Seasonal and Diurnal Regulation of Flowering *via* an Epigenetic Mechanism in *Arabidopsis thaliana*

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Summary Successful plant reproduction requires the precise control of the onset of flowering, which involves the transition from the vegetative growth phase to the reproductive growth phase. As a facultative long-day annual species, *Arabidopsis thaliana* flowers after an exposure to low temperatures under long-day conditions. This seasonal and diurnal control of flowering involves various epigenetic regulatory activities. We herein review the mechanism underlying the relevant epigenetic regulation, with a focus on the two key flowering regulatory genes, *FLOWERING LOCUS C* (*FLC*) and *FLOWERING LOCUS T* (*FT*). The expression of *FLC*, which encodes a flowering repressor, is controlled *via* a complex epigenetic mechanism involving histone modifications and long noncoding RNAs to establish the “winter memory” of plants annually exposed to winter conditions. In contrast, the expression of *FT*, which encodes a flowering activator, is temporally regulated through the diurnal binding of polycomb group proteins to the *FT* promoter to ensure day-length-dependent flowering. Thus, flowering is robustly and dynamically mediated *via* an epigenetic mechanism to ensure it occurs at the most appropriate time.

Key words Flowering, Epigenetic regulation, Histone modification.

Because plants are sessile organisms, they must be able to adapt to fluctuating environmental conditions to survive. Flowering, which is one of the most sophisticated survival mechanisms that evolved in plants, involves meiosis, which can increase the genetic diversity of progeny through recombination due to the pairing of homologous chromosomes (Gupta *et al.* 2017a, b, 2018a, b, Jeelani *et al.* 2017, Kaur *et al.* 2017, Kumar *et al.* 2017, 2018, Liébana *et al.* 2017, Lin *et al.* 2017, Rani *et al.* 2017, Saggoo and Kaur 2017, Saggoo *et al.* 2017, Singhal and Kumari 2017, Singhal *et al.* 2017, 2018a, b, Dhaliwal *et al.* 2018a, b, Farooq and Saggoo 2018, Kaur and Gupta 2018a, b, Kumari and Singhal 2018, Singh *et al.* 2018). Genetic diversity has resulted in the development of various phenotypes depending on environmental factors, and is one of the driving forces responsible for increases in the geographical distribution of certain plant species (Bordbar *et al.* 2017, Shaker *et al.* 2017, Shibata *et al.* 2017, Muakrong *et al.* 2018, Özer *et al.* 2018, Senavongse *et al.* 2018, Tapia-Pastrana *et al.* 2018).

The proper timing of flowering, which corresponds to the transition from the vegetative growth phase to the reproductive growth phase, is crucial for the reproductive success of flowering plants. This transition is precisely

controlled by developmental factors and environmental cues, and many species have evolved multiple regulatory pathways related to flowering, including the photoperiod-dependent pathway, ambient temperature-dependent pathway, and gibberellin-dependent pathway. In the regulation of flowering, a mobile flowering hormone, florigen, plays crucial roles. This hormone is transported from the leaves to the shoot apical meristem *via* phloem tissue to initiate flowering. In the model plant species *Arabidopsis thaliana*, a major component of florigen, *FLOWERING LOCUS T* (*FT*), and a major repressor of florigen gene expression, *FLOWERING LOCUS C* (*FLC*), are important for ensuring flowering is initiated at the optimal time (Michaels and Amasino 2001, Sheldon *et al.* 2000, Corbesier *et al.* 2007, Jaeger and Wigge 2007, Mathieu *et al.* 2007). Because flowering is a critical event for flowering plants, the expression levels of these floral genes are regulated by transcription factors as well as epigenetic mechanisms, including chromatin remodeling and long noncoding RNA (lncRNA) regulation. To control the flowering time, plants must monitor the onset of cold conditions in winter as well as changes in day length (Fig. 1). In this review, we focus on the regulation of two flowering genes, *FLC* and *FT*, through epigenetic mechanisms related to the seasonal and diurnal regulation of the long-day annual species *A. thaliana*.

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Seasonal regulation

Fig. 1. Epigenetic regulation of flowering in *Arabidopsis thaliana*. Before a prolonged exposure to cold conditions, FRI recruits several chromatin modifiers to the *FLC* locus to activate *FLC* expression. Additionally, *FT* expression is strongly repressed by *FLC*, which inhibits flowering. During winter, H3K27me3 is gradually deposited at the TSS by VIN3-PRC2, and *COOLAIR* represses *FLC* expression in a co-transcriptional manner. Until the following spring, H3K27me3 is enriched at the *FLC* locus because of the recruitment of PRC2 by *COOLAIR*. Therefore, the exposure to cold conditions in winter alleviates the highly repressive *FLC* effects, enabling the expression of *FT* in response to various environmental cues. Under long-day conditions, *FT* is diurnally expressed. In morning and at night, H3K27me3 is widely distributed around the *FT* locus through polycomb protein activities to maintain repressive chromatin. Toward dusk, CO gradually accumulates and CO-NF-Y-mediated chromatin looping occurs in the *FT* promoter, thereby disrupting polycomb protein-mediated repression, ultimately leading to the de-repression of *FT* expression.

Multidimensional epigenetic regulation of *FLC* mediates winter-annual flowering

The *FLC* gene encodes a MADS-box transcription factor, which is a central repressor of flowering (Sheldon *et al.* 2000, Michaels and Amasino 2001). Before winter, FRIGIDA (FRI) activates *FLC* expression to repress flowering, whereas vernalization, which refers to the prolonged exposure to cold conditions (e.g., winter weather) silences *FLC* expression through chromatin regulation and lncRNA activities to establish the "winter memory."

FRIGIDA and FRI-interacting factors are important for activating *FLC* expression. Previous investigations confirmed that FRI, which is a plant-specific protein with coiled-coil domains, functions as a scaffold protein that helps regulate *FLC* expression by interacting with various chromatin modifiers (Johanson *et al.* 2000, Crevillén and Dean 2011). The RNA polymerase II-associated factor 1 complex (PAF1c) was first identified as a FRI-interacting factor, and it is required for regulating histone modifications at the *FLC* locus (He

et al. 2004, Oh *et al.* 2004, Xu *et al.* 2008). Conserved core components of the human H3K4 methyltransferase complex called COMPASS-like complexes are involved in the regulation of H3K4me3 at the *FLC* locus along with PAF1c (Jiang *et al.* 2009, 2011). Additionally, H3K4 methyltransferases (i.e., ATX1, ATX2, ATXR3, and ATXR7) contribute to the COMPASS-mediated deposition of H3K4me3 at the region around the *FLC* transcription start site (TSS), which is where H3K4me3 is mainly accumulated (Pien *et al.* 2008, Tamada *et al.* 2009, Berr *et al.* 2011, Yun *et al.* 2012, Ding *et al.* 2012). Furthermore, the deposition of the histone variant H2A.Z at the TSS is important for the FRI-mediated induction of *FLC* expression. An ATPase chromatin-remodeling complex (i.e., SWR complex) catalyzes the replacement of H2A.Z with H2A, and this substitution around the TSS activates transcription. The SWR complex interacts with FRI, and eliminates H2A.Z to facilitate *FLC* transcription (Choi *et al.* 2007, Deal *et al.* 2007). Additionally, H3K36me2/3 and H2Bub1

regulation are required for *FLC* expression. These modifications are catalyzed by a histone methyltransferase, EARLY FLOWERING IN SHORTDAYS, and a ubiquitin-conjugating enzyme complex, respectively (Zhao *et al.* 2005, Gu *et al.* 2009, Xu *et al.* 2009). Both histone modifiers may be recruited to the *FLC* gene body *via* interactions with PAF1c and then deposit the active histone marks during transcriptional elongation (Jaehning 2010). Thus, FRI and FRI-interacting factors mediate multiple active chromatin modifications at the *FLC* locus to establish a robust repression of flowering for the maintenance of the winter-annual growth habitat.

Exposure to low temperatures during winter induces an epigenetic mechanism that gradually silences *FLC* expression. This silencing involves the polycomb group proteins and lncRNAs, and is maintained even if the temperature increases, which enables flowering in spring. Polycomb repressive complex 2 (PRC2) is highly conserved in higher eukaryotes, and the *A. thaliana* PRC2-like components are required for the deposition of H3K27me₃, which is a hallmark of transcriptional repression, during the regulation of developmental activities (Jiang *et al.* 2008, Liu *et al.* 2010, Lafos *et al.* 2011). The PRC2 complex includes H3K27 methyltransferases including CURLY LEAF (CLF) and SWINGER as well as structural subunits including FERTILIZATION-INDEPENDENT ENDOSPERM, VERNALIZATION 2, and EMBRYONIC FLOWER 2, and influences various developmental regulatory processes (Jiang *et al.* 2008, Farrona *et al.* 2011). When *A. thaliana* is exposed to prolonged cold conditions, some PRC2-interacting factors, including VERNALIZATION INSENSITIVE 3 (VIN3), which is a plant homeodomain protein, are activated and the corresponding genes are expressed only at low temperatures (Sung and Amasino 2004). The VIN3-PRC2 complex deposits H3K27me₃ around the first exon of *FLC* to silence *FLC* expression (Angel *et al.* 2011). In response to elevated temperature, VIN3 activity decreases, but the PRC2 complex without VIN3 still deposits H3K27me₃ over the entire *FLC* locus *via* mitotic activity (Finnegan and Dennis, 2007, De Lucia *et al.* 2008, Angel *et al.* 2011). These H3K27me₃ marks are recognized and bound by LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), which is a component of the *A. thaliana* Polycomb repressive complex 1, to establish the silent chromatin around the *FLC* locus (Sung *et al.* 2006, Turck *et al.* 2007). Moreover, an exposure to cold conditions transiently induces several *COOLAIR* and *COLD AIR* lncRNAs that are transcribed from the *FLC* locus. The *COOLAIR* lncRNAs are antisense *FLC* transcripts that silence *FLC* expression in a co-transcriptional manner (Swiezewski *et al.* 2009). In contrast, the *COLD AIR* lncRNAs are transcribed from the first intron of *FLC* under cold conditions, and associate with one of the PRC2 subunits to recruit this complex to the *FLC* chromatin, thereby inducing an epigenetically stable

repressive status (Heo and Sung 2011). Therefore, *FLC* expression is seasonally silenced *via* various epigenetic factors to establish the “winter memory” and to enable plants to sense diurnal cues for flowering.

Diurnal chromatin regulation of FT mediates day-length-dependent flowering

Long-day conditions induce a diurnal *FT* expression pattern in leaf phloem cells due to the long-day specific transcription activator CONSTANS (CO) (Putterill *et al.* 1995, Samach *et al.* 2000, Suárez-López *et al.* 2001, Valverde *et al.* 2004, Imaizumi 2010, Song *et al.* 2013). The *CO* gene is expressed in a diurnal manner, and CO accumulates toward dusk and interacts with the nuclear factor Ys (NF-Ys) to form a chromatin looping structure at the *FT* promoter, thereby inducing *FT* expression (Ben-Naim *et al.* 2006, Wenkel *et al.* 2006, Kumimoto *et al.* 2010, Cao *et al.* 2014, Zhao *et al.* 2017). The resulting FT protein is transported from leaves to the shoot apical meristem *via* phloem tissue. It then forms a florigen complex with the bZIP transcription factor FD to induce the expression of the floral meristem organ identity genes *LEAFY* and *APETALA1*, ultimately leading to the transition from the vegetative meristem to the reproductive meristem (Abe *et al.* 2005, Wigge *et al.* 2005). Like *FLC* regulation, various chromatin modifiers are involved in controlling *FT* expression in flowering pathways, including histone demethylases and polycomb group proteins. We herein review the regulation of H3K27me₃ at the *FT* locus involved in photoperiodic flowering.

Previous research indicated that H3K27me₃ is distributed over the *FT* locus, and helps maintain repressive chromatin (Adrian *et al.* 2010). A MjC-domain-containing protein, RELATIVE OF EARLY FLOWERING 6, is involved in the H3K27me₃ demethylation at the *FT* locus, and is required for *FT* expression (Noh *et al.* 2004). Additionally, several PRC2 complex components, including CLF, reportedly bind to *FT* chromatin, and are required for regulating the H3K27me₃ level at the *FT* locus, suggesting the PRC2 complex deposits H3K27me₃ at the *FT* locus to repress expression (Turck *et al.* 2007, Zhang *et al.* 2007, Farrona *et al.* 2008, Jiang *et al.* 2008). As mentioned above, LHP1 also recognizes and binds to H3K27me₃ at the *FT* locus to maintain repressive chromatin and inhibit precocious flowering (Takada and Goto 2003, Turck *et al.* 2007, Veluchamy *et al.* 2016). Recent studies have suggested that the formation of a CO-NF-Y-mediated chromatin loop interferes with the binding of polycomb group proteins to the *FT* locus toward dusk, leading to a decrease in the H3K27me₃ level (Luo *et al.* 2018). Because the chromatin loop develops following the accumulation of CO and the formation of the CO-NF-Y complex, H3K27me₃ is dynamically deposited under a diurnal cycle. Therefore, *FT* expression is repressed and de-repressed by both genetic and epi-

genetic processes to initiate long-day-specific flowering.

Flowering is robustly and dynamically controlled by epigenetic mechanisms under saturating environmental conditions to ensure it occurs at the most suitable time. In addition to a prolonged exposure to cold and an increase in day length, plants monitor other signals, including increases in the ambient temperature. For example, under warm conditions, a bHLH transcription factor, PHYTOCHROME INTERACTING FACTOR 4, facilitates the deposition of H2A.Z at the *FT* TSS to induce transcription (Kumar *et al.* 2012). There is considerable interest in how plants integrate these signals into flowering gene regulation through the crosstalk with genetic and epigenetic mechanisms.

In addition to controlling flowering time, plants use other processes in many developmental contexts to ensure reproductive success. Thus, further characterizing the epigenetic regulation of the flowering genes *FLC* and *FT* may be useful for elucidating the epigenetic regulatory mechanisms in other developmental processes.

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