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Review

The impact of chromatin regulation on the floral transition

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ABSTRACT

Transcription in eukaryotes is regulated by many enzymes that influence the nuclear organization of DNA in chromatin. Several of these enzymes regulate histone modifications. These modifications function as a platform for assembly of protein complexes that influence chromatin structure. Dynamic changes between chromatin states that facilitate or inhibit transcription are important in the transcriptional regulation of pathways controlling floral induction in response to environmental and developmental signals. This review focuses on the mechanistic aspects of histone modifications and chromatin changes at flowering-time genes and how these relate to the initiation of flowering.

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1. Introduction

Reproductive success of a plant depends on flowering at the right time, a decision that not only involves the perception of external cues but is also dependent on internal developmental parameters. In the model plant *Arabidopsis thaliana*, the photoperiod and vernalization pathways promote flowering through the perception of long days (LD) and prolonged cold exposure, whereas the Gibberellic acid (GA)-dependent and autonomous pathways are controlled by internal cues. Importantly, the pathways converge at the level of transcription of several genes, sometimes designated as pathway integrators, which are representatives of highly regulated and complex expression modules. The regulation of floral pathway integrators has been correlated with many chromatin-associated phenomena. Our review will focus on the mechanistic aspects of chromatin changes that are observed at flowering pathway integrator genes.

Several recent reviews have presented details on the temporal and spatial regulation of the genetic network that underlies the transition to flowering [1,2]. Here, we will briefly introduce the most important convergence points within the flowering network that will be mentioned in the context of chromatin regulation later in the text (Fig. 1). Early flowering under long summer days involves activation of transcription of *FLOWERING LOCUS T* (*FT*) toward the end of the day because the protein product of its upstream activator *CONSTANS* (*CO*) is stabilized by light at this time. FT protein

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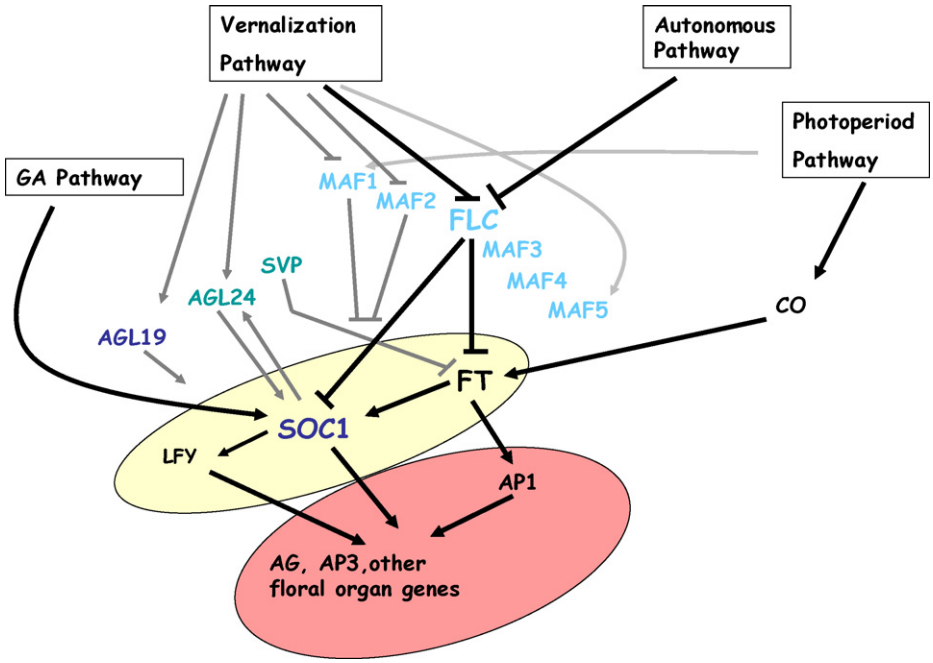


Fig. 1. Flowering pathway genes regulated by chromatin modifiers. The 4 genetic pathways to flowering are depicted in boxes. Arrows to genes indicate positive interactions, blocked lines indicated repression. Major pathway convergence points are indicated in larger size and black lines, gray lines indicate accessory pathways. MADS-box transcription factors that regulate the floral transition are indicated in color where a shared color signifies that these factors are close relatives. Genes in the yellow circle predominantly define inflorescence identity; genes in the red circle define floral identity.

moves from the site of production in the phloem companion cells of leaves to the apex where it rapidly causes the upregulation of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), which encodes a MADS box transcription factor, and less directly of *LEAFY* (*LFY*), which encodes a plant-specific transcription factor. These factors are involved in reprogramming the shoot apex from a vegetative to an inflorescence meristem. Subsequently, genes expressed specifically in the floral primordium such as *APETALA 1* (*AP1*) and floral organ identity genes such as *AGAMOUS* (*AG*) and *AP3* are expressed.

Arabidopsis accessions that require vernalization, extended exposure to low temperatures, to induce early flowering, express high levels of the MADS-box factor *FLC* due to an active allele of *FRIGIDA* (*FRI*). In these plants flowering is suppressed by direct repression of *FT* and *SOC1* by FLC [3,4]. There are five *FLC*-related loci in Arabidopsis, the *MADS AFFECTING FLOWERING* genes (*MAF 1–5*) that are co-regulated in some but not all physiological and genetic conditions. The MAFs likely also act as flowering repressors and this has been confirmed for *MAF1* (also named *FLM* and *AGL27*) and *MAF2* (also named *AGL31*) [5–8]. *FLC* expression by vernalization is stable even after a return to warmer temperatures [9–11]. Expression of *FLC* is also regulated by the autonomous pathway that represses *FLC* independently of vernalization late in development.

The GA-dependent pathway bypasses the requirement for *FT* induction and repression by FLC and promotes the expression of *SOC1* and other genes by unknown mechanisms. Several additional MADS-box factors have an impact on flowering; AGL24 and AGL19, the closest relative of SOC1, promote flowering. *AGL19* is part of an *FLC*-independent vernalization-responsive pathway [8] and *AGL24* expression is stimulated by vernalization independent of *FLC* [12]. AGL24 and SOC1 mutually stimulate each other's expression and interact to activate the expression of *LFY* [13,14]. Such interactions likely contribute to the integration of different flower promoting signals such as GA and vernalization perception. Interestingly, SHORT VEGETATIVE PHASE (SVP), the Arabidopsis protein most related to AGL24, acts as a repressor of flowering by direct repression of *FT* [15].

Transcription of floral integrator genes, like all other eukaryotic genes, requires an open chromatin structure where nucleosomes are arrayed like beads on a string. Usually, chromatin is further compacted by intranucleosomal interactions which also depend on the linker Histone 1 (H1). Several chromatin factors implicated in the regulation of floral pathway integrators act at the level of chromatin compaction.

Nucleosomes are composed of 147 base pairs of DNA wrapped around histone octamers in 1.65 superhelical turns: two H3–H4 dimers at the core flanked by two H2A–H2B dimers [16]. Even in an open chromatin conformation, nucleosomes form a strong barrier to transcription. The assembly of the large multi-protein Pre-initiation Complex (PIC) at the transcriptional start site requires a nucleosome-free region. In addition, RNA Polymerase II (RNPII) transcription through nucleosomal templates requires the removal of at least one H2A/H2B dimer from nucleosomes and the regulated repositioning of nucleosomes situated ahead of elongating RNPII to a position behind it [17,18]. Chromatin factors that participate in the transcriptional process *per se* are also crucial for the expression of floral pathway integrators.

2. Gene repression by proteins of the Polycomb Group

Methylated lysine residues of the amino-termini tail of H3, in particular at position 9 and 27, provide chromatin marks that are thought to act as an assembly platform for repressive complexes. Recent genomic studies in Arabidopsis have identified a surprisingly large number of genes (ca. 15% of all genes) that are marked by H3K27me3 [19,20]. Most H3K27me3 marked genes are lowly expressed throughout development or are expressed in a strikingly tissue-specific pattern. Many H3K27me3 targets play a confirmed role in flowering-time regulation and other developmental processes. All flowering pathway genes introduced earlier, with the notable exception of *CO*, are H3K27me3 targets.

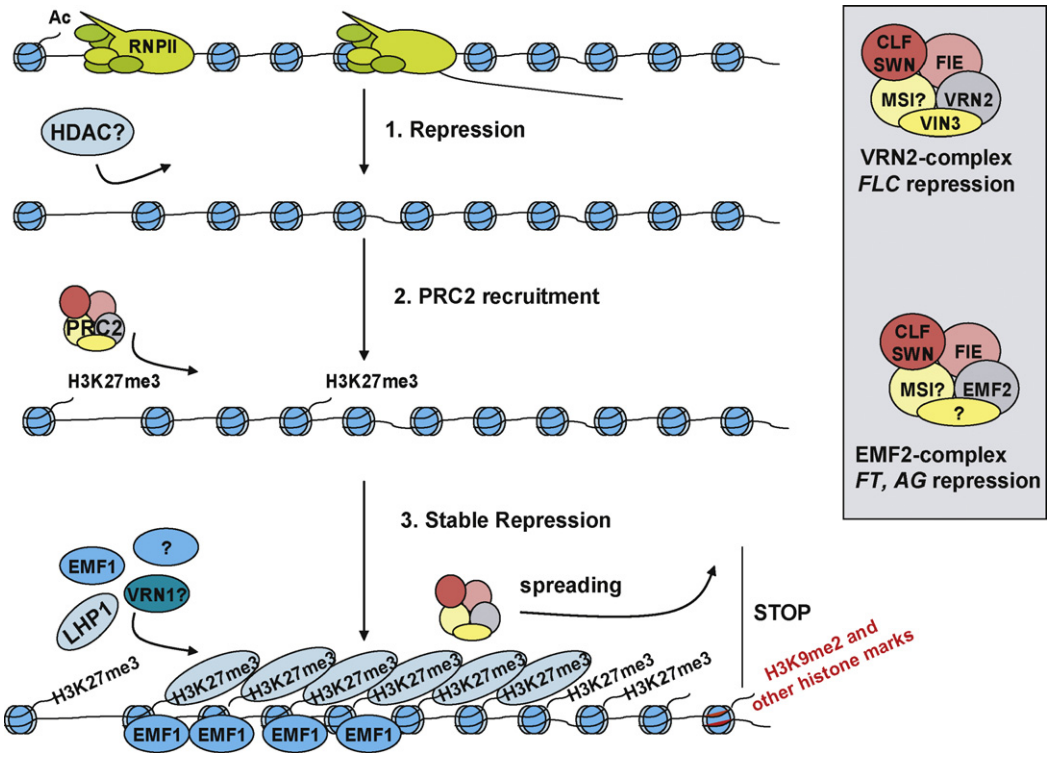


Fig. 2. PcG-mediated repression restricts access of the transcriptional machinery to target genes. (1) HDAC or other repressive complexes are targeted to a PcG-target gene for primary repression. (2) Specific PRC2 complexes, such as the VRN2 complex or the EMF2 complex (see gray box) are recruited by an unknown signal to primary target sites. The catalytic activity of PRC2 results in local trimethylation of H3 Lysine 27. (3) The H3K27me3 spreads over wide regions, sometimes encompassing several genes. Spreading is probably restricted by other Histone marks. The heterochromatic H3K9me2 mark appears to form a strong barrier for H3K27me3 [19]. Other proteins are recruited to H3K27me3 loci and participate in the stable repression. LHP1 is anchored to the target loci via its interaction with H3K27me3; EMF1 appears also to be widely associated with repressed targets, whereas VRN1 may have a more gene-specific function.

Lysine 27 methylation is functionally linked to the Polycomb Group (PcG) proteins, first discovered in genetic screens for homeotic developmental mutants in Drosophila [21]. PcG proteins are found in different complexes with distinct functions. Trimethylation of lysine 27 of H3 is carried out by Polycomb Repressive Complex 2 (PRC2), which contains the SET-domain histone methyltransferase *Enhancer of Zeste* (E(Z)) in association with *Suppressor of Zeste* (SU(Z)12) and the WD40-domain proteins *Extra sex combs* (ESC) and *Multicopy suppressor of Ira* (MSI) (SET stands for SUVAR3-9/E(Z)/Trithorax protein). H3K27me3 is recognized by PRC1, a multi-protein complex that is required for transcriptional repression. In plants, most components of the PRC2 exist as small protein families, whereas homologs of the PRC1 components are conspicuously lacking and may have been functionally replaced by unrelated proteins (Fig. 2).

2.1. The combinatorial PRC2

In *Arabidopsis*, *MEDEA* (*MEA*) [22], *CURLY LEAF* (*CLF*) [23] and *SWINGER* (*SWN* also *EZA1*) [24,25] encode homologs of E(Z). *MEA* is exclusively expressed in the gametophyte and endosperm and mutations in the gene lead to aborted development of these structures [22]. Mutations in *CLF* cause pleiotropic alterations in the sporophyte among which are homeotic transformations of floral organs, early flowering and altered leaf structure. Mutations in *SWN* alone do not cause visible alterations but enhance the effect of *mea* and *clf* mutants [24,26,27]. A decrease in H3K27me3 has been shown to occur at some genes in the *clf* and *clf swn* double mutants and this is the best experimental evidence so far of a conserved catalytic activity between the PRC2 complexes of plants and animals [26,28].

Three genes encode SU(Z)12 homologs, of which *FERTILIZATION INDEPENDENT SEED 2* (*FIS2*) possesses gametophyte and endosperm-specific expression and is essential for seedling viability [25]. *VERNALIZATION 2* (*VRN2*) [29] and *EMBRYONIC FLOWER 2* (*EMF2*) [30,31] are expressed in the sporophyte and have roles that are overlapping but not identical with *CLF* and *SWN* [31,32]. Mutations in *VRN2* specifically affect the vernalization pathway to flowering, whereas *emf2* mutants bypass the vegetative phase of development.

FERTILIZATION INDEPENDENT ENDOSPERM (*FIE*) is the only gene encoding an Arabidopsis homolog of ESC. *FIE* is an essential gene and mutations result in embryo abortion and endosperm overproliferation [33]. FIE protein is likely to be a member of all functional PRC2 complexes in *Arabidopsis*.

The small gene families encoding most PRC2 proteins in plants provide a combinatorial matrix of potentially co-existing yet distinct complexes [26]. Yeast-two-hybrid experiments and more recently co-immunoprecipitation from Arabidopsis plants expressing different combinations of epitope-tagged PcG proteins confirmed the interaction between different plant PRC2 proteins and, in part, explained the differential phenotypes of respective mutations [24,34]. CLF, SWN and FIE probably each take part in many PRC2 complexes and show a more general role in plant development [24,35,36]. In contrast, individual SU(Z)12 proteins probably have more specific roles. Therefore, a complex containing VRN2 is called the VRN2 complex and analogous EMF2 and FIS/MEA complexes have been defined [24,35,36].

In Drosophila and mammals the PRC2 also contains a MSI protein. These proteins are also part of other nuclear complexes related to chromatin regulation, such as the CHROMATIN ASSEMBLY FACTOR 1 (CAF1) or the RPD3 histone deacetylase (HDAC) complexes

[37]. There are five MSI-like proteins in Arabidopsis of which two, MSI1 and MSI4/FVE, have been related with the regulation of flowering. MSI1 is essential for gametophyte and seed development as part of the MEA complex [38] and also plays a role as an activator of flowering through an FLC-independent pathway. However, whether MSI fulfils this function as part of a PRC2 complex remains unclear [39]. Similarly, MSI4/FVE promotes flowering through the autonomous pathway by decreasing *FLC* transcript levels. This protein may be part of a HDAC complex involved in the deacetylation of *FLC* chromatin [40]. Finally, although it is expected that one MSI protein forms part of the VRN2 complex and, therefore, plays a role in *FLC* regulation, so far no data have been presented that confirm this hypothesis.

2.2. The elusive PRC 1

Although no close homologs of PRC1 components exist in plants, genomic studies have suggested some proteins that might act as PRC1-like co-repressors. The distribution of LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) to its target genes is highly correlated with H3K27me3 [19,41]. *LHP1* is also termed *TERMINAL FLOWER 2* (*TFL2*) because loss-of-function mutations cause early flowering in LD and SD, as well as the termination of the shoot in a terminal flower [42]. The correlation of LHP1 and H3K27me3 was somewhat surprising as animal HP1s typically bind lysine methylation marks of H3 occurring at position 9 rather than 27 and are therefore part of the SU(VAR)3-9 but not the PcG-pathway. However, LHP1 in contrast to HP1a recognizes both modifications without discrimination *in vitro* [19,41] and exclusively correlates with H3K27me3 but not H3K9me3 or H3K9me2 *in vivo* [19].

HP1 family proteins are defined by the presence of two domains, the chromodomain that recognizes methyl-lysine residues and the chromoshadow domain, which is a derived chromodomain and functions as a protein–protein interaction module. The animal PRC1 complex contains the protein Polycomb (Pc) that is a chromodomain-only protein with affinity for H3K27me3. LHP1 may be part of a PRC1 analogous complex and anchor the PRC1 to H3K27me3. However, Pc is an absolute requirement for PcG repression in animals, whereas LHP1 participates in the repression of only a subset of H3K27me3 target genes. Transcriptome analysis of *tfl2/lhp1* mutant plants identified only a small set of genes as upregulated compared to WT, most of which were involved in flower promoting pathways [43]. In addition, the *tfl2/lhp1* phenotype is mild if compared to that of *emf2*, *fis2*, *fie* and *clf swn* [42,44].

EMF1 is a plant-specific single copy gene whose product may also be related to PRC1 function. The EMF1 protein has no conserved domains but displays a putative ATP/GTP binding motif (P-loop motif) and a LXXLL motif that it shares with other transcriptional regulators [45]. EMF1 possesses sequence-independent nucleic acid binding activity (sdDNA, ssDNA and RNA) *in vitro* and fragments of the protein are capable of inhibiting *in vitro* transcription reactions [46]. Early flowering of *emf1* mutants is correlated with upregulation of genes that promote flowering such as *FT*, *LFY*, *AP1* and *AG* although their precise contribution is unclear since single mutations in these genes did not suppress the early flowering phenotype of *emf1* [47,48]. Transcriptome data show a significant overlap between genes that are misregulated in *emf2* and *emf1* [48] and many genes upregulated in *emf1* are also H3K27me3 targets. EMF1 and H3K27me3 co-distributed over several loci and both were shown to be dependent on *EMF2* at *AG* [46].

Although, EMF1 does not interact with EMF2, CLF and FIE [24], it physically interacts with MSI1 [46]. Since MSI1 interacts with several chromatin-related complexes it remains unclear whether EMF1 is part of the EMF2 complex or a distinct complex. In conclusion, EMF1 holds a place on the candidate list for plant-specific PRC1 components although, like LHP1, loss of EMF1 function does not cause upregulation of all PcG targets.

2.3. Lessons from vernalization

The role of PcG regulation of transcription in flowering has been most extensively studied in the context of vernalization. PcG repression of *FLC* counteracts its activation by FRI, a plant-specific coiled-coil protein of unknown function [49]. In a *vrn2* mutant the *FLC* gene is correctly repressed during the cold treatment, but this repression is not stable after the return of plants to normal growth temperatures [32]. Therefore, in *vrn2* mutants the memory mechanism is affected but not the onset of repression. Maintenance, not establishment, of *FLC* repression is also dependent on *LHP1* [50,51] and *VRN1*, which was found in a screen for mutants impaired in the vernalization pathway [29]. VRN1 contains a plant-specific B3 DNA-binding domain able to interact in a non-sequence-specific way with DNA [52]. VRN1 and VRN2 do not interact *in vivo* which makes it unlikely that VRN1 is part of the VRN2 complex [52]. Plants overexpressing *VRN1* flower earlier in SD and LD mainly due to an increase in *FT* expression that is independent of *FLC*. Therefore, *VRN1* may have additional roles in flowering [52].

The PHD-domain protein VERNALIZATION INSENSITIVE 3 (VIN3) co-purifies with the VRN2 complex [36] and directly binds to the chromatin in the 5′region of *FLC* (Table 1). However, in contrast to *vrn1*, *vrn2* and *lhp1* plants, *vin3* mutants are compromised in their ability to downregulate *FLC* during vernalization [53,54]. *VIN3* mRNA and protein levels increase during vernalization [36,54]. In fact, all proteins of the VRN2 complex accumulate during the cold treatment, although mRNA levels of *VRN2*, *FIE* and *CLF/SWN* do not change suggesting a posttranscriptional regulation of the complex at the level of protein stability [36].

Interestingly, a genomic study identified *VIN3* as a potentially PcG-regulated gene because its locus is widely covered by H3K27me3 [20]. It shares the H3K27me3 mark and transcriptional upregulation during vernalization with *MAF5*, *AGL19* and *AGL24* [5,6,55]. The upregulation of *AGL19* and *AGL24* promotes flowering and occurs independently of *FLC* [56–58]. Interestingly, VIN3 is required for *AGL19* upregulation during vernalization. VIN3 might simultaneously promote the VRN2 complex and destabilize the EMF2 complex that is thought to downregulate *AGL19* in seedlings [56].

VIN3 encodes one of 5 members of the *VEL* gene family. Mutations in a second member of this family, *VIN3 like 1*/*VERNALIZATION 5* (*VIL1/VRN5*), were recovered in two independent screens for vernalization response mutants. VIL1 physically interacts with VIN3, but its pattern of expression suggests that it has a more general role in plant development [55,59,60]. Interestingly, the repression of *MAF1* by vernalization depends on VIN3 and VIL1, but its downregulation under SD only involves VIL1. *MAF1* is not an H3K27me3 target therefore VIL1 promotes flowering through different flowering and chromatin pathways [55].

2.4. Maintenance and resetting

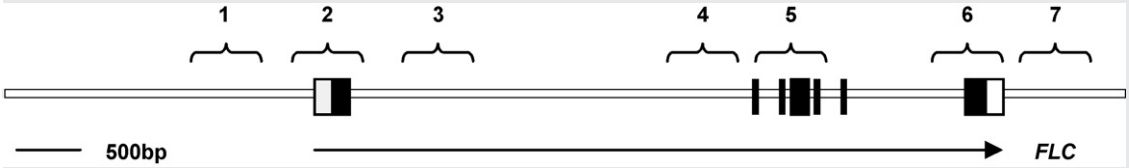
In common with PcG targets in animals *FLC* repression is maintained through mitosis; however, most plant H3K27me3 genes are more dynamically regulated. *FT* is highly upregulated in *emf2*, *clf* and *lhp1* mutant plants [31,42,44,61], but in WT plants its expression shows little indication of mitotic memory. For example, *FT* transcription is immediately induced if plants are shifted from SD to LD and rapidly falls if plants are shifted back to SD [62]. In WT plants *AG* expression is restricted to flowers and is dynamic, being expressed early in the floral primordium, followed by uniform expression in stamens and carpels, which is later restricted to a few

Table 1
Summary of histone modifications observed at *FLC*

Histone modification	FLC region	Background/ Arabidopsis line	Effect during vernalization	Mutant information	References
H3K9me2	2, 4	Col FRI	Increase	*vin3*: no increase, no *FLC* repression *vrn1*: no increase, no maintenance of *FLC* repression *vrn2*: as *vrn1*	[54]
	6		No change		
	1, 2	L*er fca-1* L*er* FRI	Increase	*vrn1*: no increase, no increase, no maintenance of *FLC* repression *vrn2*: as *vrn1*	[53]
	7	Col	–	Col: positive *dcl3-2*: 50% decrease, high *FLC* levels	[124]
H3K9me3	2, 4	Col FRI	Increase	*vin3*: no increase, no *FLC* repression *vil1-1*: no increase, reduced FLC repression	[50]
H3K27me2	2, 4	Col FRI	Increase	*vin3*: no increase, no FLC repression *vrn2*: no increase, no maintenance of *FLC* repression *vrn1*: increase, no maintenance of *FLC* repression	[54]
	6		No change		
	2	L*er fca-1* L*er* FRI	Increase	vrn2: no increase, no maintenance of *FLC* repression *vrn1*: increase, no maintenance of *FLC* repression	[53]
H3K27me3	2, 4	Col FRI	Increase	*vin3*: no increase; no *FLC* repression *vil1-1*: no increase, no *FLC* repression	[50]
	2	L*er* FRI	Increase	vin3-6: no increase, delayed *FLC* repression, no maintenance of *FLC* repression *vil1*/vrn5-1: reduced increase, reduced and delayed *FLC* repression	[59]
	1–7	L*er* WS?	–	ChIP-chip data	[19,20]
	1, 2	Col FRI	Increase		[68]
	3–7		Spreading after return to warm temperatures	Mature leaves: spreading does not occur, FLC repression is not maintained	
H3K9/K14Ac	2, 4	Col FRI	Decrease	*vin3*: no decrease, no FLC repression *vil1-1*: as *vin3* *vrn1*: decrease but not maintained after return no warm temperatures *vrn2*: as *vrn1*	[50,54]
	6		No change		
	1, 2, 4, 5	L*er fca-1* L*er* FRI	No change	*vrn1*: no change in fragment 2 *vrn2*: no change in fragment 2	[53,59]
	3		Decrease		
	2		Decrease	*vin3-6*: decrease; delayed repression of *FLC* *vil1*/*vrn5-1*: decrease; reduced repression of *FLC*	[59]
	1–7	Col FRI	Decrease	Decrease not maintained after return to warm temperatures, *FLC* repression not maintained	[68]
	1–7	Ler	–	fve: increase, high *FLC* levels	[40]
H4K5/K8/K12/K16Ac	2	Col FRI	Decrease	Mature leaves: decrease not maintained after return to warm temperatures, *FLC* repression not maintained	[68]
	2	Col	–	*fld-3*: increase, high *FLC* levels *fve-4*: increase, high *FLC* levels *fca-9*: no change, high *FLC* levels *ld-1*: no change, high *FLC* levels	[131]
H2A.Z	1–7	Col, Col FRI	–	Col: peak at transcriptional start and stop *arp6-1*: no peak, low *FLC* levels *pie1-5*: no peak, low *FLC* levels FRI: as Col, high *FLC* levels	[97]
H3K4me2	1, 2, 4, 5	L*er fca-1* L*er* FRI	No change		[53]
	3		Decrease	*vrn1*: no decrease *vrn2*: decrease	
	3, 4	Col	–	*fca-9*: increase, high *FLC* levels *fld-3*: increase, high *FLC* levels *dcl3-1*: increase, high FLC levels *fve-3*: no change, high FLC levels	[85]

Table 1 (*Continued*)

Histone modification	FLC region	Background/ Arabidopsis line	Effect during vernalization	Mutant information	References
H3K4me3	2	Col FRI	Decrease	Mature leaves: increase not maintained after return to warm temperatures, *FLC* repression not maintained	[68]
	2	Col	–	FRI: increase, high *FLC* levels *fld3*: increase; high *FLC* levels	[107]
	2	Col FRI	–	FRI: increase, high *FLC* levels *fca-9*: Increase, high *FLC* levels *efs* FRI: no increase, low *FLC* levels *efs fca*: no increase, low *FLC* levels FRI *elf7-7*: no increase, low *FLC* levels FRI *elf8-1*: no increase, low *FLC* levels	[120]
	2	Col	–	*ldl1 fld*: Increase, high *FLC* *ldl1 ldl2*: no change, high *FLC* levels	[132]
	2, 3	Col	–	*sdg8-2*: no change, low *FLC* levels *sdg26-1*: no change, high *FLC* levels *vip4*: decrease, low *FLC* levels	[118]
H3K36me1	2, 3	Col	–	*sdg8-2*: increase, low *FLC* levels *sdg26-1*: no change, high *FLC* levels *vip4*: no change, low *FLC* levels	[118]
H3K36me2	2, 3	Col	–	*sdg8-2*: decrease, low *FLC* levels *sdg26-1*: no change, high *FLC* levels *vip4*: decrease, low *FLC* levels	[118]
	1, 4	Col	–	*sdg8-1*: decrease, low *FLC* levels	[119]
	2, 3		–	*sdg8-1*: no change; low *FLC* levels	
H3K36me3	2, 3	Col	–	*sdg8-2*: decrease, low *FLC* levels *sdg26-1*: no change, high *FLC* levels *vip4*: decrease, low *FLC* levels	[118]
FCA	5	Col	–	Col and 35S::FCA Chromatin factor binding	[85]
FPA	2, 4, 5	Col	–	35S::FPA-YFP Chromatin factor binding	[125]
SUF4	1	Col	–	35S::SUF4 Chromatin? factor binding	[147]
VIN3	1, 3	Col	–	Chromatin factor binding	
VRN1	1, 3	Col		Chromatin factor binding	[54]



cells in stamens and carpels [63]. The *clf* mutant partially resembles an *AG* overexpressor and shows homeotic transformations in the flowers [23], but *AG* is normally expressed during the early stages of flower development in *clf* mutants and is ectopically expressed only during later stages [28]. Recent data suggest that DNA replication plays a role in mitotic maintenance of PcG repression. INCURVATA 2 (ICU2), the catalytic subunit of DNA polymerase , and LHP1 interact and in the *icu2-1* mutant the H3K27me3 marked genes *FT*, *AG*, *AP3*, *PI* and *SEP3* are upregulated. LHP1 shows an altered cellular localization in *icu2* plants but changes in H3K27me3 were not observed at the *AG* locus [64].

The stable repression of *FLC* and a few other targets (such as *FWA*) may require exceptional PcG regulation. Indeed heterochromatic histone marks, such as H3K27me2 and H3K9me2 occur at *FLC* (Table 1) while DNA methylation occurs at *FWA*, although neither is typically correlated with H3K27me3 in plants [19]. *FLC* repression must be erased in the next generation and this resetting was proposed to take place during the formation of gametes or during embryo development [65,66]. However, meiosis is not a prerequisite for *FLC* resetting which also occurs in various anther cell types that do not undergo meiosis [67].

"The dose alone makes the poison" (*dosis sola venenum facit*, Paracelsus), and therefore the density as well as the dynamics of the H3K27me3 mark needs to be considered. For example, H3K27me3 covers the whole *FLC* locus before vernalization [19,20], but during vernalization the density of the mark increases around the transcription start site. After the return to warm temperatures this enrichment spreads over the locus in young seedlings (Table 1). In adult leaves that do not undergo cell division, the H3K27me3 increase and *FLC* repression during vernalization occur but both are lost after a return to warm temperatures and there is no increased spreading of H3K27me3 over the locus [68]. One possibility is that variants of H3 that are recalcitrant to H3K27 trimethylation [69] and expressed at higher levels in mature leaves

are responsible for the loss of memory of the vernalization signal [68].

3. ATP-dependent chromatin remodeling machines

Chromatin remodeling machines (CRMs) are multi-protein complexes involved in the regulation of chromatin structure. These complexes alter histone–DNA interactions to allow a more fluid conformation that facilitates histone replacement and accessibility to the DNA. To carry out this function CRMs use the energy obtained from the hydrolysis of ATP and all purified CRMs share an ATPase/Helicase of the SWITCH 2/SUCROSE NONFERMENTING 2 (SWI2/SNF2) family as catalytic core. More than 40 SWI2/SNF2 proteins are present in Arabidopsis (www.chromdb.org) and, although no Arabidopsis CRMs have been purified yet, molecular and genetic data implicate CRMs in flowering regulation [70–72].

3.1. PICKLE, SPLAYED and BRAHMA

PICKLE (PKL) was the first protein of the SWI2/SNF2 family analyzed in Arabidopsis [73]. PKL forms part of the CHD-subfamily (Chromo/Helicase/DNA binding domain); furthermore, the presence of a zinc-finger domain indicates that it is an ortholog of the CHD3/Mi-2 protein of human and *Drosophila*, respectively [74]. CHD3/Mi-2 proteins are part of the nucleosome remodeling and histone deacetylase complex (NURD) that takes part in transcriptional repression and couples histone deacetylation with ATP-dependent chromatin remodeling and DNA methylation [75,76]. PKL is implicated in the GA-mediated transition from embryonic to vegetative development through repression of embryonic fate, although the pleiotropic phenotype of *pkl* mutants indicates a more general role in development. For instance, the weak late flowering phenotype of *pkl* and *gai (GA-insensitvie)* mutants in LD conditions is strongly increased in *pkl gai* double mutants suggesting that PKL could play a role in the GA flowering pathway [73,74].

Among the CRMs of yeast and animals, SWI/SNF complex is the best characterized. This is a 2 MD complex containing 8–11 subunits depending on the species. The core of the complex is formed by four subunits: an ATPase/Helicase of the SWI2/SNF2 subfamily, a SNF5-domain protein and two SWIRM-domain and SANT-domain proteins (SWI3). With the exception of the SNF5 homolog, each of these core and other putative subunits are encoded by small gene families in Arabidopsis, suggesting the possibility of several AtSWI/SNF complexes [71,77–81]. For instance, there are four SWI2/SNF2 ATPases/Helicases and two of them, SPLAYED (SYD) and BRAHMA (BRM), have been related with flowering [78,81]. BRM but not SYD contains a bromodomain. These domains preferentially interact with acetylated histones; however, although BRM bromodomain is able to bind histones *in vitro*, it does not specifically recognize this modification [82].

syd and *brm* mutants have pleiotropic phenotypes with only some redundant characteristics, which indicates specific and shared functions for these two proteins [78,81,83]. Mutations in *syd* were found as enhancers of the weak *lfy-5* mutant and *syd* single mutants show an early inflorescence to flower transition, mainly in SDs [81]. Plants with reduced *BRM* expression show precocious flowering in LD and SD, whereas *brm* mutants flower with fewer leaves but later than WT plants under both conditions. The flowering phenotype of *BRM* silenced plants correlates with increased abundance of *CO*, *FT* and *SOC1* mRNAs, and overexpression of several flower developmental genes occurs in *brm* mutants. Therefore, BRM is proposed to be involved in the photoperiod pathway, although further data are needed to demonstrate its direct role [78,79,82].

The SWI3 subunits have also been connected to the regulation of flowering. There are four members of this family in Arabidopsis (AtSWI3A-D) and a complex interaction network has been proposed among different AtSWI3 proteins and other components of the putative AtSWI/SNF complexes [79,80,83,84]. Loss-of-function *atswi3a* and *atswi3b* mutants are embryo lethal, but AtSWI3B is able to interact in yeast with FCA, a RNA-binding protein directly involved in the downregulation of *FLC* (see below) [80,85,86]. Interestingly, *atswi3c* and *atswi3d* mutants show a flowering phenotype similar to *brm*: slightly later flowering than WT plants, but flowering with fewer leaves, particularly in SDs. However, there is no clear correlation between the flowering phenotypes and the misregulation of flowering genes although *AP2*, *AP3*, *PI* and *NAP* are upregulated in *brm* and *atswic3c* mutants [80]. Taken together with the fact that AtSWI3C and BRM show the strongest interaction in yeast, these two proteins could be part of a AtSWI/SNF complex that is responsible for the regulation of these flower development genes [79,80].

3.2. The SWR1 complex and the H2A.Z isoform

PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1 (PIE1) is the only member of the SWR1 subfamily of SWI2/SNF2 proteins in Arabidopsis although initially it was misclassified as an ISWI homolog (www.chromdb.org) [87]. In yeast and humans the proteins of this subfamily are the catalytic subunit of the SWR1C and SRCAP complexes, respectively; a 10–13 protein complex involved in transcriptional regulation through the replacement of H2A by the H2A.Z variant [88–90] (Fig. 3). In Arabidopsis, genetic and molecular data suggest that the PIE complex also contains three proteins found in yeast SWR1C: ACTIN-RELATED PROTEIN 6 (ARP6 also SUPRESSOR OF FLC 3 (SUF3)/EARLY IN SD 1 (ESD1)), SWC6 (also SERRATED LEAVES AND EARLY FLOWERING (SEF)) and SWC2. *pie*, *swc6* and *arp6* mutants flower early in LD and SD, although they are still responsive to photoperiod. Early flowering is caused by the downregulation of *FLC*, *MAF4* and *MAF5* genes even in FRI or autonomous pathway mutant backgrounds [87,91–96]. Low *FLC* expression in *arp6* and *swc6* mutants has been correlated with reduced acetylation of H3 and decreased H3K4me3 levels (Table 1), although these results do not prove a direct role of the PIE complex in the deposition of these epigenetic marks and could also be indirectly caused by *FLC* downregulation [94,96] (see below). The PIE complex interacts with H2A.Z, encoded by four different genes in Arabidopsis (*HTA4*,*8*,*9* and *11*; [97,98]), through PIE, ARP6 and SWC2 [92,97,99]. In addition, plants with a reduced level of general *HTA* expression and *hta9 hta11* double mutants show a similar downregulation of *FLC* and its relatives and phenotypically resemble PIE complex mutants [92,99]. The H2A.Z variant is most abundant at the beginning and at the end of the transcribed region of the *FLC*, *MAF4* and *MAF5* loci, and this enrichment is markedly reduced in *pie* and *arp6* mutants. In addition, ChIP experiments show the binding of ARP6 and SWC6 to the *FLC* proximal promoter region [92,97]. Taken together these results indicate that the PIE complex is involved in the deposition of H2A.Z at the *FLC* locus.

What is known about the role of H2A.Z in transcriptional regulation? The presence of H2A.Z has been related to repression and activation of transcription in yeast, human cells and plants; therefore, this histone variant may be preferentially associated with silent but potentially inducible genes [99–101]. Transcriptome profiling studies in *pie1* and *hta9 hta11* mutants show that 2/3 of the genes differentially expressed in comparison to WT are upregulated, most of which are also SA responsive [97,99]. In yeast and plants many genes feature a ~150 bp nucleosome-depleted region around the transcriptional start site, which is generally

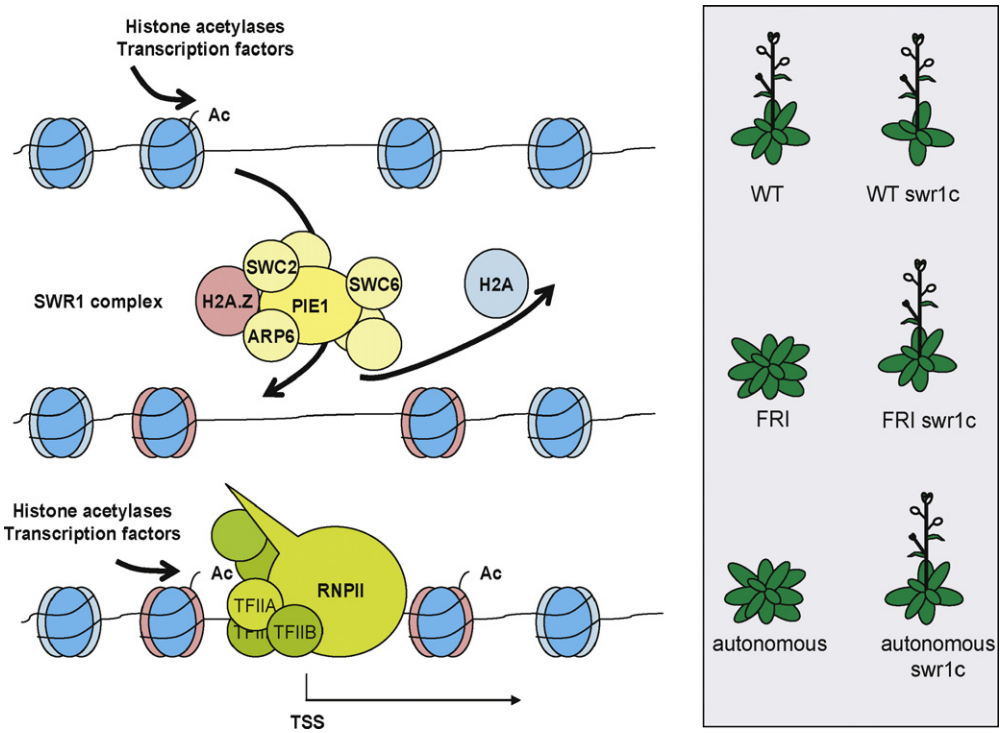


Fig. 3. Chromatin landscape at transcriptional start sites. The transcriptional start site requires a nucleosome-free region to accommodate the PIC and is flanked by nucleosomes that have exchanged H2A for H2A.Z. The precise order of events is unclear, possibly histone acetylation targets the SWR1C which subsequently exchanges H2A.Z. In yeast, H2A.Z acetylation is correlated with the transcription rate which is indicative of a function of this modification in initiation. In Arabidopsis, high *FLC* levels such as observed in FRI and autonomous pathway mutants are dependent of SWR1C, therefore mutations in genes encoding SWR1C components are epistatic to *FRI* and *fca*, *fve* and *ld* (see gray box). In contrast, many stress-induced genes are dependent on SWR1C for repression. The model is based on data from Arabidopsis and other eukaryotes.

flanked by one H2A.Z containing nucleosome in yeast [20,102]. H2A.Z is involved in the recruitment of the general transcriptional machinery and lysine 14 acetylation of H2A.Z is correlated with transcription rates in yeast [103]. However, H2A.Z in animals also correlates with specific heterochromatic regions, probably via an interaction with HP1 and Pc [104]. In contrast to other organisms, H2A.Z co-localizes exclusively with euchromatin in Arabidopsis [97,104]. In conclusion, the SWR1 complex exclusively regulates euchromatic genes in Arabidopsis, but understanding its precise participation in transcriptional activation and repression of specific genes requires further studies.

4. The PAF1 complex and its role in transcription

Genetic screens in high *FLC* expressing backgrounds for early flowering, vernalization insensitive mutants identified mutations in genes encoding 4 of the 5 subunits of the widely conserved RNPII Associated Factor 1 complex (PAF1C). The genes were designated *EARLY FLOWERING (ELF)* or *VERNALIZATION INSENSITIVE (VIP)* [105–107] and mutants in these genes show other alterations in development such as mild homeotic transformation of flower organs [108]. The severity of the abnormalities is not enhanced in double-mutants probably because deletions in one gene negatively affect accumulation of other components at the protein level. This includes the vip3 mutation [108], which may be a plant-specific addition to the PAF1C since VIP3 physically interacts with the evolutionary conserved VIP4 and VIP6 [106].

In yeast, PAF1C is recruited to RNPII during transcription initiation and persists during elongation (Fig. 4). PAF1C recruitment is dependent on Serine 5 phophorylation at the RNPII carboxy-terminal repeat domain (CTD) [18]. PAF1C orchestrates the recruitment of other complexes that participate in transcription such as histone chaperones required for the eviction and subsequent reassembly of nucleosomes on the DNA template and RAD6/Bre1 H2B-specific mono-ubiquitinase. H2B ubiquitination recruits the COMPASS complex that contains SET1 [18]. PAF1C also has an important function in transcript termination and polyadenylation (Fig. 4) [109,110].

Loss of plant PAF1C function dramatically reduces mRNA levels of *FLC* and this is the major although not the only cause of early flowering in the mutants [106,107]. Work of several groups has indicated that processes dependent on PAF1C in yeast are also important for *FLC* regulation. High expression of *FLC* in a FRI background is correlated with the increase of H3K4me3 at the 5′end of the transcription cassette [107]. In yeast, H3K4 trimethylation at transcriptional start sites is dependent on SET1 which is most closely related to the Trithorax clade of SET domain proteins in plants and animals [18]. In Arabidopsis, 6 homologs designated as *ARABIDOPSIS TRITHORAX* (*ATX*) *1–6* belong to this clade; therefore, considerable gene redundancy can be expected [111,112]. Nevertheless, mutant *atx1* plants flower early in SD and LD, have reduced expression levels of *FLC* and both effects are enhanced in a FRI background [113,114]. Furthermore, ATX1 protein directly binds to the 5′end of *FLC* and in *atx1* mutants H3K4me3 accumulation around the 5′end of the gene is reduced [114].

In addition to early flowering, *atx1* mutants show other developmental alterations such as slight dwarfism and defects in flower architecture [115]. In accordance with data from animals that indicate an antagonism between Trithorax SET domain and PcG proteins, some of the phenotypes observed in *clf* or *atx1* plants are rescued in *atx1 clf* double mutants, which are less dwarfed than both parents and have normal leaves [116]. The leaf phenotype in *clf* is caused by ectopic expression of *AG*; *AG* is a direct target of ATX1 and, not surprisingly, ectopic *AG* expression is reduced in *clf*

Fig. 4. Chromatin landscape during mRNA elongation. The model combines data from Arabidopsis and other eukaryotes. (1) During initiation, acetylated H2A.Z is evicted with assistance from Histone chaperones and PAF1C is recruited through RNPII CTD Ser 5 phosphorylation. In Arabidopsis, expression of *FLC* and several *MAFs* is strictly dependent on the presence of a functional PAF1C. In yeast the PAF1C facilitates binding of H2B-specific mono-ubiquitinases and H2B1ub recruits the COMPASS complex. A similar complex in Arabidopsis is likely to contain ATX-family proteins. ATX-family protein accumulation causes increase of the H3K4me3 histone mark at the transcriptional start which is thought to serve as recruitment signal for HAT activities associated with PHD-finger proteins. In Arabidopsis, high expression of the PcG-target genes *FLC* and *AG* is dependent on ATX1. (2) During elongation, PAF1C facilitates the recruitment of FACT histone chaperones in yeast. FACT removes and repositions nucleosomes during elongation. Elongating RNPII is phosphorylated at Ser 2, a recruitment signal for SET-domain proteins of the ASH1-family which provide H3K36 dimethylation and trimethylation. In yeast, methylated H3K36 recruits HDACs which are required to stabilize nucleosomes after the passage of RNPII. In Arabidopsis, the ASH1-related protein SDG8/EFS is required for high *FLC* expression. (3) Finally, during termination, PAF1C assists the recruitment of the polyadenylation machinery. It is possible, that the autonomous pathway component FY, a homolog of yeast polyadenylation factor pfs2p, is dependent on PAF1C in Arabidopsis.

atx1 double mutants [116]. Interestingly, CLF and ATX1 physically interact in plant cells, although the regulatory consequence of this interaction is not yet clear [116].

Similar to the amplification of the Trithorax clade, five genes encode homologs of SET2 (or ASH1 in animals) in Arabidopsis [111,112]. SET2 is recruited by Serine 2 phosphorylation at the CTD of elongating RNPII and causes dimethylation and trimethylation of H3 lysine 36, which in turn recruits HDACs. It is still controversial, whether Serine 2 phosphorylation is dependent on PAF1C in yeast and animals [18,117]. Hypoacetylation of histones in actively transcribed regions is required to stabilize nucleosomes after the passage of RNPII [17,18]. Of the Arabidopsis homologs, SDG8 shows *in vitro* methylation activity toward H3 and in *sdg8* mutants global H3K36me3 and H3K36me2 levels are greatly reduced [118]. The gene is identical to *EARLY FLOWERING IN SHORT DAYS* (*EFS*) and mutants display early flowering correlated with downregulation of *FLC*, *MAF1* and *MAF4* [119,120].

Surprisingly, loss of PAF1C affects the expression of only a relatively small subset of genes in Arabidopsis although the complex probably participates universally in transcription. A similar observation has been made in yeast, where the genes most dependent on PAF1C function are those most prone to use alternative polyadenylation sites [121]. [illegible] of polyadeny20.9(egula3.9(ei0.9(e79pe3.9(ei0.37