

Remembering Winter: Toward a Molecular Understanding of Vernalization

Sibum Sung and Richard M. Amasino

Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706;
email: sbsung@biochem.wisc.edu, amasino@biochem.wisc.edu

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Abstract

Exposure to the prolonged cold of winter is an important environmental cue that favors flowering in the spring in many types of plants. The process by which exposure to cold promotes flowering is known as vernalization. In *Arabidopsis* and certain cereals, the block to flowering in plants that have not been vernalized is due to the expression of flowering repressors. The promotion of flowering is due to the cold-mediated suppression of these repressors. Recent work has demonstrated that covalent modifications of histones in the chromatin of target loci are part of the molecular mechanism by which certain repressors are silenced during vernalization.

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INTRODUCTION

“I could while away the hours
 Conferrin’ with the flowers,
 Consultin’ with the rain.
 And my head I’d be scratchin’
 While my thoughts were busy hatchin’
 If I only had a brain.”

—Written by E.Y. Harburg and sung by
 the scarecrow in the movie version
 of “The Wizard of Oz”

Like the scarecrow, plants do not have a brain, but plants have nevertheless evolved the ability to “remember” a past exposure to winter. The flowering of many plants is either dependent on or promoted by prior exposure to the prolonged cold of a winter season. The process by which exposure to cold promotes flowering is known as vernalization. Many crop species need to undergo vernalization prior to flowering. In certain crops, for example beet and cabbage, the vernalization requirement prevents flow-

ering in the first growing season and ensures that the desirable vegetative parts of the plants proliferate. The vernalization requirement of winter cereals permits them to be planted in the fall season to take maximum advantage of the favorable growing conditions in the spring. Not surprisingly, the first reports of the effect of cold exposure on flowering are from agricultural sources. For example, in 1619 it was noted in an agricultural bulletin that winter barley planted in the fall could sometimes be destroyed by a harsh winter; therefore, as a backup in case a damaged field needed to be replanted in the spring, imbibed barley seed could be placed outdoors in the late winter to acquire the ability to flower (as cited in 43). The term vernalization was coined by the infamous Russian agriculturalist Trofim Lysenko, who referred to the process as “jarovization.” Jarovization was later translated from Russian into vernalization; vernal is derived from *vernum*, the Latin word for spring (as cited in 15, 53). There are many excellent reviews that provide a thorough coverage of the history of research on the process of vernalization (e.g., 9, 15, 53).

PHYSIOLOGY AND GENETICS OF VERNALIZATION

Physiology of Vernalization

Plants that need to experience winter to flower the next spring are either perennials, biennials, or winter annuals. Biennials and winter annuals senesce after flowering. The biennial designation is sometimes used to refer to plants with an obligate vernalization requirement, whereas winter annuals are sometimes defined as plants that exhibit a quantitative response to cold exposure (9, 15, 53). (Summer annuals do not need vernalization to flower, senesce after flowering, and complete their life cycle in one growing season.)

In many species, vernalization is not sufficient to induce flowering. Rather, vernalization renders plants competent to flower. This is illustrated by the classic experiments of Lang & Melchers with a biennial type of

henbane (*Hyoscyamus niger*) (reviewed in 53). Biennial henbane has an absolute requirement for vernalization followed by a requirement for inductive long-day photoperiods to flower. Vernalized henbane plants kept in a noninductive short-day photoperiod grow vegetatively, but do not flower. The vernalized henbane plants readily flower, however, when shifted to inductive photoperiods. Therefore, during the period of vegetative growth in noninductive photoperiods, the plants “remember” that they had been exposed to cold. The duration of this “memory” varies from days to over a year depending on species. There are vernalization-requiring plants that do not exhibit memory (in these plants, the floral transition must occur during prolonged exposure to cold) (9). A useful definition of vernalization that covers the broad range of responses was provided by Chouard (15) in the last review on this topic in this series: “the acquisition or acceleration of the ability to flower by a chilling treatment.”

Grafting and localized cooling studies demonstrate that the shoot apex is the site of cold perception during vernalization (53, 109). In most species, vernalization is a localized response; i.e., it is not graft transmissible. Yet there are examples in which the flowering of a nonvernalized shoot is promoted by grafting to a vernalized plant of the same genotype, or to a related variety of plant that does not require vernalization [e.g., pea (88) and henbane (53)]. In these graft-transmissible situations, it is possible that vernalization directly leads to the production of a flowering hormone, which is called “vernalin” (53). Alternatively, the graft donors may be producing a graft-transmissible substance that can bypass the need for vernalization.

Another classic study that demonstrated both the site of vernalization and the memory effect involved the *in vitro* regeneration of plants from various tissues of *Lunaria bien-nis* that had been exposed to a vernalizing cold treatment (108, 109). Plants regenerated from mature leaves of vernalized plants acted as if they had not been vernalized; only tissues that

contained dividing cells (including root meristems and young developing leaves) regenerated into vernalized plants. Thus, only dividing cells (or perhaps cells in which DNA replication is occurring) are capable of becoming vernalized, and these cells maintain the vernalized state through cell divisions in tissue culture during the regeneration process. This type of experiment has also been done in *Arabidopsis* (11). The mitotically stable cellular memory of the vernalized state in these experiments, and in those with henbane described above, illustrate the epigenetic nature of vernalization in many species, namely the acquisition of a state of competence to flower that is mitotically stable in the absence of the inducing signal. This memory is lost as vernalized cells pass through meiosis and into the next generation. The loss of memory ensures that the vernalization requirement is re-established in each generation in monocarpic species.

Low Temperature Response—Cold Acclimation versus Vernalization

Temperature variation is a major seasonal change in temperate climates. Except for summer-annual plants, which finish their life cycle before winter arrives, it is critical for plants to have a mechanism to survive freezing temperatures during winter. Freezing tolerance is induced by exposure to low but nonfreezing temperature. Plants usually do not exhibit freezing tolerance during the warm growing season, but as the seasons change, plants sense the falling temperatures that signal the approaching winter and develop freezing tolerance. The process of preparing to withstand freezing temperatures is known as cold acclimation (reviewed in 102, 103).

Plants typically become cold-acclimated within a short period of time. Usually, one or two days of low but nonfreezing temperature is sufficient to cause cold acclimation in most plant species that are capable of cold acclimation (29, 32, 33, 62, 72, 102). This relatively quick establishment of freezing tolerance is necessary to deal with the sudden temperature

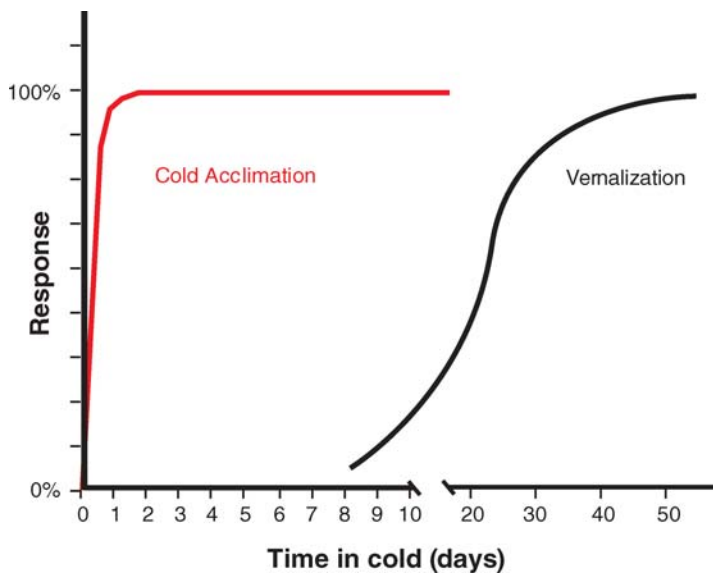


Figure 1

Typical time course of the cold acclimation and vernalization responses in *Arabidopsis thaliana*. The acquisition of freezing tolerance occurs within days, whereas vernalization requires several weeks of cold exposure.

CBF: C-repeat/
DRE-binding factor

fluctuations that are often characteristic of seasonal change. During cold acclimation, a suite of genes encoding a range of proteins are rapidly induced by cold (3, 29, 32, 33, 49, 62, 72). Comparing and analyzing promoters of these cold-induced genes have led to the discovery of a family of transcriptional activators, CBFs, that are necessary for their induction (26, 39). The CBFs are also cold induced and at least one CBF is induced by another cold-activated Myc-related transcriptional activator, ICE1 (14). Thus, cold acclimation results, at least in part, from a cascade of cold-induced transcription factors which, in turn, induce downstream genes encoding proteins involved in cold protection. As discussed above, this response is rapid: The activation of ICE1 and induction of CBFs occur within minutes of cold exposure and reach a peak after several hours (14, 25, 39, 66).

Although both cold acclimation and vernalization are responses to sensing low temperature, the duration of cold exposure that is required to initiate these responses is distinct (**Figure 1**). As discussed above, a rapid induction of cold-protective proteins is essential for surviving the colder temperatures that the plant may soon encounter. One advantage of a vernalization requirement is to prevent flowering

in the fall season, i.e., to ensure that flowering does not occur until spring. Temperatures often fluctuate in the fall and it is critical that a short cold spell followed by warm weather is not sufficient for vernalization; otherwise, flowering could be initiated before the onset of winter, which would be disastrous for most plants. Thus, plants typically achieve a vernalized state only after exposure to a period of cold of sufficient duration to indicate that winter has passed. The flowering of many vernalization-requiring plants is also promoted by long days. This provides an extra level of insurance that flowering does not occur in the fall when the day lengths become shorter.

Temperatures below $\sim -2^{\circ}\text{C}$ are typically not effective for vernalization, and thus there may be periods of extreme cold in certain climates that are not “counted” in the measurement of the cold duration (53). Different species are adapted to a variety of winter climates; therefore, it is not surprising that the range of effective cold temperatures varies among species. For example, $2\text{--}4^{\circ}\text{C}$ is optimal in *Arabidopsis*, whereas $8\text{--}17^{\circ}\text{C}$ is optimal in onion (*Allium cepa*) (53). There is also variation in the duration of cold required for effective vernalization. In the two accessions of *Arabidopsis* tested, a strong vernalization response requires 40 days of continuous exposure to 4°C (57), whereas in a variety of radish (*Raphanus sativus*), 8 days of continuous exposure to 6°C is sufficient (20). It is important to note that, in the laboratory, the duration of cold required to achieve the vernalized state is often measured as the time of continuous exposure to the optimum cold temperature, which does not mimic conditions in the field.

Another process that, in many plants, requires exposure to prolonged cold during winter is the release of bud dormancy in the spring. Vernalization and the cold-mediated release of bud dormancy were previously compared (15) and will not be further considered in this review because there have not been any molecular developments in the study of bud dormancy that relate to recent findings in vernalization. An interesting area of future research will be to

determine if there are parallels, at a molecular level, between vernalization and the release of bud dormancy.

Genetics of the Vernalization Requirement

In many species, there are both annual and biennial varieties, and crosses between annual and biennial varieties can establish the genetic basis for the vernalization requirement in a given species. In the few species that have been studied, the biennial or winter-annual habit is governed by a relatively small number of loci, either dominant or recessive depending on the species (**Table 1**). With henbane, a single dominant locus is responsible for the biennial habit (54), whereas a single recessive locus is responsible for the biennial habit in sugar beet (2). The vernalization requirement of many cereals, including wheat and barley, is controlled by one dominant and one recessive locus (18, 55, 104).

There are also summer-annual and winter-annual varieties (accessions) of *Arabidopsis thaliana*.

The summer-annual types are typically used as models to study various aspects of plant biology because the ability to flower rapidly without vernalization provides for a conveniently short life cycle. The winter-annual types exhibit a facultative vernalization response. Napp-Zinn (76) first showed that in certain crosses of summer-annual and winter-annual accessions, a single dominant locus, which he named *FRIGIDA* (*FRI*), plays a major role in conferring the winter-annual habit in *Arabidopsis* (although other loci contribute). Subsequent genetics studies comparing various winter-annual and summer-annual accessions of *Arabidopsis* have revealed that variation at one or both of two loci, *FLOWERING LOCUS C* (*FLC*) and *FRI*, can account for a large portion of the winter-annual habit in *Arabidopsis* (12, 16, 46, 58, 59). *FLC* and *FRI* synergistically delay flowering in winter-annual accessions of *Arabidopsis*, and a loss-of-function mutation in either gene results in the loss of the late-flowering phenotype (41, 68).

Cloning *FLC* (68, 96) provided the first insight into the molecular nature of vernalization in *Arabidopsis*. *FLC* is a repressor of flowering that encodes a MADS-box transcriptional regulator. The presence of a dominant allele of *FRI* elevates *FLC* expression to a level that inhibits flowering (68, 96). Vernalization overcomes the effect of *FRI* by repressing *FLC* expression, and this repression is stably maintained after plants are returned to warm growth conditions (68, 96). Thus, the epigenetic repression of *FLC* is part of the vernalization-mediated “memory of winter” in *Arabidopsis*.

FLC is expressed predominantly in mitotically active regions (31, 67, 101), such as shoot and root apices, which, as discussed above, are the sites of cold perception and the tissues that achieve the vernalized state. Although most of the flowering promotion by vernalization in *Arabidopsis* is due to *FLC* repression, there is clearly a component that is *FLC* independent (69). As discussed below, much of this *FLC*-independent component involves repressing other MADS-box genes related to *FLC*.

Table 1 Loci that confers vernalization requirement

| Species | Loci |
|--|--|
| <i>Arabidopsis thaliana</i> ^a | <i>FRI</i> * <i>FLC</i> * |
| <i>Brassica rapa</i> ^b | <i>VFR1</i> (Br <i>FLC1</i> *) <i>VFR2</i> (Br <i>FLC5</i> *) |
| <i>Brassica napus</i> ^b | <i>VFN1</i> <i>VFN2</i> |
| <i>Triticum aestivum</i> ^c (Wheat) | <i>VRN-Am1</i> (<i>VRN1</i> *) <i>VRN-Am2</i> (<i>VRN2</i> *) |
| <i>Hordeum vulgare</i> ^d (Barley) | <i>VRN-H1</i> <i>VRN-H2</i> (<i>VRN2</i> *) |
| <i>Hyocymus niger</i> ^e (Henbane) | Single dominant locus |
| <i>Beta vulgaris</i> ^f (Beet) | b (recessive) |
| <i>Pisum sativum</i> ^g (Pea) | Late flowering Vegetative 2 |

^a(12, 16, 41, 46, 58, 59, 68, 96); ^b(81, 93); ^c(104, 111, 112); ^d(55, 111); ^e(54); ^f(2); ^g(74).

*Genes have been cloned.

Summer-annual accessions of *Arabidopsis* often contain loss-of-function mutations in *FRI* (41). Therefore, some summer-annual types of *Arabidopsis* are derived from winter annuals by loss of *FRI*. Lesions in *FRI* have arisen independently several times (e.g., 23). Recently it was shown that certain summer-annual types contain an active *FRI* allele but also contain an allele of *FLC* that is not upregulated by *FRI* (23, 70). Thus, there are at least two routes by which winter-annual types of *Arabidopsis* have become summer annuals. Presumably these summer-annual-causing genetic changes resulted in an adaptation to a particular niche.

The inability of one allele of *FLC* to be upregulated by *FRI* is due to the presence of a transposable element in an *FLC* intron (70). The presence of the transposable element is associated with an island of heterochromatin (64) that may attenuate *FLC* expression by a mechanism similar to that which occurs during vernalization (see below). The idea that transposable element insertions can result in the “transposition of heterochromatin” was first noted by McClintock (65).

The facultative vernalization requirement of *Arabidopsis* can be changed to an obligate requirement simply by increasing the number of copies of the native *FLC* locus in the genome (67). This shows that there need not be a fundamental difference in the mechanism of an obligate versus a facultative requirement; rather, different levels of a repressor can be the cause.

Although the involvement of *FLC* and *FRI* in establishing the vernalization requirement seems to be conserved in other crucifers (81, 93), cereals have different genes that confer winter-annual behavior. Genetic analyses of the vernalization requirement in spring and winter varieties of wheat and barley have revealed two genes that act antagonistically. Dominant alleles of *VRN1* from wheat and barley cause a spring growth habit, whereas dominant alleles of *VRN2* from wheat and barley are necessary for a winter growth habit (55, 104, 111, 112). *VRN1* encodes an AP1-type MADS box gene that promotes flowering (112). *VRN2* encodes a ZCCT family zinc-finger protein that is downregulated by cold exposure (111). *VRN2* is a repressor that prevents flowering by repressing *VRN1* expression. Prolonged cold exposure promotes flowering by shutting off *VRN2* and relieving the repression of *VRN1*. Thus, *VRN2* may play a role analogous to that of *FLC*: Both are repressors that target genes required for flowering, and both repressors are turned off by cold exposure (Figure 2).

Dominant alleles of *VRN1* in wheat confer a spring habit because these alleles are not repressed by *VRN2* (112). One dominant *VRN1* allele contains a deletion that may remove a *cis*

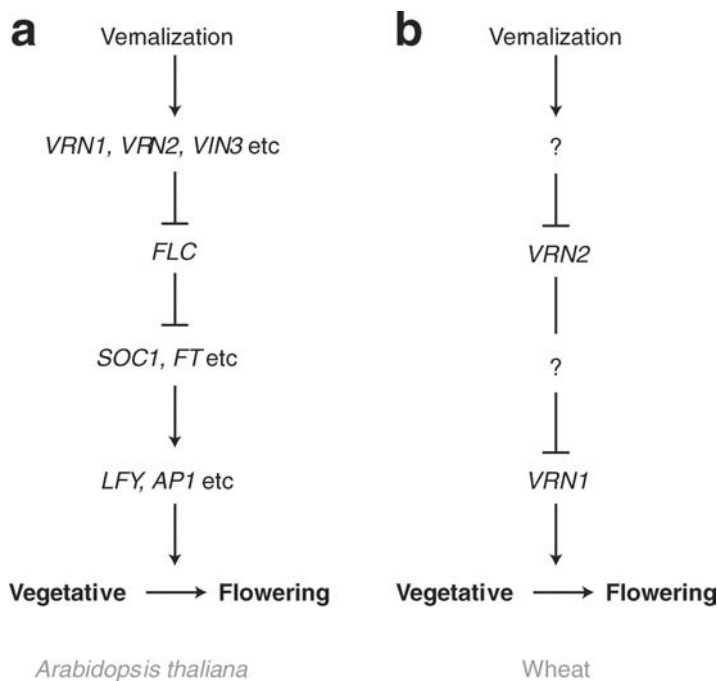


Figure 2

The status of genes currently known to be involved in the pathway from vernalization to flowering in (A) *Arabidopsis thaliana* and (B) wheat. Vernalization results in the epigenetic repression of *FLC* in *Arabidopsis* and *VRN2* in wheat. *FLC* and *VRN2* are both repressors of flowering. In *Arabidopsis*, *FLC* repression permits the activation of floral integrators such as *FT* and *SOC1* (these genes are called floral integrators because their expression is also affected by other flowering cues such as photoperiod) and, in turn, *FT* and *SOC1* activation leads to the activation of floral meristem identity genes, such as *LFY* and *API*. In wheat, the repression of *VRN2* results in *VRN1* activation and the promotion of flowering. It is not known whether *VRN1* is directly repressed by *VRN2*.

element required for *VRN2*-mediated repression (112). It is not known whether *VRN2* directly interacts with *VRN1* regulatory elements.

One definition of the vernalization pathway is the system that measures the duration of cold exposure and, when a sufficient duration of cold exposure has been perceived, initiates changes in gene expression. By this definition, the vernalization pathway of both cereals and *Arabidopsis* acts to downregulate flowering repressors, but cereals and *Arabidopsis* appear to have evolved different targets of the vernalization pathway (Figure 2). Whether the actual cold-sensing pathways are conserved between cereals and *Arabidopsis* remains to be determined. It is possible that different groups of flowering plants evolved in a warm climate in which a vernalization response was not needed. If this were the case, then the vernalization response evolved independently when different groups of plants radiated into climates in which a vernalization response was advantageous. Yet these plants had related genomic "parts catalogs" from which to assemble a cold measurement system, and it will be interesting to learn more about how this system works in a range of plants.

MOLECULAR BASIS OF VERNALIZATION

As discussed above, the identification of *FLC* in *Arabidopsis* and *VRN2* in wheat provides clues about how vernalization affects meristem competence. Expression of repressors like *FLC* and *VRN2* in the shoot apex suppresses flowering. Vernalization provides competence to flower by repressing these floral repressors.

FLC repression in *Arabidopsis* and *VRN2* repression in cereals is stably maintained through mitotic cell divisions and serves as part of the "memory of winter" in vernalization (67, 111). Some insight into the basis of this epigenetic repression has come from genetic studies of the vernalization response in *Arabidopsis*.

Unlike biennial plants, which have an obligate vernalization requirement, vernalization

promotes flowering in a facultative manner in winter-annual *Arabidopsis* accessions. The facultative nature of the promotion of flowering by vernalization in winter-annual accessions makes *Arabidopsis* an attractive system in which to study vernalization because plants containing mutations in this process will eventually flower and thus homozygous mutants are amenable to genetic studies. Genetic screens designed to find mutations that impair the vernalization response in *Arabidopsis* have led to the identification of three genes: *VRN2* (*VERNALIZATION 2*), *VRN1* (*VERNALIZATION 1*), and *VIN3* (*VERNALIZATION INSENSITIVE 3*). *VRN2* encodes a homolog of the *Drosophila* Suppressor of Zeste 12 (Su(z)12), which is a Polycomb group (PcG) transcriptional repressor present in the Enhancer of Zeste (E(z)) complex (13, 17, 24, 51, 73). *VRN1* encodes a plant-specific DNA-binding protein (60). *VIN3* encodes a PHD finger containing protein (101). PHD-finger proteins are often found in protein complexes involved in chromatin remodeling (1, 28). The nature of the proteins encoded by *VRN2* and *VIN3* provide a clue that chromatin remodeling is involved in "memory of winter" (6, 101).

The Histone Code and Vernalization

The establishment and maintenance of cell identity involves pathways that activate and silence specific sets of genes in a tissue- and development-specific manner. Maintaining stable states of gene expression is a form of cellular memory. In the early stages of animal development, the genes of the PcG and trithorax group (trxG) are part of a widely conserved cell memory system that maintains cell identity patterns via cell type-specific transcription patterns (27, 79, 82). PcG and trxG control, respectively, the repressed and active transcriptional states of many developmentally and cell cycle-regulated genes in animals. Although *FLC* is repressed in response to an environmental cue rather than a developmental program, there are several similarities between the vernalization-mediated repression of *FLC* and the PcG-mediated

PHD: plant homeodomain

repression of animal genes: (a) both are stable through mitotic cell divisions, (b) both are relieved during meiosis so that the repression system is reset for the next generation, (c) both repress genes in euchromatin, and (d) both require Su(z)12.

A breakthrough in determining how cellular memory is achieved was the discovery that Enhancer of zeste (E(z)) of the PcG complex is a histone methyltransferase (13, 17, 51, 73). Histone methyltransferases are one of many examples of enzymes that catalyze covalent modifications of the amino-terminal “tails” of histones (47). There are a range of modifications (methylations, acetylations, phosphorylations, ubiquitinations, etc.) that occur on specific amino acid residues of histone tails that can influence whether a locus assumes a transcriptionally active (euchromatin) or silent (heterochromatin) state (8, 19, 36, 40, 94, 105). The histone modifications are recognized by a variety of chromatin-associated proteins that govern transitions between active or silent chromatin (61, 107). For example, methylation of lysine 27 (K27) on histone H3 by E(z) leads to gene repression via the binding of the Polycomb Repression Complex 1 (PRC1), which contains the Polycomb protein (PC). The spectrum of histone modifications at a given locus is often called the “histone code” because the combination of modifications can create a unique state of gene activity.

During vernalization, *FLC* chromatin undergoes a series of histone modifications. Deacetylation of lysine 9 (K9) and lysine 14 (K14) on histone H3 (101) is followed by dimethylation of K27 and K9 on histone H3 (6, 101). In the *vrn1* mutant, the repressed state of *FLC* is not maintained, although dimethylation of K27 still occurs, suggesting that dimethylation of K27 is not sufficient to cause a stable repressed state of *FLC*. Vernalization-mediated dimethylation of K9 on histone H3 does not occur in *vrn1*, *vrn2*, or *vin3* mutants (6, 101), indicating that dimethylation of K9 on histone H3 plays a critical role in the maintenance of stable *FLC* repression. Thus, a series of histone modifications is associated

with encoding the “memory of winter” during vernalization.

Establishment and Maintenance of Heterochromatin on *FLC*

Although there are broad similarities between vernalization-mediated target gene repression in *Arabidopsis* and PcG-mediated gene repression in animals, some aspects of these repression systems are different. Methylation of K27 on histone H3 is likely a specific mark for PcG-mediated repression in animals, as this chromatin modification is highly enriched at PcG target sites and poorly represented in regions of constitutive heterochromatin (17). Constitutive heterochromatin refers to regions of the genome that are always maintained as heterochromatin such as centromeres and telomeres. In PcG-mediated repression, regions that were euchromatin are converted to a heterochromatin-like state (79). The mitotic stability of this heterochromatin-like state is postulated to be mediated by the binding of methylated K27 by PC (21, 22, 98). The repression of *FLC* during vernalization is another example of converting euchromatin into heterochromatin. However, dimethylation of K9 may be more important than dimethylation of K27 in the maintenance of stable *FLC* repression (6, 101).

Another difference is that homologs of PC have not been found in plants (34), raising the issue of the components required for mitotic stability of vernalization-repressed genes. PC shares a region of homology called the “chromodomain” with heterochromatin protein 1 (HP1) (10). HP1 exists both in animals and plants. The chromodomains of HP1 and PC are responsible for the recognition and binding to modified histone tails (5, 21, 38, 52, 107). In animals, HP1 recognizes and binds to methylated K9 on histone H3 and is involved mainly in maintaining constitutive heterochromatin (5, 38, 52, 92). The lack of PC in plants and the importance of dimethyl K9 raises the possibility that HP1 might be involved in the maintenance of the vernalization-mediated repressed

state of *FLC* chromatin (100). Consistent with this hypothesis, vernalization-mediated *FLC* repression is not stable in *Arabidopsis hp1* mutants (S. Sung & R.M. Amasino, unpublished). Thus, it is likely that HP1 is involved in maintaining *FLC* repression after vernalization. Su(z)12, which is a mammalian homolog of VRN2, can interact with HP1 protein in vitro, further supporting the possible role of the plant homolog of HP1 in vernalization (110).

Repression of *FLC* is quantitative (97), i.e., *FLC* can be partially repressed by shorter periods of cold exposure than that which is optimal for a maximum vernalization response. Dosage-dependent repression is characteristic of PcG repression (84). It is possible that suboptimal exposure to cold creates a situation during vernalization similar to the dosage effect in PcG repression: Partial suppression is presumably due to less than saturating levels of a component of repression. The molecular details of how partial repression of *FLC* can occur during a suboptimal exposure to cold remain to be determined, but a candidate for a component that might be limiting after shorter than optimal periods of cold exposure is VIN3 (101).

Activation of *FLC* Transcription

There are two genetic situations in which *FLC* is actively transcribed to levels that significantly delay flowering unless the plants are vernalized. One, discussed above, is due to the presence of active alleles of *FRI*. The other is due to mutations in autonomous-pathway genes (69). The autonomous pathway is defined by a group of genes involved in *FLC* repression. In the absence of *FRI*, the autonomous pathway keeps *FLC* levels low and is thus responsible for the rapid flowering, summer-annual-type habit in many accessions of *Arabidopsis*. In autonomous-pathway mutants, *FLC* repression is abrogated and such mutants exhibit the vernalization-responsive late flowering characteristic of winter annuals. The vernalization-responsiveness of autonomous-pathway mutants indicates that the vernalization-mediated suppression of *FLC* operates inde-

pendently of the autonomous pathway. Recent studies indicate that the autonomous pathway may operate at several different levels to regulate *FLC* expression (31, 45), i.e., it is not a linear pathway but a collection of genes involved in different levels of *FLC* repression. Two of the autonomous-pathway genes appear to be involved in deacetylation of *FLC* chromatin (4, 31).

When *FLC* is actively transcribed due either to the presence of *FRI* or to the absence of an autonomous-pathway gene, trimethylation of K4 on histone H3 is increased in *FLC* chromatin at the 5' region around the transcription start site (30). This situation is similar to the maintenance of gene activation by TrxG proteins in animals. The histone methyltransferases TRX and ASH1, two SET-domain-containing trxG gene products, methylate histone H3 at K4 (90). Methylation at histone H3 K4 is generally considered a mark of active genes (90). Furthermore, K4 trivalent methylation is necessary for trxG-dependent transcriptional activation, and it appears to be incompatible with the binding of PC and HP1 repressor proteins (7). Mutations in an ASH1 homolog (*EFS*) (99) in *Arabidopsis* abolish trimethylation of K4 on histone H3 in *FLC* chromatin, and *efs* mutants fail to activate transcription of *FLC* either in the presence of *FRI* or in the absence of autonomous-pathway genes (S. Michaels, Y. He & R. Amasino, unpublished).

Arabidopsis homologs of members of a complex known in yeast as the PAF1 complex (RNA polymerase II associated factor 1) are also involved in activating *FLC*. In yeast, the PAF1 complex interacts with SET1, the yeast ASH1/*EFS* homolog, resulting in recruitment of SET1 to transcription start sites and an increase in histone trimethylation in these regions (50, 77). Lesions in components of the PAF1 complex in *Arabidopsis* result in the loss of trimethylation of K4 on histone H3 and a loss of the ability to activate *FLC* transcription (30).

Recently it was shown in yeast that trimethylation of K4 on histone H3 could be recognized and bound by ISW1p (91). ISW1p contains

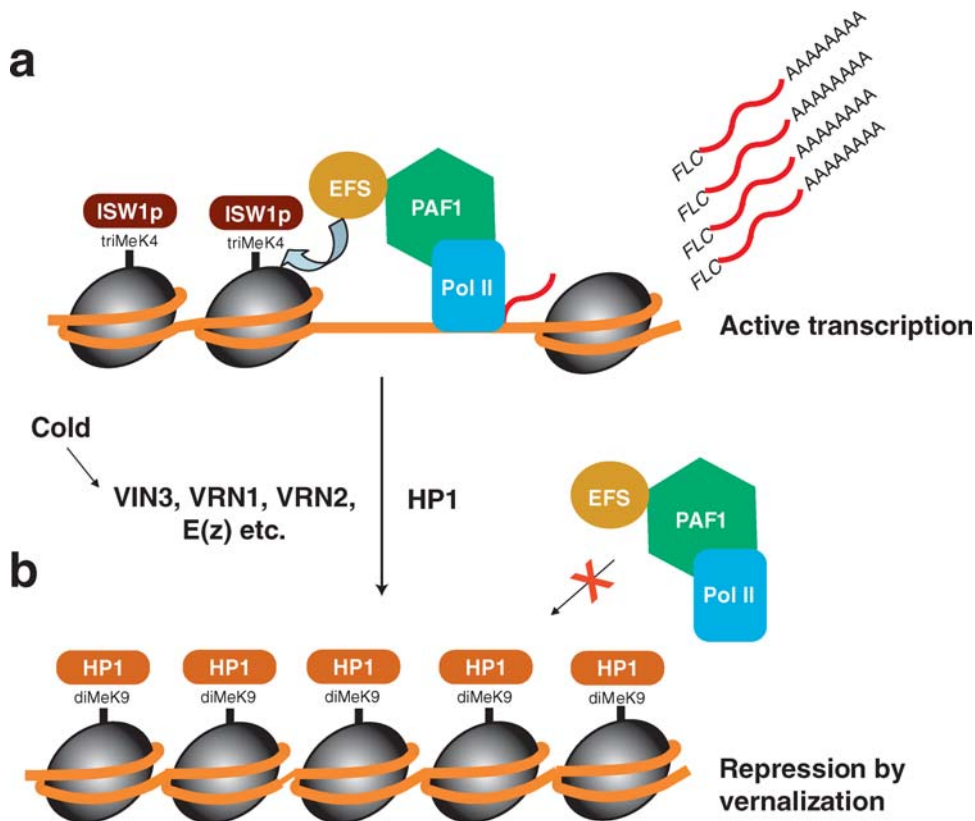


Figure 3

Maintenance of active and repressed states of *FLC* transcription by chromatin modification. (A) Activation of *FLC* transcription by either the presence of *FRI* or the loss of autonomous-pathway genes requires a suite of proteins that are involved in chromatin remodeling. (B) Vernalization results in heterochromatin formation at the *FLC* locus. Heterochromatin formation requires the induction of *VIN3* by a prolonged period of cold exposure, and involves a series of histone modifications, such as deacetylation and methylation. *VRN2* and *VRN1* are also involved in the methylation of K9 of histone H3. Enhancer of Zeste (*E(z)*) is histone methyltransferase that is responsible for methylation on histone H3 in PcG-mediated repression; there are three homologs of *E(z)* in *Arabidopsis* that might play a role in this process. *HP1* may recognize and bind to dimethyl K9 on histone H3, to participate in maintaining the heterochromatin-like state of *FLC* through mitotic cell divisions.

an ATPase domain and is a member of a class of chromatin-remodeling proteins that use the energy of ATP hydrolysis to position nucleosomes into an arrangement conducive to transcription (91). Mutations in a relative of *ISW1p* (*PIE1*) in *Arabidopsis* also result in failure to activate *FLC* transcription (78). It is possible that *PIE1* serves the same role in *Arabidopsis* as *ISW1p* does in yeast (Figure 3). However, *PIE1* is also related to yeast *SWR1*, an ATP-dependent chromatin-remodeling protein in-

involved in Histone H2A variant replacement (71a, 44a). It is possible that this type of activity is also required for *FLC* expression, and that a different *ISW1p* relative is also involved in *FLC* activation.

In both *FRI*-containing lines and in autonomous-pathway mutants, vernalization can overcome the activation of *FLC*. Interestingly, trimethylation of K4 on histone H3 in *FLC* chromatin is reduced by vernalization (Y. He & R. Amasino, unpublished). Because

expression at the mRNA level of components that are required for histone H3 K4 trimethylation of *FLC* chromatin is not repressed by vernalization, one simple model for the vernalization-mediated decrease of *FLC* H3 K4 trimethylation is that vernalization-mediated HP1 association with *FLC* chromatin (and thus the formation of heterochromatin) renders *FLC* chromatin inaccessible to the components that are required for *FLC* activation (Figure 3). This is analogous to the template exclusion of the ATP-dependent chromatin-remodeling protein hSWI/SNF by the interaction of components of PRC1 with target genes (22).

FLC-Independent Vernalization Response in *Arabidopsis*

Although a major target for vernalization in *Arabidopsis* is the repression of *FLC*, the observation that, in noninductive photoperiods, vernalization causes plants to flower earlier than nonvernalized *flc* null mutants, demonstrates that additional targets are regulated by vernalization (69). Some, perhaps all, of these additional targets are relatives of *FLC*. There are five *FLC*-related genes in *Arabidopsis*, and mutations in two of the *FLC*-related genes (*FLM/MAF1* and *MAF2*) cause earlier flowering (mutants in others have not been identified) and all five *FLC*-related genes act as floral repressors when they are ectopically expressed (85, 86, 95). Vernalization represses *FLM/MAF1* and *MAF4* (85, 86). Thus, a clad of MADS-box genes are targets for vernalization.

Measurement of Duration of Cold

Essentially nothing is known about the mechanism by which plants measure the duration of cold in processes such as vernalization or the release of bud dormancy. Unlike cold acclimation, which can be established by a very short period of cold exposure, vernalization and the release of bud dormancy typically require an extended period of cold exposure.

Although cold acclimation and vernalization differ in many aspects, it is possible that

some early events in cold signaling could be shared by both processes. However, to date, the only possible link between cold acclimation and vernalization comes from the study of *HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1)*. A mutation in *HOS1* causes elevated *CBF* expression as well as early flowering and reduced *FLC* expression (56). The *bos1* phenotype indicates that *HOS1* is a negative regulator of cold signaling. However, in a *bos1* mutant, vernalization promotes flowering by further reducing *FLC* expression (37, 56). Thus, the loss of *HOS1* either does not entirely mimic the vernalization response or the *bos1* lesion might affect *FLC* expression independently of the vernalization pathway. *HOS1* encodes a RING-finger protein, and such proteins are usually associated with the ubiquitin protein-degradation pathway. Lesions in *ESD4*, which encode small ubiquitin-related modifier (SUMO), a specific nuclear protease, also cause early flowering partly due to the reduction in *FLC* expression in a vernalization-independent manner (75, 87). Whether lesions in *SUMO* affect *CBF* expression has not been reported.

Part of the cold-acclimation process involves modifying membrane lipid composition to adjust membrane fluidity, rearrangements of microfilaments, Ca²⁺ fluxes, and changes of protein phosphorylation patterns (35, 42, 44, 48, 71, 72, 80, 83, 89, 106). These cold-induced biochemical changes occur rapidly, as expected for any process related to cold acclimation. Therefore, the timing of these changes is not likely directly involved in the system that measures the duration of cold during the vernalization response. Furthermore, constitutive expression of a *CBF1*, which causes elevated expression of downstream cold-acclimation genes and provides a degree of cold resistance without cold exposure, has no effect on the vernalization response (63). However, it is possible that some aspect of cold acclimation provides a component of the cold measurement system. For example, the altered lipid composition of a cold-acclimated membrane might provide a cold-specific substrate for a measurement system.

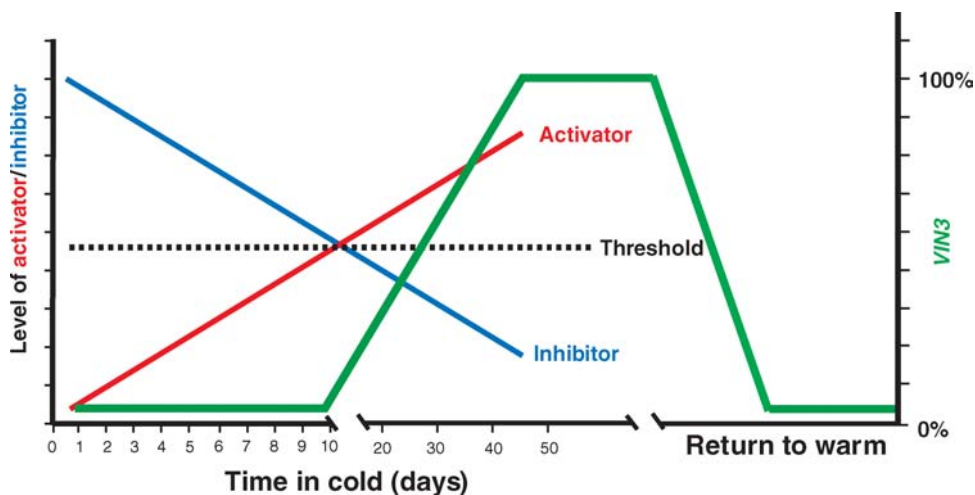


Figure 4

A general and hypothetical model for measuring the duration of cold. During exposure to low temperatures, the level of an activator (*red*) and/or inhibitor (*blue*) might slowly reach a certain threshold level (*dotted line*). When the threshold is reached in *Arabidopsis*, the induction of *VIN3* (*green*) leads to epigenetic changes in gene expression.

How might a cold measurement system operate? Nothing is known about the cold-sensing mechanism, and thus we can only speculate. General models have been proposed in which there is a slow rise or decline in the levels of a compound or compounds during prolonged cold exposure, and, when the levels cross a threshold, the processes that provide competence to flower are initiated (**Figure 4**). The time required to reach the threshold provides a system to measure the duration of cold. What might cause such a slow rise or decline to a threshold? One possibility is differential cold sensitivity of competing enzyme activities (e.g., different Q_{10} values). For example, a kinase that does not lose activity as the temperature is lowered as rapidly as does a phosphatase that acts on the same substrate would lead to the accumulation of a phosphorylated product in the cold. The substrate could be a small molecule or a protein. Another possibility is that the process of cold acclimation provides a greater amount of a specific substrate, for example a membrane lipid, which leads to an increase in the levels of a signaling molecule derived from that substrate. The system may have multiple cold-

sensing components such that a cascade of signaling events is required to measure a complete winter duration of cold.

To date, the most direct connection between the system that measures the duration of cold exposure and the acquisition of competence to flower is the induction of *VIN3* expression. *VIN3* is induced only during cold exposures of sufficient duration to affect flowering (101). Thus, the induction of *VIN3* is an output of the cold-sensing system (**Figure 4**). As discussed above, *VIN3* expression initiates a series of histone modifications of *FLC* chromatin that are required for repressing *FLC* (101). However, constitutive expression of *VIN3* in the absence of cold exposure is not sufficient to establish stable repression of *FLC* (101). Thus, cold exposure must do more than induce *VIN3* to achieve the vernalized state. Chromatin-remodeling activity is commonly mediated by protein complexes and some of the other components may be cold-induced (although the *Arabidopsis* *VRN1* and *VRN2* genes are not cold-induced). Another possibility is that cold-induced biochemical changes must accompany *VIN3* induction. *VIN3* contains a

PHD-finger motif, and this motif can bind phosphoinositides (28). It is conceivable that differences in the spectrum of phosphoinositides that arise during a vernalizing cold exposure are required to establish *FLC* repression.

CONCLUSIONS AND FUTURE DIRECTIONS

We now understand, in outline, how vernalization affects the competence of the meristem to flower in *Arabidopsis*. Competence involves the epigenetic repression of genes encoding MADS-box flowering repressors, and this epigenetic repression is associated with a series of histone modifications. A similar system appears to operate in cereals with a differ-

ent type of protein acting as the vernalization-repressed repressor. Many unanswered questions remain. For example, what is the nature of the chromatin-remodeling complexes that accomplish repression? How are these complexes targeted to *FLC* and other flowering repressors? How widespread is this type of system in plants that have a vernalization response? Do all flowering plants use either MADS-box genes or ZCCT family zinc-finger proteins to establish a vernalization requirement, or will other types of transcriptional regulators be involved in this process in different plant lineages? Furthermore, nothing is known regarding the mechanism by which the duration of cold is measured during vernalization, and this is a key area for future research efforts to address.

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LITERATURE CITED

1. Aasland R, Gibson TJ, Stewart AF. 1995. The PHD finger: implications for chromatin-mediated transcriptional regulation. *Trends Biochem. Sci.* 20:56–59
2. Abegg FA. 1936. A genetic factor for the annual habit in beets and linkage relationship. *J. Agricultural Res.* 53:493–511
3. Anderson BE, Ward JM, Schroeder JL. 1994. Evidence for an extracellular reception site for abscisic acid in commelina guard cells. *Plant Physiol.* 104:1177–83
4. Ausin I, Alonso-Blanco C, Jarillo JA, Ruiz-Garcia L, Martinez-Zapater JM. 2004. Regulation of flowering time by FVE, a retinoblastoma-associated protein. *Nat. Genet.* 36:162–66
5. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, et al. 2001. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410:120–24
6. Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* 427:164–67
7. Beisel C, Imhof A, Greene J, Kremmer E, Sauer F. 2002. Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. *Nature* 419:857–62
8. Berger SL. 2002. Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.* 12:142–48
9. Bernier G, Kinet J-M, Sachs RM. 1981. *The Physiology of Flowering*. Boca Raton, FL: CRC Press
10. Breiling A, Bonte E, Ferrari S, Becker PB, Paro R. 1999. The *Drosophila* polycomb protein interacts with nucleosomal core particles in vitro via its repression domain. *Mol. Cell. Biol.* 19:8451–60

11. Burn JE, Bagnall DJ, Metzger JD, Dennis ES, Peacock WJ. 1993. DNA methylation, vernalization, and the initiation of flowering. *Proc. Natl. Acad. Sci. USA* 90:287–91
12. Burn JE, Smyth DR, Peacock WJ, Dennis ES. 1993. Genes conferring late flowering in *Arabidopsis thaliana*. *Genetica* 90:147–55
13. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, et al. 2002. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 298:1039–43
14. Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, et al. 2003. ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. *Genes Dev.* 17:1043–54
15. Chouard P. 1960. Vernalization and its relations to dormancy. *Annu. Rev. Plant Physiol.* 11:191–238
16. Clarke JH, Dean C. 1994. Mapping *FRI*, a locus controlling flowering time and vernalization response in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 242:81–89
17. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. 2002. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* 111:185–96
18. Dubcovsky J, Luo MC, Zhong GY, Bransteitter R, Desai A, et al. 1996. Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. *Genetics* 143:983–99
19. Dutnall RN. 2003. Cracking the histone code: one, two, three methyls, you're out! *Mol. Cell.* 12:3–4
20. Erwin JE, Warner RM, Smith AG. 2002. Vernalization, photoperiod and GA3 interact to affect flowering of Japanese radish (*Raphanus sativus* Chinese Radish Jumbo Scarlet). *Physiol. Plant* 115:298–302
21. Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. 2003. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* 17:1870–81
22. Francis NJ, Saurin AJ, Shao Z, Kingston RE. 2001. Reconstitution of a functional core polycomb repressive complex. *Mol. Cell.* 8:545–56
23. Gazzani S, Gendall AR, Lister C, Dean C. 2003. Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. *Plant Physiol.* 132:1107–14
24. Gendall AR, Levy YY, Wilson A, Dean C. 2001. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in Arabidopsis. *Cell* 107:525–35
25. Gilmour S, Hajela RK, Thomashow MF. 1988. Cold acclimation in *Arabidopsis thaliana*. *Plant Physiol.* 87:745–50
26. Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF. 1998. Low temperature regulation of the Arabidopsis CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. *Plant J.* 16:433–42
27. Gould A. 1997. Functions of mammalian Polycomb group and trithorax group related genes. *Curr. Opin. Genet. Dev.* 7:488–94
28. Gozani O, Karuman P, Jones DR, Ivanov D, Cha J, et al. 2003. The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. *Cell* 114:99–111
29. Guy CL, Niemi KJ, Brambl R. 1985. Altered gene expression during cold acclimation of spinach. *Proc. Natl. Acad. Sci. USA* 82:3673–77
30. He Y, Doyle MD, 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 Dev.* 18:2774–84
31. He Y, Michaels SD, Amasino RM. 2003. Regulation of flowering time by histone acetylation in Arabidopsis. *Science* 302:1751–54

32. Hong BM, Barg R, Ho THD. 1992. Developmental and organ-specific expression of an ABA-induced and stress-induced protein in barley. *Plant Mol. Biol.* 18:663–74
33. Houde M, Danyluk J, Laliberte JF, Rassart E, Dhindsa RS, Sarhan F. 1992. Cloning, characterization, and expression of a carrier DNA encoding a 50-kilodalton protein specifically induced by cold acclimation in wheat. *Plant Physiol.* 99:1381–87
34. Hsieh TF, Hakim O, Ohad N, Fischer RL. 2003. From flour to flower: how Polycomb group proteins influence multiple aspects of plant development. *Trends Plant Sci.* 8:439–45
35. Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K. 2000. Various abiotic stresses rapidly activate Arabidopsis MAP kinases ATMPK4 and ATMPK6. *Plant J.* 24:655–65
36. Iizuka M, Smith MM. 2003. Functional consequences of histone modifications. *Curr. Opin. Genet. Dev.* 13:154–60
37. Ishitani M, Xiong L, Lee H, Stevenson B, Zhu JK. 1998. HOS1, a genetic locus involved in cold-responsive gene expression in arabidopsis. *Plant Cell* 10:1151–61
38. Jacobs SA, Khorasanizadeh S. 2002. Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* 295:2080–83
39. Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF. 1998. Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science* 280:104–6
40. Jenuwein T, Allis CD. 2001. Translating the histone code. *Science* 293:1074–80
41. 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* 290:344–47
42. Jonak C, Kiegerl S, Ligterink W, Barker PJ, Huskisson NS, Hirt H. 1996. Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. *Proc. Natl. Acad. Sci. USA* 93:11274–79
43. Kim YJ. 1998. Studies on vernalization treatment of winter barley in the 17th century that are described in Ko's "Monthly Farming Guide." *Proc. Korean Soc. Cereal Res.* 5:117–25
44. Knight MR, Campbell AK, Smith SM, Trewavas AJ. 1991. Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature* 352:524–26
- 44a. Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, et al. 2004. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol.* 2:587–99
45. Koornneef M, Alonso-Blanco C, Peeters AJ, Soppe W. 1998. Genetic control of flowering time in Arabidopsis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 49:345–70
46. Koornneef M, Blankestijn-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
47. Kouzarides T. 2002. Histone methylation in transcriptional control. *Curr. Opin. Genet. Dev.* 12:198–209
48. Kovtun Y, Chiu WL, Tena G, Sheen J. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc. Natl. Acad. Sci. USA* 97:2940–45
49. Krishna P, Sacco M, Cherutti JF, Hill S. 1995. Cold-induced accumulation of hsp90 transcripts in *Brassica napus*. *Plant Physiol.* 107:915–23
50. Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, et al. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell.* 11:721–29
51. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. 2002. Histone methyltransferase activity associated with a human multiprotein complex containing the enhancer of Zeste protein. *Genes Dev.* 16:2893–905

52. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410:116–20
53. Lang A. 1965. Physiology of flower initiation. In *Encyclopedia of Plant Physiology*, ed. W Ruhland, pp. 1371–536. Berlin: Springer-Verlag
54. Lang A. 1986. *Hyoscyamus niger*. In *CRC Handbook of Flowering*, ed. AH Halevy, pp. 144–86. Boca Raton, FL: CRC Press
55. Laurie DA. 1997. Comparative genetics of flowering time. *Plant Mol. Biol.* 35:167–77
56. Lee H, Xiong L, Gong Z, Ishitani M, Stevenson B, Zhu JK. 2001. The Arabidopsis HOS1 gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo–cytoplasmic partitioning. *Genes Dev.* 15:912–24
57. Lee I, Amasino RM. 1995. Effect of vernalization, photoperiod and light quality on the flowering phenotype of Arabidopsis plants containing the *FRIGIDA* gene. *Plant Physiol.* 108:157–62
58. Lee I, Bleecker A, Amasino R. 1993. Analysis of naturally occurring late flowering in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 237:171–76
59. Lee I, Michaels SD, Masshardt AS, Amasino RM. 1994. The late-flowering phenotype of *FRIGIDA* and *LUMINIDEPENDENS* is suppressed in the Landsberg *erecta* strain of Arabidopsis. *Plant J.* 6:903–9
60. Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C. 2002. Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. *Science* 297:243–46
61. Li Y, Kirschmann DA, Wallrath LL. 2002. Does heterochromatin protein 1 always follow code? *Proc. Natl. Acad. Sci. USA* 99(Suppl.)4:16462–69
62. Lin CT, Thomashow MF. 1992. A cold-regulated Arabidopsis gene encodes a polypeptide having potent cryoprotective activity. *Biochem. Biophys. Res. Commun.* 183:1103–8
63. Liu J, Gilmour SJ, Thomashow MF, Van Nocker S. 2002. Cold signalling associated with vernalization in Arabidopsis thaliana does not involve CBF1 or abscisic acid. *Physiol. Plant* 114:125–34
64. Liu J, He Y, Amasino RM, Chen X. 2004. siRNA targeting an intronic transposon in the regulation of natural flowering behavior in *Arabidopsis*. *Genes Dev.* 18:2873–78
65. McClintock B. 1950. The origin and behavior of mutable loci in Maize. *Proc. Natl. Acad. Sci. USA* 36:344–55
66. Medina J, Bargas M, Terol J, Perez-Alonso M, Salinas J. 1999. The Arabidopsis CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiol.* 119:463–70
67. Michaels S, Amasino R. 2000. Memories of winter: vernalization and the competence to flower. *Plant Cell Environ.* 23:1145–54
68. Michaels SD, Amasino RM. 1999. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* 11:949–56
69. Michaels SD, Amasino RM. 2001. Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13:935–41
70. Michaels SD, He Y, Scortecci KC, Amasino RM. 2003. Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 100:10102–7
71. Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, et al. 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* 93:765–69

- 71a. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. 2004. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* 303:343–48
72. Monroy AF, Castonguay Y, Laberge S, Sarhan F, Vezina LP, Dhindsa RS. 1993. A new cold-induced alfalfa gene is associated with enhanced hardening at subzero temperature. *Plant Physiol.* 102:873–79
73. Muller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, et al. 2002. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell* 111:197–208
74. Murfet IC. 1989. Flowering genes in *Pisum*. In *Plant Reproduction: From Floral Induction to Pollination*, ed. E Lord, G Bernier, pp. 10–18. Rockville, MD: Am. Soc. Plant Physiol.
75. Murtas G, Reeves PH, Fu YF, Bancroft I, Dean C, Coupland G. 2003. A nuclear protease required for flowering-time regulation in Arabidopsis reduces the abundance of SMALL UBIQUITIN-RELATED MODIFIER conjugates. *Plant Cell* 15:2308–19
76. Napp-Zinn K. 1987. Vernalization: environmental and genetic regulation. In *Manipulation of Flowering*, ed. JG Atherton, pp. 123–32. London: Butterworths
77. Ng HH, Robert F, Young RA, Struhl K. 2003. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol. Cell.* 11:709–19
78. Noh YS, Amasino RM. 2003. PIE1, an ISWI family gene, is required for FLC activation and floral repression in Arabidopsis. *Plant Cell* 15:1671–82
79. Orlando V. 2003. Polycomb, epigenomes, and control of cell identity. *Cell* 112:599–606
80. Orvar BL, Sangwan V, Omann F, Dhindsa RS. 2000. Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. *Plant J.* 23:785–94
81. Osborn TC, Kole C, Parkin IAP, Sharpe AG, Kuiper M, et al. 1997. Comparison of flowering time genes in *Brassica rapa*, *B. napus* and *Arabidopsis thaliana*. *Genet. Soc. Am.* 146:1123–29
82. Pirrotta V. 1997. PcG complexes and chromatin silencing. *Curr. Opin. Genet. Dev.* 7:249–58
83. Plieth C, Hansen UP, Knight H, Knight MR. 1999. Temperature sensing by plants: the primary characteristics of signal perception and calcium response. *Plant J.* 18:491–97
84. Poux S, Horard B, Sigrist CJ, Pirrotta V. 2002. The Drosophila trithorax protein is a coactivator required to prevent re-establishment of polycomb silencing. *Development* 129:2483–93
85. Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL. 2003. Analysis of the Arabidopsis MADS AFFECTING FLOWERING gene family: MAF2 prevents vernalization by short periods of cold. *Plant Cell* 15:1159–69
86. Ratcliffe OJ, Nadzan GC, Reuber TL, Riechmann JL. 2001. Regulation of flowering in Arabidopsis by an FLC homologue. *Plant Physiol.* 126:122–32
87. Reeves PH, Murtas G, Dash S, Coupland G. 2002. Early in short days 4, a mutation in Arabidopsis that causes early flowering and reduces the mRNA abundance of the floral repressor FLC. *Development* 129:5349–61
88. Reid JB, Murfet IC. 1975. Flowering in *Pisum*: the sites and possible mechanisms of the vernalization response. *J. Exp. Bot.* 26:860–67
89. Sangwan V, Orvar BL, Beyerly J, Hirt H, Dhindsa RS. 2002. Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. *Plant J.* 31:629–38
90. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, et al. 2002. Active genes are tri-methylated at K4 of histone H3. *Nature* 419:407–11
91. Santos-Rosa H, Schneider R, Bernstein BE, Karabetsou N, Morillon A, et al. 2003. Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. *Mol. Cell.* 12:1325–32

92. Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, et al. 2002. Central role of *Drosophila* SU(VAR)3–9 in histone H3-K9 methylation and heterochromatic gene silencing. *EMBO J.* 21:1121–31
93. Schranz ME, Quijada P, Sung SB, Lukens L, Amasino R, Osborn TC. 2002. Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. *Genetics* 162:1457–68
94. Schreiber SL, Bernstein BE. 2002. Signaling network model of chromatin. *Cell* 111:771–78
95. Scortecci KC, Michaels SD, Amasino RM. 2001. Identification of a MADS-box gene, *FLOWERING LOCUS M*, that represses flowering. *Plant J.* 26:229–36
96. Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, et al. 1999. The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *Plant Cell* 11:445–58
97. Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES. 2000. The molecular basis of vernalization: the central role of *FLOWERING LOCUS C (FLC)*. *Proc. Natl. Acad. Sci. USA* 97:3753–58
98. Simon JA, Tamkun JW. 2002. Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. *Curr. Opin. Genet. Dev.* 12:210–18
99. Soppe WJ, Bentsink L, Koornneef M. 1999. The early-flowering mutant *efs* is involved in the autonomous promotion pathway of *Arabidopsis thaliana*. *Development* 126:4763–70
100. Sung S, Amasino RM. 2004. Vernalization and epigenetics: how plants remember winter. *Curr. Opin. Plant Biol.* 7:4–10
101. Sung S, Amasino RM. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein *VIN3*. *Nature* 427:159–64
102. Thomashow MF. 1999. *PLANT COLD ACCLIMATION*: freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:571–99
103. Thomashow MF. 2001. So what's new in the field of plant cold acclimation? Lots! *Plant Physiol.* 125:89–93
104. Tranquilli G, Dubcovsky J. 2000. Epistatic interaction between vernalization genes *Vrn-Am1* and *Vrn-Am2* in diploid wheat. *J. Hered.* 91:304–6
105. Turner BM. 2002. Cellular memory and the histone code. *Cell* 111:285–91
106. Vigh L, Los DA, Horvath I, Murata N. 1993. The primary signal in the biological perception of temperature: Pd-catalyzed hydrogenation of membrane lipids stimulated the expression of the *desA* gene in *Synechocystis PCC6803*. *Proc. Natl. Acad. Sci. USA* 90:9090–94
107. Wang L, Brown JL, Cao R, Zhang Y, Kassis JA, Jones RS. 2004. Hierarchical recruitment of polycomb group silencing complexes. *Mol. Cell.* 14:637–46
108. Wellensiek SJ. 1962. Dividing cells as the locus for vernalization. *Nature* 195:307–8
109. Wellensiek SJ. 1964. Dividing cells as the prerequisite for vernalization. *Plant Physiol.* 39:832–35
110. Yamamoto K, Sonoda M, Inokuchi J, Shirasawa S, Sasazuki T. 2004. Polycomb group suppressor of *zeste 12* links heterochromatin protein 1alpha and enhancer of *zeste 2*. *J. Biol. Chem.* 279:401–6
111. Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, et al. 2004. The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* 303:1640–44
112. Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J. 2003. Positional cloning of the wheat vernalization gene *VRN1*. *Proc. Natl. Acad. Sci. USA* 100:6263–68



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ERRATA

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