

Vernalization – a cold-induced epigenetic switch

Jie Song, Andrew Angel, Martin Howard and Caroline Dean*

John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

*Author for correspondence (caroline.dean@jic.ac.uk)

Journal of Cell Science 125, 3723–3731
© 2012. Published by The Company of Biologists Ltd
doi: 10.1242/jcs.084764

Summary

Growth and development are modulated by environmental signals in many organisms. These signals are often perceived at one stage and ‘remembered’ until later in development. An increasingly well-understood example of this process in plants is provided by vernalization, which refers to the acquisition of the ability to flower after prolonged exposure to cold. In *Arabidopsis thaliana*, vernalization involves downregulation and epigenetic silencing of the gene encoding the floral repressor FLOWERING LOCUS C (FLC). This epigenetic silencing is quantitative and increases with the duration of exposure to cold. Vernalization involves a Polycomb-based switching mechanism, with localized nucleation of silencing during periods of cold, and spreading of the silencing complex over the whole gene after the exposure to cold. A number of characteristics of vernalization have recently been elaborated on through the use of mathematical modelling. This has revealed the importance of chromatin dynamics for the switching mechanism and has shown that the quantitative nature of the process is due to cell-autonomous switching of an increasing proportion of cells. The principles derived from vernalization are likely to be widely relevant to epigenetic reprogramming in many organisms.

Key words: Chromatin, Epigenetics, Flowering Locus C, Histone modification, Mathematical modelling, Polycomb, Vernalization

Introduction

A constant monitoring of the environment is central to plant growth and development. Plants adjust their metabolism and growth to daily changes in external conditions and align developmental transitions to seasonal cues. The perception of light quantity, quality and photoperiod involves a range of photoreceptors as well as the interaction with the circadian clock (Chen and Chory, 2011). The way in which plants perceive temperature cues is less well understood, but this process is likely to involve a range of sensors, including chromatin-based mechanisms (Kumar and Wigge, 2010). In some cases, these environmental cues cause changes that are mitotically stable throughout the rest of development. To date, the best-characterized example of such epigenetic memory in plants is the vernalization process (Box 1), namely the acceleration of flowering as a result of exposure to cold temperatures in winter (Chouard, 1960; Lang, 1965).

Many plants have a vernalization requirement, that is, they actively repress flowering until after a period of prolonged cold, in order to align seed production with the favourable environmental conditions of spring (Fig. 1). Vernalization occurs during the cold, but flowering only occurs many weeks or even months later when other specific conditions, including the presence of certain photoperiods and ambient temperatures, are also met (Henderson and Dean, 2004; Kim et al., 2009). Most of our knowledge about the molecular mechanisms underlying vernalization has been obtained from studies of the model plant *Arabidopsis thaliana* (hereafter referred to as *Arabidopsis*). In *Arabidopsis*, the progressive repression and stable silencing of FLOWERING LOCUS C (FLC), which encodes a MADS domain protein that acts as a repressor of flowering, is central to the vernalization mechanism (Michaels and Amasino, 1999; Sheldon et al., 1999). Other genes have also been found to show

vernalization-responsiveness in *Arabidopsis* (Schönrock et al., 2006). In other plant species, *FLC* orthologues or other target genes are central to the developmental release that enables flowering (Aikawa et al., 2010; Oliver et al., 2009; Pin et al., 2010; Wang et al., 2009).

In *Arabidopsis*, the repression of *FLC* by vernalization exhibits several well-characterized properties: (1) the prolonged exposure to cold leads to a mitotically stable state that survives even vegetative propagation of the tissue (Burn et al., 1993); (2) the epigenetic repression is limited to one locus – the genes adjacent to *FLC* are affected by the cold temperature, which is common for many genes in *Arabidopsis* (Kilian et al., 2007), but do not retain any ‘memory’ of the cold exposure (Sheldon et al., 2009); (3) there is a clear temporal separation of the establishment (during exposure to cold) and maintenance (post-exposure to cold) of silencing, which makes vernalization an ideal system to investigate how the targets that are silenced are identified, and how silencing is epigenetically maintained throughout DNA replication and in the presence of environmental noise; (4) the silencing is quantitative, whereby the degree of stable *FLC* silencing after vernalization reflects the length of cold exposure (Sheldon et al., 2000); (5) the epigenetic state is reset in every generation, which ensures that a vernalization requirement exists for each generation of plants (Choi et al., 2009; Sheldon et al., 2008).

Vernalization, thus, shares many characteristics with epigenetic silencing mechanisms found in a range of biological systems. In this Commentary, we describe the vernalization process in *Arabidopsis* and focus particularly on the new insights that have been provided by mathematical modelling of the system dynamics. The information obtained from these studies not only provides insight into how plants integrate environmental and seasonal cues into developmental decisions, but also holds generic messages for many other epigenetic systems.

Box 1. Glossary

- Vernalization: the acceleration of flowering by the prolonged cold of winter.
- FLC: a MADS box transcriptional repressor involved in silencing the genes that are required for the switch to flowering.
- PRC2: a conserved protein complex that is involved in chromatin silencing through methylation of the lysine 27 residue on histone H3.
- Histone modification: post-translational covalent modifications of histone tails, which include methylation, acetylation, phosphorylation, ubiquitylation, SUMOylation, citrullination and ADP-ribosylation; these modifications can be involved in transcriptional regulation and epigenetic silencing.
- Nucleation: in the context of this Commentary, this term refers to the build-up of a histone modification and/or protein in a localized region of the genome.
- Bistability: a property of a system that results in the system only being stable in two possible states, for example, an active and a silenced state; the system will tend to revert to one of these states if it is initially placed in an intermediate state.

The three phases of the vernalization process

Our current view of the control of vernalization in *Arabidopsis* has been developed through the identification of mutants with impaired vernalization response, the subsequent analysis of the corresponding genes and changes in chromatin biochemistry, and, more recently, through mathematical modelling. The process of vernalization can be broken down into three phases, which we will discuss in turn: setting the *FLC* expression level before exposure to cold; cold-induced *FLC* silencing; and, lastly, epigenetic silencing of *FLC* after re-exposure to warm temperatures.

Setting *FLC* expression before exposure to cold

The *FLC* expression level is set during sexual reproduction and embryogenesis (Choi et al., 2009; Sheldon et al., 2008). This ensures that every generation of newly germinated seedlings, which comes from previously vernalized parents, requires vernalization to flower and, thus, prevents premature flowering in the autumn. There are many regulators that set the initial level of *FLC* expression (Crevillén and Dean, 2011), with extensive natural variation in different *Arabidopsis* accessions. They involve antagonistic pathways, which are highlighted in Fig. 2A. These include upregulation of transcription through the conserved RNA polymerase-associated factor 1 complex (Paf1C) (Oh et al., 2004) and FRIGIDA (FRI) (Johanson et al., 2000; Kim et al., 2009), as well as downregulation by the autonomous pathway (Koornneef et al., 1991). These pathways will be described in more detail below.

Paf1C components and their role in vernalization were identified through genetic screens for early-flowering mutants with low *FLC* expression (He et al., 2004; Oh et al., 2004; Park et al., 2010; Yu and Michaels, 2010). Paf1C associates with RNA polymerase II and is required for active transcriptional elongation, as shown in yeast and mammalian cells (Selth et al., 2010). FRI is a strong upregulator of *FLC* transcription; it includes coiled-coil domains but shows little other homology to other known proteins (Johanson et al., 2000; Risk et al., 2010). It can act through a co-transcriptional mechanism that involves its direct interaction with

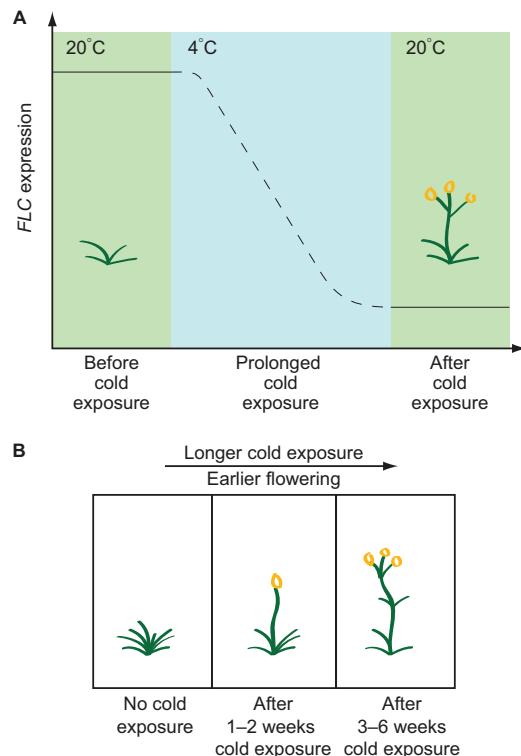


Fig. 1. Vernalization. (A) The floral repressor gene, *FLC*, which is highly expressed before the exposure to cold, is repressed when plants are exposed to the cold and, subsequently, remains stably repressed on the return to warm temperatures. (B) The repression of *FLC* is quantitative and increases with accumulating exposure to cold. This leads to progressively earlier flowering. Plant images are sketched following a fixed amount of time after exposure to cold.

the nuclear cap-binding complex (Geraldo et al., 2009). It can also function as a scaffold protein by forming a transcription-activator complex with its binding partners (Choi et al., 2011). Active FRI results in the enrichment of the WD40-repeat protein ATWDR5a at the *FLC* gene and increases trimethylation on histone H3 lysine 4 (H3K4) (Jiang et al., 2009). Full activation of *FLC* requires the function of both Set1-type (*Arabidopsis* TRITHORAX-RELATED7, ATXR7) and Trithorax-type (*Arabidopsis* ATX1 and ATX2) H3K4 methylases (Tamada et al., 2009). The Set2 methyltransferase EARLY FLOWERING IN SHORT DAYS [EFS, also known as SET domain group 8 (SDG8)] is required for di- and trimethylation of H3K36 (Xu et al., 2008). Although the function of these chromatin modifications has not been fully elucidated, they are commonly associated with actively transcribed euchromatin (Kouzarides, 2007; Wagner and Carpenter, 2012).

The autonomous pathway – a series of activities that link RNA processing with H3K4 demethylation activities – acts antagonistically to the transcriptional activators described above (Koornneef et al., 1991). Polycomb regulators are also involved in repressing the expression of *FLC* before the exposure to cold (De Lucia et al., 2008; Gendall et al., 2001; Jiang et al., 2008). Polycomb repressive complex 2 (PRC2) associates with much of the locus before, during and after cold (De Lucia et al., 2008), paralleling its behaviour at the *HOX* loci in proliferating human embryonic fibroblasts (Bracken et al., 2006). This *Arabidopsis* PRC2 complex is composed of VRN2 [a SU(Z)12 homologue],

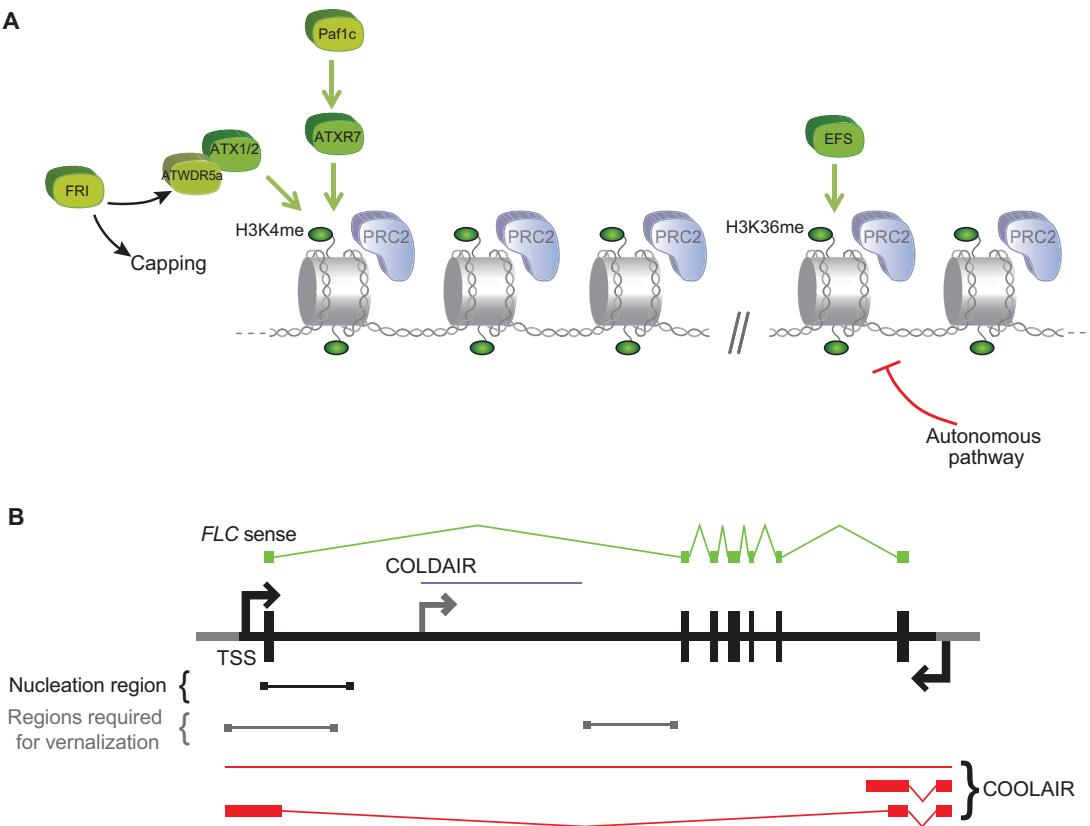


Fig. 2. Multiple antagonistic pathways regulate *FLC* expression levels before cold exposure. (A) Schematic illustration of the *FLC* locus, showing many of the known regulators that set the gene expression level and indicating whether they influence the chromatin state or affect the mRNA. Pathways believed to promote *FLC* expression are coloured in green, whereas repressive pathways are coloured in red. The components of the pathways are as follows: FRIGIDA (FRI), a strong upregulator of *FLC*; ATWDR5a, a WD40-repeat protein; ATX1 and ATX2, a Trithorax-class H3K4 methyltransferase; ATRX7, a Set1-class H3K4 methyltransferase; Paf1c, an RNA polymerase-associated factor 1 complex; EFS, a Set2 methyltransferase required for di- and trimethylation of H3K36; autonomous pathway, represses *FLC* by linking RNA processing to chromatin modification. (B) The *FLC* intron-exon structure and transcript details, including the full-length sense transcript (green); multiple, alternatively processed, antisense transcripts whose expression is upregulated by exposure to cold, collectively known as COOLAIR (red); a sense non-coding transcript COLDAIR (purple). Also marked is the nucleation region where the silencing machinery accumulates in the cold, and two regions previously shown to be important for vernalization.

SWINGER [SWN, an E(z) histone methyltransferase homologue], FERTILIZATION-INDEPENDENT ENDOSPERM [FIE, an extra sex combs (ESC) homologue] and MSI1 (a p55 homologue) (De Lucia et al., 2008). Genetic analysis has further shown that CURLY LEAF, another E(z) homologue, acts partially redundantly with SWN in the regulation of *FLC* (Chavivattana et al., 2004; Wood et al., 2006).

Cold-induced silencing

Multiple cold-induced regulatory steps, each with different timescales, are involved in vernalization. Transcription of *FLC* decreases relatively rapidly in the cold, with the reduction of nascent *FLC* transcripts becoming saturated within the first 2–3 weeks of cold exposure (Swiezowski et al., 2009) (Fig. 3A,B). Within a similar timeframe, there is a cold-dependent upregulation of non-coding antisense transcripts, referred to as COOLAIR, that are initiated immediately downstream of the poly-A site of the sense transcript (see Fig. 2B). The addition of COOLAIR promoter sequences to a reporter gene is sufficient to confer silencing of the reporter by a 2-week period of cold exposure (Swiezowski et al., 2009). COOLAIR enhances the cold-induced down-regulation of *FLC* expression as judged by

analysis of plants containing a terminator-exchange construct, which has the *FLC* sense terminator and COOLAIR promoter replaced by an alternative terminator and does not produce antisense transcripts (Q. Sun and C. Dean, unpublished data). However, disruption of COOLAIR expression by insertional mutagenesis does not disrupt vernalization in plants that have been exposed to 4 weeks of cold temperatures (Helliwell et al., 2011).

A cold-induced non-coding transcript from the sense strand of *FLC*, termed COLDAIR (Fig. 2B), has also been shown to be important for vernalization (Heo and Sung, 2011). This non-polyadenylated, non-spliced transcript is produced from intron 1 and accumulates somewhat later than COOLAIR, reaching its maximum levels after 3 weeks of exposure to cold. It has been found to interact with the histone methyltransferase subunit (CLF) of PRC2 and is thought to target PRC2 to the *FLC* locus (Heo and Sung, 2011).

Cold temperatures also induce quantitative accumulation of the Polycomb-based epigenetic-silencing complexes and histone modifications at the *FLC* locus. In the Columbia *FRI* genotype, cold-induced nucleation of a modified Polycomb complex accumulates to a maximum level after 4–6 weeks exposure to

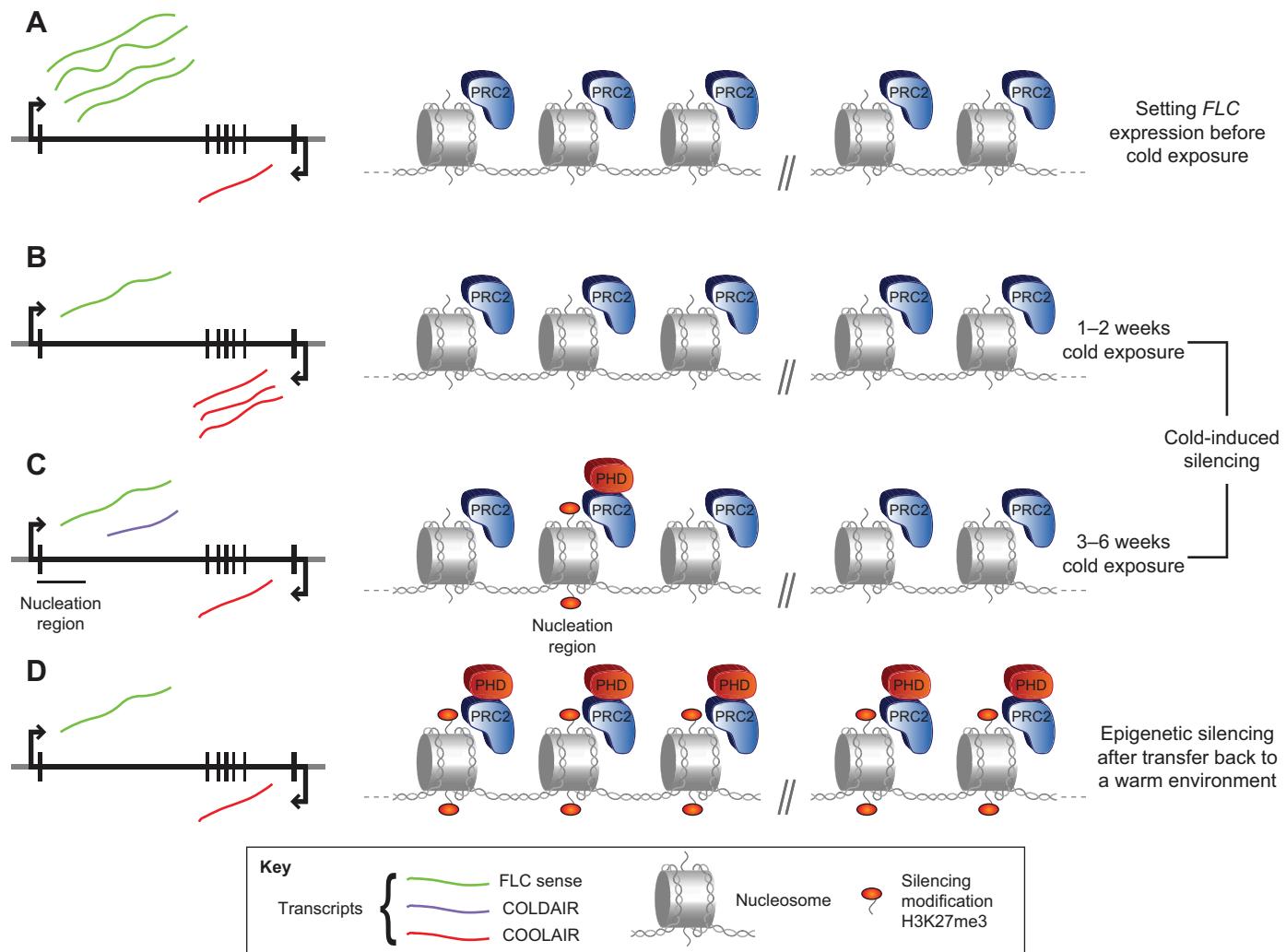


Fig. 3. *FLC* expression during different stages of vernalization. (A) Setting *FLC* expression before cold exposure. (B) Cold-induced silencing during 1–2 weeks of cold exposure. (C) Cold-induced silencing during 3–6 weeks of cold exposure. (D) Epigenetic silencing after transfer back to a warm environment. The left column shows the *FLC* locus with different transcripts: full-length sense transcripts (green); non-coding antisense transcripts, COOLAIR (red); and cold-induced non-coding sense transcripts, COLEAIR (purple). A schematic of the chromatin state of the *FLC* locus is shown on the right. PRC2 (blue) is found at the locus at all time points. Before exposure to cold, sense mRNA levels are high. Immediately after a short exposure to cold, expression of COOLAIR is upregulated and expression of *FLC* is downregulated. After exposure to a longer period of cold, COLEAIR expression is upregulated, and, the PHD–PRC2 complex accumulates at the nucleation region and causes a localized increase in H3K27 trimethylation (red dots). After exposure to long periods of cold followed by transfer to warmer temperatures, the PHD–PRC2 complex blankets *FLC* and expression is epigenetically silenced.

cold (Angel et al., 2011). The core PRC2 associates with the *FLC* locus before exposure to cold (De Lucia et al., 2008), and the cold-induced change is a result of the association of plant homeodomain (PHD) proteins with the core PRC2, to form PHD–PRC2, at a specific region within intron 1 of *FLC* (Fig. 3C). Two PHD proteins, VERNALIZATION INSENSITIVE3 (VIN3) (Sung and Amasino, 2004; Wood et al., 2006) and VERNALIZATION5 (VRN5, also known as VIL1) (Greb et al., 2007; Sung et al., 2006b), have been identified through genetic analyses, and these, together with an additional PHD protein [VIN3-LIKE 2 (VIL2), previously called VEL1], have been purified from the PHD–PRC2 complex (De Lucia et al., 2008). The PHD–PRC2 complex appears to have functional parallels with Pcl–PRC2 in *Drosophila* (Nekrasov et al., 2007) and PHF–PRC2 in mammals (Sarma et al., 2008), and its activity results in increased trimethylation of histone H3 lysine 27 (H3K27me3) in the nucleation region

(Fig. 2B; Fig. 3C) (Angel et al., 2011; De Lucia et al., 2008; Finnegan and Dennis, 2007). This nucleation region overlaps with one of the two regions in intron 1 that have previously been identified as necessary for vernalization (Fig. 2B) (Sheldon et al., 2002). The quantitative nature of vernalization is reflected in the progressive accumulation of H3K27me3 at the nucleation region with increasing lengths of cold exposure (Angel et al., 2011). What limits the accumulation of H3K27me3 to this region is currently unknown, but there are possible parallels with Polycomb response elements in *Drosophila* and the silencer elements in the silent information regulator (SIR) system in *Saccharomyces cerevisiae* (Moazed, 2011).

Epigenetic silencing after the return to warm temperatures
When plants return to warm conditions following prolonged exposure to cold temperatures, a profound and relatively rapid

change occurs at the *FLC* locus, which leads to the gene becoming epigenetically silenced. Within just a few days after the transfer to warm temperatures, the PHD–PRC2 complex is detected across the whole *FLC* locus rather than exclusively at the nucleation region (De Lucia et al., 2008) (Fig. 3D). The associated histone modification, H3K27me3, also increases substantially across the whole gene, which is a feature that is required for stable silencing throughout the rest of development (Angel et al., 2011; De Lucia et al., 2008; Finnegan and Dennis, 2007). Potentially, H3K27me3 replaces activating epigenetic mark(s) that are present before exposure to cold, but the identity of these marks remains unclear. The switching mechanism might not be operational in mature leaves, as they fail to translate the accumulation of H3K27me3 into stable repression (Finnegan and Dennis, 2007).

Defects in epigenetic silencing of *FLC* also occur in the absence of VRN1 (Levy et al., 2002), LIKE HETEROCHROMATIN PROTEIN1 (LHP1) (Mylne et al., 2006; Sung et al., 2006a) and *Arabidopsis* PROTEIN ARGinine METHYLTRANSFERASE 5 (ATPRMT5) (Schmitz et al., 2008). VRN1 encodes two plant-specific B3 DNA binding domains and associates with chromatin independently of vernalization and even during mitosis (Levy et al., 2002; Mylne et al., 2006). LHP1 is the single *Arabidopsis* homologue of the metazoan heterochromatin protein (HP1) (Mylne et al., 2006; Sung et al., 2006a), and it preferentially associates with H3K27me3 through its chromodomain (Sung et al., 2006a; Turck et al., 2007). ATPRMT5 encodes a type II protein arginine methyltransferase, which might be required for vernalization-mediated chromatin modifications (Schmitz et al., 2008). The way in which the activities of these proteins are integrated in the Polycomb switching mechanism remains largely unknown.

An important question concerning this phase of vernalization is why the system switches epigenetic states immediately on return to warm temperatures, rather than earlier during the cold. It is possible that switching requires DNA replication (Finnegan and Dennis, 2007), which is curtailed in cold conditions (Shindo et al.,

2006). DNA replication could remove active chromatin marks, thereby facilitating the switch to silencing. However, as replication occurs only once every 12–24 hours (Grandjean et al., 2004; Reddy et al., 2004), that is, far less frequently than noisy nucleosome turnover, which occurs on an hourly basis (Deal et al., 2010), its contribution is likely to be limited. An interesting possibility is that specialized components that are present during DNA replication could facilitate the switch.

Mathematical modelling of the vernalization process

The quantitative aspect of vernalization, namely how plants quantitatively ‘remember’ the duration of cold, distinguishes vernalization from many other developmental switches. This has recently been elucidated using mathematical modelling (Angel et al., 2011). These models were generated on the basis of earlier work modelling the switching of the epigenetic state at the *Schizosaccharomyces pombe* mating-type locus (Dodd et al., 2007). The model incorporates a highly dynamic chromatin environment, in which histone modifications are constantly being added and removed. One underlying assumption of the model is that the modifications are a key functional element of epigenetic memory, that is, that the overall histone modification status characterizes the transcriptional state of a locus, where H3K27me3 correlates with repression. The central idea of the model is positive feedback; histone modifications of a certain type will tend to recruit protein complexes that cause the addition of further modifications of this type in a ‘reading-and-writing’ mechanism. Under the continual action of such a mechanism, a given histone modification status in a region can become self sustaining; any histone modifications that are lost owing to noisy biological processes or DNA replication will be replaced. In particular, through cooperative long-range positive feedback of antagonistic histone modifications (Fig. 4A,B), a locus can be stably maintained in one of two states, silenced or active. Crucially, the targeted build-up (nucleation) of the silencing modification (H3K27me3) in a localized region – as observed on *FLC* – in combination with an increased activity of the PHD–PRC2

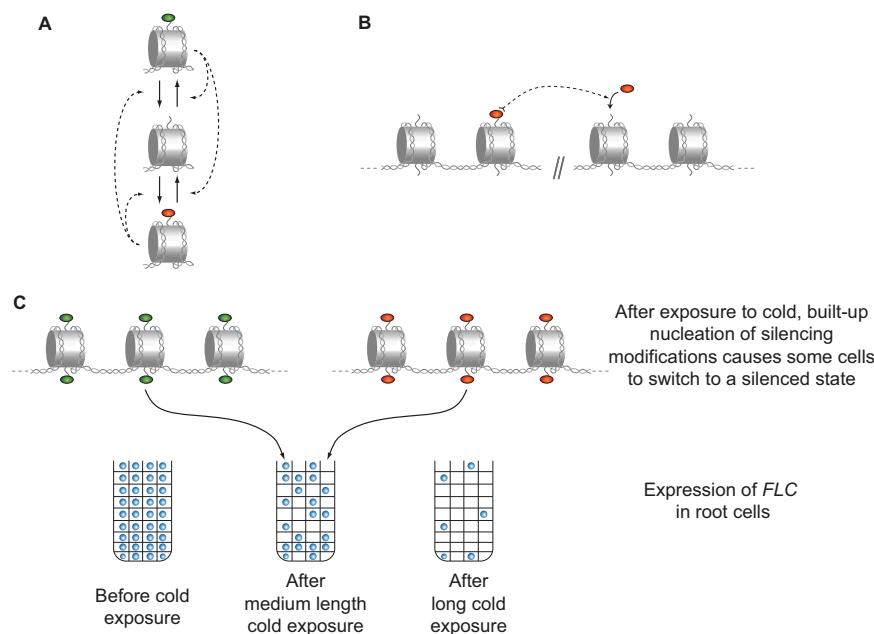


Fig. 4. Key modelling principles and the quantitative nature of the vernalization response. (A) Cooperative positive feedback. Activating histone modifications (green) encourage further activating modifications to be added or silencing modifications (red) to be removed. Similarly, silencing modifications encourage further silencing modifications and the removal of activating modifications. A requirement for two-step transitions, that is, first passing through the unmodified state, ensures non-linear co-operativity. (B) Long-range interactions. Histone modifications can alter the histone modification status anywhere on the locus. (C) During periods of cold exposure, H3K27me3 accumulates in the nucleation region. After the exposure to cold, the nucleated H3K27me3 causes some cells to switch to a silenced state, with high levels of H3K27me3 blanketing the gene. This epigenetic switch is cell-autonomous. The quantitative nature of the vernalization response is the result of an increasing number of cells switching to a silenced state after increasing cold exposure. This can be observed directly in roots by using a *proFLC::FLC-GUS* fusion gene and assessing its expression in individual cells, as shown schematically here.

complex, allows a switch of the system's state. As each locus can be maintained in only one of two expression states ('on' or 'off'), the quantitative nature of vernalization is achieved through a population average of digital cell responses; with longer exposure to cold, there is more nucleation and, consequently, *FLC* loci switch to the silenced state in more cells. The prediction from the model of a digital silencing response in individual cells has been verified by analysis of a *proFLC::FLC-GUS* reporter in cells from partially vernalized roots (Angel et al., 2011). In the following section, we will discuss the insights into vernalization that have been revealed by the model, and elaborate on the general implications of these insights on other epigenetic systems.

Generic principles that have emerged from modelling

The model of vernalization was implemented stochastically with probabilistic rates being assigned to different events, including addition and removal of histone modifications, nucleosome turnover and the effect of DNA replication (Angel et al., 2011). These processes are simulated numerically and can reproduce many of the experimentally determined features of the vernalization system. The assumptions in the model on the mechanistic basis that underlies the vernalization process lead to some interesting implications with regards to this and other epigenetic processes. Some of these have been verified, whereas others require further experimental investigation.

The basis of mitotic stability of histone modifications

Any long-term epigenetic memory must be maintained through multiple rounds of cell division. During DNA replication, histones could be shared randomly between the two daughter strands with new (presumably unmodified) nucleosomes being added (Annunziato, 2005; Dodd et al., 2007; Radman-Livaja et al., 2011). Thus, levels of any parental histone modification will be approximately halved during this process. Furthermore, nucleosomes and associated modifications are continually replaced throughout the cell cycle (Deal et al., 2010). A self-sustaining, positive feedback 'reading-and-writing' mechanism is, therefore, an attractive model to explain the mitotic stability of histone modifications, not only for Polycomb silencing but also for SIR silencing in yeast (David-Rus et al., 2009; Mukhopadhyay et al., 2010; Sedighi and Sengupta, 2007). The positive-feedback model for histone-modification is supported by experiments that have shown the preferred binding of chromatin complexes to the modifications they themselves introduce (Hansen et al., 2008; Margueron et al., 2009). However, there is much discussion in the literature about whether histone modifications are the fundamental memory element that forms the core of a positive-feedback mechanism or whether other components, for example auto-activation of a transcription factor (Ptashne, 2007), are central (Henikoff and Shilatifard, 2011). The current vernalization model proposed by Angel and colleagues (Angel et al., 2011) does not provide the answer to these questions, but does demonstrate the components that are required for a histone-based memory system to be employed. The requirements, which include cooperativity and long-range interactions, are discussed in detail below.

Cooperativity and long-range interactions

The 'read-and-write' mechanism on the basis of histone modifications is attractive in its simplicity, but for the model to

work it requires some specific features, namely cooperativity and long-range interactions, whose molecular bases are still not clearly understood (Fig. 4A,B). We envisage several ways of how cooperativity might arise; one possibility is a three-state system (with each histone possessing an activating or repressing modification, or being unmodified) (Dodd et al., 2007). The requirement for long-range interactions means that the chromatin modification machinery must be able to modify distant histones on that locus, as well as histones that are adjacent to the bound complex. This constraint could be satisfied by gene looping (Ansari and Hampsey, 2005; Hampsey et al., 2011; O'Sullivan et al., 2004), rapid remodelling of higher-order chromatin structures, a non-coding RNA 'backbone' (Spitale et al., 2011) or even transcription itself. Moreover, there must be an additional mechanism that limits the spreading of the silencing or activating modifications outside of the locus, because there are clear boundaries for the histone modifications just outside both ends of the *FLC* transcription unit (Angel et al., 2011).

How can a stable state be switched?

The modelling approach has shown that the localized nucleation of silencing histone modifications after the exposure to cold, in combination with an increased activity of PHD-PRC2 complexes, gives a bias towards silencing that is able to switch the locus to a silenced state. The fact that histone modifications in only a small region can cause this switch is because the dynamics of the system are somewhat noisy; nucleosomes are constantly being replaced, and it is expected that chromatin-modifying complexes behave stochastically. In the absence of noise, the histone modification covering the majority of the locus would always tend to induce the removal of any opposing modifications. However, fluctuations, induced by noise, can allow a small number of opposing modifications to grow and eventually switch the state of the system. Furthermore, initiation of switching requires only specificity for a small region to function – in the case of vernalization, this is ~500 bp of the nucleation region compared with ~6 Kb of the entire locus (Fig. 2B). This specificity could be provided if sequence-specific DNA-binding proteins targeted the nucleation region. The model also predicts that larger loci will require larger, or a greater number of, nucleation regions for efficient switching.

The digital nature of histone-modification-based epigenetic memory

As we have found for *FLC*, quantitative epigenetic silencing (and activating) memory mechanisms that depend on histone modifications are very likely to be on/off switches. This is based on the necessity for robustness of the system through many rounds of cell division. During DNA replication, a digital on/off memory can be straightforwardly rewritten through self-reinforcement, whereas a graded memory of an intermediate level of silencing or activation could be more easily disrupted. Thus, the most parsimonious way to build up a quantitative organism-level memory is through the population average of a number of cells, each with a digital memory. This raises the question of how the quantitative response can be acted upon at the whole organism level. For *FLC*, the averaging is likely to occur through the non-cell autonomy of immediate downstream targets of *FLC*; the FT protein moves from the leaves to the apex through the plant vasculature (Corbesier et al., 2007).

Variation in vernalization underlies differences in life history

A requirement for vernalization has a great impact on the reproductive strategy of plants. In *Arabidopsis thaliana*, some accessions do not require vernalization and have a rapid cycling (or summer annual) habit. This reproductive strategy is thought to be an adaptation to extremely harsh conditions or to allow multiple generations per year. The rapid-cycling habit has evolved independently in many *Arabidopsis* accessions through mutations in *FRI*, the upregulator of *FLC* (Gazzani et al., 2003; Johanson et al., 2000; Shindo et al., 2005). Allelic variation at *FRI* also appears to contribute to differences in vernalization requirement in different *Brassica* species (Irwin et al., 2012; Wang et al., 2011).

Variation in *Arabidopsis FLC* itself only rarely accounts for the rapid-cycling habit. However, it is a main factor contributing to the need for different winter lengths in *Arabidopsis* accessions (Werner et al., 2005). Some natural accessions of *Arabidopsis* isolated from regions that lie near the Arctic Circle need up to 14 weeks of exposure to cold, whereas plants that have adapted to more southern regions require only 4 weeks of cold exposure for vernalization (Shindo et al., 2006). Analysis so far has indicated that the accumulation of *FLC* epigenetic silencing takes much longer in northern plant types, with short vernalization leading to re-activation of expression following the transfer to warmer temperatures (Shindo et al., 2006). Recent work has elucidated the role of single-nucleotide polymorphisms in *FLC* and their effect on the different phases of vernalization (Coustham et al., 2012).

The re-activation of *FLC*, which is observed in *Arabidopsis* accessions from northerly climates when the vernalization requirement is not fully saturated (Shindo et al., 2006), could be seen as an intermediate state between annual and perennial plants. In annuals, the prolonged cold in winter satisfies the vernalization requirement; the plants flower, form and disperse seeds and then die. In some perennials, some parts of the plant do not switch to reproductive development after one winter, and remain vegetative, thereby maintaining the adult plant over many years (Fig. 5) (Albani and Coupland, 2010). Mutation analysis in the perennial *Arabis alpina*, a relative of *Arabidopsis*, revealed that the *FLC* homologue, *PERPETUAL FLOWERING 1 (PEP1)*, has an important role in the perennial habit (Wang et al., 2009). Studies of *PEP1* expression have revealed interesting regulatory

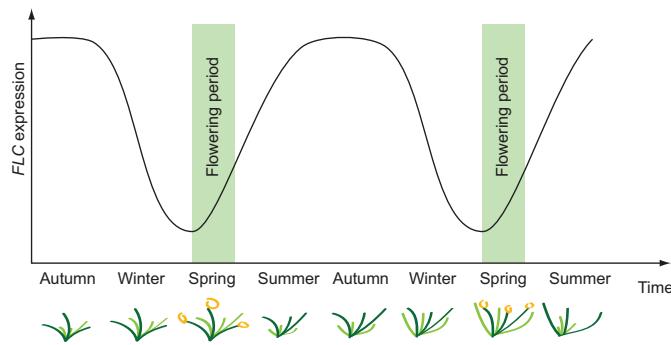


Fig. 5. Changes in epigenetic silencing underlie differences in life history. In perennial *Arabis alpina* plants, *FLC* expression is transiently but not epigenetically silenced by exposure to cold. Older meristems flower after one winter but newer meristems remain vegetative until the subsequent winter. Newer vegetative growth is shown in light green and older growth is shown in dark green.

differences when compared with *FLC* in *Arabidopsis*. During prolonged exposure to cold, expression of *PEP1* in *Arabis alpina* decreases. This enables downstream floral activators to be induced in some of the meristems, and these meristems become committed to flowering. However, after return to warm temperatures, *PEP1* expression resumes in all meristems, meaning that the silencing is not epigenetically stable. The committed meristems are able to continue flowering despite the resumption of *PEP1* expression, but the resumption of *PEP1* expression ensures that the non-committed meristems do not flower in that growing season (Albani and Coupland, 2010).

This and other practical experiences of breeding perennial-type traits into annual plant genetic backgrounds (Thomas et al., 2000) support theoretical analyses that predict small changes in initial levels of expression, rates of silencing or ability to epigenetically silence a floral repressor can have major consequences on life history (Satake, 2010). Although perennial and annual plants appear very different, the underlying evolutionary steps that separate them might have involved relatively subtle changes in expression and epigenetic silencing of floral repressors.

Conclusions

In this Commentary, we have discussed vernalization, the acceleration of flowering by prolonged exposure to cold, which is used by many plants in order to align flowering to seasonal cues. In the model plant *Arabidopsis*, the level of *FLC* expression is modulated by multiple and antagonistic pathways before vernalization. During vernalization, a quantitative epigenetic memory in the form of repression of the floral repressor gene *FLC* is generated. A combination of experimental and computational modelling approaches has suggested that a mechanism on the basis of histone modifications could contribute to this memory. This would depend on a highly dynamic system of reading and writing of modifications that is able to maintain the *FLC* locus in a stable epigenetic state. As a result of the noisy dynamics that are inherent in the system, the locus can be switched to the silent state by the nucleation of silencing histone modifications in a small region. The quantitative repression of *FLC* is achieved by switching an increasing fraction of the loci to the silent state with increasing duration of exposure to cold; the overall level of *FLC* expression is, thus, an average over the population of loci, each of which is in a silent or active state.

The inherent simplicity and robustness of the chromatin dynamics underlying vernalization would suggest that this mechanism is likely to have more general roles in biology. Measurement and memory of other environmental cues that are used to time plant developmental transitions such as germination or bud dormancy might be examples. The conservation of Polycomb and other silencing systems, which are important for gene regulation throughout higher eukaryotes, also suggests that concepts emerging from an understanding of the dynamics of vernalization have widespread relevance. They might also be more generally important in the antagonism between opposing regulatory pathways, such as the ubiquitous Polycomb and Trithorax group proteins (Ringrose and Paro, 2004; Schuettengruber et al., 2007; Schwartz and Pirrotta, 2008).

Acknowledgements

We thank all members of the Dean and Howard laboratories for useful comments on the manuscript. We apologise to all those whom we were unable to cite due to space constraints.

Funding

Research in the authors' labs is supported by the Advanced Investigator European Research Council (ENVGENE) [grant number 233039] and the Institute Strategic Programme [grant number BB/J004588/1] from the Biotechnology and Biological Sciences Research Council to the John Innes Centre.

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