimamoto, K. Hd3a protein is a mobile flowering signal in rice. *Science* **316**, 1033–1036 (2007).
37. Aki, T., Shigyo, M., Nakano, R., Yoneyama, T. & Yanagisawa, S. Nano scale proteomics revealed the presence of regulatory proteins including three FT-Like proteins in phloem and xylem saps from rice. *Plant Cell Physiol.* **49**, 767–790 (2008).
38. Giavalisco, P., Kapitza, K., Kolasa, A., Buhtz, A. & Kehr, J. Towards the proteome of *Brassica napus* phloem sap. *Proteomics* **6**, 896–909 (2006).
39. Lin, M. K. *et al.* FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. *Plant Cell* **19**, 1488–1506 (2007).
40. Liu, L. *et al. FTIP1* is an essential regulator required for florigen transport. *PLoS Biol.* **10**, e1001313 (2012).
41. Li, C. *et al.* A *cis* element within flowering locus T mRNA determines its mobility and facilitates trafficking of heterologous viral RNA. *J. Virol.* **83**, 3540–3548 (2009).
42. Lu, K. J., Huang, N. C., Liu, Y. S., Lu, C. A. & Yu, T. S. Long-distance movement of *Arabidopsis FLOWERING LOCUS T* RNA participates in systemic floral regulation. *RNA Biol.* **9**, 653–662 (2012).
43. Lifschitz, E. *et al.* The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proc. Natl Acad. Sci. USA* **103**, 6398–6403 (2006).
44. Abe, M. *et al.* FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309**, 1052–1056 (2005).
45. Wigge, P. A. *et al.* Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* **309**, 1056–1059 (2005).
This paper (and references 44 and 46) provides evidence for the formation of an activation complex between FT and FD that acts in the meristem to induce flowering.
46. Taoka, K. *et al.* 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* **476**, 332–335 (2011).
47. Yan, L. *et al.* The wheat and barley vernalization gene *VRN3* is an orthologue of FT. *Proc. Natl Acad. Sci. USA* **103**, 19581–19586 (2006).
48. Hecht, V. *et al.* The pea *GIGAS* gene is a *FLOWERING LOCUS T* homolog necessary for graft-transmissible specification of flowering but not for responsiveness to photoperiod. *Plant Cell* **23**, 147–161 (2011).
49. Hayama, R., Agashe, B., Luley, E., King, R. & Coupland, G. A circadian rhythm set by dusk determines the expression of FT homologs and the short-day photoperiodic flowering response in *Pharbitis*. *Plant Cell* **19**, 2988–3000 (2007).
50. Navarro, C. *et al.* Control of flowering and storage organ formation in potato by *FLOWERING LOCUS T*. *Nature* **478**, 119–122 (2011).
Movement of FT protein is important for floral induction in many plant species. In this publication, the authors show that in potatoes, different FT-like paralogues control flowering in the shoot and tuberization in the root.
51. Blackman, B. K., Strasburg, J. L., Raduski, A. R., Michaels, S. D. & Rieseberg, L. H. The role of recently derived FT paralogs in sunflower domestication. *Curr. Biol.* **20**, 629–635 (2010).
52. Hayama, R., Yokoi, S., Tamaki, S., Yano, M. & Shimamoto, K. Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* **422**, 719–722 (2003).
In this paper, the authors show how key genes responsible for the photoperiodic control of flowering are conserved between *A. thaliana* and rice but confer a response to short rather than long days.
53. Doi, K. *et al.* Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes Dev.* **18**, 926–936 (2004).
54. Andres, F., Galbraith, D. W., Talon, M. & Domingo, C. Analysis of PHOTOPERIOD SENSITIVITY5 sheds light on the role of phytochromes in photoperiodic flowering in rice. *Plant Physiol.* **151**, 681–690 (2009).
55. Xue, W. *et al.* Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. *Nature Genet.* **40**, 761–767 (2008).
56. Giakountis, A. *et al.* Distinct patterns of genetic variation alter flowering responses of *Arabidopsis* accessions to different daylengths. *Plant Physiol.* **152**, 177–191 (2010).
57. Alonso-Blanco, C. *et al.* What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell* **21**, 1877–1896 (2009).
58. Turner, A., Beales, J., Faure, S., Dunford, R. P. & Laurie, D. A. The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. *Science* **310**, 1031–1034 (2005).
The positional cloning and characterization of a gene that is a major determinant of photoperiodic flowering response in cultivated cereals is described.
59. Takahashi, Y., Teshima, K. M., Yokoi, S., Innan, H. & Shimamoto, K. Variations in Hd1 proteins, Hd3a promoters, and Ehd1 expression levels contribute to diversity of flowering time in cultivated rice. *Proc. Natl Acad. Sci. USA* **106**, 4555–4560 (2009).
60. Buckler, E. S. *et al.* The genetic architecture of maize flowering time. *Science* **325**, 714–718 (2009).
61. Wilczek, A. M. *et al.* Effects of genetic perturbation on seasonal life history plasticity. *Science* **323**, 930–934 (2009).
The authors carried out experiments under field conditions using *A. thaliana* mutants impaired in different signalling pathways and find that depending on the germination time some mutants behave differently from expected under laboratory conditions.
62. Donohue, K. *et al.* Niche construction through germination cueing: Life-history responses to timing of germination in *Arabidopsis thaliana*. *Evolution* **59**, 771–785 (2005).
63. Nordborg, M. & Weigel, D. Next-generation genetics in plants. *Nature* **456**, 720–723 (2008).
64. Brachi, B. *et al.* Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genet.* *6* (2010).
65. Amasino, R. Seasonal and developmental timing of flowering. *Plant J.* **61**, 1001–1013 (2010).
66. Sheldon, C. C. *et al.* The FLF MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *Plant Cell* **11**, 445–458 (1999).
67. Michaels, S. D. & Amasino, R. M. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**, 949–956 (1999).
This publication is one of the first reports (see also reference 66) describing the molecular cloning of *FLC* and its regulation by vernalization.
68. Johanson, U. *et al.* Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science* **290**, 344–347 (2000).
69. Werner, J. D. *et al.* FRIGIDA-independent variation in flowering time of natural Arabidopsis thaliana accessions. *Genetics* **170**, 1197–1207 (2005).
70. Shindo, C. *et al.* Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of *Arabidopsis*. *Plant Physiol.* **138**, 1163–1173 (2005).
71. Michaels, S. D., He, Y., Scortecci, K. C. & Amasino, R. M. Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **100**, 10102–10107 (2003).
72. Searle, I. *et al.* The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. *Genes Dev.* **20**, 898–912 (2006).
73. Deng, W. *et al.* FLOWERING LOCUS C (FLC) regulates development pathways throughout the life cycle of *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **108**, 6680–6685 (2011).
74. Theissen, G. & Saedler, H. Plant biology. Floral quartets. *Nature* **409**, 469–471 (2001).
75. Fujiwara, S. *et al.* Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. *Plant Cell* **20**, 2960–2971 (2008).
76. Lee, J. H. *et al.* Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. *Genes Dev.* **21**, 397–402 (2007).
77. Li, D. *et al.* A repressor complex governs the integration of flowering signals in *Arabidopsis*. *Dev. Cell* **15**, 110–120 (2008).
This publication describes the direct interaction between FLC and another MADS box transcription factor to form a complex that represses transcription of floral pathway integrators during the vegetative phase.
78. Liu, C., Xi, W., Shen, L., Tan, C. & Yu, H. Regulation of floral patterning by flowering time genes. *Dev. Cell* **16**, 711–722 (2009).
79. Shindo, C., Lister, C., Crevillen, P., Nordborg, M. & Dean, C. Variation in the epigenetic silencing of FLC contributes to natural variation in *Arabidopsis* vernalization response. *Genes Dev.* **20**, 3079–3083 (2006).
80. Gendall, A. R., Levy, Y. Y., Wilson, A. & Dean, C. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell* **107**, 525–535 (2001).
81. Wang, R. *et al.* PEP1 regulates perennial flowering in *Arabis alpina*. *Nature* **459**, 423–427 (2009).
The authors make use of *A. alpina*, a closely related species to *A. thaliana*, to study differences in flowering regulation between perennial and annual species.
82. Lin, S. I. *et al.* Differential regulation of expression by vernalization FLOWERING LOCUS C in cabbage and *Arabidopsis*. *Plant Physiol.* **137**, 1037–1048 (2005).

83. Yan, L. *et al.* The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* **303**, 1640–1644 (2004).
This study was a breakthrough in understanding vernalization response of cereals and in demonstrating that genes not found in *A. thaliana* confer this response in cereals.
84. Dubcovsky, J. *et al.* Effect of photoperiod on the regulation of wheat vernalization genes VRN1 and VRN2. *Plant Mol. Biol.* **60**, 469–480 (2006).
85. Yan, L. *et al.* Positional cloning of the wheat vernalization gene *VRN1*. *Proc. Natl Acad. Sci. USA* **100**, 6263–6268 (2003).
86. Trevaskis, B., Hemming, M. N., Peacock, W. J. & Dennis, E. S. HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status. *Plant Physiol.* **140**, 1397–1405 (2006).
87. Campoli, C., Drosse, B., Searle, I., Coupland, G. & von Korff, M. Functional characterisation of HvCO1, the barley (*Hordeum vulgare*) flowering time ortholog of CONSTANS. *Plant J.* **69**, 868–880 (2011).
88. Pin, P. A. *et al.* The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. *Curr. Biol.* **22**, 1095–1101 (2012).
89. Pin, P. A. *et al.* An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. *Science* **330**, 1397–1400 (2010).
The authors describe the role of two homologues of *A. thaliana FT* in the control of flowering time of cultivated sugar beet. This work provides novel insights into the vernalization process with interesting applications in breeding.
90. Kuittinen, H., Niittyvuopio, A., Rinne, P. & Savolainen, O. Natural variation in *Arabidopsis lyrata* vernalization requirement conferred by a FRIGIDA indel polymorphism. *Mol. Biol. Evol.* **25**, 319–329 (2008).
91. Aikawa, S., Kobayashi, M. J., Satake, A., Shimizu, K. K. & Kudoh, H. Robust control of the seasonal expression of the *Arabidopsis FLC* gene in a fluctuating environment. *Proc. Natl Acad. Sci. USA* **107**, 11632–11637 (2010).
92. Wang, R. *et al. Aa TFL1* confers an age-dependent response to vernalization in perennial *Arabis alpina*. *Plant Cell* **23**, 1307–1321 (2011).
93. Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R. & Coen, E. Inflorescence commitment and architecture in *Arabidopsis*. *Science* **275**, 80–83 (1997).
94. Shannon, S. & Meeks-Wagner, D. R. A. Mutation in the *Arabidopsis TFL1* gene affects inflorescence meristem development. *Plant Cell* **3**, 877–892 (1991).
95. Mohamed, R. *et al. Populus* CEN/TFL1 regulates first onset of flowering, axillary meristem identity and dormancy release in *Populus*. *Plant J.* **62**, 674–688 (2010).
96. Kotoda, N., Iwanami, H., Takahashi, S. & Abe, K. Antisense expression of *MdTFL1*, a TFL1-like gene, reduces the juvenile phase in apple. *J. Am. Soc. Hortic. Sci.* **131**, 74–81 (2006).
97. Lowry, D. B. & Willis, J. H. A. Widespread chromosomal inversion polymorphisms contributes to a major life-history transition, local adaptation, and reproductive isolation. *PLoS Biol.* **8**, e1000500 (2010).
98. Iwata, H. *et al.* The *TFL1* homologue KSN is a regulator of continuous flowering in rose and strawberry. *Plant J.* **69**, 116–125 (2011).
99. Yuceer, C., Land, S. B. Jr., Kubiske, M. E. & Harkess, R. L. Shoot morphogenesis associated with flowering in *Populus deltoides* (Salicaceae). *Am. J. Bot.* **90**, 196–206 (2003).
100. Hsu, C. Y. *et al. FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. *Proc. Natl Acad. Sci. USA* **108**, 10756–10761 (2011).
101. Bohlenius, H. *et al.* CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* **312**, 1040–1043 (2006).
102. Shalit, A. *et al.* The flowering hormone florigen functions as a general systemic regulator of growth and termination. *Proc. Natl Acad. Sci. USA* **106**, 8392–8397 (2009).
103. Hancock, A. M. *et al.* Adaptation to climate across the *Arabidopsis thaliana* genome. *Science* **333**, 83–86 (2011).
104. Adrian, J. *et al. Cis*-regulatory elements and chromatin state coordinately control temporal and spatial expression of *FLOWERING LOCUS T* in *Arabidopsis*. *Plant Cell* **22**, 1425–1440 (2010).
105. Wenkel, S. *et al.* CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. *Plant Cell* **18**, 2971–2984 (2006).
106. Ben-Naim, O. *et al.* The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and *DNA*. *Plant J.* **46**, 462–476 (2006).
107. Sawa, M. & Kay, S. A. GIGANTEA directly activates FLOWERING LOCUS T in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **108**, 11698–11703 (2011).
108. Kumar, S. V. *et al.* Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature* **484**, 242–245 (2012).
109. Liu, H. T. *et al.* Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in *Arabidopsis*. *Science* **322**, 1535–1539 (2008).
110. Kotake, T., Takada, S., Nakahigashi, K., Ohto, M. & Goto, K. *Arabidopsis TERMINAL FLOWER 2* gene encodes a heterochromatin protein 1 homolog & represses both *FLOWERING LOCUS T* to regulate flowering time and several floral homeotic genes. *Plant Cell Physiol.* **44**, 555–564 (2003).
111. Turck, F. *et al. Arabidopsis* TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. *PLoS Genet.* **3**, e86 (2007).
112. Helliwell, C. A., Wood, C. C., Robertson, M., James Peacock, W. & Dennis, E. S. The *Arabidopsis* FLC protein interacts directly *in vivo* with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. *Plant J.* **46**, 183–192 (2006).
113. Castillejo, C. & Pelaz, S. The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. *Curr. Biol.* **18**, 1338–1343 (2008).
114. Mathieu, J., Yant, L. J., Murdter, F., Kuttner, F. & Schmid, M. Repression of *Flowering* by the miR172 target SMZ. *PLoS Biol.* **7**, e1000148 (2009).
115. Borner, R. *et al.* A MADS domain gene involved in the transition to flowering in *Arabidopsis*. *Plant J.* **24**, 591–599 (2000).
116. Samach, A. *et al.* Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. *Science* **288**, 1613–1616 (2000).
117. Torti, S. *et al.* Analysis of the *Arabidopsis* shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. *Plant Cell* **24**, 444–462 (2012).
118. Melzer, S. *et al.* Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. *Nature Genet.* **40**, 1489–1492 (2008).
119. Lee, J., Oh, M., Park, H. & Lee, I. SOC1 translocated to the nucleus by interaction with AGL24 directly regulates leafy. *Plant J.* **55**, 832–843 (2008).
120. Yamaguchi, A. *et al.* The microRNA-regulated SBP-box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. *Dev. Cell* **17**, 268–278 (2009).
121. Jung, J. H., Ju, Y., Seo, P. J., Lee, J. H. & Park, C. M. The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in *Arabidopsis*. *Plant J.* **69**, 577–588 (2011).
122. Wang, J. W. Czech, B. & Weigel, D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* **138**, 738–749 (2009).
This publication and reference 120 describe the role of miRNA-regulated SPL transcription factors in controlling flowering in an age-dependent manner.
123. Benlloch, R. *et al.* Integrating long-day flowering signals: a LEAFY binding site is essential for proper photoperiodic activation of APETALA1. *Plant J.* **67**, 1094–1102 (2011).
124. Shannon, S. & Meekswagner, D. R. Genetic interactions that regulate inflorescence development in *Arabidopsis*. *Plant Cell* **5**, 639–655 (1993).
125. Liljegren, S. J., Gustafson-Brown, C., Pinyopich, A., Ditta, G. S. & Yanofsky, M. F. Interactions among APETALA1, LEAFY, and TERMINAL FLOWER1 specify meristem fate. *Plant Cell* **11**, 1007–1018 (1999).
126. Hanano, S. & Goto, K. *Arabidopsis*, *TERMINAL FLOWER1* is involved in the regulation of flowering time and inflorescence development through transcriptional repression. *Plant Cell* **23**, 3172–3184 (2011).
127. Swiezewski, S., Liu, F. Q., Magusin, A. & Dean, C. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* **462**, 799–802 (2009).
These authors, together with those of reference 130, show that cold-induced non-coding transcripts at *FLC* are expressed before the epigenetic silencing of the gene.
128. Helliwell, C. A., Robertson, M., Finnegan, E. J., Buzas, D. M. & Dennis, E. S. Vernalization-repression of *Arabidopsis* FLC requires promoter sequences but not antisense transcripts. *PLoS ONE* **6**, e21513 (2011).
129. Hornyik, C., Terzi, L. C. & Simpson, G. G. The Spen Family Protein, F. P. A. Controls alternative cleavage and polyadenylation of RNA. *Dev. Cell* **18**, 203–213 (2010).
130. Heo, J. B. & Sung, S. Vernalization-mediated epigenetic silencing by a long intronic noncoding *RNA*. *Science* **331**, 76–79 (2011).
131. Goodrich, J. *et al.* A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature* **386**, 44–51 (1997).
132. De Lucia, F., Crevillen, P., Jones, A. M., Greb, T. & Dean, C. A. PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. *Proc. Natl Acad. Sci. USA* **105**, 16831–16836 (2008).
133. Finnegan, E. J. & Dennis, E. S. Vernal ization-induced trimethylation of histone H3 lysine 27 at FLC is not maintained in mitotically quiescent cells. *Curr. Biol.* **17**, 1978–1983 (2007).
134. Angel, A., Song, J., Dean, C. & Howard, M. A. Polycomb-based switch underlying quantitative epigenetic memory. *Nature* **476**, 105–108 (2011).
135. Sung, S. & Amasino, R. M. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* **427**, 159–164 (2004).
136. Fornara, F. *et al.* Arabidopsis DOF transcription factors act redundantly to reduce CONSTANS expression and are essential for a photoperiodic flowering response. *Dev. Cell* **17**, 75–86 (2009).
137. Valverde, F. *et al.* Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* **303**, 1003–1006 (2004).
138. Yang, H. Q. *et al.* The C termini of *Arabidopsis* cryptochromes mediate a constitutive light response. *Cell* **103**, 815–827 (2000).
139. Wang, H. Y., Ma, L. G., Li, J. M., Zhao, H. Y. & Deng, X. W. Direct interaction of *Arabidopsis* cryptochromes with COP1 in light control development. *Science* **294**, 154–158 (2001).
140. Itoh, H., Nonoue, Y., Yano, M. & Izawa, T. A pair of floral regulators sets critical day length for Hd3a florigen expression in rice. *Nature Genet.* **42**, 635–U115 (2010).
141. Osugi, A., Itoh, H., Ikeda-Kawakatsu, K., Takano, M. & Izawa, T. Molecular dissection of the roles of phytochrome in photoperiodic flowering in rice. *Plant Physiol.* **157**, 1128–1137 (2011).
142. Trevaskis, B., Bagnall, D. J., Ellis, M. H., Peacock, W. J. & Dennis, E. S. MADS box genes control vernalization-induced flowering in cereals. *Proc. Natl Acad. Sci. USA* **100**, 13099–13104 (2003).

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