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# Role of plant *CBP/p300-like* genes in the regulation of flowering time

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**Summary**

**CREB-binding protein (CBP) and its homolog p300 possess histone acetyltransferase activity and function as key transcriptional co-activators in the regulation of gene expression that controls differentiation and development in animals. However, the role of *CBP/p300-like* genes in plants has not yet been elucidated. Here, we show that Arabidopsis *CBP/p300-like* genes promote flowering by affecting the expression of a major floral repressor *FLOWERING LOCUS C* (*FLC*). Although animal CBP and p300 generally function as co-activators, Arabidopsis CBP/p300-like proteins are required for the negative regulation of *FLC*. This CBP/p300-mediated *FLC* repression may involve reversible protein acetylation independent of histone modification within *FLC* chromatin.**

**Keywords: CREB-binding protein/p300, histone acetyltransferase, Histone deacetylase, *FLOWERING LOCUS C*, flowering, transcription.**

## Introduction

Accurate control of differentiation and development in higher eukaryotes largely depends on the transcriptional regulation of spatial and temporal gene expression in response to developmental or environmental signals. Transcriptional gene regulation is a complex process and requires the orchestration of many transcription factors and multi-functional co-activators/co-repressors (Spiegelman and Heinrich, 2004). CREB-binding protein (CBP) and p300 are well-known transcriptional co-activators in animals and are closely related in their sequences and functions (Kalkhoven, 2004; Ogryzko *et al.*, 1996). CBP/p300 consist of multiple structural domains through which they interact with a wide spectrum of nuclear proteins, including basic transcription factors such as TATA box-binding proteins and TFIIB, as well as various other transcription factors (Goodman and Smolik, 2000; Kalkhoven, 2004). Thus, CBP/p300 function as scaffolds connecting the transcriptional activators to the basic transcriptional machinery (Goodman and Smolik, 2000; Kalkhoven, 2004).

An interesting feature of CBP/p300 is that they possess intrinsic histone acetyltransferase (HAT) activities (Bannister and Kouzarides, 1996; Ogryzko *et al.*, 1996). CBP/p300 acetylate histones within the proximal regions of the promoters; this alters the chromatin structure to be permissive for transcription factor binding (Bannister and Kouzarides, 1996; Korzus *et al.*, 2004; Ogryzko *et al.*, 1996). In addition to their ability to acetylate histones, both CBP and p300 are able to acetylate and affect the transcriptional activity of non-histone nuclear proteins such as chromatin-associated proteins, transcription factors and transcription co-factors (Goodman and Smolik, 2000; Kalkhoven, 2004; Sterner and Berger, 2000). The loss of CBP/p300 function induces multiple developmental defects and abnormal cell growth in humans, mice and *Drosophila* due to the misregulation of their target genes (Goodman and Smolik, 2000; Kalkhoven, 2004). This is often linked to a genetic disorder known as Rubinstein–Taybi syndrome (Petrij *et al.*, 1995) or other malignancies (Kalkhoven, 2004).

*CBP/p300-like* genes are also found in plants (Pandey *et al.*, 2002). Of these gene products, the Arabidopsis CBP/p300-like protein HAC1 was demonstrated to possess HAT activity (Bordoli *et al.*, 2001) and implicated in the transcriptional activation of a heat-shock-inducible gene in a protoplast system (Bharti *et al.*, 2004). However, the relevance of

such observations to the biological roles of *HAC1* has not been addressed.

In plants, flowering is a major switch from the vegetative to the reproductive phase of development. Because flowering is closely associated with plant reproductive strategy, different plant species have distinct flowering times that are optimized to ensure reproductive success. Flowering time is regulated by the convergence of multiple endogenous and environmental signals, including developmental status, hormone signaling, light period and quality, and vernalization (reviewed in Boss *et al.*, 2004; Simpson and Dean, 2002). Molecular genetics studies have identified major floral regulatory pathways in Arabidopsis, namely the photoperiod pathway, the gibberellin (GA)-dependent pathway, and the *FLOWERING LOCUS C* (*FLC*)-dependent pathway. The photoperiod pathway mediates the effect of day length on flowering. In Arabidopsis, flowering is promoted by long days (LD) but repressed by short days (SD). The GA-dependent pathway functions as a default floral promotion pathway in non-inductive SD in Arabidopsis.

*FLC* is a central floral repressor in Arabidopsis and acts as a convergence point of multiple floral regulatory pathways. *FRIGIDA* (*FRI*) functions as a transcriptional activator of *FLC* in winter-annual Arabidopsis. However, this positive effect of *FRI* on *FLC* is antagonized by a long-term cold treatment known as vernalization. Genes in the autonomous pathway negatively regulate *FLC* expression in summer-annual Arabidopsis (Noh and Noh, 2006). In these plants, mutations in the autonomous pathway genes result in increased *FLC* expression and late flowering, similar to winter-annual Arabidopsis. Studies using an *FRI*-containing winter-annual Arabidopsis have identified numerous factors required for the elevated expression of *FLC*. Many of these factors resemble proteins that are involved in chromatin modifications in other organisms (reviewed in He and Amasino, 2005; Noh and Noh, 2006). However, the molecular mechanisms of *FLC* regulation exerted by the genetically identified *FLC* repressors and activators are yet to be elucidated.

Here, we report that Arabidopsis CBP/p300-like proteins control flowering in the autonomous floral regulatory pathway by negatively affecting the expression of a major floral repressor *FLC*. Further, we show that this Arabidopsis CBP/p300-mediated *FLC* regulation might involve reversible protein acetylation independent of histone modification within *FLC* chromatin.

## Results

### *Identification of T-DNA insertional mutants of the Arabidopsis* CBP/p300-like *genes*

The Arabidopsis genome encodes five CBP/p300-like proteins, namely HAC1, HAC2, HAC4, HAC5 and HAC12 (Figure 1) (Pandey *et al.*, 2002). The domain composition and organization are conserved in these five proteins, although the HAC2 protein lacks the N-terminal TAZ-type ZnF domain. However, the domain organization and composition differ between animal CBP/p300 and Arabidopsis HATs of the CBP family (HACs). For example, the CREB transcription factor binding domain and the bromodomain that is known to bind to the acetylated histone tails (Dhalluin *et al.*, 1999) are not detected in HACs; this suggests that the biochemical roles of plant and animal CBP/p300 might be different.

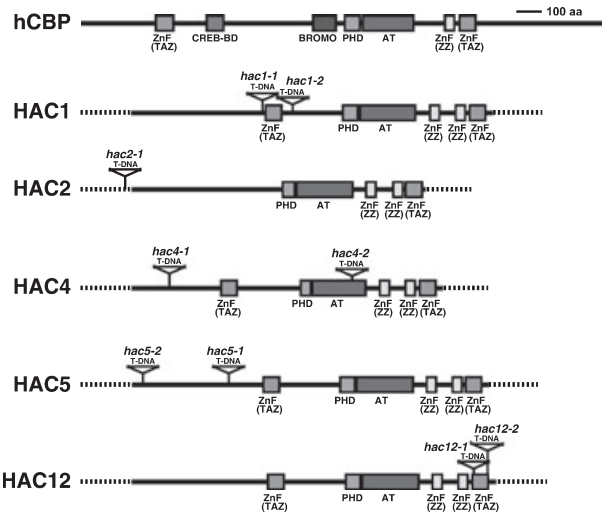



**Figure 1.** The domains of the proteins encoded by Arabidopsis *CBP*/*p300-like* genes and the T-DNA insertion sites in the corresponding mutants.
AT, acetyltransferase; PHD, plant homeodomain zinc finger; CREB-BD, CREB binding domain; ZnF (TAZ), transcriptional adaptor zinc finger; ZnF (ZZ), zinc finger present in dystrophin and CBP (Ponting *et al.*, 1996). The T-DNA insertion sites in the genomic sequences are marked at the corresponding positions of the translated protein products. Dotted lines: 5′/3′ untranslated regions. hCBP, human CBP; aa, amino acids. The protein domains were predicted by SMART (http://smart.embl-heidelberg.de/).

To elucidate the biological roles of the CBP/p300-like proteins in plants, we obtained T-DNA insertion lines of these *CBP*/*p300-like* genes from the SALK T-DNA collection. The T-DNA insertion site in each line was defined by sequencing the PCR product obtained using a T-DNA border primer and a gene-specific primer in order to confirm the presence of T-DNA. As shown in Figure 1, the T-DNAs were inserted within the coding regions of the *HAC* genes in all alleles, except for the *hac2-1* allele in which the T-DNA was inserted 80 bp upstream from the start codon. In case of the *hac12-2* allele, the T-DNA was inserted 8 bp upstream from the stop codon. RT-PCR analyses confirmed the absence of full-length messages in the *hac1-1*, *hac1-2*, *hac4-1*, *hac4-2*, *hac5-1*, *hac5-2* and *hac12-1* mutants (data not shown).

### *Mutations in* HAC1 *cause late flowering*

Phenotypes of the *hac1-1*, *hac1-2*, *hac4-1*, *hac4-2*, *hac5-1*, *hac5-2* and *hac12-1* homozygous mutants were examined

throughout their development. The only single mutant with an observable defect was *hac1*. Both *hac1-1* and *hac1-2* flowered later than the wild-type (wt) in LD (16 h light/8 h dark) as well as SD (8 h light/16 h dark) (Figure 2a–c). The delayed flowering was not due to a delayed leaf initiation rate (Supplementary Figure S1) but was due to delayed transition of the shoot apical meristem (SAM) from the vegetative to the reproductive phase as characterized by a higher number of rosette leaves at the onset of flowering (Figure 2b,c). The photoperiod-independent late-flowering phenotypes of *hac1* suggested that HAC1 might play a role in the *FLC*-dependent pathway. The late flowering of *hac1* was also effectively suppressed by vernalization (wt and *hac1-1* flowered with $12.5 \pm 0.9$ and $13.2 \pm 0.8$ rosette leaves, respectively, in LD after 50 days of vernalization), similar to other late-flowering mutants in the *FLC*-dependent pathway (e.g. Michaels and Amasino, 2001).

In order to address the molecular mechanism underlying the late flowering of *hac1*, we first examined the expression levels of various floral pathway genes. These included *FLC* (a floral repressor in the autonomous and vernalization pathways), *CONSTANS* (*CO*; a floral activator in the photoperiod pathway), and *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*; downstream floral integrators). The *FLC* mRNA levels were increased in *hac1-1* and *hac1-2*, whereas there was no detectable difference in the *CO* mRNA levels between wt and *hac1*. Consistent with these observations, the *FT* and *SOC1* mRNA levels were decreased in the mutants (Figure 2d). Furthermore, the late-flowering phenotype of *hac1* was completely suppressed by an *flc* null mutation (Figure 2e,f), thereby demonstrating that *HAC1* controls flowering by negatively regulating *FLC* expression.

### *Arabidopsis* CBP/p300*-like genes have redundant functions in flowering time regulation*

Although morphological phenotypes were not detected among the *hac5* or *hac12* single mutants (Figure 3c), the conserved domain organizations (Figure 1) and high sequence similarities among HAC1, HAC5 and HAC12 (Supplementary Figure S2) suggest the possibility of functional redundancy among these genes. In order to test this possibility, we generated double mutants between the three genes (*hac1-1 hac5-1*, *hac1-1 hac12-1* and *hac5-1 hac12-1*) and examined their phenotypes. Additional loss of HAC5 activity or, to a lesser extent, loss of HAC12 activity in the *hac1-1* background dramatically delayed flowering compared to that in wt or the *hac1* single mutants (Figure 3a). However, the *hac5-1 hac12-1* double mutants flowered slightly later than wt but earlier than the *hac1* single mutants (Figure 3a; wt, *hac5-1 hac12-1* and *hac1-1* flowered with $10.1 \pm 0.7$, $13 \pm 1.3$, and $16.1 \pm 1.4$ rosette leaves, respectively).

For the following two reasons, it was unlikely that the strong late-flowering phenotypes of *hac1-1 hac5-1* and *hac1-1 hac12-1* were caused by mutations independent of the lesions in *HAC1*, *HAC5* and *HAC12*. Firstly, the two independent mutant alleles of *HAC1* (*hac1-1* and *hac1-2*) showed consistent late-flowering phenotypes, and this phenotype was enhanced by mutations in either *HAC5* or *HAC12*, two homologous genes of *HAC1*. Secondly, it is very unlikely that both *hac5-1* and *hac12-1* alleles carry the same-type of mutations that enhance the late-flowering phenotype of *hac1-1* in regions outside of HAC5 and HAC12, respectively. Taken together, these results suggest that (i) *HAC1*, *HAC5* and *HAC12* are functionally redundant in the regulation of flowering, (ii) *HAC1* plays a dominant role over the other functionally redundant HAC genes, and (iii) *HAC5* has a more significant role than *HAC12*.

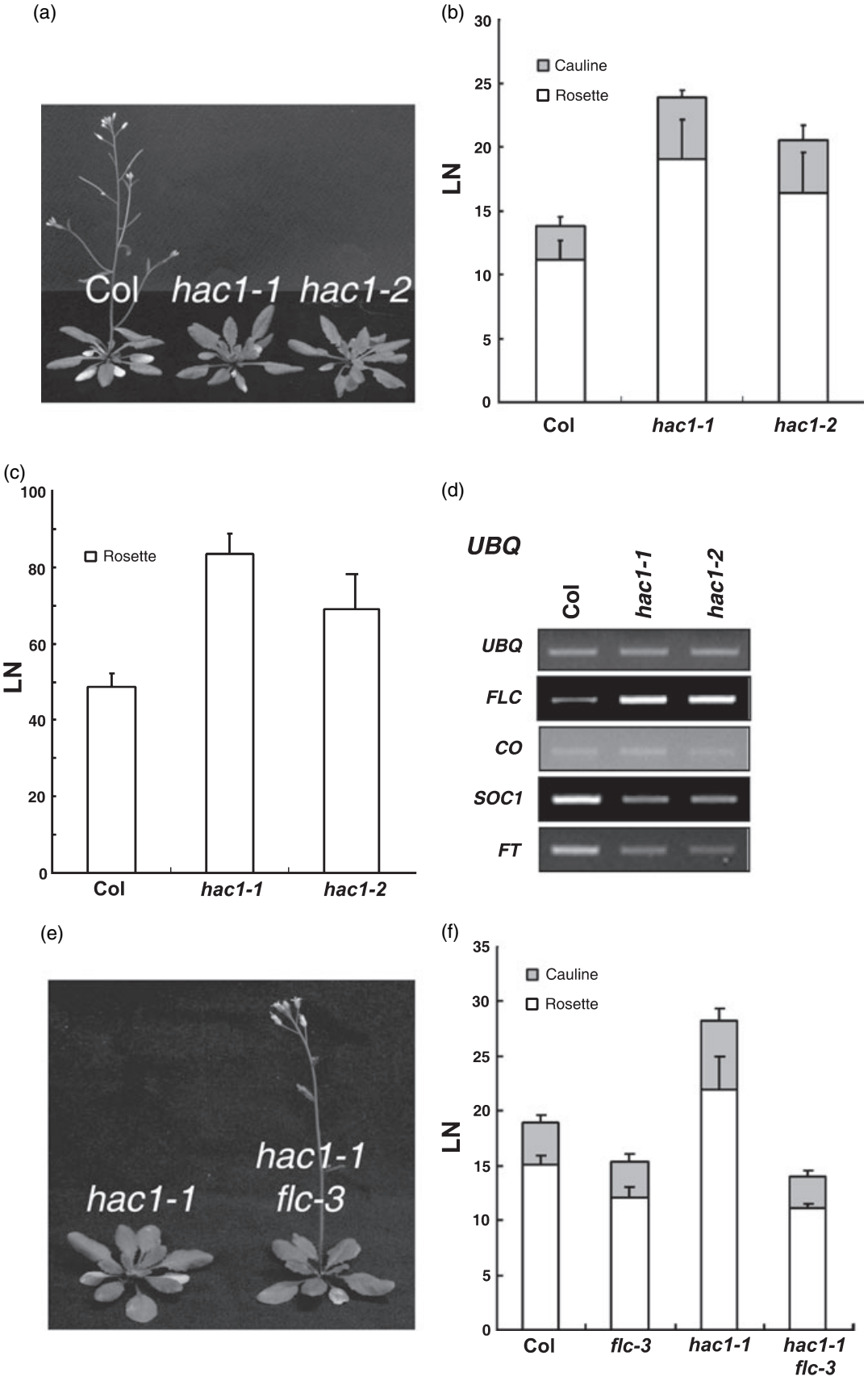
Consistent with the flowering phenotypes, the *FLC* mRNA levels in the *hac1 hac5* and *hac1 hac12* double mutants were considerably increased compared to those in the *hac1* single mutants (Figure 3b). The Arabidopsis genome has five genes encoding MADS box proteins that are highly related to *FLC* (*MAF1–MAF5*; Parenicova *et al.*, 2003). Previous studies showed that a portion of these genes are co-regulated with *FLC* (Deal *et al.*, 2005; He *et al.*, 2004). Therefore, we examined whether HACs are also involved in the transcriptional regulation of these *FLC* paralogs. Among these genes, the expression of *MAF3*, *MAF4* and *MAF5* was also weakly but significantly elevated in the *hac1* single mutants and in the *hac1 hac5* and *hac1 hac12* double mutants (Figure 3b).

### *Multiple roles of HACs in Arabidopsis development*

In addition to differences in flowering time, the *hac1 hac5* and *hac1 hac12* double mutants also displayed multiple developmental and morphological defects that were not observed in any of the single mutants. The plant size of the double mutants was smaller than that of the wt, particularly at younger stages (Figure 3c). In addition, *hac1 hac5* mutants developed round cotyledons and rosette leaves that were not observed in *hac1 hac12*, which suggests that *HAC* genes have distinct functions with respect to certain developmental programs. Similarly, human *CBP* and *p300* are not completely redundant in their functions but possess unique roles (Kalkhoven, 2004; Kawasaki *et al.*, 1998).

Flowers of the two double mutants displayed a gradient of morphological aberrancy. In a majority of the double mutant flowers, the pistils were enlarged and elongated, resulting in their protrusion from the perianth. This protrusion was not because the sepals and petals of the double mutant flowers were smaller than those of wt flowers but because the pistils of the double mutant flowers were longer than those of wt flowers (Figure 3d). In severe cases, the *hac1 hac5* double mutant flowers contained twisted and enlarged pistils, underdeveloped petals and shorter filaments than those of wt flowers, with aberrantly developed pollen grains



(Figure 3d). However, the numbers of each floral organ were not changed even in severe cases. Therefore, our data show that the functionally redundant Arabidopsis *CBP/p300-like* genes are required for the control of a variety of developmental processes including flowering.

### *Expression patterns of* HACs

To gain a deeper insight into the functional redundancy among *HAC* genes, we examined their expression patterns by RT-PCR using RNAs obtained from various tissues such as roots, leaves, leaves with SAMs, flowers and seedlings. All five *HAC* genes were expressed in all the tissues examined; however, the expression patterns varied slightly (Figure 4a). A more careful examination of the spatial expression patterns of *HAC1*, *HAC5* and *HAC12* by using transgenic plants carrying promoter:GUS fusion constructs revealed GUS-staining patterns that were comparable to the RT-PCR results (Figure 4b). In general, all three genes showed similar expression patterns, and there was a good agreement between the gene expression sites and the organs that displayed morphological abnormalities in the mutants. The expression patterns of the three genes at the seedling stage were similar to the spatial expression pattern of *FLC* (Figure 4b), which was consistent with their function as *FLC* repressors. On the other hand, the absence of *FLC* expression in the anthers and stigmas, where *HAC* genes are highly expressed, and the high level of *FLC* expression in the styles, where the expression of *HAC* genes is undetectable (Figure 4b), suggest the possibility of *FLC* repression by *HAC* genes in these floral tissues. It was observed that the green fluorescent protein (GFP) fused with the full-length HAC1 protein was localized in the nucleus when transiently expressed in onion epidermal cells (Figure 4c). This suggests the role of HACs as transcriptional co-factors.

### *No reciprocal transcriptional regulation occurs between* HAC *genes and other* FLC *regulators*

In order to understand the molecular mechanism underlying *HAC*-mediated *FLC* repression, we first tested whether *HAC* expression is affected by *FRI* (Johanson *et al.*, 2000) or by the autonomous pathway *FLC* repressors, namely *FCA* (Macknight *et al.*, 2002), *FY* (Simpson *et al.*, 2003), *FLK* (Lim *et al.*, 2004), *FPA* (Schomburg *et al.*, 2001), *LD* (Lee *et al.*, 1994), *FLD* (He *et al.*, 2003), *FVE* (Ausín *et al.*, 2004) and *REF6* (Noh *et al.*, 2004a,b). However, the transcript levels of *HAC1*, *HAC5* and *HAC12* were not altered by *FRI* or by mutations in the autonomous pathway genes (Figure 5a).

As CBP and p300 are generally known as transcriptional co-activators, we tested whether the expression of the autonomous pathway *FLC* repressors is reduced by *hac* mutations. No difference was observed in the mRNA levels of the *FLC* repressors between wt and the *hac* mutants (Figure 5b), indicating that the HAC-mediated *FLC* repression does not involve an increased mRNA level of other *FLC* repressors. Additionally, we examined the expression of previously identified *FLC* activators, namely *ABH1* (Bezerra *et al.*, 2004), *EFS* (Kim *et al.*, 2005), *ELF5* (Noh *et al.*, 2004a,b), *ELF7* (He *et al.*, 2004), *ESD4* (Reeves *et al.*, 2002), *FRL1* and *FRL2* (Michaels *et al.*, 2004), *HUA2* (Doyle *et al.*, 2005), *PIE1* (Noh and Amasino, 2003), *SE* (Bezerra *et al.*, 2004; Prigge and Wagner, 2001), *SUF3* (Choi *et al.*, 2005), and *VIP3–VIP6* (Oh *et al.*, 2004; Zhang *et al.*, 2003) in wt and the *hac* mutants. The transcript levels of the *FLC* activators were not altered by the *hac* mutations (Figure 5c). Therefore, HACs might directly affect *FLC* transcription, or the HAC-mediated control of *FLC* expression might involve the regulation of so far unidentified *FLC* regulators.

### *HAC-mediated* FLC *repression might involve reversible protein acetylation independent of histone acetylation within* FLC *chromatin*

It is unlikely that HACs repress *FLC* transcription through histone acetylation of *FLC* chromatin because this process is correlated with the transcriptional activation rather than repression of *FLC* (Ausín *et al.*, 2004; He *et al.*, 2003; Noh *et al.*, 2004a,b), and this is consistent with the general concept of the relationship between histone acetylation and transcriptional activation (Eberharter and Becker, 2000; Kalkhoven, 2004). In fact, chromatin immunoprecipitation (ChIP) assays with antibodies specific to penta-acetylated histone H4 or tetra-acetylated histone H3 revealed no detectable differences in the acetylation levels of the H3 and H4 histones within *FLC* chromatin in the *hac* mutants when compared with that in the wt (Figure 6b).

Therefore, what is the mechanism by which HACs repress *FLC* transcription? A growing body of evidence has shown that certain HATs, including CBP/p300, are also capable of acetylating non-histone transcription-related proteins and regulate their function (Bereshchenko *et al.*, 2002; Gu and

**Figure 2.** HAC1 acts as an *FLC* repressor.
(a) Wild-type (Col) and *hac1* plants grown for 35 days under long days (LD).
(b) Flowering time of *hac1* under LD. LN, leaf number.
(c) Flowering time of *hac1* under SD.
(d) Increased expression of *FLC* but not *CO* mRNA in *hac1*. The mRNA expression was studied by RT-PCR analyses using RNAs isolated from 10-day-old seedlings grown under LD. *Ubiquitin* (*UBQ*) was used as the control.
(e) *hac1-1* and *hac1-1 flc-3* plants grown for 24 days under LD.
(f) Suppression of *hac1*-mediated late flowering by *flc-3* (Michaels and Amasino, 2001).

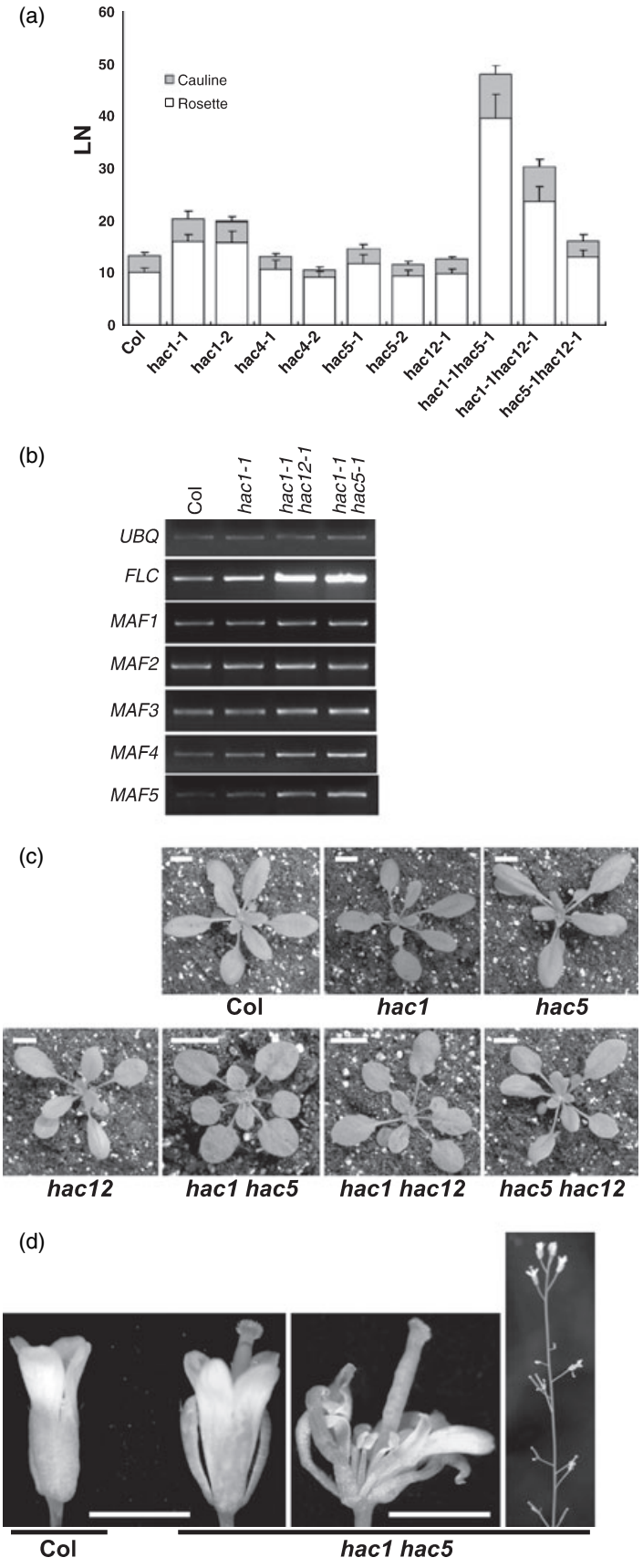



**Figure 3.** HAC1, HAC5 and HAC12 are functionally redundant.
(a) Flowering time of the single and double *hac* mutants grown under long days (LD).
(b) Expression of *FLC* and *FLC* homologs. The mRNA expression of the marked genes was studied by RT-PCR analyses using RNAs isolated from 7-leaf-stage whole plants grown under LD.
(c) Phenotypes of the *hac* mutants grown for 23 days under LD. Bar = 1 cm.
(d) Flower and inflorescence of *hac1-1 hac5-1*. Left, a flower of the *hac1 hac5* double mutant compared with that of wild-type Col; middle, a flower of *hac1 hac5* with severe phenotypes; right, an inflorescence of *hac1 hac5*. Bar = 2 mm.

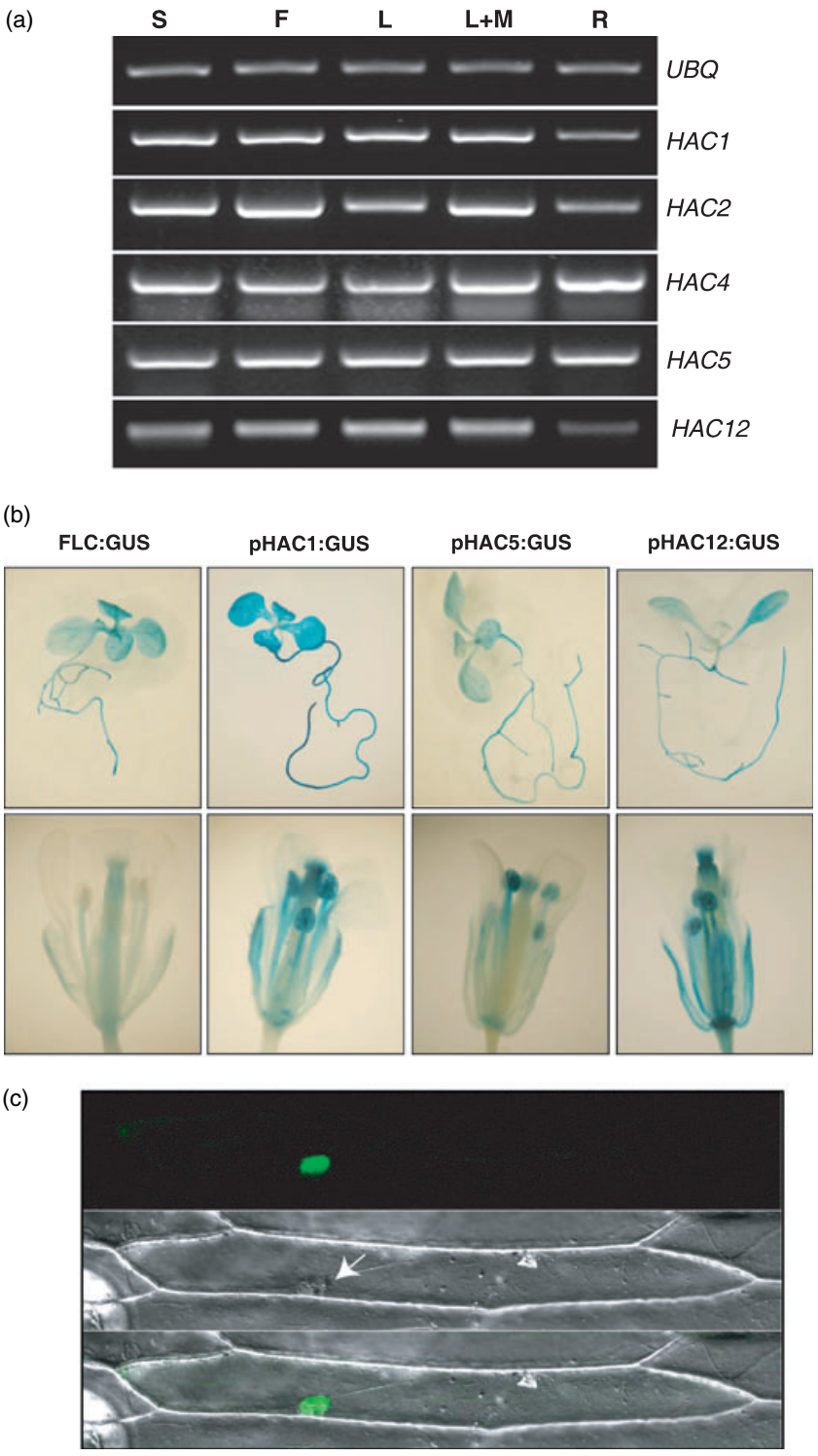



**Figure 4.** Expression of the *HAC* genes.
(a) mRNA expression of the *HAC* genes in various tissues. Tissues were collected from 10-day-old seedlings (S), floral buds and open flowers (F), adult leaves (L), entire shoots including the shoot apical meristems (L + M), and roots (R). The expression was studied by RT-PCR analyses.
(b) Histochemical GUS staining of transgenic Arabidopsis containing the marked GUS fusion constructs. The 5′ upstream promoter sequences were used to drive the GUS expression for *HAC1*, *HAC5* and *HAC12*. The *FLC*:GUS construct has been described previously (Michaels *et al.*, 2005).
(c) Nuclear localization of the HAC1:GFP fusion protein. Onion epidermal cells transiently expressing HAC1:GFP, as a green florescence image (top), a bright-field image (middle), and a merged image (bottom). The arrow indicates the nucleus.

Roeder, 1997; Guidez *et al.*, 2005; Kalkhoven, 2004; Korzus *et al.*, 2004; Yuan *et al.*, 2005). It is possible that such post-translational acetylation is involved in the HAC-mediated *FLC* repression. As the activity of HATs on either histones or non-histone proteins is dynamically counterbalanced by the opposing activity of histone deacetylases (HDACs) in order to attain appropriate transcription levels (Korzus *et al.*, 2004; Martínez-Balbás *et al.*, 2000; Yuan *et al.*, 2005), we studied whether the loss of HAT activity in the *hac* mutants is compensated by the elimination of HDAC activity, and whether the *hac* mutations increase *FLC* expression by causing hyperacetylation of a target protein other than

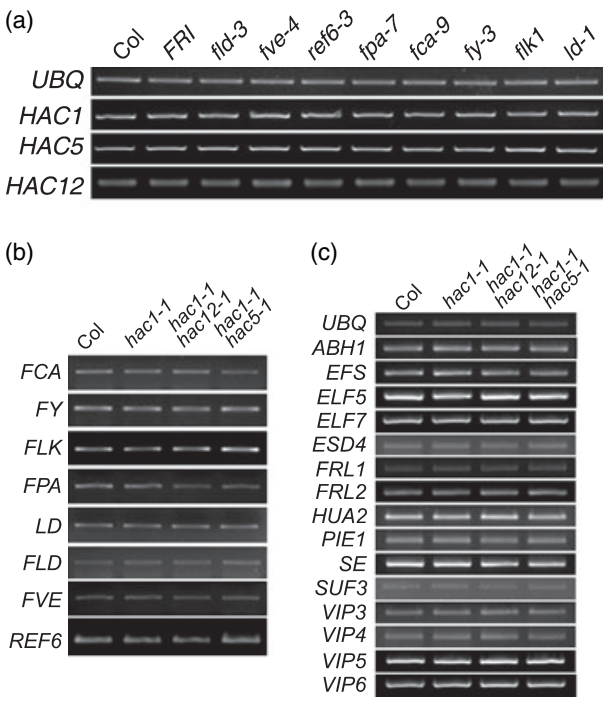



**Figure 5.** There is no reciprocal transcriptional regulation between *HAC* genes and other *FLC* regulators.
(a) Transcription of *HAC1, 5* and *12* is not regulated by other *FLC* repressors. RT-PCR analyses were performed as described in Figure 2(d).
(b) mRNA expression of the autonomous pathway *FLC* repressors in wt, *hac1*, *hac1 hac12* and *hac1 hac5*. Expression was studied as described in Figure 3(b).
(c) mRNA expression of the *FLC* activators in wt, *hac1*, *hac1 hac12* and *hac1 hac5*. Expression was studied as described in Figure 3(b).

histones within *FLC* chromatin. When *hac1* and *hac1 hac12* were treated with the HDAC inhibitor trichostatin A (TSA), the elevated *FLC* transcript levels in the mutants decreased (Figure 6c), although the histone acetylation levels within *FLC* chromatin increased (Figure 6d). *hac1 hac12* rather than *hac1 hac5* double mutants were used for the TSA treatment of seedlings because of the severe sterility of *hac1 hac5* mutant plants.

This result indicates that HDAC activity is required for the activation of *FLC* transcription as well as for the deacetylation of histones within *FLC* chromatin that has been implicated in the repression of *FLC* transcription (Ausín *et al.*, 2004; He *et al.*, 2003; Noh *et al.*, 2004a,b). Thus, *FLC* transcription appears to be affected by the balance between HAC-mediated HAT activity and HDAC activity for substrates other than histones, and it may be suggested that another layer of protein acetylation/deacetylation independent of histone modifications is required for the regulation of *FLC* transcription. Alternatively, HACs might control *FLC* expression through a modification of histones within the chromatin of so far unidentified *FLC* regulators. In future studies, the identification of HAC-interacting proteins or genome-wide expression analyses using the *hac* mutants might be useful in elucidating the biochemical mechanisms of the HAC-mediated *FLC* regulation.

## Discussion

CBP and p300 are well-known transcriptional co-factors that are involved in multiple aspects of animal development (reviewed in Goodman and Smolik, 2000; Kalkhoven, 2004). Although the HAT activity (Bordoli *et al.*, 2001) and transcriptional co-activator function in a protoplast system (Bharti *et al.*, 2004) of the plant CBP/p300-like protein HAC1 have been demonstrated, the *in vivo* roles of plant CBPs have not yet been addressed. In this study, we demonstrated the *in vivo* roles of Arabidopsis CBP family genes using a reverse genetics approach. We found that three (*HAC1*, *HAC5* and *HAC12*) of the five Arabidopsis *CBP/p300-like* genes have redundant roles in the promotion of flowering through the repression of mRNA expression of the central floral repressor *FLC* (Figure 3a,b). These genes also have other roles in Arabidopsis development as evidenced from the formation of smaller round leaves in *hac1 hac5* (Figure 3c) and the abnormal flower development and reduced fertility in *hac1 hac5* and *hac1 hac12* (Figure 3d). Thus, our study reports multiple *in vivo* roles of plant CBPs in Arabidopsis development. One of their prominent roles is to promote flowering through the repression of *FLC*.

Eight members (*LD*, *FCA*, *FPA*, *FY*, *FLD*, *FVE*, *FLK* and *REF6*) of the autonomous floral regulatory pathway that promote flowering through *FLC* repression have been identified using forward genetics approaches. In this study, we report the ninth functionally redundant member of the autonomous floral regulatory pathway by using a reverse genetics approach. We found that *HAC1* specifically represses *FLC* (Figure 2d,f), and this repressive role is shared by two other members of the Arabidopsis *CBP/p300-like* family (Figure 3a,b). Based on previous studies using forward molecular genetics approaches, we believe that the mild late-flowering phenotype of the *hac1* single mutants (Figures 2 and 3a) might prevent the identification of *HAC1* as an *FLC* repressor by these methods. Further, it might not have been possible to determine the roles of *HAC5* and *HAC12* as autonomous pathway floral regulators by using forward genetics approaches because their single mutants display normal flowering phenotypes (Figure 3a). Therefore, our study suggests that there might be more unidentified members in the autonomous pathway as well as in other floral regulatory pathways, and that the identification of such novel floral regulators by approaches other than forward genetics could lead to a better understanding of flowering time regulation.

In eukaryotic cells, histone acetylation and deacetylation have generally been implicated in the positive and negative regulation of transcription, respectively. Accordingly, HATs and HDACs have been identified as components of tran-

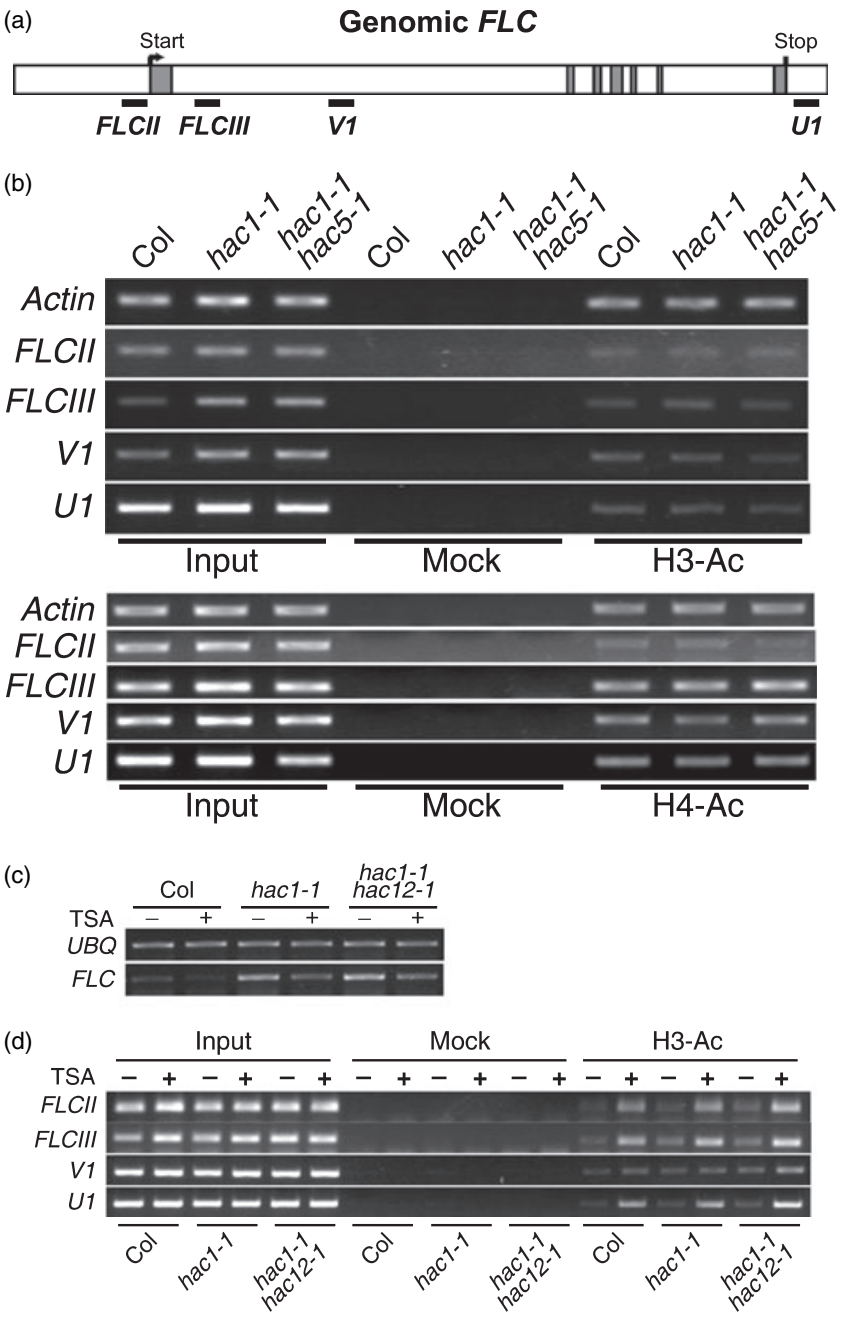



**Figure 6.** Regulation of *FLC* transcription by HACs is independent of histone acetylation within *FLC* chromatin.
(a) Schematic structure of *FLC*. *FLCII*, *FLCIII*, *V1* and *U1* indicate regions in which histone H3 or H4 acetylation states were examined by ChIP and have been described previously (He *et al.*, 2003; Sung and Amasino, 2004). The translation start and stop points are indicated. The gray boxes represent exons, and the open boxes represent introns or untranslated regions.
(b) ChIP analyses of *FLC* chromatin using antibodies against hyperacetylated histones H3 and H4. 'Input' indicates the chromatins before immunoprecipitation. 'Mock' refers to the control samples lacking the antibody. *Actin* served as an internal control.
(c) TSA represses *FLC* transcription. *FLC* expression was studied by RT-PCR using RNAs isolated from seedlings either treated with (+) or without (–) TSA.
(d) TSA increases histone acetylation levels within *FLC* chromatin.

scriptional activator and repressor complexes, respectively (reviewed in Lee and Young, 2000). This positive relationship between histone acetylation and transcription was also found in *FLC* transcription (Ausín *et al.*, 2004; He *et al.*, 2003; Noh *et al.*, 2004a,b). Therefore, it was predicted that HATs and HDACs might play activator and repressor roles, respectively, in the transcriptional regulation of *FLC*.

Contrary to our expectations, we found that the CBP/p300-like HATs (HAC1, HAC5 and HAC12) function as transcriptional repressors of *FLC* (Figures 2d and 3b). One of the explanations for this reverse role of HACs is that CBP/p300 might activate the transcription of other *FLC* repressors that in turn repress *FLC* transcription. However, no change was observed in the mRNA expression of the eight previously identified *FLC* repressors (Figure 5b) and the known *FLC* activators (Figure 5c) in the *hac1* single mutants and the *hac1 hac12* and *hac1 hac5* double mutants. Therefore, HACs might directly affect *FLC* transcription or be required for the regulation of so far unidentified *FLC* regulators.

Although the mechanism underlying HAC-mediated *FLC* repression remains unclear, our data suggest that HATs and HDACs might be involved in at least two different modes of

transcriptional regulation of *FLC*. Firstly, the pharmacological inhibition of HDAC activities reduced *FLC* expression (Figure 6c) but induced the hyperacetylation of histones within *FLC* chromatin (Figure 6d). Secondly, the loss of HAT activities in the *hac* mutants caused increased transcript levels of *FLC* (Figure 3b) independently of histone acetylation within *FLC* chromatin (Figure 6b).

It is possible that HACs affect *FLC* expression by affecting the expression of unidentified *FLC* repressors. However, an aspect of CBP/p300 function suggests another possible mechanism by which *FLC* repression is directly mediated by HACs. It has been reported that HATs acetylate non-histone nuclear proteins as well as histones, resulting in either increased or decreased DNA–protein or protein–protein interactions for the non-histone proteins (Bereshchenko *et al.*, 2002; Gu and Roeder, 1997; Guidez *et al.*, 2005; Kalkhoven, 2004; Korzus *et al.*, 2004; Yuan *et al.*, 2005). Thus, it is possible that HACs might affect *FLC* transcription by modifying the activity of other *FLC* transcriptional regulators.

Our data show that the open configuration of *FLC* chromatin induced by increased histone acetylation is not sufficient for the transcriptional activation of *FLC* (Figure 6c,d). These results could be explained by the hypothesis that the reduced activity of HDAC might increase the pool of acetylated active *FLC* repressors as well as acetylated histones. In addition, the open configuration of *FLC* chromatin induced by histone acetylation might increase the accessibility of DNA to the acetylated active repressors as well as to the activators. Alternatively, the inhibition of HDAC activity might decrease the pool of functionally active deacetylated *FLC* transcriptional activators.

Although previous studies on *FLD* (He *et al.*, 2003), *FVE* (Ausín *et al.*, 2004) and *REF6* (Noh *et al.*, 2004a,b) showed positive relationships between histone acetylation and the expression level of *FLC* mRNA, biochemical evidence recently reported shows that amine oxidases homologous to FLD are actually demethylases specific for di-methylated histone H3 lysine 4 (Shi *et al.*, 2004), and that the Jumonji C omains that REF6 has possess demethylase activities specific for histone H3 lysine 36 and/or lysine 9 (Tsukada *et al.*, 2006; Whetstine *et al.*, 2006; Yamane *et al.*, 2006). The biochemical function of FVE is not yet clear. Therefore, the significance of the hyperacetylation of histones at the *FLC* locus observed in *fld*, *ref6* and *fve* mutants is yet to be clarified, and it would be premature to claim that the hyperacetylation of histones is indeed a cause for the higher level of expression of *FLC* mRNA in those mutants. It is possible that the hyperacetylation is an indirect effect of altered methylation levels of histones at the *FLC* locus or an event accompanying the transcriptional activation of *FLC*. Our data in Figure 6 are consistent with the possibility that the hyperacetylation is not a direct cause of the transcriptional activation of *FLC*. We propose that HACs might contribute to the regulation of *FLC* transcription by acetylating another *FLC* transcriptional activator or repressor to alter its DNA-binding affinity or its ability to recruit other transcriptional co-regulators.

## Experimental procedures

### *Plant materials*

The following *hac* T-DNA insertion lines were obtained from the SALK collection (http://signal.salk.edu/): *hac1-1*, SALK_082116; *hac1-2*, SALK_070277; *hac2-1*, SALK_049434; *hac4-1*, SALK_051750; *hac4-2*, SALK_006923; *hac5-1*, SALK_074472; *hac5-2*, SALK_024278; *hac12-1*, SALK_052490; *hac12-2*, SALK_071102. *fy-3* is SALK_053604. All the transgenic and mutant plants used in this study are in the Col background. The late-flowering autonomous pathway mutants have been described previously (Ausín *et al.*, 2004; He *et al.*, 2003; Johanson *et al.*, 2000; Lee *et al.*, 1994; Lim *et al.*, 2004; Macknight *et al.*, 2002; Noh *et al.*, 2004a,b; Schomburg *et al.*, 2001; Simpson *et al.*, 2003). All the plants were grown under 100 $\mu$E $m^{-2}$ $sec^{-1}$ cool white fluorescent light at 22°C. For the TSA treatment, the seedlings were incubated in the presence of 20 $\mu$M TSA (Sigma, St Louis, MO, USA) for 2 h, and then harvested for RNA or chromatin preparation.

### *Flowering time analyses*

The flowering times were measured as the number of rosette and cauline leaves (LN) formed by the primary meristem, and the data are presented as means ± SD for at least 12 plants for each genotype.

### *RT-PCR analyses*

Total RNA was isolated from 10-day-old or 7-leaf-stage plants using the TRI reagent (Sigma) according to the manufacturer's instructions. Reverse transcription was performed using Superscript II (Invitrogen Life Technologies, Carlsbad, CA, USA) followed by quantitative PCR of the first-strand DNA with ExTaq polymerase (TaKaRa Bio, Otsu, Shiga, Japan). Sequences of the RT-PCR primers used to study the expression of the *HAC* genes are provided in Supplementary Table S1. Sequences of the RT-PCR primers used to study the expression of the flowering genes other than *HAC* genes are available on request.

### *ChIP assays*

ChIP was performed as described previously (Noh *et al.*, 2004a,b) with a few modifications. To purify DNA from immunoprecipitated complexes, the QIAquick Spin Column (Qiagen, Hilden, Germany) was used instead of the phenol/chloroform extraction method. The details and sequences of the primers that were used to amplify different *FLC* regions in the ChIP assays have been described previously (He *et al.*, 2003; Sung and Amasino, 2004).

### *Histochemical GUS staining*

In order to create the *HAC* promoter:GUS fusion constructs, DNA fragments containing the regions from −1187 to −1, −4370 to −1 and −3070 to −1 of *HAC1*, *HAC5* and *HAC12*, respectively, were cloned into the pPZP211-GUS vector (Noh and Amasino, 2003). Each DNA construct was introduced into *Agrobacterium tumefaciens* strain

ABI, followed by transformation of Col wt plants (Clough and Bent, 1998). Histochemical GUS staining was performed on the transgenic plants as previously described (Noh *et al.*, 2001). Primers used for DNA construction are listed in Supplementary Table S2. The seedlings shown in Figure 4(b) were grown for 8 days under LD before staining.

### *Subcellular localization assay*

The 5076 bp *HAC1* cDNA was obtained by RT-PCR using HAC1GFP-F and HAC1GFP-R (Supplementary Table S2) as primers. The cDNA was cloned into the JJ461 binary vector in the region between the CaMV 35S promoter and *GFP*, thereby creating a C-terminal translational fusion of *GFP* to *HAC1*. For the transient expression of *HAC1:GFP*, the plasmid DNA was introduced into onion epidermal cells by DNA-coated gold particle bombardment. After incubation at 25°C for 1 day, the cells were observed using a confocal microscope (LSM 510 META; Carl Zeiss, Jena, Germany).

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## Supplementary material

The following supplementary material is available for this article online:

**Figure S1.** Leaf initiation rate of the *hac1-1* and *hac1-2* mutants compared with the wt (Col).

**Figure S2.** Sequence comparison of HAC1, 2, 4, 5 and 12.

**Table S1** Primers used for RT-PCR analyses of *HAC* expression

**Table S2** Primers used for the plasmid DNA constructions

## References

**Ausín, I., Alonso-Blanco, C., Jarillo, J., Ruiz-García, L. and Martínez-Zapater, J.M.** (2004) Regulation of flowering time by FVE, a retinoblastoma-associated protein. *Nature Genet.* **36**, 162–166.

**Bannister, A.J. and Kouzarides, T.** (1996) The CBP co-activator is a histone acetyltranscferase. *Nature*, **384**, 641–643.

**Bereshchenko, O.R., Gu, W. and Dalla-Favera, R.** (2002) Acetylation inactivates the transcriptional repressor BCL6. *Nature Genet.* **32**, 606–613.

**Bezerra, I.C., Michaels, S.D., Schomberg, F.M. and Amasino, R.M.** (2004) Lesions in the mRNA cap-binding gene ABA HYPERSENSITIVE 1 suppress FRIGIDA-mediated delayed flowering in Arabidopsis. *Plant J.* **40**, 112–119.

**Bharti, K., von Koskull-Döring, P., Bharti, S., Kumar, P., Tintschl-Körbitzer, A., Treuter, E. and Nover, L.** (2004) Tomato heat stress transcription coactivator with a histone-like motif interacting with the plant CREB binding protein ortholog HAC1. *Plant Cell*, **16**, 1521–1535.

**Bordoli, L., Netsch, M., Lüthi, U., Lutz, W. and Eckner, R.** (2001) Plants orthologs of p300/CBP: conservation of a core domain in metazoan p300/CBP acetyltransferase-related proteins. *Nucl. Acids Res.* **29**, 589–597.

**Boss, P.K., Bastow, R.M., Mylne, J.S. and Dean, C.** (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. *Plant Cell*, **16**, S18–S31.

**Choi, K., Kim, S., Kim, S.Y., Kim, M., Hyun, Y., Choe, S., Kim, S.G., Michaels, S. and Lee, I.** (2005) SUPPRESSOR OF FRIGIDA3 encodes a nuclear ACTIN-RELATED PROTEIN6 required for a floral repression in Arabidopsis. *Plant Cell*, **17**, 2647–2660.

**Clough, S.J. and Bent, A.F.** (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735–743.

**Deal, R.B., Kandasamy, M.K., Mckinney, E.C. and Meagher, R.B.** (2005) The nuclear actin-related ARP6 is a pleiotropic development regulator required for the maintenance of *FLOWERING LOCUS C* expression and repression of flowering in Arabidopsis. *Plant Cell*, **17**, 2633–2646.

**Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K. and Zhou, M.M.** (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature*, **399**, 491–496.

**Doyle, M.R., Bizzell, C.M., Keller, M.R., Michaels, S.D., Song, J., Noh, Y.-S. and Amasino, R.M.** (2005) *HUA2* is required for the expression of floral repressors in *Arabidopsis thaliana*. *Plant J.* **41**, 376–385.

**Eberharter, A. and Becker, P.B.** (2000) Histone acetylation: a switch between repressive and permissive chromatin. *EMBO Rep.* **3**, 224–229.

**Goodman, R.H. and Smolik, S.** (2000) CBP/p300 in cell growth, transformation, and development. *Genes Dev.* **14**, 1553–1577.

**Gu, W. and Roeder, R.G.** (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell*, **90**, 595–606.

**Guidez, F., Howell, L., Isalan, M. *et al.*** (2005) Histone acetyltransferase activity of p300 is required for transcriptional repression by the promyeolocytic leukemia zinc finger protein. *Mol. Cell. Biol.* **25**, 5552–5566.

**He, Y. and Amasino, R.M.** (2005) Role of chromatin modification in flowering-time control. *Trends Plant Sci.* **10**, 30–35.

**He, Y., Michaels, S.D. and Amasino, R.M.** (2003) Regulation of flowering time by histone acetylation in *Arabidopsis*. *Science*, **302**, 1751–1754.

**He, Y., Doyle, M.R. and Amasino, R.M.** (2004) PAF1-complex-mediated histone methylation of *FLOWERING LOCUS C* chromatin is required for the vernalization responsive, winter annual habit in Arabidopsis. *Genes Dev.* **18**, 2774–2784.

**Johanson, U., West, J., Lister, C., Michaels, S., Amasino, R. and Dean, C.** (2000) Molecular analysis of *FRIGIDA*, a major determinant of natural variation in Arabidopsis flowering time. *Science*, **290**, 344–347.

**Kalkhoven, E.** (2004) CBP and p300: HATs for different occasions. *Biochem. Pharmacol.* **68**, 1145–1155.

**Kawasaki, H., Eckner, R., Yao, T.P., Taira, K., Chiu, R., Livingston, D.M. and Yokoyama, K.K.** (1998) Distinct roles of the co-activators p300 and CBP in retinoic-acid-induced F9-cell differentiation. *Nature*, **393**, 284–289.

**Kim, S.Y., He, Y., Jacob, Y., Noh, Y.-S., Michaels, S. and Amasino, R.** (2005) Establishment of the vernalization-responsive, winter-annual habit Arabidopsis requires a putative histone H3 methyl transferase. *Plant Cell*, **17**, 3301–3310.

**Korzus, E., Rosenfeld, M.G. and Mayford, M.** (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron.* **42**, 961–972.

**Lee, T.I. and Young, R.A.** (2000) Transcription of eukaryotic protein-coding genes. *Annu. Rev. Genet.* **34**, 77–137.

**Lee, I., Aukerman, M.J., Gore, S.L., Lohman, K.N., Michaels, S.D., Weaver, L.M., John, M.C., Feldmann, K.A. and Amasino, R.M.** (1994) Isolation of *LUMINIDEPENDENS*: a gene involved in the control of flowering time in Arabidopsis. *Plant Cell*, **6**, 75–83.

**Lim, M.-H., Kim, J., Kim, Y.-S., Chung, K.-S., Seo, Y.-H., Lee, I., Kim, J., Hong, C.B., Kim, H.-J. and Park, C.-M.** (2004) A new Arabidopsis gene, *FLK*, encodes an RNA binding protein with K homology motifs and regulates flowering time via *FLOWERING LOCUS C*. *Plant Cell*, **16**, 731–740.

**Macknight, R., Durox, M., Laurie, R., Dijkwel, P., Simpson, G. and Dean, C.** (2002) Functional significance or the alternative transcript processing of the Arabidopsis floral promoter *FCA*. *Plant Cell*, **14**, 877–888.

**Martínez-Balbás, M.A., Bauer, U.-M., Nielson, S.J., Brehm, A. and Kouzarides, T.** (2000) Regulation of E2F1 activity by acetylation. *EMBO J.* **19**, 662–671.

**Michaels, S.D. and Amasino, R.M.** (2001) Loss of *FLOWERING LOCUS C* activity eliminates the late flowering of *FRIGIDIA* and autonomous pathway mutants but not responsiveness to vernalization. *Plant Cell*, **13**, 935–941.

**Michaels, S.D., Bezerra, I.C. and Amasino, R.M.** (2004) *FRIGIDA*-related genes are required for the winter-annual habit in Arabidopsis. *Proc. Natl Acad. Sci. USA*, **9**, 3281–3285.

**Michaels, S.D., Himelblau, E., Kim, S.Y., Schomburg, F.M. and Amasino, R.M.** (2005) Integration of flowering signals in winter-annual Arabidopsis. *Plant Physiol.* **137**, 149–156.

**Noh, Y.S. and Amasino, R.M.** (2003) *PIE1*, an ISWI family gene, is required for *FLC* activation and floral repression in *Arabidopsis*. *Plant Cell*, **15**, 1671–1682.

**Noh, B. and Noh, Y.-S.** (2006) Chromatin-mediated regulation of flowering time in Arabidopsis. *Physiol. Plant*, **126**, 484–493.

**Noh, B., Murphy, A.S. and Spalding, E.P.** (2001) Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *Plant Cell*, **13**, 2441–2454.

**Noh, B., Lee, S.H., Kim, H.-J., Yi, G., Shin, E.-A., Lee, M., Jung, K-J., Doyle, M.R., Amasino, R.M. and Noh, Y.-S.** (2004a) Divergent roles of a pair of homologous jumonji/zinc-finger-class transcription factor proteins in the regulation of flowering time. *Plant Cell*, **16**, 2602–2613.

**Noh, Y.S., Bizzel, C.M., Noh, B., Schomberg, F.M. and Amasino, R.M.** (2004b) *EARLY FLOWERING 5* acts as a floral repressor in Arabidopsis. *Plant J.* **38**, 664–672.

**Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H. and Nakaratani, Y.** (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell*, **87**, 953–959.

**Oh, S., Zhang, H., Ludwig, P. and van Nocker, S.** (2004) A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the Arabidopsis *FLC*/*MAF* MADS box gene family. *Plant Cell*, **16**, 2940–2953.

**Pandey, R., Muller, A., Napoli, C., Selinger, D.A., Pikaard, C.R., Richards, E.J., Bender, J., Mount, D.W. and Jorgenson, R.A.** (2002) Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucl. Acids Res.* **30**, 5036–5055.

**Parenicova, L., de Folter, S., Kieffer, M. *et al.*** (2003) Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in Arabidopsis: new openings to the MADS world. *Plant Cell*, **15**, 1538–1551.

**Petrij, F., Giles, R.H., Dauwerse, H.G. *et al.*** (1995) Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature*, **376**, 348–351.

**Ponting, C.P., Blake, D.J., Davies, K.E., Kendrick-Jones, J. and Winder, S.J.** (1996) ZZ and TAZ: new putative zinc fingers in dystrophin and other proteins. *Trends Biochem. Sci.* **21**, 11–13.

**Prigge, M.J. and Wagner, D.R.** (2001) The Arabidopsis SERRATE gene encodes a zinc-finger protein required for normal shoot development. *Plant Cell*, **13**, 1263–1279.

**Reeves, P.H., Murtas, G., Dash, S. and 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.

**Schomburg, F.M., Patton, D.A., Meinke, D.W. and Amasino, R.M.** (2001) *FPA*, a gene involved in floral induction in Arabidopsis, encodes a protein containing RNA-recognition motifs. *Plant Cell*, **13**, 1427–1436.

**Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., Casero, R.A. and Shi, Y.** (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, **119**, 941–953.

**Simpson, G.C. and Dean, C.** (2002) Arabidopsis, the Rosetta stone of flowering time? *Science*, **296**, 285–289.

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

**Spiegelman, B.M. and Heinrich, R.** (2004) Biological control through regulated transcriptional coactivators. *Cell*, **119**, 157–167.

**Sterner, D.E. and Berger, S.L.** (2000) Acetylation of histone and transcription-related factors. *Microbiol. Mol. Biol. Rev.* **64**, 435–459.

**Sung, S. and Amasino, R.M.** (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature*, **427**, 159–164.

**Tsukada, Y.-I., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P. and Zhang, Y.** (2006) Histone demethylation by a family of JmjC domain-containing proteins. *Nature*, **439**, 811–816.

**Whetstine, J.R., Nottke, A., Lan, F. *et al.*** (2006) Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell*, **125**, 467–481.

**Yamane, K., Toumazou, C., Tsukada, Y., Erdjument-Bromage, H., Tempst, P., Wong, J. and Zhang, Y.** (2006) JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell*, **125**, 483–495.

**Yuan, Z.-L., Guan, Y.-J., Chatterjee, D. and Chin, Y.E.** (2005) Stat3 dimerization regulated by reversible acetylation of single lysine residue. *Science*, **307**, 269–273.

**Zhang, H., Ransom, C., Ludwig, P. and van Nocker, S.** (2003) Genetic analysis of early flowering mutants in Arabidopsis defines a class of pleiotropic developmental regulator required for expression of the flowering-time switch *FLOWERING LOCUS C*. *Genetics*, **164**, 347–358.

 