Vernalization: Winter and the Timing of Flowering in Plants

Dong-Hwan Kim,1 Mark R. Doyle,2 Sibum Sung,1 and Richard M. Amasino2

1Section of Molecular Cell and Developmental Biology and the Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas 78712
2Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706-1544; email: kdhinchrist@mail.utexas.edu, markdoyle@biochem.wisc.edu, sbsung@mail.utexas.edu, amasino@biochem.wisc.edu

Key Words
Arabidopsis, cereals, histone modifications, epigenetics, Polycomb, Trithorax

Abstract
Plants have evolved many systems to sense their environment and to modify their growth and development accordingly. One example is vernalization, the process by which flowering is promoted as plants sense exposure to the cold temperatures of winter. A requirement for vernalization is an adaptive trait that helps prevent flowering before winter and permits flowering in the favorable conditions of spring. In Arabidopsis and cereals, vernalization results in the suppression of genes that repress flowering. We describe recent progress in understanding the molecular basis of this suppression. In Arabidopsis, vernalization involves the recruitment of chromatin-modifying complexes to a clade of flowering repressors that are silenced epigenetically via histone modifications. We also discuss the similarities and differences in vernalization between Arabidopsis and cereals.
INTRODUCTION

Like all organisms, species of flowering plants have evolved mechanisms to maximize their reproductive success. One component of optimizing this success is the proper timing of the transition from vegetative to reproductive growth. Proper timing is important for many reasons; the following are a few examples. A wide range of plants require cross-pollination for successful reproduction, and thus flowering must occur synchronously within individuals of the same species. For many plants, flowering must also occur at a time when pollinators are present. In many parts of the world, flowering must coincide with weather conditions that will permit flowers, which are relatively delicate structures, to develop properly, followed by conditions that will permit seeds to mature properly.

To ensure that flowering occurs within a particular season, plants rely primarily on environmental cues. One such cue is photoperiod, the relative change in the length of day and night that occurs throughout the course of a year. Plants that respond to lengthening days and flower in the spring or early summer are known as long-day (LD) plants. Short-day (SD) plants flower in the late summer or autumn in response to shortening days and lengthening nights (Thomas & Vince-Prue 1997). In temperate climates, many winter-annual, biennial, and perennial plants also use cold as an environmental cue to flower at the proper time of year. Certain plant species need to experience a period of winter cold to overcome a block to flowering. This occurs through a process known as vernalization. A requirement for vernalization is an adaptation to temperate climates that prevents flowering prior to winter and permits flowering in the favorable conditions of spring. Many vernalization-requiring plants are LD plants. A LD requirement coupled to a vernalization requirement further ensures that precocious flowering does not occur during the decreasing day lengths of fall and favors flowering as day length increases during spring.

The physiology of vernalization in a wide range of species has been intensively studied for many decades, and there are several comprehensive reviews of vernalization physiology (e.g., Bernier et al. 1981, Chouard 1960, Lang 1965). The effective temperature and the length of cold exposure required to achieve the vernalized state vary among different plant species and different varieties within a species. This variation is expected for a response that provides an adaptation to a range of different environmental niches. A vernalization response occurs only at temperatures around or above freezing; temperatures below freezing are not effective, which is not surprising because vernalization is an active process that requires changes in gene expression during cold exposure, as discussed below. The focus of this article is to discuss recent progress in understanding the molecular basis of vernalization. The link between classic physiological studies and more recent molecular genetic analyses have been reviewed elsewhere (Amasino 2004, Sung & Amasino 2005).
Floral Integrators and Environmental Sensing

Flowers result from the expression of regulatory genes known as floral meristem-identity genes, which specify that certain cells in the growing tips of the plant (the shoot apical meristems) differentiate into a floral meristem and ultimately form a flower (Coen & Meyerowitz 1991). Many of these genes are conserved throughout the plant kingdom including MADS-box transcription factors like APETALA1 (AP1), FRUITFUL (FUL), and LEAFY (LFY), which is a protein unique in the plant lineage (reviewed in Soltis et al. 2007). Upstream of the floral meristem-identity genes are a group of genes known as floral integrators such as FT and FD (Figure 1); i.e., the floral integrators are involved in the regulation of meristem-identity genes. The floral integrators are so named because their expression is in turn regulated by flowering pathways that sense environmental cues, such as photoperiod and cold, and/or developmental cues such as the levels of the hormone gibberellin; i.e., the regulation of these genes serves to integrate a range of environmental and developmental cues (Figure 1).

There is not a precise demarcation between a floral integrator and a meristem-identity gene, and a gene can have properties of both. For example, LFY is a key meristem-identity gene, but because it is activated directly by gibberellin (Figure 1) (Blazquez et al. 1998), it can also be considered a floral integrator. In this review, we discuss how the circuitry of photoperiod and vernalization pathways are connected to floral integrators in the well-studied model Arabidopsis thaliana.

Studies of the photoperiod control of flowering indicated the existence of a common floral stimulus in plants, sometimes referred to as florigen (Zeevaart 1976). Physiological studies revealed that the floral stimulus was produced in leaves exposed to inductive photoperiods and traveled to the meristem and caused flowering. Recent studies in Arabidopsis and rice have made a strong case that florigen, or at least a component of the floral stimulus, is the floral integrating factor FT. The FT gene is expressed in leaves, and the protein travels to the meristem where it interacts with another integrator, FD, to initiate the floral transition (reviewed in Turck et al. 2008, Zeevaart 2008). FT-like genes are ubiquitous in plants and have been found to regulate flowering in a variety of species including wheat and poplar (reviewed in Turck et al. 2008). [Note: Several genes discussed in this review such as FT, FD, FVE, FCA, FY, and FPA were given two- or three-letter designations but not longer names (Koornneef et al. 1991).]
There is also a significant level of conservation in the molecular mechanisms plants use to sense photoperiod. *Arabidopsis* is a facultative LD plant; i.e., it flowers most rapidly in LD, but it also flowers eventually in SD. In *Arabidopsis*, the perception of day length is a function of coincidence between light and expression of the circadian-regulated gene *CONSTANS (CO)* (reviewed in Turck et al. 2008). In LD-grown *Arabidopsis* plants, *CO* transcription extends into the daylength phase. Light stabilizes *CO* protein, which in turn leads to increased expression of *FT* (*Figure 1*) (Corbesier et al. 2007, Hepworth et al. 2002, Wenkel et al. 2006). The photoperiodic induction system, in which *CO* levels are affected by day length and translated into the regulation of *FT* (or *FT* homologs such as *Hd3a* in rice or *VRN3* in cereals), appears to be well conserved among flowering plants (reviewed in Turck et al. 2008).

Much of what we know about the molecular mechanism of the vernalization response comes from studies of *Arabidopsis* and temperate cereals. These studies of vernalization indicate that this process is not conserved at a biochemical level like the photoperiodic flowering pathway. However, as discussed in detail below, the floral integrator *FT/VRN3* is one of the targets of the vernalization pathway in both *Arabidopsis* and temperate cereals, and vernalization alleviates the repression of *FT/VRN3* expression. Thus, the control of *FT/VRN3* expression may be conserved as an integration point of the photoperiod and vernalization pathways.

In *Arabidopsis*, there are additional floral integrators including the MADS-box genes *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)*, *AGAMOUS-LIKE 19 (AGL19)*, and *AGL24* (Lee et al. 2000, Liu et al. 2008, Michaels et al. 2003, Samach et al. 2000, Schonrock et al. 2006). *FT* and *FD* partner to activate *SOC1* in the meristem, and *SOC1* expression is also controlled by vernalization, as discussed below. Thus, *SOC1* regulation integrates inputs from multiple flowering pathways (*Figure 1*). *SOC1* also functions with *FUL* to maintain the meristem in a floral state (i.e., as an inflorescence meristem) (Melzer et al. 2008), and therefore, *SOC1* can also be considered a meristem-identity gene. *SOC1* and *AGL24* positively regulate each other (Liu et al. 2008, Michaels et al. 2005), interact at the protein level, and are both required for proper activation of *LFY* (Lee et al. 2008). Indeed, there is an intricate network of positive feedback loops among floral integrators and meristem-identity genes to ensure that once flowering is initiated in *Arabidopsis*, it is maintained in the absence of environmental and developmental cues. It is beyond the scope of this review to discuss the details of these loops. Hereafter we focus on vernalization and note the connections to the integrators.

**Vernalization in Arabidopsis**

The identification of genes involved in the vernalization response in *Arabidopsis* began with the study of natural variation in flowering found among different isolates collected from a range of locations worldwide. Most commonly used lab strains of *Arabidopsis* do not require vernalization to flower rapidly; however, many isolates flower very late unless first vernalized (Burn et al. 1993, Clarke & Dean 1994, Lee et al. 1993, Napp-Zinn 1987). The rapid-flowering types are sometimes referred to as summer annuals, which indicates that their life cycle is likely to be completed in one growing season. Plants with a strong vernalization requirement are often called winter annuals, because they may not complete their life cycle until the second growing season after an intervening winter. However, the actual life cycle in nature is clearly influenced by environment as well as genotype; in one environment a genotype might take two seasons to flower, whereas in a different environment the same genotype might flower in a single season (e.g., Wilczek et al. 2009).

In *Arabidopsis*, studies of natural variation demonstrated that the vernalization requirement is conferred by two dominant genes, *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC)* (Koornneef et al. 1994, Lee et al. 1994). *FRI* encodes a nuclear protein found only in plants (Johanson et al. 2000) that may interact...
with the mRNA cap-binding protein to regulate the level of FLC mRNA (Geraldo et al. 2009). FLC encodes a MADS-box DNA binding protein that functions as a repressor of flowering (Michaels & Amasino 1999, Sheldon et al. 1999). Genetic studies have shown that in the context of vernalization FRI acts solely to up-regulate FLC (Michaels & Amasino 2001).

The level of FLC expression is the primary determinant of the vernalization requirement in Arabidopsis. FLC represses flowering, in part, by repressing expression of floral integrators such as FT, FD, and SOC1 (Hellwell et al. 2006, Hepworth et al. 2002, Lee et al. 2000, Samach et al. 2000, Searle et al. 2006). FLC binds to promoter regions of SOC1 and FD and to the first intron of FT (Searle et al. 2006). This binding likely attenuates the ability of the photoperiod pathway to activate these integrators. Indeed, the expression of integrators from constitutive promoters bypasses the repressive effects of FLC and the vernalization requirement (Lee et al. 2000, Michaels et al. 2005).

There are many examples of MADS-box proteins acting in multimeric complexes with other MADS-box proteins (de Folter et al. 2005, Honma & Goto 2001, Pelaz et al. 2000). Recent studies show that FLC interacts directly with another MADS-box protein SHORT VEGETATIVE PHASE (SVP) (Fujinawa et al. 2008, Li et al. 2008). Genetic studies indicate that this interaction is biologically relevant: loss of SVP provides partial suppression of the FLC-mediated delay in flowering (Li et al. 2008). However, the loss of SVP cannot fully suppress the FLC-mediated delay of flowering, which indicates that FLC may redundantly interact with other MADS-box proteins. Although SVP and other interaction partners may be critical for FLC-mediated repression of flowering, SVP levels or activity do not appear to be involved in the regulation of flowering like FLC. For example, SVP mRNA levels are not affected by vernalization, whereas vernalization results in the stable repression of FLC (Bastow et al. 2004, Michaels & Amasino 1999, Sheldon et al. 1999, Sung & Amasino 2004). Moreover, overexpression of FLC alone is sufficient to cause extremely late flowering (Michaels & Amasino 2001), which indicates that the levels of binding partners are not limiting in vivo.

The circadian clock has an influence on SVP levels; SVP protein accumulates to higher levels in a double mutant of two clock components, LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (C1), than in wild type (Fujinawa et al. 2008). Thus, there may be cross talk in the circuitry between the photoperiod pathway and FLC-dependent repression in Arabidopsis; however, no effect of different photoperiods on SVP levels has been reported.

The Autonomous Pathway

Most commonly used types of Arabidopsis are null mutants for FRI (e.g., Columbia, Landsberg, Wassilewskija) and thus do not require vernalization for rapid flowering. Historically, a group of late-flowering mutants derived from such rapid-flowering accessions have been referred to as the autonomous pathway (AP) of floral promotion (Henderson & Dean 2004). AP mutants are characterized by delayed flowering in both LD and SD, which is in contrast to photoperiod-pathway mutants that show delayed flowering in inductive LD photoperiods only. The flowering behavior of AP mutants is similar to FRI-containing winter-annual accessions (i.e., both have strong FLC expression, are late flowering, and require vernalization for rapid flowering) (Michaels & Amasino 2001). To date, eight AP genes have been identified: LUMINIDEPENDENS (LD), FCA, FY, FPA, FLOWERING LOCUS D (FLD), FVE, FLK, and REF6 (Noh et al. 2004, Simpson 2004). FCA, FPA, FY, and FLD encode proteins that are predicted to be involved in RNA metabolism (Lim et al. 2004, Macknight et al. 1997, Schomberg et al. 2001, Simpson et al. 2003); however, there is no evidence to suggest that these components directly interact with FLC mRNA. FVE, FLD, and REF6 have domains common to chromatin-modifying components. FVE (also known as MSH4) is a member of the...
MSI1-like protein family (MSI1–MSI5) in *Arabidopsis* (Ausin et al. 2004, Kim et al. 2004); MSI proteins are found in several chromatin-modifying complexes in eukaryotes (Hennig et al. 2005). *FLD* and *REF6* are predicted to encode two different types of histone demethylases (He et al. 2003, Jiang et al. 2007, Noh et al. 2004).

The repression of flowering by AP genes acts primarily through *FLC* because *flc* null mutants completely suppress the delayed-flowering phenotypes of AP mutants (Michaels & Amasino 2001). Recent studies demonstrate that many AP genes are also involved in the regulation of other genes that are not involved in flowering (Baurle et al. 2007, Veley & Michaels 2008). Indeed, most of the AP genes do not appear to be specific to flowering, nor do they comprise a pathway in the typical sense. Instead, these genes are involved in a range of repressive mechanisms that act on *FLC* and other genes. For example, the RNA-binding proteins FPA and FCA are broadly required for RNA-mediated silencing, which raises the possibility that their role in *FLC* repression involves RNA-mediated silencing (Baurle et al. 2007). In addition, all tested AP mutants showed derepression of certain transposons (Baurle et al. 2007, Veley & Michaels 2008), and double mutant analysis among the AP mutants revealed a wide range of nonflowering phenotypes (Veley & Michaels 2008). It is interesting to note that genetic analysis showed FCA requires FVE for the repression of certain transposons (Baurle & Dean 2008), whereas *FLD* is necessary for FCA to repress *FLC* (Liu et al. 2007). Thus, there appears to be a range of mechanisms and combinatorial interactions by which AP genes affect gene expression.

Recent work provides additional support for the involvement of RNA-mediated silencing in *FLC* repression. Double mutants of *dicer-like 1* and *dicer-like 3* (*dll*/3) have phenotypes similar to that of AP mutants: elevated levels of *FLC* expression and delayed flowering that is suppressed by both *flc* mutations and vernalization (Schmitz et al. 2007). Because late flowering in *dll*/3 and AP mutants is overcome by vernalization, *FLC* repression by these genes occurs via a vernalization-independent mechanism. Perhaps RNA-mediated silencing plays a role in setting the prevernalization basal level of *FLC* expression.

Notably, there is an intermediate-size, noncoding RNA that arises from beyond 3’UTR of *FLC* (Swiezewski et al. 2007). Mutations in the region of this RNA have a small effect on flowering relative to *dll*/3 or other AP mutants, and the relationship of this RNA to other flowering pathways is not known.

**Vernalization as an Epigenetic Switch**

Chouard (1960) provided a useful definition of vernalization as “the acquisition or acceleration of the ability to flower by a chilling treatment.” As defined, vernalization does not necessarily induce flowering. Rather, prolonged chilling provides competence to flower. As noted above, in many vernalization-requiring plant species, other endogenous and/or environmental conditions, such as inductive photoperiods, are also required for flowering. A classic experiment illustrates that this competence to flower can be stable through mitotic cell divisions. Lang and Melchers used a biennial variety of *Hyoscyamus niger* (henbane) that requires both vernalization and inductive photoperiods (LD) to initiate flowering (reviewed by Lang 1965). When plants are first exposed to cold (vernalized) and then kept in a noninductive photoperiod (SD) in a normal, warm growth temperature, the plants grow vegetatively but do not flower. However, when the previously vernalized plants are moved to inductive photoperiods, even after many months in noninductive conditions, flowering occurs. Thus, the vernalization-mediated change in competence is stable through a large number of mitotic divisions in the apical meristem. Mitotic stability in the absence of the inducting signal (cold in the case of vernalization) is arguably an epigenetic switch (e.g., Amasino 2004, Dennis & Peacock 2007, Wu & Morris 2001). The molecular basis of competence in *Arabidopsis* is the mitotically stable repression of *FLC* and some *FLC* relatives discussed below.
It is worth noting that not all plant species exhibit behavior that fits the stable acquisition of competence model described above; for example, there are species that need to flower during cold exposure presumably because competence is not stably maintained through mitotic cell divisions after cold exposure ceases (Bernier et al. 1981). Indeed, there are mutants in *Arabidopsis* in which *FLC* repression occurs during cold exposure but it is not stably maintained upon a return to warm; not surprisingly, these mutants affect chromatin-modifying proteins (Gendall et al. 2001, Levy et al. 2002, Mylne et al. 2006, Sung et al. 2006a).

**Genetic Analysis of the Vernalization Response**

As discussed above, winter-annual accessions of *Arabidopsis* exhibit a delayed-flowering phenotype if they are not vernalized, and most of the natural variation in the vernalization requirement is due to allelic variation at *FRI* and *FLC*. The molecular mechanism by which *FLC* is epigenetically repressed by vernalization in *Arabidopsis* has been addressed by molecular genetic analyses. Using winter-annual accessions as a parental line, one can screen for mutants that can no longer be vernalized; such vernalization-insensitive mutants have lesions in genes that participate in the vernalization pathway. To date, these screens have revealed five genes: *VERNALIZATION 1* (?VRN1), *VRN2*, *VERNALIZATION INSENSITIVE 3* (?VIN3), ?VRN5/ ?VIN3-LIKE 1 (?VIL1), and at?PRMT5 (Bastow et al. 2004, Gendall et al. 2001, Greb et al. 2007, Schmitz et al. 2008, Sung & Amasino 2004, Sung et al. 2006b). As expected, given the nature of the genetic screen, all mutants fail to stably repress *FLC* by vernalization. All of these genes are constitutively expressed except for ?VIN3. ?VIN3 is expressed specifically during a vernalizing cold treatment, and expression is completely abolished when plants are returned to a warm temperature (Sung & Amasino 2004). The cold induction and transient nature of ?VIN3 expression indicates that ?VIN3 may be a part of the trigger to set in motion the molecular events that stably repress *FLC* during vernalization.

**Vernalization-Mediated Changes in FLC Chromatin**

The stable nature of the vernalized state is consistent with a role for chromatin modification of target genes. In *Arabidopsis*, *FLC* repression is the molecular basis of vernalization-induced competence. Thus, *FLC* is a likely target for chromatin modification. The first molecular evidence for chromatin modification in *Arabidopsis* vernalization came from the identification of ?VRN2 and ?VIN3. ?VRN2 encodes a homolog of Suppressor of zeste (Su(z)12), a component of Polycomb repression complex 2 (PRC2) that was first identified in animals (Gendall et al. 2001). ?VIN3 encodes a PHD (plant homeodomain) protein (Sung & Amasino 2004); the PHD domain is commonly found in a wide range of complexes that are involved in chromatin-level regulation (Mellor 2006).

Vernalization results in an increase in two repressive histone modifications at *FLC* chromatin (**Figure 2**): histone H3 Lys 9 (H3K9) and histone H3 Lys 27 (H3K27) methylation (Bastow et al. 2004, Sung & Amasino 2004). In ?vin3 and ?vrn2 mutants, H3K9 and H3K27 methylation are not enriched at *FLC* following a sufficient cold treatment, and *FLC* expression is not repressed (Bastow et al. 2004, Sung & Amasino 2004). The loss of ?VRN1, which encodes a DNA-binding protein containing two plant-specific B3 domains, shows distinctive chromatin modifications from those of ?vin3 and ?vrn2 mutants. Whereas methylation of both H3K9 and H3K27 are affected in ?vin3 and ?vrn2 mutants, only H3K9 methylation fails to occur in ?vrn1 during and after vernalization (Bastow et al. 2004, Sung & Amasino 2004), which suggests the two methylation events can occur independently and that ?VRN1 is involved specifically in H3K9 methylation (**Figure 2**).

?VIL1/VRN5 was identified independently by a yeast two-hybrid screen for ?VIN3-interacting proteins (Sung et al. 2006b) and by a
Figure 2
Vernalization-mediated changes in FLC chromatin. (a) Prior to cold exposure, FLC is actively expressed. The complexes that maintain this active chromatin conformation include the PAF complex, which methylates histone 3 tails at lysine 4 and 36 (H3K4triMe and H3K36triMe), a SWR1-like complex, which deposits a histone 2A variant in the nucleosomes of FLC chromatin, and H2B ubiquitinases like HUB1 and HUB2 that ubiquitinate histone 2B tails (H2Bub1). Although FLC is in an active state, there are repressive complexes present such as Polycomb Repression Complex 2 and some degree of lysine 27 methylation of histone 3 (H3K27triMe—a repressive modification) (b) During cold exposure, FLC repression is initiated. VIN3 is induced, VIN3 and VIL1/VRN5 associate with the Polycomb complex, the density of repressive chromatin modifications such as lysine 27 methylation of histone 3 increases, and repressors such as LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) assemble on FLC chromatin. (c) As vernalization proceeds, the density of repressive modifications, particularly H3K27triMe and lysine 9 methylation of histone 3 [H3K9triMe; mediated by an unknown H3K9 methyltransferase (HMTase)] increases. (d) Eventually, a mitotically stable state of repression that no longer requires VIN3 is achieved. This mitotically stable state is likely to involve positive feedback loops in which the repressive chromatin modifications serve to recruit the chromatin-modifying complexes including VRN1 to maintain a repressive state. As the FLC locus passes to the next generation, the active chromatin state represented in (a) is re-established.
step, but how this translates into a change in the activities of chromatin-modifying complexes at the FLC locus is not at all understood. Even if the PHD domain of VIN3 binds to specific histone modifications characteristic of FLC in the pre-vernalized state (see below), such modifications are not likely to be unique to FLC chromatin. Clearly, there is much to learn about targeting specificity.

In addition to vernalization-mediated methylation at H3K9 and H3K27 at FLC chromatin, histone arginine methylation also plays a role in maintaining stable repression of FLC (Schmitz et al. 2008). Mutations in a Type II protein arginine methyltransferase gene atPRMT5, reduced the vernalization response. atPRMT5 is required for the symmetric methylation of Arg 3 of histone H4 (sMeH4R3), and the level of this methylation is increased at FLC chromatin by vernalization. Furthermore, sMeH4R3 appears to be required for the vernalization-mediated enrichment of both H3K9 and H3K27 methylation (Schmitz et al. 2008).

Polycomb Repression Complexes and Vernalization-Mediated FLC Repression

As discussed above, the identification of an Arabidopsis homolog of Su(z)12, VRN2, in a vernalization-insensitive mutant screen suggested the involvement of a PRC2-like complex in FLC repression (Gendall et al. 2001). In animals, there are two distinct PRC complexes, PRC2 and PRC1 (reviewed in Schuettengruber et al. 2007). Polycomb group (PcG) proteins were first identified in Drosophila as essential components for maintaining HOMEOTIC (HOX) genes in a repressed state. The PRC2 complex is involved in the initial di- or trimethylation of H3K27 at target chromatin. Then, in many systems, methylated H3K27 is bound by the PRC1 complex, which acts to maintain chromatin in a repressed state and to maintain H3K27 methylation through mitotic cell divisions (Cao et al. 2002, Muller et al. 2002). Core components of PRC2 are well conserved from plants to animals (Hsieh et al. 2003), but, as discussed below, PRC1 components are not.

In Drosophila, core components of PRC2 consist of Enhancer of zeste [E(z)], Extra Sex Combs (ESC), and Suppressor of zeste Su(z)12 (Cao et al. 2002). Arabidopsis and other plants contain multiple copies of many PRC2 components (Hsieh et al. 2003, Wood et al. 2006). For example, in Arabidopsis, there are three homologs of the histone methyltransferase E(z). These include CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA) (Hsieh et al. 2003). CLF was first identified as a repressor of the floral homeotic gene AGAMOUS (AG) (Goodrich et al. 1997), whereas MEA was identified in a screen for mutants that have altered endosperm development (Grossniklaus et al. 1998). SWN is partially redundant with CLF (Chanvivattana et al. 2004). None of the Arabidopsis E(z) homologs were identified in screens for vernalization-insensitive mutants, which is likely due to the functional redundancy among these proteins with regard to FLC repression. In fact, VRN2 is the only PRC2 component identified as being involved in vernalization from mutant screens.

Biochemical approaches revealed that Arabidopsis PRC2 complexes include VIN3 during cold exposure (De Lucia et al. 2008, Wood et al. 2006). PRC2 complexes in Arabidopsis can also include VIL1/VRN5; purification of proteins associated with a VIL1/VRN5-TAP fusion revealed components of PRC2, which include VRN2, SWN, MSI1, and FERTILIZATION INDEPENDENT ENDOSPERM (FIE; an Arabidopsis ESC homolog) (De Lucia et al. 2008). The theme of PHD domain-containing proteins associating with PRC2 may be widespread in eukaryotes; PHD–domain proteins also associate with PRC2 in Drosophila and humans (Cao et al. 2008a, Nekrasov et al. 2007, Sarma et al. 2008).

Analyses of the occupancy of PRC2 components at FLC chromatin showed that VRN2 is already present at FLC chromatin prior to vernalization (De Lucia et al. 2008). In cold, VIN3 is induced and VIN3 protein is present at
a region encoding the first intron of FLC. Association of VIL1/VRN5 is VIN3-dependent, and thus VIN3 may serve as a guiding factor for the recruitment of VIL1/VRN5 to FLC (De Lucia et al. 2008). Whereas the enrichment of VIL1/VRN5 is restricted to the first intron of FLC in cold temperatures, VIL1/VRN5 spreads throughout the FLC chromatin when plants return to warm temperatures. This spreading may require warm temperatures, or warm temperatures may simply increase the rate of these chromatin changes. Presumably, the spreading of VIL1/VRN5 across FLC chromatin maintains the repressed state of FLC (Figure 2) (De Lucia et al. 2008).

Although the presence of PRC2 components in plants is well established, Arabidopsis lacks components with an overall similarity to PRC1 components (Hsieh et al. 2003, Schubert et al. 2005). Instead, LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) likely binds to and stably maintains the PRC2-mediated modified histones at FLC following vernalization (Mylne et al. 2006, Sung et al. 2006a). In lhp1 mutants, the active histone mark, H3K4triMe, is only transiently reduced when plants are kept in cold (Sung et al. 2006a). Interestingly, lhp1 mutants were not found in screens for vernalization-insensitive mutants because the loss of LHP1 also results in the derepression of flowering promoters such as FT, and thus lhp1 mutants are early flowering without vernalization (Kotake et al. 2003, Sung et al. 2006a). LHP1 is likely to play a broad role in gene repression in plants and may be part of a protein complex that serves a similar role to that of PRC1 in animals.

**Activation of FLC Is Associated with Specific Chromatin Modifications**

As discussed above, FLC repression is associated with dynamic changes in histone composition, which are initiated during prolonged cold exposure. Conversely, the high levels of FLC that create a vernalization requirement are associated with active histone marks. Mutant screens for the loss of the vernalization requirement (i.e., plants that flower early without cold) in winter-annual Arabidopsis have revealed many of the components required for FLC activation.

H3K4triMe is associated with active chromatin in most eukaryotes (Schneider et al. 2004). A specific Saccharomyces cerevisiae HMTase, Set1, is responsible for mono- to trimethylation of H3K4, and mutations in set1 cause diverse phenotypic defects such as slow growth and rDNA derepression (Briggs et al. 2001, Fingerman et al. 2005). Yeast Set1 is an essential component of a complex called COMPASS (complex proteins associated with Set1) (Krogan et al. 2003). Another complex, the RNA polymerase II-Associated Factor 1 (PAF1)-containing complex, is necessary for H3K4triMe enrichment at target chromatin. These two complexes, COMPASS and PAF1, physically associate to coordinate the transcription of target genes (Krogan et al. 2003).

Screens for rapidly flowering mutants identified two Arabidopsis homologs of the yeast PAF1 complex components PAF1 and CTR9: EARLY FLOWERING 7 (ELF7) and ELF8, respectively (He & Amasino 2005). ELF7 and ELF8 are required for high FLC expression and for H3K4triMe enrichment at FLC chromatin (He et al. 2004). Other Arabidopsis genes that encode relatives of PAF1 and COMPASS complex components are VERNALIZATION INDEPENDENCE 3 (VIP3, Arabidopsis homolog of human bSki8), VIP4 (Arabidopsis homolog of yeast Leo1), VIP5 (Arabidopsis homolog of yeast Rtf1), ARABIDOPSIS TRITHORAX-LIKE 1 (ATX1), and ATX2 (see below for roles of ATX1 and ATX2) (Alvarez-Venegas et al. 2003, He et al. 2004, Oh et al. 2004, Pien et al. 2008, Saleh et al. 2008, Zhang et al. 2003, Zhang & van Nocker 2002). Mutations in these genes typically cause reduced H3K4triMe deposition at FLC chromatin and early-flowering phenotypes similar to elf7 and elf8 (Alvarez-Venegas et al. 2003, He et al. 2004, Pien et al. 2008, Saleh et al. 2008, Zhang & van Nocker 2002). Methylation of H3K4 at FLC chromatin appears to be mediated by ATX1 and ATX2, which are homologous to Drosophila Trithorax (Trx). Trx is a H3K4
methyltransferase that belongs to the Trithorax group of proteins (TrxG), which are activators of Drosophila homeotic genes (a role opposite to that of PcG proteins) (Pien et al. 2008, Saleh et al. 2008). ATX1 mediates trimethylation on H3K4, whereas ATX2 dimethylates H3K4 in vitro (Saleh et al. 2008). The involvement of TrxG- and PcG-like genes in the regulation of FLC indicates that FLC is controlled by an evolutionarily conserved mechanism that involves a dynamic balance between PcG and TrxG. A recent study reveals that this dynamic balance occurs throughout the genome in plants (Oh et al. 2008).

In Drosophila, TrxG activator proteins as well as components of PRC2 repressive complexes are constitutively bound to HOX target chromatin; i.e., both repressive and activating modifiers can reside at the same locus (Papp & Muller 2006). As discussed above, PRC2 is detectable at FLC chromatin prior to vernalization (De Lucia et al. 2008). Furthermore, H3K27triMe is present at FLC chromatin prior to vernalization when FLC is actively transcribed, but the level of H3K27triMe is much lower than when FLC is repressed (Pien et al. 2008). This indicates that a threshold level of H3K27triMe enrichment is necessary to establish repressed FLC chromatin.

Another Trx-like SET domain protein is also required for proper FLC activation. EARLY FLOWERING IN SHORT DAYS (EFS) encodes a SET domain protein, which mediates di- and trimethylation of histone H3 Lys 36 (H3K36) on target chromatin (Xu et al. 2008). Mutants with EFS lesions exhibit reduced FLC expression and early flowering similar to mutations in PAF1-COMPASS components (Kim et al. 2005, Xu et al. 2008, Zhao et al. 2005). In winter-annual strains of Arabidopsis, EFS may also be required for the enrichment of H3K4triMe at FLC chromatin (Kim et al. 2005). Thus, the three Arabidopsis HMTases, ATX1, ATX2, and EFS, appear to act coordinately to mediate the transcriptional activation of FLC.

Monoubiquitination at lysine 123 of Histone H2B (H2Bub1), like H3K4, is a histone modification associated with active transcription. In yeast, a complex that contains RAD6 (with E2-ubiquitin-conjugating activity) and BRE1 (with E3-ubiquitin ligase activity) acts to monoubiquitinate histone H2B at specific target chromatin (Tennen & Shilatifard 2005). H2Bub1 is an important prerequisite for the proper enrichment of H3K4triMe and for transcriptional activity of target genes (Wood et al. 2003). In Arabidopsis, H2B monoubiquitination of FLC chromatin is also required for proper activation of FLC. Investigators identified two Arabidopsis BRE1 homologs, HISTONE MONOUBIQUITINATION 1 (HUB1) and HISTONE MONOUBIQUITINATION 2 (HUB2) (Cao et al. 2008b, Gu et al. 2009), and found that mutations in either hub1 or hub2 result in early flowering and the loss of H3K4triMe enrichment at the promoter region of FLC. Arabidopsis has three RAD6 homologs: UBIQUITIN-CONJUGATING ENZYME 1, 2, and 3 (AtUBC1, AtUBC2, and AtUBC3) (Cao et al. 2008b, Gu et al. 2009). AtUBC1 and AtUBC2 are involved in flowering and act redundantly for the enrichment of H2Bub1 at FLC chromatin, whereas AtUBC3 does not play a role in FLC activation (Xu et al. 2009). Although the enrichment of H2Bub1 is required for the proper activation of FLC, over-enrichment of H2Bub1 results in the failure of FLC activation; a mutation in a H2B deubiquitinase, UBIQUITIN-SPECIFIC PROTEASE26 (UBP26), results in a rapid-flowering phenotype due to the loss of FLC expression (Schmitz et al. 2009). Interestingly, ubp26 mutants have a reduced level of H3K36 trimethylation at FLC chromatin without altered enrichment of H3K4triMe (Schmitz et al. 2009). Thus, altered levels of H2Bub1 at FLC chromatin result in a phenotype similar to atx1, atx2, and efs mutants, which indicates the presence of an intricate regulatory loop among histone modifiers in FLC activation.

In addition to modifications of histone proteins, the exchange of certain histone variants also plays a role in achieving proper levels of FLC expression. The replacement of H2A with its variant H2AZ is mediated by the SWR1
Generational Resetting of FLC

Although FLC repression in Arabidopsis is stably maintained throughout mitotic cell divisions following vernalization, FLC is reactivated at some point as the locus is passed to the next generation. This reactivation re-establishes the requirement for vernalization (Figure 2). The resetting of FLC expression in each generation distinguishes this type of epigenetic repression from heritable (i.e., not reset) epigenetic silencing that involves DNA methylation and small interfering RNAs (siRNAs) (e.g., Henderson & Jacobsen 2007). Changes in DNA methylation do not appear to be involved in the resetting of FLC expression (Finnegan et al. 2005).

Studies of expression of a vernalized FLC locus can determine the time by which FLC resetting must have occurred (Sheldon et al. 2008, Choi et al. 2009). However, it is important to note that a locus can be reset prior to actual expression; for example, key chromatin changes can occur before the transcription factors necessary for expression are present. Expression studies reveal that FLC is transiently expressed during male gametogenesis (Sheldon et al. 2008), but there is no expression during female gametogenesis (Sheldon et al. 2008, Choi et al. 2009). Whether this transient expression during male gametogenesis represents resetting or a transient state in which there is some expression of repressed gene remains to be determined. In the next generation, FLC is clearly re-expressed in the mid- to late stages of embryonic development (i.e., in the early stages of the next generation) (Sheldon et al. 2008, Choi et al. 2009). Interestingly, the earliest stages of FLC expression during early embryogenesis are dependent on the presence of PIE1 but not on the presence of FRI; however, later maintenance of FLC expression requires both FRI and PIE1 (Choi et al. 2009). Perhaps reduced levels of some of the key players in FLC repression in pollen, including VRN1 and LHP1, may contribute to the reactivation of FLC during gametogenesis (Mylne et al. 2006). Indeed, a majority of FLC regulators, which includes FLC activators as well as repressors, are poorly expressed in pollen (Choi et al. 2009). Perhaps any FLC reactivation mechanism that occurs in male gametogenesis might be expected to apply to the female gamete as well. The mechanism of FLC resetting will be an interesting area to explore.

Other Targets of Vernalization in Arabidopsis

In Arabidopsis there are five paralogs of FLC (often called the FLC clade) FLOWERING LOCUS M (FLM)/MADS AFFECTING FLOWERING 1 (MAF1), MAF2, MAF3, MAF4, and MAF5. FLM/MAF1, MAF2, and MAF4 act as floral repressors (Ratcliffe et al. 2001, 2003; Scortechi et al. 2001). It is likely that these proteins repress the expression of floral integrators in a manner that is biochemically redundant with that of FLC (Figure 1).
In the presence of FRI, FLC provides the majority of the repressive activity, but in rapid-flowering lines that lack FRI, other clade members such as FLMAFI can provide a greater amount of repression than FLC under certain environmental conditions (Ratcliffe et al. 2001, Scortecchi et al. 2001, Werner et al. 2005). Likewise, when FRI is present, the repression of FLC accounts for the majority of the vernalization response. However, vernalization clearly promotes flowering in flc null mutants in SD. This vernalization response has been referred to as an FLC-independent vernalization pathway (Michaels & Amasino 2001). The repression of FLC paralogs is likely to account for some, and possibly all, of the vernalization response that remains in flc null mutants. Given the presumably functionally overlapping FLC paralogs, it is perhaps simpler to think of vernalization as repressing FLC and several other FLC-like genes rather than being comprised of FLC-dependent and independent pathways. Indeed, at least one other member of the FLC clade, FLMAFI, undergoes the same VIN3-dependent, vernalization-mediated chromatin changes as FLC (Sung et al. 2006b). Whether there is an effect of vernalization in Arabidopsis that is independent of repression of all members of the FLC clade remains to be determined. For example, it will be interesting to determine whether the induction of genes such as AGL19 (Alexandre & Henning 2008, Schonrock et al. 2006) or AGL24 (Michaels et al. 2003) by vernalization is independent of not only FLC but of other FLC clade members as well.

Vernalization in Cereals

The cereals of the grass subfamily Pooidae, particularly wheat and barley, are the only other group of plants in which vernalization has been characterized molecularly. There are winter varieties of wheat and barley that possess a clear vernalization requirement and spring varieties that flower without vernalization. As in Arabidopsis, the genetic differences between winter and spring varieties have been explored, and genes responsible for the winter/spring difference have been cloned (reviewed in Colasanti & Coneva 2009, Distelfeld et al. 2009, Trevaskis et al. 2007). As discussed below, although some aspects of the circuitry of the vernalization pathway are similar in Arabidopsis and cereals, the differences indicate that the respective vernalization pathways evolved independently. Genetic studies comparing winter and spring cultivars of wheat and barley have identified three loci that play a role in the vernalization response: VRN1, VRN2, and VRN3 (Figure 3). VRN1 and VRN2 are not homologous to the Arabidopsis genes with the same name. VRN1 encodes a MADS-box transcription factor that resembles the Arabidopsis floral meristem-identity genes AP1 and FUL.
Constitutive expression of VRN3, the wheat/barley homolog of FT, bypasses the vernalization requirement (Yan et al. 2006), just as the constitutive expression of VRN3, which is the Arabidopsis VIN3-like gene, activates VRN3 (Turner et al. 2005). VRN3, in turn, is also induced by a vernalizing environment (Li & Dubcovsky 2008). VRN2, as noted above, acts to repress the expression of VRN3 under LD conditions. This repression prevents the activation of VRN3 by PPD1 and establishes a requirement for vernalization in LD (Hemming et al. 2008). VRN2 expression, like that of FLC, is repressed by cold (Yan et al. 2004).

As discussed above, the induction of VIN3 by a long exposure to cold is key to FLC repression in Arabidopsis. In wheat, prolonged cold elevates the expression level of VIN3-like genes; however, the change in expression is not as defined as that seen for Arabidopsis VIN3, and none of the wheat homologs to date have been linked to the control of flowering time (Fu et al. 2007). In cereals, VRN1 is also induced by a vernalizing cold exposure, and VRN1 acts to repress VRN2 expression (Loukoianov et al. 2005, Trevaskis et al. 2006). Thus, VRN1 serves a similar role to VIN3 in cereal vernalization, but that does not rule out the existence of other components that play a VIN3-like role in cereals.

Thus, there are three general similarities in the vernalization circuitry between cereals and Arabidopsis: (a) a block to flowering results from a repressor (FLC in Arabidopsis or VRN2 in wheat) that represses a homologous floral integrator in both groups of plants (FT/VRN3), (b) the repressors of flowering (FLC or VRN2) are downregulated in the cold, which permits FT/VRN3 to be expressed, and (c) downregulation of FLC and VRN2 is mediated by upregulation in the cold of VIN3 and VRN1 in Arabidopsis and cereals, respectively (Figures 1 and 3).

There are, however, some distinct differences in both the vernalization circuitry and components between Arabidopsis and cereals. As discussed above, the repressors (FLC and VRN2) are not related proteins. With respect to circuitry, the VRN1 gene in cereals plays a dual role in the regulation of flowering. VRN1 is both a promoter of flowering downstream of VRN3 and a cold-activated repressor upstream of VRN2. This dual role of VRN1 in cereals creates a situation not found in Arabidopsis: a positive flowering feedback loop that involves vernalization components (Figure 3). Such a positive feedback loop ensures that once flowering initiates, it continues in the absence of environmental cues. The regulation of flowering in Arabidopsis also incorporates positive feedback loops as discussed above, but these involve components that are downstream of vernalization such as the reciprocal reinforcement of LFY and API expression (Figure 4) (Sablowski 2007).

Another circuitry difference is the convergence of photoperiod and vernalization on the floral repressor VRN2 in cereals (Figure 3). In addition to being repressed by cold, VRN2 expression is also governed by day length. In SD, VRN2 expression is greatly reduced, and this reduction occurs rapidly upon transfer of LD-grown plants to SD (Dubcovsky et al. 2006, Trevaskis et al. 2006). Cold and SD appear to repress VRN2 by distinct mechanisms. VRN2 repression via cold is mediated by VRN1, but VRN1 is not expressed in SD (Dubcovsky et al. 2006, Trevaskis et al. 2006). Wheat and barley are LD plants, so even though SD removes VRN2, LD is still required to activate VRN3. SD and cold are indicators of winter, and cereals can apparently use both as cues to remove a
block to flowering, whereas in *Arabidopsis* only cold is a winter cue. Grasses such as wheat and barley may have evolved in a region where winters can be too mild to induce floral competence. Thus, there may be adaptive value in linking a photoperiod response with vernalization because, regardless of severity, winter always coincides with short days.

That vernalization alleviates repression of the homologous genes *FT* in *Arabidopsis* and *VRN3* in cereals is perhaps not surprising. As discussed above, *FT*-like genes are ubiquitous in plants and have been found to initiate the transition to flowering in all angiosperms examined (Turck et al. 2008). Because the role of *FT/VRN3* homologs as a key flowering initiator is conserved among flowering plants, it makes a logical target for the repression of flowering; i.e., selection for this target may be an example of convergent evolution in cereals and *Arabidopsis*.

It is important to note that vernalization has been more thoroughly studied in *Arabidopsis* than in cereals. As more of the molecular components of vernalization in grasses are identified, it is possible that additional similarities between the vernalization processes in these two groups may be revealed.

**FUTURE DIRECTIONS**

In cereals and *Arabidopsis*, the only two systems in which vernalization has been studied at a molecular level, the first known molecular event of vernalization is the induction of genes by cold. Upstream of these genes there must be a biochemical process that serves as a cold sensor. The mechanisms of cold sensing and subsequent gene activation are not known. There are a variety of possibilities for how cold sensing might operate during vernalization [see Sung & Amasino (2005) for a partial list], but in the absence of data these possibilities remain speculative. Indeed, little is known about the molecular basis of any cold-induced process in plants. A challenge for the future will be to understand how plants sense cold.

Another challenge will be to explore the range of vernalization mechanisms that exist in flowering plants. There are some striking differences between the circuitry and components of vernalization in *Arabidopsis* and cereals. Perhaps the lack of conservation in vernalization pathways compared with the photoperiod pathway is not surprising. Flowering plants began to diversify less than 200 mya (Solds et al. 2008) when the climate was warmer and the locations of continents were quite different than at present. The major groups of angiosperms arose before continental drift, and a changing climate created environments in which a vernalization response would have had adaptive value. In contrast, evolving a mechanism to sense photoperiod would have had adaptive value much earlier as seasonal changes in day length occur nearly everywhere. Moreover, the molecular bases of other long-term cold responses such as bud dormancy remain to be determined. In many perennials, buds become dormant in the fall season and, once dormant, buds of many species must be exposed to a long period of cold before they become competent to exit dormancy and resume growth (e.g., Chouard 1960). Exploring the cold response pathways in a range of species will hopefully provide insight into the molecular mechanisms that plants have evolved to coordinate their life cycles with the changing seasons.

**DISCLOSURE STATEMENT**

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

**ACKNOWLEDGMENTS**

R.M.A. is grateful to the National Institutes of Health, the National Science Foundation, the U.S. Department of Agriculture National Research Initiative Competitive Grants Program, the
College of Agricultural and Life Sciences, and the Graduate School of the University of Wisconsin for their generous support of our flowering research. S.S. is grateful to the College of Natural Sciences and the Institute for Cellular and Molecular Biology of the University of Texas for their generous start-up support. We apologize to those in the flowering field whose work was not cited due to length limits.

LITERATURE CITED


Koornneef M, Blankestein-de Vries H, Hanhart C, Soppe W, Peeters T. 1994. The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg erecta wild-type. *Plant J.* 6:911–19
Martin-Trillo M, Lazaro A, Poethig RS, Gomez-Mena C, Pineiro MA, et al. 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 133:1241–52
Mellor J. 2006. It takes a PHD to read the histone code. Cell 126:22–24

Schmitz RJ, Hong L, Fitzpatrick KE, Amasino RM. 2007. DICER-LIKE 1 and DICER-LIKE 3 redundantly


Arabidopsis

Sablowski R. 2007. Flowering and determinacy in

Ratcliffe OJ, Nadzan GC, Reuber TL, Riechmann JL. 2001. Regulation of flowering in

Preston JC, Kellogg EA. 2006. Reconstructing the evolutionary history of paralogous APETALA1/

Schubert D, Clarenz O, Goodrich J. 2005. Epigenetic control of plant development by Polycomb-group

Papp B, Muller J. 2006. Histone trimethylation and the maintenance of transcriptional ON and OFF states

by trxG and PcG proteins. Genes Dev. 20:2041–54


FLOWERING LOCUS C activation via histone 3 lysine 4 trimethylation. Plant Cell 20:580–88

Preston JC, Kellogg EA. 2006. Reconstructing the evolutionary history of paralogous APETALA1/

FRUITFULL-like genes in grasses (Poaceae). Genetics 174:421–37


FLOWERING gene family: MAF2 prevents vernalization by short periods of cold. Plant Cell 15:1159–69


homologue. Plant Physiol 126:122–32


trithorax ATX1 and ATX2 encode proteins with divergent biochemical functions. Plant Cell 20:568–79


target genes in reproductive development of Arabidopsis. Science 288:1613–16


Schmitz RJ, Hong L, Fitzpatrick KE, Amasino RM. 2007. DICER-LIKE 1 and DICER-LIKE 3 redundantly

act to promote flowering via repression of FLOWERING LOCUS C in Arabidopsis thaliana. Genetics

176:1359–62

Schmitz RJ, Sung S, Amasino RM. 2008. Histone arginine methylation is required for vernalization-induced


transcriptional activation of FLOWERING LOCUS C and for proper control of flowering in Arabidopsis. 

Plant Physiol. 149:1196–204


lysine 4 methylation patterns in higher eukaryotic genes. Nat. Cell Biol. 6:73–77

Schomburg FM, Patron DA, Meinke DW, Amasino RM. 2001. FPA, a gene involved in floral induction in


floral activator AGL19 in the FLC-independent vernalization pathway. Genes Dev. 20:1667–78

Schubert D, Clarens O, Goodrich J. 2005. Epigenetic control of plant development by Polycomb-group

proteins. Curr. Opin. Plant Biol. 8:553–61


Plant Cell 20:580–88

Scortecci KC, Michaels SD, Amasino RM. 2001. Identification of a MADS-box gene, FLOWERING LOCUS C, 

that represses flowering. Plant J. 26:229–36


response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes 

Dev. 20:898–912


Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ. 2008. Resetting of FLOWERING LOCUS 


identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. J. Biol. Chem. 282:2450–55

Shitsukawa N, Ikari C, Shimada S, Kitagawa S, SakaMoto K, et al. 2007. The einkorn wheat (Triticum mono-

coccum) mutant, maintained vegetative phase, is caused by a deletion in the VRN1 gene. Genes Genet. Syst. 

82:167–70

www.annualreviews.org • Vernalization 297
Simpson GG, Dijkwel PP, Quesada V, Henderson I, Dean C. 2003. FY is an RNA 3′ end-processing factor that interacts with FCA to control the Arabidopsis floral transition. Cell 113:777–87
Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. 2003. The Pa1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J. Biol. Chem. 278:34739–42


