

Mechanisms underlying vernalization-mediated *VIN3* induction in Arabidopsis

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VERNALIZATION INSENSITIVE 3 (*VIN3*) is required for vernalization-mediated repression of *FLOWERING LOCUS C (FLC)* in Arabidopsis. The induction of *VIN3* by long-term exposure to cold is one of earliest events in vernalization response. However, molecular mechanisms underlying for the *VIN3* induction are poorly understood. Recently, we reported that the constitutive repression of *VIN3* in the absence of the cold exposure is due to multiple repressive chromatin modifying components, including a transposable element (TE)-derived sequence, LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) and POLYCOMB REPRESSION COMPLEX 2 (PRC2). In addition, the maximum level of *VIN3* induction requires *EARLYFLOWERING 7 (ELF7)* and *EARLY FLOWERING IN SHORDDAYS (EFS)*, which are components of activating chromatin modifying complexes. Furthermore, dynamic changes in histone modifications at *VIN3* chromatin are observed during the course of vernalization. Thus, mechanisms underlying the induction of *VIN3* include changes at the level of chromatin.

Vernalization can be defined as “the acquisition or acceleration of the ability to flower by a chilling treatment.”¹ To maximize floral reproductive capability, plants in temperate climates have evolved a vernalization requirement to prevent flowering before the winter season and to ensure flowering in the spring. Vernalization response provides plants with competence to flower, rather than to induce flowering

itself, through changes that remain stable even after cold exposure. This process is an epigenetic switch, whereby molecular changes remain stable throughout subsequent mitotic divisions despite the absence of initiating stimulus, cold exposure.²⁻⁴ Vernalization response can be thought of as being comprised of two phases. The first is a cold perception system that measures the cumulative time of exposure to cold. The second phase is essentially the output of cold perception system: when a sufficient duration of cold has been perceived, a series of changes of gene expression ensue, ultimately leading to the epigenetic repression of *FLC*.

Measurement of Duration of Cold in Arabidopsis

Although a vernalization response may only be elicited at near or above freezing temperatures, the exact temperatures and length of cold exposure required to efficiently prompt a response varies among plant species.⁵ Since cold perception by plants involves the measurement of the length of exposure, it is the duration of cold that creates two distinct responses—cold acclimation and vernalization. Entering a state of cold acclimation requires a short period of time, typically within hours of low and non-freezing temperatures.⁶ The low threshold required to elicit a cold acclimation response is quite critical in dealing with fluctuating temperatures. Conversely, achieving a vernalized state in plants occurs only after a sufficient and prolonged exposure to cold (e.g., weeks to

Key words: vernalization, flowering, chromatin

Submitted: 08/25/10

Accepted: 08/26/10

Previously published online:

www.landesbioscience.com/journals/psb/article/13465

DOI: 10.4161/psb.5.11.13465

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Addendum to: Kim DH, Zografos BR, Sung S. Vernalization-mediated *VIN3* induction overcomes the LIKE-HETEROCHROMATIN PROTEIN 1/Polycomb Repression Complex 2-mediated epigenetic repression. *Plant Physiology* 2010; 154:949–57 PMID: 20671111; DOI:10.1104/pp.110.161083.

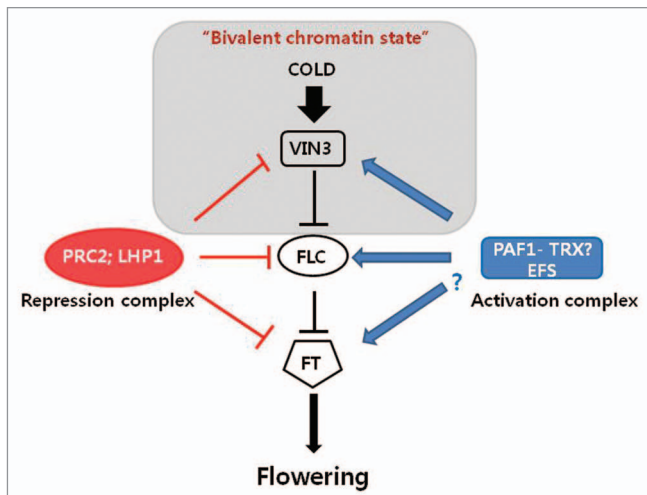


Figure 1. Common regulatory networks to control the VIN3-FLC-FT pathway to control flowering time by vernalization. Common repression and activation complexes are likely involved in regulations of *VIN3*, *FLC* and *FT*. Bivalent chromatin status at *VIN3* chromatin is achieved by vernalizing cold, resulting in transient induction of *VIN3*.

months) has occurred, indicative of the passing of winter. Notably, the molecular components tested to date that affect cold acclimation (a short-term cold response) have no effect on vernalization, suggesting that the cold perception system during vernalization response represents a distinct regulatory process specifically responsible for long-term cold sensing.

Regulation of *VIN3* at the Level of Chromatin

As of yet, the most direct link between measurement of the duration of cold and the output of vernalization response is the induction of *VIN3* expression, which is tightly linked to prolonged cold exposure and whose expression is completely abrogated upon return to warm temperatures.⁷ *VIN3* encodes a PHD (plant homeodomain) protein, a motif routinely found in a variety of proteins involved in modifying chromatin.⁸ After induction, *VIN3* expression initiates a series of repressive histone modifications such as methylations at Histone H3 Lys 9 (H3K9) and Histone H3 Lys 27 on *FLC*, a potent floral repressor.^{2,3} *FLC* repression by vernalization requires components of Poycomb Repression Complex 2 (PRC2) and LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1).⁹⁻¹² Interestingly, *VIN3* is also de-repressed in the absence

of LHP1 and components of PRC2 prior to the cold exposure, suggesting that common components are necessary for the repression of both *VIN3* and its target, *FLC*. Both LHP1 and PRC2 increase their associations with *FLC* chromatin to repress *FLC* during the course of vernalization.⁹⁻¹³ On the other hand, LHP1 and PRC2 are constantly associated with *VIN3* chromatin during the course of vernalization; LHP1 and PRC2 are enriched at *VIN3* chromatin before vernalization and remain associated even when *VIN3* is induced during vernalization. Thus, *VIN3* induction overcomes the repressive effects of LHP1 and PRC2.

Screens for rapidly flowering mutants in Arabidopsis revealed *ELF7* and *ELF8*, two homologs of the yeast PAF1 complex, which are required for high *FLC* expression and Histone H3 Lys 4 trimethylation (H3K4me3) enrichment at *FLC* chromatin.^{14,15} Induced levels of *VIN3* by vernalization are significantly lower in *elf7* and *efs* mutants compared to the wild-type, implicating their function in *VIN3* activation. Concomitant with *ELF7* involvement in *VIN3* induction, H3K4me3 is enriched during vernalizing cold treatment, when *VIN3* is induced. Thus, activating complex components (e.g., PAF1 and Trithorax-like proteins) are likely required for the full extent of *VIN3* induction by vernalization.

Although H3K27me3 and H3K9me2 are constantly enriched at *VIN3* chromatin during the course of vernalization, an activation mark, H3K4me3 becomes enriched when *VIN3* is induced under the cold exposure, creating a bivalent state characterized by both repressive and active histone marks concomitantly. Such bivalent chromatin states are typical of genes that are prepared to be activated or repressed, thus providing elasticity for gene expression in undifferentiated cells.^{16,17} It is considerable that this bivalent state of repressive marks and active marks of *VIN3* chromatin could contribute to a rapid re-repression of *VIN3* when plants return to warm growth temperature.

Common Machineries for Histone Modifications of Genes Involved in Arabidopsis Flowering

The direct association of PRC2 and LHP1 with *VIN3* chromatin suggests these common regulators play roles in the repression of both *VIN3* and its target, *FLC*. Furthermore, PRC2 and LHP1 are also required for the repression of *FLOWERING LOCUS T (FT)*, a target of *FLC*.¹⁸⁻²⁰ In addition, common activators, including *PAF1* and *EFS*, are also necessary for the full extent of both *VIN3* and *FLC*. Thus, the *VIN3-FLC-FT* regulatory network to control flowering by vernalization has common regulatory components as well as specific regulatory mechanisms to shift the status of genes regulated by these common regulators (Fig. 1). One example is vernalization-mediated repression of *FLC*. Prolonged cold exposure induces *VIN3* expression, which overcomes repressive activity of PRC2-LHP1 on *VIN3* locus. After induction, *VIN3* is apparently required for the repression of *FLC* by PRC2. *VIN3* biochemically co-purifies with PRC2, suggesting that the formation of *VIN3*-PRC2 complex enhances the repressive activity of PRC2 on *FLC*. Therefore, the induced nature of *VIN3* by vernalization shifts the activity of the *VIN3-FLC-FT* network towards the repression of *FLC* and the activation of *FT*, which results in promotion of floral transition (Fig. 1).

Components Specific for the Induction of *VIN3* by Vernalization Are Not Known

VIN3 induction by vernalization still occurs in the absence of either PAF1 or EFS and de-repression of *VIN3* in the absence of repressive complexes (i.e., PRC2 and LHP1) is not sufficient for the complete induction. Thus, there must be unknown cold-induced activators and/or cold-repressed repressors responsible for maximum *VIN3* induction during vernalization. Together, these unknown factors along with PAF1 and EFS are likely responsible for the full extent of *VIN3* induction during prolonged cold exposure, which overcomes repression by PRC2 and LHP1. Since *VIN3* is required for vernalization response, it is conceivable that we would recover mutants that affect induction of *VIN3* from vernalization mutant screens. To date, however, no mutant that affects vernalization response impairs *VIN3* induction by vernalization. Although it is possible that the lack of such mutants is simply a matter of lack of saturating mutagenesis, it is also possible that involvement of common regulators in the *VIN3-FLC-FT* network impedes the recovery of such mutants based on flowering times. Thus, the efforts to study the *VIN3* regulation may need to focus on the *VIN3* expression itself

during the course of vernalization rather than on conventional flowering mutant screens. Further study on this unknown initial factor is necessary to fully understand on mechanisms underlying vernalization-mediated *VIN3* induction and it will shed insights on long-term cold sensing mechanism of plants.

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