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BACHELOR'S THESIS

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The role of histone post-translational modifications in the regulation of gene expression in plants

Úloha posttranslačných modifikácií histónov v regulácii génovej expresie u rastlín

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Prehlásenie

Prehlasujem, že som záverečnú prácu spracoval samostatne a že som uviedol všetky použité informačné zdroje a literatúru. Táto práca ani jej podstatná časť nebola predložená k získaniu iného alebo rovnakého akademického titulu.

V Prahe, dňa .....

Jakub Chromý

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Abstract

Chromatin structure, gene expression and consequently many important aspects of the plant development are under control of epigenetic regulation. Within epigenetic regulation, histones and their modifications play a pivotal role. The N-terminal tails of histones are dynamically modified by covalent post-translational modifications (PTMs). These modifications are key regulators modulating chromatin structure and thus regulating gene expression. In angiosperms, one of the processes finely regulated at the epigenetic level is the flowering. Flowering represents a very complex process, that is relevant for the study of epigenetic regulation as well as for practical application. In this work, I summarize current knowledge of the role of histone PTMs in the regulation of gene expression in plants, focused predominantly on two key regulators of flowering in *Arabidopsis* – *FLC* and *FT*.

Keywords: *epigenetics, flowering, gene expression, histone modification, plants, transcriptional regulation*

Abstrakt

Štruktúra chromatínu, génová expresia a následne i mnohé dôležité aspekty rastlinného vývinu podliehajú epigenetickej regulácii. V rámci epigenetickej regulácie, históny a ich modifikácie zohrávajú ústrednú úlohu. N-konce histónov sú dynamicky modifikované kovalentnými posttranslačnými modifikáciami (PTM). Tieto modifikácie sú kľúčové pri modulácii a regulácii štruktúry chromatínu a tak i génovej expresie. Jedným z procesov citlivo regulovaných na epigenetickej úrovni je práve kvitnutie krytosemenných rastlín. Kvitnutie predstavuje veľmi komplexný dej, zaujímavý ako z pohľadu štúdia epigenetickej regulácie, tak i praktického uplatnenia. V tejto práci zhrňujem doterajšie poznatky o úlohe posttranslačných modifikácií histónov v regulácii génovej expresie u rastlín, zameranej predovšetkým na dvoch ústredných regulátorov kvitnutia u *Arabidopsis* – *FLC* a *FT*.

Kľúčové slová: *epigenetika, kvitnutie, génová expresia, histónová modifikácia, rastliny, regulácia transkripcie*

Contents

List of Abbreviations

AG	AGAMOUS
ATX	ARABIDOPSIS HOMOLOG OF TRITHORAX
CLF	CURLY LEAF
COMPASS	COMPLEX PROTEINS ASSOCIATED WITH SET1
CO	CONSTANS
EMF	EMBRYONIC FLOWER
FLC	FLOWERING LOCUS C
FLD	FLOWERING LOCUS D
FRI	FRIGIDA
FT	FLOWERING LOCUS T
H2Bub1	monoubiquitination of histone H2B
HATs	histone acetyltransferases
HDACs	histone deacetylases
HDMs	histone demethylases
HKMTs	histone lysine methyltransferases
HXLYme1/me2/me3	mono-/ di-/ tri-methylation of Lys Y of histone X
LHP1	LIKE HETEROCHROMATIN PROTEIN 1
MAF	MADS AFFECTING FLOWERING, homologues of FLC
MRG	MORF4-RELATED GENE
MSI	MULTICOPY SUPPRESSOR OF IRA
PAF1	RNA POLYMERASE-ASSOCIATED FACTOR 1
PHD	PLANT HOMEODOMAIN
Pol II, IV, V	DNA-dependent RNA polymerase II, IV, V
PRC	POLYCOMB REPRESSIVE COMPLEX
PTMs	post-translational modifications
RdDM	RNA-directed DNA methylation
SDG	SET DOMAIN GROUP
SET domain	Su(var)3–9, Enhancer-of-zeste and Trithorax domain
SOC1	SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1
SRA domain	SET and RING Associated domain
SUVH	SU(VAR)3–9 HOMOLOG
SWI/SNF	SWITCH/SUCROSE NONFERMENTING
TSS	transcriptional start site
VIN3	VERNALIZATION INDEPENDENT 3
VRN	VERNALIZATION

Introduction

Chromatin structure and consequently gene expression are a subject of extensive epigenetic regulation. Many major breakthroughs in epigenetics were made in past two decades and although still not completely understood, it is now clear, that epigenetic regulation significantly modulates gene expression. Within epigenetic regulation, histone post-translational modifications (PTMs) play a pivotal role, linking several epigenetic regulatory pathways and determining chromatin state.

Histone PTMs represent a very diverse and in terms of function still quite elusive epigenetic modification. A great number of histone variants, histone modifications and possible positions of residues modified all together give rise to so-called 'histone code'. Deciphering of this histone code would enlighten the complex regulatory mechanism of eukaryotic systems (Strahl & Allis, 2000). It is therefore no surprise, that histone PTMs and their roles in gene expression regulation are in a focus of intensive research. Histone PTMs participate in a whole variety of processes in plants. They are profoundly involved in chromatin management and genome integrity maintenance, as well as in plant development and responses to environment, including seed, root and leaf development, flowering time control, or responses to environmental stresses (Berr *et al.*, 2011; Liu *et al.*, 2016b).

One of the best understood example of the role of histone PTMs in gene regulation in plants is that of the *Arabidopsis FLOWERING LOCUS C* (*FLC*) gene, which participates in the control of the transition from vegetative growth to flowering (Deal & Henikoff, 2011). Ensuring reproductive success, flowering represents one of the most critical parts of angiosperm life cycle. The precise timing of transition from the vegetative to the generative state must therefore be thoroughly controlled and regulated. In *Arabidopsis*, flowering time is regulated by several flowering-promoting pathways, including autonomous, vernalization, photoperiod and gibberellin pathways (Henderson & Dean, 2004).

In this work, I summarize our current knowledge of the role of histone PTMs in the regulation of gene expression with special focus on two key regulators of flowering in *Arabidopsis* – *FLOWERING LOCUS C* (*FLC*) and *FLOWERING LOCUS T* (*FT*).

1 | Chromatin Structure

In eukaryotic cell nuclei, genomic DNA is packaged in a highly organized nucleoprotein complex known as chromatin. The basic repeating unit of chromatin is the nucleosome. The nucleosome consists of ∼146 base pairs (bp) of DNA wrapped approximately 1.65 times around an octameric histone core, composed of two copies of each of the H2A, H2B, H3 and H4 histone proteins (Luger *et al.*, 1997). In between each two nucleosomes, there is a linker DNA ranging between 20 to 90 bp. The linker histone H1, which can be present between neighbouring nucleosomes, interacts with both the linker DNA and histone cores (Figure 1). Core histones typically have their N-terminal tails protruding from the histone core, allowing them to effectively interact with other nucleosomes and chromatin-associated proteins (Szerlong & Hansen, 2011; Luger *et al.*, 2012).

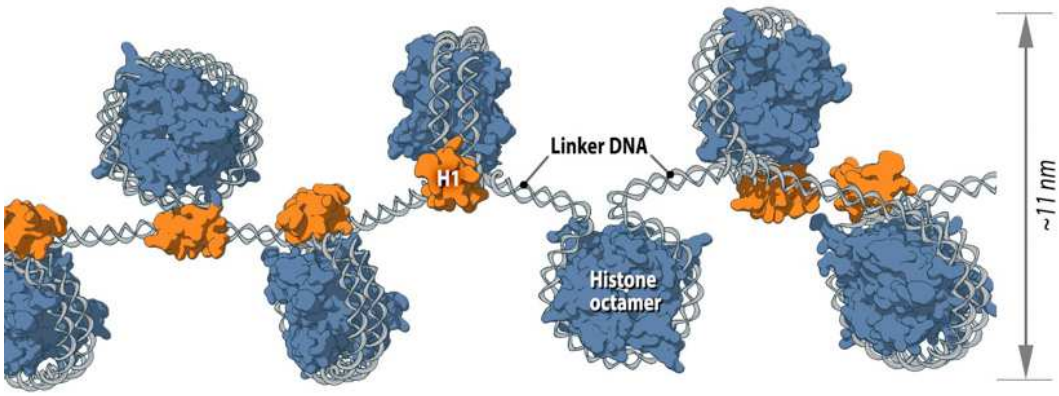

Figure 1: Primary chromatin structure. *The first level of chromatin compaction is given by the wrapping of DNA double-strand around the histone octamers, resulting in the linear array of nucleosomes linked by the linker DNA and bound by the linker histone H1 (adapted from* MBInfo contributors*, 2014).*

Under the physiological conditions, the linear array of nucleosomes is helically folded to produce more compact chromatin fibre, for instance, the 30 nm fibre. Two principal *in vitro* conformational models of 30 nm fibre were described – solenoid and zigzag models (Dorigo *et al.*, 2004; Luger *et al.*, 2012). It is rather probable that chromatin *in vivo* exists as a heteromorphic fibre of multiple conformations (Luger *et al.*, 2012). Further compaction of chromatin is driven mainly by fiber-fiber interactions and by attachment of the 30nm fiber to a central proteinaceous scaffold (also referred to as nuclear or matrix scaffold, or karyoskeleton) to produce helically arranged looped chromatin, which can be further folded during chromosome condensation in prophase to produce maximally compacted metaphase chromosomes (Woodcock & Ghosh, 2010).

2 | Chromatin Regulation

Chromatin is a dynamic structure, strongly affected by epigenetic modifications of its components, including histone post-translational covalent modifications (PTMs), DNA methylation and placement of histone variants. ATP-dependent chromatin remodelling and regulation by non-coding RNAs mediate chromatin structure modulation (Pikaard & Scheid, 2014).

2.1 DNA Methylation

DNA methylation is a heritable epigenetic mark playing an important role in genome management and regulation of gene expression. The most prevalent type of DNA methylation in eukaryotes occurs on cytosine, resulting in 5-methyldeoxycytosine (5mC). Much less common methylation occurs also on adenine, resulting in N6-methyldeoxyadenine (6mA) (Huang *et al.*, 2015). DNA methylation in plants is performed by three distinct methyltransferase families, specificity of which depends particularly on the sequence context of the target cytosines. (Finnegan & Kovac, 2000; Gouil & Baulcombe, 2016).

METHYLTRANSFERASE 1 (MET1) recognizes the hemimethylated state of a newly synthesized double strand after replication cycle and maintains methylation pattern preferentially in CpG dinucleotides (Finnegan & Kovac, 2000). Second, plant-specific family of CHROMOMETHYLASEs (CMTs), is characterized by the presence of chromodomain (chromatin organization modifier domain) (Finnegan & Kovac, 2000). Chromodomain recognizes specific methylation marks on histones and enables interaction between histones and CMTs. This makes CMTs mediators, capable of methylation of DNA in response to histone methylation. CMT3 catalyses methylation in the symmetrical CpHpG (where H stands for A, C or T) context, while CMT2 catalyses methylation in non-symmetrical CpHpH context (Gouil & Baulcombe, 2016). Finally, DOMAIN REARRANGED METHYLTRANSFERASEs (DRMs) are key *de novo* methyltransferases, mediating sRNA pathway to maintain methylation pattern after replication or to realize RNA-directed *de novo* methylation (RdDM) in all sequential contexts (Zhong *et al.*, 2014). In the RdDM pathway, plant-specific DNA-dependent RNA polymerase IV (Pol IV) generates precursors for siRNA production. AGO4 with loaded siRNA then guides DRM2 to the target DNA by binding scaffold RNA generated by another plant-specific Pol V (Liu *et al.*, 2014).

There are also other proteins required for DNA methylation in plants. DECREASE IN DNA METHYLATION 1 (DDM1), a member of the SWI2/SNF2-like family of DNA-dependent chromatin remodelling ATPase proteins, binds to H1-containing heterochromatin and might facilitate a better access for DNA methyltransferases to the substrate (Zemach *et al.*, 2013). DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1 (DRD1), another member of the

SWI2/SNF2-like family, cooperates with plant-specific Pol V to facilitate RdDM and silencing of homologous DNA in chromatin without histone H1 (Huettel *et al.*, 2006; Cho *et al.*, 2016).

2.2 Histone Variants

In addition to the canonical histones, deposited into chromatin during or after DNA replication, eukaryotes possess also a variety of their less abundant analogues, so-called histone variants. These variants differ in a number of amino acid residues and their incorporation into chromatin is replication-independent (Talbert & Henikoff, 2014). Histone variants impart novel structural and functional properties to the nucleosomes they are part of, subsequently affecting chromatin remodelling and histone post-translational modifications. Variants of histones H2A and H3 are the most common variants in eukaryotes. Variants H2A.Z and H3.3 play major roles in transcriptional regulation in plants (Deal & Henikoff, 2011).

The H3.3 variant requires histone chaperone HISTONE REGULATOR A (HIRA) for its deposition during transcription as well as for its deposition at DNA damage site (Talbert & Henikoff, 2014). In general, H3.3 is enriched in active chromatin, predominantly within promoters, transcribed regions of expressed genes and at gene regulatory elements, where nucleosomes are rapidly disrupted and replaced (Deal & Henikoff, 2011).

The H2A.Z variant is the most evolutionary conserved variant of the largest histone H2A family and an essential histone variant found in all higher eukaryotes (Suto *et al.*, 2000). H2A.Z acts as a key player in transcriptional regulation, inhibition of antisense transcription, telomere silencing, maintenance of genome integrity and in defining of heterochromatin boundaries (Jarillo & Piñeiro, 2015). H2A.Z is relatively abundant throughout genomes. Recent studies suggest a model of the dual role of H2A.Z, where H2A.Z within gene bodies has rather repressive effect on transcription and promotes a higher variability in expression patterns, while H2A.Z in nucleosomes occupying the transcriptional start site (TSS) is important for maintaining the activity of some genes (Coleman-Derr & Zilberman, 2012; Sura *et al.*, 2017). H2A.Z at TSS appears to promote transcription by destabilizing nucleosome core and thus facilitating access for transcriptional machinery (March-Díaz & Reyes, 2009). DNA methylation has been shown to prevent H2A.Z incorporation, especially in bodies of highly or constitutively expressed genes (Coleman-Derr & Zilberman, 2012).

In plants, H2A.Z deposition and removal is controlled by SWR1-like ATP-dependent nucleosome remodelling complex [SWI2/SNF2-Related 1, also called PIE complex (a member of the SWI2/SNF2-like family)]. SWR1-c functions through partially unwrapping the nucleosome and replacing H2A/H2B dimer with H2A.Z/H2B dimer (March-Díaz *et al.*, 2008). Multiple H2A.Z isoforms together with an SWR1-c are involved in both development and environmental responses in plants, including the flower transition (March-Díaz & Reyes, 2009; Deal & Henikoff, 2011). Recent studies suggest H2A.Z to be thermosensitive

and therefore to be involved in the regulation of thermosensory response, including the vernalization-dependent pathway of flowering (Jarillo & Piñeiro, 2015).

3 Histone Post-translational Modifications

Histone N-terminal tails are a subject of active and dynamic covalent post-translational modifications (PTMs). The most common modifications encompass acetylation, methylation, phosphorylation and ADP-ribosylation, as well as addition of relatively large peptides such as SUMO and ubiquitin (Kouzarides, 2007; Berger, 2007).

In general, histone PTMs function through two basic mechanisms. First, some modifications, such as acetylation, can directly alter histone physical properties and subsequently affect chromatin conformation. Second, PTMs generate novel docking sites, which may serve as a platform for recruitment of specific 'reader' proteins, including chromatin remodelling complexes, modifying enzymes or other specific non-histone effector proteins (Kouzarides, 2007). These 'reader' proteins then determine the transcriptional outcome of the target genes (Xu *et al.*, 2014). There is a dynamic cross-regulation between various PTMs (Latham & Dent, 2007), contributing to the complexity of so-called 'histone code'.

So far, histone methylation and acetylation and their roles in the gene regulation are best described, and their functions are conserved in both animals and plants (Deal & Henikoff, 2011).

3.1 Histone Methylation

Histone methylation is profoundly involved in various biological processes, from transcriptional regulation to heterochromatin formation. Histone methylation is a very complex and versatile epigenetic mark, occurring at different degrees, i.e. mono-, di-, and tri-methylation, on various, either lysine or arginine residues (Liu *et al.*, 2016a).

Methylation functions either through elevating hydrophobicity and thus affecting intra- or intermolecular interactions, or through creating new binding sites for other effector proteins (Liu *et al.*, 2010). These effector chromatin-binding proteins typically contain conserved chromodomain or chromo-like domain (e.g. Tudor or PWWP domains), which recognize and bind to methylated-amino acid residues at the histone tails (Berger, 2007).

Histone methylation pattern is dynamic. Methyl residues are established by histone lysine methyltransferases (HKMTs) or protein arginine methyltransferases (PRMTs), and conversely, are removed by histone demethylases (HDMs) (Liu *et al.*, 2010).

3.1.1 Lysine Methylation

The most substantial lysine methylation in *Arabidopsis* occurs at Lys4 (K4), Lys9 (K9), Lys27 (K27) and Lys36 (K36) of histone H3. Lysine methylation can have both activating and repressing outcomes, depending on the specific context and residues modified. In general, methylation of H3K9 and H3K27 are considered repressive marks, while methylation of H3K4 and H3K36 are associated with actively transcribed genes (Berger, 2007; Liu *et al.*, 2016a).

HKMTs possessing catalytic SET (Su(var)3–9, Enhancer-of-zeste and Trithorax) domain mediate histone lysine methylation (Pontvianne *et al.*, 2010; Liu *et al.*, 2010).

H3K9

The histone H3K9 methylation occurs in *Arabidopsis* predominantly as H3K9me1 and H3K9me2, which are typically enriched within highly condensed heterochromatic chromocenters (Jackson *et al.*, 2004). Especially H3K9me2 plays a key role in the transcriptional repression, being strongly associated with DNA methylation and helping to maintain constitutive heterochromatin status. In contrast, H3K9me3 is present in *Arabidopsis* at very low levels (Jackson *et al.*, 2004) and is rather associated with euchromatin and active genes (Charron *et al.*, 2009).

KRYPTONITE or SU(VAR)3–9 homolog 4 (KYP/SUVH4) is the major histone H3K9 methyltransferase (Jackson *et al.*, 2004; Du *et al.*, 2015). In addition to C-terminal SET domain (Du *et al.*, 2015), KYP/SUVH4 possesses also N-terminal SRA (SET and RING associated) domain, which recognizes methylated DNA (Du *et al.*, 2014). KYP/SUVH4 and its close homologues SUVH5 and SUVH6 recognize mCHG and mCHH and catalyse H3K9 methylation of adjacent nucleosomes. H3K9me2 is then recognized by CMT3 or CMT2, which in turn catalyse the methylation of CHG (in the case of CMT3) or CHH (in the case of CMT2) DNA sites in the corresponding region. This creates a self-reinforcing, positive feedback loop (Du *et al.*, 2014; Du *et al.*, 2015; Figure 2), that ensures proper methylation pattern maintenance and constitutively silenced chromatin status.

Furthermore, two catalytically inactive homologues SUVH2 and SUVH9 with conserved SRA domain link DNA methylation with the RdDM pathway. SUVH2 and SUVH9 bind to methylated DNA at RdDM loci and act as adaptor proteins mediating the occupancy of PolV and thus DNA methylation at these loci (Liu *et al.*, 2014). SUVH2 and SUVH9 have been also shown to participate in RdDM-

mediated heterochromatin condensation via interaction with MORC1-MORC6 complex (Jing *et al.*, 2016).

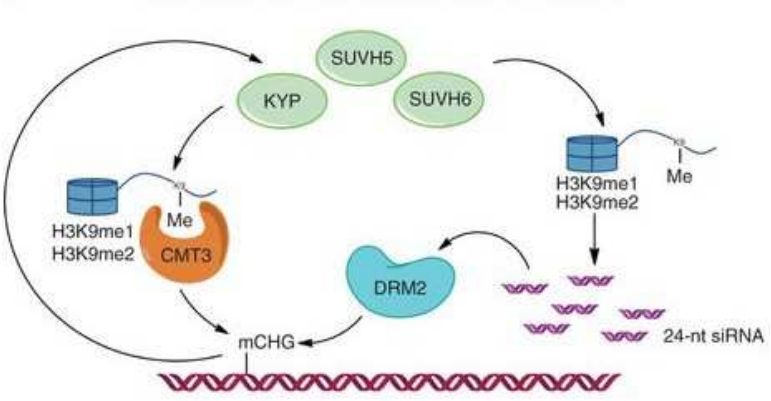

Figure 2: Histone and DNA methylation loop. *KYP/SUVH4 (and its homologues SUVH5 and SUVH6) and CMTs engage in a self-reinforcing loop between H3K9me1/me2 and DNA methylation. H3K9me1/me2 and DNA methylation are also recognized by SHH1 and by SUVH2 and SUVH9 in the RdDM pathway respectively, resulting in de novo DNA methylation by DRM2 (adapted from* Stroud *et al., 2014).*

H3K9me2 is recognized also by SAWADEE HOMEODOMAIN HOMOLOG 1 (SHH1). SHH1 associates with Pol IV, upstream in the RdDM pathway. SHH1 possesses Tudor-like folded SAWADEE domain, which functions as a dual lysine reader, probing for both unmethylated H3K4 and H3K9me2 – typical heterochromatin pattern. SHH1 therefore mediates the recruitment of Pol IV to a large subset of the RdDM targets, which results in production of siRNA from these loci (Law *et al.*, 2013).

In addition, SRA domain-containing VARIANT IN METHYLATION (VIM) proteins have been shown to collaborate with MET1 and might link DNA methylation with H3K9 methylation to regulate epigenetic gene silencing primarily in heterochromatic regions (Kim *et al.*, 2014).

H3K27

H3K27me1 is established by ARABIDOPSIS TRITHORAX RELATED 5 (ATR5) or ATR6 histone monomethyltransferases. Similarly to H3K9me1/me2, H3K27me1 is also enriched in constitutive heterochromatin, but in contrast to DNA methylation-dependent H3K9me1/me2, there is no mutual relationship between H3K27me1 and DNA methylation (Jacob *et al.*, 2009). H3K27me1 HKMTs have been proposed to control replication of heterochromatin DNA (Thorstensen *et al.*, 2011).

On the other side, H3K27me3 is found in euchromatin and is considered the major repressive modification involved in the regulation of a large number of developmentally important genes in both animals and plants, including *FLC*, *FT* and *AGAMOUS* (*AG*) genes in *Arabidopsis* (Gan *et al.*, 2015). The maintenance of H3K27me3 seems to be largely independent of other epigenetic pathways, including DNA methylation and RNAi pathway (Zhang *et al.*, 2007).

H3K27me3, PRC2 and PRC1

In animals, trimethylation of H3K27 is established by ENHANCER OF ZESTE [E(Z)], a SET domain histone methyltransferase within POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) complex. H3K27me3 set by the PRC2 complex is then recognized by the chromodomain of POLYCOMB (Pc), a core component of the PRC1 complex (Pien & Grossniklaus, 2007). PRC1 complex demonstrates ubiquitin ligase activity and mediates downstream monoubiquitination of histone H2AK119 to establish a stable repression of PcG (POLYCOMB GROUP) target genes by chromatin condensation (Wang *et al.*, 2004; Figure 3).

The PRC2 complex is conserved in both plants and animals and the *Arabidopsis* genome encodes homologues of all members of the PRC2 complex. *Arabidopsis* E(Z) proteins, MEDEA (MEA), CURLY LEAF (CLF) and SWINGER (SWN), function within PRC2 to mediate H3K27 trimethylation (Thorstensen *et al.*, 2011). At least three distinct PRC2-like complexes are found in *Arabidopsis* – the FERTILIZATION-INDEPENDENT SEED 2 (FIS)-, EMBRYONIC FLOWER 2 (EMF2)- and VERNALIZATION 2/PLANT HOMEODOMAIN (VRN2/PHD)-containing PRC2 complexes, of which the last two play a major role in the regulation of floral regulatory genes, including *FLC* and *FT* (Liu *et al.*, 2010; Mozgova *et al.*, 2015).

In contrast to PRC2, the PRC1 function was long thought to be absent in plants and only recently, PRC1-like activity was confirmed also in *Arabidopsis*. LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) [also known as TERMINAL FLOWER 2 (TFL2)] possesses chromodomain and acts as a plant-specific H3K27me3 reader, similarly to Pc. Although LHP1 was at first thought to recognize H3K27me3 and to recruit plant PRC1, LHP1 has been recently shown to co-purify with PRC2 components and to assist the recruitment of PRC2 to target sites for maintaining the H3K27me3 levels upon replication (Derkacheva *et al.*,

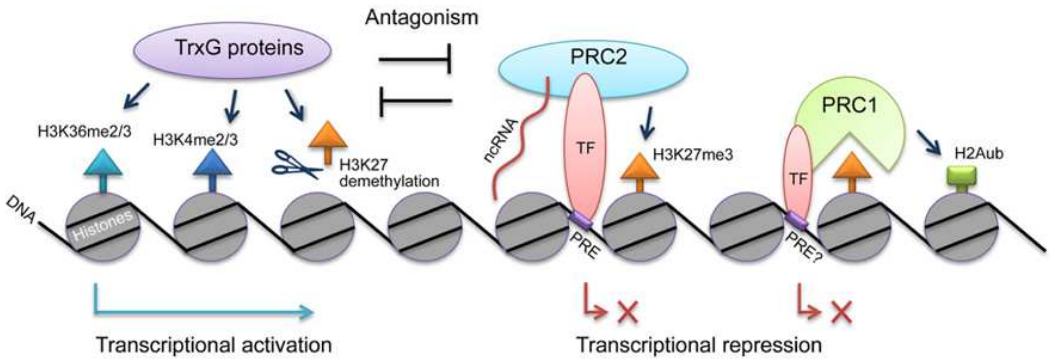

Figure 3: Mutually antagonistic TrxG and PcG complexes. *TrxG-mediated gene activation and PcG-mediated gene silencing provide a dynamic regulation of gene expression (adapted from* He *et al., 2013).*

2013; Merini & Calonje, 2015). EMF1 has been proposed as another PRC1-like component acting downstream of the PRC2 complexes in stabilizing gene repression. EMF1 has been shown to cooperate with PRC2 in the repression of

the floral homeotic gene *AG* during vegetative development and to be required for H3K27 trimethylation, as *emf1-2* mutants showed decreased levels of H3K27me3 at many genes. Furthermore, the most recently characterized PRC1 RING-finger proteins have also been shown to interact with PRC2 and to mediate H2A monoubiquitination in plants. Overall, recent studies show that PRC1 not necessarily acts downstream of the PRC2 and that PRC1 activity might actually be required for the PRC2 recruitment and subsequent H3K27me3 marking (reviewed in Merini & Calonje, 2015). Both PRC2 and PRC1-like complexes are profoundly involved in different aspects of flower development, including flowering time control and flower organ patterning and development (Molitor & Shen, 2013; Mozgova *et al.*, 2015).

H3K4

The histone H3K4 methylation is considered the major activating histone modification in eukaryotes. In contrast to animals, in *Arabidopsis* only H3K4me3 has been implicated in gene transcriptional activation. TRITHORAX group (TrxG) methyltransferases catalyse H3K4 methylation in eukaryotes including plants (Figure 3). SET DOMAIN GROUP 2 (SDG2) has been shown to be necessary for global genome-wide H3K4me3 deposition in *Arabidopsis* and SDG2-dependent H3K4me3 is critical for regulating gene expression and plant development (Guo *et al.*, 2010; Yao *et al.*, 2013). Furthermore, *Arabidopsis* COMPASS-related (Complex Proteins Associated with SET1) protein complex containing ARABIDOPSIS HOMOLOG OF TRITHORAX 1 (ATX1) SET-domain methyltransferase, has been shown to bind to chromatin of *FLC* and its homologues *MAF*s (*MADS AFFECTING FLOWERING* and to deposit H3K4me3 in order to activate their expression (Jiang *et al.*, 2011). ATX1/AtCOMPASS might function to both facilitate preinitiation complex (PIC) assembly and to generate H3K4me3 as an activating mark for the transcriptional elongation on target genes (Fromm & Avramova, 2014). In addition, H3K4me3 facilitates transcription through being recognized by multiple protein complexes, including histone acetyltransferases (HATs), as well as the chromatin remodelling complexes, which support ongoing transcription. (Deal & Henikoff, 2011).

H3K36

In *Arabidopsis*, two homologues of yeast SET2 histone methyltransferase, SET DOMAIN GROUP 8 (SDG8) and SDG26 (members of the TrxG HKMT family) catalyse di- and trimethylation of H3K36. H3K36me2/me3 correlate with transcription activation. SDG8 [also known as EFS (EARLY FLOWERING IN SHORT DAYS) or ASHH2 (ABSENT, SMALL, OR HOMEOTIC DISCS 1 HOMOLOG 2)] is required for deposition of H3K36me2/me3 for active transcription of *FLC* and its homologues (Zhao *et al.*, 2005; Xu *et al.*, 2008) and is epistatic to SDG26 (Liu *et al.*, 2016b). SDG26 (also known as ASHH1) binds to *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*) chromatin and is required for deposition of H3K4me3 and H3K36me3 for active transcription of *SOC1*, which in turn promotes flowering (Berr *et al.*, 2015; Liu *et al.*, 2016b). SDG26 might be also involved in the H3K36me1 deposition. SDS26/ASHH1 has

been shown to interact with two distinct HKMTs – ATX1 and SDG8, and may act in a common multiprotein complex with them, thus linking together H3K4 and H3K36 methylation (Valencia-Morales *et al.*, 2012).

3.1.2 Arginine Methylation

Histone arginine methylation is involved in a whole variety of cellular processes. Arginine methylation is catalysed by a family of Protein arginine methyltransferases (PRMTs). Two best-characterized plant PRTMs, the type I (catalysing asymmetric R dimethylation) PRMT4, and type II (catalysing symmetric R dimethylation) PRMT5, function redundantly and co-regulate the expression and splicing of key regulatory genes associated with transcription, RNA processing, responses to light, flowering, and abiotic stress tolerance (Hernando *et al.*, 2015).

Both type I and type II appear to regulate flowering time in *Arabidopsis* likely through the *FLC*-dependent pathway, based on late-flowering phenotypes of *prmt5*, *prmt10* mutants and *prmt4a;4b* double mutant (Hernando *et al.*, 2015).

3.1.3 Histone Demethylases

Two distinct families of histone demethylases remove methyl residues from lysines. First, LYSINE DEMETHYLASE 1 (KDM1) demethylates only mono- and dimethylated lysines (Liu *et al.*, 2010). The *Arabidopsis* genome encodes four KDM1 homologues - FLOWERING LOCUS D (FLD), LSD1-LIKE 1 (LDL1), LDL2, and LDL3. LDL1 functions as an H3K4 demethylase, reducing the levels of H3K4me1/me2 in chromatin of *FLC* and the sporophytically silenced floral repressor *FWA*. FLD, LDL1 and LDL2 act with partial redundancies in the silencing of *FLC* expression (Jiang *et al.*, 2007).

Second, JUMONJI-DOMAIN CONTAINING (JmjC) demethylases catalyse demethylation of mono-, di-, and trimethylated amino acid residues (Liu *et al.*, 2010; Jeong *et al.*, 2015). Two JmjC members, EARLY FLOWERING 6 (ELF6) and RELATIVE OF EARLY FLOWERING 6 (REF6) have been shown to have opposite functions in flowering time regulation. ELF6 participates in the reactivation of silenced *FLC* during embryogenesis (Crevillén *et al.*, 2014), whereas REF6 removes repressive H3K27me3 from *FT* (Lu *et al.*, 2011) and *SOC1* (Hou *et al.*, 2014), promoting their expressions. Another JmjC demethylase JMJ30 is involved in the circadian systems and the thermosensory pathway of flowering control (Gan *et al.*, 2015).

3.2 Histone Acetylation

Reversible histone acetylation and deacetylation play a crucial role in the regulation of gene expression. Acetylation of histone lysines effectively neutralizes positive charges, weakening the interaction between the histone octamer and the negatively charged DNA. This may result in a local chromatin structure opening,

which enables transcriptional regulators better access to the DNA. Furthermore, acetylation of lysine residues generates a binding platform for bromodomain-containing effector proteins (Kouzarides, 2007), including chromatin-remodelling factors and transcription factors. In general, hyperacetylation of histones relaxes chromatin and induces transcription, whereas hypoacetylation of histones leads to chromatin compaction and consequently gene repression (Liu *et al.*, 2016a).

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyse histone acetylation and deacetylation, respectively, and are involved in various developmental processes and responses to environment, including the flowering time control. Four families of HATs and three families of HDACs have been identified in plants (reviewed in Liu *et al.*, 2016a).

Two HDACs, HDA5 and HDA6 have been shown to form an HDAC complex with FLD and MULTICOPY SUPPRESSOR OF IRA1 4 (MSI4/FVE) to repress *FLC* expression by histone deacetylation and H3K4 demethylation (Luo *et al.*, 2015). Under the non-inductive photoperiod, HDA9 has been shown to repress *AGAMOUS-LIKE 19* (*AGL19*), an up-stream activator of FT, through the histone deacetylation (Kang *et al.*, 2015). Furthermore, SAP30 FUNCTION-RELATED 1 (AFR1) and AFR2 are involved in circadian deacetylation of *FT* chromatin (Gu *et al.*, 2013b). HAC family of HATs have also been indicated to be involved in FLC-dependent flowering time control (Deng *et al.*, 2007; Han *et al.*, 2007).

Furthermore, chromodomain-containing proteins MORF RELATED GENE 1 (MRG1) and MRG2 link H3K36 methylation and histone acetylation. MRG1 and MRG2 recognize H3K36me3 set by SDG8/EFS and interact with the histone H4K5-specific acetyltransferases HAM1 and HAM2 to ensure the high transcription levels of two flowering time genes with opposing functions, *FLC* and *FT* (Xu *et al.*, 2014).

3.3 Histone Ubiquitination

Another important histone PTM is the ubiquitination. Histone ubiquitin ligases and histone deubiquitinases catalyse deposition and removal of the small protein ubiquitin, respectively. The most common histone ubiquitination occurs as the monoubiquitination of H2A and H2B histones. Both H2Aub1 and H2Bub1 are involved in the transcriptional regulation. H2Aub1 occupation is rather associated with the transcriptional repression, with H2A ubiquitin ligases being found in various repressor complexes, including PRC1 complex (Cao & Yan, 2012).

In contrast, H2Bub1 occupation is strongly associated with gene expression. H2Bub1 facilitates Pol II elongation by promoting the H2A/H2B-dimer replacement from the core nucleosomes of the gene body (Cao & Yan, 2012). In addition, H2Bub1 might promote other activating histone PTMs. H2Bub1 has been proposed to cooperate with acetylated H4 in inhibition of chromatin compaction (Fierz *et al.*, 2011). Furthermore, H2Bub1 might also promote H3K4 dimethylation in the chromatin of specific genes (Cao *et al.*, 2015).

4 | Histone PTMs in the Regulation of Flowering Time

Flowering is a very complex process, that occurs in response to various developmental and environmental cues (Figure 4). In *Arabidopsis*, flowering time is regulated by multiple pathways, including the autonomous, vernalization, photoperiod and gibberellin pathways (Henderson & Dean, 2004). FLOWERING LOCUS C (FLC), a MADS-box transcription factor, acts as the main repressor of the flowering (Michaels & Amasino, 1999). FRIGIDA (FRI) elevates the expression of *FLC*, to levels that suppress flowering (Johanson *et al.*, 2000). *FLC* is expressed during the vegetative growth and acts to repress the expression of downstream genes that promote flowering, including *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). *FLC* must be repressed for flowering to occur (Crevillén & Dean, 2011).

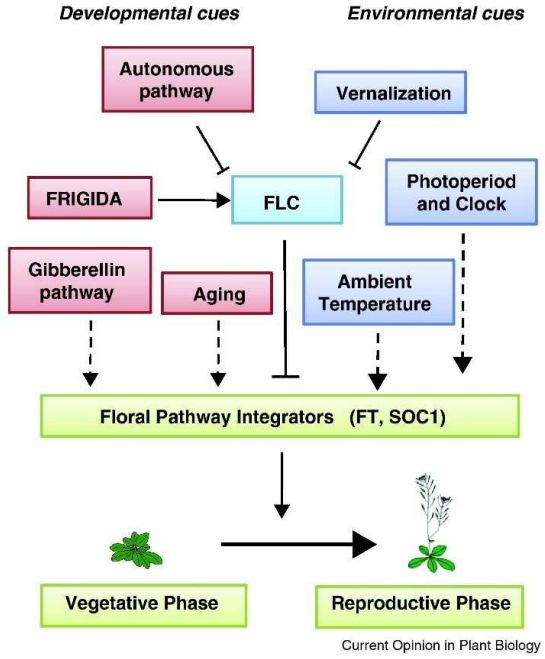

Figure 4: Flowering regulatory pathways. *Different flowering-promoting pathways converge onto the so-called 'floral pathway integrators', including FT and SOC1. FLC, the main repressor of the flowering stands upstream of these integrator genes (adapted from* Crevillén & Dean, *2011).*

4.1 Chromatin-mediated Regulation of *FLC* Expression

Several regulatory pathways converge onto *FLC* to coordinate the fine-tuned regulation of flowering time (Figure 4, Crevillén & Dean, 2011). The regulation of the *FLC* gene expression and silencing is an excellent example of the interplay between various epigenetic mechanisms, with histone PTMs playing a pivotal role.

4.1.1 FRI-dependent Activation of *FLC*

In initiation phase, Pol II engages and quickly pauses transcription of *FLC*, forming the preinitiation complex (PIC). A plant-specific scaffold protein FRI-GIDA (FRI) interacts with several other *FLC*-specific regulators to form the transcription activator complex (FRI-c – Choi *et al.*, 2011). FRI-c binds to the *FLC* promoter and recruits chromatin modifiers, including SWR1-c (for the H2A.Z deposition around the *FLC* TSS), SDG8/EFS (for H3K36me2/me3 – Xu *et al.*, 2008; Choi *et al.*, 2011; Yang *et al.*, 2014) and WDR5a, a component of COMPASS (for H3K4me3 – Jiang *et al.*, 2009), in order to upregulate *FLC* expression. RNA POLYMERASE-ASSOCIATED FACTOR 1 complex (PAF1-c) associated with Pol II and HUB-UBC complex (required for genome-wide H2Bub1 deposition) may also cooperate or directly associate with FRI-c in order to recruit or enrich SWR1-c, SDG8/EFS and COMPASS modifiers at the *FLC* locus (Figure 5, He, 2012).

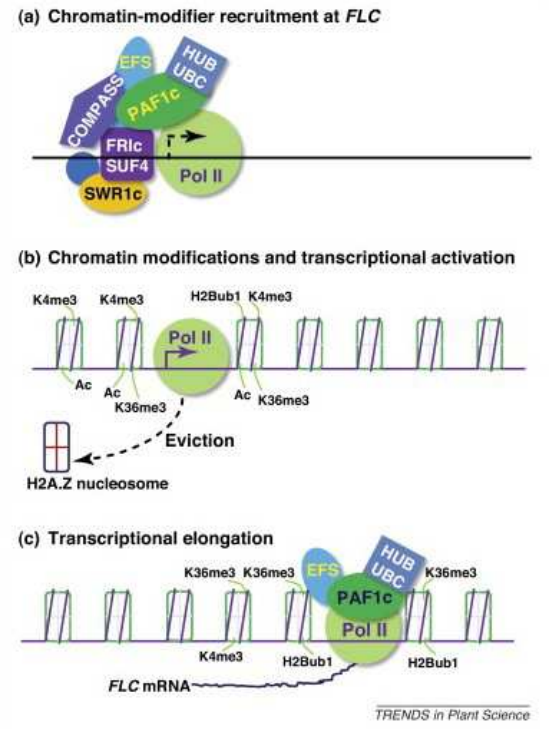

Figure 5: FRI-dependent *FLC* activation. *(a) FRI-c binds to the FLC promoter and recruits chromatin modifiers SWR1-c, COMPASS and EFS. FRI-c also interacts with PAF1c associated with Pol II and HUB-UBC. (b) Nucleosomes around FLC TSS region are modified with active marks, incl. acetylation, H3K4me3, H3K36me3 and H2Bub1. Incorporation of H2A.Z into the nucleosome occupying TSS leads to its destabilization and subsequent eviction to facilitate the transcription by Pol II. (c) PAF1-c travels with Pol II and may recruit EFS and HUB-UBC, modifying the nucleosomes of the FLC gene body (adapted from* He, *2012).*

During transcription activation, SWR1-c replaces H2A with the H2A.Z variant, while SDG8/EFS deposits H3K36me2/me3. SDG8/EFS has been shown to mediate the recruitment of FRI-c at the *FLC* locus (Ko *et al.*, 2010). Furthermore, SDG8/EFS has been found to physically interact with H3K27 demethylase EARLY FLOWERING 6 (ELF6), which removes the repressive H3K27me3 mark from *FLC* (Yang *et al.*, 2016). H3K36me3 set by SDG8/EFS is then recognized by MRG1 or MRG2, which in turn may recruit H4K5-specific acetyltransferase HAM1 and HAM2 complexes (Xu *et al.*, 2014). Acetylation of H2A.Z-containing nucleosomes might lead to their destabilization and removal to facilitate transcription (He, 2012).

To escape preinitiation complex and to proceed to the elongation phase, Pol II requires the additional activities of chromatin modifying complexes – PAF1-c,

COMPASS and HUB-UBC (Crevillén & Dean, 2011). Pol II associates and travels with the conserved PAF1-c complex, which orchestrates the recruitment of SDG8/EFS, COMPASS and HUB-UBC complex, to catalyse H3K36me3, H3K4me3 and H2Bub1 within the *FLC* gene body, promoting on-going transcription (Cao *et al.*, 2008; Ko *et al.*, 2010; He, 2012, Figure 5). COMPASS has been shown to catalyse H3K4me3 at *FLC* (Jiang *et al.*, 2011). HUB-UBC mediates histone H2B monoubiquitination at the *FLC* locus (Schmitz *et al.*, 2009). H2Bub1 is required for the enhancement of H3K4me3 and H3K36me2/me3. In contrast to the H2Aub1, catalysed by PRC1-like and necessary for the gene silencing, HUB-UBC complex works together with PAF1-c, COMPASS and Pol II to facilitate transcription elongation (Cao *et al.*, 2008). The histone chaperone FACILITATES CHROMATIN TRANSCRIPTION (FACT) complex mediates the H2A/H2B dimer replacement and nucleosome reassembly, which facilitate the movement of transcription machinery through nucleosomes in the body of gene (Lolas *et al.*, 2010).

4.1.2 Autonomous Silencing of *FLC*

Work on rapid-flowering *Arabidopsis* accessions lacking a functional *FRI* allele (such as Col or Ler) shows, that *FLC* is repressed vernalization-independently by the autonomous or constitutive *FLC* repressors, including FVE/MSI4 and MSI5, FLOWERING LOCUS D (FLD), HISTONE DEACETYLASE 6 (HDA6) and HDA5, and PcG components. These components directly interact with the *FLC* locus and mediate *FLC* chromatin silencing, thus promoting the flowering (He, 2012). The FLD-HDA6-HDA5-FVE/MSI4 or MSI5 complex deacetylates histones H3 and demethylates H3K4me3 to repress *FLC* expression (Yu *et al.*, 2011; Gu *et al.*, 2011; Luo *et al.*, 2015; Figure 6)

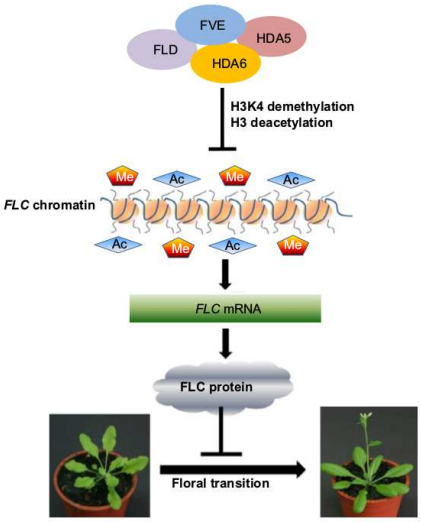

Figure 6: Autonomous *FLC* repression by FLD complex. *FLD, FVE, HDA5 and HDA6 form a complex, that represses FLC expression through H3K4 demethylation and histone H3 deacetylation (adapted from* Liu *et al., 2016b).*

The activity of PRC2 complex is also required for autonomous *FLC* silencing. The EMF2-containing PRC2 complex, composed of the CURLY LEAF (CLF)/SWINGER (SWN) H3K27 methyltransferases and structural subunits EMBRYONIC FLOWER 2 (EMF2, a VRN2 homolog), FERTILIZATION-INDEPENDENT ENDOSPERM (FIE), and MULTICOPY SUPPRESSOR OF IRA 1 (MSI1), is thought to interact with both *FLC* and *FT* (as CLF mediates H3K27me3 deposition at both these genes – Jiang *et al.*, 2008), however, EMF2-PRC2 has been shown to be re-

cruited only to *FLC* (Kim *et al.*, 2010b). In *emf* mutants, floral regulatory genes *FLC* and *FT* were up-regulated (Jiang *et al.*, 2008; Kim *et al.*, 2010b) and the floral organ identity genes showed ectopic expression (Moon *et al.*, 2003). Consistently, H3K27me3 levels were dramatically decreased in both *emf1* and *emf2* mutants (Calonje *et al.*, 2008). Overall, although repressing *FLC* too, *EMF* genes repress the flower program, resulting in early-flowering *emf* mutants (Moon *et al.*, 2003). The EMF2-PRC2 complex might act in concert with FLD complex to demethylate H3K4, deacetylate H3 histones and deposit repressive H3K27me3, in order to establish chromatin environment that represses *FLC* expression (He, 2012).

Functional *FRI* allele revokes the function of the autonomous pathway genes and mediates the chromatin activation of *FLC* expression (Choi *et al.*, 2011; He, 2012). FRI appears to suppress binding of EMF2-PRC2 (specifically of CLF subunit) to *FLC*, thus reducing the level of H3K27me3 (Doyle & Amasino, 2009), and FRI-c might overcome the FLD demethylase activity by elevating the H3K4me3 through the action of COMPASS (Jiang *et al.*, 2009). Thus, autonomous *FLC* silencing pathway alone might not be sufficient enough in plants with functional *FRI*. *Arabidopsis* has therefore also the vernalization pathway to silence *FLC* and induce flowering.

4.1.3 Vernalization-dependent Silencing of *FLC*

Many plants acquire the competence to flower only after a prolonged cold exposure, in the process called vernalization. Vernalization silences the expression of *FLC* and its homologues, to promote the flowering upon the return of *Arabidopsis* to warm conditions, usually after winter (Kim *et al.*, 2009). The vernalization-dependent *FLC* silencing involves non-coding RNAs (Heo & Sung, 2011) and PcG activity (De Lucia *et al.*, 2008). The VRN2/PHD-containing PRC2 complex and PRC1-like complex induce and maintain mitotically stable, repressed *FLC* chromatin state. Such epigenetically silenced *FLC* provides *Arabidopsis* with the 'memory of winter' (Angel *et al.*, 2011) during the rest of its life until the *FLC* chromatin state is reset during gametogenesis and embryogenesis (Choi *et al.*, 2009; Iwasaki, 2015; Figure 7).

During prolonged cold exposure a PHD-PRC2 complex is formed, composed of core PRC2 components VRN2, CLF (or SWN), FIE and MSI1, and three related PHD finger proteins, VRN5/VIL1 (VIN3-LIKE 1), VERNALIZATION INDEPENDENT 3 (VIN3) and VEL1 (De Lucia *et al.*, 2008). Induction of *VIN3* is the earliest known step in vernalization. *VIN3* mRNA levels increase simultaneously with the length of cold exposure and *VIN3* expression is completely repressed upon the return into warm conditions (Sung & Amasino, 2004). Before and also during vernalization, *VIN3* is also constitutively repressed by LHP1 and PRC2. The LHP1/PRC2-mediated repression is overcome by still not completely clear, distinct cold–response mechanism, that induces H3K4 trimethylation (Zografos & Sung, 2012) and changes in H3 and H4 acetylation (Bond *et al.*, 2009), and requires PAF1 and SDG8/EFS activity (Kim *et al.*, 2010a). VIN3 binds to the *FLC* chromatin and interacts with other members of PRC2

(De Lucia *et al.*, 2008). H3K4me3 at *FLC* likely suppresses VIN3 binding and might need to be first removed for vernalization-dependent silencing to occur (Kim & Sung, 2017a).

In the initial phase of vernalization, the transcribed *FLC* gene loop is disrupted (Crevillén *et al.*, 2013), coinciding with the increased expression of prolonged cold-induced antisense and non-coding RNAs originating from the *FLC* locus. First, antisense *COOLAIR* transcripts, derived from the 3' region of the *FLC* locus, physically associate with the *FLC* chromatin and facilitate the cold-induced replacement of H3K36 methylation with H3K27me3 (Csorba *et al.*, 2014). However, *COOLAIR* is not essential for the vernalization-mediated *FLC* silencing (Helliwell *et al.*, 2011). Additionally, two non-coding sense RNAs *COLDAIR* (derived from a cryptic promoter in the first intron of *FLC* – Heo & Sung, 2011) and newly characterized *COLDWRAP* (derived from the proximal promoter of *FLC* – Kim & Sung, 2017b) associate with CLF or SWN catalytic subunit of the PRC2 complex and guide PHD-PRC2 to *FLC* chromatin (Figure 7).

PHD-PRC2 deposits H3K27me3 at the nucleation region around the first exon of *FLC* in order to initiate *FLC* silencing (Angel *et al.*, 2011). At the same time, the level of H3K36me3 significantly decreases (Yang *et al.*, 2014). Upon the return of plants to warm conditions, the PHD-PRC2 complex (now without VIN3) spreads across the *FLC* gene and continues to deposit H3K27me3, thus spreading H3K27me3 across the whole *FLC* locus (Angel *et al.*, 2011). Mitotic activity, i.e. DNA replication is required for H3K27me3 spreading and maintenance of vernalization-induced *FLC* repression (Finnegan & Dennis, 2007). The MSI1 subunit of PRC2 has been shown to interact with LHP1 (a component of the *Arabidopsis* PRC1-like complex), thus linking the actions of PHD-PRC2 and PRC1-like for the functional plant PcG repressive system (Derkacheva *et al.*, 2013). LHP1 recognizes and binds H3K27 mark and is required for the maintenance of stable *FLC* repression, stabilizing increased levels of the repressive marks H3K27me3 and H3K9me2 (Mylne *et al.*, 2006; Sung *et al.*, 2006). The LHP1-MSI1-mediated positive feedback loop allows the recruitment of PRC2 to chromatin carrying H3K27me3, possibly ensuring further H3K27me3 spreading and stability during replication (Derkacheva *et al.*, 2013; Figure 7). Consistently, newly characterized ENHANCER OF LHP1 (EOL1) has been shown to interact with PcG components CLF, SWN, and LHP1, to ensure faithful inheritance of the H3K27me3 modification through replication (Zhou *et al.*, 2017). The repressed *FLC* alleles have been shown to physically cluster within the nucleus (Rosa *et al.*, 2013; Zhu *et al.*, 2015).

Sufficient period of cold is necessary for the stable vernalization-dependent repression of *FLC* in a sufficient number of cells to avoid the re-activation of *FLC* transcription in the subsequent warm period. The quantitative increase in the H3K27me3 levels has been shown to reflect the percentage of the cells that have switched *FLC* to the epigenetically silenced state (Angel *et al.*, 2011; Yang *et al.*, 2014).

To ensure the vernalization requirement in the progeny, *FLC* locus is reactivated during gametogenesis and embryogenesis. *FLC* epigenetic reset requires the H3K27me3 JmjC-domain demethylase EARLY FLOWERING 6 (ELF6), which presumably counteracts the silencing activity of embryo-specific PRC2 (Crevillén *et al.*, 2014; Figure 7).

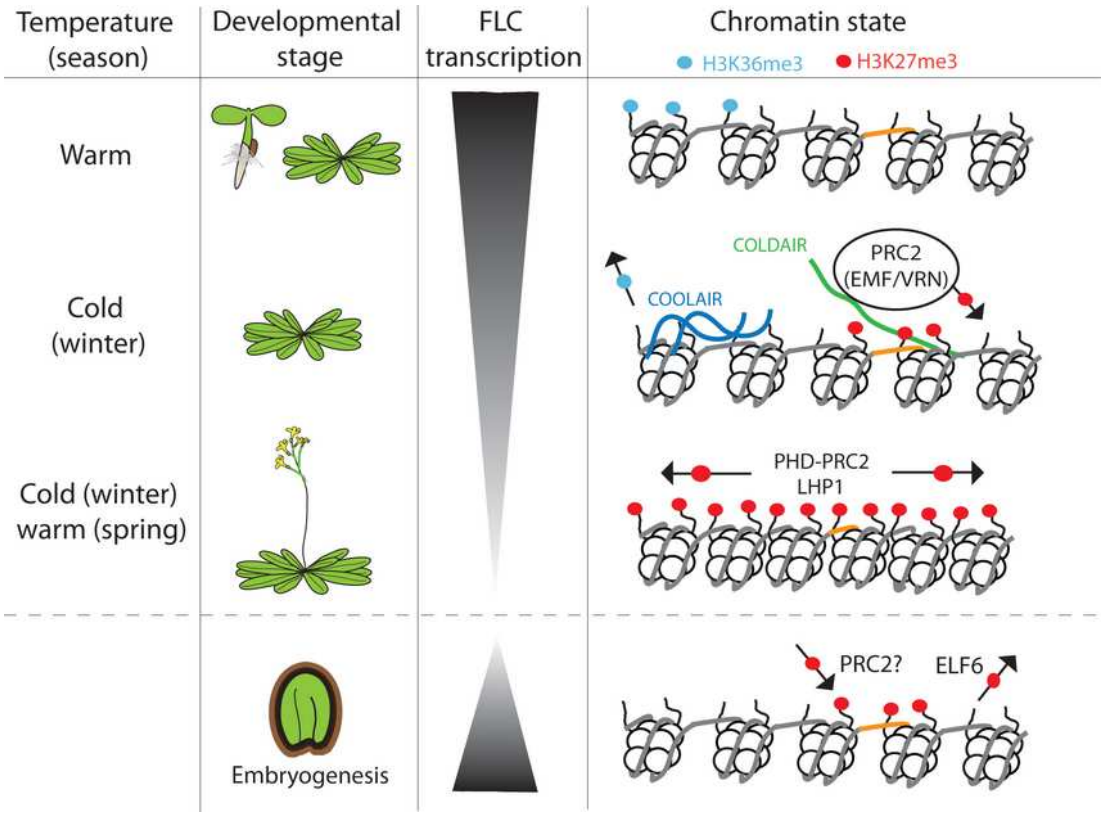

Figure 7: The vernalization-dependent *FLC* repression. *In response to prolonged cold exposure, FLC expression is reduced. COOLAIR facilitates H3K36me3 replacement for repressive H3K27me3. COLDAIR guides the recruitment of PRC2 complex to the nucleation region at the FLC chromatin (orange segment). Finalized PHD-PRC2 then spreads H3K27me3 across the FLC locus. The silenced FLC chromatin is further stabilized and maintained by the LHP1 cooperating with PHD-PRC2. FLC chromatin state and expression are reactivated during embryogenesis through the action of H3K27me3 demethylase ELF6 (adapted from* Mozgova *et al., 2015).*

4.2 Chromatin-mediated Regulation of *FT* Expression

Beside autonomous and temperature-dependent vernalization pathways, flowering is controlled also by the change in photoperiod. In *Arabidopsis*, the inductive long-day photoperiods trigger the production of a systemic mobile flowering signal, so-called florigen. FLOWERING LOCUS T (FT) is a major component of the mobile florigen signal (Gu *et al.*, 2013b). *FT* is expressed in the leaf phloem and is then transported into the shoot apical meristem (SAM) to activate *SOC1* (Lee & Lee, 2010) and the floral-meristem identity genes *AP1* and *LFY*, thus

inducing the flowering (Wigge, 2011; Gu *et al.*, 2013b). Under non-inductive conditions, *Arabidopsis FT* is repressed by MADS-box transcriptional repressors FLC and FLC-related FLOWERING LOCUS M (FLM; Gu *et al.*, 2013a).

FT repression requires the PcG activity. FLC has been shown to interact with EMF1, recruiting the PRC1-like EMF1 complex (EMF1-c) at the *FT* locus. EMF1-c contains EMF1, LHP1 and H3K4me3 demethylase PKDM7B/JMJ14, which removes the activating H3K4me3 mark from *FT* chromatin (Lu *et al.*, 2010; Wang *et al.*, 2014). The PRC1-like EMF1-c might therefore mediate the initial silencing of *FT* (Mozgova *et al.*, 2015). PRC2 recruited by the interaction with LHP1 is likely to maintain the repressed *FT* chromatin (analogically to the maintenance of silenced *FLC* described in section 4.1.3; Derkacheva *et al.*, 2013). The EMF2-PRC2 complex, containing EMF2, FIE, CLF/SWN, has been suggested to mediate H3K27 trimethylation of *FT* chromatin, as CLF has been shown to deposit the repressive H3K27me3 mark at the *FT* locus to repress its expression and in *emf2* mutants, *FT* and *FLC* were up-regulated (Jiang *et al.*, 2008). Despite this fact, direct EMF2 binding to *FT* chromatin was not observed (Kim *et al.*, 2010b). However, recent studies have shown, that catalytic subunit EARLY IN SHORT DAYS 7 (ESD7) of DNA polymerase ϵ interacts with CLF, EMF2 and MSI1, components of PRC2, and that ESD7 is necessary for the PRC2 recruitment to *FT* and *SOC1* chromatin, negatively regulating their expression. ESD7 might therefore link DNA replication with the PcG complexes (delOlmo *et al.*, 2016). *FT* chromatin has a bivalent epigenetic labelling, carrying simultaneously two antagonistically acting marks - activating H3K4me3 and repressive H3K27me3, and the relative levels of both play a critical role in the regulation of *FT* expression (He, 2012; a similar situation can be found in the regulation of the floral homeotic gene *AG* - Saleh *et al.*, 2007).

As described above, EMF1-c and presumably EMF2-PRC2 are required for continuous *FT* repression in leaf vasculature along day/night cycles. On the other side, *FT* expression is rhythmically activated at the end of long days (LDs) by the photoperiod-pathway output CONSTANS (CO). In response to inductive LD photoperiod set by circadian clock, light-stabilized CO protein accumulates towards the LD's end and suppresses EMF1 (EMF1c) binding to FT chromatin (Gu *et al.*, 2013b; Wang *et al.*, 2014). MRG1 and MRG2 have been shown to physically interact with CO and to mediate CO binding to *FT*. MRG2 recognizes H3K4me3/H3K36me3 and associates with the chromatin of *FT* promoter in a CO-dependent manner (Bu *et al.*, 2014). MRG1 and MRG2 may then recruit HAM1 and HAM2 H4K5 acetyltransferases (in a similar manner as in *FLC* activation described in 4.1.1; Derkacheva *et al.*, 2013; Figure 8) and the repressive H3K27me3 mark set by PcG is removed by the H3K27 JmjC-domain methyltransferase RELATIVE OF EARLY FLOWERING 6 (REF6 - Lu *et al.*, 2011; Gan *et al.*, 2015) in order to up-regulate *FT* expression. FT protein produced at the end of long day then moves to shoot apical meristem to induce the flowering.

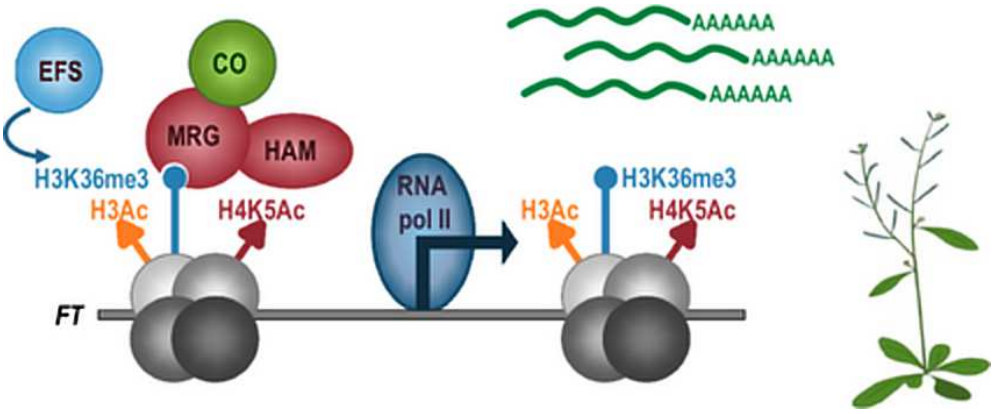

Figure 8: Photoperiod-dependent activation of *FT*. *MRGs recognize the H3K36me3 marks on FT chromatin and mediate the recruitment of long day-photoperiod induced CO and H4K5 acetylase HAM, in order to upregulate FT mRNA expression at the end of long day. Leaf vasculature-expressed FT is then transported to shoot apical meristem to induce flowering (adapted from* Jarillo & Piñeiro*, 2015).*

During the night, CO is rapidly degraded by the proteasome, EMF1-c binds back to *FT* chromatin (Wang *et al.*, 2014) and the MADS-domain transcription factor AGAMOUS LIKE 18 (AGL18) recruits the SAP30 FUNCTION-RELATED 1 (AFR1) and AFR2 HDACs to *FT* chromatin to catalyse histone deacetylation upon *FT* activation, suppressing *FT* expression again. Such mechanism efficiently regulate flowering in response to photoperiod in *Arabidopsis* (Gu *et al.*, 2013b).

FT expression is also induced by ambient temperature rise. Histone variant H2A.Z deposited by SWR1-c near the *FT* TSS has been shown to mediate the thermosensory response (Kumar & Wigge, 2010; Jarillo & Piñeiro, 2015).

Conclusion

Histone post-translational modifications are the key components of epigenetic regulation. By modulating the chromatin structure, they affect gene expression at transcriptional level and consequently control many aspects of the plant development, including flowering. Regulation of flowering time is a very complex process due to a great number of regulatory pathways and factors involved. In this work, I have summarized current knowledge of histone PTMs with special impact on the control of flowering time in *Arabidopsis*. Although regulatory mechanisms of flowering can vary between different plant species, it appears that many characteristics of this regulation are shared (Khan *et al.*, 2014). Flowering is a very attractive topic from the research and practical application point of view. Recent research of histone-dependent and epigenetic regulation of flowering in general is conducted mainly on *Arabidopsis* and rice, and shows a lot of promise for better understanding of epigenetics and for a development of crops with improved properties.

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