HISTORICAL PERSPECTIVE ESSAY

Vernalization, Competence, and the Epigenetic Memory of Winter

Vernalization is the process by which prolonged exposure to cold temperatures promotes flowering. Over the past century, this process has been studied extensively at the physiological level. Recent studies have provided some insight into the molecular basis of vernalization. The rich history of vernalization research has been discussed in detail in many reviews (Chouard, 1960; Lang, 1965; Bernier et al., 1981). I will briefly summarize some highlights and classic experiments that I would like to relate to recent molecular advances.

HISTORY OF VERNALIZATION RESEARCH

The first papers describing exposure to cold as the specific climatic aspect of winter that was necessary for flowering in some species were published in the latter half of the 19th century. However, the work of Gassner (1918) is usually cited as the first report that a wide range of plant species require cold exposure to flower (Chouard, 1960; Lang, 1965).

There are several ways to classify the vernalization responsiveness of plants. One is whether a requirement for exposure to the prolonged cold of winter to flower influences the plant's life history. Monocarpic species senesce after flowering and setting seed. Monocarpic plants that require vernalization to flower thus typically require two seasons to complete the life cycle and are usually classified as biennials or winter annuals. The term biennial is often used for plants that have an obligate requirement for cold exposure to flower, and the term winter annual is often used for plants with a quantitative cold requirement (Lang, 1965; Figure 1A). Monocarpic species that flower in one growing season without a vernalizing cold treatment are often called summer annuals. Many polycarpic species (i.e., perennials) also require a vernalizing cold treatment to enable flowering.

The distinction between summer annuals and winter annuals or biennials is not always absolute. It is possible that genetically identical plants could behave as summer annuals in one location and as winter annuals in a different location with a different climate. Furthermore, these classifications do not imply fundamental differences in the mechanisms that control flowering. In Arabidopsis, for example, single-gene changes can convert plants without a vernalization requirement into plants that have either a quantitative or obligate requirement or vice versa; therefore, the relevant molecular differences between plants in various categories can be minor.

Many winter annuals and biennials become established in the fall, taking advantage of the cool and moist conditions

Figure 1. Examples of Plants Requiring Vernalization.

(A) A biennial cabbage (Brassica oleracea) variety with an obligate vernalization requirement that had been growing for five years without cold exposure. The small plant in my daughter's hands is a summer-annual variety of B. oleracea that flowers rapidly without vernalization.

(B) and (C) Summer annual and vernalization-requiring types of henbane (B) and Arabidopsis (C). In both examples, a single-dominant gene is responsible for the vernalization-requiring habit. All plants were grown in long days (inductive photoperiods) without vernalization. The rapid-flowering summer annuals (which have initiated flowering) are at left and the winter-annual types at right. (Henbane images courtesy of Jan Zeevaart.)
optimal for their growth. The vernalization requirement of such plants prevents flowering until spring has actually arrived. Weather is often variable, so for a vernalization requirement to work as intended, plants must not only sense cold exposure but also have a mechanism to measure the duration of cold exposure. For example, if a plant is exposed to a short period of cold in the fall season, followed by a return of warm temperatures later that fall or in early winter, it is important for the plant not to perceive the brief exposure to cold and the following warm weather as spring. One mechanism to determine that spring has in fact arrived is to measure the duration of cold and to permit flowering only after a period of cold that is sufficient to ensure that winter has passed. Sensing the increasing daylengths in the spring can also play a role. In many perennial species, the release of buds from dormancy only after perception of a sufficient duration of cold exposure is, like vernalization, designed to measure the duration of a winter season. Processes that require prolonged exposure to cold, such as vernalization and the cold-induced release of bud dormancy, stand in contrast with cold acclimation—a process designed to respond to cold as rapidly as possible (Thomashow, 2001).

Within a given species, there can be variation in the extent to which vernalization affects flowering time. In some species there are varieties that require vernalization and others that do not, such as winter and spring varieties of cereals (e.g., winter wheat and spring wheat). In fact, the term vernalization comes from studies of flowering in cereals. The infamous Russian geneticist Trofim Lysenko, who studied the effect of cold on flowering, coined the term jarovization to describe what we now call vernalization. Spring cereals are called jarovoe in Russian (derived from Jar, the god of spring), and cold exposure causes a winter cereal to behave like a jarovoe (i.e., to flower rapidly). Jarovization was trans-
a recessive fri allele that confers early flowering. Subsequent studies demonstrated that FRI is required for the winter-annual habit in many additional accessions (Burn et al., 1993; Lee et al., 1993; Clarke and Dean, 1994; Koornneef et al., 1994). In addition, a dominant allele of another gene, FLOWERING LOCUS C (FLC), is necessary for FRI to confer a winter-annual habit (Koornneef et al., 1994; Lee et al., 1994). Thus, in Arabidopsis, FRI and FLC act together to block the ability of a nonvernalized shoot apex to flower; that is, a requirement for vernalization results from the synergistic interaction of dominant alleles of FRI and FLC.

WHAT WE HAVE LEARNED FROM GENE IDENTIFICATION

The molecular characterization of FLC provided a clue as to how vernalization affects competence to flower in Arabidopsis (Michaels and Amasino, 1999; Sheldon et al., 1999). FLC encodes a MADS domain protein that acts as a potent repressor of flowering. Expression of FLC alone (i.e., without FRI) from a heterologous promoter is sufficient to block flowering. The role of FRI is to elevate the expression of FLC to levels that block flowering. Vernalization promotes flowering by repressing FLC expression.

The repression of FLC by vernalization does not occur via FRI regulation; rather, vernalization overrides the effect of FRI by repressing FLC via a pathway acting in parallel to the activation of FLC by FRI. An additional pathway that negatively regulates FLC is the autonomous floral promotion pathway. Autonomous-pathway mutants in a fri null mutant background (i.e., in a summer-annual parental background) behave as winter annuals because mutations in autonomous-pathway genes cause elevated FLC expression similar to dominant alleles of FRI (Michaels and Amasino, 2001). Vernalization effectively promotes flowering by repressing FLC in a fri autonomous-pathway double mutant, indicating that the vernalization-mediated repression of FLC also occurs independently of the autonomous pathway. An outline of the pathways that regulate flowering in Arabidopsis is presented in Figure 2A.

The vernalization-mediated repression of FLC is epigenetic in the sense discussed above: The repressed state of FLC is maintained after vernalized plants are returned to warm growing conditions. Thus, in Arabidopsis, vernalization provides competence to flower by repressing the expression of a flowering repressor. As expected,
FLC expression is on again in the next generation. This resetting of the epigenetic switch during passage to the next generation is reminiscent of genomic imprinting in animals (e.g., de la Casa-Esperon and Sapienza, 2003). But the unique aspect of this switch is that the on-to-off direction of the switch is set by perception of the environment, whereas the off-to-on direction is set by passage to the next generation.

Recent work has provided an outline of the mechanism by which vernalization represses FLC. Screens for Arabidopsis mutants that can no longer respond to vernalization have revealed three genes involved in this process: VERNALIZATION1 (VRN1; Levy et al., 2002), VERNALIZATION2 (VRN2; Gendall et al., 2001), and VERNALIZATION INSENSITIVE3 (VIN3; Sung and Amasino, 2004). VRN1 belongs to a class of plant-specific DNA binding proteins, VRN2 is a relative of the polycomb-group protein SUPPRESSOR OF ZESTE-12, and VIN3 contains a PHD domain. In animals and yeast, proteins related to VRN2 and VIN3 are involved in chromatin-remodeling complexes. Such complexes often catalyze the covalent modification of specific histone residues. The spectrum of histone modifications and their effects on gene expression are referred to as the histone code (Jenuwein and Allis, 2001; Iizuka and Smith, 2003; Lachner et al., 2003). Examination of FLC chromatin has revealed vernalization-mediated changes. During and after vernalization, the levels of certain modifications associated with active genes are reduced, such as acetylation of histone 3 (H3) at Lys 9 and 14 (K9 and K14; Sung and Amasino, 2004). By contrast, the level of two other modifications, methylation of H3K9 and H3K27, are increased by vernalization (Bastow et al., 2004; Sung and Amasino, 2004). Elevated H3K9 and H3K27 methylation is typically associated with the formation of stable heterochromatin. Thus, the vernalization-mediated formation of heterochromatin at FLC appears to account, at least in part, for the epigenetic nature of the vernalized state. It is important to note that deciphering the histone code in eukaryotes and identifying the vernalization-mediated changes in FLC chromatin are works in progress; there are many potential histone modifications for which FLC chromatin has not been evaluated, and new modifications of eukaryotic chromatin and their effect on gene expression continue to be discovered.

As discussed above, deacetylation is one modification of FLC chromatin that occurs during vernalization. Two components of the autonomous pathway, FVE and FLOWERING LOCUS D (FLD), are also involved in deacetylation of FLC chromatin (He et al., 2003; Austin et al., 2004). Fld and fve mutants exhibit a normal vernalization response, and, thus, FVE/FLD-mediated deacetylation is not required for vernalization.

Some types of Arabidopsis are rapid flowering despite the presence of an active FRI allele because their allele of FLC is not upregulated by FRI (Gazzani et al., 2003; Michaels et al., 2003). Such FRI-resistant FLC alleles can result from the insertion of a transposable element in an FLC intron (Michaels et al., 2003). The mechanism by which insertions of transposable elements reduce expression of two different FLC alleles appears to be via an alteration of chromatin structure mediated by silencing RNAs directed against the transposable element (J. Liu, Y. He, R. Amasino, and X. Chen, unpublished data). Specifically, the transposable element creates an island of a chromatin modification (H3K9 methylation) characteristic of vernalization-induced heterochromatin at FLC. This is an example of the prescient idea of Barbara McClintock that insertion of a transposable element can result in “the transposition of heterochromatin” (McClintock, 1950, p. 354). Thus, transposable element insertions may create novel FLC alleles that can convert a winter annual into a rapid-flowering type, and the presence of these alleles in natural populations indicates that the resulting change in flowering behavior may have had adaptive value.

The cloning of FRI revealed that the recessive alleles found in many rapid-flowering types of Arabidopsis are loss-of-function mutations. Therefore, many of the widely used rapid-flowering types, such as Columbia, have been derived from ancestral winter-annual types (Johanson et al., 2000; Gazzani et al., 2003). There are relatives of FRI in Arabidopsis, and at least one of them, FRI-LIKE1 (FRL1), is required for FRI to upregulate FLC (Michaels et al., 2004). Genetic evidence is consistent with FRI and FRL1 acting in a complex (Michaels et al., 2004).

How FRI and FRL1 elevate FLC mRNA levels is not known, and the sequence does not provide any clues—FRI and FRL1 encode plant-specific proteins (Johanson et al., 2000; Michaels et al., 2004). The presence of FRI leads to an increased level of another chromatin modification at FLC, H3K4 trimethylation (Y. He and R. Amasino, unpublished data). However, increased H3K4 trimethylation at FLC is also found in autonomous-pathway mutants in a fri null background. Thus, FRI activity is not directly required for this FLC chromatin modification, although it is possible that FRI prevents an autonomous-pathway component from preventing H3K4 trimethylation of FLC chromatin. Unlike the repressive K9 and K27 methylations associated with vernalization, H3K4 trimethylation is associated with gene activation (Santos-Rosa et al., 2002). After vernalization, the increased H3K4 trimethylation of FLC in FRI-containing lines or in autonomous-pathway mutants is reduced, consistent with the vernalization-mediated repressed state of FLC overriding the ability of FRI or autonomous-pathway mutations to cause activation of FLC (Figure 2). One model to account for this hierarchy of regulation is that the heterochromatin-like state of FLC that results from vernalization blocks the access of activators involved in H3K4 trimethylation to FLC.

COMPARATIVE STUDIES

In wheat, two genes for which allelic variation accounts for the spring versus winter habit have recently been identified. These genes are called VRN1 and VRN2, but there is no relationship to the Arabidopsis genes with the same name. Wheat VRN1 encodes a MADS domain protein that promotes flowering (Yan et al., 2003). In many winter varieties of wheat, VRN1 is induced by cold exposure (Danyuk et al., 2003; Murai et al., 2003; Trevaskis et al., 2003; Yan et al.,
2003). VRN2 is a repressor of VRN1, and VRN2 expression is repressed by vernalization (Yan et al., 2004; Figure 2B). Wheat VRN2 encodes a protein with a putative zinc-finger domain, and there are no homologs of VRN2 in Arabidopsis or rice (Yan et al., 2004). Spring varieties have an allele of VRN1 that is not repressed by VRN2. Therefore, cold exposure is not required for expression of the spring-type VRN1 allele.

The closest relative of VRN1 in Arabidopsis is the MADS domain protein APETALA1 (AP1), a protein that promotes the formation of flowers (Mandel and Yanofsky, 1995). Thus, in both Arabidopsis and wheat, these relatives appear to play the same role—promoting flowering. However, unlike the situation in wheat, there are no examples in Arabidopsis of allelic variation at AP1 causing a difference in the vernalization requirement.

Although they are not related at the amino acid level, wheat VRN2 and Arabidopsis FLC play similar roles: Both repress genes involved in the promotion of flowering and both are repressed by vernalization (Figure 2). In winter wheat, VRN2 represses VRN1 (Figure 2B). In Arabidopsis, FLC represses the floral promoters SOC1 and FT, which are two genes that are also positively regulated by the photoperiod pathway (Figure 2A). SOC1 and FT activate LEAFY and AP1—genes that promote floral meristem identity. Thus, FLC indirectly represses floral meristem-identity genes. Whether wheat VRN1, which is similar to Arabidopsis AP1, is acting as a floral meristem-identity gene or as a more upstream regulator of flowering like SOC1 is not known, nor is it known whether VRN2 directly or indirectly represses VRN1.

Did the vernalization response evolve independently in the crucifers and cereals? As discussed above, evidence for this is that genes unrelated at the sequence level (FLC and VRN2) play similar roles as vernalization-repressed repressors in wheat and Arabidopsis and that allelic variation in an AP1-like gene (VRN1) plays a role in the vernalization requirement in wheat but not in Arabidopsis. Indeed, if major groups of flowering plants evolved in a warm climate in which a vernalization response was not needed, the vernalization response would have had to evolve independently as different groups of plants radiated into regions with a winter season. Homologs of FLC have not been found in wheat or other cereals, and the similar roles of Arabidopsis FLC and wheat VRN2 may be an example of convergent evolution.

In comparative studies, it is important to acknowledge how little is known at a molecular level about the vernalization process in any species. In fact, if the vernalization pathway is strictly defined as the system that senses prolonged cold and transduces the prolonged cold signal to a downstream target, components of the vernalization pathway have not yet been found in any species. By this definition, only targets of the vernalization pathway are known. In Arabidopsis, the most upstream target of the vernalization pathway found to date is VIN3, and FLC is a downstream target of VIN3 (Sung and Amasino, 2004; Figure 2A). In wheat, the most upstream target of the vernalization pathway found to date is VRN2 (Yan et al., 2004; Figure 2A). Thus, Arabidopsis and wheat may have different upstream targets of the vernalization pathway, but it is possible that in cereals a VIN3-like target is upstream of VRN2 just as VIN3 is upstream of FLC in Arabidopsis. Furthermore, because we do not know anything about the system that senses prolonged cold and transduces the prolonged cold signal to a downstream target, we cannot address whether this system is conserved among flowering plants.

FUTURE PROSPECTS

One intriguing area is the mechanism by which plants measure the duration of cold during vernalization. For example, how can a plant distinguish a few days of cold (which typically has no effect on flowering) from several weeks of cold? One link to cold measurement is the unique expression pattern of VIN3 in Arabidopsis (Sung and Amasino, 2004). VIN3 is only expressed after several weeks of cold exposure. Furthermore, VIN3 is only expressed in the cold; VIN3 mRNA rapidly becomes undetectable after plants are returned to warm conditions (i.e., VIN3 does not undergo a stable, epigenetic switch of gene expression like FLC).

Currently, we do not know any details of how the duration of cold is measured. What is the cold sensor? In cold-sensing neurons, the cold sensors are cold-responsive calcium channels that transduce the cold signal via altered calcium flux (Story et al., 2003). However, cold-responsive calcium channels might not be expected to be involved in measuring the duration of cold because cells typically readjust to ion fluxes (although a cold-responsive channel in plants might be involved in rapid cold responses such as cold acclimation). The classic models of vernalization postulate that during prolonged cold, the levels of some factor(s) slowly decline or increase until a threshold is reached that is then transduced into the acquisition of competence to flower. The duration of cold exposure that is required to reach this threshold determines how long the plant must experience winter to flower in the spring. In such a threshold model, the cold sensor could simply be an enzyme that is more or less active than a balancing enzymatic activity in the cold (i.e., enzymes with antagonistic activities that have different Q10 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. One challenge for the future is to understand the nature of the measurement of cold duration at a molecular level.

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Richard Amasino
Department of Biochemistry
University of Wisconsin
Madison, WI 53706-1544
amasino@biochem.wisc.edu

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