




Research progress on the autonomous flowering time pathway in *Arabidopsis*

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Abstract The transition from vegetative to reproductive growth phase is a pivotal and complicated process in the life cycle of flowering plants which requires a comprehensive response to multiple environmental aspects and endogenous signals. In *Arabidopsis*, six regulatory flowering time pathways have been defined by their response to distinct cues, namely photoperiod, vernalization, gibberellin, temperature, autonomous and age pathways, respectively. Among these pathways, the autonomous flowering pathway accelerates flowering independently of day length by inhibiting the central flowering repressor FLC. FCA, FLD, FLK, FPA, FVE, FY and LD have been widely known to play crucial roles in this pathway. Recently, AGL28, CK2, DBP1, DRM1, DRM2, ESD4, HDA5, HDA6, PCFS4, PEP, PP2A-B'γ, PRMT5, PRMT10, PRP39-1, REF6, and SYP22 have also been shown to be involved in the autonomous flowering time pathway. This review mainly focuses on *FLC* RNA processing, chromatin modification of *FLC*, post-translational modification of FLC and other molecular mechanisms in the autonomous flowering pathway of *Arabidopsis*.

Keywords Flowering time · Autonomous pathway · FLC · RNA processing · Chromatin modification · Post-translational modification · *Arabidopsis*

Introduction

The transition from vegetative growth (producing stems and leaves) to reproductive development (producing flowers) is a major and complex process in the life cycle of flowering plants, which was widely regulated by multiple environmental aspects and endogenous signals (Higgins et al. 2010; Abou-Elwafa et al. 2011).

There is an extensive progress in the gene and molecular mechanisms of flowering controlling process recently in *Arabidopsis* (Srikanth and Schmid 2011; Zhang et al. 2014) and varieties of crop species (Kim et al. 2009). In *Arabidopsis*, which requires for the condition of vernalization and long days facultatively, several regulatory pathways have been defined that is different in their response to distinct internal and external cues, including vernalization, photoperiod, gibberellin (GA), temperature, age and autonomous pathways (He and Amasino 2005; Michaels 2009; Srikanth and Schmid 2011; Zhang et al. 2014). The autonomous pathway functions promote the flowering independently of day length by repressing the central flowering repressor and vernalization regulatory gene *FLOWERING LOCUS C* (*FLC*) (also termed as *AGAMOUS-LIKE 25/AGL25*, *FLOWERING LOCUS F/FLF*, *REDUCED STEM BRANCHING 6/RSB6*) (Michaels et al. 2003; Pazhouhandeh et al. 2011; Abou-Elwafa et al. 2011; Yan et al. 2010). Comparisons between *Arabidopsis* and rice have revealed that rice contains several homologues of many of the known flowering time regulatory genes, and moreover, that certain aspects of the photoperiodic and autonomous flowering pathways are well conserved in this species (Lagercrantz 2009). Understanding the regulation of the floral transition in different plant species provides with important perceptiveness for the possible ancestral

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control of flowering and the alternatively evolutionary mechanisms in different plants (Higgins et al. 2010).

Based on protein sequence similarity, FLC belongs to a six-gene sub-family of the MADS-box class of transcription factors. Through inhibiting the expression of the floral primordium identity genes *FLOWERING LOCUS T (FT)*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1/AGAMOUS-LIKE 20 (SOC1/AGL20)*, *LEAFY (LFY)*, *APETALA 1 (AP1)* and floral organ identity genes *AGAMOUS (AG)* and *AP3*, FLC hinders the floral transition at a quantitative style (Boss et al. 2004; Kobayashi and Weigel 2007; Michaels 2009; Pazhouhandeh et al. 2011).

Autonomous pathway genes repress *FLC* and thus promote the floral transition. Several genes are known to be involved in this pathway such as *FLOWERING LOCUS CA (FCA)*, *FLOWERING LOCUS D (FLD)*, *FLOWERING LOCUS KH DOMAIN (FLK)*, *FLOWERING LOCUS PA (FPA)*, *FLOWERING LOCUS VE (FVE)*, *FLOWERING LOCUS Y (FY)*, and *LUMINIDEPENDENS (LD)* (Simpson 2004; Marquardt 2006; Srikanth and Schmid 2011). Most mutants for above genes are recessive and showed late-flowering phenotype under both long- and short-day conditions; however, the related phenotype can be recovered by treatment with vernalization (Abou-Elwafa et al. 2011).

Since the recent reviews about the autonomous flowering pathway (Simpson 2004; Marquardt 2006), considerable progresses in this research field have been made. Hence, evidence now exists to indicate that additional genes including *AGL28 (AGAMOUS LIKE 28)*, *CK2 (Casein kinase II)*, *DBP1 (DNA-binding protein phosphatase 1)*, *DRM1 (Developmentally Retarded Mutant1)*, *DRM2 (DOMAINSREARRANGED METHYLTRANSFERASE2)*, *ESD4 (EARLY IN SHORT DAYS 4)*, *HDA5 (HISTONE DEACETYLASE 5)*, *HDA6 (HISTONE DEACETYLASE 6)*, *PCFS4 (PCF11P-SIMILAR PROTEIN 4)*, *PEP (PEPPER)*, *PP2A-B γ (protein phosphatase 2A-B γ)*, *PRMT5 (protein arginine methyltransferase 5)*, *PRMT10 (protein arginine methyltransferase 10)*, *PRP39-1 (Pre-mRNA Processing Protein 39-1)*, *REF6 (RELATIVE OF EARLY FLOWERING 6)*, and *SYP22 (ARABIDOPSIS THALIANA SYNTAXIN OF PLANTS 22)*; Table 1) are also likely to be participated in the autonomous flowering time pathway. The present reviews mainly focused on RNA processing and chromatin modification of *FLC*, post-translational modification of FLC and other mechanisms of FLC regulation in *Arabidopsis*.

FCA, FLK, FPA, FY, PCFS4, and PEP are involved in *FLC* RNA processing

Autonomous pathway genes regulate the levels of *FLC* mainly through RNA-based post-transcriptional regulation mechanisms and chromatin epigenetic modification

(Simpson 2004; Boss et al. 2004; Quesada et al. 2005; Bäurle et al. 2007; Bäurle and Dean 2008). Nine proteins are known to mediate RNA regulating processes: FCA, FLK, FPA, FY, PAPS1, PAPS2, PAPS4, PCFS4 and PEP (Fig. 1).

Through genetic dissection of autonomous pathway in *Arabidopsis*, highly conserved RNA 3'-end processing factors that is plant-specific have been identified (Rataj and Simpson 2014). FCA and FPA, two proteins with a plant-specific RNA-recognition motif-type RNA-binding domain, regulate alternative polyadenylation of antisense RNAs and 3'-end formation at the *FLC* (Macknight et al. 1997; Schomburg et al. 2001; Liu et al. 2007; Hornyk et al. 2010b; Liu and Mara 2010). FCA is physically and genetically correlated with the RNA 3'-end processing protein FY (Simpson et al. 2003). This interaction is facilitated through 2 proline-rich (PPLPP) motifs in the carboxyl terminal of FY and the WW (typified by two conserved tryptophan residues) domain of FCA and is necessary for both the correct processing of transcripts derived from *FCA* itself, and the downregulation of *FLC* expression (Quesada et al. 2003; Simpson et al. 2003; Srikanth and Schmid 2011). Moreover, *FPA* and *FCA* interact with *FLD* on genetical level, which encodes a histone demethylase, linking RNA processing to chromatin changes in autonomous pathway (Liu et al. 2007). At least the effect of *FCA* and *FPA* on levels of *FLC* and flowering transition partially depends on *FLD* (Liu et al. 2007; Bäurle and Dean 2008). Hence, *FPA* and *FCA* seem to link chromatin-level regulation with RNA processing of *FLC* (Abou-Elwafa et al. 2011). Functionally, *FPA* has been linked to *FVE* in the flowering regulation network. However, it is less sensitive to FLC gene dosage than *FVE* (Marquardt 2006).

PCFS4 was identified as a new component in the autonomous flowering pathway that regulates the alternative polyadenylation of FCA to promote flowering (Xing et al. 2008). It is hypothesized that PCFS4 interacts with FCA, which regulates alternative RNA processing (Yan et al. 2010), and thus a protein complex consisting of PCFS4, FCA, FPA and FY may regulate *FLC* expression (Fig. 1).

FLK possesses 3 KH (K-homology) RNA-binding domains and has only been found in plants (Mockler et al. 2004; Lim et al. 2004). The precise molecular mechanism of FLK regulation of *FLC* expression has yet to be revealed. However, FLK may suppress *FLC* partially at transcriptional level or through RNA-directed chromatin regulation (Veley and Michaels 2008; Ripoll et al. 2009). Other KH-domain containing proteins in *Arabidopsis* include RS2-INTERACTING KH PROTEIN (RIK) and HUA ENHANCER 4 (HEN4), which are the part of protein complexes mediating pre-mRNA processing of *AG* (Cheng et al. 2003).

Table 1 Summary of important regulators in autonomous flowering time pathway

Gene	Gene Name	Locus	Function	References
<i>FCA</i>	<i>FLOWERING LOCUS CA</i>	<i>AT4G16280</i>	RNA processing	Macknight et al. (1997), Schomburg et al. (2001), Quesada et al. (2003), Marquardt (2006), Liu et al. (2007), Bäurle and Dean (2008), Hornyik et al. (2010b), Liu and Mara (2010), Abou-Elwafa et al. (2011)
<i>FLK</i>	<i>FLOWERING LOCUS KH DOMAIN</i>	<i>AT3G04610</i>	RNA processing	Cheng et al. (2003), Lim et al. (2004), Mockler et al. (2004), Veley and Michaels (2008), Ripoll et al. (2009)
<i>FPA</i>	<i>FLOWERING LOCUS PA</i>	<i>AT2G43410</i>	RNA processing	Schomburg et al. (2001), Liu et al. (2007), Bäurle and Dean (2008), Hornyik et al. (2010b), Liu and Mara (2010), Abou-Elwafa et al. (2011)
<i>FY</i>	<i>FLOWERING LOCUS Y</i>	<i>AT5G13480</i>	RNA processing	Quesada et al. (2003), Simpson et al. (2003)
<i>PCFS4</i>	<i>PCF11P-SIMILAR PROTEIN 4</i>	<i>AT4G04885</i>	RNA processing	Xing et al. (2008), Yan et al. (2010)
<i>PEP</i>	<i>PEPPER</i>	<i>AT4G26000</i>	RNA processing	Ripoll et al. (2006), Ripoll et al. (2009)
<i>DRM2</i>	<i>DOMAINS REARRANGED METHYLTRANSFERASE 2</i>	<i>AT5G14620</i>	Chromatin modification	Zhong et al. (2014)
<i>FLD</i>	<i>FLOWERING LOCUS D</i>	<i>AT3G10390</i>	Chromatin modification	He et al. (2003), Jiang et al. (2007)
<i>FVE</i>	<i>FLOWERING LOCUS VE</i>	<i>AT2G19520</i>	Chromatin modification	Kenzior and Folk (1998), He et al. (2003), Amasino (2004), Ausin et al. (2004), Kim et al. (2004), Hennig et al. (2005)
<i>HDA5</i>	<i>HISTONE DEACETYLASE 5</i>	<i>AT5G61060</i>	Chromatin modification	Luo et al. (2015)
<i>HDA6</i>	<i>HISTONE DEACETYLASE 6</i>	<i>AT5G63110</i>	Chromatin modification	Yu et al. (2011)
<i>LD</i>	<i>LUMINIDEPENDENS</i>	<i>AT4G02560</i>	Chromatin modification	Lee et al. (1994), Aukerman et al. (1999), Blázquez et al. (2001), Kim et al. (2006), Domagalska et al. (2007)
<i>PRMT5</i>	<i>PROTEIN ARGININE METHYLTRANSFERASE 5</i>	<i>AT4G31120</i>	Chromatin modification	Niu et al. (2007), Pei et al. (2007)
<i>PRMT10</i>	<i>PROTEIN ARGININE METHYLTRANSFERASE 10</i>	<i>AT1G04870</i>	Chromatin modification	Niu et al. (2007), Pei et al. (2007)
<i>REF6</i>	<i>RELATIVE OF EARLY FLOWERING 6</i>	<i>AT3G48430</i>	Chromatin modification	Noh et al. (2004), Hornyik et al. (2010a)
<i>CK2</i>	<i>CASEIN KINASE II</i>	<i>AT2G23080</i>	Post-translational modification	Mulekar, et al. (2012), Mulekar and Huq (2015)
<i>PP2A-B'γ</i>	<i>PROTEIN PHOSPHATASE 2A-B'γ</i>	<i>AT4G15415</i>	Post-translational modification	Mulekar, et al. (2012), Heidari et al. (2013), Mulekar and Huq (2015)
<i>SYP22</i>	<i>ARABIDOPSIS THALIANA SYNTAXIN OF PLANTS 22</i>	<i>AT5G46860</i>	Vacuolar and/or endocytic trafficking	Ebine et al. (2012)
<i>DRM1</i>	<i>DEVELOPMENTALLY RETARDED MUTANT 1</i>	<i>Need cloning</i>	Molecular mechanism undefined	Zhu et al. (2005)
<i>AGL28</i>	<i>AGAMOUS LIKE 28</i>	<i>AT1G01530</i>	Molecular mechanism undefined	Yoo et al. (2006)
<i>ESD4</i>	<i>EARLY IN SHORT DAYS 4</i>	<i>AT4G15880</i>	Molecular mechanism undefined	Reeves et al. (2002), Son et al. (2014), Villajuana-Bonequi et al. (2014)
<i>PRP39-1</i>	<i>Pre-mRNA PROCESSING PROTEIN 39-1</i>	<i>AT1G04080</i>	Molecular mechanism undefined	Wang et al. (2007)
<i>DBP1</i>	<i>DNA-binding protein phosphatase 1</i>	<i>AT2G25620</i>	Molecular mechanism undefined	Zhai et al. (2016)

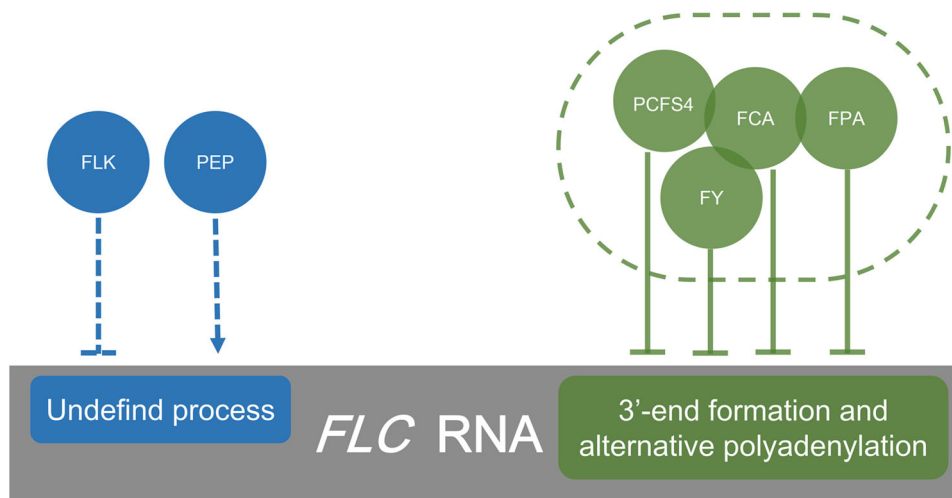


Fig. 1 Six proteins are known to mediate *FLC* RNA regulatory process: FCA, FLK, FPA, FY, PCFS4 and PEP. FPA interacts with FCA, and both control alternative polyadenylation of antisense RNAs and 3'-end formation at *FLC*. FCA interacts with the RNA 3'-end processing protein FY. PCFS4 interacts with FCA and regulates the alternative polyadenylation of FCA. These 4 proteins may present in a

protein complex and interplay to repress *FLC* expression via RNA regulatory processes. FLK which is a plant-specific protein with 3 KH RNA-binding domains suppresses *FLC* expression and its paralogue PEP promotes *FLC* expression, but the precise molecular mechanism for both needs to be explored further

PEPPER (PEP) is a paralogue of FLK and positively regulates expression of *FLC* in *Arabidopsis* (Ripoll et al. 2009). In *flk* mutant, disruption of *PEP* can rescue the delayed-flowering transition with a concomitant *FLC* transcripts decrease. Consistent with this, *PEP* overexpression resulted in high *FLC* transcript levels and delayed flowering (Ripoll et al. 2006). The function of FLK and PEP in the autonomous pathway is found to be independent of *FCA* through genetic and molecular analyses. In addition, *PEP* may influence *FLC* RNA level at both transcriptional and post-transcriptional levels. Taken together, *PEP* is a novel upregulation factor for *FLC*, emphasizing the importance of RNA-binding activities during flowering transition (Bäurle et al. 2007; Liu et al. 2007; Bäurle and Dean 2008). However, as for FLK, the precise molecular mechanism of PEP in regulating *FLC* expression needs to be explored further.

Chromatin modification of *FLC* expression is mediated by DRM2, FLD, FVE, HDA5, HDA6, LD, PRMT5, PRMT10 and REF6

Through chromatin modification, DRM2, FLD, FVE, HDA5, HDA6, LD, PRMT5, PRMT10 and REF6 can regulate *FLC* expression in *Arabidopsis* (Fig. 2). Active *FLC* expression results from a high level of methylated H3K4 around the initiation site of transcription (He and Amasino 2005). FLD, FVE, HDA5, HDA6, LD and REF6 repress *FLC* expression through histone modification including H3K4 demethylation and H3 or H4 deacetylation

in chromatin of *FLC* (He et al. 2003; Domagalska et al. 2007; Yu et al. 2011; Luo et al. 2015). PRMT5 and PRMT10 play an important role in asymmetric histone arginine methylation to control the floral transition by activating other repressors of *FLC* indirectly (Niu et al. 2007; Pei et al. 2007). DRM2 is involved in a conserved epigenetic regulatory mechanism through DNA methylation in *Arabidopsis* (Zhong et al. 2014).

FVE, also termed as MSI4 (MULTICOPY SUPPRESSOR OF IRA1 4) and ACG1 (ALTERED COL-DRESPONSIVE GENE EXPRESSION 1), is orthologous to yeast MSI1 (MULTICOPY SUPPRESSOR OF IRA1) and animal retinoblastoma-associated proteins, which is a component of a histone-acetylation complex. *Arabidopsis* FVE belongs to a family of MSI1-like WD40 repeat proteins (Kenziar and Folk 1998; Kim et al. 2004; Ausin et al. 2004; Hennig et al. 2005). Together with FLD, FVE may be part of a larger protein complex that represses *FLC* expression through histone deacetylation (He et al. 2003; Ausin et al. 2004; Amasino 2004). *FLD* encodes a histone demethylase which is homologous to the human histone H3K4 demethylase LSD1 (LYSINE-SPECIFIC HISTONE DEMETHYLASE1) (Jiang et al. 2007; Yu et al. 2011).

Histone deacetylase HDA5 and HDA6 regulate gene expression cooperately, which display its deacetylase activity by binding to the chromatin of *FLC* (Yu et al. 2011; Luo et al. 2015). In BiFC (bimolecular fluorescence complementation) and CHIP (Chromatin Immunoprecipitation) assays, HDA5 interacts with HDA6, HDA6 interacts with FLD and FLD interacts with FVE (Yu et al. 2011; Luo et al. 2015). The results indicate that these proteins

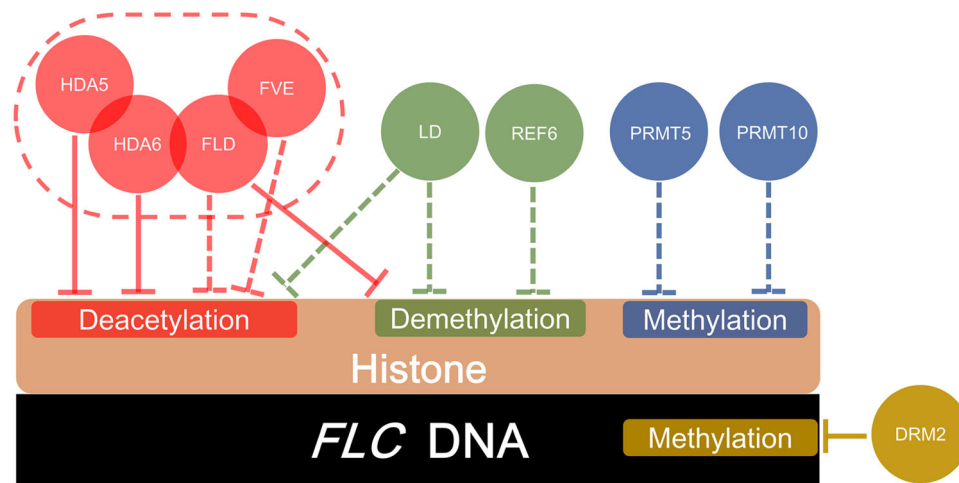


Fig. 2 Through chromatin modification, DRM2, FLD, FVE, HDA5, HDA6, LD, PRMT5, PRMT10 and REF6 can regulate *FLC* expression. FVE interacts with the histone demethylase FLD and both have been implicated in histone deacetylation complexes, however, the mechanism is unknown recently. The histone deacetylase HDA5 interacts with HDA6 and both display deacetylase

may present in a co-repressor complex to regulate *FLC* expression (Fig. 2), suggesting the functional interplay between histone deacetylation and demethylation.

LD is a unique protein possessing a homeodomain-like domain and locates to nucleus in *Arabidopsis* (Lee et al. 1994; Aukerman et al. 1999). LD was initially considered to be a transcriptional regulator (Blázquez et al. 2001) but was later found to repress *FLC* expression through a negatively regulatory interaction with SUF4 (SUPPRESSOR OF FRIGIDA 4), a transcriptional activator of *FLC* (Kim et al. 2006). Until just recently, LD was shown to repress *FLC* expression via histone modification such as H3 deacetylation and H3K4 demethylation (Domagalska et al. 2007).

Previously, REF6 has been shown to be critical in the regulation of *Arabidopsis* flowering and act as a *FLC* repressor. REF6 suppresses *FLC* transcription through histone modifications in *FLC* chromatin, suggesting that this class of proteins play the activity of transcriptional regulation by remodeling chromatin (Noh et al. 2004). Recently, REF6 was found to act primarily on *FLC* antisense RNA (Horniyk et al. 2010a) through its JMJC domain (Jumonji C domain). This domain has been found in human histone demethylases JHDM (jumonji C domain-containing histone demethylase) 2A and JHDM1, and specifically causes H3K9me and H3K36me, respectively (Noh et al. 2004).

Histone H3 lysine methyltransferases are known to be pivotal in gene silencing and developmental control in plants. Recent studies have found that PRMT5 is a type II histone arginine methyltransferase that plays an important role in promoting growth and flowering (Pei et al. 2007). *Arabidopsis* PRMT10, the *Arabidopsis* ortholog of plant histone arginine methyltransferase 10 (PHRMT10), a

activity. HDA6 interacts with FLD. These 4 proteins may present in a protein complex and interplay to repress *FLC* expression via histone modification including demethylation and deacetylation. REF6 acts as a histone demethylase. PRMT5 functions independently of PRMT10, but both play an important role in asymmetric histone arginine methylation. DRM2 functions in methylation of DNA

dimeric plant-specific histone H4 methyltransferase in cauliflower; was shown to be a type I PRMT. PRMT10 is found to react in the autonomous pathway and may act as a modulator by activating other repressors of *FLC* indirectly to control the floral transition. Disruption of *PRMT10* resulted in late flowering through the upregulation of *FLC* transcript levels. Genetically, *PRMT10* functions independently of *PRMT5*, but both act to fine-tune the expression of *FLC*. This result also indicates the importance of asymmetric arginine methylation in plant development and flowering-time regulation (Niu et al. 2007).

DNA methylation is a classical epigenetic gene regulatory mechanism in the autonomous flowering time pathway. DRM2 (DOMAINSREARRANGED METHYLTRANSFERASE 2) is a key *de novo* methyltransferase and is component of a complex possessing the siRNA effector ARGONAUTE4 (AGO4) and preferentially methylating one DNA strand, which likely acts as the template for RNA polymerase V mediated non-coding RNA transcripts. The DNA methylation is positively correlated with the accumulation of strand-biased siRNA. These data indicate that AGO4-siRNA leads DRM2 to its target, and the later is involved in the siRNAs-associated base pairing (Zhong et al. 2014).

Post-translational modification of *FLC* is mediated by CK2 and PP2A-B γ

Post-translational modification of proteins has been shown to be indispensable in the regulation of all aspects of plant development including flowering. Casein kinase II (CK2)

and Protein phosphatase 2A (PP2A) repress FLC to drive flowering through phosphorylation and dephosphorylation (Mulekar, et al. 2012; Mulekar and Huq 2015).

Protein kinases modify their substrate protein by adding one or more phosphate groups to it, which frequently affects its cellular function and/or abundance. Phosphatases can remove the phosphate group from the substrate proteins. This reversible phosphate group transfer results in post-translational modification of target proteins and allows cells to rapidly respond to an internal cue and/or external stimulus. Casein kinase II (CK2) is a necessary and highly-conserved Ser/Thr kinase that regulates proteins in the post-translational process in all eukaryotes. Evidence from several prediction algorithms show that the majority of the autonomous pathway components, including FLC, have multiple CK2 phosphorylation sites, which may modulate their activity or stability and thus drive flowering (Mulekar et al. 2012; Mulekar and Huq 2015). Protein phosphatase 2A (PP2A) comprises 3 types of subunits: scaffolding (A), regulatory (B) and catalytic (C) subunit. The knockdown lines of *PP2A-b'γ* displayed a late flowering phenotype in *Arabidopsis*. The function of PP2A-B'γ in the autonomous pathway is to repress the main flowering inhibitor, *FLC*. The knockout lines *PP2A-b55α* and *PP2A-b55β* flowered earlier than wild type. These results demonstrate that PP2A acts as both a positive and negative regulator of flowering time, depending on which types of regulatory B subunit is involved (Heidari et al. 2013).

Other regulatory mechanisms in the autonomous flowering pathway

The *SYP22* gene encodes a vacuolar N-ethyl-maleimide sensitive factor attachment protein receptor (SNARE) that plays a role in vacuolar and endocytic trafficking pathways. Disruption of *SYP22* increases expression of *FLC* and leads to the late flowering phenotype in *Arabidopsis* (Ebine et al. 2012). Also the elevated levels of *FLC* transcripts accumulated in *doc1-1* (DARK OVER-EXPRESSION OF CAB 1, DOC1) mutant, and the *syp22* phenotypes were enhanced in the *syp22 doc1-1* (Ebine et al. 2012). This elevated expression of *FLC* and the phenotype were suppressed by *ara6-1*, a mutation in the gene encoding a Rab GTPase involved in endosomal trafficking, indicating the involvement of vacuolar and/or endocytic trafficking in the *FLC* regulation of flowering (Ebine et al. 2012).

Other 5 genes have also been shown to involve in the autonomous pathway, however the molecular mechanism underlining the regulation need to be elucidated. It is necessary for plant growth and development to be post-translational modified through attachment of a small ubiquitin-like modifier (SUMO) (Villajuana-Bonequi et al.

2014). In *Arabidopsis*, early genetic analysis indicated that *ESD4* (*EARLY IN SHORT DAYS 4*) is involved in the autonomous pathway. Furthermore, mRNA levels of *FLC* are decreased by the *esd4* mutation, and the expression of flowering-time genes known to be repressed by *FLC*, are increased in the *esd4* mutants (Reeves et al. 2002). Recent research has revealed that *ESD4* encodes a SUMO protease, and mutation in this gene causes hyperaccumulation of conjugates formed between SUMO and its substrates. *FLC* has been shown to interact with the SUMO ligase and to be subsequently modified (Son et al. 2014). Thus, *FLC*-mediated flowering repression might be positively regulated by sumoylation, mediated by *ESD4*.

DRM1 (*Developmentally Retarded Mutant1*) is a novel flowering-promoting locus. The *drm1* mutation is a single recessive nuclear mutation, and is late flowering under all photoperiod conditions. Moreover, vernalization treatment could restore its late flowering phenotype significantly, suggesting that *drm1* is a typical late-flowering mutant and most likely involves in the autonomous flowering pathway. The expression of 3 important repressors, *FLC*, *EMF* and *TFL1*, were increased, in *drm1* mutant, implying that these repressors act in parallel pathways in the *drm1* mutant to regulate flowering. It also suggests that *DRM1* might be a upstream regulator for these repressors (Zhu et al. 2005).

PRP39-1 (*Pre-mRNA Processing Protein 39-1*) has been identified and appears to promote flowering indirectly through RNA processing. Mutant lines of *PRP39-1* in *Arabidopsis* show increased expression of *FLC* accompanied by downregulation of *FT* and *SOC1* (Wang et al. 2007).

Although *AGL28* is known to act in the vegetative growth, overexpression of *Arabidopsis AGL28* causes early flowering through increasing *FCA* and *LD* expression. Hence, *AGL28* promotes the autonomous flowering transition (Yoo et al. 2006). However, disruption of *AGL28* does not lead to any obvious flowering phenotype, suggesting that *AGL28* might be functionally redundant.

DNA-binding protein phosphatase 1 (DBP1) was shown to bind with DNA and displayed protein phosphatase activity in vitro (Carrasco et al. 2006). Zhai et al. (2016) reported that *DBP1* was involved in the flowering time regulation via the autonomous pathway and the photoperiod pathway by modifying the transcript levels of several important integrators, such as *CO*, *SOC1*, *LFY*, *FT* and *FLC*.

Conclusions and perspective

This review summarizes recent research progress in the autonomous pathway of flowering time regulation in *Arabidopsis*. Autonomous pathway constituents participate in repressing the main flowering inhibitor *FLC* and thus,

indirectly promote floral transition. Key regulators in this pathway include AGL28, CK2, DBP1, DRM1, DRM2, ESD4, FCA, FLD, FLK, FPA, FVE, FY, HDA5, HDA6, LD, PCFS4, PEP, PP2A-B γ , PRMT5, PRMT10, PRP39-1, REF6 and SYP22 (Table 1). The molecular mechanisms underlying the regulation of flowering by the autonomous pathway members are primarily concerned with *FLC* RNA processing mediated by FCA, FLK, FPA, FY, PCFS4 and PEP (Table 1; Fig. 1) and chromatin modification mediated by DRM2, FLD, FVE, HDA5, HDA6, LD, PRMT5, PRMT10 and REF6 (Table 1; Fig. 2); and finally, post-translational modification of *FLC* mediated by CK2 and PP2A-B γ (Table 1).

In the future, it can be predicted that other additional members of the autonomous pathway will be identified, and the molecular mechanisms behind previously undefined mediated by AGL28, DBP1, SYP22, ESD4, PRP39-1, DRM1 (Table 1) and newly-discovered members will be revealed. These studies extend our existing understanding of the molecular mechanisms of the autonomous flowering time pathway and may reveal new, as yet undefined, regulatory mechanisms.

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Author's contribution TCE planned and designed the work and wrote some parts. CJZ drawn the figures and wrote some parts. ZYP wrote some parts. LTX and XCP contributed critically to the improvement and editing the manuscript. All authors contributed to improving the paper and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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