FLOWERING NEWSLETTER REVIEW

Vernalization-mediated chromatin changes

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Abstract

Proper flowering time is vital for reproductive fitness in flowering plants. In Arabidopsis, vernalization is mediated primarily through the repression of a MADS box transcription factor, FLOWERING LOCUS C (FLC). The induction of a plant homeodomain-containing protein, VERNALIZATION INSENSITIVE 3 (VIN3), by vernalizing cold is required for proper repression of FLC. One of a myriad of changes that occurs after VIN3 is induced is the establishment of FLC chromatin at a mitotically repressed state due to the enrichment of repressive histone modifications. VIN3 induction by cold is the earliest known event during the vernalization response and includes changes in histone modifications at its chromatin. Here, the current understanding of the vernalization-mediated chromatin changes in Arabidopsis is discussed, with a focus on the roles of shared chromatin-modifying machineries in regulating VIN3 and FLC gene family expression during the course of vernalization.

Key words: Chromatin, cold response, epigenetic, flowering, LHP1, PRC2, vernalization.

Introduction

Originally, vernalization was an agricultural practice defined as ‘the acquisition or acceleration of the ability to flower by a chilling treatment’ (Chouard, 1960). A prolonged period of cold during the winter season serves as an environmental stimulus by which plants sense seasonal changes. For plants that reside in temperate climates, a vernalization requirement plays a role in optimizing reproductive fitness in flowering plants by prohibiting the floral transition before the winter season and promoting the transition from vegetative to reproductive growth in the spring. Plants with a vernalization requirement typically also need proper photoperiod – another environmental stimulus – to initiate flowering (Kim et al., 2009). Photoperiod is the relative change in the length of day and night that occurs throughout the course of a year and thus serves as an indicator for seasonal changes (Amasino, 2010).

In Arabidopsis, CONSTANS (CO) acts to promote the expression of floral integrator genes, such as FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CO I (SOC1), under proper photoperiods (i.e. long days). Floral integrator genes are integral to promoting the floral transition of plants, as they are responsible for integrating a range of environmental and developmental cues and subsequently activating downstream floral meristem identity genes (Moon et al., 2003, 2005; Wigge et al., 2005; Lee and Lee, 2010). These genes, in turn, act to promote the process of flowering by specifying certain cells in the growing tips of the plant (shoot apical meristems) to differentiate into a floral meristem and eventually form a flower (Amasino, 2010; Amasino and Michaels, 2010).

Some strains of Arabidopsis require a vernalizing cold treatment in addition to the proper photoperiod in order to accelerate the transition to a reproductive state. Vernalization provides these accessions with the competence to flower in the future through changes that remain stable even after plants are removed from the environmental stimulus – exposure to winter cold. Due to the stability of these molecular changes throughout subsequent mitotic divisions, this process is an epigenetic event whose effect on cellular processes is permanent even after the initiating environmental stimulus is removed (Dennis and Peacock, 2007; Kim et al., 2009).
The vernalization response is comprised of two distinct phases: cold perception and its output, acceleration of flowering. The first phase includes a cold perception network that acts to sense and measure the duration of cumulative cold exposure during the winter. The second phase acts as an output of the cold perception system: a series of gene expression changes occur in response to a sufficient length of cold exposure, ultimately leading to the repression of downstream floral repressors. This review discusses two distinct phases of the vernalization response — cold perception and vernalization response output — with a focus on chromatin changes at loci known to be involved in these two processes.

Sensing prolonged cold

Although a vernalization response may only be elicited at near or above freezing temperatures, optimal temperatures, and lengths of cold exposure required to efficiently cause a response vary among plant species (Lang, 1965). Two related yet distinct responses to low temperatures in plants are cold acclimation and vernalization (Fig. 1). Plants initiate establishment of a freezing tolerance through a process known as cold acclimation within hours of exposure to low, non-freezing temperatures (Thomasaw, 2001). However, achieving a vernalized state in plants takes a markedly longer time, resulting only from sufficient prolonged exposure to cold (e.g. weeks to a month). It is only prolonged cold exposure that is sufficient to indicate the passing of winter to plants: short-term cold is not sufficient to elicit a vernalization response, imposing a higher threshold on the plant to recognize the winter season. The lower threshold required to elicit a cold acclimation response is critical in coping with the unpredictable fluctuations in temperature associated with the transition to the coming winter. Given that both responses are triggered by non-freezing cold stimulus, it is not unreasonable to speculate that there are common early events in sensing cold temperature for both cold acclimation and vernalization (Fig. 1). However, the components tested to date that affect cold acclimation (a short-term cold response) have no effect on the vernalization response (Bond et al., 2011). Thus, the regulatory networks governing cold acclimation and vernalization response may be distinct. In addition, the quantitative nature of vernalization response suggests that there may be a process to measure the duration of cold exposure.

To date, the most direct correlation between measurement of the duration of cold and the cascade of gene expression changes governing the vernalization response in Arabidopsis is the induction of VERNALIZATION INSENSITIVE 3 (VIN3) (Sung and Amasino, 2004). VIN3 is a plant homeodomain (PHD) motif containing protein that is part of a family consisting of four additional members: VIN3-LIKE 1/VERNALIZATION 5 (VIL1/VRN5) through VIL4, or VERNALIZATION5/VIN3-Like 1–3 (VEL1–3) (Sung et al., 2006a; Greb et al., 2007). PHD motifs are mostly found in a range of proteins that are typically involved in chromatin modifications and serve to recognize certain modified histones (Mellor, 2006). Levels of induced VIN3 mRNA directly correlate with the vernalization response (i.e. flowering time) (Sung and Amasino, 2004). VIN3 is required for both Histone H3 Lys 9 (H3K9) and Histone H Lys 27 (H3K27) methylation at FLOWERING LOCUS C (FLC) chromatin, ultimately leading to its repression (Bastow et al., 2004; Sung and Amasino, 2004). FLC is a MADS box DNA-binding protein that acts as a potent floral repressor in Arabidopsis (Michaels and Amasino, 1999). Like VIN3, FLC is a member of a family of related MADS box proteins, including five MADS AFFECTING FLOWERING (MAF) proteins – FLM (or MADS AFFECTING FLOWERING 1, MAF1) and MAFs 2–5 – which possess 53–87% amino acid sequence identity among each other (Ratcliffe et al., 2001, 2003; Scoleteci et al., 2001; De Bodt et al., 2003). MADS box proteins are a transcription factor family responsible for regulating many developmental processes in Arabidopsis as well as in animals (De Bodt et al., 2003). Interestingly, some members of FLC gene family are responsive to vernalizing cold treatment, suggesting their possible roles in the vernalization response (Ratcliffe et al., 2001, 2003; Sheldon et al., 2009).

Vernalization-mediated FLC clade gene repression

FLC

FLC, a MADS box gene, is largely responsible for conferring the vernalization requirement in Arabidopsis (Michaels and Amasino, 1999, 2001). FLC is highly expressed prior to cold exposure. FLC serves to directly repress downstream floral integrators, FT, and SOC1, thus preventing floral transition (Hellwell et al., 2006; Searle et al., 2006). Before prolonged cold exposure, FLC chromatin is in an active state, whereby active histone marks (e.g. histone H3 Lys 4 (H3K4), histone H3 Lys 36 (H3K36) methylation, and histone H3 acetylation are present (He et al., 2003; Kim et al., 2005; Zhao et al., 2005). Furthermore, homologues of the yeast RNA polymerase II (Pol II) Associated Factor 1 (PAF1) complex, a putative H3K4 methyltransferase known as ARABIDOPSIS TRITHORAX-RELATED7 (ATXR7), and homologues of the human COMPASS and COMPASS-like H3K4 methyltransferase complex are involved in FLC activation before cold (He et al., 2004; Tamada et al., 2009; Jiang et al., 2011).
Vernalization results in the mitotically stable epigenetic repression of FLC transcription after the induction of VIN3 transcription (Sung and Amasino, 2004). VIN3, along with POLYCOMB REPRESSIVE COMPLEX2 (PRC2) – a protein complex possessing H3K27 methyltransferase activity – establish the enrichment of a series of repressive chromatin modifications at the FLC locus in response to cold (Dennis and Peacock, 2007; Kim et al., 2009). In particular, H3K27 methylation (H3K27me) and H3K9 methylation (H3K9me) are enriched at FLC during the vernalization response (Bastow et al., 2004; Sung and Amasino, 2004). VIN3-LIKE 1 (VIL1)/VERNALIZATION 5 (VRN5), a PHD-containing protein and a member of the VIN3 protein family, also appears to play a prominent role in regulating FLC by vernalization. Unlike VIN3, whose mRNA levels increase concomitantly with the length of cold exposure (Sung and Amasino, 2004), prolonged cold exposure appears to have little effect on VIL1/VRN5 mRNA levels (Sung et al., 2006a; Greb et al., 2007). However, it is clear that VIL1/VRN5 is required for the maintenance of vernalization-mediated FLC repression. The ability to add both H3K9 and H3K27 methylation to FLC chromatin – hallmarks of the vernalized state – are largely impaired in the absence of VIL1/VRN5 (Greb et al., 2007; Sung et al., 2006a). Thus, both VIL1/VRN5 and VIN3 – two related proteins – are required for modifying the histone architecture of a MADS box floral repressor, FLC, in response to prolonged cold exposure in Arabidopsis.

The quantitative nature of FLC silencing underlying the vernalization response appears to be due to a small subpopulation of cells that initially change to a silenced state during early period of cold exposure (Angel et al., 2011). Prolonged cold exposure triggers stable silencing of FLC in more cells and thus results in overall silencing of FLC. In this model, the quantitative nature of FLC repression during the course of vernalization is not due to gradual decrease of FLC expression in each cell, rather it is due to increasing fraction of cells in which FLC is stably silenced. Consistent with this model, a smaller fraction of cells with stable FLC repression is observed with suboptimal duration of cold exposure (Angel et al., 2011).

Concomitant with FLC repression is the enrichment of H3K27me3 at this locus, originating from a particular region, termed the nucleation peak, and spreading as the length of cold exposure increases (Angel et al., 2011). During the course of vernalization, an increasing number of cells also begin to repress FLC through H3K27me3 enrichment emanating from the nucleation region. The nucleation region corresponds to the VIN3 enrichment region at FLC chromatin, suggesting that VIN3 enrichment marks FLC chromatin to be repressed. It would be interesting to determine whether the quantitative nature of VIN3 induction by vernalization (Sung and Amasino, 2004) is also cell autonomous – i.e. whether increasing numbers of cells express VIN3 with increasing duration of cold exposure. Both LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) – a component of Arabidopsis Polycomb Repression Complex 1 – like complex (Bratze et al., 2010) – and PRC2 also increase their association with FLC chromatin in order to stably silence this locus by vernalization (Schubert et al., 2006; Sung et al., 2006b; Wood et al., 2006; De Lucia et al., 2008). Although VIN3 is present only during the cold exposure, LHP1, PRC2 and VIL1 are constitutively expressed. Along with LHP1 and PRC2, VIL1 enrichment after cold, when VIN3 disappears, is necessary to maintain silenced FLC chromatin (Angel et al., 2011).

A plethora of recent reports indicate that long noncoding RNAs (lncRNAs) plays various roles in gene regulation in eukaryotes. LncRNA has also been shown to play an important role in aiding the epigenetic repression of FLC. One class of lncRNAs was observed to originate from the 3’ region of FLC (Liu et al., 2007; Swiezewski et al., 2009; Hornyik et al., 2010; Liu et al., 2010). These lncRNAs are transcribed in an antisense direction compared to FLC transcripts. The expression of these FLC antisense transcripts, termed as COOLAIR, increases upon cold exposure (Swiezewski et al., 2009). Although it is interesting that COOLAIR expression is induced by cold exposure and may play a role in helping to downregulate FLC transcription, it appears that these antisense transcripts are largely dispensable for the vernalization-mediated FLC repression (Helliwell et al., 2011). Instead, stable maintenance of vernalization-mediated FLC repression is dependent upon a long intronic noncoding RNA, COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR) (Heo and Sung, 2011). COLDAIR is transcribed from the first intron of FLC during the cold exposure and physically interacts with PRC2. When COLDAIR is knocked down, the enrichment of PRC2 at FLC chromatin is largely impaired, resulting in the unstable silencing of FLC (Heo and Sung, 2011). Thus, COLDAIR is required for establishing stable FLC repression through its direct interaction with PRC2 during the vernalization response.

FLC/MAF1 and MAF2–5

Although FLC plays the major role in the repression of flowering, FLC-independent vernalization responses exist (Michaels and Amasino, 2001; Moon et al., 2005; Schonrock et al., 2006). One of the routes to achieve FLC-independent vernalization response is through its-related genes, known as FLC clade. FLOWERING LOCUS M (FLM)/MADS AFFECTING FLOWERING 1 (MAF1) is a transcription factor that shares 62% amino acid sequence identity with FLC and 82% identity to the FLC MADS box DNA-binding region (Ratcliffe et al., 2001; Scortecci et al., 2001). Similar to FLC expression before cold, FLM is expressed in areas prone to mitosis, such as root and shoot apices and in young leaves (Scortecci et al., 2001). As is the case with FLC, FLM also plays a role in regulating the floral transition, acting as a floral repressor in Arabidopsis to prevent flowering before prolonged cold has been perceived (Ratcliffe et al., 2001; Scortecci et al., 2001).

Previously, it was found that FLM repression is alleviated in vil1 mutants (Sung et al., 2006a). VIN3 physically interacts with VIL1 and they are involved in H3K9me3 and H3K27me3 enrichments at FLM chromatin by vernalization, as they are involved with changes to FLC chromatin after cold (Sung et al., 2006a). Since VIN3 and VIL1 are biochemically co-purify with the PRC2 complex, it is probable that PRC2 activity is required for regulating FLM expression as well.

As is the case with both FLC and FLM, MAF2 through MAF5 were also responsive to cold treatment (Ratcliffe et al., 2001;
Vernalization-mediated changes in \textit{VIN3} chromatin architecture

To date, the most direct link between the measurement of cold duration and the output of the vernalization response is the induction of \textit{VIN3} expression, which is tightly linked to duration of cold exposure and whose expression is completely abrogated upon return to warm temperatures (Sung and Amasino, 2004). Interestingly, chromatin-modifying complexes found to be involved in the regulation of \textit{FLC} also play roles in the regulation of \textit{VIN3} (Fig. 2).

Before vernalization, both LHP1 and PRC2 are present at \textit{VIN3} chromatin (Kim et al., 2010; Finnegan et al., 2011). Furthermore, repressive histone modifications, H3K27me3 and H3K9me2, are enriched at \textit{VIN3} chromatin prior to cold exposure (Kim et al., 2010; Finnegan et al., 2011). Interestingly, both PRC2 and LHP1 remain associated with \textit{VIN3} chromatin throughout vernalization cold exposure. Thus, induction of \textit{VIN3} must overcome the physical presence of these two repressive protein complexes during the course of vernalization (Kim et al., 2010; Finnegan et al., 2011). Although levels of H3K27me3, which is mediated by PRC2, are not changed at \textit{VIN3} chromatin during the course of vernalization, an active histone mark, histone H3 Lys 4 trimethylation (H3K4me3), becomes enriched at \textit{VIN3} chromatin, creating a bivalent state at \textit{VIN3} chromatin (Kim et al., 2010; Finnegan et al., 2011). It is tempting to speculate that the constitutive association of repressive complexes may render \textit{VIN3} chromatin poised for repression and \textit{VIN3} induction has to overcome the activity of these repressive chromatin-modifying complexes. Consistent with that, hyperinduction of \textit{VIN3} is observed in \textit{lhp1} mutants (Kim et al., 2010). However, it is important to note that even in the absence of LHP1 or PRC2, \textit{VIN3} is not significantly induced unless vernalized (Kim et al., 2010; Finnegan et al., 2011), suggesting that cold-specific events must occur to trigger \textit{VIN3} induction.

Increases in the level of H3K4me3 enrichment at \textit{VIN3} chromatin during the cold exposure suggests that activating chromatin-remodeling complexes may participate in the induction of \textit{VIN3} by vernalization. Although the level of \textit{VIN3} induction by vernalization is reduced in the absence two activating chromatin remodeling complexes, PAF1 and EFS, significant \textit{VIN3} induction is still observed (Kim et al., 2010), again indicating the presence of cold-specific events for the induction of \textit{VIN3}. It has also yet to be determined whether activating chromatin-remodeling complexes are recruited to \textit{VIN3} chromatin upon cold exposure or if the increase of H3K4me3 enrichment is a result of active transcription (Fig. 2). To date, no cold-specific factors have been identified that are involved in the induction of \textit{VIN3}. Mutants that affect cold acclimation do not affect the induction of \textit{VIN3} (Bond et al., 2011), suggesting distinct mechanisms for two cold responses in plants. Since the most upstream event so far in the vernalization response is the induction of \textit{VIN3}, it will be of great interest to characterize cold-perception mechanisms in vernalization response.

\textbf{Perspectives}

The vernalization pathway is comprised of two steps: perception of an environmental stimulus (prolonged cold) and the subsequent response output, characterized by a cascade of chromatin changes. In \textit{Arabidopsis}, a winter-annual flowering species, output of cold perception results in epigenetic changes in floral repressors. Given the similarity of vernalization physiology across winter-annual/biennial flowering species (i.e. stable maintenance of the effect of vernalization), it would not be surprising to observe similar epigenetic mechanisms to govern vernalization response. In perennial flowering species, where flowering must occur several times over their lifetime, however, the effect of vernalization is not permanent. An \textit{FLC} orthologue, \textit{PEP1}, from a perennial flowering plant,
Arabidopsis alpina, acts as a floral repressor. Consistent with its vernalization physiology, PEPI is only transiently repressed during the cold exposure (Wang et al., 2009). It is interesting to note that the increase in H3K27 methylation is still observed at PEPI chromatin, although the increased level of H3K27 methylation is not maintained after the cold. Stability of the chromatin changes associated with vernalization may be a part of mechanisms governing differences between perennial and winter-annual/biennial flowering behaviour.

It is important to note that homologues of FLC or its clade do not appear to be widespread among vernalization-responsive flowering plants. In particular, no obvious homologue of FLC has been identified in grasses to date. In beet, there are related FLC homologues with limited floral repressor activity (Reeves et al., 2007). However, a recent study shows that vernalization mainly acts to repress a different gene in beets (Pin et al., 2010). In Beta vulgaris (sugar beet), flowering time is regulated by the antagonistic relationship between a pair of FT homologues, where one locus, BvFT1, behaves as a potent floral repressor (FLC-like) and the other locus, BvFT2, is essential for flowering (FT-like) (Pin et al., 2010). Repression of BvFT1 is stably maintained after plants move to warm temperature, showing mitotically stable epigenetic repression. Whether chromatin changes occur at these particular loci during the vernalization response have yet to be determined. The epigenetic repression of floral repressors in response to winter cold may be a conserved mechanism among vernalization-responsive flowering plants, although floral repressors themselves are not conserved.

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References


