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RESEARCH COMMUNICATION

Role of *SVP* in the control of flowering time by ambient temperature in *Arabidopsis*

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Plants must perceive and rapidly respond to changes in ambient temperature for their successful reproduction. Here we demonstrate that *Arabidopsis SHORT VEGETATIVE PHASE* (*SVP*) plays an important role in the response of plants to ambient temperature changes. The loss of *SVP* function elicited insensitivity to ambient temperature changes. *SVP* mediates the temperature-dependent functions of *FCA* and *FVE* within the thermosensory pathway. SVP controls flowering time by negatively regulating the expression of a floral integrator, *FLOWERING LOCUS T* (*FT*), via direct binding to the CArG motifs in the *FT* sequence. We propose that this is one of the molecular mechanisms that modulate flowering time under fluctuating temperature conditions.

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Plants are sessile organisms and are, consequently, exposed to a wide variety of environmental stresses, both abiotic and biotic, exerted by their surroundings. The most common of these is temperature. Within the range of temperatures tolerable to plants, the response to low temperature, particularly near-freezing temperature, is well understood. Plants have evolved a number of adaptive mechanisms to meet the challenge of low temperature. In *Arabidopsis,* flowering is accelerated by prolonged exposure to cold, a process called vernalization. The epigenetic silencing of the *FLOWERING LOCUS C* (*FLC*) (Michaels and Amasino 1999; Sheldon et al. 1999) is central to the vernalization process (Sung and Amasino 2005), and this silencing has been attributed to the activities of the *VERNALIZATION1* (*VRN1*), *VERNALIZATION2* (*VRN2*), and *VERNALIZATION INSENSITIVE3* (*VIN3*) genes (Gendall et al. 2001; Levy et al. 2002; Sung and Amasino 2004). Cold acclimation is another well-characterized response to low temperature (Guy 1990). Plants become tolerant to freezing temperatures by being previously exposed to short periods of low but nonfreezing temperatures. Analyses of mutant plants have identified C-Repeat-binding factor (*CBF*)-dependent and *CBF*-independent signaling pathways in cold acclimation (Sharma et al. 2005), suggesting that plants use distinct mechanisms to respond to low temperature.

There is increasing concern about the potential impact of global temperature changes, which significantly affect ambient temperature, on plant development. Several lines of evidence suggest that the recently observed alterations in the flowering times of many plant species and the increase in plant respiration rates are closely associated with these changes in ambient temperature (Fitter and Fitter 2002; Atkin and Tjoelker 2003). Although a great deal of progress has been made in our understanding of the regulation of plant development by low temperature, less is currently known about the molecular mechanisms underlying the responses of plants to changes in ambient temperature (Coupland and Prat Monguio 2005; Samach and Wigge 2005). Here, we show that the *SHORT VEGETATIVE PHASE* (*SVP*) gene mediates ambient temperature signaling in *Arabidopsis* and that the *SVP*-mediated control of *FLOWERING LOCUS T* (*FT*) expression is one of the molecular mechanisms evolved by plants to modulate the timing of the developmental transition to flowering phase in response to changes in the ambient temperature.

Results and Discussion

As a first step to determining the mechanism underlying the perception and transduction of ambient temperature signaling in plants, we assessed mutants in known flowering time genes for their insensitivity to changes in ambient growth temperature. Of the flowering time mutants tested, one with a lesion in *svp* was indeed insensitive to such changes. The flowering of the majority of these flowering time mutants was noticeably delayed at 16°C, with flowering time ratios (16°C/23°C) ranging from 1.1 to 2.0 (Fig. 1A), the exception being *ld-1.* However, *svp-31* and *svp-32* mutants, the T-DNA alleles of *SVP* (Supplementary Fig. 1; Hartmann et al. 2000), manifested almost identical flowering times at 23°C and 16°C (Fig. 1A). *svp* mutants were early flowering, especially at 16°C, suggesting that a reduction in *SVP* activity significantly decreased plant response to lower temperature and that the loss of *SVP* activity would result in the loss of the effects of low temperature. In contrast, *SVP* overexpressor plants were late flowering, especially at 23°C, suggesting that overexpression of *SVP* can mimic the effect of low temperature. Reduced *FT* expression was likely responsible for this late flowering phenotype of *35S::SVP* plants (Supplementary Fig. 2). The weak temperature response seen in *35S::SVP* plants can be explained by the differential expression of *FT,* such that *FT* expression at 23°C was higher than that at 16°C in *35S::SVP* plants. *SVP* probably performs a nonredundant role in ambient temperature sensing, as loss of the function of *AGL24* (Yu et al. 2002), the closest homolog of *SVP,* did not induce temperature insensitivity.

Characterization of the pattern of *SVP* expression at different temperatures in wild-type plants by real-time PCR analyses revealed that *SVP* expression slightly in-

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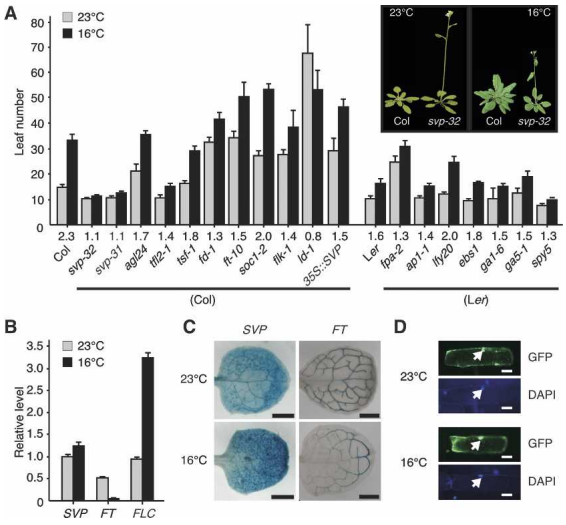

Figure 1. Role of *SVP* in the temperature-dependent control of flowering in *Arabidopsis*. (*A*) Flowering time of a group of flowering time mutants at 23°C and 16°C under long-day conditions. The numbers listed *above* the genotypes denote the ratios of flowering time at 16°C and 23°C (16°C/23°C). Error bars indicate the standard deviation. The *inset* shows wild-type Columbia (Col) plants and *svp-32* plants grown at 23°C and 16°C. (*B*) Effects of low temperature on *SVP* expression in wild-type plants (Col). *SVP, FLC,* and *FT* expression levels were measured by real-time PCR in the leaf of 10-d-old seedlings grown at the indicated temperatures. The average of three technical replicates is shown. *FLC* and *FT* were used as control genes, the expressions of which are thermo-regulated (Blázquez et al. 2003). Tubulin was used as an internal control. (*C*) Histochemical analysis of 10-d-old seedlings of *SVP*::*GUS* and *FT*::*GUS* plants grown at 23°C and 16°C. Bars, 500 µm. (*D*) Nuclear localization of SVP-GFP fusion protein in onion epidermal cells incubated at 16°C and 23°C. The nucleus is indicated by an arrow. DAPI (4′-6-Diamidino-2-phenylindole) was used for nuclear staining. Bars, 10 µm.

creased in the leaf at 16°C (Fig. 1B). In contrast, *FT* expression was strongly repressed in the leaf at 16°C. Histochemical β-glucuronidase (GUS) analysis detected both *SVP* and *FT* expression throughout the expanded leaves, but *SVP* expression was up-regulated and *FT* expression was down-regulated (Fig. 1C). Since this up-regulation of *SVP* may not be significant in itself in explaining this dramatic down-regulation of *FT* expression, it is possible that the post-transcriptional regulation of *SVP* or altered protein–protein interaction of SVP at the lower temperature may also be responsible for the reduction in *FT* transcription. Considering that *SVP* acts as a floral repressor (Hartmann et al. 2000), these data suggested that additional flower-inhibitory factors exist in the leaf at lower temperature. Subcellular localization analysis showed that the SVP-green fluorescence protein (GFP) fusion protein localized in the nucleus at both 16°C and 23°C (Fig. 1D). Taken together, these results indicate that *SVP* expression is weakly temperature dependent, similar to the thermosensory genes of other species (Johansson et al. 2002).

Ambient temperature is perceived via a genetic pathway (thermosensory pathway) that requires both *FCA* and *FVE* in *Arabidopsis* (Blázquez et al. 2003). An analysis of the genetic interaction of *svp* mutants with *fca* and *fve* mutants was conducted to ascertain whether or not *SVP* operates within the same genetic pathway as *FCA* and *FVE*. The late flowering phenotypes observed in the *fca-9* and *fve-3* mutants under long-day conditions were largely masked by the loss of *SVP* function (Fig. 2A), demonstrating that *svp* is epistatic to the *fca* and *fve* mutants. In addition, the temperature insensitivity induced by the *fca* and *fve* mutations persisted even in the absence of *SVP* function, which suggests that *SVP* functions downstream from *FCA* and *FVE* within the thermosensory pathway and that *SVP* mediates temperature signaling. Consistent with this view, *SVP* expression was elevated in the *fca* and *fve* mutants (Fig. 2B), but not in other autonomous pathway mutants (Supplementary Fig. 3), and the *flk* and *fpa* mutants were temperature sensitive (Fig. 1A). *SVP* expression, however, was regulated neither by vernalization nor by *CONSTANS* (*CO*) (Samach et al. 2000), a central regulator of the long-day pathway. The observation that both the *svp-32* mutants and wild-type plants responded similarly to gibberellin (GA) treatment or to differing light conditions (Supplementary Fig. 4) supports the premise that *SVP* functions primarily within the thermosensory pathway.

The genetic interaction of *svp* mutants with *flc* mutants was assessed in an attempt to determine whether or not *SVP* interacts with *FLC*, since *FLC* is an important regulator that mediates vernalization effects in the autonomous pathway (Michaels and Amasino 1999), and both *FLC* and *SVP* function downstream from *FCA* and *FVE* (Fig. 2A,B). The results indicate that *SVP* acts independently of *FLC* at the transcriptional level within the thermosensory pathway: *SVP* expression was unchanged in the presence of functional alleles of *FRI* or *FLC* (Fig. 2C) and *FLC* expression remained unaffected by increases or reductions in *SVP* activity (Fig. 2D). Based on these results, we propose that *SVP* is very likely a thus-far unidentified repressor that mediates the temperature-

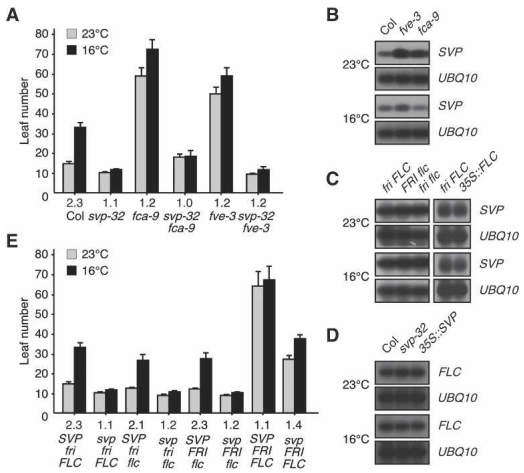

Figure 2. Genetic interaction of *SVP* with *FCA, FVE,* and *FLC*. (*A*) Flowering time of the *svp-32 fca-9*, and *svp-32 fve-3* double mutants at 23°C and 16°C under long-day conditions. The numbers listed *below* denote the ratios of flowering time (16°C/23°C). (*B*) Effects of *fca* and *fve* mutations on *SVP* expression in 10-d-old seedlings. (*C*) *SVP* expression in 10-d-old seedlings of loss- and gain-of-function alleles of *FLC*. The *FRI* locus originates from the Sf2 ecotype, and *flc* is *flc-3* (Michaels and Amasino 1999). *35S*::*FLC* plants were used rather than an allele harboring functional *FRI* and *FLC*. (*D*) *FLC* expression in 10-d-old seedlings of the loss- and gain-of-function mutants of *SVP*. (*E*) Flowering time of mutants harboring various combinations of *svp, fri,* and *flc* mutations at 23°C and 16°C. *SVP fri FLC* and *svp fri FLC* indicate wild-type Columbia plants and *svp-32* plants, respectively. The numbers listed *below* denote the ratios of flowering time (16°C/23°C).

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dependent role of *FCA* and *FVE* (Blázquez et al. 2003). *SVP* appears to function, at least in part, downstream from *FLC* by modulating flowering time in response to ambient temperatures. The flowering of *fri flc, FRI flc,* and *FRI FLC* mutants was accelerated by the *svp-32* mutation (Fig. 2E). Conversely, the temperature responsiveness exhibited by the *fri flc* and *FRI flc* mutants disappeared in the absence of *SVP* function, thereby suggesting that *SVP* exerts its effects principally within the thermosensory pathway. Interestingly, at flowering, *FRI FLC* plants had a similar number of leaves at 23°C and 16°C (64 vs. 67 leaves), which was also found in *fca* and *fve* mutants in which *FLC* levels were elevated even in the absence of functional *FRI*. A possible explanation of this flowering time phenotype of *FRI FLC* plants is that the floral repressive activity of *FLC* may be highly elevated in *FRI FLC* mutants and, consequently, further floral repression at 16°C may be masked. Consistent with this premise, temperature responses were restored in *fve flc* and *fca flc* double mutants to a level similar to that shown by *flc* single mutants (Blázquez et al. 2003; Balasubramanian et al. 2006). Of particular interest is that the severe late flowering of *FRI FLC* plants was largely suppressed by the *svp-32* mutation, suggesting that *FLC* requires *SVP* to inhibit flowering. Considering that the MADS-box proteins are known to interact physically in a protein complex (Riechmann and Meyerowitz 1997), a possible scenario to explain this suppression by the *svp-32* mutation is that SVP and FLC proteins may interact in a complex during temperature signaling. This proposal is supported by recent findings that FLC is a component of a multimeric protein complex in vivo and that SVP interacts with several MADS-box proteins (de Folter et al. 2005; Helliwell et al. 2006).

The conclusion that *SVP* functions as a floral repressor (Hartmann et al. 2000) raises an important question: On which flowering time gene does *SVP* exert its negative effects in the transduction of ambient temperature signaling? An analysis of the expression levels of known flowering time genes in the *svp* mutants revealed that the expression levels of *FT* (Kardailsky et al. 1999; Kobayashi et al. 1999), a floral integrator, were substantially elevated in the *svp-32* mutants at both 23°C and 16°C (Fig. 3A). A similar up-regulation of *FT* in the *svp-32* mutants was observed at a series of defined growth stages (Supplementary Fig. 5; Boyes et al. 2001). These observations indicated that the thermosensory signaling pathway functions, at least in part, via *FT* (Blázquez et al. 2003; Halliday et al. 2003). A reporter assay, carried out to confirm the negative regulation of *FT* expression effected by *SVP*, revealed profound ectopic *pFT::GUS* expression in both the leaves and vascular root tissues of the *svp-32* mutants (Fig. 3B). This suggests that *SVP* is required for the stable repression of *FT* in the ground tissues of the leaves of wild-type plants. Considering that *FT* is the major output of *CO* (Schmid et al. 2003; Wigge et al. 2005; Yoo et al. 2005) and that *FT* mRNA is an important component of the long-distance signaling mechanism that triggers flowering (Huang et al. 2005), the early flowering phenotypes observed in the *svp-32* mutants can be explained as follows: The absence of *SVP* activity induces the accumulation of *FT* mRNA in the leaf transportable to the shoot apex, thereby triggering floral development. Consistent with a role of *FT* downstream from *SVP*, the loss of *FT* function partially suppressed the early flowering of the *svp-32* mutants, the

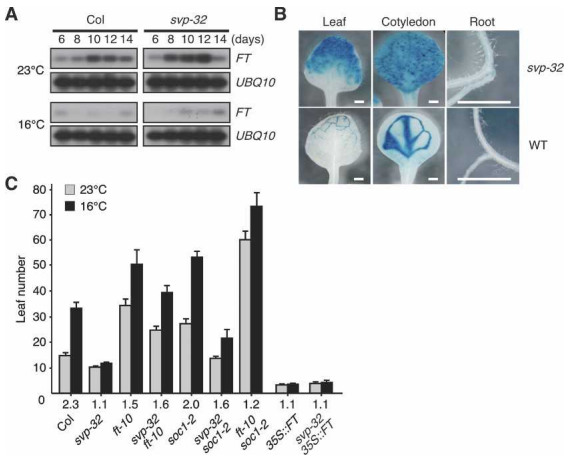

Figure 3. Role of *SVP* as an *FT* repressor. (*A*) Time-course expression of *FT* in wild-type (Col) and *svp-32* plants at 23°C and 16°C. *FT* expression level was monitored in 6-, 8-, 10-, 12-, and 14-d-old seedlings. (*B*) *pFT::GUS* expression patterns in 10-d-old seedlings of wild-type (Col) and *svp-32* plants at 23°C. Bars, 500 µm. (*C*) Flowering time of *svp-32 ft-10*, *svp-32 35S::FT*, and *ft-10 soc1-2* double mutants at 23°C and 16°C. The numbers listed *below* denote the flowering time ratios (16°C/23°C).

constitutive expression of *FT* masked the phenotype in the *svp-32* mutants (Fig. 3C), and *FT* expression was significantly reduced in *35S::SVP* plants (Supplementary Fig. 2). Importantly, *svp-32 ft-10* double mutants showed a weak temperature response, as did *ft-10* mutants (flowering time ratio = 1.6 vs. 1.5, respectively), although *svp-32* single mutants showed temperature insensitivity. Similar phenotypic masking by temperature-sensitive mutants has been observed in *fca flc* and *fve flc* mutants (Blázquez et al. 2003; Balasubramanian et al. 2006). One possible scenario explaining why *svp-32 ft-10* mutants were more responsive than *svp-32* single mutants is that *svp-32* mutants display a temperature-insensitive phenotype as the result of increased *FT* activity, the floral-promoting effects of which are more profound at 16°C. When *FT* function is absent in the double mutants, the floral-promoting effect by *FT* at 16°C is not present and, therefore, temperature sensitivity may be restored to a level similar to that found in *ft-10* single mutants. The observation that *svp-32 ft-10* mutants were—albeit weakly—temperature sensitive indicates that the ambient temperature signaling mechanism of *SVP* requires *FT* and an additional downstream target(s). One possible target candidate is *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), since the *soc1-2* mutation additively reduced the temperature sensitivity of *ft-10* mutants (flowering time ratio of *ft-10 soc1-2* double mutants = 1.2) (Fig. 3C), although *soc1-2* single mutants responded to temperature changes. Consistent with this redundant role of *FT* and *SOC1*, neither the *ft-10* nor *soc1-2* single mutations completely suppressed the early flowering phenotypes of the *svp-32* plants (Fig. 3C). Rather, the early flowering of the *svp-32* mutants is masked, in large part, by the *ft soc1* double mutation (T. Mizoguchi, pers. comm.). Nevertheless, we cannot exclude the possibility that *TWIN SISTER OF FT* (*TSF*) (Yamaguchi et al. 2005) and *FD* (Abe et al. 2005; Wigge et al. 2005) may be also the target of *SVP*.

SVP is a member of the MADS-box proteins, which function as transcriptional regulators via their DNA-

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binding motifs (Riechmann and Meyerowitz 1997). As such, it appears likely that the negative regulation of *FT* expression by the SVP protein can be achieved via direct binding to the *FT* sequence. This hypothesis was bolstered by the findings that the 1.8-kb promoter region of *FT* harbors six variants of CArG motifs (vCArG) (Fig. 4A; Tang and Perry 2003), the consensus binding sequences of the MADS-box proteins, and that the first intron of *FT* harbors a CArG motif to which FLC proteins directly bind (Helliwell et al. 2006; Searle et al. 2006). Chromatin immunoprecipitation (ChIP) assays using *Arabidopsis* protoplasts were carried out to evaluate this hypothesis. Using chromatin immunoprecipitated with HA antibodies, we detected amplified products from fragments harboring vCArG III/IV, vCArG V, and CArG VII (Fig. 4A), indicating that SVP and FLC proteins bind to these motifs in vivo. The vCArG III/IV and vCArG V motifs were more efficiently precipitated by SVP-HA. The CArG VII motif, which is present in the first intron of *FT*, was strongly enriched by FLC-HA proteins, which is consistent with previous findings (Helliwell et al. 2006; Searle et al. 2006). This motif was also precipitated by SVP-HA proteins, but SVP's binding affinity appeared to be weaker than that of FLC. It therefore appears likely that SVP preferentially binds to the vCArG motifs of the *FT* promoter and that FLC preferentially binds to the CArG VII of the first intron of *FT*. As the vCArG III/IV and V motifs were observed to bind efficiently, we verified the direct binding of the SVP proteins to these motifs in vivo by conducting a transient expression assay in protoplasts transfected with SVP-HA proteins and *FT*-promoter-driven luciferase (LUC) reporters (Fig. 4B). An abundance of SVP protein (35S::SVP-HA) effected a reduction of *FT::LUC* activity. This reduction disappeared when SVP protein was used without its MADS domain (35S::SVP ΔM-HA), thereby indicating that the reduction in luciferase activity was induced by the binding of SVP to the *FT* promoter via the MADS domain. A subsequent assay aimed at assessing the ability of SVP-HA to repress the activity of an *FT* promoter harboring a mutation in the vCArG motif revealed that SVP-HA failed to reduce the expression of *FT::LUC* harboring mutations in vCArG III (*m3FT::LUC*). This result suggests that vCArG III is required for the SVP-mediated negative regulation of *FT* expression. Coupled with the mapping of the SVP-binding site in the *FT* promoter, our findings support our hypothesis that SVP binds directly to CArG motifs, thereby regulating *FT* expression to modulate flowering time in response to ambient temperature changes.

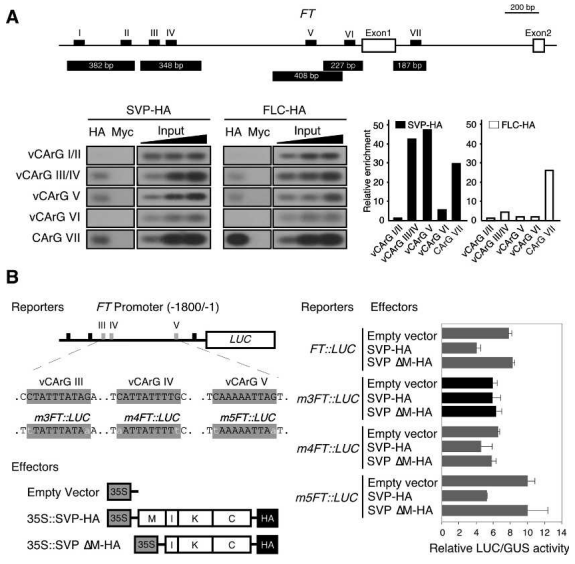

Figure 4. Binding of SVP protein to the vCArG III in the *FT* promoter. (*A*) A ChIP assay using protoplasts transfected with *SVP-HA* and *FLC-HA* constructs. The location of six vCArG motifs (vCArG I to vCArG VI) identified in a 1.8-kb *FT* promoter and the different fragments analyzed by PCR are represented. A CArG motif to which FLC is known to bind within the first intron of *FT* (Helliwell et al. 2006; Searle et al. 2006) is designated as CArG VII. A fourfold dilution series of the input DNA was used as a semiquantitative standard. Relative enrichment indicates the amplified signal value normalized against that of input DNA. The value of enrichment in vCArG I/II was set to 1 for SVP-HA and FLC-HA. Similar results were obtained from five independent experiments. (Input) Total input chromatin DNA; (HA) DNA selected using HA antibodies; (Myc) DNA selected using Myc antibodies. (*B*) The effects of SVP-HA protein on the *FT* promoter activities. A schematic representation of the reporters and the effectors used in this assay is shown. vCArG motifs are shaded in gray and mutations introduced in vCArG motifs are indicated in lowercase. *m3FT::LUC* indicates the *FT::LUC* construct harboring a mutated vCArG III. Luciferase activities were normalized by GUS activities. This experiment was repeated five times, with similar results.

In conclusion, based on the results reported here, ambient temperature signaling in *Arabidopsis* is mediated by *SVP*, which functions within the thermosensory pathway, but only partially within the *FT* pathway (Blázquez et al. 2003; Wigge et al. 2005). SVP represses *FT* expression via direct binding to the vCArG III motif in the *FT* promoter. The *SVP*-mediated control of *FT* gene expression (Fig. 4) may represent a mechanism used by the plant to adjust the timing of flower development under fluctuating temperature conditions, although we cannot dismiss the possibility that altered interactions between MADS-box proteins, including SVP, FLC, and FLM (de Folter et al. 2005; Balasubramanian et al. 2006; Helliwell et al. 2006; Searle et al. 2006), effect the adjustment of flowering times at different ambient temperatures. We propose that the genetic evidence reported here is a valuable supplement to current knowledge on the manner in which plants integrate environmental signals to modulate development.

Materials and methods

Plant materials, growth conditions, and measurement of flowering time

All mutations used in this study were in the Columbia (Col) background, unless otherwise noted. *svp-31* (SALK_026551) and *svp-32* (SALK_072930), both T-DNA insertion lines of *SVP*, were obtained from the *Arabidopsis* Biological Resource Center (ABRC) (Alonso et al. 2003). To confirm the T-DNA insertion sites of these alleles, we sequenced the PCR products amplified using left border primers and gene-specific primers. *SVP* overexpressor plants obtained from H. Sommer (Max Planck Institut, Köln, Germany) have been described previously (Masiero et al. 2004). The mutant lines used in this study are described in Supplementary Table S1. The plants were grown in soil or MS medium at 23°C or 16°C under long-day (LD) conditions (16 h light/8 h dark) with light provided at an intensity of 120 μmol m^{-2} sec^{-1}. The homozygosity of the double mutants was verified via PCR genotyping. The details of the genotyping procedures are available on request. The flowering times of the plants are expressed as the total number of primary leaves of at least 12 plants.

Expression analysis

Expression levels of the flowering time genes were determined via semiquantitative reverse transcriptase-meditated PCR or real-time PCR. To-

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tal RNA was extracted using Trizol reagent (Invitrogen), and 1 µg of total RNA was used to synthesize the complementary DNA. The primer sequences and amplification conditions are available on request. The real-time PCR analysis was performed using an ABI PRISM 7900HT sequence detection system (Applied Biosystems), and expression levels were normalized against that of tubulin. For the histochemical GUS analysis, we generated a *SVP*::*GUS* translational fusion construct. The 4.9-kb *SVP* genomic region was amplified using JH2929 (5′-GTGGTCGACACTTT TTATTTTACTCTGG-3′) and JH2985 (5′-GGATCCGCACCACCATA CGGTAAGCTGC-3′), and then fused with the GUS reporter gene. *FT*::*GUS* plants (Takada and Goto 2003) were obtained from K. Goto (Research Institute for Biological Sciences, Okayama, Japan). *SVP* cDNA-GFP chimeric constructs were used as a reporter to examine the localization pattern of SVP. To generate the 35S promoter-driven *SVP* cDNA-GFP construct, the GFP sequence was in-frame-fused to the C-terminal region of a *35S*::*SVP* chimeric plasmid. A particle bombardment system (PDS-1000/He; Bio-Rad) was utilized for the delivery of DNA-coated tungsten particles into onion epidermal cells. After 24 h of incubation at 23°C or 16°C, the subcellular localization pattern was observed under a fluorescence microscope (Carl Zeiss).

ChIP assay

ChIP assays were conducted as described (Tang and Perry 2003) with minor modifications. The *Arabidopsis* protoplasts were transfected with either *SVP* cDNA fused to HA tags or *FLC* cDNA fused to HA tags and then incubated for 24 h at room temperature. The expressions of the SVP-HA and FLC-HA proteins were determined by protein blots using extracts from the protoplasts. After formaldehyde fixation, the chromatin of the protoplasts was isolated and sheared via sonication. Mouse anti-HA antibodies (Santa Cruz Biotechnology) or anti-Myc 9B11 antibodies (Cell Signaling Technology) were used to immunoprecipitate the genomic fragments. Five sequence fragments spanning six vCArG motifs within the promoter and a CArG motif in the first intron of *FT* were amplified from the immunoprecipitated genomic DNA. PCR products were visualized after 35 cycles using DNA purified from chromatin immunoprecipitated with antibodies against HA or Myc. Nonselected input DNA and Myc antibody-selected DNA were used as PCR templates for the positive and negative controls, respectively. Quantitation of the enrichment of CArG motifs by the SVP-HA and FLC-HA proteins were performed on PhosphorImager plates (Fujifilm BAS 2500; Fuji). The primers used for the ChIP assays are described in Supplementary Table S2. Detailed descriptions of the protocols of these experiments are available on request.

Luciferase reporter assay

To generate the *FT*::*LUC* construct, we amplified 1.8 kb of the *FT* promoter fragment using JH3096 (5′-TGAACACTAACATGATTGAATGA CA-3′) and JH2865 (5′-GATCTTGAACAAACAGGTGGT-3′) and fused this to luciferase. The luciferase reporter constructs harboring the mutated vCArG motifs within the *FT* promoter were used as reporters to examine the effects of the vCArG motifs on the specific binding of SVP to the *FT* promoter. Site-directed mutagenesis was utilized to generate the *FT*::*LUC* constructs harboring the mutated vCArG motifs, using the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene), in accordance with the manufacturer's instructions. The primers used in this mutagenesis protocol are shown in Supplementary Table S3. Mutations introduced into the vCArG motifs in these constructs were verified via sequencing. SVP—with or without its MADS domain (35S::SVP-HA and 35S::SVP ΔM-HA, respectively)—was used as an effector. A reporter and an effector were cotransfected into the protoplasts. The *35S*::*GUS* construct was used as an internal control. Luciferase activities were normalized by GUS activities. Detailed descriptions of the protocols are available upon request.

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