Journal of Experimental Botany, Vol. 59, No. 6, pp. 1127–1135, 2008
doi:10.1093/jxb/ern070 Advance Access publication 4 April, 2008

Journal of Experimental Botany
www.jxb.oxfordjournals.org

FLOWERING NEWSLETTER REVIEW

FLC or not *FLC*: the other side of vernalization

Cristina Madeira Alexandre and Lars Hennig*

Institute of Plant Sciences and Zurich-Basel Plant Science Center, ETH Zurich, CH-8092 Zurich, Switzerland

Received 19 December 2007; Revised 11 February 2008; Accepted 15 February 2008

Abstract

Vernalization is the promotion of the competence for flowering by long periods of low temperatures such as those typically experienced during winters. In *Arabidopsis*, the vernalization response is, to a large extent, mediated by the repression of the floral repressor *FLC*, and the stable epigenetic silencing of *FLC* after cold treatments is essential for vernalization. In addition to *FLC*, other vernalization targets exist in *Arabidopsis*. In grasses, vernalization seems to be entirely independent of *FLC*. Here, the current understanding of *FLC*-independent branches of the vernalization pathway in *Arabidopsis* and vernalization without *FLC* in grasses is discussed. This review focuses on the role of *AGL19*, *AGL24*, and the *MAF* genes in *Arabidopsis*. Interestingly, vernalization acts through related molecular machineries on distinct targets. In particular, protein complexes similar to *Drosophila* Polycomb Repressive Complex 2 play a prominent role in establishing an epigenetic cellular memory for cold-regulated expression states of *AGL19* and *FLC*. Finally, the similar network topology of the apparently independently evolved vernalization pathways of grasses and *Arabidopsis* is discussed.

Key words: *AGL19*, *Arabidopsis*, chromatin, epigenetics, *FLC*, flowering time, polycomb, PRC2, vernalization.

Introduction

Early observations reported that prolonged exposure to low temperatures can accelerate flowering in a broad range of plant species (for a review, see Chouard, 1960). This effect is termed vernalization, and constitutes a major determinant in the switch from vegetative to reproductive development. For non-perennials in temperate climates, where the winter season lasts for several months, it is crucial that flowering occurs at the appropriate time, such as in early spring when environmental conditions favour reproductive success. In order to cope with this challenge, plants have devised vernalization mechanisms whereby cold is used as an enabling signal to induce the competence to flower.

One of the distinguishing features of vernalization is the uncoupling between stimulus and effect (Chouard, 1960). This uncoupling is both temporal, because often several months separate the initiation of the vernalization response and the actual transition to flowering, as well as developmental, because even imbibed seeds can become vernalized and retain the vernalized state throughout development until the adult phase. Although many of the physiological aspects of vernalization are still elusive, much progress has been achieved recently at deciphering the molecular basis of the underlying cellular-memory mechanism(s).

In *Arabidopsis thaliana*, the vernalization requirement is largely conferred by the MADS-box gene *FLOWERING LOCUS C* (*FLC*) (Michaels and Amasino, 1999; Sheldon *et al*., 1999), which is in most vernalization-requiring accessions transcriptionally activated by *FRIGIDA* (*FRI*) (Napp-Zinn, 1957; Michaels and Amasino, 1999; Sheldon *et al*., 1999; Johanson *et al*., 2000). FLC then acts both in leaves and in the apical meristem to repress downstream floral integrators such as *FT* and *SOC1*, thereby acting as a floral repressor to delay flowering (Helliwell *et al*., 2006; Searle *et al*., 2006).

FLC is not exclusively regulated by vernalization (Fig. 1). A substantial number of additional unrelated positive and negative regulators have been described (for reviews, see Simpson, 2004; He *et al*., 2005; Quesada *et al*., 2005; Sung and Amasino, 2006; Schmitz and Amasino, 2007).

Vernalization acts at the epigenetic level to stably reduce *FLC* expression (for a review, see Schmitz and

* To whom correspondence should be addressed. E-mail: lars.hennig@ipw.biol.ethz.ch
Abbreviations: CArG-box, $CC(A/T)_6GG$ motif, MADS-domain protein-binding element; H3K27me3, histone 3 lysine 27 trimethylation; LD, long days; PRC2, polycomb repressive complex 2; SD, short days.

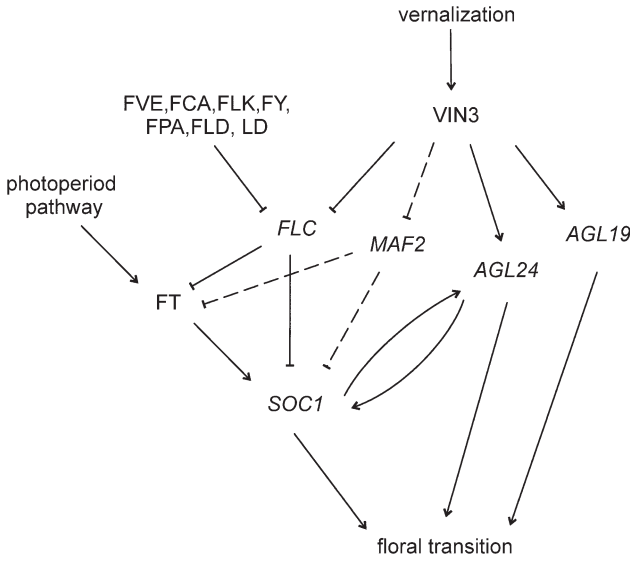

Fig. 1. The vernalization pathway in *Arabidopsis*. Three independent molecular vernalization responses have been identified in *Arabidopsis*, defining the *FLC*-, *AGL24*-, and *AGL19*-branches. Similar to FLC, its homologue MAF2 might act to repress *SOC1* and *FT*, but this is not firmly established. Downstream factors of *AGL19* and *AGL24* remain unidentified. It is possible that, at least in part, *AGL24* acts through *SOC1*, because positive regulation between *AGL24* and *SOC1* occurs. Dashed lines represent hypothetical genetic links.

Amasino, 2007), and this effect overrides other types of regulation such as by the autonomous pathway. Once *FLC* is repressed by vernalization, it can only be reactivated in the next generation. The mechanism of *FLC* repression during vernalization has been studied extensively. The most upstream molecular effect of vernalization is induction of expression of the PHD-domain protein VIN3 (Sung and Amasino, 2004). *VIN3* is only expressed at low temperatures, and both transcript and protein levels increase gradually with the length of exposure. Once sufficient amounts of VIN3 protein are present, the cold signal is transduced into chromatin modifications at the *FLC* locus.

It has been reported that VERNALIZATION 2 (VRN2), a homologue of one subunit of metazoan Polycomb-group Repressive Complex 2 (PRC2), is required for epigenetic maintenance of *FLC* silencing after vernalization (Gendall *et al*., 2001; Bastow *et al*., 2004). More recently, a PRC2-like complex including at least the VRN2, FERTILIZATION INDEPENDENT ENDOSPERM (FIE), and CURLY LEAF (CLF)/SWINGER (SWN) subunits has been reported to associate with VIN3 at the *FLC* locus (Wood *et al*., 2006). PRC2 complexes are conserved between plants and animals, and usually have a histone methyltransferase activity that deposits histone 3 lysine 27 trimethylation (H3K27me3) marks on target genes (for a review, see Schwartz and Pirrotta, 2007). Increased levels of H3K27me3 can be observed at *FLC* following vernalization (Shindo *et al*., 2006; Sung *et al*., 2006*b*; Finnegan and Dennis, 2007; Greb *et al*., 2007). Subsequently, H3K27me3 is thought to recruit additional repressors such as VRN1 and LHP1, which together assist in the maintenance of a stably silenced state (Levy *et al*., 2002; Mylne *et al*., 2006; Sung *et al*., 2006*a*). Notably, it has long been known that vernalization requires mitotic activity (Wellensiek, 1962, 1964), and a recent report suggests that both H3K27me3 at *FLC* and *FLC* repression cannot be efficiently maintained in non-dividing tissue after transfer to ambient temperature (Finnegan and Dennis, 2007).

Much of the knowledge on the molecular basis of vernalization has come from the studies of *FLC* regulation, and it is established that *FLC* is responsible for a considerable part of the vernalization response in *Arabidopsis*. Nonetheless, an *FLC*-independent vernalization response exists, because *flc* null mutants still have a vernalization-sensitive phenotype (Michaels and Amasino, 2001; Moon *et al*., 2005). In the *flc* null mutant, both *FT* and *SOC1* are up-regulated after vernalization and this suggests that *FLC*-dependent and -independent vernalization branches share common targets (Moon *et al*., 2005) (Fig. 1). Here, the current knowledge about the *FLC*-independent vernalization response is reviewed.

FLC-independent responses in *Arabidopsis*

FLC-*related repressors of flowering*

Arabidopsis FLC is a member of a small family of closely related MADS-domain proteins. Besides FLC, this family contains the five MADS AFFECTING FLOWERING (MAF) proteins with 53–87% identity (Bodt *et al*., 2003; Ratcliffe *et al*., 2003). FLOWERING LOCUS M (FLM, also called MAF1) is a repressor of flowering (Ratcliffe *et al*., 2001; Scortecci *et al*., 2001), and repression of *FLM* seems to contribute to the acceleration of flowering by elevated growth temperatures (Werner *et al*., 2005; Li *et al*., 2006). It is possible that all MAF proteins function as repressors of flowering (Ratcliffe *et al*., 2003), but this hypothesis needs experimental testing. So far, a role for MAF1 in the vernalization response has not been reported, but the recent findings that *MAF1* can be suppressed by vernalization (Sung *et al*., 2006*b*) might bring new insights into MAF1 functions.

FLC and some of the *MAF* genes share several regulators, such as VIP5 and ELF8/VIP6 (He *et al*., 2004; Oh *et al*., 2004) or ESD1 (Martin-Trillo *et al*., 2006). In addition, plants with reduced DNA methylation have reduced expression of *FLC* and *MAF1-5*, suggesting that DNA methylation alters the expression of a common *trans*-acting regulator (Finnegan *et al*., 2005). By contrast, some other regulators were found to affect only a subset of the *FLC*/*MAF* genes (He *et al*., 2004; March-Diaz *et al*., 2007), but it is possible that, at least in some cases,

this reflects assay resolution rather than true biological differences. Similar to *FLC*, *MAF* genes are usually regulated by vernalization. Vernalization represses *MAF1*, *MAF2*, and *MAF3*, but it induces *MAF5* and does not strongly affect *MAF4* (Ratcliffe *et al*., 2003; Sung *et al*., 2006*b*). Furthermore, some natural variability occurs in the regulation of *MAF* expression, because differences between Col and two late-flowering accessions (Pitztal and Stockholm) have been reported (Ratcliffe *et al*., 2003). Although H3K27me3 has been found to be essential for *FLC* repression by vernalization, it is not clear whether this histone mark is involved in the regulation of *MAF* genes as well. Examination of published data about genome-wide H3K27me3 distribution (Zhang *et al*., 2007) revealed that *MAF4* and *MAF5* but not *MAF1*, *MAF2*, or *MAF3* are decorated with H3K27me3 (Fig. 2). However, in contrast to *FLC*, which is covered over its entire length by H3K27me3, *MAF4* and *MAF5* carry H3K27me3 only in certain regions. It should be noted that this genome-wide dataset is based on non-vernalized seedlings and that *FLC* carries considerable H3K27me3 even under these conditions. Because it is possible that after vernalization H3K27me3 marks accumulate also at *MAF1–MAF3*, more directed experiments are needed to address the potential involvement of this chromatin mark in *MAF* regulation.

In addition to *FLC* and *MAF1*/*FLM*, mutant studies have so far only revealed a function for *MAF2*. *maf2* mutants flower slightly earlier than wild type, but still retain a normal response to 6 weeks of vernalization (Ratcliffe *et al*., 2003). However, if plants were submitted to only 10 d of cold, which is insufficient to elicit a vernalization response in the wild type, a significant acceleration of flowering was observed. In fact, in *maf2* mutants a 21 d cold treatment can accelerate flowering to a similar extent as an 85 d cold treatment in wild type, suggesting that *MAF2* might regulate the delayed establishment of vernalization (Fig. 3). Such a specific function could prevent, for example, a few days of cold in autumn triggering precocious flowering during winter. This induction of flowering by short periods of low temperature seems to be independent of *FLC* as no significant decrease in *FLC* expression could be detected after 10 d of cold treatment (Ratcliffe *et al*., 2003). Furthermore, *35S::MAF2* plants are unable to respond to vernalization due to continuous *SOC1* repression, even when *FLC* expression is normally reduced (Ratcliffe *et al*., 2003). It is possible that an ancestor of the FLC/MAF family was a repressor of *SOC1* and that at least MAF2 and FLC retained this original function. It will be important to establish if MAF3–5 also repress *SOC1* expression and flowering.

The flowering promoter AGL24

Arabidopsis AGAMOUS-LIKE 24 (*AGL24*) and its paralogue *SVP* belong to the ancient *StMADS11* clade of MADS-box genes (Bodt *et al*., 2003). Curiously, the separation of the *AGL24*/*SVP* branch involved strong positive Darwinian selection, and the same was observed for *FLC*-like genes (Martinez-Castilla and Alvarez-Buylla, 2003). Thus it seems that flowering time control is a specialized function acquired separately in different

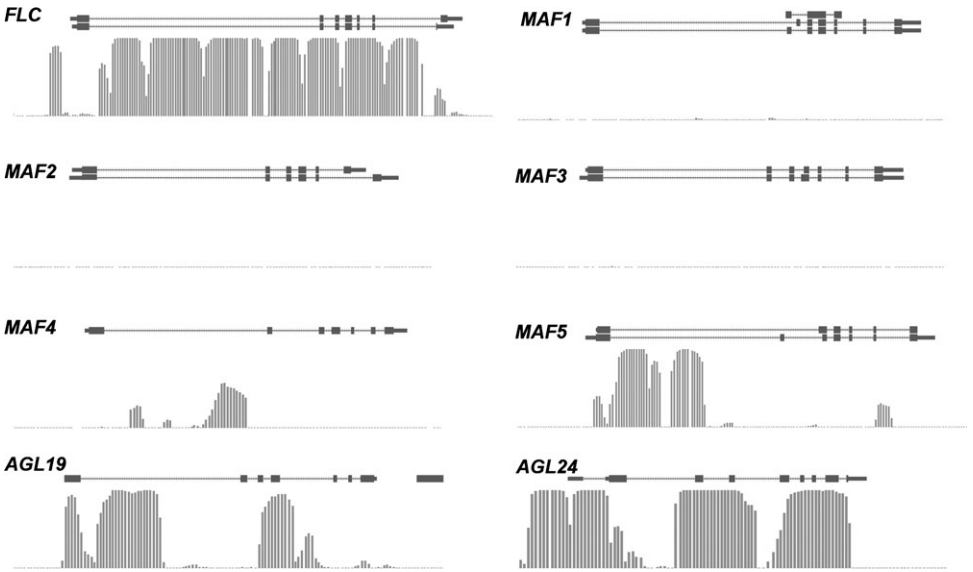

Fig. 2. Distribution of H3K27me3 marks on vernalization-related loci in *Arabidopsis*. Whole-genome analysis of H3K27me3 on wild-type seedlings (Zhang *et al*., 2007) shows that *AGL19*, *AGL24*, *MAF4*, *MAF5*, and *FLC* all carry this repressive mark, although with very distinct profiles. Whereas for *AGL19*, *MAF4*, and *MAF5* the methylation is restricted to one or two defined regions, in both *FLC* and *AGL24*, H3K27me3 covers nearly the entire locus. Experimental evidence for a function of H3K27me3 exists only for *FLC* and *AGL19*. The lines represent gene models and the bars indicate the probability of the deposition of H3K27met3 at a particular position.

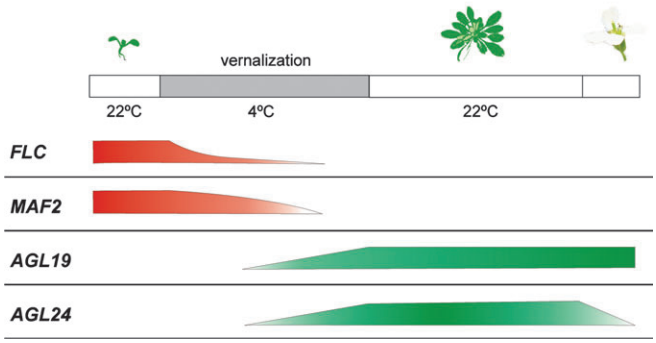

Fig. 3. Vernalization-regulated genes respond to different lengths of exposure to cold. The flowering activators *AGL24* and *AGL19* require long cold exposures to be activated, and are mostly functional from the late stages of vernalization on. The flowering repressors *FLC* and *MAF2* function before and during early stages of vernalization. *MAF2* has been proposed to prevent induction of flowering by short periods of cold, which could repress *FLC* (Ratcliffe *et al*., 2003). The upper bar represents the different events in the plant development until flowering; red represents repressive functions and green activating functions; the shape is intended to depict gene function in relation to the duration of the cold period.

MADS-box gene lineages, and maintained most likely through its direct impact on plant fitness (Martinez-Castilla and Alvarez-Buylla, 2003).

AGL24 functions as an activator of flowering in response to vernalization (Yu *et al*., 2002; Michaels *et al*., 2003). *agl24* mutants are late-flowering, and this phenotype is only slightly suppressed by vernalization much as in *soc1* mutants. *SOC1* and *AGL24* activate each other's expression but can promote flowering independently as well (Yu *et al*., 2002; Michaels *et al*., 2003). Furthermore, unlike *SOC1*, *AGL24* is activated following vernalization in an *FLC*-independent manner.

In addition to its role in flowering-time control, *AGL24* has other developmental functions. Over-expression of *AGL24* is correlated with several floral abnormalities consistent with a role in establishing inflorescence meristem identity (Yu *et al*., 2004). In fact, *AGL24* is generally expressed in vegetative organs before the floral transition, but gets progressively cleared as floral development proceeds and eventually becomes confined to the two inner whorls of the flower, the carpels and stamens (Michaels *et al*., 2003; Yu *et al*., 2004; Gregis *et al*., 2006). Recently, it has been shown that repression of the flowering time genes *AGL24*, *SVP*, and *SOC1* by AP1 is an essential step in the establishment of floral meristem identity (Liu *et al*., 2007). The emerging picture is that the same developmental regulators can assume distinct roles at different moments in the plant's life cycle.

The flowering promoter AGL19

Recently, another *FLC*-independent branch of the vernalization pathway in *Arabidopsis* was identified (Schönrock *et al*., 2006). Again, the key regulator responding to the vernalization treatment is a MADS-box gene, a close homologue of *SOC1* from the *TM3*-clade—*AGAMOUS-LIKE 19* (*AGL19*).

Initially described as root-specific, a novel role was assigned to *AGL19* after it had been found to be involved in controlling the flowering transition. When ectopically expressed, *AGL19* is a potent activator of flowering, but unlike *AGL24* only mild floral abnormalities were observed. This suggests that *AGL19* has a more restricted role in flowering time control. Also, in contrast to *AGL24*, *AGL19* does not affect *SOC1* levels, indicating that it probably acts independently of *SOC1* (Schönrock *et al*., 2006). *AGL19* and *SOC1* share a conserved similar CArG-box (for CC-A rich GG) motif in their upstream regions. FLC binds the *SOC1* CArG-box and represses transcription (Hepworth *et al*., 2002). In *AGL19*, the CArG-box sequence differs in a nucleotide that is essential for the *FLC*-binding to the *SOC1* promoter (Schönrock, 2006). Thus, it is likely that FLC cannot bind and repress *AGL19*. Indeed, *AGL19* expression levels are independent of FLC. Furthermore, genetic evidence also supports an *FLC*-independent function of *AGL19* because the double mutant *agl19 flc* has an additive impairment of the vernalization response (Schönrock *et al*., 2006).

Interestingly, although *AGL19* acts as an activator of flowering, it shares a common regulatory mechanism with the flowering repressor *FLC*. Both are regulated at the chromatin level by vernalization in a VIN3-dependent manner. Chromatin immunoprecipitation assays demonstrated that *AGL19* chromatin is enriched in repressive H3K27me3 before, but much less after, vernalization (Schönrock *et al*., 2006). While *FLC* is permanently silenced after vernalization, *AGL19* is temporarily silenced before vernalization. It has been reported that *FLC* silencing involves not only H3K27me3 but also repressive H3K9me2 marks (Bastow *et al*., 2004; Sung and Amasino, 2004). By contrast, H3K9me2 marks could not be found on *AGL19* chromatin. In plants, H3K9me2 is usually associated with stable heterochromatic silencing while H3K27me3 is usually associated with more transient euchromatic silencing (Fuchs *et al*., 2006), and it is possible that the stable *FLC* silencing involves additional, heterochromatin-like mechanisms not needed for the transient *AGL19* silencing.

H3K27me3 is thought to be deposited by PRC2-like complexes, and H3K27me3 at *AGL19* is most likely deposited by a complex of EMF2, CLF/SWN, FIE, and MSI1 (Schönrock *et al*., 2006). By contrast, H3K27me3 at *FLC* is most likely deposited by a complex of VRN2, CLF/SWN, FIE, and possibly VIN3 (Wood *et al*., 2006) (Fig. 4). How the same signal, i.e. prolonged cold, can differentially affect distinct PRC2-like complexes is not known. It is possible that VIN3 assists in assembling the VRN2 complex on *FLC* (Wood *et al*., 2006). Because VIN3 is needed for *AGL19* regulation as well, it would be

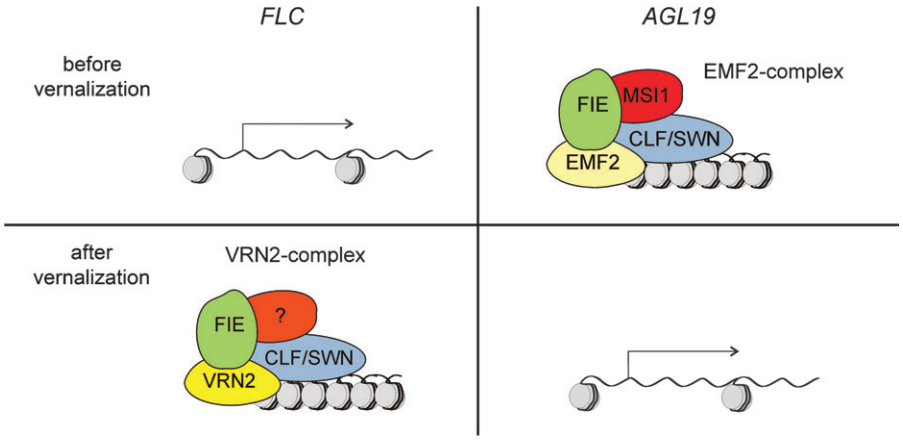

Fig. 4. Distinct polycomb-group complexes are required for vernalization-dependent regulation of *AGL19* and *FLC*. *AGL19* and *FLC* have opposite roles in promoting the switch to flower development and are differentially affected by vernalization, but both are regulated by polycomb-group complexes. For *FLC*, vernalization is thought to initiate recruitment of the VRN2 complex to *FLC* chromatin, leading to stable repression. For *AGL19*, vernalization is thought to initiate dissociation of the EMF2 complex from *AGL19* chromatin enabling gene expression.

highly interesting to investigate whether VIN3 could mediate disassembly of the EMF2 complex associated with *AGL19*.

Clearly, many details of the cellular memory of vernalization in *Arabidopsis* still need to be discovered, but it is exciting to see that stable gene repression by PRC2-complexes is a recurrent scheme. Notably, similar to *FLC* and *AGL19*, *AGL24* is also decorated with H3K27me3 marks, which are most likely deposited by a PRC2-like complex (Fig. 2). The prominent role of PRC2-like complexes and the H3K27me3 modifications raise the question whether other species use similar mechanisms in their vernalization response.

Vernalization pathways in other species

Initially, *FLC*-like genes were believed to be restricted to the Brassicaceae (Becker and Theissen, 2003), but recent work by Reeves *et al.* (2007) suggests that the strong positive Darwinian selection acting on these genes might have compromised their identification in other species and that the *FLC* clade actually originated early in the diversification of the eudicots. Reeves *et al.* (2007) identified the sugar beet (*Beta vulgaris* ssp. *vulgaris*) *FLC* homologue *BvFLC*. *BvFLC* is repressed by extended cold and delays flowering when expressed in transgenic *Arabidopsis* plants. Further research is needed to clarify how important *FLC*-like genes are for vernalization responses in non-Brassicaceae species.

The process of vernalization was first discovered in grasses, where vernalization is of great agronomic importance (for historical reviews, see Chouard, 1960; Amasino, 2004). Interestingly, many grasses are short-day–long-day (SD-LD) plants that need to be exposed first to short-day photoperiods and subsequently to long-day photoperiods to flower efficiently (Heide, 1994). In many winter varieties, the initial SD treatment can substitute for the effect of prolonged cold on the induction of the competence to flower (McKinney *et al.*, 1935; Evans, 1987).

Much has been learned about the genetics and molecular mechanisms of vernalization responses in grasses (for a review, see Trevaskis *et al.*, 2007*a*). Work in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) led to a model of vernalization that includes four central genes: *VRN1*, *VRN2*, *VRN3*, and *VRT2*, and genetic data strongly substantiate the importance of grass *VRN1-3* for vernalization (Fig. 4). Importantly, despite identical names, wheat and barley *VRN1* (also called *TmAP1/TaVRT-1* and *HvVRN1*, respectively) and *VRN2* (also called *TmZCCT1* and *HvZCCTa/HvZCCTb*, respectively) do not share any sequence similarity with *Arabidopsis VRN1* and *VRN2*. Instead, grass VRN1 is a homologue of the meristem identity MADS-domain protein APETALA1 (AP1) in *Arabidopsis* (Schmitz *et al.*, 2000; Danyluk *et al.*, 2003; Trevaskis *et al.*, 2003; Yan *et al.*, 2003; von Zitzewitz *et al.*, 2005), and grass VRN2 shares the CO, CO-like, and TOC1 (CCT) domain with the flowering time regulator CONSTANS (CO) from the photoperiod pathway of *Arabidopsis* (Yan *et al.*, 2004). Grass VRN3 is a homologue of *Arabidopsis* FT, a major component of florigen in *Arabidopsis* (Yan *et al.*, 2006). In grasses, VRN1 and its homologue FUL are thought to be direct activators of flowering (Preston and Kellogg, 2007), and diploid einkorn wheat *T. monococcum* mutants that lack *VRN1* (*TmAP1*) do not flower (Shitsukawa *et al.*, 2007). The vernalization response in wheat might include yet another gene, VRT2, a homologue of the *Arabidopsis* flowering-time genes *AGL24* and *SVP*. VRT2 binds to the *VRN1* promoter *in vitro*, and can recruit VRN2 (Kane *et al.*, 2005); VRT2 and VRN2 together can repress *VRN1* (*TaVRN1*) in a tobacco reporter assay (Kane *et al.*, 2007).

Both vernalization and SD photoperiods can repress VRN2 and thus lift *VRN1* repression (Yan *et al*., 2004; Dubcovsky *et al*., 2006). However, for efficient activation of *VRN1*, VRN3 is also needed. *VRN3* is repressed by VRN2 and activated by LD photoperiods (Yan *et al*., 2006). According to this model, vernalization or SD photoperiods are needed to repress *VRN2* and thus lift the repression from *VRN3* and *VRN1*. If subsequently LD photoperiods are present, VRN3 will activate *VRN1* to induce flowering.

In this model, the signalling network of vernalization in grasses has the same topology as the FLC network in *Arabidopsis*: Two activators (FT/SOC1 and VRN3/VRN1), which transduce the LD signal for induction of flowering, are repressed by a negative regulator (FLC and VRN2) (Fig. 5). The two activators belong to the same protein families in *Arabidopsis* and grasses, respectively: FT and VRN3 are both Raf kinase inhibitor-domain proteins (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999; Yan *et al*., 2006), and SOC1 and VRN1 are both MADS-domain proteins (Borner *et al*., 2000; Lee *et al*., 2000; Danyluk *et al*., 2003; Trevaskis *et al*., 2003; Yan *et al*., 2003). By contrast, the repressor that is under negative control by vernalization differs: FLC is a MADS-domain protein (Michaels and Amasino, 1999; Sheldon *et al*., 1999), but VRN2 is a CCT-domain protein (Yan *et al*., 2004). This reflects the likely evolutionary history of the signalling networks: FT-like proteins seem to be ancient floral activators that transmit day-length signals in *Arabidopsis* (LD plant), rice (SD plant), and poplar (perennial tree) but function also in tomato (day-neutral plant) (Bohlenius *et al*., 2006; Lifschitz and Eshed, 2006; Corbesier *et al*., 2007; Jaeger and Wigge, 2007; Mathieu *et al*., 2007; Tamaki *et al*., 2007). It is believed that vernalization requirement developed independently in *Arabidopsis* and grasses. In the former, a MADS-box transcription factor evolved to repress *FT* (and *SOC1*) in the absence of vernalization. In the latter, a CCT-domain protein evolved to repress *VRN3* (and *VRN1*) in the absence of vernalization.

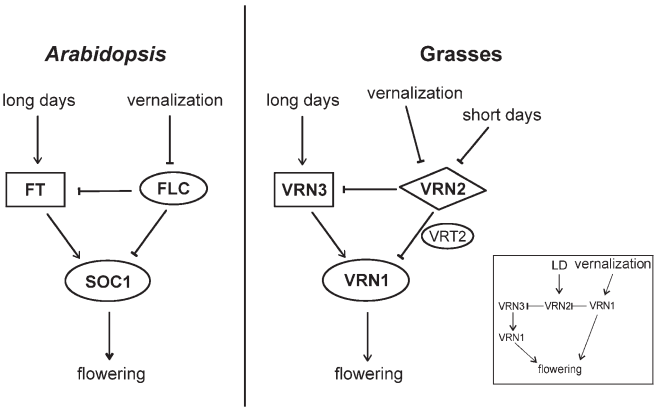

Fig. 5. Common topologies for vernalization pathways in *Arabidopsis* and in grasses. Vernalization networks evolved independently in distant plant groups, but have similar topology in *Arabidopsis* and the grasses. In both cases, two activators are sensitive to long-day photoperiod signals (FT/SOC1 and VRN3/VRN1), and are repressed by a negative regulator (FLC and VRN2). In *Arabidopsis* and grasses, the activator functions are executed by one FT-like and one MADS-domain protein. The repressor is the MADS-domain protein FLC in *Arabidopsis* and the CCT-domain protein VRN2 in grasses. The shapes represent protein families: boxes for FT-like Raf-kinase-inhibitor-domain proteins, ovals for MADS-domain proteins, and diamonds for CCT-domain proteins. Inset: an alternative model to explain the vernalization response in grasses was proposed, where the primary target of cold is VRN1 and not VRN2 (Trevaskis *et al*., 2007).

An alternative model suggests that vernalization in grasses acts primarily on VRN1, which represses *VRN2* (Fig. 5) (Trevaskis *et al*., 2006, 2007*a*, *b*). Given that FT is a mobile signal and that FLC acts both in the leaves and in the meristem, it will be important to establish in grasses in which organs and tissue VRN1, VRN2, and VRN3 function. In addition, the role of *VRT2* and its homologues needs further investigation. Recently it was proposed that barley *VRT2*-like genes do not participate in vernalization-mediated repression of *VRN1* but rather function in a similar way to *Arabidopsis AGL24* and *SVP* to inhibit floral meristem identity (Trevaskis *et al*., 2007*b*).

Another open question is the nature of the cellular memory of vernalization in grasses. While chromatin-based mechanisms, which involve PRC2-like complexes, are needed for the maintenance of silencing in the *Arabidopsis FLC* and *AGL19* branches, it is not clear to which extent such mechanisms control vernalization in grasses. Two lines of evidence suggest that this might be the case: First, three wheat *VIN3*-like (*VIL*) genes (*TmVIL1–3*) were described (Fu *et al*., 2006). Similar to *Arabidopsis VIN3*, *TmVIL1–3* transcripts accumulate after 4–6 weeks of cold treatment, but return rapidly to pre-vernalization levels after the shift to ambient temperatures. It remains to be tested whether VIL proteins mediate the vernalization response in grasses. Second, transcriptional profiling of the vernalization response in perennial rye grass (*Lolium perenne*) identified not only a MADS-box gene and a *VRN2*-like CCT-domain gene but also a JUMONJI (JmjC)-like gene, LpJMJC (Ciannamea *et al*., 2006). Although JmjC-domain proteins have not yet been found in the *Arabidopsis* vernalization response, the JmjC-protein REF6 is a repressor of *FLC*, possibly by modifying *FLC* chromatin (Noh *et al*., 2004). LpJMJC is not a close homologue of REF6, but it may also act through chromatin remodelling. This idea is supported by the recent finding that JmjC-domain proteins are often histone-demethylases (Klose *et al*., 2006). More research is needed to establish whether JmjC proteins such as LpJMJC mediate epigenetic regulation of vernalization-responsive genes in grasses.

Conclusions

The ability to be vernalized is an important adaptive trait in plants. Evolution found multiple answers to the old

question: 'how to check that the winter is over and that a ''better life'' can begin?' (Becker *et al*., 2003). Mechanisms of *FLC*-dependent vernalization are understood best, but it remains to be tested how widely they are used outside of the Brassicaceae. At least in grasses a different vernalization pathway evolved. Interestingly, even within a single species, vernalization signalling can follow multiple branches. It is possible that, after the first vernalization pathways evolved, selection favoured the addition of more robust branches to the vernalization signalling network. In the case of *Arabidopsis*, vernalization involves *FLC*-, *AGL24*-, and *AGL19*-dependent branches, and it is currently not clear which of the three is most ancient and which is most recent. Despite the multiple appearances of pathways for vernalization during evolution, there are recurrent themes. First, vernalization networks consist of similar network motifs and are of similar topology. This suggests that certain network structures evolve easily and give a robust performance. Second, epigenetic memory mechanisms are used both in the *FLC*- and the *AGL19*-branches of the *Arabidopsis* vernalization pathway. Such epigenetic mechanisms ideally serve the purpose of stable maintenance of previously established gene expression states. It will be exciting to see whether epigenetic mechanisms such as PRC2-mediated gene silencing participate in other vernalization pathways as well.

Acknowledgements

We thank Vivien Exner for critical reading of the manuscript, and two anonymous reviewers for their insightful comments. Work in the authors' laboratory is supported by SNF project 3100AO-116060 and ETH project TH-16/05-2.

References

Amasino R. 2004. Vernalization, competence, and the epigenetic memory of winter. *The Plant Cell* **16,** 2553–2559.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* **427,** 164–167.

Becker A, Theissen G. 2003. The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Molecular Phylogenetics and Evolution* **29,** 464–489.

Bodt SD, Raes J, Peer YVd, Theissen G. 2003. And then there were many: MADS goes genomic. *Trends in Plant Science* **8,** 475–483.

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

Borner R, Kampmann G, Chandler J, Gleissner R, Wisman E, Apel K, Melzer S. 2000. A MADS domain gene involved in the transition to flowering in Arabidopsis. *The Plant Journal* **24,** 591–599.

Chouard P. 1960. Vernalization and its relations to dormancy. *Annual Review of Plant Physiology* **11,** 191–238.

Ciannamea S, Busscher-Lange J, de Folter S, Angenent GC, Immink RG. 2006. Characterization of the vernalization response in *Lolium perenne* by a cDNA microarray approach. *Plant Cell Physiology* **47,** 481–492.

Corbesier L, Vincent C, Jang S, *et al*. 2007. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316,** 1030–1033.

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 Physiology* **132,** 1849–1860.

Dubcovsky J, Loukoianov A, Fu D, Valarik M, Sanchez A, Yan L. 2006. Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2*. *Plant Molecular Biology* **60,** 469–480.

Evans LT. 1987. Short day induction of inflorescence initiation in some winter wheat varieties. *Australian Journal of Plant Physiology* **14,** 277–286.

Finnegan EJ, Dennis ES. 2007. Vernalization-induced trimethylation of Histone H3 Lysine 27 at *FLC* is not maintained in mitotically quiescent cells. *Current Biology* **17,** 1978–1983.

Finnegan EJ, Kovac KA, Jaligot E, Sheldon CC, Peacock WJ, Dennis ES. 2005. The downregulation of *FLOWERING LOCUS C (FLC)* expression in plants with low levels of DNA methylation and by vernalization occurs by distinct mechanisms. *The Plant Journal* **44,** 420–432.

Fu D, Dunbar M, Dubcovsky J. 2006. Wheat *VIN3*-like PHD finger genes are up-regulated by vernalization. *Molecular Genetics and Genomics* **277,** 301–313.

Fuchs J, Demidov D, Houben A, Schubert I. 2006. Chromosomal histone modification patterns: from conservation to diversity. *Trends in Plant Science* **11,** 199–208.

Gendall AR, Levy YY, Wilson A, Dean C. 2001. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in Arabidopsis. *Cell* **107,** 525–535.

Greb T, Mylne JS, Crevillen P, Geraldo N, An H, Gendall AR, Dean C. 2007. The PHD finger protein VRN5 functions in the epigenetic silencing of Arabidopsis FLC. *Current Biology* **17,** 73–78.

Gregis V, Sessa A, Colombo L, Kater MM. 2006. *AGL24*, *SHORT VEGETATIVE PHASE*, and *APETALA1* redundantly control *AGAMOUS* during early stages of flower development in Arabidopsis. *The Plant Cell* **18,** 1373–1382.

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

He Y, Doyle MR, Amasino RM. 2004. PAF1-complex-mediated histone methylation of *FLOWERING LOCUS C* chromatin is required for the vernalization-responsive, winter-annual habit in Arabidopsis. *Genes & Development* **18,** 2774–2784.

Heide OM. 1994. Control of flowering and reproduction in temperate grasses. *New Phytology* **128,** 347–362.

Helliwell CA, Wood CC, Robertson M, Peacock WJ, 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. *The Plant Journal* **46,** 183–192.

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 Journal* **21,** 4327–4337.

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

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.

Kane NA, Agharbaoui Z, Diallo AO, Adam H, Tominaga Y, Ouellet F, Sarhan F. 2007. TaVRT2 represses transcription of the wheat vernalization gene *TaVRN1*. *The Plant Journal* **51,** 670–680.

Kane NA, Danyluk J, Tardif G, Ouellet F, Laliberte JF, Limin AE, Fowler DB, Sarhan F. 2005. *TaVRT-2*, a member of the *St*MADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. *Plant Physiology* **138,** 2354–2363.

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D. 1999. Activation tagging of the floral inducer *FT*. *Science* **286,** 1962–1965.

Klose RJ, Kallin EM, Zhang Y. 2006. JmjC-domain-containing proteins and histone demethylation. *Nature Reviews Genetics* **7,** 715–727.

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.

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I. 2000. The AGAMOUS-like 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. *Genes & Development* **14,** 2366–2376.

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C. 2002. Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. *Science* **297,** 243–246.

Li Y, Roycewicz P, Smith E, Borevitz JO. 2006. Genetics of local adaptation in the laboratory: flowering time quantitative trait loci under geographic and seasonal conditions in Arabidopsis. *PLoS ONE* 1, e105.

Lifschitz E, Eshed Y. 2006. Universal florigenic signals triggered by *FT* homologues regulate growth and flowering cycles in perennial day-neutral tomato. *Journal of Experimental Botany* **57,** 3405–3414.

Liu C, Zhou J, Bracha-Drori K, Yalovsky S, Ito T, Yu H. 2007. Specification of Arabidopsis floral meristem identity by repression of flowering time genes. *Development* **134,** 1901–1910.

March-Diaz R, Garcia-Dominguez M, Florencio FJ, Reyes JC. 2007. SEF, a new protein required for flowering repression in Arabidopsis, interacts with PIE1 and ARP6. *Plant Physiology* **143,** 893–901.

Martinez-Castilla LP, Alvarez-Buylla ER. 2003. Adaptive evolution in the Arabidopsis MADS-box gene family inferred from its complete resolved phylogeny. *Proceedings of the National Academy of Sciences, USA* **100,** 13407–13412.

Martin-Trillo M, Lazaro A, Poethig RS, Gomez-Mena C, Pineiro MA, Martinez-Zapater JM, Jarillo JA. 2006. *EARLY IN SHORT DAYS 1 (ESD1)* encodes ACTIN-RELATED PROTEIN 6 (AtARP6), a putative component of chromatin remodelling complexes that positively regulates *FLC* accumulation in Arabidopsis. *Development* **133,** 1241–1252.

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

McKinney HH, Sando WJ. 1935. Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. *Journal of Agricultural Research* **51,** 621–641.

Michaels SD, Amasino RM. 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *The 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. *The Plant Cell* **13,** 935–941.

Michaels SD, Ditta G, Gustafson-Brown C, Pelaz S, Yanofsky M, Amasino RM. 2003. AGL24 acts as a promoter of flowering in Arabidopsis and is positively regulated by vernalization. *The Plant Journal* **33,** 867–874.

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

Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C. 2006. LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of *FLC*. *Proceedings of the National Academy of Sciences, USA* **103,** 5012–5017.

Napp-Zinn K. 1957. Untersuchungen über das Vernalisationsverhalten einer winterannuellen Rasse von *Arabidopsis thaliana*. *Planta* **50,** 177–210.

Noh B, Lee SH, Kim HJ, Yi G, Shin EA, Lee M, Jung KJ, Doyle MR, Amasino RM, Noh YS. 2004. Divergent roles of a pair of homologous jumonji/zinc-finger-class transcription factor proteins in the regulation of Arabidopsis flowering time. *The Plant Cell* **16,** 2601–2613.

Oh S, Zhang H, Ludwig P, van Nocker S. 2004. A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the Arabidopsis *FLC*/*MAF* MADS box gene family. *The Plant Cell* **16,** 2940–2953.

Preston JC, Kellogg EA. 2007. Discrete developmental roles for temperate cereal grass VRN1/FUL-like genes in flowering competency and the transition to flowering. *Plant Physiology* **146,** 265–276.

Quesada V, Dean C, Simpson GG. 2005. Regulated RNA processing in the control of Arabidopsis flowering. *The International Journal of Developmental Biology* **49,** 773–780.

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. *The Plant Cell* **15,** 1159–1169.

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

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.

Schmitz J, Franzen R, Ngyuen TH, Garcia-Maroto F, Pozzi C, Salamini F, Rohde W. 2000. Cloning, mapping and expression analysis of barley MADS-box genes. *Plant Molecular Biology* **42,** 899–913.

Schmitz RJ, Amasino RM. 2007. Vernalization: a model for investigating epigenetics and eukaryotic gene regulation in plants. *Biochimica et Biophysica Acta* **1769,** 269–275.

Schönrock N. 2006. Polycomb group proteins repress the floral activator AGL19 in the FLC-independent vernalization pathway. PhD thesis, ETH Zurich.

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 & Development* **20,** 1667–1678.

Schwartz YB, Pirrotta V. 2007. Polycomb silencing mechanisms and the management of genomic programmes. *Nature Reviews Genetics* **8,** 9–22.

Scortecci KC, Michaels SD, Amasino RM. 2001. Identification of a MADS-box gene, *FLOWERING LOCUS M*, that represses flowering. *The Plant Journal* **26,** 229–236.

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G. 2006. The transcription factor FLC

confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes & Development* **20,** 898–912.

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. *The Plant Cell* **11,** 445–458.

Shindo C, Lister C, Crevillen P, Nordborg M, Dean C. 2006. Variation in the epigenetic silencing of FLC contributes to natural variation in Arabidopsis vernalization response. *Genes & Development* **20,** 3079–3083.

Shitsukawa N, Ikari C, Shimada S, *et al*. 2007. The einkorn wheat (*Triticum monococcum*) mutant, *maintained vegetative phase*, is caused by a deletion in the *VRN1* gene. *Genes and Genetic Systems* **82,** 167–170.

Simpson GG. 2004. The autonomous pathway: epigenetic and post-transcriptional gene regulation in the control of Arabidopsis flowering time. *Current Opinion in Plant Biology* **7,** 570–574.

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

Sung S, Amasino RM. 2006. Molecular genetic studies of the memory of winter. *Journal of Experimental Botany* **57,** 3369–3377.

Sung S, He Y, Eshoo TW, Tamada Y, Johnson L, Nakahigashi K, Goto K, Jacobsen SE, Amasino RM. 2006*a*. Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. *Nature Genetics* **38,** 706–710.

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

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

Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis ES. 2003. MADS box genes control vernalization-induced flowering in cereals. *Proceedings of the National Academy of Sciences, USA* **100,** 13099–13104.

Trevaskis B, Hemming MN, Dennis ES, Peacock WJ. 2007*a*. The molecular basis of vernalization-induced flowering in cereals. *Trends in Plant Science* **12,** 352–357.

Trevaskis B, Hemming MN, Peacock WJ, Dennis ES. 2006. *HvVRN2* responds to daylength, whereas *HvVRN1* is regulated by vernalization and developmental status. *Plant Physiology* **140,** 1397–1405.

Trevaskis B, Tadege M, Hemming MN, Peacock WJ, Dennis ES, Sheldon C. 2007*b*. Short vegetative phase-like MADS-box genes inhibit floral meristem identity in barley. *Plant Physiology* **143,** 225–235.

von Zitzewitz J, Szucs P, Dubcovsky J, Yan L, Francia E, Pecchioni N, Casas A, Chen TH, Hayes PM, Skinner JS. 2005. Molecular and structural characterization of barley vernalization genes. *Plant Molecular Biology* **59,** 449–467.

Wellensiek SJ. 1962. Dividing cells as the locus for vernalization. *Nature* **195,** 307–308.

Wellensiek SJ. 1964. Dividing cells as the prerequisite for vernalization. *Plant Physiology* **39,** 832–835.

Werner JD, Borevitz JO, Warthmann N, Trainer GT, Ecker JR, Chory J, Weigel D. 2005. Quantitative trait locus mapping and DNA array hybridization identify an *FLM* deletion as a cause for natural flowering-time variation. *Proceedings of the National Academy of Sciences, USA* **102,** 2460–2465.

Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA. 2006. The *Arabidopsis thaliana* vernalization response requires a Polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. *Proceedings of the National Academy of Sciences, USA* **103,** 14631–14636.

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*. *Proceedings of the National Academy of Sciences, 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*. *Proceedings of the National Academy of Sciences, USA* **100,** 6263–6268.

Yu H, Ito T, Wellmer F, Meyerowitz EM. 2004. Repression of *AGAMOUS-LIKE 24* is a crucial step in promoting flower development. *Nature Genetics* **36,** 157–161.

Yu H, Xu Y, Tan EL, Kumar PP. 2002. AGAMOUS-LIKE 24, a dosage-dependent mediator of the flowering signals. *Proceedings of the National Academy of Sciences, USA* **99,** 16336–16341.

Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, Jacobsen SE. 2007. Whole-genome analysis of Histone H3 Lysine 27 trimethylation in Arabidopsis. *PLoS Biology* **5,** 1026–1035.