Regulation of the floral repressor gene *FLC*: the complexity of transcription in a chromatin context

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The genetic pathways regulating the floral transition in *Arabidopsis* are becoming increasingly well understood. The ease with which mutant phenotypes can be quantified has led to many suppressor screens and the molecular identification of the underlying genes. One focus has been on the pathways that regulate the gene encoding the floral repressor *FLC*. This has revealed a set of antagonistic pathways comprising evolutionary conserved activities that link chromatin regulation, transcription level and co-transcriptional RNA metabolism. Here we discuss our current understanding of the transcriptional activation of *FLC*, how different activities are integrated at this one locus and why *FLC* regulation seems so sensitive to mutation in these conserved gene regulatory pathways.

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**Introduction**

One of the most exciting aspects of plant science today is how much it contributes to general concepts in biology. The ability to combine a genetic analysis with detailed molecular and biochemical approaches offers real advantages for mechanistic analysis. Of all the developmental transitions in plants flowering has been the best studied and over the last 15 years a concentrated effort in *Arabidopsis thaliana* has provided a good understanding of how different environmental and endogenous cues are integrated to cause the developmental switch [1]. Several flowering time pathways converge on *FLOWERING LOCUS C (FLC)*, a MADS box transcriptional regulator that represses the floral transition (Figure 1). Vernalization, the acceleration of flowering by prolonged cold, epigenetically silences *FLC* through the action of Polycomb proteins which deposit the repressive histone mark H3K27me3 [2]. Acting in parallel to vernalization is the autonomous pathway, a series of activities that involve RNA-mediated chromatin silencing of *FLC* [3]. These repressive pathways have received much attention and involve a complex regulation of sense and antisense *FLC* transcripts [4, 5] that we shall review in a future COPB.

Here we focus on the transcriptional activating mechanisms and discuss how they might be co-ordinated to regulate *FLC* expression. Some of these activities clearly involve transcriptional processes common to many organisms, whilst others appear to involve proteins with no clear homologues in animals. How these activities can be fine-tuned in response to different inputs is likely to provide an important paradigm for gene regulation in many eukaryotes.

**Conserved chromatin complexes required for transcriptional activation of *FLC***

Many genetic screens have identified suppressors of high-level *FLC* expression. Molecular characterization of these has identified a series of activities that link chromatin regulation and transcriptional control, which have been characterized extensively in yeast and metazoans (Table 1). Mutation of these generic factors would be expected to have catastrophic effects for the plant but remarkably many are still viable. A common phenotype is early flowering due to reduced expression of *FLC* and in many cases also of its homologues, the *MAF* genes [1, 2]. What are these generic chromatin complexes and what is their function in transcription? The nucleosomal structure of chromatin imposes problems for the large RNA polymerase complex during transcription, necessitating activities by chromatin complexes mediating histone modifications, histone variant exchange and chromatin remodelling [6, 7]. At many genes the polymerase engages and then quickly pauses, a state often characterized by accumulation of a peak of H3K4me3, a histone mark associated with active transcription. Transition into the elongation phase requires RNA polymerase-associated factor 1 complex (Paf1C); RAD6-BRE1, important for histone H2B ubiquitination; and Set complexes, important for histone methylation. A schematic overview of their functions in the transcription initiation and elongation is given in Figure 2. Below we describe what we know of these complexes at *FLC* and how their functions are integrated.

**Paf1 complex**

The Paf1 complex accompanies the RNA polymerase II (PolII) from the promoter to the 3' end of the mRNA. All
the components of the Arabidopsis Paf1C (Table 1) have emerged from screens for early flowering mutants suggesting flowering time regulation by FLC is very sensitive to changes in Paf1C function [8–11,12,13]. There is still extensive discussion and research into the precise role it plays but loss of Paf1C in yeast and mammalian cells affects transcriptional elongation, a number of histone modifications/chromatin-remodelling activities and 3’ end processing factor recruitment [7]. It is therefore still not clear whether Paf1C is primarily a platform on PolII that coordinates association of many factors, or if the complex plays a more direct role in one or more key steps in transcription.

At the FLC locus, paf1c mutations cause reduction in transcription, loss of H3K4me3 at the 5’ end and H3K36me2/3 in the main body of the gene. This is accompanied by increases in H3K27me3 over the locus [14]. Most of the mutations also caused floral organ phenotypes and reduced plant size, however mutants in AtdeC73 (also called PHP) showed less pleiotropy [12,13]. Why FLC expression is apparently more sensitive to loss of AtCDC73 is not clear but the human homologue, also called Parafibromin, is required for correct transcript processing likely through a direct interaction with the CPSF-CstF RNA processing complex [15]. This may provide an interesting connection to the autonomous pathway function where the same 3’ processing complex is important for alternative processing the FLC antisense transcript [5]. Antagonistic effects on co-transcriptional RNA metabolism of FLC could account for why AtdeC73 shows different interactions with mutants of the autonomous pathway [12].

Histone 2B ubiquitination

Mutations disturbing ubiquitination levels of histone H2B also result in early flowering phenotypes. In yeast H2B monoubiquitination requires RAD6-BRE1 activities; Paf1C is necessary for RAD6-BRE1 dependent ubiquitination and in turn H2Bub1 is necessary for H3K4me3 (Figure 2). Arabidopsis has two E3 ubiquitin ligases homologous to BRE1 (HU1B and HUB2) and three E2 carrier proteins homologous to RAD6 (UBC1 to UBC3) [16]. hub1 and hub2 mutants, and ubc1 ubc2 double mutant show loss of H2Bub1, reduction of H3K4me3 and H3K36me3, early flowering and misregulation of FLC [16,17,18]. They also show pleiotropic phenotypes and determine genome-wide levels of H2Bub1 but not H3K4me3 [18]. The ubiquitin modification enhances the movement of PolII through nucleosomes, possibly via Facilitates Chromatin Transcription (FACT) complex dependent removal of H2A/H2B dimer. In Arabidopsis, mutations in the FACT subunits SSRP1 and SPT16 also produce severe phenotypes including early bolting due to FLC misregulation [19]. In some cases, full transcription requires cycling of both H2B ubiquitination and deubiquitination although the exact function of the later is not well known [6]. Arabidopsis UBP26 catalyses the deubiquitination reaction and ubp26 is also early flowering [20].

Histone K4 and K36 methyltransferase complexes

Arabidopsis genome sequence analysis initially identified 29 SET domain proteins [21] and several have now been shown to function at FLC. EFS/SDG8 functions as the homologue of methyltransferase SET2 delivering H3K36 methylation to the 3’ end of FLC [22,23]. The Trithorax homologues ATX1 and ATX2 deposit H3K4me3/me2 respectively at the 5’ end of FLC [24,25]. And mutants in the Set1 homologue ATXR7/SDG25 show reduced FLC, decreased H3K4me3, slightly decreased H3K36me2 and increased H3K27me3 [26,27]. Interestingly, ATX1, ATX2 and ATXR7 are not fully redundant and have additive roles regulating FLC expression. These histone methyltransferases also regulate a number of targets genome wide, for example about 900 genes with changed expression levels were found in atx1 mutant plants [25]. FLC is also target of the Arabidopsis homologue of the human WD40 domain-containing protein WDR5, namely AtWDR5a, which is a conserved core
component of the H3K4 methyltransferase COMPASS/MLL complex. Recombinant AtWDR5a binds H3K4-methylated peptides and directly interacts with ATX1 [28**]. Current data support that FRIGIDA, a profound up regulator of FLC levels (see below), is required for the enrichment of AtWDR5a at the FLC locus and increases in H3K4me3 [28**].

**SWR1 complex**

Another series of early flowering mutants identified a major role for the SWR1 complex in FLC expression. The SWR1/SRCAP complex is a chromatin-remodelling complex that has been shown to be involved in substitution of histone H2A by the histone variant H2AZ. Mutations in PIE1 [29], ARP6 (also known as SUF3 and ESD1 in Arabidopsis) [30–32], SEF/AtSW6C [33–35] all gave early flowering and ARP6 and PIE1 were found to be required for H2AZ deposition at FLC [36]. H2AZ is frequently enriched at the 5′ and 3′ end of genes, and is associated with both gene activation and gene repression. It is thought to lead to relative nucleosome instability so aiding in nucleosome displacement by transcription machinery and this could be the mechanism by which it promotes FLC transcription.

**Requirements for FLC activation by FRIGIDA**

In the 1950s it was established that the overwintering habit of Arabidopsis could be mapped predominantly as a monogenic trait to the FRIGIDA (FRI) locus. Arabidopsis laboratory accessions like Columbia and Landsberg erecta carry non-functional fri alleles, but addition of an active FRI very strongly upregulates FLC expression so determining a vernalization requirement on plants [2]. The majority of FRI suppressors identified components required for high FLC expression as described above. Some, however, identified components that had much weaker effects on autonomous pathway mutations or were specifically required for FRI-dependent increases. Since FRI is a novel protein with coiled-coil domains but little other homology to other known proteins [37] these proteins could represent a plant specific function or perhaps more likely a function with poorly conserved components. Mutations in five genes fall into this group (Table 1).

**FRIGIDA-like genes (FRL1 and FRL2)**

The Arabidopsis FRI family has seven members and is conserved among other plant species [37]. FRL1 was isolated as an FRI suppressor in a mutagenesis screen of a Columbia-FRI line [38]. FRL1 shares only a low level of homology with FRI throughout the length of the proteins and they do not function redundantly. The different FRL proteins may have specific roles as the fri1 fri2 double mutant in Columbia flowers earlier than fri1. Interestingly, FRL1-Ler is non-functional and its function is replaced by a strong FRL2-Ler allele [39].

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</table>
FRIGIDA ESSENTIAL 1 (FES1)
A CCCH zinc finger protein expressed in shoot and root apices and the vascular system and conserved in other plant species [40]. CCCH zinc finger proteins can bind RNA so FES1 may function co-transcriptionally on FLC.

SUPPRESSOR OF FRIGIDA 4 (SUF4)
This contains a BED-ZP207 zinc finger domain (DNA binding) and proline-rich region (often important for protein interactions) [41,42]. SUF4 appears to be nuclear-localized, expressed more widely than FLC and the transcript is alternatively spliced [42]. SUF4 interacts with FRI and FRL1 and binds to the FLC promoter, and may provide specificity to the putative FRI complex. [41]

FLC EXPRESSOR (FLX)
An alpha-helix protein that forms part of a small family of related proteins in Arabidopsis [43]. FLX function is unknown but its leucine zipper domain shares homology with the yeast nuclear proteins SMC and NUF1 [43].

All these mutants are genetically epistatic with fri suggesting that they function in the same pathway. There have also been reported protein-protein interactions between FRI or FRL1 with SUF4 [41], and between FRI and FRL1 [41] supporting the idea that they physically interact and form a protein complex (Figure 3). How such a complex might activate FLC transcription is still a matter of speculation. However, like the autonomous pathway it could involve co-transcriptional processes. Mutations in the Arabidopsis CAP binding complex (CBC) subunits CBP80 and CBP20 suppress FRI and CBP20 interacts directly with FRI in yeast and in planta [44,45]. The data suggest that FRI upregulates FLC expression through a co-transcriptional mechanism involving direct physical interaction with the nuclear CBC with concomitant effects on FLC transcription [44*]. This is not the only example of late flowering phenotype suppression linked to RNA metabolism; mutations in HUA2 [46], a putative DExH-box RNA helicase required for the processing of AGAMOUS pre-mRNA, and PEP-PER [47], a protein with three KH RNA-binding domains, also suppress late flowering due to high FLC levels. There are now many examples in yeast and metazoans indicating a tight connection between transcriptional elongation, RNA processing, and export [48].
Reactivation of FLC during the late reproductive phase

After Arabidopsis has been vernalized, generally in the vegetative phase, FLC expression is silenced and this state is epigenetically maintained throughout most of the rest of the life-cycle. However, to ensure each generation of plants requires vernalization, FLC expression is reactivated during gametogenesis and embryo development [51*,52*]. Current data suggest a default program for reactivation of FLC expression even in non-vernalized plants [52*] indicating that FLC ‘resetting’ could be part of a genome-wide epigenetic reprogramming at plant embryogenesis. The precise role of all the chromatin regulators and FRI-accompanying proteins in this process is still unknown. But pipel mutation impairs FLC::GUS transgene expression at all embryonic states whereas FRI and SUF4 are only required to maintain FLC::GUS expression during late embryogenesis [52*]. High FLC expression during later stages of embryo development has been shown to be crucial for late flowering [51*], so the identity of the regulatory factors activating FLC during embryogenesis remains an important, unresolved question.

Conclusions

Analysis of mutations that activate or silence expression of the floral regulator gene FLC have revealed conserved gene regulatory pathways that link chromatin regulation and transcription. This system therefore provides an excellent vehicle to combine genetic with biochemical analysis and fully define those activities whilst exploring the conservation in gene regulatory pathways. An interesting question that emerges from this analysis is why FLC is apparently so sensitive to mutations in pathways that probably regulate most of the genome. Could this be due to FLC transcriptional output being set by a balance between the antagonistic pathways that promote or repress — a molecular sumo wrestle? The effect of loss of one of the activities is magnified by the increased activity of the opposing effect. Mutations in components leading to loss of H3K4me3 often result in increased H3K27me3, and mutants with high levels of FLC and H3K4me3 show reduced H3K27me compared to wild type plants. [53]. Continued analysis of mutants that affect FLC expression will allow the interactions of these antagonistic pathways to be understood — what determines which activity is predominant and how does this change in natural variants? How is that interaction influenced by different environmental cues? The conservation of the pathways involved suggests lessons from these studies will be widely relevant to gene regulation generally in plants. Given the importance and remarkable conservation of co-transcriptional mechanisms across eukaryotes, they will keep interest in plant biology strong.

Update

While preparing this manuscript new data addressing EFS role in FLC up-regulation has appeared (Ko JH et al., EMBO J 2010). This excellent paper shows that EFS is crucial for FRI recruitment to FLC and FRI enhances EFS histone methyltransferase activity. The authors propose a model of how flowering time may be regulated by the balance between different histone methylation and demethylation activities.

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References and recommended reading

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

• of special interest

•• of outstanding interest

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12. Yu X, Michaels SD: The Arabidopsis PAF1c complex component CDC73 participates in the modification of FLOWERING LOCUS C chromatin. Plant Physiol 2010, 153:1074-1084. In ref. [12] and [13] the Arabidopsis CDC73/Parafibromin homologue is described. In contrast to the previous results in Arabidopsis, PAF1c mutants isolated, which show pleiotropic phenotypes, cdc73 is only defective in flowering time and affects a limited subset of genes including FLC.

13. Park S, Oh S, Ek-Ramos J, van Nocker S: PLANT HOMOLOGOUS TO PARAFIBROMIN is a component of the PAF1 complex and assists in regulating expression of genes within H3K27me3-enriched chromatin. Plant Physiol 2010, 153:821-831. In ref. [12] and [13] the Arabidopsis CDC73/Parafibromin homologue is described. In contrast to the previous results in Arabidopsis, PAF1c mutants isolated, which show pleiotropic phenotypes, cdc73 is only defective in flowering time and affects a limited subset of genes including FLC.


28. Jiang D, Gu X, He Y: Establishment of the winter-annual growth habit via FRIGIDA-mediated histone methylation at FLOWERING LOCUS C in Arabidopsis. Plant Cell 2009, 21:1733-1746. The authors describe the role of a homologue of WDR5, a core component of the human COMPASS-like complex, in regulating FLC in a PAF1-dependent manner. They also provide biochemical data showing that AtWDR5a binds to H3K4 and interacts with ATX1 protein.


This paper pursues why mutations in the CAP Binding Complex (CBC) suppress the effect of FRIGIDA on FLC up-regulation. It provides data showing direct physical interaction of FRIGIDA and CBP20, that FRIGIDA decreased the proportion of FLC transcripts lacking the 5′ cap and suggests that the FRIGIDA complex may function through a co-transcriptional mechanism linking the CBC, splicing and transcription.


This paper and ref. [52] characterize the expression changes in FLC during reproductive and embryonic phases of development using an FLC::GUS transgene. This is when FLC expression is reactivated, following the silencing caused by vernalization, a process necessary to ensure a vernalization requirement in every generation.


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