Physiol Mol Biol Plants (July–September 2017) 23(3):477–485
DOI 10.1007/s12298-017-0458-3

REVIEW ARTICLE

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

Jing-Zhi Cheng[1] · Yu-Ping Zhou[1] · Tian-Xiao Lv[1] · Chu-Ping Xie[1] · Chang-En Tian[1]

Received: 13 April 2017 / Revised: 6 June 2017 / Accepted: 13 June 2017 / Published online: 26 June 2017

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*

✉ Chang-En Tian
changentian@aliyun.com

[1] School of Life Sciences, Guangzhou University, Guangzhou 510006, China

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

control of flowering and the alternatively evolutional 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* (*DOMAINSREARRANGEDMETHYLTRANSFERASE2*), *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; Hornyik 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
FCA	*FLOWERING LOCUS CA*	*AT4G16280*	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)
FLK	*FLOWERING LOCUS KH DOMAIN*	*AT3G04610*	RNA processing	Cheng et al. (2003), Lim et al. (2004), Mockler et al. (2004), Veley and Michaels (2008), Ripoll et al. (2009)
FPA	*FLOWERING LOCUS PA*	*AT2G43410*	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)
FY	*FLOWERING LOCUS Y*	*AT5G13480*	RNA processing	Quesada et al. (2003), Simpson et al. (2003)
PCFS4	*PCF11P-SIMILAR PROTEIN 4*	*AT4G04885*	RNA processing	Xing et al. (2008), Yan et al. (2010)
PEP	*PEPPER*	*AT4G26000*	RNA processing	Ripoll et al. (2006), Ripoll et al. (2009)
DRM2	*DOMAINS REARRANGED METHYLTRANSFERASE 2*	*AT5G14620*	Chromatin modification	Zhong et al. (2014)
FLD	*FLOWERING LOCUS D*	*AT3G10390*	Chromatin modification	He et al. (2003), Jiang et al. (2007)
FVE	*FLOWERING LOCUS VE*	*AT2G19520*	Chromatin modification	Kenzior and Folk (1998), He et al. (2003), Amasino (2004), Ausin et al. (2004), Kim et al. (2004), Hennig et al. (2005)
HDA5	*HISTONE DEACETYLASE 5*	*AT5G61060*	Chromatin modification	Luo et al. (2015)
HDA6	*HISTONE DEACETYLASE 6*	*AT5G63110*	Chromatin modification	Yu et al. (2011)
LD	*LUMINIDEPENDENS*	*AT4G02560*	Chromatin modification	Lee et al. (1994), Aukerman et al. (1999), Blázquez et al. (2001), Kim et al. (2006), Domagalska et al. (2007)
PRMT5	*PROTEIN ARGININE METHYLTRANSFERASE 5*	*AT4G31120*	Chromatin modification	Niu et al. (2007), Pei et al. (2007)
PRMT10	*PROTEIN ARGININE METHYLTRANSFERASE 10*	*AT1G04870*	Chromatin modification	Niu et al. (2007), Pei et al. (2007)
REF6	*RELATIVE OF EARLY FLOWERING 6*	*AT3G48430*	Chromatin modification	Noh et al. (2004), Hornyik et al. (2010a)
CK2	*CASEIN KINASE II*	*AT2G23080*	Post-translational modification	Mulekar, et al. (2012), Mulekar and Huq (2015)
PP2A-B' γ	*PROTEIN PHOSPHATASE 2A-B'γ*	*AT4G15415*	Post-translational modification	Mulekar, et al. (2012), Heidari et al. (2013), Mulekar and Huq (2015)
SYP22	*ARABIDOPSIS THALIANA SYNTAXIN OF PLANTS 22*	*AT5G46860*	Vacuolar and/or endocytic trafficking	Ebine et al. (2012)
DRM1	*DEVELOPMENTALLY RETARDED MUTANT 1*	*Need cloning*	Molecular mechanism undefined	Zhu et al. (2005)
AGL28	*AGAMOUS LIKE 28*	*AT1G01530*	Molecular mechanismundefined	Yoo et al. (2006)
ESD4	*EARLY IN SHORT DAYS 4*	*AT4G15880*	Molecular mechanism undefined	Reeves et al. (2002), Son et al. (2014), Villajuana-Bonequi et al. (2014)
PRP39-1	*Pre-mRNA PROCESSING PROTEIN 39-1*	*AT1G04080*	Molecular mechanism undefined	Wang et al. (2007)
DBP1	*DNA-binding protein phosphatase 1*	*AT2G25620*	Molecular mechanism undefined	Zhai et al. (2016)

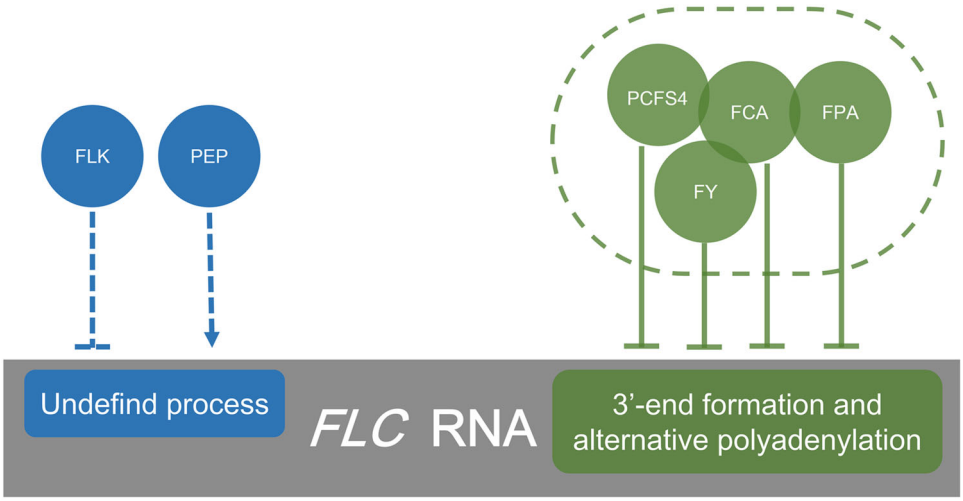

Fig. 1 Six proteins are known to mediate FLC RNA regulatory process: FCA, FLK, FPA, FY, PCFS4 and PEP. FPA interacts with FCA, and both control alternative polyadenylation of antisense RNAs and 3′-end formation at *FLC*. FCA interacts with the RNA 3′-end processing protein FY. PCFS4 interacts with FCA 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 COLDRESPONSIVE 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 (Kenzior 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

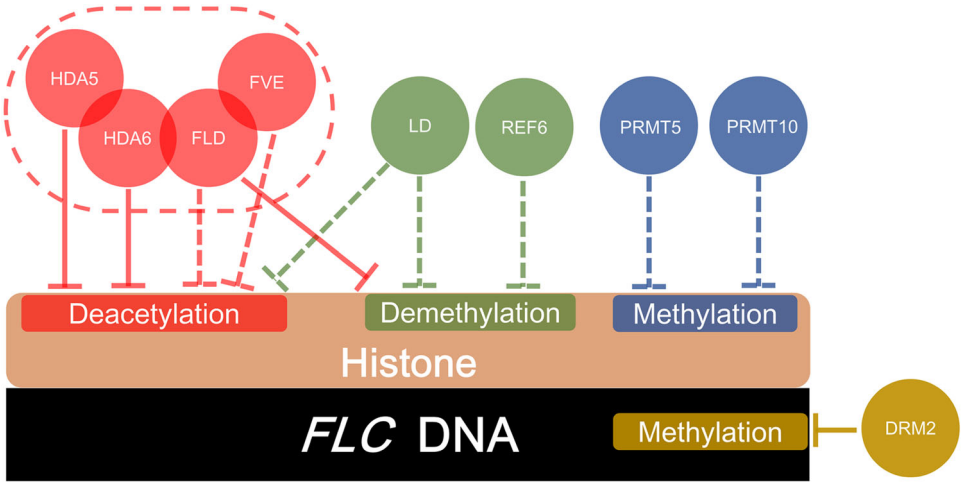

Fig. 2 Through chromatin modification, DRM2, FLD, FVE, HDA5, HDA6, LD, PRMT5, PRMT10 and REF6 can regulate FLC expression. FVE interacts with the histone demethylase FLD and both have been implicated in histone deacetylation complexes, however, the mechanism is unknown recently. The histone deacetylase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

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 (Blázquezet 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 (Hornyik 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 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 (DOMAINSREARRANGEDMETHYLTRANSFERASE2) 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 in 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 aninternal 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 involvment 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,.impliying 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.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Nos. 30570151 and 30870212), the Science and Technology Planning Project of Guangzhou Municipality (Nos. 201504281716332) and the Science and Technology Planning Project of the Education Bureau of Guangzhou Municipality (No. 12A001G) to Dr. Chang-En Tian.

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.

References

Abou-Elwafa SF, Büttner B, Chia T, Schulze-Buxloh G, Hohmann U, Mutasa-Göttgens E, Jung C, Müller AE (2011) Conservation and divergence of autonomous pathway genes in the flowering regulatory network of *Beta vulgaris*. J Exp Bot 62:3359–3374

Amasino R (2004) Take a cold flower. Nat Genet 36:111–112

Aukerman MJ, Lee I, Weigel D, Amasino RM (1999) The *Arabidopsis* flowering-time gene LUMINIDEPENDENS is expressed primarily in regions of cell proliferation and encodes a nuclear protein that regulates LEAFY expression. Plant J 18:195–203

Ausin I, Alonso-Blanco C, Jarillo JA, Ruiz-Garcia L, Martinez-Zapater JM (2004) Regulation of flowering time by FVE, a retinoblastoma-associated protein. Nat Genet 36:162–166

Bäurle I, Dean C (2008) Differential interactions of the autonomous pathway RRM proteins and chromatin regulators in the silencing of *Arabidopsis* targets. PLoS ONE 3:e2733

Bäurle I, Smith L, Baulcombe DC, Dean C (2007) Widespread role for the flowering-time regulators FCA and FPA in RNA-mediated chromatin silencing. Science 318:109–112

Blázquez M, Koornneef M, Putterill J (2001) Flowering on time: genes that regulate the floral transition. Workshop on the molecular basis of flowering time control. EMBO Rep 2:1078–1082

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell 16:S18–S31

Carrasco JL, Castelló MJ, Vera P (2006) 14-3-3 mediates transcriptional regulation by modulating nucleocytoplasmic shuttling of tobacco DNA-binding protein phosphatase. J Biol Chem 281:22875–22881

Cheng Y, Kato N, Wang W, Li J, Chen X (2003) Two RNA binding proteins, HEN4 and HUA1, act in the processing of AGAMOUS pre-mRNA in *Arabidopsis thaliana*. Dev Cell 4:53–66

Domagalska MA, Schomburg FM, Amasino RM, Vierstra RD, Nagy F, Davis SJ (2007) Attenuation of brassinosteroid signaling enhances *FLC* expression and delays flowering. Development 134:2841–2850

Ebine K, Uemura T, Nakano A, Ueda T (2012) Flowering time modulation by a vacuolar SNARE via FLOWERING LOCUS C in *Arabidopsis thaliana*. PLoS ONE 7:42239

He YH, Amasino RM (2005) Role of chromatin modification in flowering-time control. Trends Plant Sci 10:30–35

He YH, Michaels SD, Amasino RM (2003) Regulation of flowering time by histone acetylation in *Arabidopsis*. Science 302:1751–1754

Heidari B, Nemie-Feyissa D, Kangasjärvi S, Lillo C (2013) Antagonistic regulation of flowering time through distinct regulatory subunits of protein phosphatase 2A. PLoS ONE 8:e67987

Hennig L, Bouveret R, Gruissem W (2005) MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. Trends Cell Biol 15:295–302

Higgins JA, Bailey PC, Laurie DA (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. PLoS ONE 5:e10065

Hornyik C, Duc C, Rataj K, Terzi LC, Simpson GG (2010a) Alternative polyadenylation of antisense RNAs and flowering time control. Biochem Soc Trans 38:1077–1081

Hornyik C, Terzi LC, Simpson GG (2010b) The spen family protein FPA controls alternative cleavage and polyadenylation of RNA. Dev Cell 18:203–213

Jiang D, Yang W, He Y, Amasino RM (2007) *Arabidopsis* relatives of the human lysine-specific demethylase 1 repress the expression of *FWA* and *FLOWERING LOCUS C* and thus promote the floral transition. Plant Cell 19:2975–2987

Kenzior AL, Folk WR (1998) AtMSI4 and RbAp48 WD-40 repeat proteins bind metal ions. FEBS Lett 440:425–429

Kim HJ, Hyun Y, Park JY, Park MJ, Park MK, Kim MD, Lee MH, Moon J, Lee I, Kim J (2004) A genetic link between cold responses and flowering time through *FVE* in *Arabidopsis thaliana*. Nat Genet 36:167–171

Kim S, Choi K, Park C, Hwang HJ, Lee I (2006) SUPPRESSOR OF FRIGIDA4, encoding a C2H2-type zinc finger protein, represses flowering by transcriptional activation of *Arabidopsis* FLOWERING LOCUS C. Plant Cell 18:2985–2998

Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. Annu Rev Cell Dev Biol 25:277–299

Kobayashi Y, Weigel D (2007) Move on up, it's time for change–mobile signals controlling photoperiod-dependent flowering. Genes Dev 21:2371–2384

Lagercrantz U (2009) At the end of the day: a common molecular mechanism for photoperiod responses in plants? J Exp Bot 60:2501–2515

Lee I, Aukerman MJ, Gore SL, Lohman KN, Michaels SD, Weaver LM, John MC, Feldmann KA, Amasino RM (1994) Isolation of LUMINIDEPENDENS: a gene involved in the control of flowering time in *Arabidopsis*. Plant Cell 6:75–83

Lim MH, Kim J, Kim YS, Chung KS, Seo YH, Lee I, HongCB Kim HJ, Park CM (2004) A new *Arabidopsis* gene, *FLK*, encodes an RNA binding protein with K homology motifs and regulates flowering time via *FLOWERINGLOCUS C*. Plant Cell 16:731–740

Liu Z, Mara C (2010) Regulatory mechanisms for floral homeotic gene expression. Semin Cell Dev Biol 21:80–86

Liu F, Quesada V, Crevillén P, Bäurle I, Swiezewski S, Dean C (2007) The *Arabidopsis* RNA-binding protein FCA requires alysine-specific demethylase 1 homolog to downregulate FLC. Mol Cell 28:398–407

Luo M, Ready T, Yu CW, Yang SG, Chen CY, Lin WD, Wolfgang S, Wu KQ (2015) Regulation of flowering time by the histone deacetylase HDA5 in *Arabidopsis*. Plant J 82:925–936

Macknight R, Bancroft I, Page T, Lister C, Schmidt R, Love K, Westphal L, Murphy G, Sherson S, Cobbett C, Dean C (1997) *FCA*, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. Cell 89:737–745

Marquardt S (2006) Additional targets of the *Arabidopsis* autonomous pathway members. FCA and FY. J Exp Bot 57(13):3379–3386

Michaels SD (2009) Flowering time regulation produces much fruit. Curr Opin Plant Biol 12:75–80

Michaels SD, He Y, Scortecci KC, Amasino RM (2003) Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. Proc Natl Acad Sci USA 100:10102–10107

Mockler TC, Yu X, Shalitin D, Parikh D, Michael TP, Liou J, Huang J, Smith Z, Alonso JM, Ecker JR, Chory J, Lin C (2004) Regulation of flowering time in *Arabidopsis* by K homology domain proteins. Proc Natl Acad Sci USA 101:12759–12764

Mulekar JJ, Huq E (2015) Developmental pathways in a functionally overlapping manner. Plant Sci 236:295–303

Mulekar JJ, Bu QY, Chen FL, Huq E (2012) Casein kinase II α subunits affect multiple developmental and stress responsive pathways in *Arabidopsis*. Plant J 69:343–354

Niu LF, Lu FL, Pei YX, Liu CY, Cao XF (2007) Regulation of flowering time by the protein arginine methyltransferase *AtPRMT10*. EMBO Rep 8:1190–1195

Noh B, Lee SH, Kim HJ, Yi G, Shin EA, Lee M, Jung KJ, Doyle MR, Amasino RM, Noh YS (2004) Divergent roles of a pair of homologous jumonji/zinc-finger-class transcription factor proteins in the regulation of *Arabidopsis* flowering time. Plant Cell 16:2601–2613

Pazhouhandeh M, Molinier J, Berr A, Genschik P (2011) MSI4/FVE interacts with CUL4-DDB1 and a PRC2-like complex to control epigenetic regulation of flowering time in *Arabidopsis*. Proc Natl Acad Sci USA 108:3430–3435

Pei Y, Niu L, Lu F, Liu C, Zhai J, Kong X, Cao X (2007) Mutations in the type II protein arginine methyltransferase AtPRMT5 result in pleiotropic developmental defects in *Arabidopsis*. Plant Physiol 144:1913–1923

Quesada V, Macknight R, Dean C, Simpson GG (2003) Autoregulation of FCA pre-mRNA processing controls *Arabidopsis*flowering time. EMBO J 22:3142–3152

Quesada V, Dean C, Simpson GG (2005) Regulated RNA processing in the control of *Arabidopsis* flowering. Int J Dev Biol 49:773–780

Rataj K, Simpson GG (2014) Message ends: RNA 3′ processing and flowering time control. J Exp Bot 65:353–363

Reeves PH, Murtas G, Dash S, Coupland G (2002) Early in short days 4, a mutation in *Arabidopsis* that causes early flowering and reduces the mRNA abundance of the floral repressor FLC. Development 129:5349–5361

Ripoll JJ, Ferrándiz C, Martínez-Laborda A, Vera A (2006) *PEPPER*, a novel K-homology domain gene, regulates vegetative and gynoecium development in *Arabidopsis*. Dev Biol 289:346–359

Ripoll JJ, Rodríguez-Cazorla E, González-Reig S, Andújar A, Alonso-Cantabrana H, Perez-Amador MA, Carbonell J, Martínez-Laborda A, Vera A (2009) Antagonistic interactions between *Arabidopsis* K-homology domain genes uncover PEPPER as a positive regulator of the central floral repressor*FLOWERING LOCUS C*. Dev Biol 333:251–262

Schomburg FM, Patton DA, Meinke DW, Amasino RM (2001) *FPA*, a gene involved in floral induction in *Arabidopsis*, encodes a protein containing RNA-recognition motifs. Plant Cell 13:1427–1436

Simpson GG (2004) The autonomous pathway: epigenetic and posttranscriptional gene regulation in the control of *Arabidopsis* flowering time. Curr Opin Plant Biol 7:570–574

Simpson GG, Dijkwel PP, Quesada V, Henderson I, Dean C (2003) FY is an RNA 3′ end-processing factor that interacts with FCA to control the *Arabidopsis* floral transition. Cell 113:777–787

Son GH, Park BS, Song JT, Seo HS (2014) FLC-mediated flowering repression is positively regulated bysumoylation. J Exp Bot 65:339–351

Srikanth A, Schmid M (2011) Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci 68:2013–2037

Veley KM, Michaels SD (2008) Functional redundancy and new roles for genes of the autonomous floral-promotion pathway. Plant Physiol 147:682–695

Villajuana-Bonequi M, Elrouby N, Nordström K, Griebel T, Bachmair A, Coupland G (2014) Elevated salicylic acid levels conferred by increased expression of ISOCHORISMATE SYNTHASE 1 contribute to hyperaccumulation of SUMO1 conjugates in the *Arabidopsis* mutant early in short days 4. Plant J 79:206–219

Wang CX, Tian Q, Hou ZL, Mucha M, Aukerman M, Olsen OA (2007) The *Arabidopsis thaliana AT PRP39-1* gene, encoding a tetratricopeptide repeat protein with similarity to the yeast pre-mRNA processing protein PRP39, affects flowering time. Plant Cell Rep 26:1357–1366

Xing D, Zhao H, Xu R, Li QQ (2008) *Arabidopsis PCFS4*, a homologue of yeast polyadenylation factor Pcf11p, regulates *FCA* alternative processing and promotes flowering time. Plant J 54:899–910

Yan Z, Liang D, Liu H, Zheng G (2010) *FLC*: a key regulator of flowering time in *Arabidopsis*. Russ J Plant Physiol 57:177–185

Yoo SK, Lee JS, Ahn JH (2006) Overexpression of *AGAMOUS-LIKE 28* (*AGL28*) promotes flowering by upregulating expression of floral promoters within the autonomous pathway. Biochem Biophys Res Commun 348:929–936

Yu CW, Liu XC, Luo M, Chen CY, Lin XD, Tian G, Lu Q, Cui YH, Wu KQ (2011) HISTONE DEACETYLASE6 interacts with FLOWERINGLOCUS D and regulates flowering in *Arabidopsis*. Plant Physiol 156:173–184

Zhai H, Ning W, Wu H, Zhang X, Lü S, Xia Z (2016) DNA-binding protein phosphatase AtDBP1 acts as a promoter of flowering in *Arabidopsis*. Planta 243:623–633

Zhang YN, Zhou YP, Chen QH, Huang XL, Tian CE (2014) Molecular basis of flowering time regulation in *Arabidopsis*. Chin Bull Bot 49:469–482

Zhong X, Du J, Hale CJ, Gallego-Bartolome J, Feng S, Vashisht A, Chory J, Wohlschlegel J, Patel D, Jacobsen S (2014) Molecular mechanism of action of plant DRM de novo DNA methyltransferases. Cell 157:1050–1060

Zhu Y, Zhao HF, Ren GD, Yu XF, Cao SQ, Kuai BK (2005) Characterization of a novel developmentally retarded mutant (*drm1*) associated with the autonomous flowering pathway in *Arabidopsis*. Cell Res 15:133–140