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Epigenetic regulation of flowering

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The acceleration of flowering by prolonged low temperature treatment (vernalization) has unique properties including the floral transition occurring at a time separate from the vernalization treatment. This implies the vernalization condition is inherited through mitotic divisions, but this vernalized state is not inherited from one generation to the next. FLC, the key gene mediating this response in the Arabidopsis is repressed by histone modifications involving the VRN2 protein complex. Other protein complexes participate in activating the gene. While many plant species depend on vernalization for optimising flowering time, the genes involved differ between dicot and monocot plants in both Arabidopsis and cereals, vernalization regulates photoperiod control of flowering by preventing the induction of the floral promoter FT by long days in autumn but allowing induction of FT in spring and hence flowering occurs at an optimal time in the annual life cycle.

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Introduction

Epigenetic controls of gene expression have been explored in the genomes of the animal kingdom over the past few years. In plants, one epigenetically controlled developmental process is the promotion of flowering as a consequence of exposure of the developing plant to a period of low temperature (vernalization). Vernalization induction of flowering is operative in many plant species, particularly those growing in temperate regions; the low temperature exposure can be applied to the developing plant and in many cases to germinating seed. In all cases, the period of exposure to low temperature results, at a time later in plant development, in the transformation of the vegetative meristem of the plant to a reproductive meristem, which produces the inflorescence with flowers and ultimately seed for the next generation.

Vernalization generally acts in concert with a long day photoperiod cue to ensure flowering and seed formation occurs in spring and summer.

There are two striking properties of the vernalization response (reviewed in [1]). The low temperature induced activity state of key flowering genes is inherited through successive mitotic divisions that occur during development of the plant until the reproductive transformation at the apical meristem occurs. The second property is that the mitotic memory of the vernalized state is reset to the original activity state of the key genes in the next sexual generation.

The mode of action of vernalization promotion of flowering has been a puzzle for many years, primarily because of the separation in time of the initial response to the exposure to low temperature and the final result of flowering, often many weeks later. In recent years Arabidopsis has provided an understanding of the vernalization response at a molecular level. The key gene in the vernalization response in Arabidopsis is *FLOWERING LOCUS C* (*FLC*) [2,3]. The vernalization-induced state of repressed transcriptional activity at the *FLC* locus is achieved through specific modifications of the histones associated with the chromatin of this gene segment [4••,5••].

In this review we present details of epigenetic control of gene activity in the response but we are still faced with the intriguing puzzle of the mitotic memory and just how the epigenetic state of gene activity is promulgated through successive mitotic divisions. We are now able to say something about the timing of resetting of the activity state of *FLC* in the next sexual generation, but again, there is little information as to the detailed molecular mechanism involved.

Regulation of FLC

FLC is a MADS box gene producing a protein which maintains the vegetative state of the growing apex of the plant. There are a number of 'loci', which affect the regulation of *FLC* activity, the major activator being *FRIGIDA* (*FRI*) [6]. Recently *SUPPRESSOR OF FRIGIDA4* (*SUF4)* has been shown to be necessary for the *FRI*-associated late flowering, which results from a high level of *FLC* activity [7••,8•]. SUF4 contains a nuclear localisation signal, two C_2H_2-type zinc finger domains and a proline rich domain; it interacts with FRI in a complex, which binds to a specific region of the *FLC* promoter previously identified as a positive regulation of *FLC*. Two other proteins, FRIGIDA ESSENTIAL1 (FES1) and

FRIGIDA-LIKE1 (FRL1), are likely to be in this same complex [9•,10] (Figure 1).

Homologues of the proteins of the yeast PAF1 complex (RNA polymerase II associated factor) are the VERNALIZATION INDEPENDENT PROTEINS (VIPs1–7 EARLY FLOWERING 8 (ELF8) is a synonym of VIP6) which together with ELF7 are required for transcriptional activity of *FLC* [11,12]. In yeast, PAF1 is required for full expression of a number of genes and is involved in the initiation and elongation of transcripts. The PAF1 complex also interacts with the histone methyl transferases SET1 and SET2, which are involved, respectively, in methylation of histone H3K4 and H3K36 residues, marks of transcriptionally active chromatin [13] (Figure 1).

In *Arabidopsis*, mutations in *ELF7*and *8* result in reduced H3K4 trimethylation in *FLC* chromatin and generate earlier flowering [11]. EARLY FLOWERING IN SHORT DAYS (EFS, synonym SET DOMAIN GROUP 8, SDG8), a putative H3K36 methyltransferase, causes, in its mutant form, early flowering in short days [14,15].

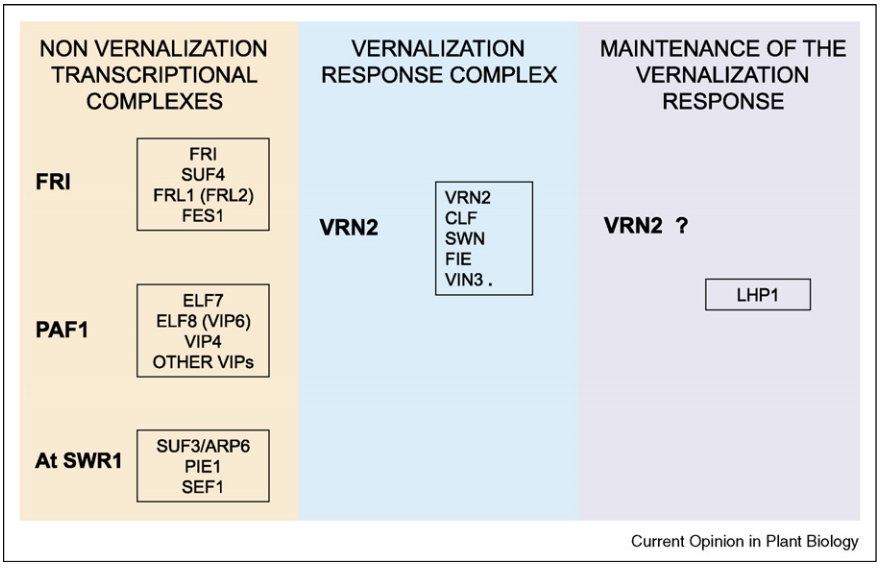
The histone variant H2A.Z is necessary for transcriptional activity of FLC [16••]. Three genes, PHOTOPERIOD INDEPENDENT EARLY FLOWERING (PIE1), ACTIN RELATED PROTEIN 6 (ARP6; synonym, SUPPRESSOR OF FLOWERING 3 (SUF3)) and SERRATED LEAVES AND EARLY FLOWERING (SEF) [17] are likely to be involved in the insertion of H2A.Z into FLC chromatin. These proteins are orthologues of proteins of the SWR1 complex of yeast, which is involved in inserting H2A.Z molecules into chromatin [16••]. In Arabidopsis, null mutants of ARP6, PIE1 or SEF eliminate FLC activity and the non-active chromatin has no H2A.Z associated with it. In the active gene, the H2A.Z molecules are in nucleosomes primarily in the promoter and 3′ regions of the gene. Orthologues of other proteins of the SWR1 complex have been identified and it is likely that they will also be involved in the regulation of FLC activity (Figure 1).

A role for small RNAs in regulating the level of *FLC* activity has recently been suggested with the description of 30 and 24 nucleotide small RNAs complementary to the *FLC* sense strand and located 3′ to the major poly A addition site [18•]. The presence of these RNAs is dependent on polymerase IVa. Disruption of the region encoding the RNAs led to increased expression of *FLC* and later flowering. How these RNAs regulate *FLC* activity is not yet known.

The molecular basis of the vernalization response at the FLC locus

The vernalization-induced repression of *FLC* activity is the key component of low temperature-promoted flowering. Repression of *FLC* activity is dependent upon the low temperature induction of *VERNALIZATION INSENSITIVE 3* (*VIN3*) [5••] which is recruited to a protein complex, the VRN2 Polycomb Repressive Complex 2 (PRC2) associated with the *FLC* gene segment [19••] (Figure 1). There is a positive correlation between the level of induction of *VIN3*, the length of exposure to low temperature, the extent of promotion of flowering and a

Figure 1



Relationship between environmental cues and gene action in flowering in Arabidopsis and barley and wheat. In the long days of autumn FT is repressed (by FLC in Arabidopsis and VRN2 in wheat and barley). In winter low temperatures repress FLC in Arabidopsis or in wheat and barley VRN1 is induced. VRN2 is repressed by short days. In the long days of spring, FT is induced and causes flowering in both systems.

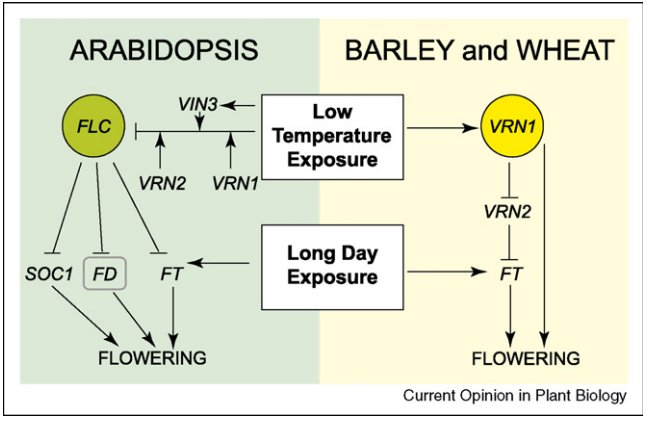
series of modifications to particular residues in the histones associated with the *FLC* gene. It is possible that the amount of VIN3 protein that is recruited to the VRN2 complex determines the proportion of histone molecules modified, decreasing transcriptional activity at the *FLC* segment. However, when *VIN3* is expressed under the 35S promoter it is not able to repress *FLC* so some modification of the protein or another factor must be required (Figure 2).

The protein constituents of the VRN2 complex are VRN2, CURLY LEAF (CLF1), SWINGER (SWN1), FERTILIZATION INDEPENDENT ENDOSPERM (FIE1), all being homologues of proteins of the PRC2 complex of Drosophila [18•] (Figure 1). During vernalization, there is an increase in the PRC2 complex components.

VIN3 is associated with a histone deacetylation activity, which occurs during the low temperature exposure. Other modifications include the removal of the transcriptionally active marks of H3K4me3 and H3K36me and the acquisition of the repressive marks H3K27me3 and H3K9me2 [4••,5••]. Trimethylation of H3K27 is carried out by CLF and SWN proteins. Another protein interacting with VIN3, VIN3-like1 (VIL1, synonym VRN5), is also critical for the accumulation of the repressive histone modifications during the low temperature treatment [20,21].

The repression of transcription at the *FLC* locus by low temperature is dominant in its effect over all of the positive transcriptional activities involved in the regulation of *FLC*. One reason for the overriding influence of the vernalization-induced changes could be that the VRN2 complex may mediate a heterochromatinization of *FLC* chromatin. This change in chromatin architecture could result in the displacement of the transcriptional activation complexes and promote the loss of activity of the gene. During vernalization genes adjacent to *FLC* are co-ordinately downregulated along with *FLC*; an *NPTII* gene inserted next to *FLC* is also downregulated. If *FLC* is moved to other chromosomal locations the *NPTII* gene next to *FLC* on the construct is also downregulated by vernalization. Thus *FLC* can confer responsiveness to vernalization on adjacent chromatin segments [22].

Figure 2



Complexes involved in regulating FLC. PAF1, FRI, AtSWRI and PARO complexes are necessary for activity of FLC before vernalization. During vernalization the VRN2 PRCII associates with VIN3 and represses FLC. Following vernalization the repressed state is maintained by LHP1 and probably the VRN2 complex without VIN3.

Action of FLC

A molecular interaction between meristem and leaf cells involves leaf derived FLOWERING LOCUS T (FT) protein transported to the cells of the meristem [23••]. In leaves, *FLC* activity suppresses the induction of *FT* until the low temperature treatment removes this block, allowing *FT* to respond to long days [24••]. The low temperature repression of *FLC* also removes the inhibition of the *SOC1* and *FD* genes. FLC binds to the *SOC1* promoter and to a region well upstream of the *FD* translation start and to a site within the first intron of *FT* [24••,25•]. The interaction of low temperature removal of FLC repression of *FT* and *SOC1* in the leaves and of *SOC1* and *FD* in the meristem, links the low temperature cue with the long day cue for induction of *FT*. In the apex, FT and FD physically interact inducing *AP1* [26•]. The activation of these two genes initiates inflorescence development and floral morphogenesis (Figure 2).

The importance of the tissue specificity of *FLC* expression was demonstrated using a series of promoters with particular patterns of tissue specific activities driving *FLC* coding region. Promoters active in the meristem, *KNAT1* and *UFO*, and in phloem, *SUC2* and *ROLC* induced late flowering [24••]. Plants with one of the phloem constructs and one of the meristem constructs showed even later flowering than plants containing just one of these constructs.

These results demonstrate that *FLC* activity preventing the long day induction of *FT* in leaves is removed in the low temperature response and show how the two environmental cues, low temperature and photoperiod long days, are linked to ensure that the flowering meristem develops at an optimum time in the annual life cycle of the plant.

The memory component of the vernalization response

The observation that the low levels of *FLC* activity following low temperature exposure are maintained during the subsequent stages of plant development, poses the question as to the mechanism of maintenance of that induced state. Two genes, *VRN1* and *VRN2*, were suggested to be involved in maintenance of the residual level of activity [27,28]. However, Sheldon *et al.* [29] using *vrn1* and *vrn2* mutants did not find evidence for involvement of the two genes in the maintenance of the activity state, but con-

firmed they were important in the extent of the initial downregulation.

Recently *LIKE-HETEROCHROMATIN PROTEIN1* (*LHP1*) [30] has been suggested to be involved in maintenance of the repressed *FLC* chromatin state (Figure 1). In Arabidopsis, LHP1 is widely distributed throughout the interphase nucleus. During vernalization the association of LHP1 with *FLC* chromatin increases. This association is maintained after the low temperature exposure [31••]. The regions of *FLC* chromatin in which LHP1 is enriched during vernalization are those in which H3K9 dimethylation occurs. A 2.8 kb region of intron1 is required for the stable maintenance of the induced repression of *FLC* [32] and Sung *et al.* [31••] further defined the essential region with a 289 bp deletion in the intron; this Vernalization Response Element (VRE) prevents the maintenance of the reduced *FLC* activity and may be the region for LHP1 binding.

In Drosophila the level of gene activity associated with the action of the PRC2, is maintained through the action of another complex, PRC1, which is recruited to the Polycomb Response Element (PRE) [33]. In Arabidopsis there is no specific definition of the PRC2 binding element. LHP1 may act in lieu of the PRC1 complex but it may be associated with as yet unknown proteins.

A recent paper casts doubts on the H3K9me binding of LHP1 as, *in vitro*, LHP1 binds to H3K9me2 or me3 and to H3K27me2 or me3, but *in vivo*, it associates almost exclusively with H3K27me3 [34••]. There is little overlap between *LHP1* binding and H3K9me2 in the genome. Most LHP1 targets co-localise with H3K27me3 sites suggesting that LHP1 may bind to the H3K27 methylated histones and maintain the repressed state. In the *lhp1* mutant H3K27me3 is still present in the *FLC* chromatin indicating that the LHP1 protein is not involved in the production of the repressive mark.

The second stage of the vernalization response is the faithful transference of the changed state of *FLC* chromatin during the mitotic divisions subsequent to the low temperature treatment. The composition of the modified histones in the nucleosomes of the FLC region is in some way repeated in the daughter cells of each mitosis, even though only half of the pre-existing histone molecules would be present in each daughter chromosome. At present there is no evidence as to the mechanism of this mitotic memory of the chromatin architecture associated with the inactive state of the *FLC* gene.

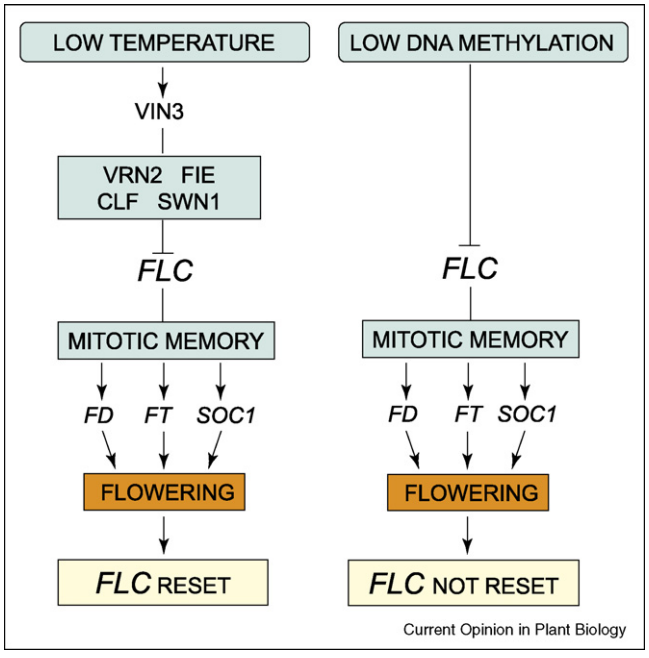
Epigenetic control associated with DNA methylation

Reduced FLC activity and earlier flowering can be induced through the action of another epigenetic mechanism, a reduced level of DNA methylation. Low genome methylation has been achieved experimentally either by a reduction in the activity of DNA Methyl Transferase (MET1) [35], which methylates CpG nucleotides, or by treatment with 5aza-cytidine which prevents DNA methylation [36]. DNA methylation levels as low as 15% of normal throughout the life cycle of the plant produce early flowering. 5aza-cytidine treatment applied as a pulse during germination also results in early flowering in vernalization requiring lines.

In a segregating F1 population from a parent with one copy of the mutant or antisense *MET1*, the level of *FLC* activity is low in segregants that receive the *MET1* antisense construct but also in those which do not. This implies that the methylation pattern within the genome is probably set in the early zygote but must be dependent on the methylation pattern inherited from the previous generation. Mutations in the methyl DNA binding protein AtMBD9 also result in lower FLC activity and earlier flowering [37] (Figure 3).

In the low methylation induced reduction in FLC activity, there is deacetylation of both histone H3 and histone H4 molecules at the FLC locus equivalent to that occurring in the vernalization response. The reduced methylation does not result in the induction of VIN3 so the origin of the deacetylation may differ in the two responses [38].

Figure 3



A comparison of vernalization and low DNA methylation pathways for FLC repression. Low methylation does not require VIN3 or members of the polycomb repressive complex II for repression. FLC activity is not reset in the progeny in the low methylation pathway.

The *FLC* gene itself has only one methylated cytosine residue in intron1, and this is not altered by vernalization. The conclusion is that the reduction of FLC activity by low methylation must be dependent on a change of methylation status at another locus, which regulates *FLC*.

Resetting of FLC activity

FLC activity following vernalization treatment of the parent plant is detectable in the early embryo but *FLC* activity is lacking in the developing endosperm. (CC Sheldon Abstract SYM-3.3-03 8th International Society of Plant Molecular Biology, Adelaide, S.A. August 2006, CC Sheldon, personal communication). Mylne *et al.* [39•] have reported that in contrast to chromosomes in mitotic divisions, there is no VRN1 associated with the chromosomes in the male meiotic divisions. VRN1 is the only protein known to be associated with *FLC* regulation and the vernalization response of *FLC* that has been shown to have a difference in presence between mitosis and meiosis.

The absence of VRN1 from the chromatin may predispose *FLC* to reset to the normal level of activity in the new embryo. The S phase of the second pollen mitosis occurs in the pollen tube and this may be the time when a new complement of histones associate with the *FLC* segment re-establishing the normal or default state of *FLC* activity.

Other species

It is likely that FLC represses long day induction of *FT* in *Brassica napus* and probably in other Brassica species. The *FLC* orthologues from *B. napus* delay flowering when introduced into Arabidopsis [40].

Vernalization treatment of *B. napus* downregulates its *FLC* orthologues' activity and promotes flowering. *B. napus* has a long day response so it is likely that the same interactions between FT, FD and SOC hold in this species. *FLC-like* genes have been identified in sugarbeet (*Beta vulgaris*) and in tomato, potato and grape. In sugarbeet the *FLC-like* gene (*BvFLI*) represses flowering when introduced into Arabidopsis. The downregulation of *BvFLI* is not stable, as its activity is upregulated on return to normal temperatures, a point of difference to the situation in the Brassicaceae [41].

The only detailed analysis of genes involved in vernalization and long day responses in plants other than Arabidopsis has been in the monocot cereals, wheat and barley, particularly in barley. Varieties of wheat and barley, termed winter varieties, require low temperature exposure in order that flowering and grain development occurs in the spring and summer. In these cereals the mitotic memory of the vernalization response is present, as is the resetting to normal gene activity levels in the next generation.

The cereal terminal apex presents a clear morphological series of the different transition states of the apical meristem. The sequence moves from a vegetative meristem producing leaf primordia to a reproductive transition state triggered by the vernalization conditions; this state establishes a new pattern of development converting the vegetative meristem to an inflorescence meristem which develops a double ridge morphology where reproductive structures are formed in the axils of the leaf primordia.

Genetic analyses have identified three loci associated with the vernalization response in barley and wheat, *VRN1*, *VRN2* and *VRN3* (*VRN1* and *VRN2* have no relationship to the *VRN1* and *VRN2* genes of Arabidopsis). The key low temperature response gene is the *VRN1* gene, a MADS box gene which is induced by the low temperature treatment [42,43]. The induction of the *VRN1* gene is parallelled by a repression of the *VRN2* gene [44]. VRN2, a repressor of flowering, is active only in long days, [45•], and whilst present, prevents the long day induction of the *VRN3* gene which is homologous to the Arabidopsis *FT* locus [46••]. *VRN3 (FT)*, when induced by long days, enhances reproductive development of the apex (Figure 2). With the induction of *FT* under long day conditions, the apex develops into a full flowering meristem producing the grain head and the seed of the next sexual generation (Figure 3).

As in Arabidopsis, low methylation induced by 5aza-cytidine in wheat can partially substitute for vernalization [47].

Epigenetic regulation of gene activity—an early strategy in angiosperm evolution

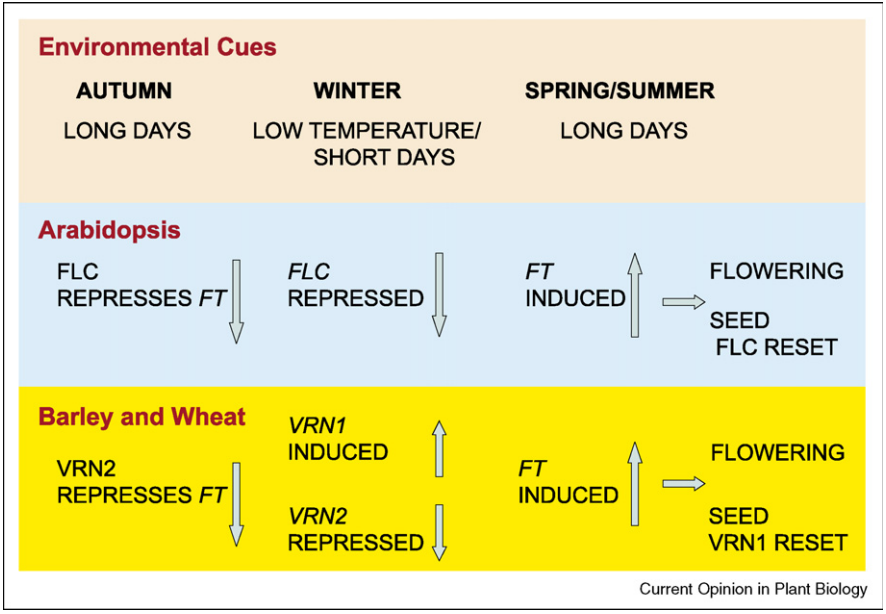
Arabidopsis, a dicotyledonous plant and the cereals, wheat and barley, monocotyledonous plants, show that these groups of plants which diverged some 150 million years ago have both employed epigenetic control of gene action to regulate flowering, a critical phase in plant development.

Vernalization induction of flowering in both plant groups has similar basic properties, the mitotic memory of the low temperature response involves a change in the state of activity of a key gene, and the resetting of that gene to the default situation in the next sexual generation. The key genes involved in the vernalization response, *FLC* in Arabidopsis and *VRN1* in cereals, are both MADS box transcription factors, a family of genes, many of which are associated with aspects of reproductive processes in plants. The vernalization induced response of these two genes present contrasting situations; *VRN1* is induced by low temperature and *FLC* is repressed by the low temperature treatment.

FT is of central importance in both groups. Induction of *FT* by long day photoperiod in the autumn is prevented by *VRN2* in cereals and by *FLC* in Arabidopsis (Figure 4).

It is not known whether the low temperature induction of the cereal *VRN1* gene involves similar protein complexes and histone modifications featured in the repression of

Figure 4



A comparison of vernalization pathways between Arabidopsis and barley and wheat. While the FT gene is sequenced and functionally very similar between wheat and barley and Arabidopsis, VRN1 and VRN2 are very different in the two categories.

the *FLC* gene. Since the mitotic memory and resetting characteristics are similar, we suspect that *VRN1* induction will be shown to involve histone modifications with appropriate marks of methylation and acetylation on particular residues.

The polycomb complex/histone modification mode of chromatin control of gene activity is likely to apply in at least one other major process of plant development—seed development. The FIS protein complex shows similar but not completely identical composition and behaviour to the VRN2 complex. It acts to repress the induction of seed development before a double fertilization event. Loss of activity of one or more of the proteins in the FIS complex leads to precocious development of endosperm but not of embryo formation [48].

A third system surrounding the activity of the *AGAMOUS* gene may also have epigenetic controls which are associated with the onset of flowering but there are no detailed analyses as yet [49].

Epilogue

We discussed reduced levels of genomic methylation as another mode of epigenetic regulation and repression of *FLC*. Although DNA methylation has mainly been shown to control transposable element activities in plants, the finding that some 30% of genes in Arabidopsis have methylated C residues in their coding regions, may indicate that DNA methylation is widely important for the fine tuning of gene expression. Methylation levels do not seem to be reversible as is histone modification. Patterns of methylation are not reset in successive sexual generations and it is more likely that changes in gene activity associated with a change in the methylation status of a gene are normally involved in control of activity in different somatic tissues, which do not contribute to subsequent generations. There are demethylases that are known to act on particular loci in certain tissues [50].

So why do epigenetic modes of control of gene activity exist alongside the gene-by-gene controls mediated through transcriptional activators? One possible reason for the evolution of the chromatin control is that it is reversible from generation to generation. This could be important for a plant exposed to the exigencies of climate in the different seasons of the year, ensuring that the key processes of flowering and seed development occur in the most propitious times of each annual or biennial cycle ensuring the ongoing existence of the species.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lang A: In *Physiology of Flower Initiation Encyclopedia of Plant Physiology.* Edited by Ruhland W. Berlin: Springer-Verlag; 1965.

2. Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES: **The FLF MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation**. *Plant Cell* 1999, **11**:445-458.

3. Michaels SD, Amasino RM: **FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering**. *Plant Cell* 1999, **11**:949-956.

4. •• Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C: **Vernalization requires epigenetic silencing of FLC by histone methylation**. *Nature* 2004, **427**:164-167.
Characterisation of the histone modifications that occur at the FLC chromatin during and after vernalization.

5. •• Sung SB, Amasino RM: **Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3**. *Nature* 2004, **427**:159-164.
Characterisation of the histone modifications that occur at the FLC chromatin during and after vernalization. *VIN3* is identified as critical for the vernalization response. *VIN3* is induced by low temperature treatment and represses *FLC*.

6. Napp-Zinn K: **Population genetical and gene geographical aspects of germination and flowering in Arabidopsis thaliana**. *Arabidopsis Inform Serv* 1976, **13**:56.

7. •• Kim S, Choi, Park KC, Hwang HJ, Lee I: **SUPPRESSOR OF FRIGIDA4, encoding a C_2H_2-type zinc finger protein. Represses flowering by transcriptional activation of Arabidopsis FLOWERING LOCUS C**. *Plant Cell* 2006, **18**:2985-2998.
Description of a complex that includes FRI, FRL1 and FES. This complex activates *FLC* by binding to the *FLC* promoter at a region previously identified as being a positive regulatory segment.

8. • Kim SY, Michaels SD: **SUPPRESSOR OF FRI 4 encodes a nuclear-localized protein that is required for delayed flowering in winter-annual Arabidopsis**. *Development* 2006, **133**:4699-4707.
See annotation for Ref. [7••].

9. • Schmitz RJ, Hong L, Michaels S, Amasino RM: **FRIGIDA-ESSENTIAL 1 interacts genetically with FRIGIDA and FRIGIDA-LIKE 1 to promote the winter-annual habit of Arabidopsis thaliana**. *Development* 2005, **132(24)**:5471-5478.
See annotation for Ref. [7••].

10. Michaels SD, Bezerra IC, Amasino RM: **FRIGIDA-related genes are required for the winter-annual habit in Arabidopsis**. *Proc Natl Acad Sci U S A* 2004, **101**:3281-3285.

11. He Y, Doyle MR, Amasino RM: **PAF1-complex-mediated histone methylation of FLOWERING LOCUS C chromatin is required for the vernalization-responsive, winter-annual habit in Arabidopsis**. *Genes Dev* 2004, **18**:2774-2784.

12. Oh S, Zhang H, Ludwig P, van Nocker S: **A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the Arabidopsis FLC/MAF MADS box gene family**. *Plant Cell* 2004, **16**:2940-2953.

13. Rondón AG, Gallardo M, García-Rubio M, Aguilera A: **Molecular evidence indicating that the yeast PAF complex is required for transcription elongation**. *EMBO Rep* 2004, **5(1)**:47-53.

14. Zhao Z, Yu Y, Meyer D, Wu C, Shen WH: **Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36**. *Nat Cell Biol* 2005, **7**:1256-1260.

15. Kim SY, He Y, Jacob Y, Noh YS, Michaels S, Amasino R: **Establishment of the Vernalization-responsive, winter-annual habit in *Arabidopsis* requires a putative histone H3 methyl transferase**. *Plant Cell* 2005, **17**:3301-3310.

16. •• Deal RB, Topp CN, McKinney EC, Meagher RB: **Repression of flowering in Arabidopsis requires activation of FLOWERING LOCUS C expression by the histone variant H2A**. *Z. Plant Cell* 2007, **19**:74-83.
Demonstrates that a histone variant, H2A.Z, incorporated into *FLC* chromatin is critical for *FLC* activity. A number of previously described loci which are necessary for *FLC* activity encode proteins essential for the insertion of H2A.Z into the FLC chromatin.

17. March-Diaz R, Garcia-Domingues M, Flrencio FJ, Reyes JC: **SEF, a new protein required for flowering repression in Arabidopsis. Interacts with PIE1 and ARP6**. *Plant Physiol* 2007, **143**:893-901.

18. • Swiezewski S, Crevillen P, Liu F, Ecker JR, Jerzmanowski A, Dean C: **Small RNA-mediated chromatin silencing directed to the 3′ region of the Arabidopsis gene encoding the developmental regulator, FLC**. *Proc Natl Acad Sci U S A* 2007, **104**:3633-3638.
Identifies small RNAs that may play a role in regulating FLC. These RNAs do not mediate the downregulation of FLC by vernalization.

19. •• Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA: **The Arabidopsis thaliana vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3**. *Proc Natl Acad Sci U S A* 2006, **103(39)**:14631-14636.
Identified the components of the VRN2 polycomb complex and showed the VIN3 is recruited to the complex during low temperature treatment.

20. Sung S, Schmitz RJ, Amasino RM: **A PHD finger protein involved in both the vernalization and photoperiod pathways in Arabidopsis**. *Genes Dev* 2006, **20**:3244-3248.

21. Greb T, Mylne JS, Crevillen P, Geraldo N, An H, Gendall AR, Dean C: **The PHD finger protein VRN5 functions in the epigenetic silencing of Arabidopsis FLC**. *Curr Biol* 2007, **17(1)**:73-78.

22. Finnegan EJ, Sheldon CC, Jardinaud FM, Peacock WJ, Dennis ES: **A cluster of Arabidopsis genes with a co-ordinate response to an environmental stimulus**. *Curr Biol* 2004, **14**:911-916.

23. •• Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G: **FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis**. *Science* 2007, **316**:1030-1033.
Shows that it is the FT protein that is translocated.

24. •• Searle I, He Y, Turck F, Vincent C, Fornara F, Kröber S, Amasino RA, Coupland G: **The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis**. *Genes Dev* 2006, **20**:898-912.
Describes the binding of FLC to SOC1, FT and FD. Shows that FLC, if expressed in the phloem or apex, can cause late flowering. A combination of phloem and apex expression causes even later flowering.

25. • Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES: **The Arabidopsis FLC protein interacts directly *in vivo* with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex**. *Plant J* 2006, **46**:183-192.
Shows that FLC binds directly to the promoter of SOC1 and the first intron of FT and presumably this is the mechanism of repression of these genes by FLC.

26. • Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T: **FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex**. *Science* 2005, **309**:1052-1056.
Identifies FD as a bzip protein that interacts with FT to activate flowering.

27. Gendall AR, Levy YY, Wilson A, Dean C: **The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in, Arabidopsis**. *Cell* 2001, **107**:525-535.

28. Levy Y, Mesnage S, Mylne JS, Gendall AR, Dean C: **Multiple roles of *Arabidopsis* VRN1 in vernalization and flowering time control**. *Science* 2002, **297**:243-246.

29. Sheldon CC, Finnegan EJ, Peacock WJ, Dennis ES: **Quantitative effects of vernalization on FLC and SOC1 expression**. *Plant J* 2006, **45**:871-883.

30. Nakahigashi K, Jasencakova Z, Schubert I, Goto K: **The Arabidopsis heterochromatin protein1 homolog (TERMINAL FLOWER2) silences genes within the euchromatic region but not genes positioned in heterochromatin**. *Plant Cell Physiol* 2005, **46**:1747-1756.

31. •• Sung SB, He YH, Eshoo TW, Tamada Y, Johnson L, Nakahigashi K, Goto K, Jacobsen SE, Amasino RM: **Epigenetic maintenance of the vernalized state in Arabidopsis thaliana requires LIKE HETEROCHROMATIN PROTEIN 1**. *Nat Genet* 2006, **38**:706-710.
Shows that LHP1 is essential for maintenance of the repressed state of *FLC*. A 289bp region of the first intron of FLC (the Vernalization Responsive Element) is also essential for the maintenance.

32. Sheldon CC, Conn AB, Dennis ES, Peacock WJ: **Different regulatory regions are required for the vernalization-induced repression of FLOWERING LOCUS C and for the epigenetic maintenance of repression**. *Plant Cell* 2002, **14**:2527-2537.

33. Ringrose L, Paro R: **Polycomb/Trithorax response elements and epigenetic memory of cell identity**. *Development* 2007, **134**:223-232.

34. •• Turck F, Roudier F, Farrona S, Martin-Magniette ML, Guillaume E, Buisine N, Gagnot S, Martienssen RA, Coupland G, Colot V: **Arabidopsis TFL2/LHP1 Specifically Associates with Genes Marked by Trimethylation of Histone H3 Lysine 27**. *PLoS Genet* 2007:e86.eor doi: 10.1371/journal.pgen.0030086.eor.
A genomic analysis of the binding properties of LHP1. The authors conclude that *in vivo* LHP1 binds to H3K27me3, not H3K9me2.

35. Finnegan EJ, Genger RK, Kovac K, Peacock WJ, Dennis ES: **DNA methylation and the promotion of flowering by vernalization**. *Proc Natl Acad Sci U S A* 1998, **95**:5824-5829.

36. Burn JE, Bagnall DJ, Metzger JD, Dennis ES, Peacock WJ: **DNA methylation, vernalisation and the initiation of flowering**. *Proc Natl Acad Sci U S A* 1993, **90**:287-291.

37. Peng MS, Cui YH, Bi YM, Rothstein SJ: **AtMBD9: a protein with a methyl-CpG-binding domain regulates flowering time and shoot branching in Arabidopsis**. *Plant J* 2006, **46(2)**:282-296.

38. Finnegan EJ, Kovac KA, Jaligot ES, Sheldon CC, Peacock WJ, Dennis ES: **The down regulation of FLOWERING LOCUS C (FLC) in plants with low levels of DNA methylation and by vernalization occurs by distinct mechanisms**. *Plant J* 2005, **44**:420-442.

39. • Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C: **LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of FLC**. *Proc. Natl Acad Sci U S A* 2006, **103**:5012-5017.
Shows that LHP1 is required for the maintenance of the FLC repressed state following vernalization. VRN1 is not expressed in male meiosis.

40. Tadege M, Sheldon CC, Helliwell CA, Stoutjesdijk P, Dennis ES, Peacock WJ: **Control of flowering time by FLC orthologues in Brassica napus**. *Plant J* 2001, **28**:545-553.

41. Reeves PA, He Y, Schmitz RJ, Amasino RM, Panella LW, Richards CM: **Evolutionary conservation of the *FLC*-mediated vernalization response: evidence from the sugar beet (*Beta vulgaris*)**. *Genetics* 2007, **176**:295-307.

42. Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J: **Positional cloning of wheat vernalization gene. VRN1**. *Proc Natl Acad Sci U S A* 2003, **100**:6263-6268.

43. Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis ES: **MADS box genes control vernalization induced flowering in cereals**. *Proc Natl Acad Sci U S A* 2003, **100(22)**:3099-13104.

44. Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J: **The wheat VRN2 gene is a flowering repressor downregulated by vernalization**. *Science* 2004, **303**:1640-1644.

45. • Trevaskis B, Hemming MN, Peacock WJ, Dennis ES: **HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status**. *Plant Physiol* 2006, **140**:1397-1405.
Shows that the VRN2 is regulated by long days and is not critical for vernalization occurring in short days.

46. •• Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J: **The wheat and barley vernalization gene VRN3 is an orthologue of FT**. *Proc Natl Acad Sci U S A* 2006, **103**:19581-19586.
The gene known from classical genetics as *VRN3* in wheat and barley is shown to be the cereal orthologue of *FT*.

47. Brock RD, Davidson JL: **5-Azacytidine and gamma-rays partially substitute for cold treatment in vernalizing winter-wheat**. *Environ Exp Bot* 1994, **34(2)**:195-199.

48. Kohler C, Makarevich G: **Epigenetic mechanisms governing seed development in plants**. *EMBO Rep* 2006, **7(12)**:1223-1227.

49. Schubert D, Primavesi L, Bishopp A, Roberts G, Doonan J, Jenuwein T, Goodrich J: **Silencing by plant Polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27**. *EMBO J* 2006, **25(19)**:4638-4649.
Shows for a number of systems that H3K27me3 is critical for polycomb repression.

50. Penterman J, Zilberman D, Huh JH, Ballinger T, Henikoff S, Fischer RL: **DNA demethylation in the Arabidopsis genome**. *Proc Natl Acad Sci U S A* 2007, **104**:6752-6757.