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REVIEW ARTICLE

Current understanding of flowering pathways in plants: focusing on the vernalization pathway in Arabidopsis and several vegetable crop plants

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Abstract
Over the decades, extensive studies have been performed to elucidate the molecular mechanisms underlying the floral transition process in model plants, as well as in crop plants. It has been demonstrated that floral integrator genes, such as *FLOWERING LOCUS T* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1*, are highly conserved in most of the flowering plants. This finding has accelerated the identification and functional analyses of these orthologues involved in floral transition in flowering plant species. Even though the upstream regulator networks of the floral integrator genes seem to be quite diverged among plant species, they share four conserved flowering pathways, including the photoperiod, autonomous, gibberellin, and vernalization pathways. The comprehensive knowledge of the molecular mechanisms underlying floral transitions in the model plant *Arabidopsis thaliana* has helped us explore and elucidate the molecular mechanisms controlling floral transitions in other crop plants. This review highlights the current understandings of the flowering pathways elucidated in Arabidopsis, and mainly focuses on understanding the vernalization pathway in Arabidopsis as well as in several horticultural crop plants, including those of the genus *Brassica*.

Keywords *CONSTANS* · Chromatin · *FLOWERING LOCUS C* · Flowering time · Vernalization

1 Introduction

In the life cycle of a plant, the floral transition is important for survival as well as reproductive success. Environmental cues, such as photoperiod (day/night) and temperature, trigger the floral transition. Environmental signals are interpreted by multiple regulatory networks in plants (Fig. 1). Intensive genetic and molecular analyses have identified four major flowering pathways, namely the photoperiod, autonomous, gibberellin, and vernalization pathways, in plants (Kim et al. 2009; Amasino and Michaels 2010; Blazquez et al. 2001; Song et al. 2013; Capovilla et al. 2015). Even though the recent integration of several additional pathways, such as sugar-, hormone-, and ambient temperature-dependent pathways, have been reported (Bolouri Moghaddam and Van den Ende 2013; Wahl et al. 2013; Rolland et al. 2002; Conti 2017; Seo et al. 2011; D'Aloia et al. 2011; Tsai and Gazzarrini 2012; Li et al. 2016; Susila et al. 2018), it is beyond the scope of this review to discuss the details of these pathways. This review discusses the current understanding of each flowering pathway operating in the model plant *Arabidopsis thaliana*. Furthermore, the current understanding of the vernalization pathway is provided in the latter part of this review.

2 Floral induction by the photoperiod pathway

Plants utilize photoperiod signals to perceive a seasonal change. Photoperiod (or day length) regulates flowering time in many plants. The core components involved in the

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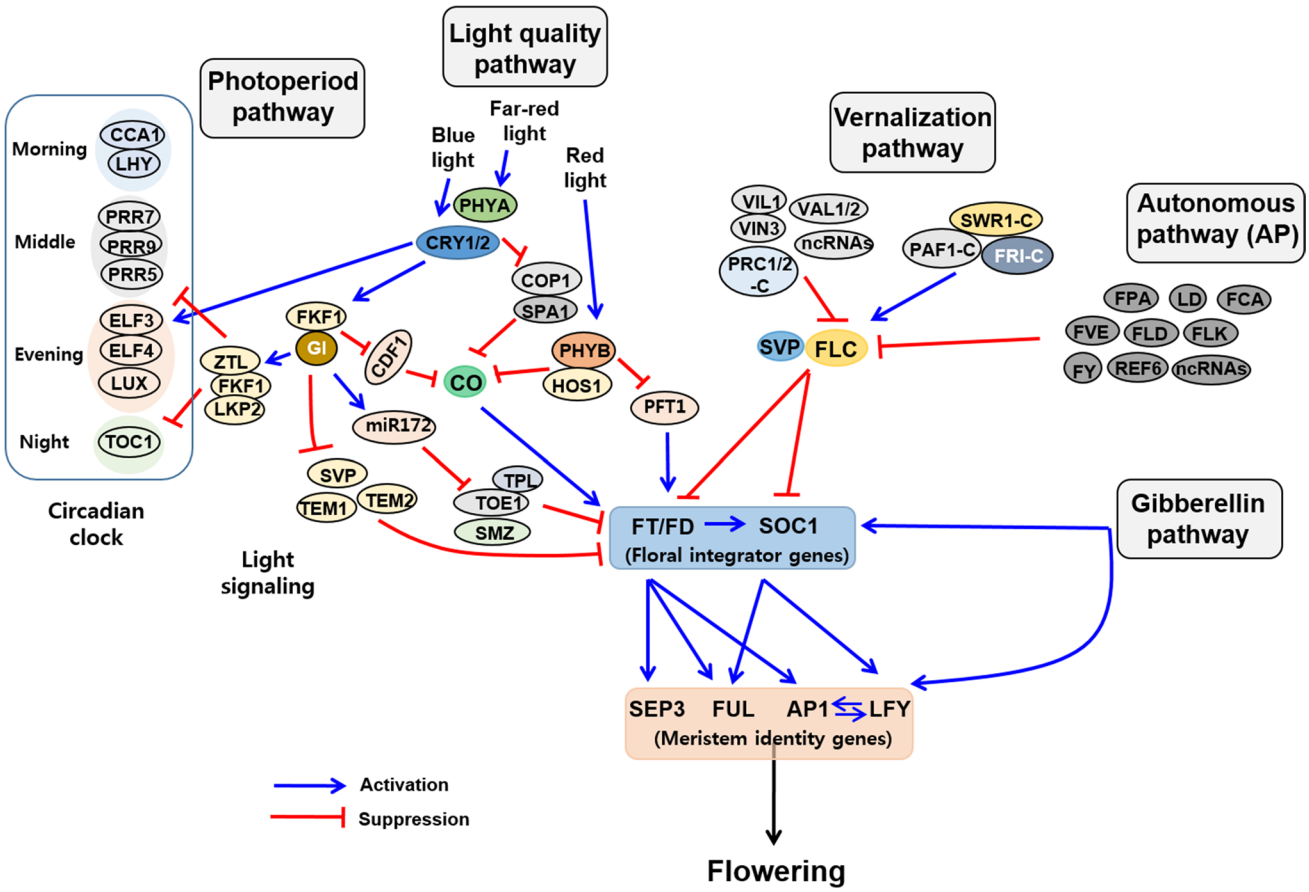

Fig. 1 Schematic of the flowering pathways in Arabidopsis plants. The components involved in each flowering pathway are shown below the corresponding pathway name. The floral integrator genes, including *FT* and *SOC1*, act as floral inducers in plants. The leaf-derived FT protein interacts with FD to form an FT-FD dimer (Turck et al. 2008), which activates *SOC1* and several meristem identity genes, such as *SEP3*, *FUL*, *AP1*, and *LFY*, to initiate the floral transition in the Arabidopsis plants (Abe et al. 2005). FLC suppresses floral transition by inhibiting the transcription of *FT* and *SOC1* (Kim et al. 2009; Helliwell et al. 2006). Meanwhile, CO acts to activate the *FT* and *SOC1* genes (Hepworth et al. 2002; Wenkel et al. 2006; Samach et al. 2000). The photoperiod pathway involves the circadian clock that comprises four complicated and intricate complexes and is tightly connected to the light quality pathway (Gardner et al. 2006; Guerriero et al. 2014; Huang and Nusinow 2016). GI acts in both CO-dependent (i.e., by repressing *CDF1*) and CO-independent ways (i.e., by suppressing the SVP and TEM factors or by promoting *miR172* expression) to control the flowering program. The light quality pathway also operates in both CO-dependent and CO-independent ways to regulate of *FT* expression (Cerdan and Chory 2003; Backstrom et al. 2007). PHYB acts to suppress CO protein activity, whereas PHYA, CRY1, and CRY2 function to enhance the activity of CO. In parallel, PHYB affects *FT* transcription by suppressing PFT1, an upstream activator of *FT* (Inigo et al. 2012). The autonomous pathway contains eight components and a recently-identified antisense non-coding RNA (ncRNA) called *COOLAIR* (Swiezewski et al. 2009). The plant hormone, gibberellic acid (GA), was shown to directly affect the transcription of *SOC1* and *LFY*. Before vernalization, the level of *FLC* mRNA is highly activated by three cooperative complexes named as the SWR1-complex, PAF1-complex, and FRI-complex (Choi et al. 2011). The vernalization pathway includes two PHD-finger domain proteins, VIN3 and VIL1, and two polycomb complexes, PRC1 and PRC2 (Kim and Sung 2014b). The ncRNAs, *COOLAIR* and *COLDWRAP*, were also shown to be involved in the vernalization pathway (Heo and Sung 2011; Kim and Sung 2017). The PHD-PRC2 complex was also shown to associate with other transcriptional regulators, such as VAL1 and VAL2 (Yuan et al. 2016; Questa et al. 2016). The red bar indicates the suppression or negative effect, and the blue arrows indicate the activation or positive effect

photoperiod pathway, such as *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*), are well conserved among many plant species (Kobayashi and Weigel 2007). In Arabidopsis, the level of *FT* mRNA expression determines the timing of bolting (Turck et al. 2008; Corbesier et al. 2007). As a facultative long-day (LD) plant, Arabidopsis exhibits accelerated expression of *FT* under LD conditions, but lowered expression under short-day (SD) conditions. Thus, the transcriptional activation of *FT* is critical for the induction of floral transition in the photoperiod pathway (Searle and Coupland 2004).

The photoperiodic induction of flowering appears to operate via a system in which the *CO* expression levels are affected by day length (Corbesier et al. 2007; Kobayashi and

Weigel 2007; Notaguchi et al. 2008). The *CO* gene, which encodes a BBX domain protein, is a main upstream activator of *FT* in Arabidopsis (Adrian et al. 2010). In LD conditions, the Arabidopsis *CO* expression extends to the daytime phase, and light enhances CO protein stability (Yanovsky and Kay 2002; Valverde et al. 2004). The stabilized CO protein directly binds to the proximal promoter region of *FT* and stimulates the transcription of *FT* in the inductive LD condition (Hepworth et al. 2002; Wenkel et al. 2006; Samach et al. 2000) (Fig. 1).

The levels of both *CO* transcripts and CO protein are tightly coordinated by several signaling systems, such as circadian clock and light signaling, including photoreceptors (Fig. 1). In light signaling, the transcription of *CO* is directly repressed by the upstream regulator, CYCLING DOF FACTOR 1 (CDF1). Blue light promotes the transcriptional activation of *CO* with the help of a blue-light photoreceptor F-box protein, FLAVIN-BINDING, KELCH REPEAT, F BOX 1 (FKF1) and GIGANTEA (GI) protein. The transcript and protein levels of both *FKF1* and *GI* display a diurnal rhythm and show the highest abundance at the afternoon, which coincides with the peak time of CO protein expression. Abundant amounts of FKF1 or GI physically interact with CDF1, subsequently triggering CDF1 degradation (Fig. 1). Consequently, the reduction of CDF1 releases *CO* from repression under LD conditions, thus inducing the floral transition (Fornara et al. 2009; Imaizumi et al. 2005; Song et al. 2012b).

In the dark (night) condition, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and members of the SUPPRESSOR OF PHYA-105 (SPA) family (SPA1, SPA3, and SPA4) are abundant and physically interact with one another to form the COP1/SPA complex, which acts to destabilize CO by ubiquitination in the dark condition (Laubinger et al. 2006; Liu et al. 2008). In the blue-light condition, however, two blue-light receptors, CRYPTOCHROME 1 (CRY1) and CRY2, interact with COP1 and SPA and prevent the physical interaction of COP1/SPA with CO, thus stabilizing the CO protein in the blue-light condition (Fig. 1).

In Arabidopsis plants, there are five red/far-red light photoreceptors, called phytochromes (PHYA–PHYE) (Quail 2010), which are produced in the cytosol of plant cells. They can be reversibly converted from an inactive form (Pr) to an active form (Pfr) and vice versa, depending on the presence of light. The inactive Pr form of PHYs in the dark can be converted into their active Pfr form in the light, which is subsequently translocated into the nucleus to trigger light-induced photomorphogenesis (Bae and Choi 2008). Among the red-light receptors, while PHYB acts to delay floral transition by destabilizing the CO protein through ubiquitination, PHYA has an opposite function to enhance the stability of CO by inhibiting the COP1/SPA1-destabilizing activity (Valverde et al. 2004). As a result, the *phyA* mutants exhibit late flowering, whereas the *phyB* mutants show early flowering compared to the wild-type plants. Unlike the case of PHYA, the PHYB-mediated control of CO stability does not involve the COP1/SPA1 components (Valverde et al. 2004; Jang et al. 2008). Instead, it was recently proposed that a RING-finger-containing E3-ligase protein, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1) is involved in the PHYB-mediated destabilization of CO (Lazaro et al. 2015). PHYB was shown to physically interact with HOS1 and CO in vivo and *in planta*, implying that they might form a complex and function to coordinate the precise abundance of CO in the light condition in Arabidopsis. Interestingly, PHYB functions not only in a CO-dependent manner, but also in a CO-independent manner. For instance, PHYB suppresses PHYTOCHROME AND FLOWERING TIME 1 (PFT1)/MEDIATOR 25 (MED25), which acts to activate *FT* expression in optimal light conditions (Cerdan and Chory 2003; Backstrom et al. 2007).

In several recent studies, it has been proposed that microRNAs (miRNAs) are involved in floral transition in a CO-independent manner (Hong and Jackson 2015; Zhu and Helliwell 2011; Teotia and Tang 2015; Aukerman and Sakai 2003). Two AP2-type transcription factors, TARGET OF EARLY ACTIVATION TAGGED 1 (TOE1) and TOE2, directly bind and repress *FT* expression. As plants age, both *TOE1* and *TOE2* are post-transcriptionally inhibited by the action of *miR172* (Aukerman and Sakai 2003). TOE1 was shown to block the direct binding of CO to the promoter of *FT* (Zhang et al. 2015). TOE1 acts together with the transcriptional repressor TOPLESS (TPL) to suppress the *FT* gene (Fig. 1). In addition, another AP2 transcription factor, SCHLAFMUTZE (SMZ), and its paralogous protein, SNARCHZAPFEN (SNZ), function to suppress *FT* expression. In a similar way, these two factors were also targeted by *miRNA172* in an age-dependent manner (Mathieu et al. 2009; Wu et al. 2009). These data imply that the expression of *FT* is not only controlled by a light signal, but also by the age-dependent flowering pathway.

Light signaling is indeed connected to the intrinsic circadian clock (Gardner et al. 2006; Guerriero et al. 2014). Different light wavelengths, including red, far-red, blue, and UV, can give rise to different developmental outputs. Light information perceived by photoreceptors is delivered to the circadian clock system to modulate gene sets involved in the floral transition process in Arabidopsis plants (Nakamichi 2011; Imaizumi 2010). Most of the components of the circadian clock act as transcriptional repressors. The circadian clock system in plants comprises four distinctive, but interconnected regulatory complexes: (1) morning complex, (2) middle complex, (3) evening complex, and (4) night complex (Huang and Nusinow 2016; Hsu and Harmer 2014; Shim and Imaizumi 2015; Seo and Mas 2014) (Fig. 1). At the dawn stage, two morning complex components,

CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), are abundant and directly suppress the other three complex genes (Adams et al. 2015; Gendron et al. 2012). The middle complex contains several *PSEUDO RESPONSE REGULATOR (PRR)* genes. The evening complex genes include *EARLY FLOWERING 3* (*ELF3*), *ELF4*, and *LUX ARRYTHMO* (*LUX*) (Kamioka et al. 2016; Nusinow et al. 2011). The night complex includes TIMING OF CAB EXPRESSION 1 (TOC1, also known as PRR1), a transcriptional repressor acting on genes belonging to the other three complexes (Gendron et al. 2012). These components in the circadian clock system are interconnected and form a feedback regulation loop with one another at the transcriptional level.

In early morning, the expression of *CCA1* and *LHY* is robust and directly suppresses the other middle, evening, and night complex genes. CCA1 and LHY were previously shown to recognize and bind to the cis-acting Evening Element (EE) and CCA1-binding sites (CBS). In addition, a genome-wide chromatin immunoprecipitation sequencing (ChIP-seq) assay found that the CCA1-containing complex binds approximately 449 loci and recognizes additional DNA elements, such as G-box and CT repeats, which might be recognized with the help of a binding partner interacting with CCA1 (Kamioka et al. 2016).

From morning to the early evening stage, the PRR proteins PRR7, PRR9, and PRR5 are highly expressed and act to suppress *CCA1* and *LHY* expression (Nakamichi et al. 2010; Liu et al. 2016). This repressive action of the PRR proteins on the *CCA1* and *LHY* genes allows the evening complex genes to be eventually accumulated in the evening time. As a result, the evening complex proteins become dominant at the evening time and then the ELF3-ELF4-LUX evening complex suppresses the middle complex genes through direct binding to the promoter regions of *PRR9* and *LUX* (Dixon et al. 2011; Helfer et al. 2011; Nusinow et al. 2011). At night time, the level of TOC1 becomes abundant and it acts to repress *CCA1* and *LHY* as well as other complex genes (Gendron et al. 2012; Huang et al. 2012). As time progresses toward the end of night, the TOC1-mediated repression is abolished by the action of E3-ubiquitin ligases, ZEITLUPE (ZTL), FKF1, and LOV KELCH PROTEIN 2 (LKP2) (Baudry et al. 2010; Mas et al. 2003). These ZTL family E3-ubiquitin ligases target TOC1 for ubiquitin-mediated degradation at night. GI plays an important role in the ZTL-mediated degradation of TOC1. Indeed, there is a complicated and intricate feedback loop regulation among these circadian clock components. Since it is beyond the scope of this review to describe the details of this network, please refer to other recent reviews (Shim et al. 2017).

During daytime, GI physically interacts with ZTL and protects ZTL from degradation by sequestering the ZTL protein in a GI-ZTL complex (Kim et al. 2007; Kiba et al. 2007; Mas et al. 2003; Pokhilko et al. 2010). At night, ZTL is released from the GI-ZTL complex and then TOC1 and PRR5 are targeted for degradation. As mentioned above, GI interacts with FKF1 and the GI-FKF1 complex acts to increase *CO* transcription by triggering the degradation of CDF1, the upstream transcriptional repressor of the *CO* gene. In parallel, GI can regulate the transcription of *FT* independent of CO in at least two different ways. First, GI physically interacts with a couple of repressors of *FT,* such as SHORT VEGETABLE PHASE (SVP), TEMPRANILLO 1 (TEM1), and TEM2 (Sawa and Kay 2011). The GI and *FT* repressors (SVP-TEM1-TEM2) proteins were shown to compete for binding to the promoter site of *FT*. Thus, it is likely that GI can directly bind and activate *FT* expression by out-competing the binding of *FT* repressors to the promoter of *FT* during the daytime. The second way involves *miRNA172*, which, as mentioned above, inhibits the expression of AP2-like transcription factors, such as *TOE1*, *TOE2*, *SMZ*, and *SNZ*, acting to repress *FT* transcription (Zhang et al. 2015; Mathieu et al. 2009). Interestingly, GI has been shown to enhance the expression of *miRNA172*, which can subsequently cause a reduction in the AP2 repressor gene transcripts, thus resulting in a switch from transcriptional repression to activation of *FT* (Jung et al. 2007; Mathieu et al. 2009). These data indicate that there are multiple layers of regulation via a CO-dependent or CO-independent pathway to correctly respond to the photoperiod to optimize plant growth according to the environmental conditions. The information on the individual components involved in the photoperiod pathway is provided in Supplementary Table 1.

In conclusion, light signaling and the circadian clock are integrated to ensure that the CO and FT proteins accumulate only in optimal light conditions (LD), thus allowing plants to correctly respond to a seasonal change.

3 Floral induction by gibberellic acids (GAs)

Gibberellins (GAs) are plant hormones, which are involved in many aspects of plant growth and development, including seed germination, hypocotyl elongation, chlorophyll biosynthesis, and flowering induction (Yamaguchi 2008). GAs are biosynthesized from an initial compound, *ent*-kaurenoic acid, and the activity of GIBBERELLIN 3-beta-DIOXYGENASE (GA3ox) is important for the production of bioactive GAs, such as GA_4, whereas GA2ox converts the bioactive GA_4 into its inactive form in Arabidopsis plants (Olszewski et al. 2002). Treatment with GA can initiate the floral transition in many plant species, including Arabidopsis. Mutations in the genes involved in either GA biosynthesis or the GA signaling pathway result in alterations in flowering time (Blazquez et al. 1998, 2002). For instance, the mutant *ga1-3*, which does not produce GA, fails to flower under SD

conditions and displays a delay in flowering under LD conditions (Mutasa-Gottgens and Hedden 2009). GA seems to act independently of the photoperiod pathway because the delayed flowering in *ga1* mutants is relatively minor under LD conditions compared to SD conditions. Furthermore, a double mutant of *ga1* with the photoperiod mutant *co* displays an additive phenotype of extremely delayed flowering in LD conditions (Reeves and Coupland 2001). However, GA seems to be not inherently required for vernalization because when the *ga1* mutation was introduced into vernalization-requiring plants, the plants still retained a complete vernalization response in LD conditions (Borner et al. 2000; Michaels and Amasino 1999). Instead, it is suggested that GA promotes flowering by promoting the expression of the floral integrators, such as *LEAFY(LFY)* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), via a DELLA-dependent mechanism (Moon et al. 2003; Achard et al. 2004). Thus, the photoperiod- and GA-induced flowering pathways act independently to promote floral transition by activating the expression of floral integrators such as *LFY* and *SOC1* (Fig. 1).

4 Floral induction by the autonomous pathway (AP)

A handful of late-flowering mutants were derived from the summer annual, rapid-flowering Arabidopsis accessions, like *Columbia-0* (*Col-0*) plants, and were referred to as defining the autonomous pathway (AP) of floral promotion (Simpson 2004; Amasino and Michaels 2010) (Fig. 1). The AP promotes flowering independently of environmental conditions (Amasino and Michaels 2010). The AP mutants, characterized by delayed flowering in both LD and SD conditions, are distinctive from the photoperiod pathway mutants, which exhibit delayed flowering only under inductive LD photoperiods in Arabidopsis. To date, eight AP genes have been identified: *LUMINIDEPENDENS* (*LD*), *FLOWERING CONTROL LOCUS A* (*FCA*), *FLOWERING LOCUS Y* (*FY*), *FPA*, *FLOWERING LOCUS D* (*FLD*), *FVE/MIS4 (MULTICOPY SUPPRESSOR OF IRA1 4)*, *FLOWERING LOCUS K* (*FLK*), and *RELATIVE OF EARLY FLOWERING 6* (*REF6*) (Simpson 2004; Lim et al. 2004; Schomburg et al. 2001; Noh et al. 2004). *FCA*, *FPA*, *FLK*, and *FY* encode proteins that are predicted to be involved in RNA metabolism (Macknight et al. 1997; Schomburg et al. 2001; Simpson et al. 2003; Lim et al. 2004). FVE, FLD, LD, and REF6 have domains commonly found in chromatin-modifying proteins: *FVE* encodes a WD-repeat protein, which is found in various chromatin-remodeling complexes (Ausin et al. 2004), and *FLD* and *REF6* have been predicted to encode two different types of histone demethylases (He et al. 2003; Jiang et al. 2007; Noh et al. 2004). A major target of these AP proteins is *FLOWERING LOCUS C* (*FLC*), a floral repressor. For example, the delayed flowering phenotypes of the AP mutants were completely suppressed in the *flc* null mutant background, indicating that AP acts to repress a common target, the *FLC* gene, which inhibits floral transition (Michaels and Amasino 2001; Sheldon et al. 1999). Thus, it is likely that they are involved in floral transition by regulating *FLC* expression via RNA processing and/or chromatin modification of *FLC*.

Some of the AP group members were shown to control the expression of *FLC* by modulating the expression of the antisense non-coding RNA (ncRNA), *COOLAIR*, which is derived from the 3′ region of the sense *FLC* locus (Swiezewski et al. 2009). For example, both FCA and FPA contain a plant-specific RNA-recognition motif (RRM)-type RNA-binding domain and regulate alternative polyadenylation of the antisense RNA, *COOLAIR* (Liu et al. 2010). For example, FCA and FPA were shown to promote the cleavage and polyadenylation event at the proximal sites of the *COOLAIR* antisense RNA at the end of the sense *FLC* transcript. In the absence of FPA and FCA, the 3′-end formation occurs at the distal sites of *COOLAIR*, reaching the proximal promoter region of the sense *FLC* gene.

FCA was shown to physically interact with another RNA-processing factor, FY. This interaction is mediated by the PPLPP motifs in FY and the WW domain within FCA (Henderson et al. 2005). In addition, FCA and FPA require FLD, a histone H3K4 demethylase, to downregulate the *FLC* mRNA level, showing a link between RNA processing and chromatin modification (Liu et al. 2007).

FLK encodes a plant-specific K-homology (KH) RNA-binding protein (Lim et al. 2004). The mutants of the *FLC* gene displayed a severe late flowering phenotype under both LD and SD conditions. Consistent with this, *FLC* expression was highly upregulated in the *FLC* mutants. The delayed flowering phenotype was suppressed by the GA and vernalization treatments, indicating that FLK acts in the autonomous pathway. However, the molecular function of FLK in *FLC* expression is not clearly determined.

FVE, also referred to as MULTICOPY SUPPRESSOR OF IRA1 4 (MSI4), acts in a large chromatin-modifying complex, POLYCOMB REPRESSIVE COMPLEX 2 (PRC2), which catalyzes a repressive histone mark, the tri-methylation of histone H3-lysine 27 (H3K27me3). The PRC2 complex is described in detail in the section describing the vernalization pathway in this review.

The LD protein contains a homeodomain-like domain and is localized in the nucleus (Aukerman et al. 1999; Lee et al. 1994). It negatively inhibits the transcriptional activity of SUPPRESSOR OF FRIGIDA 4 (SUF4), which activates the transcription of *FLC* in a FRIGIDA (FRI)-containing protein complex in Arabidopsis. Even though it is obvious that

LD acts to repress *FLC* expression, its detailed mechanism remains to be clarified.

REF6 belongs to a group of jumonji (Jmj)-domain family proteins (Noh et al. 2004). The Jmj-domain proteins act in a large complex, the chromatin demethylase complex, which functions to remove a certain methyl group from the target gene chromatins. It has been recently demonstrated that REF6 is an H3K27me3 demethylase acting on *FLC* in Arabidopsis plants (Lu et al. 2011; Li et al. 2016). Another recent study suggested that REF6 might act primarily on antisense RNAs with some other AP RNA-binding proteins (Hornyik et al. 2010), confirming that REF6 might link chromatin demethylation and RNA processing in the regulation of *FLC* expression. However, it is still not clear how a chromatin demethylase, REF6, can participate in the regulation of the transcript levels of the antisense RNA, *COOLAIR*. The H4 acetylation levels in the *FLC* chromatin decreased in the *ref6* mutants compared to the wild-type plants, indicating that REF6 enables a close relationship between histone demethylation and acetylation. Thus, it might be an interesting topic to identify the chromatin regulatory proteins that interact with REF6 for histone demethylation and acetylation of *FLC* by biochemical analyses, such as an immunoprecipitation (IP) assay. The individual components of the autonomous pathway are described in Supplementary Table 1.

It is evident that the AP group genes act primarily on *FLC* because a mutation in the *FLC* gene completely suppresses the late flowering phenotype. However, recent studies reported that some AP genes are also involved in other developmental processes, including seed germination (Baurle et al. 2007; Veley and Michaels 2008; Auge et al. 2018). For instance, the mutants of some AP genes displayed abnormal seed germination frequencies (Auge et al. 2018). In addition, FCA and FPA were shown to be, at least partly, involved in RNA-mediated transposon silencing. Thus, it is likely that there might be still unidentified functions of the AP genes in plant developmental programs.

5 Floral induction by the vernalization pathway

Vernalization is a process in which plants acquire the competence to flower in the following spring through exposure to long-term cold temperatures (Kim et al. 2009; Amasino and Michaels 2010; Amasino 2004; Kim and Sung 2014a; Song et al. 2012a). Unlike the cold acclimation response, vernalization is not immediately triggered by a short-term cold stimulus (Amasino 2005; Sung and Amasino 2005; Amasino 2004). Rather, as a result of vernalization, accelerated flowering appears when the original stimulus (low temperatures) is removed, in other words, when plants are re-exposed to warm temperatures in the following spring. This epigenetic nature of vernalization indicates that low temperatures during winter establish stable changes that last until the following spring to evoke floral transition (Kim and Sung 2014a; Amasino et al. 2017; Amasino 2018).

Arabidopsis plants can be grouped into summer-annual and winter-annual plants based on their vernalization requirement (Amasino 2004, 2018). Previous genetic and molecular studies identified that two major factors, FLC and FRI, provide the vernalization requirement in the winter-annual ecotype plants. Both FLC and FRI repress flowering in Arabidopsis plants (Henderson et al. 2003). FRI acts to upregulate the expression of the floral repressor *FLC* in winter-annual plants, thus inducing delayed flowering, whereas summer-annual plants harbor a genomic deletion in the *FRI* allele, failing on the upregulation of *FLC* and consequently showing an early flowering phenotype (Amasino 2005; Sheldon et al. 1999; Michaels and Amasino 2001; Johanson et al. 2000). FLC, a MADS-box protein, functions to suppress flowering by directly inhibiting the expression of the floral integrator genes, such as *FT* and *SOC1* (Kim et al. 2009; Helliwell et al. 2006). Prior to vernalization, *FLC* is highly expressed to prevent the floral transition. The *FLC*-mediated inhibition of the floral transition is most pronounced in winter-annual plants because of the presence of the functional *FRI* allele in Arabidopsis. A prolonged exposure to cold (i.e., the winter season) eventually represses *FLC* expression. The cold-triggered repressed state of *FLC* is stably maintained throughout subsequent mitotic cell divisions even in warm growth temperatures of the following spring season. The repression of *FLC* releases the repression of *FT* and *SOC1* to initiate the floral transition after return to warm conditions (Bastow et al. 2004; De Lucia et al. 2008). Therefore, the vernalization-mediated stable repression of *FLC* is a prerequisite for floral transition in the next spring season, wherein the CO-mediated photoperiod pathway strongly promotes the expression of the floral integrator genes, *FT* and *SOC1* (Fig. 1).

6 Polycomb group proteins (PcG): PRC1 and PRC2

Polycomb group proteins (PcG) are evolutionarilly conserved multi-protein complexes that play important roles in the epigenetic control of gene expression in plants as well as other eukaryotes (Kim et al. 2009; Molitor and Shen 2013). The PcG genes were first isolated from the genetic mutant screening of *Drosophila* to identify the genes involved in controlling homeotic gene expression (Simon and Kingston 2013). To date, 18 PcG proteins have been identified in *Drosophila* and 18–37 homologs were shown to exist by multiple duplication events in mammals. An increasing number of proteins exist as members of the PcG complexes,

providing an additional layer of complexity in their function (Di Croce and Helin 2013). These PcG proteins have long been one of the excellent models to elucidate the epigenetic mechanisms of cell development (Schwartz and Pirrotta 2007; Simon and Kingston 2013). They are classified into two different groups of multi-protein complexes, polycomb repressive complex 2 (PRC2) and polycomb repressive complex 1 (PRC1) (Schatlowski et al. 2008; Margueron and Reinberg 2011). The PRC2 and PRC1 complexes repress gene expression through covalent histone modifications H3 methylation and H2A ubiquitination, respectively.

7 Polycomb components involved in vernalization

The Arabidopsis core PRC2 complex containing CURLY LEAF (CLF) regulate floral transition by silencing the expression of the floral repressor *FLC* (De Lucia et al. 2008; Kim and Sung 2013; Wood et al. 2006). Through vernalization, the PRC2 complex is substantially enriched at *FLC* chromatin (Fig. 2). Through genetic screening, which was aimed to isolate the mutants resistant to the vernalization treatment, several components have been identified to date. One of these components is *VERNALIZATION INSENSITIVE 3* (*VIN3*), which encodes a PLANT HOMEODOMAIN (PHD) finger domain protein (Bastow et al. 2004; Sung and Amasino 2004). VIN3 is temporarily expressed upon long-term exposure to cold and rapidly disappears upon return to warm growth conditions, implying that VIN3 might be one of the early factors triggering the vernalization response. A PHD finger domain within the VIN3 protein was shown to bind preferentially to H3K9me2, a repressive histone mark, in an in vitro histone peptide assay (Kim and Sung 2013). The enrichments of H3K9me2 and H3K27me3, which accumulate during vernalization, were significantly impaired in the *vin3* mutants. The repression of the expression of *FLC* was alleviated in the *vin3* mutants during and after vernalization, indicating that VIN3 is essentially required for the proper repression of *FLC* during vernalization. These data suggest that VIN3 plays a pivotal role in the reduction of *FLC* expression in vernalization via modulating epigenetic histone modifications.

The biochemical and genetic analyses demonstrated that another PHD finger protein, VIN3-LIKE 1 (VIL1)/VERNALIZATION5 (VRN5), physically interacts with VIN3 in vitro, as shown a by yeast two-hybrid assay, and associates with the VIN3-containing PRC2 complex (Fig. 2)

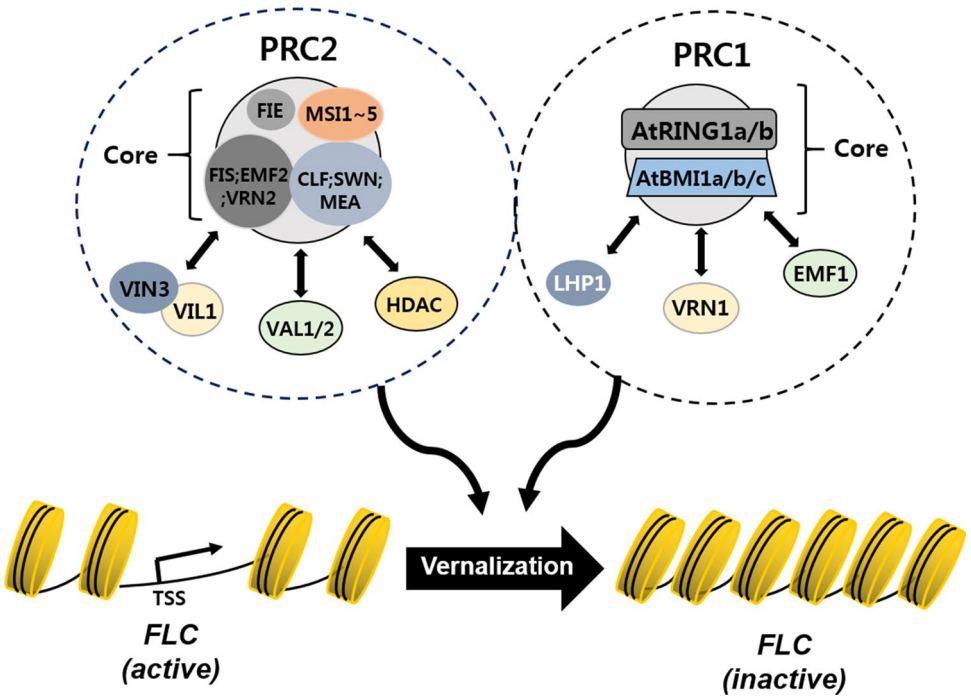

Fig. 2 Schematic model of transcriptional repression by polycomb complexes, PRC1 and PRC2. Upon exposure to long-term cold, a potent floral repressor, *FLC*, is repressed by both multi-protein complexes, PRC1 and PRC2. Each polycomb complex has several core components, which are highly conserved among higher eukaryotes. In addition, each polycomb complex physically associates with several other regulators. For instance, two PHD-finger domain proteins (VIN3 and VIL1), two B3 domain proteins (VAL1 and VAL2), and several HDAC proteins, including HDA19, were reported to associate with the PRC2 core complex. Furthermore, the PRC1 core complex components associate with LHP1, VRN1, and EMF1 to completely suppress *FLC* expression in Arabidopsis

(Sung et al. 2006b; Greb et al. 2007). Like the case of VIN3, VIL1/VRN5 is also involved in the vernalization-mediated repression of *FLC*. The *vil1/vrn5* mutants displayed the de-repressed expression of *FLC* and a delayed flowering time after vernalization. Thus, these two PHD-finger domain proteins, VIN3 and VIL1/VRN5, act together in a PRC2 complex (often referred to as PHD-PRC2) for repressing *FLC* expression during vernalization.

The Arabidopsis PRC1 complex comprises two core members, RING1 proteins and Arabidopsis homolog of B Lymphoma Mo-MLV Insertion Region 1(AtBMI1) proteins (Fig. 2) (Kim and Sung 2014b; Merini et al. 2017). Two E3-ubiquitin ligases, RING1A and RING1B, catalyze mono-oubiquitylation at histone H2A lysine 121 (H2AK121ub) of target chromatins in Arabidopsis (Bratzel et al. 2010). AtRING1A (AT5G44280) and AtRING1B (AT1G03770), the homologs of human RING1A and RING1B, respectively (Kim and Sung 2014b; Sanchez-Pulido et al. 2008), were shown to redundantly regulate key developmental genes related to embryo development and shoot and root meristem development (Molitor and Shen 2013; Chen et al. 2016; Xu and Shen 2008). While AtRING1A/B executes the enzymatic activity for H2AK121ub modification, three Arabidopsis homologs of human BMI1, AtBMI1A (At2g30580), AtBMI1B (At1g06770), and AtBMI1C (At3g23060), act to stimulate the enzymatic activity of RING1A/B in the Arabidopsis PRC1 complex. The double or triple mutants of the *AtBMI1A/B/C* genes displayed detrimental defects in a diversity of plant developmental programs, including embryogenesis, seed dormancy, flower and root development, among others (Chen et al. 2010; Merini et al. 2017; Pico et al. 2015).

LIKE-HETEROCHROMATIN PROTEIN 1(LHP1)/TERMINAL FLOWER2 (TFL2) was shown to accumulate at the *FLC* chromatin as a result of vernalization (Fig. 2) (Sung et al. 2006a; Mylne et al. 2006). In the *lhp1* mutants, the vernalization-mediated repression of *FLC* is not stably maintained because of the impaired enrichment of the H3K27me3 mark on the *FLC* chromatin. Recently, it was reported that LHP1 interacts with two B3 domain DNA-binding proteins, VP1/ABI3-LIKE 1 (VAL1) and VAL2, to achieve the stable repression of *FLC* in vernalization (Fig. 1) (Yuan et al. 2016; Questa et al. 2016). Like LHP1, VAL1 and VAL2 were also shown to preferentially bind to the H3K27me2/3 histone mark via its PHD-L-domain. VAL1 and VAL2 were shown to bind to the Sph/RY-like (-TTCTGCATGG-) motifs located in the first intron of the *FLC* genomic region (Questa et al. 2016; Yuan et al. 2016). They were also co-purified with the VIN3-containing PRC2 complex and HDA19-containing HDAC (histone deacetylase) complex, indicative of their linker role in connecting the LHP1-PRC1, PHD-PRC2, and HDAC complexes (Fig. 2). Most of all, the loss of the *VAL1* gene resulted in a de-repressed level of *FLC* expression and a lower level of the H3K27me3 mark deposited by PRC2 and a higher level of the H3K27ac mark after vernalization when compared to the wild-type plants. Thus, it is likely that VAL1 acts as a recruiter of three distinct repressive complexes, LHP1-PRC1, PHD-PRC2, and HDAC, which coordinate the proper repression of *FLC* via H3K27me3 deposition and H3K27ac removal. However, even though VAL1, potentially with VAL2, is suggested to work as a sequence-specific repressor in the PRC2 complex for *FLC*, it is still ambiguous how VAL1/2 induces vernalization-mediated *FLC* repression because both *VAL1* and *VAL2* are consistently expressed, and they appear to bind to the *FLC* chromatin, irrespective of the cold condition. Further studies are needed to address this question.

Another B3 domain protein, VERNLAIZATION 1 (VRN1), is considered as a member of the non-canonical PRC1 complex in Arabidopsis and contributes to vernalization-mediated repression of *FLC* in Arabidopsis (Fig. 2) (Levy et al. 2002). VRN1 was shown to bind DNA in a non-sequence specific manner. In *vrn1* mutants, the enrichment of H3K9me2 on *FLC* chromatin is severely compromised, but the histone modification of H3K27me3 was not impaired, indicating that VRN1 might be specifically involved in H3K9me2 deposition on target chromatin. Even though the *vrn1* mutants displayed significantly delayed flowering time in vernalization, its function in vernalization is not well addressed to date. Another component of core PRC1, AtBMI1C was shown to be involved in the repression of *FLC* through its H2Aub1 activity (Li et al. 2011). However, it is not clarified yet whether AtBMI1C is involved in the vernalization-mediated suppression of *FLC*. Detailed information on the individual components of these polycomb complexes is provided in Supplementary Table 1. In conclusion, the Arabidopsis core PRC1 and PRC2 complex closely interacts with other chromatin regulators (e.g., HDA19) and transcription factors (e.g., VAL1) to precisely repress *FLC* in vernalization.

8 Vernalization response in other flowering plants

Although Arabidopsis plants have served as an excellent model system to understand the molecular mechanisms of the vernalization response, it has not been clearly understood whether other vernalization-required species might use similarly conserved or different gene regulatory circuitries for their vernalization response. Here, I briefly describe the current understanding on molecular circuitries working in the vernalization response in other flowering plant species.

8.1 Alphine rock-cress (*Arabis alpina* L.)

Arabis alpina, a perennial relative of Arabidopsis, is distinctive in its vernalization response compared to the annual/biennial Arabidopsis accessions (Ansell et al. 2008; Koch et al. 2006). In contrast to the annual Arabidopsis plants, the *A. alpina* plants do not require an inductive photoperiod (Wang et al. 2009; Bergonzi et al. 2013). Thus, young, juvenile-stage *A. alpina* plants, which have only immature meristems, do not respond to vernalization for floral transition (Bergonzi et al. 2013).

Annual plants initiate floral transition in all apical meristems at the same time during their life time; this phenomenon is known as monocarpy. In contrast, perennial plants bloom in spring and summer seasons, but arrest flowering in the later seasons. Perennial plants resume vegetative growth in the fall and repeatedly undergo vernalization. Therefore, perennial plants flower and set seeds many times in their life time (known as polycarpy). The *A. alpina* plants repeat the cycle of vegetative and reproductive growth phases. Similar to Arabidopsis, an ortholog of *FLC*, *PERPETUAL FLOWERING 1* (*PEP1*), functions as a major floral repressor in *A. alpina* plants (Wang et al. 2009). The expression of *PEP1* is repressed by vernalization, thus allowing plants to bloom (Fig. 3a). However, unlike annual Arabidopsis plants, *PEP1* is de-repressed when plants are returned to warm growth temperatures. Reactivated PEP1 in warm conditions represses *A. alpina FT* orthologs (*AaFT1* and *AaFT3*), which promotes flowering in a way similar to annual Arabidopsis plants (Hyun et al. 2019). This fluctuating nature of the repression of *PEP1* allows *A. alpina* plants to display polycarpic flowering behavior in their lifespan. Consistent with the fluctuating pattern of *PEP1* mRNA expression, a repressive histone mark, H3K27me3, is enriched at the *PEP1* chromatin during the cold exposure, but depleted when plants are exposed to warm temperatures.

In Arabidopsis plants, *TERMINAL FLOWER1* (*TFL1*) represses the floral transition and regulates inflorescence architecture. In a similar way, the ortholog of *TFL1* (*AaTFL1*) in *A. alpina* is also shown to be involved in the response to the vernalization-mediated floral transition (Wang et al. 2011a). A mutation in *AaTFL1* resulted in early flowering and determinate inflorescences, which prematurely terminate the flowering program after the formation of a few flower buds. The silencing of *AaTFL1* in *A. alpina* did not eliminate the vernalization requirement for floral transition, but allowed the plants to respond to a relatively shorter duration of cold. These data indicate the genetic connection between the duration of the vernalization requirement and inflorescence determinacy in *A. alpina* plants. In addition, AaTFL functions as a repressor of flowering to guarantee that plants spend a certain period of developmental growth before floral transition in *A. alpina* plants.

An APETALA2 (AP2)-type transcription factor, *PEP2* (*Arabis* ortholog of Arabidopsis AP2), also functions to repress flowering in *A. alpina* plants (Bergonzi et al. 2013). PEP2 acts to up-regulate *PEP1* in order to prevent flowering prior to vernalization. Interestingly, *A. alpina* plants respond to vernalization only when they reach a certain mature age. This age-dependent response to vernalization is achieved by a miRNA group termed as *miRNA156* (Bergonzi et al. 2013). When plants are at the young and juvenile vegetative stage, *miRNA156* is abundantly expressed and inhibits flowering by degrading the transcripts of the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) floral activators, which induce the expression of *AaFT*, the *A. alpina* homolog of *FT*. However, as plants age, the *miRNA156* levels eventually decline, resulting in an increase in the expression levels of the *SPL* floral activators. The increased amounts of SPL protein acts to upregulate the level of *miRNA172*, which targets the mRNA of a group of floral repressors, including *TOE1* and *TOE2*, thus providing competence to flowering at the adult vegetative stage (Fig. 3a). Thus, the *miRNA156*-targeted *SPL* repression module allows only adult vegetative-stage plants to respond to vernalization.

Previously, a genome-wide analysis found that PEP1 directly binds to the promoter of *SPL15* in *A. alpina* plants (Mateos et al. 2017). Most recently, *SPL15* was shown to play a key role in the age-dependent vernalization response in perennial *A. alpina* plants (Hyun et al. 2019). The activity of *SPL15* is uniquely confined to older shoots and branches during vernalization, allowing only adult-stage meristems to respond to cold. The annual vernalization response was recapitulated in *A. alpina* perennial plants by using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing tools and interspecific gene transfer methods, confirming that the age-dependent vernalization response can be conferred by PEP1-mediated repression of the *SPL15* module in perennial *A. alpina* plants.

Altogether, two tightly interlocked modules, vernalization (PEP2-PEP1 module) and juvenility (*miRNA156*-mediated repression of the SPL15 module) function cooperatively to ensure that *A. Alpina* plants become competent to flower only when they have reached the appropriate vegetative stage and have been exposed to vernalization (Fig. 3a).

8.2 Beet (*Beta vulgaris* L.)

In beet, the *FT* homologs, *BvFT1* and *BvFT2*, which encode the proteins of the phosphatidylethanolamine-binding protein (PEBP) family, act antagonistically in flowering (Fig. 3b). While *BvFT1* represses the floral transition, *BvFT2* activates flowering (Pin et al. 2010). It has been shown that vernalization acts to decrease the expression of the floral repressor, *BvFT1*. The vernalization-triggered repression

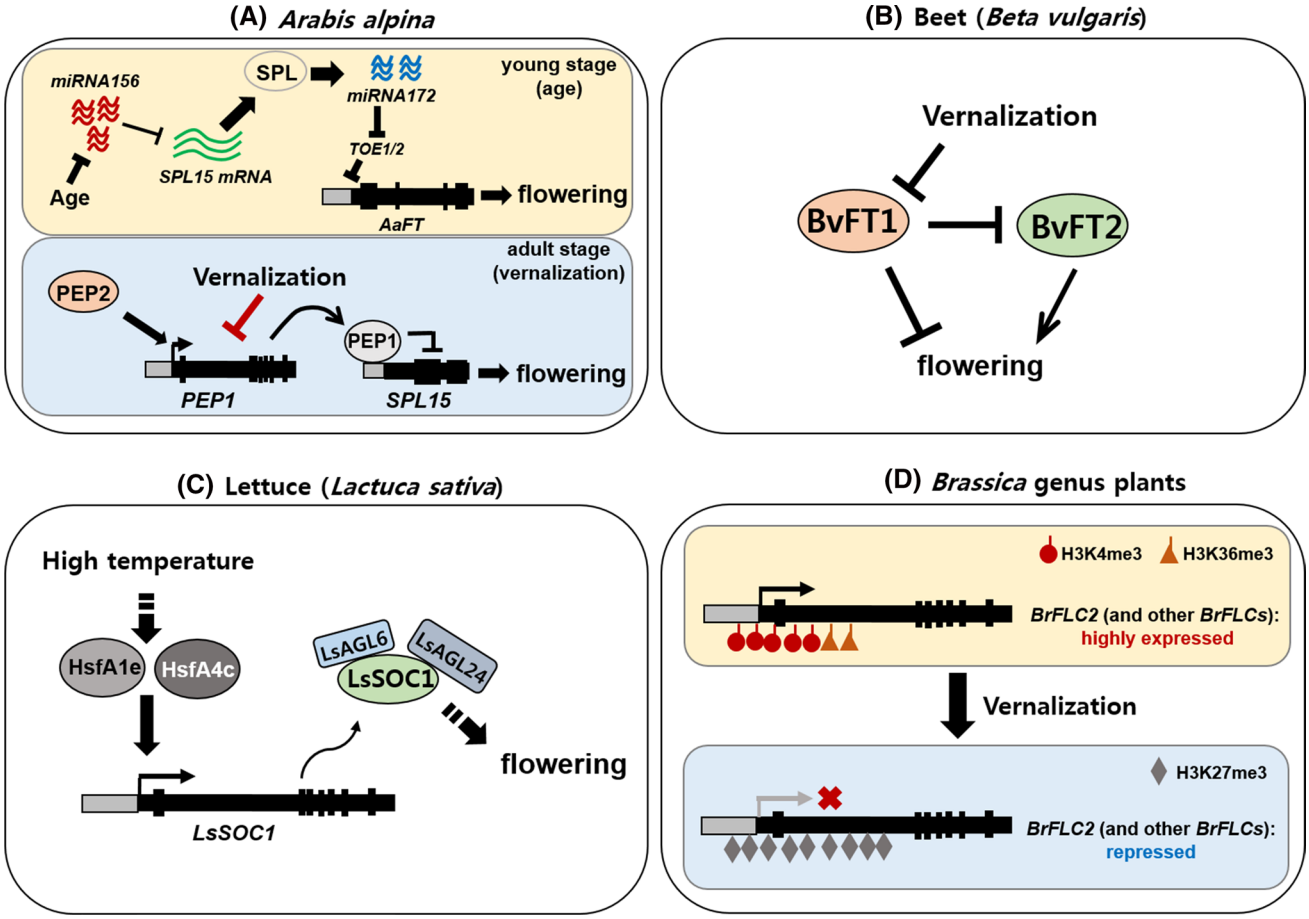

Fig. 3 Outline of flowering pathways in several crop plants. **a** *Arabis alpina* is a perennial species related to Arabidopsis. In its young vegetative stage (upper panel), the juvenile-to-adult vegetative transition is strictly prohibited by *miRNA156* action. *miRNA156* is abundantly expressed in the early seedling stage and blocks the juvenile-to-adult vegetative transition by degrading the mRNA of a group of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes, which play a role in developmental transition from the vegetative to reproductive stage via activating the floral activator, *Arabis alpina FLOWERING LOCUS T* (*AaFT*) (Wang et al. 2009; Hyun et al. 2019; Bergonzi et al. 2013). Thus, the miRNA156-targeted silencing of *SPL15* plays a major role in inhibiting floral transition in the early vegetative stage of the *A. alpina* plants. As time progresses, the levels of *miRNA156* decrease and, conversely, SPL accumulates abundantly and provides partial competence to flowering plants. However, even in the adult vegetative stage (bottom panel), a MADS-box domain protein, named PERPETUAL FLOWERING 1 (PEP1), which is a homolog of Arabidopsis *FLC*, actively acts to prohibit floral transition. The *A. alpina* ortholog of Arabidopsis AP2, PERPETUAL FLOWERING 2 (PEP2), enhances the expression of *PEP1* in the vernalization pathway, thus blocking floral transition. Vernalization triggers the silencing of *PEP1* to induce the floral transition by, at least in part, activation of *AaFT*. **b** In beet, the regulation of floral transition is shown to be controlled by the antagonistic interplay of two homologs of the Arabidopsis FT (Pin et al. 2010). BvFT2 acts as a floral activator, similar to the function of Arabidopsis FT. In contrast, BvFT1 functions to repress floral transition by suppressing *BvFT2* expression. Thus, the vernalization pathway in beets targets *BvFT1* for downregulation in order to induce flowering. **c** In lettuce, high temperature triggers the expression of two heat-shock transcription factors, HsfA1e and HsfA4c, which directly bind to the promoter region of *Lactuca sativa* SOC1 (*LsSOC1*) and induce heat-promoted bolting. *LsSOC1* encodes a MADS-box protein and acts as a floral activator in lettuce (Chen et al. 2018). Two other MADS-box domain proteins, LsAGL6 and LsAGL24, physically interact with LsSOC1, forming a multi-protein complex that plays a key role in floral induction in lettuce. **d** In *Brassica* species, including *B. rapa*, the vernalization pathway acts to reduce the expression of *BrFLC2*, as well as other *BrFLC* paralogous genes, via epigenetic histone modifications in a manner similar to that in Arabidopsis plants (Kawanabe et al. 2016). Active histone marks, H3K4me3 and/or H3K36me3, were highly enriched at the *BrFLC* chromatin before vernalization, providing robust *BrFLC* expressions to block floral transition. The vernalization treatment removes the active histone marks from the *BrFLC* chromatin, and promotes the high-level accumulation of repressive histone marks, such as H3K27me3, at the *BrFLC* region, which is stably maintained even after return to the normal growth temperature. This indicates that epigenetic histone modifications play an important role in changing the expression levels of the *BrFLC* genes in *B. rapa*

of *BvFT1* is consistently maintained even after plants are returned to warm growth temperatures, indicating that *BvFT1* is a functional equivalent of the Arabidopsis *FLC* gene. The vernalization requirement in beet is provided by one dominant allele named *BvBTC1*, which regulates the downstream targets, *BvFT1* and *BvFT2* (Pin et al. 2012) (Fig. 3b). Some annual beets not requiring vernalization have a dominant allele of *BvBTC1*, whose expression is increased by long days and which promotes flowering by reducing the expression of *BvFT1* and activating the transcription of *BvFT2* under LD conditions. As a result, the annual beet plants carrying a functional *BvBTC1* gene exhibit rapid flowering and do not require vernalization for flowering. Meanwhile, the biennial beet plants possess a partial loss-of-function allele of *BvBTC1*, *Bvbtc1*, which is not substantially induced under LD conditions without the vernalization treatment. The *Bvbtc1* allele is only gradually activated by vernalization and is able to reach to the level sufficient to suppress the floral repressor gene, *BvFT1*, and activate the floral activator gene, *BvFT2*.

8.3 Lettuce (*Lactuca sativa* L.)

A cold-temperature (4 °C) treatment does not promote the floral transition in lettuce, whereas a high-temperature treatment at the late seedling stage accelerates this progress (Fukuda et al. 2011). Thus, lettuce plants are considered as so-called "non-low temperature vernalization" plants. Floral transition in lettuce is promoted under high-temperature conditions. Upon bolting, lettuce loses its economic value because its leaves accumulate a bitter taste. Thus, understanding the mechanism of bolting in lettuce is important for breeding for high-value traits. Recently, the molecular mechanism underlying the floral transition in lettuce was elucidated (Chen et al. 2018). A homolog of Arabidopsis *SOC1*, *LsSOC1*, was identified as one of the key factors responsible for heat-promoted floral transition (Fig. 3c). *LsSOC1* encodes a MADS-box protein, which acts as a floral activator in lettuce. In addition, it was shown that two other MADS-box domain proteins, lettuce homolog of Arabidopsis AGAMOUS-LIKE 6 (LsAGL6) and LsAGL24, physically interact with LsSOC1, forming a multi-protein complex for floral induction (Fig. 3c). Furthermore, two heat-shock transcription factors, HsfA1e and HsfA4c, were shown to directly bind to the promoter region of *LsSOC1* and induce heat-promoted bolting.

8.4 *Brassica rapa* and *B. oleracea*

Brassica is a genus belonging to the family *Brassicaceae* and contains 37 flowering plant species, including *Brassica rapa* and *B. oleracea*. Similar to Arabidopsis, they flower early under LD conditions (Leijten et al. 2018). They commonly have spring- and winter-type plants. The spring-type plants do not require vernalization and display early flowering (Qi et al. 2015; Yi et al. 2014). They have been grown in geographical areas with severe winter climates or in subtropical climates. The winter-type plants require vernalization for inducing flowering and have been grown in areas with moderate winter climates. A comparative phylogenic analysis of the *Brassica* plants identified three *FLC* clades, which reflect the occurrence of genome-wide triplication events during the evolution of their genomes (Razi et al. 2008; Schranz et al. 2002; Zou et al. 2012).

The *B. rapa* crops, including Chinese cabbage, pak choi, and turnip, are cultivated worldwide and are most popular in Asian countries (Wang et al. 2011b; Leijten et al. 2018; Lee et al. 2015; Kim et al. 2014; Takada et al. 2019). Several quantitative trait loci (QTL) influencing flowering time in *B. rapa* were identified using the F_2 mapping population between an annual and a biennial cultivar (Teutonico and Osborn 1995; Osborn et al. 1997). In the genome of the *B. rapa* cultivar 'Chiifu-401-42,' four *FLC* homologs were identified and named as *BrFLC1* (Bra009055), *BrFLC2* (Bra028599), *BrFLC3* (Bra006051), and *BrFLC5* (Bra022771). *BrFLC1* and *BrFLC2* were shown to be linked to the QTLs controlling flowering time using an F_2 mapping population (Li et al. 2009; Yuan et al. 2009). In addition, a QTL analysis using another F_2 mapping population between early-flowering and late-flowering cultivars reported that *BrFLC2* was co-localized to a major QTL (Zhao et al. 2010; Xiao et al. 2013; Wu et al. 2012). In a different F_2 population (Early × Tsukena No. 2), *BrFLC2* and *BrFLC3* were co-localized to the QTLs affecting flowering time after the vernalization treatment (Kakizaki et al. 2011). These two genes were detected to have large insertions in the first intron, suggesting that the sequence element in the first intron might be responsible for the repression of the *BrFLC2* and *BrFLC3* genes upon vernalization (Kitamoto et al. 2014). It was shown that the protein-coding sequences of these four *BrFLC* genes are highly conserved among the *Brassica* species, although *BrFLC5* is considered to be a pseudogene because it lacks two exons. However, the genomic sequence of the upstream part and intron region of the *FLC* homologs were relatively divergent among the *Brassica* species (Zou et al. 2012), suggesting that the sequence divergence of these non-coding regions might account for different expression patterns in the vernalization response of the *B. rapa* species. The expression of all four *BrFLC* homologs was decreased by vernalization and stably maintained at low levels even after plants were exposed to warm temperatures (Fig. 3d) (Kawanabe et al. 2016). Active histone marks, H3K4me3 and/or H3K36me3, were highly enriched at the *BrFLC* chromatins before vernalization, providing robust *BrFLC* expressions to block the floral transition (Fig. 3d). The vernalization treatment removes the active histone marks from

the *BrFLC* chromatin, and represses histone marks, such as H3K27me3, which are highly accumulated at the *BrFLC* region, which is stably maintained even after return to normal growth temperatures. This indicates that similar to the case of Arabidopsis, epigenetic histone modifications play an important role in changing the expression levels of the *BrFLC* genes in *B. rapa* varieties.

The *B. oleracea* plants include many commercially important vegetables and can be categorized according to their edible parts. For example, cabbage, kohlrabi, and kale are harvested at the vegetative stage, while broccoli and cauliflower are harvested for their curd (edible flower head part of the plant) after bolting. Therefore, the regulation of the flowering time of these *Brassica* species is of great interest and importance. Owing to the limitation of space, this review focuses on the vernalization of *B. oleracea* plants, such as cabbage (*B. oleracea* L. var. *capitata*), cauliflower (*B. oleracea* L. var. *botrytis*), and broccoli (*B. oleracea* L. var. *italica*).

The orthologs of *FLC* were also identified from *B. oleracea* plants and reported to be involved in floral transition (Lin et al. 2005; Leijten et al. 2018). Through intensive QTL analyses, *BoFLC4* (also known as *BoFLC2*) was identified as a major locus conferring the vernalization requirement in broccoli (Okazaki et al. 2007; Irwin et al. 2016), cabbage (Okazaki et al. 2007), and cauliflower (Ridge et al. 2015). A genomic fragment containing the whole sequence of the *BoFLC4* gene was transformed into the Arabidopsis *FRI_flc2* mutant background (Michaels and Amasino 1999). This heterologous transformation of *BoFLC4* complemented an early flowering phenotype of *FRI_flc2,* suggesting that *BoFLC4* might contribute to provide the vernalization requirement in *B. oleracea* plants (Irwin et al. 2016). In another QTL analysis, *BoFLC3*, *BoFLC5*, and *BoFLC1* were also found to co-localize with a QTL (Razi et al. 2008). Recently, *BoFLC3* was shown to be involved in curd induction variation in the subtropical broccoli breeding lines under a subtropical environment (Lin et al. 2018). Interestingly, another recent study reported that a *FLC* homolog, named the *BoFLC1.C9* locus, contains an insertion of 67 nucleotides in the second intron in late-flowering cultivars, which seems to be originated from two DNA fragments of the Arabidopsis *FLC* sequence (Abuyusuf et al. 2019). Two *FT* loci (*BoFT.C2* and *BoFT.C6*) have been identified and shown to exhibit an increased expression pattern after the vernalization treatment, in a manner similar to Arabidopsis *FT* (Lin et al. 2005; Ridge et al. 2015; Irwin et al. 2016). Even though the *BoFLC* clade genes are well conserved and demonstrated to be involved in flowering time control in *B. oleracea*, a detailed understanding of the role of these *BoFLC* genes is still lacking. A method using the CRISPR/Cas9 genome editing tools might be a good approach to study the detailed role of each *BoFLC* member involved in flowering time control of *B. oleracea* plants. Additionally, through F_2 mapping and a candidate gene approach, a recent paper reported that a gene with a peroxidase domain, named *BolPrx.2*, contributes to the variation of flowering time in cabbage (Abuyusuf et al. 2018). Interestingly, an intron of *BolPrx.2* displayed 76% sequence similarity to the Arabidopsis *FLC* sequence. In addition, an early flowering accession used in this study showed a 27-bp insertion and a 2-bp deletion in the intron region, which might result in the variation of bolting time. It might be an interesting topic to investigate the biological role of these inserted/deleted DNA fragments in terms of their recruitment of the polycomb complex and epigenetic gene silencing processes on flowering-related genes in cabbage.

In Arabidopsis plants, the proximal promoter and the first intron region of *FLC* are important for its stable repression (Sheldon et al. 2002; Helliwell et al. 2011). More recently, two Arabidopsis non-coding RNAs, *COLDAIR* and *COLDWRAP*, were shown to originate from the first intron and the proximal promoter region of *FLC*, respectively. They are involved in the vernalization-mediated intragenic gene loop formation (Heo and Sung 2011; Kim and Sung 2017). As mention above, a large insertion in the first intron of *BrFLC2* and *BrFLC3* caused a defect in vernalization-mediated repression, suggesting that the sequence elements in the first intron of *BrFLC2* and *BrFLC3* might be required for the vernalization-mediated repression of the *BrFLC* genes. However, the sequences similar to *COLDAIR* or *COLDWRAP* have not been identified yet in Chinese cabbage (*B. rapa* subsp. pekinensis) and remain to be further studied. The absence or mutation of the *COLDAIR* and *COLDWRAP* sequences in *A. thaliana* resulted in a defect in intragenic loop formation between the proximal promoter and the end of the first intron region. It resulted in the de-repression of Arabidopsis *FLC* and displayed an extremely late-flowering phenotype (Kim and Sung 2017). Thus, it would be an interesting topic to investigate whether the *BrFLC* genes also undergo an intragenic chromatin conformational change because of the vernalization treatment. Another antisense ncRNA group, *COOLAIR*, which originates from the 3′ region of *FLC*, was also suggested to be involved in the regulation of *FLC* in Arabidopsis (Swiezewski et al. 2009). In *B. rapa*, *COOLAIR-like* transcripts were detected in the *BrFLC2* gene. Because overexpression of the *COOLAIR-like* transcripts resulted in the reduced expression of *FLC* and an early-flowering phenotype, the *COOLAIR-like* transcripts in *B. rapa* might be involved in the repression of *BrFLC2* and possibly other *BrFLC* genes. Taken together, *BrFLC2* might play a major role in the vernalization response. DNA element(s) responsible for the stable repression of *BrFLC2* in the vernalization response remain to be further identified.

9 Conclusion

Flowering time is an agriculturally important trait. Knowledge of the mechanisms underlying flowering time control in plants can be applied to improve important crop traits. For example, the leafy vegetables of the genus *Brassica*, such as Chinese cabbage, eventually lose their commercial value after bolting because energy and metabolites are reallocated to reproductive tissues, causing the devaluation of their leafy tissues. In contrast, canola (*B. napus*) is mainly cultured to harvest seeds for oil. Therefore, an appropriate control of flowering time can maximize the productivity and quality of leaf, flower, or seed tissues in crop plants. In this regard, understanding the molecular mechanisms underlying floral transition is of particular interest in agricultural breeding programs.

This review describes the current understanding of the molecular mechanisms revealed in the model plant Arabidopsis and several crop plants. Intensive studies on this topic greatly increased our knowledge on the genes involved in these pathways, protein–protein interactions, and the molecular interaction networks among these genes. In addition, the epigenetic chromatin regulators (i.e., PRC complexes) of the key genes involved in flowering time expanded our understanding of how plants optimize their growth and development according to changing environmental cues. However, some areas still remain unclear. For example, it is not fully understood how plants accurately measure the duration of cold in the winter season and prevent premature flowering even in fluctuating temperature variations during winter. The mechanisms by which plants can count the length of the vernalizing cold temperatures should be independent of the cold acclimation pathway.

Even though our understanding of flowering pathways in crop plants has been greatly enhanced, the molecular mechanisms underlying these pathways remain poorly understood. Therefore, it is required to explore and elucidate the molecular mechanisms controlling flowering pathways in crop plants. We expect that the comparative study based on the model plant Arabidopsis will help us acquire more comprehensive understanding on the molecular details underlying plant flowering programs. Especially, the computation analysis using the next-generation sequencing data and genomics tools for map-based cloning will highly accelerate the identification of agriculturally important loci and genes in many crop species. Thus, we highly expect that these current approaches will help us identify essential DNA elements required for the vernalization-mediated floral transition. Furthermore, a recently developed genome-editing tool, CRISPR-Cas9, can be utilized to modify the identified DNA elements to engineer crop traits, including flowering time, to enhance the commercial value of crop plants.

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Compliance with ethical standards

Conflict of interest The author declare that I have no conflict of interest.

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