Genetic and Epigenetic Understanding of the Seasonal Timing of Flowering

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ABSTRACT

The developmental transition to flowering in many plants is timed by changing seasons, which enables plants to flower at a season that is favorable for seed production. Many plants grown at high latitudes perceive the seasonal cues of changing day length and/or winter cold (prolonged cold exposure), to regulate the expression of flowering-regulatory genes through the photoperiod pathway and/or vernalization pathway, and thus align flowering with a particular season. Recent studies in the model flowering plant Arabidopsis thaliana have revealed that diverse transcription factors engage various chromatin modifiers to regulate several key flowering-regulatory genes including FLOWERING LOCUS C (FLC) and FLOWERING LOCUS T (FT) in response to seasonal signals. Here, we summarize the current understanding of molecular and chromatin-regulatory or epigenetic mechanisms underlying the vernalization response and photoperiodic control of flowering in Arabidopsis. Moreover, the conservation and divergence of regulatory mechanisms for seasonal flowering in crops and other plants are briefly discussed.

Key words: vernalization, photoperiodism, flowering time, FLOWERING LOCUS C, FLOWERING LOCUS T, chromatin modification

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INTRODUCTION

The developmental transition from a vegetative phase to reproduction, i.e., flowering, is a major developmental switch in the plant life cycle, and the timing of this switch is critical for reproductive success. The decision of when to flower, or flowering time, is genetically determined by the integration of endogenous factors (e.g., age and developmental state) and environmental inputs, such as day length, light quality, and temperature (Amasino, 2010; Andres and Coupland, 2012). Flowering is often timed by seasonal cues, which enables plants to flower at the right season and thus maximize seed production. Many plants grown at high latitudes perceive seasonal cues including changing day length and winter cold (prolonged cold exposure) to align flowering with a particular season (Amasino, 2010; Andres and Coupland, 2012; Song et al., 2015).

Decades of molecular genetic studies have revealed that winter cold, through a process termed vernalization, typically represses the expression of floral repressors that block flowering before winter cold exposure, and thus enable plants to become competent to flower in spring or early summer in response to increasing day length, i.e., long-day (LD) signals (Figure 1) (Amasino, 2010; Andres and Coupland, 2012; Kim and Sung, 2014; Xu and Chong, 2018). Changing day length (long days or short days) through the photoperiod pathway can induce flowering (Andres and Coupland, 2012; Song et al., 2015). These seasonal responses are integrated to confer seasonal timing of flowering in many plants (Michaels, 2009; Amasino, 2010; Andres and Coupland, 2012). Both the vernalization pathway and the photoperiod pathway have been well characterized in the model plant Arabidopsis thaliana. Here, we first describe the molecular epigenetic mechanisms for the vernalization pathway in Arabidopsis, followed by a brief discussion on vernalization responses in other over-wintering plants. We then review molecular genetic mechanisms underlying the photoperiodic regulation of flowering in Arabidopsis, followed by a brief summary...
The production of FT length. Winter cold, through the vernalization pathway, represses temperate regions flower in late spring in response to increasing day length. FT expression and thus relieves these blocks to flowering (Amasino, 2010; Andres and Coupland, 2012). In many over-wintering plants grown in temperate climates, including winter annuals, biennials, and perennials, floral repressors act to inhibit flowering from occurring before winter cold exposure (continuous low temperature for at least a few weeks), and winter cold exposure through the vernalization pathway represses expression of these genes or enables plants to overcome and winter cold exposure. This enables long-day induction of FT expression by the photoperiod pathway to promote flowering, FT feedback represses FLC expression.

Vernalization: Enabling Over-Wintering Plants to Become Competent to Flower in the Following Spring

In many over-wintering plants grown in temperate climates, the vernalization pathway represses expression of these genes or enables plants to overcome these blocks to flowering (Amasino, 2010; Andres and Coupland, 2012; Xu and Chong, 2018). The repressive state at floral repressors, or “vernalized state,” is maintained during subsequent growth and development for a certain period of time when the temperature rises in the following spring, enabling the plants that experienced cold to flower (Amasino, 2010; Andres and Coupland, 2012; He and Li, 2018). In Arabidopsis winter cold induces silencing of the potent floral repressor FLC, which encodes a MADS-box transcription factor (TF) (Michaels and Amasino, 1999; Sheldon et al., 1999), by chromatin-associated Polycomb group (PcG) proteins through deposition of repressive chromatin modifications at the FLC locus in a process called Polycomb silencing (Kim and Sung, 2014; He and Li, 2018). This results in a repressive chromatin state that is maintained during subsequent growth and development when the temperature rises, but is reset in next generation during early embryogenesis, ensuring that each generation requires winter cold exposure before flowering (Kim and Sung, 2014; He and Li, 2018).

Establishment of the Vernalization-Required Growth Habit

Based on their flowering responses to winter cold, Arabidopsis accessions are classified into two types: vernalization-responsive winter annuals and rapid-cycling/flowering summer annuals (Gazzani et al., 2003; Michaels et al., 2003). The winter annual growth habit is conferred by FRIGIDA (FRI) and FLC (Gazzani et al., 2003; Michaels et al., 2003). FRI encodes a plant-specific scaffold protein and functions dominantly to upregulate FLC expression to a high level that inhibits flowering (Johanson et al., 2000; Choi et al., 2011). In summer annuals, defects in either FRI or FLC result in a lack of FLC activity and thus rapid flowering without cold (Gazzani et al., 2003; Michaels et al., 2003).

Before winter cold exposure, the scaffold protein FRI forms a large multiprotein complex, the FRI supercomplex (FRIscc), that establishes a local chromosomal environment around the FLC locus with chromatin modifications (e.g., H3K4 and H3K36 trimethylation) and chromatin topology (5’ to 3’ FLC gene looping) conducive to active gene expression (Li et al., 2018b). This active chromatin environment promotes and coordinates transcriptional activation, rapid elongation, and efficient splicing of FLC premRNAs, resulting in a high level of mature FLC mRNA production (Li et al., 2018b). FLC directly represses the expression of FT (which encodes a major florigen) and a MADS-box TF, SUPPRESSOR OF OVEREXPRESSSION OF CONSTANS1 (SOC1), that acts to promote the floral transition, and thus prevents flowering (Helliwell et al., 2006; Searle et al., 2006; Andres and Coupland, 2012). This establishes the vernalization-responsive winter annual growth habit in Arabidopsis.

Formation of the Vernalized State

Winter cold induces PcG-mediated chromatin silencing at FLC through the vernalization pathway (Amasino, 2010; He and Li, 2018). PcG proteins assemble into several chromatin-associated complexes including Polycomb repressive complex 1 (PRC1) and PRC2 that mediate repressive chromatin modifications to repress target gene expression in plants and animals (Molitor and Shen, 2013; Mozgova and Henning, 2015). The histone 3 lysine 27 (H3K27) methyltransferase complex PRC2 is evolutionarily conserved, and the core PRC2 consists of four subunits including a H3K27 methyltransferase and three structural subunits (Mozgova and Henning, 2015). Molecular genetic analyses have revealed that in Arabidopsis the H3K27 methyltransferases CURLY LEAF (CLF) and SWINGER (SWN), the structural subunit VERNALIZATION 2 (VRN2), a zinc-finger
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protein, and two WD40 repeat proteins known as FIE and MS1 are involved in the silencing of FLC by vernalization (Wood et al., 2006; De Lucia et al., 2008). Prolonged cold exposure (weeks of low temperature), but not a short period of cold, induces the expression of VERNALIZATION INSENSITIVE 3 (VIN3), which encodes a PLANT HOMEODOMAIN (PHD) domain protein (Sung and Amasino, 2004). VIN3 and VIN3 LIKE 1 (VIL1, also known as VRN5; Greb et al., 2007; Sung et al., 2006b), together with the core PRC2 subunits, form a vernalization/cold-specific PHD (VIN3)-PRC2 complex that catalyzes the deposition of repressive H3K27me3 marks on FLC chromatin in cold conditions (Wood et al., 2006; De Lucia et al., 2008).

PHD (VIN3)-PRC2 is recruited specifically to a three-nucleosome region around the junction between the first exon and the first intron of FLC, the nucleation region of H3K27 methylation and Polycomb silencing (Angel et al., 2011). There is a cis-regulatory DNA element with two 6-bp canonical (RY) motifs localized in the nucleation region, which are recognized by two homologous DNA-binding proteins, VIVIPAROUS1/AB3-LIKE 1 (VAL1) and VAL2, that mediate FLC silencing (Questa et al., 2016; Yuan et al., 2016). This cis-regulatory element mediates FLC silencing during and after winter cold exposure, and thus is named the Cold Memory Element (CME) (Yuan et al., 2016). The trans-acting VAL1/2 proteins contain a plant-specific DNA-binding domain, B3 (which recognizes the RY motifs in the CME), and several chromatin-associated domains (Suzuki et al., 2007; Yuan et al., 2016). VAL1/2 bind to the CME, and they also physically associate with PcG proteins to enrich the PHD (VIN3)-PRC2 complex at the CME-bearing nucleation region, resulting in H3K27me3 accumulation in this region during vernalization (Yuan et al., 2016). This converts the active FLC chromatin established by FR1scl to a silenced state with H3K27me3 marks, leading to FLC silencing during vernalization (Questa et al., 2016; Yuan et al., 2016).

Several long non-coding RNAs (IncRNAs) have been reported to be involved in FLC silencing during winter cold exposure (Csorba et al., 2014; Kim et al., 2017; Kim and Sung, 2017). Three types of cold-upregulated IncRNAs, COLDWRAP, COLDAIR, and COOLAIR, are transcribed from the FLC locus (Csorba et al., 2014; Kim et al., 2017; Kim and Sung, 2017). COOLAIR is a group of antisense transcripts initiated from the 3’ region immediately downstream of FLC (Csorba et al., 2014). Before winter cold, the FR1scl promotes looping between the 5’ proximal promoter and a 3’ region downstream of FLC, and coordinates the expression of sense FLC with the antisense COOLAIR (Li et al., 2018b). A few days of cold exposure strongly upregulates COOLAIR expression, but the regulatory role of this upregulation is unclear as COOLAIR expression is not required for vernalization-mediated FLC silencing (Helliwell et al., 2011; Csorba et al., 2014). COLDWRAP and COLDAIR are sense transcripts transcribed from a proximal 5’ promoter region and around the middle of the first intron, respectively, and both are cold-upregulated but expressed at a low level (Heo and Sung, 2011; Kim et al., 2017; Kim and Sung, 2017). COLDWRAP and COLDAIR have been reported to bind FLC chromatin and interact with PHD-PRC2 to mediate FLC silencing during vernalization (Heo and Sung, 2011; Kim et al., 2017; Kim and Sung, 2017). It seems that, upon winter cold exposure, IncRNAs may function collaboratively with the CME-VAL1/2 regulatory module to recruit the PHD (VIN3)-PRC2 complex to the Polycomb nucleation region at FLC, to mediate H3K27 trimethylation.

“Remembering Winter” in Warm Seasons

After return to warmth (e.g., temperature rise in the following spring), winter cold-induced H3K27me3 accumulation in the Polycomb nucleation region encompassing the CME is spread across the entire FLC locus and maintained through cell divisions during subsequent growth and development, resulting in a stably silenced chromatin state at FLC (Finnegan and Dennis, 2007; Jiang and Berger, 2017; Yang et al., 2017). This enables plants to remember the past winter cold experience under warm growth conditions. The epigenetic maintenance of the vernalized state throughout mitotic cell divisions in warm seasons constitutes the “epigenetic memory of winter cold” (Kim et al., 2009; He and Li, 2018).

In Arabidopsis, the H3K27me3 mark is recognized and read by LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) and two BAH domain-bearing proteins EARLY BOLTING IN SHORT DAYS (EBS) and SHORT LIFE (SHL) (Turck et al., 2007; Zhang et al., 2007; Li et al., 2018a; Yang et al., 2018). It has been shown that LHP1 is moderately enriched at the Polycomb nucleation region in cold and spreads to the entire FLC locus after return to warm temperatures (Sung et al., 2006a; Yang et al., 2017). In addition to reading H3K27me3, LHP1 physically associates with VAL1/2 and the PRC2 subunits, and is expected to function together with PRC2 in the deposition of H3K27me3 on adjacent histones at FLC in a positive feedback manner (Derkacheva et al., 2013; Yuan et al., 2016). Consistent with this expectation, LHP1 is partly required for the spread of H3K27me3 at FLC in warm temperatures (Yang et al., 2017). Other H3K27me3 readers may function partially redundantly with LHP1 to mediate silencing of FLC by vernalization.

The cold-induced H3K27me3 marks on FLC chromatin in a parental cell are maintained through cell divisions and thus are present on daughter chromatin. This process involves LHP1, PHD-PRC2, and CHROMATIN ASSEMBLY FACTOR 1 (CAF-1) (Yuan et al., 2016; Jiang and Berger, 2017; Yang et al., 2017). After the return to warm temperatures, VIN3 expression is turned off swiftly (Sung and Amasino, 2004), and thus the cold-specific PHD (VIN3)-PRC2 is not involved in H3K27me3 spreading and maintenance when the temperature rises. However, the VIN3 homolog VIL1 remains associated with the core PRC2 and the PHD (VL1)-PRC2 complex mediates H3K27me3 deposition on FLC chromatin in warm temperatures (De Lucia et al., 2008; Kim and Sung, 2013). Interestingly, both H3K27 methyltransferases CLF and SWN are associated with FLC chromatin during and after prolonged cold exposure (Wood et al., 2006; Heo and Sung, 2011; Yang et al., 2017), and function partially redundantly to mediate silencing of FLC by vernalization, indicating that several PHD-PRC2 complexes may function at FLC to catalyze deposition of H3K27me3 marks during and after cold exposure.

Following DNA replication, the H3K27me3 marks on parental histones are diluted two times on the histones assembled on daughter DNA strands. CAF-1 assembles the nucleosomes
following DNA replication and plays a critical role in restoring H3K27me3 on FLC chromatin (Jiang and Berger, 2017). Following its deposition by CAF-1 at the replication fork, the canonical H3 variant H3.1 is methylated by PRC2 (Wollmann et al., 2012; Jiang and Berger, 2017). On one hand, CAF-1 physically associates with LHP1, which presumably recognizes H3K27me3 on a parental histone; on the other hand, CAF-1 recruits PRC2 for H3K27me3 deposition on newly assembled histones, leading to the maintenance of parental H3K27me3 marks on daughter chromatin at the FLC locus in subsequent cell divisions following vernalization (Jiang and Berger, 2017). In addition to CAF-1, the cis-regulatory element CME is essential for the maintenance of H3K27me3 and FLC silencing in warm temperatures (Yuan et al., 2016). CME mutations cause a loss of H3K27me3 at FLC, and this loss is associated with FLC re-activation following prolonged cold exposure (Yuan et al., 2016). VAL1/2 directly interacts with LHP1, and the CME-VAL1/2-LHP1 regulatory module not only recognizes a specific DNA sequence (CME), but also reads the silenced chromatin state at FLC (Yuan et al., 2016). This conceivably provides DNA sequence- and chromatin state-specific information that guides CAF-1 and PHD-PRC2 to specific regions for maintaining H3K27me3 following DNA replication. In short, the collaborative functions of CME-VAL1/2, LHP1, CAF-1, and PHD-PRC2 lead to the spread and maintenance of H3K27me3 on FLC chromatin and the consequent stable FLC silencing during subsequent growth and development in warm temperatures following winter cold exposure.

**Erasing Parental “Memory of Winter Cold” During Embryogenesis**

The memory of past winter experience in winter annuals or biennials is stable through mitotic cell divisions, but is lost in the next generation (Kim et al., 2009; He and Li, 2018). Thus, each generation must over-winter before flowering in spring. In Arabidopsis, the cold memory is erased or reset in early embryogenesis by an embryonic regulatory pathway, referred to as the LEC pathway, consisting of three master TFs involved in embryogenesis, namely LEAF COTYLEDON 1 (LEC1), LEC2, and FUSCA 3 (FUS3) (Tao et al., 2017, 2019).

Following parental vernalization, FLC remains silenced in both mature male and female gametes (Sheldon et al., 2008; Choi et al., 2009), and shortly after fertilization, de novo FLC re-activation is initiated by the LEC1 TF (Tao et al., 2017). The embryo-specific LEC1, which encodes a B subunit of the trimeric nuclear factor Y (NF-Y) (Pelletier et al., 2017), is activated shortly after fertilization, and initiates embryonic FLC re-activation (Tao et al., 2017). LEC1/NF-Y acts as a pioneer TF and binds to nucleosomal DNA, and is enriched in a 5′ promoter region of FLC (Tao et al., 2017), which may lead to an open chromatin configuration that facilitates subsequent binding of additional active TFs to FLC chromatin. Following embryonic LEC1 re-activation, the expression of LEC2 and FUS3, which encode three domain TFs (Stone et al., 2001; Santos-Mendoza et al., 2008), is re-activated within 2 DAP (for days after pollination) and 3 DAP, respectively (Tao et al., 2019). Crystal structural studies revealed that the B3 domains of LEC2, FUS3 and VAL1 bind to the cis-regulatory CME at FLC through a nearly identical binding interface (Tao et al., 2019). The binding of LEC1 to FLC chromatin enables LEC2 and FUS3 to bind the CME in early embryogenesis (Tao et al., 2019), perhaps through chromatin opening. LEC2 and FUS3 compete against VAL1/2 and are enriched at the CME region in early embryogenesis, leading to a disruption of VAL1/2-mediated Polycomb silencing inherited from the vernalized parents (Tao et al., 2019). Because cells divide rapidly in early embryogenesis, the H3K27me3 marks on parental FLC chromatin from gametes are greatly diluted by several rounds of cell division occurring within a very short time period, and thus the parental memory of winter cold is erased in early embryos (Tao et al., 2019). Furthermore, upon binding to the CME at FLC both LEC2 and FUS3 directly interact with the plant-specific scaffold protein FRI and recruit it as well as its associated active chromatin modifiers (in the FRIs), among which are the H3K36 methyltransferase EFS and the H3K4 methyltransferase complex COMPASS-like, leading to the formation of an active chromatin state with H3K4 and H3K36 trimethylation and other active modifications (Tao et al., 2019). These two functions of LEC2 and FUS3 result in the conversion of the H3K27me3-bearing silenced FLC chromatin to an active chromatin state in early embryogenesis (Tao et al., 2019). This resets the silenced FLC to an active expression state.

The vernalized state of FLC appears to be fully reset in embryos within 6 DAP by the LEC pathway in Arabidopsis (Tao et al., 2017, 2019). The active expression state of FLC is sustained through embryo development and seed maturation (Tao et al., 2017). All three genes in the LEC pathway are turned off in post-seed development stages, and thus this embryonic pathway is not functional throughout vegetative growth and development; however, the embryonic active FLC chromatin is transmitted to post-embryonic tissues upon seed germination (Tao et al., 2017, 2019). During the seedling stage, FRIs remains associated with FLC chromatin to maintain the active state, and FLC is highly expressed to prevent flowering before winter cold exposure (Li et al., 2018b), ensuring that this generation needs to experience winter to become competent to flower in the following spring. In temperate climates, the seeds of Arabidopsis winter annuals typically germinate in autumn, and plants encounter winter cold at early seedling stages where the LEC pathway is developmentally silenced (Armasino, 2010; Tao et al., 2019). This enables VAL1/2 to recognize and bind to the CME upon prolonged cold exposure, leading to FLC silencing again by vernalization (Tao et al., 2019).

In short, a subfamily of the plant-specific B3 domain transcriptional regulators mediates the dynamic epigenetic control of the expression of the potent floral repressor FLC throughout the Arabidopsis life cycle (Figure 2). Through binding to the cis-regulatory element CME, these B3 proteins switch on, off, and on again the expression of FLC during the embryo-to-plant-to-embryo cycle. This ensures that plants adapt to seasonal temperature changes.

**Convergent Evolution of Vernalization Responses in Angiosperms**

Flowering plants diversified around several hundred million years ago in regions where the climate was relatively warm (i.e., winter cold likely not encountered) (Armasino, 2010; Li et al., 2019). In various vernalization-responsive plants including crucifers,
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Figure 2. Dynamic Epigenetic Regulation of FLC Expression throughout the Arabidopsis Life Cycle.

In early embryogenesis, FLC is re-activated or reset by the embryonic LEC pathway consisting of three transcription factors (TFs): the pioneer TF LEC1 (for LEAFY COTYLEDON 1) and two B3 domain TFs including LEC2 and FUSCA 3 (FUS3). These TFs function together with the FRI supercomplex (FRisc) containing active chromatin modifiers (e.g., the EFS H3K36 methyltransferase) to establish an active chromatin state at FLC, which is transmitted to young seedlings following seed germination (often in autumn under field conditions). The LEC pathway is developmentally silenced during post-seed development. When seedlings encounter winter cold, the B3 domain proteins VIVIPAROUS1/AB3-LIKE 1 (VAL1)/VAL2 bind to the Cold Memory Element (CME) to mediate FLC repression by PcG proteins (e.g., LHP1 and PRC2). This results in a silenced state that is subsequently maintained upon return to warmth in the following spring, enabling long-day induction of flowering in late spring in temperate regions.

Arabidopsis is a facultative LD plant, and thus is a good genetic model to dissect the photoperiodic regulation of flowering. Earlier molecular genetic studies of Arabidopsis mutants that flower later than wild-type in LDs, but at the same time as wild-type in SDs, have identified a few flowering-regulatory genes that define the photoperiod pathway (Koomen et al., 1991). There are four major regulatory genes in this pathway, namely FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1), GIGANTEA (GI), CONSTANS (CO), and FT (Andres and Coupland, 2012; Song et al., 2015). GI and the blue light receptor FKF1 form a dimeric ubiquitin ligase complex (Sawa et al., 2007), and CO bears a DNA-binding CCT (for CONSTANS, CONSTANS-LIKE, and TOC1) domain and activates the expression of FT, which encodes a major mobile florigen that induces flowering (Wenkel et al., 2006; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). All four genes are expressed in the vascular tissues of leaves and regulated by both the endogenous circadian clock and light signals that are perceived in leaves (Andres and Coupland, 2012; Song et al., 2015). The photoperiod pathway functions in leaf veins to induce the expression of the florigenic FT or FT homologs in response to seasonal changes in day length (Andres and Coupland, 2012; Song et al., 2015).

Induction of flowering by seasonal day-length changes through the photoperiod pathway

Over-wintering vernalization-responsive plants typically flower late in the following spring or in early summer at high latitudes (Amasino, 2010; Andres and Coupland, 2012; Xu and Chong, 2018). Turning off the floral repressors through the vernalization pathways in response to winter cold and subsequent maintenance of the vernalized state when the temperature rises enable plants to become competent for floral induction by increasing day length in late spring/early summer through the photoperiod pathway (Amasino, 2010; Andres and Coupland, 2012). In addition to winter cold, many plants perceive another seasonal cue—changing day length—to align flowering time with changing seasons (Andres and Coupland, 2012; Song et al., 2015). Many vernalization-responsive plants are LD plants, which flower when the day length becomes longer than a threshold; there are also short-day (SD) plants, which flower when day length becomes shorter than a threshold, such as rice and soybean (Andres and Coupland, 2012; Song et al., 2015).

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**Transcriptional Regulation of CO mRNA Expression**

The output of the photoperiod pathway is increased expression of CO, which directly promotes FT expression to induce flowering under inductive LD conditions in *Arabidopsis* (Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002; Gnesutta et al., 2017). CO mRNA is expressed at a high level from afternoon until night in LDs, and CO expression is tightly regulated by FKFI, GI, CYCLING DOF FACTORS (CDFs), and FLOWERING BHLH (FBH) (Suarez-Lopez et al., 2001; Valverde et al., 2004; Sawa et al., 2007; Fornara et al., 2009; Ito et al., 2012; Song et al., 2012). CDFs, consisting of CDF1 to CDF5, are plant-specific DOF TFs that directly repress CO expression (Fornara et al., 2009). In the morning, two MYB TFs CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), which are components of the circadian clock oscillator (Schaffer et al., 1998; Wang and Tobin, 1998), function to activate CDF expression, resulting in repression of CO expression (Nakamichi et al., 2007).

The circadian-regulated GI and FKFI genes have similar diurnal expression patterns, and the proteins encoded by both genes accumulate from afternoon to dusk in LDs (David et al., 2006; Kim et al., 2007, 2013; Sawa et al., 2007). Upon perception of blue light by its LOV (for light, oxygen, or voltage domain, FKFI associates with GI to form a dimeric E3 ubiquitin ligase complex that accumulates in the afternoon and toward dusk (Kim et al., 2007, 2013; Sawa et al., 2007). FKFI-GI targets CDF proteins for degradation by the proteasome, and thus disrupts CDF-mediated CO repression (Sawa et al., 2007). Subsequently, four basic helix-loop-helix (bHLH) TFs, namely FLOWERING BHLH 1 (FBH1), FBH2, FBH3, and FBH4, access the CO promoter through the E-box cis-element to promote CO expression (Ito et al., 2012), resulting in a high level of CO expression in the afternoon and at night (Figure 3A).

**Control of CO Protein Abundance via External Cues**

The stability of the CO protein is controlled by light signals, and under inductive LDs, CO accumulates in late afternoon and peaks at dusk through the coincidence of a high level of CO mRNA with light exposure in the late afternoon (Andres and Coupland, 2012; Song et al., 2015) (Figure 3B). The CO protein is degraded via the ubiquitin-dependent proteasome pathway (Valverde et al., 2004). In the morning, the RING-finger E3 ubiquitin ligase, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1), targets CO for degradation (Lazaro et al., 2012); in addition, the red-light photoreceptor Phytochrome B (PHYB) functions to destabilize the CO protein in the morning in LDs (Valverde et al., 2004). At night (in both LDs and SDs), another RING-finger E3 ubiquitin ligase, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), interacts with members of the SUPPRESSOR OF PHYA-105 (SPA) family including SPA1, SPA3, and SPA4 to form a complex that targets CO for degradation by the proteasome (Laubinger et al., 2006; Liu et al., 2008b; Jang et al., 2008).

In late afternoon in LDs, far-red light and blue light stabilize the CO protein, enabling its accumulation toward dusk (Valverde et al., 2004; Liu et al., 2008b). Upon perception of far-red light in the afternoon, Phytochrome A (PHYA) functions to stabilize the CO protein (Valverde et al., 2004). Perception of blue light by the Cryptochrome 2 (CRY2) receptor leads to its phosphorylation (Tan et al., 2013), and phosphorylated CRY2 physically associates with SPAs, which suppresses the activity of the COP1-SPA1/3/4 complex and thus inhibits CO degradation (Zuo et al., 2011). In addition, blue light also stabilizes the CO protein via FKFI. The circadian-regulated FKFI protein accumulates in the late afternoon, and upon perceiving blue light, FKFI physically associates with CO to stabilize it (Song et al., 2012). In short, the coincidence of a high level of CO mRNA with these light-dependent CO stabilization mechanisms in the late afternoon results in CO accumulation toward dusk in LDs. This leads to a daily rhythmic activation of FT expression around dusk in LDs to induce flowering in response to LD signals (Andres and Coupland, 2012; Song et al., 2015).

**Regulation of seasonal flowering**

**Long-Day Induction of FT Expression**

FT expression is largely constitutively repressed by Polycomb silencing over a 24-h LD cycle except around dusk when the CO protein abundance in leaf veins reaches a threshold and relieves Polycomb silencing at the FT locus (Figure 3C) (Wang et al., 2014; Luo et al., 2018). This results in daily rhythmic FT de-repression/activation specifically in leaf veins, with de-repression occurring at around dusk (under controlled growth conditions) (Luo et al., 2018). In SDs, FT expression is constitutively repressed by Polycomb silencing and thus the floral transition is inhibited (Wang et al., 2014). Under LD conditions, PcG complexes act to repress FT expression from morning through late afternoon and at night (Luo et al., 2018; Jing et al., 2019a). A CLF-bearing H3K27 methyltransferase complex, CLF-PRC2, catalyzes the deposition of repressive H3K27me3 marks on FT chromatin; these marks are read by LHP1, in association with the plant-specific EMBRYONIC FLOWER 1 (EMF1) protein, which compacts nucleosomes and represses transcription (Calonje et al., 2008; Jiang et al., 2008; Adrian et al., 2010; Wang et al., 2014). LHP1 and EMF1, together with the H3K4 demethylase JMJ14, form a PRC1-like complex called LHP1-EMF1c that demethylates H3K4 and reads and further maintains H3K27me3 marks and chromatin silencing at FT (Wang et al., 2014). Recently, the BAH domain H3K27me3 readers EBS and SHL were shown to form a complex with EMF1, bind to FT chromatin, and function partially redundantly to repress FT expression (Li et al., 2018a; Yang et al., 2018). Thus, the concerted action of PRC2 and the PRC1-like EMF1c complexes (LHP1-EMF1c and BAH-EMF1c) represses FT expression over a 24-h LD cycle except around dusk.

Before winter exposure, FLC is highly expressed in winter annuals and inhibits FT expression (Michaels, 2009; Amasino, 2010). FLC and FLC clade members, such as FLM (for FLOWERING LOCUS M) and MAF3 (for MADS AFFECTING FLOWERING 3), together with another MADS-box TF SHORT VEGETATIVE PHASE (SVP), form a multiprotein MADS-box complex that recognizes several CArG motifs in the FT promoter and gene body and represses FT expression (Lee et al., 2007, 2013; Li et al., 2008; Gu et al., 2013a; Pose et al., 2013). Furthermore, both FLC and FLM physically associate with EMF1c and presumably recruit/engage Polycomb complexes for FT repression (Wang et al., 2014), and thus prevent the floral transition in the absence of vernalization.
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Figure 3. Long-Day Induction of FT Expression by CO in Arabidopsis under Controlled Growth Conditions.

(A) Transcriptional regulation of CO mRNA expression in LDs. The morning-expressed MYB TFs CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) activate the expression of CYCLING DOF FACTORS (CDFs), which directly repress CO expression in the morning. In the afternoon, upon perception of blue light FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) forms a dimeric ubiquitin ligase complex with GIANTETA (GI) to degrade CDFs; subsequently, the FLOWERING BHLH (FBH) family TFs bind to a CO promoter region to promote CO transcription.

(B) Control of CO protein accumulation in the leaf vasculature in LDs. The E3 ubiquitin ligase HOS1 (for HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1) and the red-light photoreceptor Phytochrome B (PHYB) function to destabilize the CO protein in the morning. In the afternoon, CO is stabilized by the blue light photoreceptors FKF1 and Cryptochrome 2 (CRY2), and the far-red light photoreceptor Phytochrome A (PHYA). The blue light-dependent association of FKF1 with CO functions to stabilize the CO protein. In addition, the blue-light activated CRY2 physically associates with the E3 ubiquitin ligase complex, resulting in CO degradation by the proteasome.

(C) Transcriptional regulation of FT expression in leaf veins in LDs. FT expression is largely constitutively repressed by PcG proteins together with repressive TFs (e.g., the FLC family), over a 24-h LD cycle except around dusk. Upon CO protein accumulation toward dusk, the DNA-binding CO associates with a dimer of NF-YB2/3 and NF-YC3/4/9 to form a trimeric NF-CO complex. NF-CO and the trimeric NF-Y complex (NF-YA with an NF-YB and NF-YC dimer) bind to proximal and distal FT promoter regions, respectively, to disrupt PcG enrichment on FT chromatin. The chromatin-remodeler PICKLE (PKL) physically associates with CO to facilitate its binding to the FT promoter, and PKL-CO recruits the H3K4 methyltransferase ATX1 (for ARABIDOPSIS HOMOLOG OF TRITHORAX 1) and H3K4me3/H3K36me3 readers including MORF RELATED GENE 1 (MRG1) and MRG2 to promote deposition of the active H3K4me3 mark on FT chromatin specifically at dusk. In addition, CO directly interacts with CIB1 (for CRY2-INTERACTING bHLH) and the ARABIDOPSIS HOMOLOG OF TRITHORAX 1 and the red-light photoreceptor Phytochrome A (PHYA). The blue light-dependent association of FKF1 with CO functions to stabilize the CO protein. In addition, the blue-light activated CRY2 physically associates with the E3 ubiquitin ligase complex, resulting in CO degradation by the proteasome.

Upon the accumulation of CO protein toward dusk in LDs, CO associates with a histone fold domain (HFD) dimer of NF-YB2/3 and NF-YC3/4/9 to form a trimeric NF-CO complex (Wenkel et al., 2006; Cao et al., 2014; Gnesutta et al., 2017). NF-CO, through the CCT domain of CO, recognizes the CCACA motifs in the CO-responsive element (CORE) located in a proximal FT promoter region (Jing et al., 2019b). The HFD dimer of NF-YB2/3 and NF-YC3/4/9 also associates with the DNA-binding NF-YA subunit to form a trimeric NF-YC complex that recognizes a CCAAT motif located in a distal FT promoter region (Adrian et al., 2010; Cao et al., 2014). This distal region physically contacts the proximal FT promoter region, leading to promoter looping at around dusk in LDs, possibly through the association of NF-CO with NF-Y (Cao et al., 2014; Liu et al., 2014; Luo et al., 2018). The binding of NF-CO and NF-Y to the FT promoter and the reconfiguration of the chromosomal conformation at FT result in a strong reduction in the enrichment of PcG factors including PRC2 and EMF1c on FT chromatin at dusk in LDs (Liu et al., 2018a; Luo et al., 2018). This results in FT de-repression in leaf veins at dusk. Notably, the specific reduction in PcG protein enrichment on FT chromatin at around dusk is unlikely due to active FT expression per se, given that elevated FT expression in a FT repressor mutant background has no effect on the reduction in PcG enrichment at FT at dusk (Luo et al., 2018).

FT de-repression/activation at around dusk in inductive LDs partly requires active chromatin modifications. Under LD conditions, FT chromatin is bivalently marked by PcG-mediated H3K27me3 deposition and Trithorax group (TrxG) protein-mediated H3K4me3 deposition (Jiang et al., 2008) from morning through late afternoon, and at night FT chromatin is marked predominantly with repressive H3K27me3 (Wang et al., 2014; Luo et al., 2018). PcG and TrxG proteins antagonize each other to regulate target gene expression in plants and animals; PcG proteins function to repress gene expression, whereas TrxG proteins mediate transcriptional activation.

CONSTITUTIVE PHOTOMORPHOGENIC 1 (COPI1)-SPA1 (for SUPPRESSOR OF PHYA-105) (FLC), which physically associates with CRY2 under blue light, and the CO-CIB1-CRY2 complex promotes FT expression at dusk, likely by facilitating active chromatin modifications on FT chromatin. CO-mediated disruption of Polycomb silencing at FT, together with the active chromatin modifications made by CO partners, results in FT de-repression/activation specifically at dusk in LDs. Upon FT de-repression at dusk, the histone deacetylase complex AF-AR-HDAC dampens the level of FT expression to prevent precocious flowering.

Upon the accumulation of CO protein toward dusk in LDs, CO associates with a histone fold domain (HFD) dimer of NF-YB2/3 and NF-YC3/4/9 to form a trimeric NF-CO complex (Wenkel et al., 2006; Cao et al., 2014; Gnesutta et al., 2017). NF-CO, through the CCT domain of CO, recognizes the CCACA motifs in the CO-responsive element (CORE) located in a proximal FT promoter region (Tiwari et al., 2010; Gnesutta et al., 2017). In addition, the ATPase-dependent chromatin-remodeling factor PICKLE (PKL) associates with CO and facilitates the access of CO to the CORE, leading to the enrichment of NF-CO at the FT proximal promoter (Jing et al., 2019b). The HFD dimer of NF-YB2/3 and NF-YC3/4/9 also associates with the DNA-binding NF-YA subunit to form a trimeric NF-YC complex that recognizes a CCAAT motif located in a distal FT promoter region (Adrian et al., 2010; Cao et al., 2014). This distal region physically contacts the proximal FT promoter region, leading to promoter looping at around dusk in LDs, possibly through the association of NF-CO with NF-Y (Cao et al., 2014; Liu et al., 2014; Luo et al., 2018). The binding of NF-CO and NF-Y to the FT promoter and the reconfiguration of the chromosomal conformation at FT result in a strong reduction in the enrichment of PcG factors including PRC2 and EMF1c on FT chromatin at dusk in LDs (Liu et al., 2018a; Luo et al., 2018). This results in FT de-repression in leaf veins at dusk. Notably, the specific reduction in PcG protein enrichment on FT chromatin at around dusk is unlikely due to active FT expression per se, given that elevated FT expression in a FT repressor mutant background has no effect on the reduction in PcG enrichment at FT at dusk (Luo et al., 2018).
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Sanchez et al., 2015; Mozgova and Hennig, 2015). Through physical association, CO and the chromatin-remodeler PKL promote each other’s binding to FT chromatin at dusk, and PKL further recruits a TrxG protein, the H3K4 methyltransferase ARABIDOPSIS HOMOLOG OF TRITHORAX 1 (ATX1) (Alvarez-Venegas et al., 2003), to FT chromatin resulting in elevated H3K4me3 deposition specifically at around dusk (Jing et al., 2019b, 2019c). The H3K4me3/H3K36me3 readers MORF RELATED GENE 1 (MRG1) and MRG2 bind to FT chromatin at dusk in LDs, and associate with the CO protein to further enrich it at FT (Bu et al., 2014; Xu et al., 2014), possibly through a positive feedback loop. In addition, CO directly interacts with the bHLH TF CRY2-INTERACTING bHLH1 (CIB1), which physically associates with CRY2 under blue light (Liu et al., 2013, 2018b). The CO-CIB1-CRY2 complex binds to the FT proximal promoter to promote FT expression at around dusk in LDs (Liu et al., 2018b), likely through facilitating active chromatin modifications on FT chromatin. In short, upon CO accumulation toward dusk in inductive LDs, CO not only mediates disruption of Polycomb silencing, but also promotes TrxG-mediated active chromatin modifications at FT, resulting in FT de-repression/activation specifically at dusk.

FT protein uploading is compromised upon loss of function of FTIP1, QRY, or SYP121 (Liu et al., 2012, 2019).

Following the uploading of FT from the companion cells to sieve elements, the long-distance transport of FT in the phloem stream from leaf veins to the shoot apex is facilitated by the NaKR1 protein (Zhu et al., 2016). NaKR1 is expressed in phloem and activated by CO at dusk in response to inductive LDs (Zhu et al., 2016). FT physically associates with NaKR1 in the sieve elements, and formation of the NaKR1-FT complex facilitates FT transport through the sieve elements from leaves to the shoot apical meristem (SAM) where FT encounters and forms a complex with the basic leucine zipper domain TF FLOWERING D (FD) (Abe et al., 2005; Wigge et al., 2005; Taoka et al., 2011; Zhu et al., 2016). The FT-FD complex activates the expression of the floral meristem identity genes to induce flower formation (Abe et al., 2005; Wigge et al., 2005).

Induction of Flowering by FT in the Shoot Apex

Upon FT transport from the companion cells in leaf veins to the SAM, the SAM-expressed FD directly interacts with FT (Abe et al., 2005; Wigge et al., 2005). The FT-FD complex may associate with a 14-3-3 molecular chaperone to form a transcription-regulatory complex (Taoka et al., 2011; Collani et al., 2019). This complex directly binds to a 5’ promoter region of the potent floral repressor FLC and feedback represses its expression (Luo et al., 2019). Furthermore, this transcription-regulatory complex directly activates the expression of the MADS-box TF SOC1 (Collani et al., 2019). The SOC1 protein further interacts with another MADS-box TF, AGL24, to promote the expression of the floral meristem identity gene LEAFY (LFY) (Liu et al., 2008a; Lee et al., 2008). In addition, the FT-FD complex directly activates the expression of another floral meristem identity gene, APETALA 1 (AP1) (Wigge et al., 2005; Collani et al., 2019). The transcriptional activation of LFY and AP1 leads to the initiation of flower development in the SAM, i.e., the transition to flowering (Wigge et al., 2005; Collani et al., 2019).

Conservation and Variation of the Photoperiod Pathway across Flowering Plants

The major regulatory genes in the photoperiod pathway identified in Arabidopsis are evolutionarily conserved in other flowering plants, suggesting the ancient nature of this pathway (Amasino, 2010; Andres and Coupland, 2012). Understanding of the photoperiodic regulation of flowering in Arabidopsis has provided a molecular framework for the induction of flowering by day-length changes in other plants.

The florigenic FT is highly conserved across angiosperms (Andres and Coupland, 2012; Ho and Weigel, 2014). In response to inductive day-length changes (LDs or SDs), the expression of FT or a direct FT homolog is induced in the leaf vasculature through the photoperiod pathway to mediate discrete flowering responses to different day-length signals in diverse plants (Andres and Coupland, 2012; Shrestha et al., 2014; Xu and Chong, 2018). Furthermore, the molecular mechanisms through which the FT protein or FT homologs induce flower formation at the SAM appear to be evolutionarily
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conserved; FT, in complex with FD and the molecular chaperone 14-3-3 proteins, activates the expression of floral meristem identity genes and thus initiates flower formation in diverse plants from the dicots Arabidopsis and soybean to the monocots rice and temperate cereals (Andres and Coupland, 2012; Shrestha et al., 2014; Song et al., 2015).

There are variations in the regulatory mechanisms that control of FT expression in response to different day lengths across flowering plants (Shrestha et al., 2014; Song et al., 2015). In addition to the evolutionarily conserved components in the photoperiod pathway, additional subpathways may have evolved to confer species-specific flowering characteristics in certain plants or plant lineages (Shrestha et al., 2014; Song et al., 2015). For instance, in the well-studied SD plant rice, the conserved GI-CO-FT pathway (OsGI-Hd1-Hd3a) acts to induce flowering in response to inductive SD signals; however, there is a unique Ghd7 (for Grain number, plant height, and heading date) subpathway that induces the expression of Hd3a and its paralog RTF1 and consequent flowering in various day-length conditions (Shrestha et al., 2014; Hori et al., 2016).

The timing of flowering is finely tuned in diverse plants to maximize reproductive success in response to seasonal cues and other developmental inputs. Diverse flowering behaviors in different plants certainly involve conserved as well as diverse/unique regulatory mechanisms, which are awaiting to be uncovered in future research. Most of the flowering time studies in Arabidopsis have been conducted under controlled growth conditions. Under field conditions, plants encounter more complex environmental conditions; hence, additional layers of regulation in flowering responses may occur in response to seasonal changes, as revealed in a recent study of flowering regulation under natural LD conditions in Arabidopsis (Song et al., 2018).

Molecular, genetic, and epigenetic understanding of the seasonal timing of flowering has broad applications in agricultural production including the development of crops with wide geographic adaptation and with reproductive success maximized to increase yields. For instance, the global staple food rice, first domesticated in a subtropical region in China (Huang et al., 2012; Choi et al., 2017), has been bred to adapt to a range of geographical areas from Southern to Northern latitudes largely through the selection of flowering-regulatory genes (Shrestha et al., 2014). Rice varieties that can flower under LD conditions have spread to Northern latitudes including Northern China, Japan, and Europe (Shrestha et al., 2014). During domestication and genetic improvement of another major crop, soybean, a SD plant that was first domesticated in East Asia (Hyten et al., 2006), various genes involved in photoperiodic regulation of soybean flowering have been intensively selected to broaden soybean cultivation to a wide range of geographic regions (Zhang et al., 2017). A major challenge for genetic improvement of soybean is to breed elite cultivars that can adapt to discrete photoperiodic settings at different latitudes; for example, tropical soybean cultivars with a late-flowering trait (Zhang et al., 2017). Flowering responses to seasonal cues have thus far been well studied in the LD plant Arabidopsis, but to a lesser degree in crops including rice and soybean. With the availability of molecular, genetic, and genomic tools, it is time now to dissect the diverse flowering-regulatory mechanisms in crops and further apply the knowledge to manipulate flowering time to broaden crop geographic adaptation and maximize crop production under a given environment.

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