Winter Memory throughout the Plant Kingdom: Different Paths to Flowering[1][OPEN]

Frédéric Bouché, Daniel P. Woods, and Richard M. Amasino*

Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706 (F.B., D.P.W., R.M.A.); and United States Department of Energy Great Lakes Bioenergy Research Center, Madison, Wisconsin 53726 (D.P.W., R.M.A.)

ORCID IDs: 0000-0002-8017-0071 (F.B.); 0000-0002-1498-5707 (D.P.W.); 0000-0003-3068-5402 (R.M.A.).

Plants have evolved a variety of mechanisms to synchronize flowering with their environment to optimize reproductive success. Many species flower in spring when the photoperiod increases and the ambient temperatures become warmer. Winter annuals and biennials have evolved repression mechanisms that prevent the transition to reproductive development in the fall. These repressive processes can be overcome by the prolonged cold of winter through a process known as vernalization. The memory of the past winter is sometimes stored by epigenetic chromatin remodeling processes that provide competence to flower, and plants usually require additional inductive signals to flower in spring. The requirement for vernalization is widespread within groups of plants adapted to temperate climates; however, the genetic and biochemical frameworks controlling the response are distinct in different groups of plants, suggesting independent evolutionary origins. Here, we compare and contrast the vernalization pathways in different families of plants.

The timing of flowering is an important adaptive trait that often involves integrating multiple environmental cues to ensure reproductive success. In many species, the perception of daylength (photoperiod) is an essential environment cue as it provides reliable information about seasonal shifts (e.g. Song et al., 2015; Shim et al., 2017). In Arabidopsis (*Arabidopsis thaliana*), the so-called photoperiodic pathway is coupled to the sensing of ambient temperatures as warmer growth conditions accelerate flowering (Balasubramanian et al., 2006; Verhage et al., 2014). The perception of these environmental signals is superimposed on an internal developmental program that prevents flowering in young seedlings and promotes the transition to reproductive development in older plants (e.g. Yu et al., 2015). In many species adapted to temperate climates, the perception of seasonal changes also involves the acquisition of the competence to flower in response to an extended cold period, a process referred to as vernalization (e.g. Chouard, 1960; Preston and Sandve, 2013; Fig. 1A). In addition, some species acquire floral competence when exposed to the shorter photoperiod of winter (Purvis and Gregory, 1937; Wellensiek, 1985), but the molecular mechanisms controlling the so-called "short-day vernalization" are still unknown. Vernalization is adaptive in that it ensures that flowering does not occur before the freezing temperatures of winter, which would reduce reproductive success. After vernalization, however, many plants still require subsequent exposure to additional inductive signals to initiate reproductive development (e.g. Amasino, 2010).

Whether vernalization is required for flowering as well as the duration of cold exposure required to fulfill the vernalization requirement varies considerably among species and even within a species (Amasino, 2010; Duncan et al., 2015). Genotypes with a vernalization requirement are typically referred to as either winter annuals or biennials. There is not a sharp distinction between winter annuals and biennials, but the difference often relates to the extent to which the plant

ADVANCES

- Recent advances highlight the existence of distinct vernalization pathways throughout the plant kingdom, although many more species with a vernalization requirement remain largely unexplored.
- The acquisition of winter memory results from independent evolutionary events, suggesting its important role in the adaptation of growth habits to fit new environments.
- The lack of winter memory appears to be an essential factor conferring a perennial behavior to the Brassicaceae species A. alpina and C. flexuosa.

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* Address correspondence to amasino@biochem.wisc.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Richard M. Amasino (amasino@biochem.wisc.edu).

F.B., D.P.W., and R.M.A. wrote the article.

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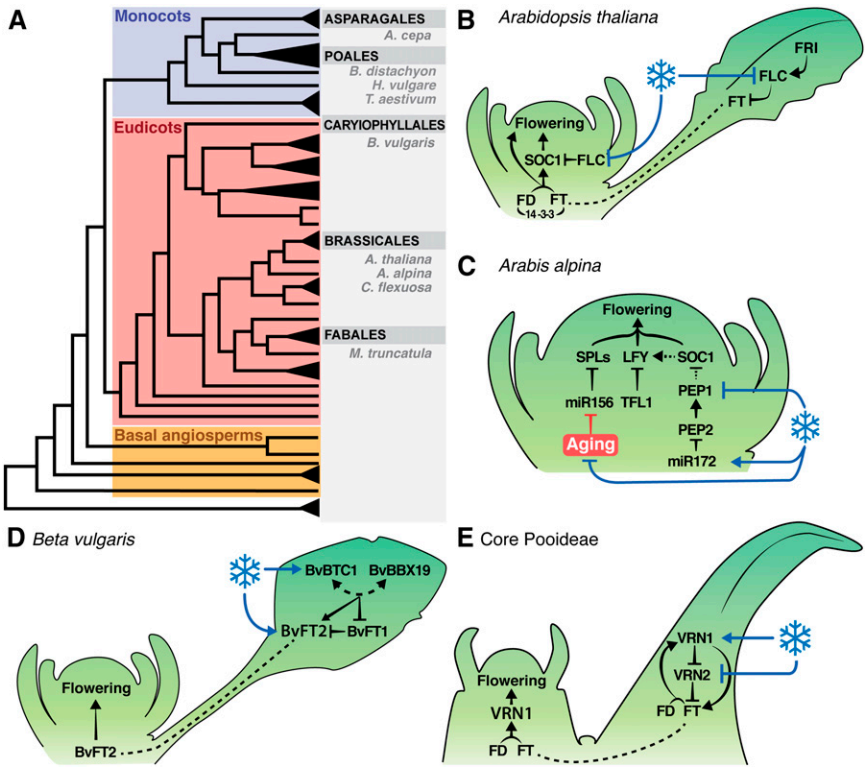

Figure 1. Vernalization pathways in different plant groups. A, Phylogenetic tree of angiosperms (data from Jansen et al. [2007]) showing, on the right, the vernalization-responsive species mentioned in the main text. B to E, Schematic representation of the mechanisms governing vernalization in Arabidopsis (B), *A. alpina* (C), sugar beet (D), and core Pooideae (E).

develops before winter and/or whether there is an obligate requirement for cold exposure to flower (e.g. Salisbury and Ross, 1992). When there is variation within a species for a vernalization requirement, the vernalization-requiring genotypes are often classified as winter annuals (or winter varieties), whereas the genotypes without vernalization requirement are often referred to as spring annuals or spring varieties because they will readily flower when planted after winter in the spring. These behavioral differences have a considerable influence on agricultural practices and are key to the adaptation of plant varieties to distinct climates. Here, we review the recent progress made in our understanding of the molecular mechanisms controlling vernalization with a focus on three different plant groups, Brassicales, Caryophyllales, and Poales.

THE MEMORY OF WINTER IN ARABIDOPSIS

The first insights into the molecular mechanisms controlling flowering were obtained in the model Brassicaceae, Arabidopsis (e.g. Pajoro et al., 2014). We know from studies in this model that the timing of flowering is a complex process that involves many genes in networks coordinating the initiation of flowering with environmental cues and developmental programs (e.g. Bouché et al., 2016). An essential downstream step of floral induction involves the up-regulation of *FLOWERING LOCUS T* (*FT*), a gene that encodes a small protein with similarity to phosphatidylethanolamine-binding proteins (Kobayashi et al., 1999). The FT protein, traditionally called "florigen," is produced in leaf vascular tissues and moves through the phloem to the shoot apical meristem (SAM), where it interacts with the bZIP transcription factor FD and 14-3-3 proteins (Fig. 1B; Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007; Taoka et al., 2011; Ho and Weigel, 2014). Together, these proteins form a floral activator complex that triggers the expression of several downstream targets, including *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), resulting in switching the fate of the SAM from initiating leaves to the production of flowers (Moon et al., 2005; Yoo et al., 2005). Pathways controlling flowering, including the vernalization pathway in Arabidopsis, act primarily through the modulation of the activity of the floral integrators *FT* and *SOC1*.

In Arabidopsis, natural diversity of the vernalization requirement is largely due to allelic variation at *FRIGIDA* (*FRI*) and its downstream target *FLOWERING LOCUS C* (*FLC*); winter accessions bear dominant (i.e. active) alleles of both genes (Michaels and Amasino, 1999; Sheldon et al., 1999; Johanson et al., 2000; Gazzani et al., 2003). FRI is part of a complex involved in activating *FLC*, and *FLC* encodes a MADS-box protein that represses flowering by preventing the transcription of *FT* in leaves and *SOC1* in the SAM (Michaels and Amasino, 1999; Sheldon et al., 2000; Hepworth et al., 2002; Helliwell et al., 2006; Searle et al., 2006). Thus, FLC repression of both leaf and meristem flowering pathways ensures a tight repression of flowering prior to cold in winter accessions. Upon cold exposure, the expression of *FLC* is stably repressed, thus

conferring a molecular "memory" of the past winter (e.g. Amasino, 2010). Interestingly, the exposure to cold temperatures triggers a rapid decrease in *FLC* expression levels (i.e. within a few days), but only extended periods of cold ensure stable repression upon return to warmer growth temperatures (e.g. Finnegan, 2015). Although *FLC* repression is maintained throughout the plant's life cycle, the repressed state of *FLC* is reset to an active state in the following generation, resulting in the re-establishment of the vernalization requirement (e.g. Schmitz and Amasino, 2007).

How cold represses *FLC* has been a long-standing question on which many studies have been focused. These studies have revealed multiple components of cold-mediated repression, including epigenetic modifications and antisense transcription (e.g. Kim and Sung, 2014), but the mechanisms controlling the initial decrease in *FLC* levels are not yet fully understood (Helliwell et al., 2015). The first vernalization-related, cold-induced change identified to date is the peak of expression of antisense *FLC* transcripts, collectively called COOLAIR, which are conserved in Arabidopsis relatives (Swiezewski et al., 2009; Castaings et al., 2014; Marquardt et al., 2014). The experimental reduction of COOLAIR expression prevents the vernalization-induced decrease in some activating chromatin marks at the *FLC* locus (Csorba et al., 2014), whereas the disruption of its promoter by T-DNA does not prevent the overall repression of *FLC* by vernalization (Helliwell et al., 2011). The peak of COOLAIR is followed by the increase in the expression of a sense noncoding RNA originating from the first intron of *FLC*, called COLDAIR (Heo and Sung, 2011). Although COLDAIR appears to be less evolutionary conserved than COOLAIR in Arabidopsis relatives (Castaings et al., 2014), the knock-down of its expression results in an increase of *FLC* expression associated with late flowering and reduced vernalization response (Heo and Sung, 2011). Following the expression of these noncoding RNAs, a key event is the cold-mediated induction of the gene encoding VERNALIZATION-INSENSITIVE3 (Sung and Amasino, 2004). This protein, which is necessary for the deposition of H3K27me3 repressive marks at the *FLC* locus, participates in the stable repression of *FLC* by the polycomb remodeling complex PRC2, as extensively reviewed elsewhere (e.g. Kim and Sung, 2014; Berry and Dean, 2015; Hepworth and Dean, 2015). The mitotic stability of vernalization-mediated *FLC* repression, as well as the subsequent resetting in the next generation, has provided a system to explore multiple aspects of the epigenetic control of gene expression. Other mechanisms have been postulated to regulate *FLC* at a molecular level, such as alternative splicing (Mahrez et al., 2016), and possibly posttranslational protein stabilization (Kwak et al., 2016). However, the extent to which these mechanisms participate in the control of the vernalization response in natural conditions is not well understood.

The repression of *FLC* has received much attention, but the regulation of additional genes appears to also contribute to the vernalization response in Arabidopsis as the flowering time of an *flc* null mutant is still accelerated by exposure to prolonged cold temperatures (Michaels and Amasino, 2001). Some obvious candidate genes to fulfill such a role are the paralogs of *FLC*, called *FLOWERING LOCUS M* (*FLM*) and *MADS AFFECTING FLOWERING2-5* (*MAF2-5*), which also control flowering by repressing *FT* expression (Gu et al., 2013). Although initial studies reported somewhat contradictory results (Ratcliffe et al., 2001, 2003; Sung et al., 2006; Sheldon et al., 2009), the thorough characterization of the expression of the *FLC* family genes showed that they all respond to vernalizing treatments, albeit with different kinetics (Kim and Sung, 2013): *FLC* expression decreases rapidly upon cold exposure, whereas *FLM* and *MAF2-3* expression only decreases after the cold period has ended, and *MAF4-5* expression peaks during cold. The role of *FLM* and *MAF2* in the vernalization response seems to be marginal (Kim and Sung, 2013); instead, these genes appear to be key to the repression of flowering at low ambient temperatures (Posé et al., 2013; Lee et al., 2013a; Rosloski et al., 2013; Airoldi et al., 2015; Sureshkumar et al., 2016). The *maf3* single mutant does not show any phenotype, but the *maf4* and *maf5* single mutants are induced to flower by shorter cold periods, suggesting that *MAF4* and *MAF5* normally ensure that vernalization is not achieved by suboptimal durations of cold exposure (Kim and Sung, 2013). AGL19, another MADS-box protein closely related to SOC1, might also play a role in the vernalization pathway, as the *agl19* and *flc* mutations show additive impairment of the vernalization response (Schönrock et al., 2006). *AGL19* appears to be a floral activator up-regulated upon cold exposure through *FLC*-independent processes (Schönrock et al., 2006; Kang et al., 2015). Although further work is needed to assess the role of these additional components in the vernalization response, a recent study suggests that they might participate in environmental adaptation as their differential regulation is correlated with the flowering time of different accessions originating from an altitudinal gradient (Suter et al., 2014). In conclusion, there is still much to learn about *FLC*-independent vernalization events in Arabidopsis.

VERNALIZATION IN PERENNIAL BRASSICACEAE

In contrast to the annual habit of Arabidopsis, perennials live for many years and flower repeatedly throughout their lives. Critical to this life history strategy is that not all meristems are converted to inflorescences because some meristems must be reserved for next season's growth. Indeed, the perennial life history of *Arabis alpina*, a close relative of Arabidopsis in the Brassicaceae, relies on the transient floral competence to ensure that not all meristems flower in a growing season. Some meristems undergo the floral transition in spring, while others remain vegetative to resume growth the following year. In *A. alpina*, the repression of flowering prior to vernalization is mediated by an *FLC*

ortholog called *PERPETUAL FLOWERING1* (*PEP1*), and repression of *PEP1* during vernalization leads to increased expression of *SOC1* and *LEAFY*, two essential promoters of flowering (Fig. 1C; Wang et al., 2009b, 2011a). As the gene name suggests, *pep1* mutants flower rapidly without vernalization, and allelic variation at *PEP1* contributes to the natural variation in flowering responses that exists among different accessions of *A. alpina* (Wang et al., 2009b; Albani et al., 2012). Unlike *FLC* in Arabidopsis, the expression of *PEP1* is only transiently repressed by cold, and the meristems that transition to flowering during cold become inflorescences, whereas meristems at an immature stage remain vegetative (Wang et al., 2009b). Although *FLC* and *PEP1* share several regulatory mechanisms, including chromatin remodeling and antisense transcription (Wang et al., 2009b; Castaings et al., 2014), the repressive H3K27me3 marks at *PEP1* return to their original levels a few weeks after the end of the cold period, correlating with its transient repression (Wang et al., 2009b). Although it is not surprising that *FLC* and orthologs such as *PEP1* are the basis of vernalization requirement in Brassicaceae, this difference in memory at the *AtFLC* locus versus lack of memory at the *PEP1* locus is likely to be crucial for the perennial nature of *A. alpina* versus the annual habit of Arabidopsis.

The ability of a meristem to transition to flowering is also controlled by an age-dependent pathway. In the early stages of Arabidopsis development, the age-regulated miR156 promotes juvenility and represses flowering by posttranscriptionally down-regulating the expression of genes from the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*s) family, which are positive regulators of flowering (Cardon et al., 1997). As plants age, miR156 levels decrease, leading to an up-regulation of SPLs, which in turn induce miR172 expression; the levels of miR172 are thus negatively correlated with those of miR156 (Wu and Poethig, 2006; Wang et al., 2009a). Although this balance is conserved in many species, including rice, maize, and poplar (Chuck et al., 2007; Wang et al., 2011b; Xie et al., 2012), the levels of miR156 and miR172 are uncoupled in *A. alpina*, and exposure to cold temperatures triggers the expression of miR172 independently of miR156 (Bergonzi et al., 2013; Fig. 1C). *PEP2*, also identified through the screening for perpetual-flowering mutants, encodes a miR172-regulated APETALA2-like transcription factor that positively regulates *PEP1* expression, thus contributing to the maintenance of the vegetative stage (Bergonzi et al., 2013). The cold-mediated induction of miR172 thus leads to the repression of PEP2 and, concomitantly, a decrease of PEP1 levels. In young meristems, however, high levels of miR156 block flowering, even in the absence of a PEP1-repressing effect. As observed in Arabidopsis, miR156 levels decrease as meristems age (Bergonzi et al., 2013), and only meristems with low miR156 levels can be induced to flower by prolonged exposure to cold temperatures. Interestingly, the decline in miR156 is blocked by cold, ensuring that flowering occurs only in plants that had previously grown rapidly under warmer ambient temperatures. In addition, *TERMINAL FLOWER1* (*TFL1*), a paralog of *FT*, represses flowering in immature meristems (Kobayashi et al., 1999; Wang et al., 2011a). The experimental down-regulation of *AaTFL1* allows the vernalization-mediated floral induction of younger meristems and a response to shorter periods of cold exposure (Wang et al., 2011a). Such a role for TFL1 in the repression of flowering in young meristems has been observed in other perennial species, such as apple trees (Kotoda et al., 2006). TFL1 appears to set a minimal threshold of inductive signal that is required to trigger flowering, and participates in the selective induction of flowering in mature meristems only.

In another perennial Brassicaceae, *Cardamine flexuosa*, the mechanisms involved in the age-dependent ability to become vernalized appear to be less complex. As in Arabidopsis, the balance between miR156 and miR172 is maintained, and the age-driven decrease of miR156 is associated with a concomitant increase of miR172. In *C. flexuosa*, the prevention of flowering in young nonvernalized plants is ensured by two potent floral repressors, *FLC* and *TARGET OF EAT1* (*TOE1*), an *APETALA2-like* transcription factor that is posttranscriptionally regulated by miR172 (Zhou et al., 2013). As the plants age, miR172 levels increase to repress *TOE1*, conferring to the meristem the competence to flower when exposed to a vernalizing treatment that transiently represses *FLC* expression (Zhou et al., 2013). Hence, distinct mechanisms using a similar framework evolved to confer perennial behavior in Brassicaceae.

VERNALIZATION SYSTEMS IN OTHER EUDICOTS

The key regulators of the vernalization pathway appear to be conserved within Brassicaceae; however, there is no strong evidence that similar components are involved in the vernalization systems of other eudicot families. Although the heterologous expression of *FLC*-like genes from different families is able to repress flowering in Arabidopsis *flc* mutants, their functional relevance in the control of vernalization response in their respective species is not clear (e.g. Reeves et al., 2007; Périlleux et al., 2013). Moreover, in *Medicago truncatula*, a legume with a vernalization response that diverged 90 to 100 million years ago from Arabidopsis (Zeng et al., 2014), neither *FRI* nor *FLC* orthologs have been identified (Hecht et al., 2005). However, a mutation of *MtVERNALIZATION2* (*MtVRN2*), a member of the Polycomb Group Repressive Complex (VRN2-PRC2) responsible for the deposition of H3K27me3 repressive marks at the *FLC* locus in Arabidopsis (De Lucia et al., 2008), bypasses the vernalization requirement and leads to early flowering (Jaudal et al., 2016). Under long-day conditions, this phenotype requires a functional allele of the florigen *FTa1* (Jaudal et al., 2016). Interestingly, *FTa1* is up-regulated in the *Mtvrn2* mutant, but does not display H3K27me3 alterations, suggesting that MtVRN2 represses genes upstream of *FTa1* (Jaudal et al., 2016).

VRN2 thus plays distinct roles in the control of flowering in different plant groups, acting as an essential component of the cold-mediated acquisition of the competence to flower in Arabidopsis and participating in the establishment of the vernalization requirement in *M. truncatula*.

To date, the best characterized vernalization system in a eudicot family other than Brassicaceae comes from sugar beet (*Beta vulgaris*), a Caryophyllales species that diverged from Arabidopsis soon after the eudicot-monocot split, about 110 million years ago (Zeng et al., 2014). Cultivated sugar beet is a biennial root crop that requires vernalization to flower, but many wild relatives behave as annuals. Unlike in the Brassicaceae, the ortholog of *FLC* from sugar beet plays at best a minor role in vernalization (Vogt et al., 2014). The biennial behavior of cultivated beet is associated with a recessive allele at the bolting locus *B*. This locus encodes the pseudo-response regulator (PRR) BOLTING TIME CONTROL1 (BvBTC1), a protein related to AtPRR7 (Pin et al., 2012), and the reduction of *BvBTC1* activity by RNAi is sufficient to convert annual varieties into biennials. Recessive alleles of another bolting locus, called *B2*, confer a biennial behavior to plants homozygous for the annual allele *BTC1* (Büttner et al., 2010). This locus encodes the DOUBLE B-BOX TYPE ZINC FINGER protein BvBBX19, which is orthologous to an Arabidopsis protein that negatively influences the induction of *FT* (Dally et al., 2014; Wang et al., 2014). Interestingly, the expression of both *BvBBX19* and *BvBTC1* is diurnally regulated (Pin et al., 2012; Dally et al., 2014), and although the functional relationship between these two proteins is still unclear, they both participate in the regulation of an antagonistic pair of FT-like proteins, BvFT1 and BvFT2 (Fig. 1D; Pin et al., 2010, 2012; Dally et al., 2014). The short-day-expressed *BvFT1* represses floral transition and negatively influences *BvFT2* levels, whose expression is promoted by long days (Pin et al., 2010). In biennial beets that require vernalization, cold stably represses *BvFT1* expression possibly through BvBTC1, relieving its repressive effect on *BvFT2*; BvFT2, in turn, is able to trigger flowering if plants are exposed to long days (Pin et al., 2010, 2012). The pathway through which BvBBX19 and BvBTC1 regulate the expression of *BvFT* genes is not known. Transcriptomic studies have provided some new candidate genes possibly involved in the vernalization pathway (Mutasa-Göttgens et al., 2012), and the publication of the sugar beet genome will undoubtedly open new perspectives for the dissection of the molecular mechanisms controlling its floral induction (Dohm et al., 2014).

VERNALIZATION SYSTEMS IN MONOCOTS

Vernalization responsiveness is also common in different species of monocots (Chouard, 1960; Brewster, 1987; Preston and Sandve, 2013). A few years ago, some key components of the system governing bulb formation and floral induction in onion (*Allium cepa*), a biennial species that belongs to the Asparagales order, were identified (Lee et al., 2013b). In the model proposed by Lee et al. (2013b), the initiation of bulbing and flowering are both controlled by *FT*-like genes. After planting in spring, high AcFT4 activity inhibits bulb formation by repressing *AcFT1*. Later in the season, inductive photoperiods down-regulate *AcFT4*, allowing the induction of the bulb-promoting *AcFT1*. During winter, vernalization leads to the up-regulation of another *FT*-like gene, *AcFT2*, which is necessary to promote flowering the next summer (Lee et al., 2013b). Further experiments are required to confirm and expand this model, but these preliminary results suggest a mechanism distinct from the vernalization system in Brassicales, Caryophyllales, and, as discussed below, Pooideae.

In Pooideae (temperate grasses), a grass subfamily that includes crown pooid crops such as wheat, oats, rye, and barley, allelic variation and functional studies have advanced the understanding of the molecular basis of vernalization. As in Arabidopsis, the timing of flowering is an important trait that is tightly controlled by genetic networks that integrate environmental cues, such as photoperiod and vernalization. After the identification of *FLC* in Arabidopsis, many efforts were directed toward the identification of its ortholog in vernalization-sensitive monocots, and a recent study identified *ODDSOC2* as an ortholog in cereals (Ruelens et al., 2013). Although *ODDSOC2* expression is suppressed by cold (Greenup et al., 2010), its specific role in the vernalization response is not clear. Instead, like in sugar beet, an *FLC*-independent pathway appears to be the major contributor to the vernalization requirement and response to prolonged cold exposure in winter cereal varieties.

Our current understanding is that the core regulatory mechanisms of vernalization in cereals includes three genes called *VRN1*, *VRN2*, and *VRN3* (Yan et al., 2003, 2004, 2006). VRN3 is an ortholog of FT that interacts with an FD-like protein to trigger the expression of downstream targets, including the *AP1/FUL* ortholog *VRN1* (Li and Dubcovsky, 2008; Li et al., 2015). *VRN4*, which resulted from a duplication of *VRN1*, displays a distinct expression pattern and appears to be associated with a weaker vernalization requirement (Kippes et al., 2015). As in Arabidopsis, the control of *FT* expression in leaves is key to the acquisition of the competence to flower in response to vernalization. Prior to cold, in winter cereal varieties, *VRN1* is expressed at low levels, and the *CONSTANS*-like *VRN2* gene plays a repressive role on *FT* (Fig. 1E; Danyluk et al., 2003; Yan et al., 2004; Chen and Dubcovsky, 2012). The role of *VRN2* in conferring the vernalization requirement is supported by the fact that nonfunctional *VRN2* alleles can confer a spring habit in diploid wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), and the development of a triple *VRN2* mutant in hexaploid wheat also results in rapid flowering and a reduced vernalization response (Kippes et al., 2016). In cereals, prolonged cold temperatures cause the repression of *VRN2* and a quantitative induction of *VRN1*, which directly binds to the *FT* promoter to trigger its expression and also to *VRN2* to presumably repress its expression (Yan et al., 2004; Shimada et al., 2009; Deng et al., 2015). The induction of

VRN1 by cold is associated with removal of H3K27me3 repressive marks and deposition of H3K4me3 activating marks (Oliver et al., 2009), and also possibly involves posttranscriptional mechanisms (Xiao et al., 2014). The initial down-regulation of *VRN2* during the cold occurs independently of *VRN1*, but *VRN1* is critical for the stable repression of *VRN2* after cold exposure ends (Chen and Dubcovsky, 2012). When induced, *FT* reinforces the expression of *VRN1*, thus creating a positive feedback loop that ensures the transition to the reproductive stage (Li and Dubcovsky, 2008). Hence, vernalization results in decreased *VRN2* expression and increased *VRN1* expression, facilitating the induction of *FT* expression upon extension of the photoperiod.

The photoperiodic activation of *FT* involves the PRR PHOTOPERIOD1 (PPD1), which is responsible for a large part of the natural variation in flowering observed in wheat and barley; varieties with active PPD1 contain high levels of FT and flower early, whereas *ppd1* varieties have low FT and flower late (Turner et al., 2005; Beales et al., 2007; Kitagawa et al., 2012; Shaw et al., 2012). How PPD1 controls *FT* expression in grasses is not known, but it possibly acts through the transcriptional regulation of *CONSTANS* (Turner et al., 2005; Shaw et al., 2012). More recently, the light receptor for the photoperiodic pathway was identified as PHYTOCHROME C (PHYC); *phyC* mutants flower very late under inductive photoperiods (Chen et al., 2014; Woods et al., 2014b).

Although there has been great progress in understanding vernalization at a molecular level from studying wheat and barley, much remains to be learned, including identifying additional components within the vernalization pathway and the extent to which vernalization pathways are conserved throughout the grasses. Pooideae is a diverse grass subfamily comprising ~3,800 species with a large geographical range, predominantly in higher latitudes (Mannion, 1997; Grass Phylogeny Working Group, 2001). Recently it has been shown that vernalization responsiveness is widespread throughout Pooideae, although there have been several independent losses of a vernalization requirement (McKeown et al., 2016; Woods et al., 2016). Furthermore, studies in the small temperate grass model *Brachypodium distachyon*, which is an early diverging pooid sister to the crown pooid clade, have contributed to understanding the evolution of vernalization systems in pooids (Woods and Amasino, 2015). Like wheat and barley, *B. distachyon* is a long-day plant that exhibits an extensive natural variation of flowering behavior across accessions with respect to photoperiod and vernalization responses (Ream et al., 2014; Tyler et al., 2016) and has a "memory" of winter (Woods et al., 2014a). Additionally, *B. distachyon* contains orthologs of all of the *VRN* genes discovered in wheat and barley, and these genes also likely contribute to natural variation in flowering responses among different accessions of *B. distachyon* (Higgins et al., 2010; Woods et al., 2016, 2017; Bettgenhaeuser et al., 2017). Genetic and physiological characterizations carried out in different Pooideae species, including *B. distachyon*, confirms the conservation of *VRN1* and *VRN3/FT* as promoters and *VRN2* as a repressor of flowering (Lv et al., 2014; Ream et al., 2014; Woods et al., 2016). However, the cold- and *VRN1*-mediated repression of *VRN2* is restricted to core Pooideae, such as wheat and barley, suggesting that the establishment of the VRN1-VRN2 loop occurred late in the diversification of temperate grasses (Woods et al., 2016). In contrast, the induction of *VRN1* by cold evolved early in the diversification of Pooideae, and *VRN1* is induced following cold exposure even under noninductive conditions (McKeown et al., 2016). Hence, whereas the memory of winter in Arabidopsis is controlled by the stable repression of the floral inhibitor *FLC*, this memory appears to rely, in large part at least, on the stable activation of the floral promoter *VRN1* in Pooideae (Woods et al., 2014a).

CONCLUDING REMARKS

The exploration of the vernalization systems in different plant groups reveals distinct pathways that share a common principle: in vernalization-responsive species, there is a block to flowering (a vernalization requirement), and cold provides competence to flower (overcomes the block to flowering), whether it is via the suppression of a floral repressor (e.g. Arabidopsis) or the activation of a floral promoter (e.g. Pooideae). Moreover, the induction of flowering typically requires additional inductive signals. In most vernalization-requiring species, the transition to floral development upon perception of these signals may occur long after return to warmer growth temperatures, revealing a memory of the past winter. Other species, such as the Brassicaceae *A. alpina*, exhibit a transient response to cold temperatures, and this

OUTSTANDING QUESTIONS

- The mechanisms governing winter memory in agronomically important grasses is still largely unknown, and a better understanding of these processes might help fine-tune flowering to improve yields.
- Exploring the relationship between life habits and winter memory in different plant groups may provide insights into the molecular mechanisms governing perenniality across different species.
- How plants sense the duration of cold in order to saturate their vernalization response remains unknown. Might there be a "cold clock"? If so, the components of the cold clock may be distinct across different plant groups.
- In many grass species, the memory of winter is triggered not only by cold, but also by short days. The molecular mechanisms governing short-day vernalization and how it interacts with cold perception are still unknown and provide a yet unexplored field of investigation.

absence of winter memory is key to its perennial growth habit because only meristems that are mature at the start of the cold exposure will undergo floral transition, while the other meristems remain vegetative to maintain growth from year to year. Evolution of unique vernalization systems is key to the establishment of a species life history strategy. Further dissection of the vernalization pathways throughout angiosperm lineages is likely to uncover new mechanisms establishing the memory of winter (see "Outstanding Questions") and shed light on the role of vernalization in the diversification of plant groups.

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LITERATURE CITED

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

Airoldi CA, McKay M, Davies B (2015) *MAF2* is regulated by temperature-dependent splicing and represses flowering at low temperatures in parallel with *FLM*. PLoS One **10:** e0126516

Albani MC, Castaings L, Wötzel S, Mateos JL, Wunder J, Wang R, Reymond M, Coupland G (2012) PEP1 of *Arabis alpina* is encoded by two overlapping genes that contribute to natural genetic variation in perennial flowering. PLoS Genet **8:** e1003130

Amasino R (2010) Seasonal and developmental timing of flowering. Plant J **61:** 1001–1013

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent induction of *Arabidopsis thaliana* flowering by elevated growth temperature. PLoS Genet **2:** e106

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

Bergonzi S, Albani MC, Ver Loren van Themaat E, Nordström KJV, Wang R, Schneeberger K, Moerland PD, Coupland G (2013) Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. Science **340:** 1094–1097

Berry S, Dean C (2015) Environmental perception and epigenetic memory: mechanistic insight through *FLC*. Plant J **83:** 133–148

Bettgenhaeuser J, Corke FMK, Opanowicz M, Green P, Hernández-Pinzón I, Doonan JH, Moscou MJ (2017) Natural variation in *Brachypodium* links vernalization and flowering time loci as major flowering determinants. Plant Physiol **173:** 256–268

Bouché F, Lobet G, Tocquin P, Périlleux C (2016) FLOR-ID: an interactive database of flowering-time gene networks in *Arabidopsis thaliana*. Nucleic Acids Res (D1) **44:** D1167–D1171

Brewster JL (1987) Vernalization in the onion - a quantitative approach. *In* JG Atherton, ed, Manipulation of Flowering. Butterworths, London, pp 171–183

Büttner B, Abou-Elwafa SF, Zhang W, Jung C, Müller AE (2010) A survey of EMS-induced biennial Beta vulgaris mutants reveals a novel bolting locus which is unlinked to the bolting gene B. Theor Appl Genet **121:** 1117–1131

Cardon GH, Höhmann S, Nettesheim K, Saedler H, Huijser P (1997) Functional analysis of the *Arabidopsis thaliana* SBP-box gene *SPL3*: a novel gene involved in the floral transition. Plant J **12:** 367–377

Castaings L, Bergonzi S, Albani MC, Kemi U, Savolainen O, Coupland G (2014) Evolutionary conservation of cold-induced antisense RNAs of *FLOWERING LOCUS C* in *Arabidopsis thaliana* perennial relatives. Nat Commun **5:** 4457

Chen A, Dubcovsky J (2012) Wheat TILLING mutants show that the vernalization gene *VRN1* down-regulates the flowering repressor *VRN2* in leaves but is not essential for flowering. PLoS Genet **8:** e1003134

Chen A, Li C, Hu W, Lau MY, Lin H, Rockwell NC, Martin SS, Jernstedt JA, Lagarias JC, Dubcovsky J (2014) Phytochrome C plays a major role in the acceleration of wheat flowering under long-day photoperiod. Proc Natl Acad Sci USA **111:** 10037–10044

Chouard P (1960) Vernalization and its relations to dormancy. Annu Rev Plant Physiol **11:** 191–238

Chuck G, Cigan AM, Saeteurn K, Hake S (2007) The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. Nat Genet **39:** 544–549

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

Csorba T, Questa JI, Sun Q, Dean C (2014) Antisense COOLAIR mediates the coordinated switching of chromatin states at *FLC* during vernalization. Proc Natl Acad Sci USA **111:** 16160–16165

Dally N, Xiao K, Holtgräwe D, Jung C (2014) The *B2* flowering time locus of beet encodes a zinc finger transcription factor. Proc Natl Acad Sci USA **111:** 10365–10370

Danyluk J, Kane NA, Breton G, Limin AE, Fowler DB, Sarhan F (2003) TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. Plant Physiol **132:** 1849–1860

De Lucia F, Crevillen P, Jones AME, Greb T, Dean C (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of *FLC* during vernalization. Proc Natl Acad Sci USA **105:** 16831–16836

Deng W, Casao MC, Wang P, Sato K, Hayes PM, Finnegan EJ, Trevaskis B (2015) Direct links between the vernalization response and other key traits of cereal crops. Nat Commun **6:** 5882

Dohm JC, Minoche AE, Holtgräwe D, Capella-Gutiérrez S, Zakrzewski F, Tafer H, Rupp O, Sörensen TR, Stracke R, Reinhardt R, et al (2014) The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). Nature **505:** 546–549

Duncan S, Holm S, Questa J, Irwin J, Grant A, Dean C (2015) Seasonal shift in timing of vernalization as an adaptation to extreme winter. eLife **4:** e06620

Finnegan EJ (2015) Time-dependent stabilization of the +1 nucleosome is an early step in the transition to stable cold-induced repression of *FLC*. Plant J **84:** 875–885

Gazzani S, Gendall AR, Lister C, Dean C (2003) Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. Plant Physiol **132:** 1107–1114

Grass Phylogeny Working Group (2001) Phylogeny and subfamilial classification of the grasses (Poaceae). Ann Mo Bot Gard **88:** 373–457

Greenup AG, Sasani S, Oliver SN, Talbot MJ, Dennis ES, Hemming MN, Trevaskis B (2010) *ODDSOC2* is a MADS box floral repressor that is down-regulated by vernalization in temperate cereals. Plant Physiol **153:** 1062–1073

Gu X, Le C, Wang Y, Li Z, Jiang D, Wang Y, He Y (2013) *Arabidopsis* FLC clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. Nat Commun **4:** 1947

Hecht V, Foucher F, Ferrándiz C, Macknight R, Navarro C, Morin J, Vardy ME, Ellis N, Beltrán JP, Rameau C, et al (2005) Conservation of Arabidopsis flowering genes in model legumes. Plant Physiol **137:** 1420–1434

Helliwell CA, Anderssen RS, Robertson M, Finnegan EJ (2015) How is *FLC* repression initiated by cold? Trends Plant Sci **20:** 76–82

Helliwell CA, Robertson M, Finnegan EJ, Buzas DM, Dennis ES (2011) Vernalization-repression of Arabidopsis *FLC* requires promoter sequences but not antisense transcripts. PLoS One **6:** e21513

Helliwell CA, Wood CC, Robertson M, James Peacock W, Dennis ES (2006) 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

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science **331:** 76–79

Hepworth J, Dean C (2015) Flowering Locus C's lessons: conserved chromatin switches underpinning developmental timing and adaptation. Plant Physiol **168:** 1237–1245

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-time gene *SOC1* by CONSTANS and FLC via separate promoter motifs. EMBO J **21:** 4327–4337

Higgins JA, Bailey PC, Laurie DA (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. PLoS One **5:** e10065

Ho WWH, Weigel D (2014) Structural features determining flower-promoting activity of *Arabidopsis* FLOWERING LOCUS T. Plant Cell **26:** 552–564

Jansen RK, Cai Z, Raubeson LA, Daniell H, Depamphilis CW, Leebens-Mack J, Müller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, et al (2007)

Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc Natl Acad Sci USA **104:** 19369–19374

Jaudal M, Zhang L, Che C, Hurley DG, Thomson G, Wen J, Mysore KS, Putterill J (2016) *MtVRN2* is a Polycomb *VRN2-like* gene which represses the transition to flowering in the model legume *Medicago truncatula.* Plant J **86:** 145–160

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. Science **290:** 344–347

Kang M-J, Jin H-S, Noh Y-S, Noh B (2015) Repression of flowering under a noninductive photoperiod by the *HDA9-AGL19-FT* module in Arabidopsis. New Phytol **206:** 281–294

Kim DH, Sung S (2013) Coordination of the vernalization response through a *VIN3* and *FLC* gene family regulatory network in *Arabidopsis.* Plant Cell **25:** 454–469

Kim D-H, Sung S (2014) Genetic and epigenetic mechanisms underlying vernalization. The Arabidopsis Book **12:** e0171, doi/10.199/tab.0171

Kippes N, Chen A, Zhang X, Lukaszewski AJ, Dubcovsky J (2016) Development and characterization of a spring hexaploid wheat line with no functional *VRN2* genes. Theor Appl Genet **129:** 1417–1428

Kippes N, Debernardi JM, Vasquez-Gross HA, Akpinar BA, Budak H, Kato K, Chao S, Akhunov E, Dubcovsky J (2015) Identification of the *VERNALIZATION 4* gene reveals the origin of spring growth habit in ancient wheats from South Asia. Proc Natl Acad Sci USA **112:** E5401–E5410

Kitagawa S, Shimada S, Murai K (2012) Effect of *Ppd-1* on the expression of flowering-time genes in vegetative and reproductive growth stages of wheat. Genes Genet Syst **87:** 161–168

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science **286:** 1960–1962

Kotoda N, Iwanami H, Takahashi S, Abe K (2006) Antisense expression of *MdTFL1*, a *TFL1-like* gene, reduces the juvenile phase in apple. J Am Soc Hortic Sci **131:** 74–81

Kwak JS, Son GH, Kim S-I, Song JT, Seo HS (2016) Arabidopsis HIGH PLOIDY2 sumoylates and stabilizes Flowering Locus C through its E3 ligase activity. Front Plant Sci **7:** 530

Lee JH, Ryu HS, Chung KS, Posé D, Kim S, Schmid M, Ahn JH (2013a) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. Science **342:** 628–632

Lee R, Baldwin S, Kenel F, McCallum J, Macknight R (2013b) *FLOWERING LOCUS T* genes control onion bulb formation and flowering. Nat Commun **4:** 2884

Li C, Dubcovsky J (2008) Wheat FT protein regulates *VRN1* transcription through interactions with FDL2. Plant J **55:** 543–554

Li C, Lin H, Dubcovsky J (2015) Factorial combinations of protein interactions generate a multiplicity of florigen activation complexes in wheat and barley. Plant J **84:** 70–82

Lv B, Nitcher R, Han X, Wang S, Ni F, Li K, Pearce S, Wu J, Dubcovsky J, Fu D (2014) Characterization of *FLOWERING LOCUS* T1 (*FT1*) gene in *Brachypodium* and wheat. PLoS One **9:** e94171

Mahrez W, Shin J, Muñoz-Viana R, Figueiredo DD, Trejo-Arellano MS, Exner V, Siretskiy A, Gruissem W, Köhler C, Hennig L (2016) BRR2a affects flowering time via *FLC* splicing. PLoS Genet **12:** e1005924

Mannion AM (1997) Global Environmental Change: A Natural and Cultural Environmental History. Routlege, Abingdon-on-Thames, UK

Marquardt S, Raitskin O, Wu Z, Liu F, Sun Q, Dean C (2014) Functional consequences of splicing of the antisense transcript *COOLAIR* on *FLC* transcription. Mol Cell **54:** 156–165

McKeown M, Schubert M, Marcussen T, Fjellheim S, Preston JC (2016) Evidence for an early origin of vernalization responsiveness in temperate Pooideae grasses. Plant Physiol **172:** 416–426

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell **11:** 949–956

Michaels SD, Amasino RM (2001) Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. Plant Cell **13:** 935–941

Moon J, Lee H, Kim M, Lee I (2005) Analysis of flowering pathway integrators in *Arabidopsis.* Plant Cell Physiol **46:** 292–299

Mutasa-Göttgens ES, Joshi A, Holmes HF, Hedden P, Göttgens B (2012) A new RNASeq-based reference transcriptome for sugar beet and its application in transcriptome-scale analysis of vernalization and gibberellin responses. BMC Genomics **13:** 99

Oliver SN, Finnegan EJ, Dennis ES, Peacock WJ, Trevaskis B (2009) Vernalization-induced flowering in cereals is associated with changes in histone methylation at the *VERNALIZATION1* gene. Proc Natl Acad Sci USA **106:** 8386–8391

Pajoro A, Biewers S, Dougali E, Leal Valentim F, Mendes MA, Porri A, Coupland G, Van de Peer Y, van Dijk ADJ, Colombo L, et al (2014) The (r)evolution of gene regulatory networks controlling *Arabidopsis* plant reproduction: a two-decade history. J Exp Bot **65:** 4731–4745

Périlleux C, Pieltain A, Jacquemin G, Bouché F, Detry N, D'Aloia M, Thiry L, Aljochim P, Delansnay M, Mathieu A-S, et al (2013) A root chicory MADS box sequence and the Arabidopsis flowering repressor *FLC* share common features that suggest conserved function in vernalization and de-vernalization responses. Plant J **75:** 390–402

Pin PA, Benlloch R, Bonnet D, Wremerth-Weich E, Kraft T, Gielen JJL, Nilsson O (2010) An antagonistic pair of *FT* homologs mediates the control of flowering time in sugar beet. Science **330:** 1397–1400

Pin PA, Zhang W, Vogt SH, Dally N, Büttner B, Schulze-Buxloh G, Jelly NS, Chia TYP, Mutasa-Göttgens ES, Dohm JC, et al (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. Curr Biol **22:** 1095–1101

Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RGH, Schmid M (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature **503:** 414–417

Preston JC, Sandve SR (2013) Adaptation to seasonality and the winter freeze. Front Plant Sci **4:** 167

Purvis ON, Gregory FG (1937) Studies in vernalisation of cereals: I. A comparative study of vernalisation of winter rye by low temperature and by short days. Ann Bot (Lond) **1:** 569–592

Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL (2003) Analysis of the Arabidopsis *MADS AFFECTING FLOWERING* gene family: *MAF2* prevents vernalization by short periods of cold. Plant Cell **15:** 1159–1169

Ratcliffe OJ, Nadzan GC, Reuber TL, Riechmann JL (2001) Regulation of flowering in Arabidopsis by an *FLC* homologue. Plant Physiol **126:** 122–132

Ream TS, Woods DP, Schwartz CJ, Sanabria CP, Mahoy JA, Walters EM, Kaeppler HF, Amasino RM (2014) Interaction of photoperiod and vernalization determines flowering time of *Brachypodium distachyon.* Plant Physiol **164:** 694–709

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

Rosloski SM, Singh A, Jali SS, Balasubramanian S, Weigel D, Grbic V (2013) Functional analysis of splice variant expression of *MADS AFFECTING FLOWERING 2* of *Arabidopsis thaliana.* Plant Mol Biol **81:** 57–69

Ruelens P, de Maagd RA, Proost S, Theißen G, Geuten K, Kaufmann K (2013) FLOWERING LOCUS C in monocots and the tandem origin of angiosperm-specific MADS-box genes. Nat Commun **4:** 2280

Salisbury F, Ross C (1992) Plant Physiology, Ed 4. Wadsworth, Belmont, CA

Schmitz RJ, Amasino RM (2007) Vernalization: a model for investigating epigenetics and eukaryotic gene regulation in plants. Biochim Biophys Acta **1769:** 269–275

Schönrock N, Bouveret R, Leroy O, Borghi L, Köhler C, Gruissem W, Hennig L (2006) Polycomb-group proteins repress the floral activator *AGL19* in the *FLC*-independent vernalization pathway. Genes Dev **20:** 1667–1678

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

Shaw LM, Turner AS, Laurie DA (2012) The impact of photoperiod insensitive *Ppd-1a* mutations on the photoperiod pathway across the three genomes of hexaploid wheat (*Triticum aestivum*). Plant J **71:** 71–84

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell **11:** 445–458

Sheldon CC, Finnegan EJ, Peacock WJ, Dennis ES (2009) Mechanisms of gene repression by vernalization in Arabidopsis. Plant J **59:** 488–498

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES (2000) The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). Proc Natl Acad Sci USA **97:** 3753–3758

Shim JS, Kubota A, Imaizumi T (2017) Circadian clock and photoperiodic flowering in Arabidopsis: CONSTANS is a hub for signal integration. Plant Physiol **173:** 5–15

Shimada S, Ogawa T, Kitagawa S, Suzuki T, Ikari C, Shitsukawa N, Abe T, Kawahigashi H, Kikuchi R, Handa H, et al (2009) A genetic network of flowering-time genes in wheat leaves, in which an *APETALA1/FRUITFULL*-like gene, *VRN1*, is upstream of *FLOWERING LOCUS T*. Plant J **58:** 668–681

Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T (2015) Photoperiodic flowering: time measurement mechanisms in leaves. Annu Rev Plant Biol **66:** 441–464

Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature **427:** 159–164

Sung S, Schmitz RJ, Amasino RM (2006) A PHD finger protein involved in both the vernalization and photoperiod pathways in *Arabidopsis*. Genes Dev **20:** 3244–3248

Sureshkumar S, Dent C, Seleznev A, Tasset C, Balasubramanian S (2016) Nonsense-mediated mRNA decay modulates FLM-dependent thermosensory flowering response in *Arabidopsis*. Nat Plants **2:** 16055

Suter L, Rüegg M, Zemp N, Hennig L, Widmer A (2014) Gene regulatory variation mediates flowering responses to vernalization along an altitudinal gradient in *Arabidopsis*. Plant Physiol **166:** 1928–1942

Swiezewski S, Liu F, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. Nature **462:** 799–802

Taoka K, Ohki I, Tsuji H, Furuita K, Hayashi K, Yanase T, Yamaguchi M, Nakashima C, Purwestri YA, Tamaki S, et al (2011) 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature **476:** 332–335

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

Tyler L, Lee SJ, Young ND, DeIulio GA, Benavente E, Reagon M, Sysopha J, Baldini RM, Troìa A, Hazen SP, et al (2016) Population structure in the model grass *Brachypodium* distachyon is highly correlated with flowering differences across broad geographic areas. Plant Genome **9:** 2

Verhage L, Angenent GC, Immink RGH (2014) Research on floral timing by ambient temperature comes into blossom. Trends Plant Sci **19:** 583–591

Vogt SH, Weyens G, Lefèbvre M, Bork B, Schechert A, Müller AE (2014) The *FLC-like* gene *BvFL1* is not a major regulator of vernalization response in biennial beets. Front Plant Sci **5:** 146

Wang CQ, Guthrie C, Sarmast MK, Dehesh K (2014) BBX19 interacts with CONSTANS to repress *FLOWERING LOCUS T* transcription, defining a flowering time checkpoint in *Arabidopsis*. Plant Cell **26:** 3589–3602

Wang J-W, Czech B, Weigel D (2009a) miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. Cell **138:** 738–749

Wang J-W, Park MY, Wang L-J, Koo Y, Chen X-Y, Weigel D, Poethig RS (2011b) miRNA control of vegetative phase change in trees. PLoS Genet **7:** e1002012

Wang R, Albani MC, Vincent C, Bergonzi S, Luan M, Bai Y, Kiefer C, Castillo R, Coupland G (2011a) Aa *TFL1* confers an age-dependent response to vernalization in perennial *Arabis alpina*. Plant Cell **23:** 1307–1321

Wang R, Farrona S, Vincent C, Joecker A, Schoof H, Turck F, Alonso-Blanco C, Coupland G, Albani MC (2009b) *PEP1* regulates perennial flowering in *Arabis alpina*. Nature **459:** 423–427

Wellensiek SJ (1985) Campanula medium. *In* AH Halevey, ed, CRC Handbook of Flowering, Vol 2. CRC Press, Boca Raton, FL, pp 123–126

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

Woods DP, Amasino RM (2015) Dissecting the control of flowering time in grasses using *Brachypodium distachyon*. *In* JP Vogel, ed, Genetics and Genomics of Brachypodium. Springer, New York, pp 259–273

Woods DP, Bednarek R, Bouché F, Gordon SP, Vogel JP, Garvin DF, Amasino RM (2017) Genetic architecture of flowering-time variation in *Brachypodium distachyon*. Plant Physiol **173:** 269–279

Woods DP, McKeown MA, Dong Y, Preston JC, Amasino RM (2016) Evolution of *VRN2/Ghd7-Like* genes in vernalization-mediated repression of grass flowering. Plant Physiol **170:** 2124–2135

Woods DP, Ream TS, Amasino RM (2014a) Memory of the vernalized state in plants including the model grass *Brachypodium distachyon*. Front Plant Sci **5:** 99

Woods DP, Ream TS, Minevich G, Hobert O, Amasino RM (2014b) PHYTOCHROME C is an essential light receptor for photoperiodic flowering in the temperate grass, *Brachypodium distachyon*. Genetics **198:** 397–408

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

Xiao J, Xu S, Li C, Xu Y, Xing L, Niu Y, Huan Q, Tang Y, Zhao C, Wagner D, et al (2014) O-GlcNAc-mediated interaction between VER2 and TaGRP2 elicits *TaVRN1* mRNA accumulation during vernalization in winter wheat. Nat Commun **5:** 4572

Xie K, Shen J, Hou X, Yao J, Li X, Xiao J, Xiong L (2012) Gradual increase of miR156 regulates temporal expression changes of numerous genes during leaf development in rice. Plant Physiol **158:** 1382–1394

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc Natl Acad Sci USA **103:** 19581–19586

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

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA **100:** 6263–6268

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

Yu S, Lian H, Wang J-W (2015) Plant developmental transitions: the role of microRNAs and sugars. Curr Opin Plant Biol **27:** 1–7

Zeng L, Zhang Q, Sun R, Kong H, Zhang N, Ma H (2014) Resolution of deep angiosperm phylogeny using conserved nuclear genes and estimates of early divergence times. Nat Commun **5:** 4956

Zhou CM, Zhang TQ, Wang X, Yu S, Lian H, Tang H, Feng ZY, Zozomova-Lihová J, Wang JW (2013) Molecular basis of age-dependent vernalization in *Cardamine flexuosa*. Science **340:** 1097–1100