SHORT VEGETATIVE PHASE reduces gibberellin biosynthesis at the *Arabidopsis* shoot apex to regulate the floral transition

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In *Arabidopsis thaliana* environmental and endogenous cues promote flowering by activating expression of a small number of integrator genes. The MADS box transcription factor SHORT VEGETATIVE PHASE (SVP) is a critical inhibitor of flowering that directly represses transcription of these genes. However, we show by genetic analysis that the effect of SVP cannot be fully explained by repressing known floral integrator genes. To identify additional SVP functions, we analyzed genome-wide transcriptome data and show that *GIBBERELLIN 20 OXIDASE 2*, which encodes an enzyme required for biosynthesis of the growth regulator gibberellin (GA), is upregulated in *svp* mutants. GA is known to promote flowering, and we find that *svp* mutants contain elevated levels of GA that correlate with GA-related phenotypes such as early flowering and organ elongation. The *ga20ox2* mutation suppresses the elevated GA levels and partially suppresses the growth and early flowering phenotypes of *svp* mutants. In wild-type plants, *SVP* expression in the shoot apical meristem falls when plants are exposed to photoperiods that induce flowering, and this correlates with increased expression of *GA20ox2*. Mutations that impair the photoperiodic flowering pathway prevent this downregulation of *SVP* and the strong increase in expression of *GA20ox2*. We conclude that SVP delays flowering by repressing GA biosynthesis as well as integrator gene expression and that, in response to inductive photoperiods, repression of SVP contributes to the rise in GA at the shoot apex, promoting rapid induction of flowering.

In plants, the transition from vegetative growth to flowering is regulated by a complex combination of environmental and internal signals. This developmental transition is controlled by environmental cues, such as seasonal changes in day length (photoperiod) or winter cold (vernalization) as well as ambient conditions including light intensity and spectral quality (1). Furthermore, endogenous signals such as the age of the plant or hormone levels influence flowering time. In *Arabidopsis thaliana* the genetic architecture of the pathways mediating these effects has been partially elucidated. Defined pathways conferring flowering responses to photoperiod and vernalization have been described (1), whereas the growth regulator gibberellin (GA) and age-related changes in expression of particular microRNAs represent endogenous flowering pathways (2, 3). These diverse pathways converge to regulate the transcription of a small number of integrator genes that promote the floral induction program. Notable among these genes are *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). *FT* is transcribed in the leaves and encodes a small protein related to phosphatidylethanolamine-binding proteins that is transported to the shoot apex where it promotes the transcriptional reprogramming of the meristem to initiate flowering (4–10). *SOC1* encodes a MADS box transcription factor that is expressed in the shoot meristem during floral induction and is the earliest gene shown to be upregulated by environmental cues such as day length (11–13).

Floral integrator gene expression is repressed by the MADS box transcription factor SHORT VEGETATIVE PHASE (SVP), an inhibitor of flowering. Mutations in *SVP* cause early flowering under noninductive short days (SD) and under long days (LDs) (14), which correlates with increased levels of the mRNAs of *FT*, its paralogue *TWIN SISTER OF FT* (*TSF*) and *SOC1* (15–17). In wild-type plants, the repressive function of SVP is overcome by exposure to LDs, indicating that SVP increases the amplitude of the photoperiodic response by preventing premature flowering under SDs. SVP plays a similar role in response to vernalization where it forms a heterodimer with the MADS box transcription factor FLOWERING LOCUS C (FLC) to strongly repress flowering before exposure to cold (17, 18). Repression of SVP activity also contributes to the early flowering observed under high ambient temperatures (19, 20). Patterns of naturally occurring allelic variation at *SVP* also suggest that SVP plays a role in adapting flowering time to local conditions (21). Thus, SVP represents a critical node in the

Significance

In plants the transition from vegetative growth to flowering is induced by environmental cues. The amplitude of these responses is enhanced by repressors that strongly delay flowering under non-inductive conditions. In *Arabidopsis thaliana*, the transcription factor SHORT VEGETATIVE PHASE (SVP) has a major role among these repressors. We show that SVP has an unrecognized function in repressing biosynthesis of the plant growth regulator gibberellin (GA) at the shoot apex. Under inductive photoperiods, *SVP* expression falls, contributing to increased expression of a GA biosynthetic enzyme that accelerates flowering. These results link GA biosynthesis to the established regulatory network controlling flowering and illustrate one of the mechanisms by which the levels of growth regulators are synchronized with the floral transition.

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control of flowering time of *A. thaliana*. Genomic studies proposed several hundred SVP direct targets based on ChIP-chip or ChIP-seq analysis (22, 23). This global analysis together with specific ChIP-PCR experiments demonstrated that repression of some flowering genes by SVP, including *FT* and *SOC1*, is direct (16, 17). However, further functional analysis of the processes downstream of SVP are required to understand how this transcription factor so effectively represses flowering and thereby increases the amplitude of flowering responses to different environmental cues.

Here we show that an important previously unrecognized function of SVP is to reduce levels of GA by reducing expression of *GA20-OXIDASE 2* (*GA20ox2*), which encodes a rate-limiting enzyme in GA biosynthesis (24–26). We show that *svp* mutants contain elevated levels of GA and propose that repression of *SVP* transcription during floral transition leads to an increase in *GA20ox2* expression. Our data indicate that the resulting increase in GA levels, for example, during photoperiodic flowering, increases the mRNA levels of genes encoding SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) transcription factors, stably inducing the floral transition. This analysis demonstrates a mechanism for how GA biosynthesis is increased at the shoot apex by environmental cues through the well-established regulatory network that controls flowering (27).

Results

Inhibition of Floral Induction by *SVP* Cannot Be Fully Explained by Repression of *FT*, *TSF*, *SOC1*, and *FUL*. The MADS box transcription factor SVP regulates flowering under SDs and LDs by repressing transcription and reducing steady-state mRNA levels of the floral integrators *FT*, *TSF*, and *SOC1*, which are all required for the photoperiodic flowering response (28). By contrast, the mRNA abundance of *FRUITFULL* (*FUL*), which also encodes a MADS box transcription factor that acts in the photoperiod pathway and is partially genetically redundant with *SOC1* (9, 29), is affected only by *SVP* under LDs (Fig. S1 *A–D*). The relevance of the increase in *FT*, *TSF*, *SOC1*, and *FUL* mRNA levels for the early flowering phenotype of *svp* mutants was tested by genetic analysis using the *svp-41* null allele (14). The *svp-41 ful-2 soc1-2* and *svp-41 ft-10 tsf-1* triple mutants flowered significantly later than *svp-41* mutants but much earlier than the *ful-2 soc1-2* or *ft-10 tsf-1* double mutants, respectively (9, 15) (Fig. 1*A*). Therefore, *FUL SOC1* and *FT TSF* contribute to the early flowering of *svp-41* mutants, but these pairs of genes are not responsible for the full early flowering phenotype of *svp-41*. To test whether this early flowering can be fully explained by all four genes, the quintuple mutant *svp-41 ft-10 tsf-1 soc1-2 ful-2* was constructed and its flowering time compared with that of the quadruple mutant *ft-10 tsf-1 soc1-2 ful-2*. Under inductive LDs the quadruple mutant flowered after forming around 85 leaves, whereas the quintuple mutant flowered after producing around 50 leaves (Fig. 1 *A* and *B*). Therefore, the *svp-41* mutation causes earlier flowering even in the absence of functional *FT TSF SOC1 FUL* genes.

***SVP* Reduces Levels of the GA Growth Regulator by Repressing Transcription of the Gene Encoding the GA-Biosynthetic Enzyme *GA20-oxidase 2*.** Genome-wide transcriptome analysis was used to identify additional genes regulated by *SVP* that could contribute to the early flowering of *svp-41 ft-10 tsf-1 soc1-2 ful-2* plants. Previously, hybridization of Affymetrix tiling arrays was used to identify genes deregulated in *svp-41* mutants compared with wild type (23). Among the genes differentially expressed in *svp-41* mutants compared with wild type were several that contribute to the biosynthesis, catabolism, or signaling pathway for the growth regulator GA (Fig. 2*A*), which promotes flowering of *A. thaliana*. Expression of genes involved in GA catabolism and signaling was upregulated in *svp-41* mutants whereas those contributing to GA biosynthesis were downregulated. A striking exception to this trend was *GIBBERELLIN 20-OXIDASE 2* (*GA20ox2*), which encodes a GA biosynthetic enzyme and showed an increase in mRNA abundance in *svp-41* compared with wild type. SVP acts as a transcriptional repressor, and therefore whether it binds directly to the *GA20ox2* genomic region in vivo was tested. Mutant *svp-41* plants in which the mutation was complemented by a *SVP::SVP:GFP* were used for ChIP-qPCR. No enrichment of the *GA20ox2* locus was detected after ChIP, although positive controls with the known SVP target *SEP3* clearly detected binding of SVP:GFP (Fig. S2 *A–C*). Therefore, SVP reduces the transcription of *GA20ox2*, but probably does not bind directly to the gene.

Increased expression of *GA20ox2* mRNA in *svp-41* mutants suggested that these plants might contain higher levels of the growth regulator GA than wild-type plants and that this could contribute to the early flowering of *svp-41*. Consistent with this idea, comparisons of the *svp-41* and wild-type plants revealed that the mutants exhibit phenotypes that resemble those of plants over-accumulating GA. For example, in addition to early flowering, *svp-41* mutants display a larger rosette radius, lower chlorophyll content, and a longer stem (Fig. 2*B* and Table S1).

If *svp-41* plants are altered in their GA content, then their responses to exogenously applied GA might differ from those of wild-type plants. Treatment of SD-grown wild-type plants with GA_4 accelerated flowering and reduced chlorophyll content; by contrast, no significant changes in these phenotypes were observed after application of GA_4 to *svp-41* mutants (Fig. 2 *C* and *D* and Fig. S2*D*). The insensitivity of *svp-41* to exogenous

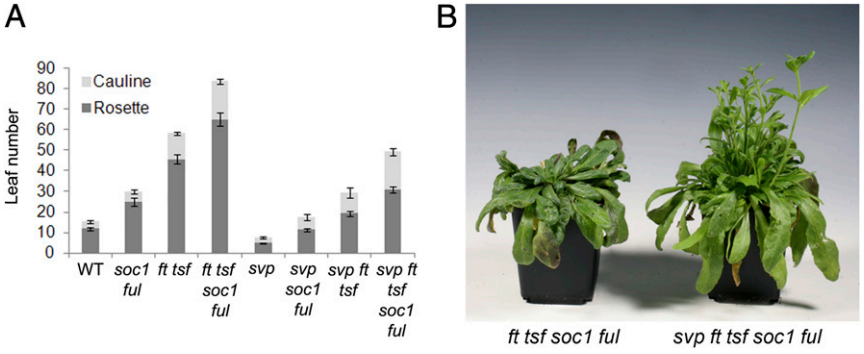

Fig. 1. The *svp-41* mutation accelerates flowering in the absence of functional *FT TSF SOC1 FUL* genes. (*A*) Leaf number at flowering of plants grown under LD condition. Data are mean ± SD of at least 10 individual plants. (*B*) Phenotypes of the quadruple *ft-10 tsf-1 soc1-2 ful-2* and of the quintuple *svp-41 ft-10 tsf-1 soc1-2* mutant plants around 60 d after germination growing under LDs. See also Fig. S1.

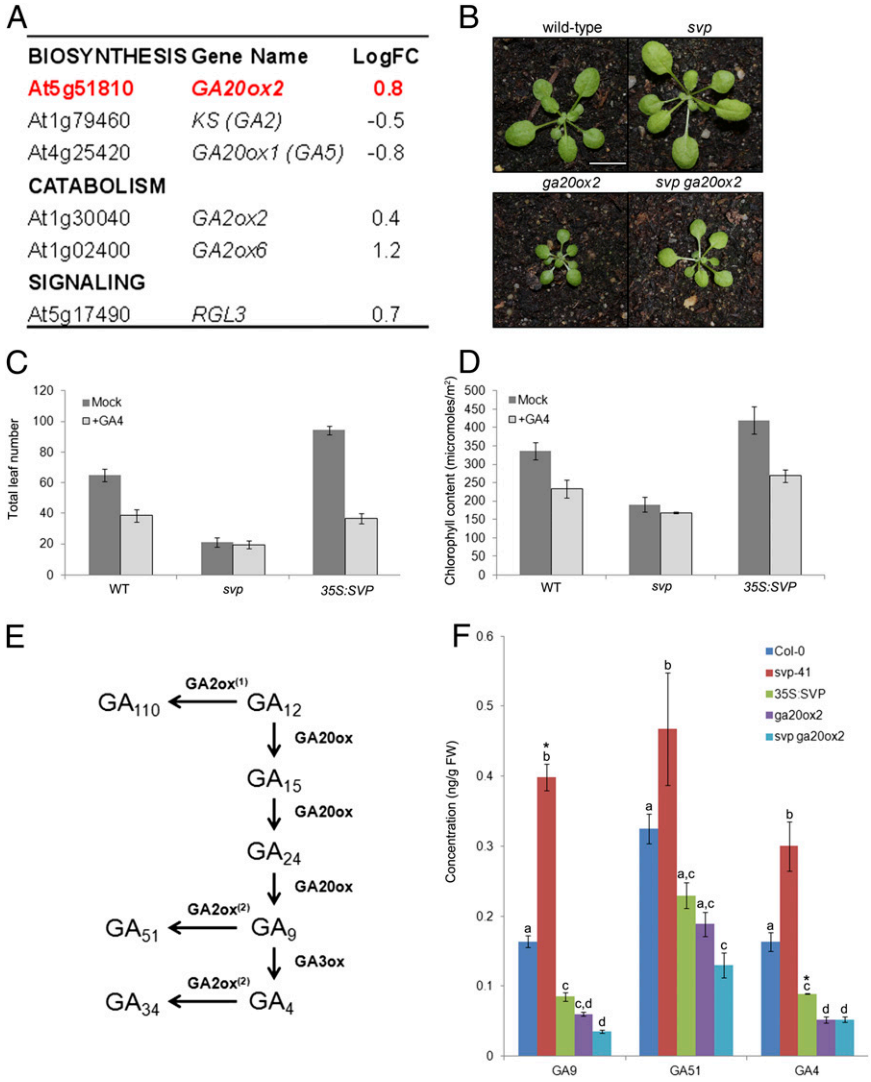

Fig. 2. *SVP* reduces GA content through the transcriptional repression of *GA20ox2*. (*A*) GA-related genes differentially expressed in *svp-41* mutant compared with wild-type plants according to the microarray experiments described (23). (*B*) Phenotype of seedlings of wild-type and *svp-41* mutant (*Upper*) and *ga20ox2-1* mutant and *svp-41 ga20ox2-1* double mutants (*Lower*). Bar = 10 mm. (*C*) Flowering time and (*D*) chlorophyll content measurement of wild-type, *svp-41*, and *35S::SVP* plants after treatments with GA_4 (light bars) or mock (dark bars). All plants in *A–D* were grown under SDs. n = 10–12. (*E*) Schematic representation of the non–13-hydroxylated GA-biosynthetic pathway in *Arabidopsis* (adapted from Yamaguchi, ref. 32) [(1)]GA2ox7 and -8; [(2)]GA2ox1, -2, -3, -4, and -6. (*F*) Concentration of GAs in aerial part of seedlings grown for 2 wk under SDs. The values are the mean ± SEM of three biological replicates (ng/g fresh weight). Letters shared in common between the genotypes indicate no significant difference in GA concentration (pairwise multiple comparison procedures, Student–Newman–Keuls method, $P < 0.05$). *Two biological replicates. See also Fig. S2 and Table S1.

application of GA_4 is consistent with *svp-41* mutants containing high endogenous levels of the hormone that saturate downstream responses. By contrast, flowering time and chlorophyll content of *35S::SVP* plants were hypersensitive to GA_4 treatment (Fig. 2 *C* and *D*), suggesting that phenotypes associated with high expression of *SVP* are at least partially due to unusually low levels of GA.

Further support for *svp-41* containing increased levels of GA was obtained by direct quantification of GA and by analysis of expression of *GA20ox1* (*GA5*), which is regulated by GA via negative-transcriptional feedback control (30, 31). The microarray data showed that levels of *GA20ox1* mRNA were significantly lower in *svp-41* mutants than in wild-type plants, consistent with the mutant containing elevated levels of GA (Fig. 2*A*). To explore this idea further, we quantified the concentration of GA forms belonging to the non–13-hydroxylated pathway that contributes mainly to the biosynthesis of GA_4 (Fig. 2*E*) (32). The levels of the final GA products of this pathway (GA_9, GA_{51}, and G_{A4}) were significantly increased in *svp-41* and reduced in *35S::SVP* compared with wild type (Fig. 2*F*).

Whether increased expression of *GA20ox2* contributes to the over-accumulation of GA and the early flowering phenotype of the *svp-41* mutant was then tested. As shown in Fig. 3*A* and Fig. S2*E*, the loss-of-function *ga20ox2-1* mutant flowered slightly later than wild type (14.6 and 1.9% more leaves under SDs and LDs, respectively); however, when this mutation was introduced into the *svp-41* mutant, it strongly delayed flowering (35.5 and 32.5% more leaves under SDs and LDs, respectively). Moreover, the GA over-accumulation phenotypes observed in *svp-41*, including the leaf radius and chlorophyll content, were largely suppressed in the *svp-41 ga20ox2-1* double mutant (Fig. 2*B* and Table S1). In addition, GA quantification analyses demonstrated that *GA20ox2* was the main contributor to the GA_9, GA_{51}, and GA_4 over-accumulation in the *svp-41* mutant because the levels of these forms

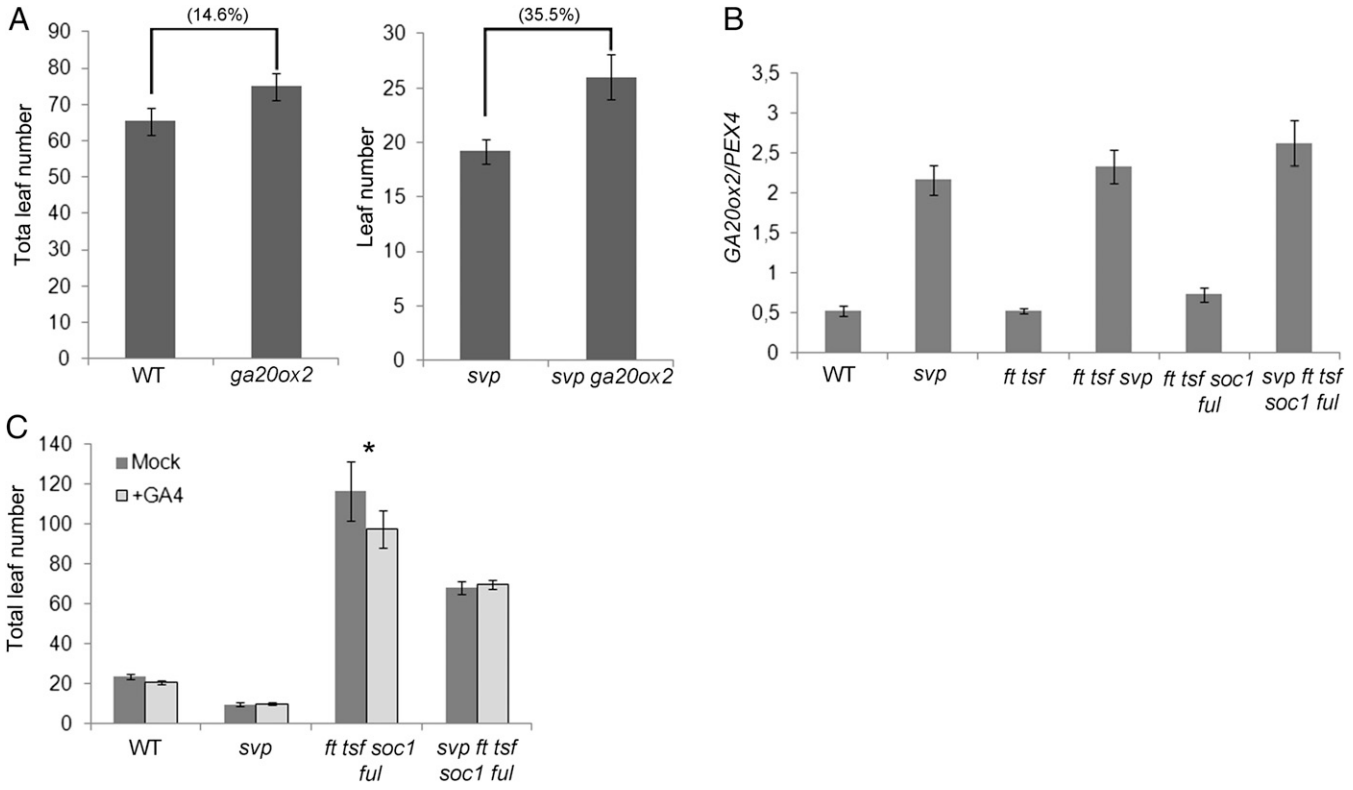

Fig. 3. *SVP* regulates flowering time through transcriptional regulation of *GA20ox2*. (*A*) Flowering time of wild-type plants compared with *ga20ox2-1* (*Left*) and *svp-41* compared with *svp-41 ga20ox2-1* plants (*Right*) grown under SDs. The numbers in parentheses indicate the differences in flowering time expressed as a percentage. The ANOVA analysis showed that this difference is statistically significant (Holm–Sidak test, $P = 0.003$). (*B*) *GA20ox2* mRNA levels in 2-wk-old seedlings of *ft-10 tsf-1* and *soc1-2 ful-2* in the presence or absence of *SVP*. Wild-type and *svp-41* plants were used as controls. Samples were collected 8 h after dawn under SDs. (*C*) Effect of GA_4 treatment on flowering phenotype of *svp-41*, *ft-10 tsf-1 soc1-2 ful-2*, and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutants growing under LDs. Treatment was carried out with at least 10 individual plants, and wild type was used as control. The asterisk indicates that there is a statistically significant difference between the treated and untreated *ft-10 tsf-1 soc1-2 ful-2* plants ($P = 0.007$).

were strongly reduced in the *svp-41 ga20ox2-1* double mutant (Fig. 2*E*). Therefore, repression of *GA20ox2* is an important aspect of the role of *SVP* in modulating GA biosynthesis and the phenotypes controlled by this pathway, including flowering time.

The increase in *GA20ox2* mRNA was also detected in the *svp-41 soc1-2 ful-2 ft-10 tsf-1* quintuple mutant compared with the *soc1-2 ful-2 ft-10 tsf-1* quadruple, consistent with it contributing to the earlier flowering phenotype of the quintuple (Fig. 3*B*). Whether GA20ox2 activity is responsible for all of the residual flowering in the quintuple mutant requires construction of the hextuple mutant *svp-41 soc1-2 ful-2 ft-10 tsf-1 ga20ox2*, and so far we have not been able to test the flowering time of this genotype. Nevertheless, support for the role of GAs in promoting flowering independently of *FT*, *TSF*, *SOC1*, and *FUL* was obtained by applying GA4 to the quadruple and quintuple mutants. Strikingly, GA4 treatment accelerated flowering of the quadruple mutant (Fig. 3*C*), but had no effect on flowering time of the quintuple mutant (Fig. 3*C*). Taken together, these results suggest that GAs promote flowering by acting either downstream or in parallel to the photoperiodic pathway containing *FT*, *TSF*, *SOC1*, and *FUL* and that this process is regulated by the floral repressor *SVP*.

***SVP* Regulates Flowering and the Expression of *GA20ox2* in the SAM.** SVP represses *FT* and *TSF* in the leaves and *SOC1* and *FUL* in the SAM. In the absence of *FT TSF* photoperiodic signals produced in the leaves, the *svp-41* mutation still accelerates flowering, and this is associated with an increase of *GA20ox2* mRNA. Therefore, *SVP* might act downstream of *FT* and *TSF* to repress *GA20ox2* in the SAM. We tested this possibility by quantifying the expression of *GA20ox2* mRNA in different plant organs. As shown in Fig. 4*A*, *GA20ox2* mRNA is more abundant in apices than in leaves of wild-type and *svp-41* seedlings.

The effect of misexpression of *SVP* in the SAM on *GA20ox2* expression was also tested. A *pKNAT1::SVP* transgene that drives *SVP* expression in the shoot meristem was introduced into the *svp-41* mutant. The transgenic plants showed a significant delay of flowering under LDs and SDs compared with the *svp-41* mutant, indicating that *SVP* expression in the SAM is sufficient to repress flowering (Fig. 4*B* and Fig. S3 *C* and *D*). In addition, the *GA20ox2* mRNA level was lower in apices of these transgenic plants than in apices of *svp-41* mutants, confirming that *SVP* represses the transcription of *GA20ox2* in the SAM (Fig. 4*C*) and that this is associated with delayed flowering. Thus, in wild-type plants *SVP* represses *GA20ox2* expression at the shoot apex. However, when *SVP* is expressed specifically in leaves by using the phloem-specific promoter *pSUC2*, it only delays flowering under LDs probably by repressing the transcription of *FT* and *TSF* (Fig. S4 *A*–*C*).

During Photoperiodic Induction of Flowering, FT Signaling Mediates the Downregulation of *SVP* and the Induction of GA Biosynthesis. *SVP* mRNA levels are reduced in the shoot apical meristem during floral induction (15) and are absent in the inflorescence meristem (33). Our data show that this correlates with increased *GA20ox2* mRNA abundance and higher GA levels. To test the dynamics of *SVP* downregulation, we studied the temporal and spatial expression patterns of *SVP* mRNA at the SAM of wild-type

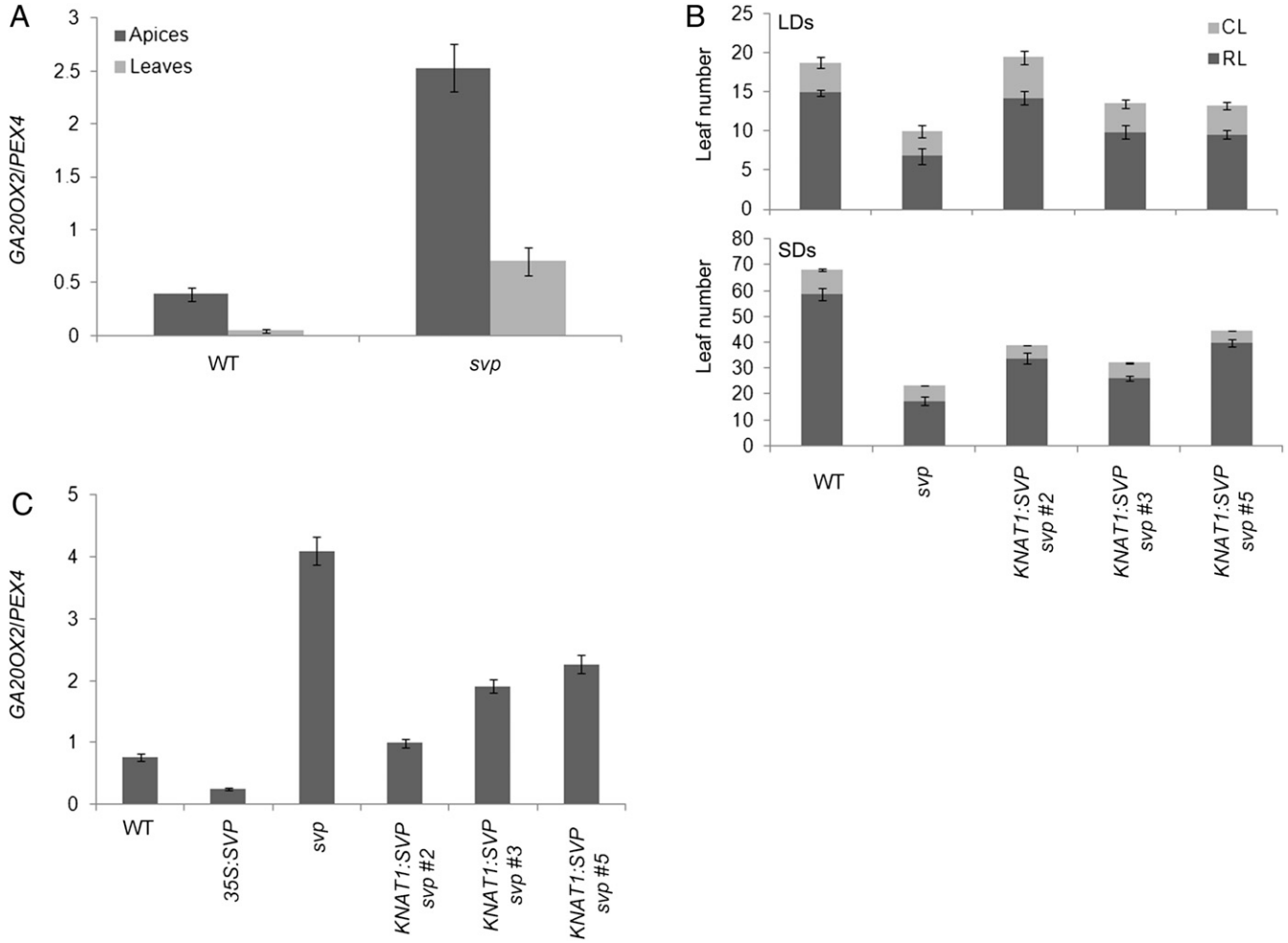

Fig. 4. SVP controls floral transition and *GA20ox2* transcription in the SAM. (*A*) Levels of *GA20ox2* mRNA in apices and leaves of wild-type and *svp-41* plants. (*B*) Effect of the misexpression of *SVP* in the SAM on flowering time under LDs (*Upper*) and SDs (*Lower*). CL: cauline leaves; RL: rosette leaves. (*C*) Levels of *GA20ox2* mRNA in apices of transgenic plants misexpressing *SVP* compared with WT and *svp-41* mutant grown for 2 wk under SDs.

plants grown in SDs and then transferred to inductive LDs. The *SVP* mRNA was strongly detected at the meristem of wild-type plants under SDs in agreement with the function of *SVP* as a repressor of flowering (Fig. 5*A*). However, after transferring plants to LDs, *SVP* mRNA decreased from the center of the meristem, and it was detectable only in floral primordia at 5 and 7 LDs, representing a later function of *SVP* in floral development (34, 35). Thus, during photoperiodic induction LD signals repress activity of the floral repressor *SVP* in the shoot apical meristem. To test whether this reduction is associated with changes in the levels of *GA20ox2* mRNA, quantitative RT-PCR (qRT-PCR) was performed with cDNA extracted from apices of wild-type plants transferred from SDs to LDs. The levels of *GA20ox2* mRNA significantly increased at the apex of these plants after exposure to 3, 5, and 7 LDs, consistent with the idea that reduced *SVP* mRNA level is associated with increased expression of *GA20ox2* at the apex (Fig. 5*B*).

To characterize the *GA20ox2* spatial expression pattern at the SAM of wild-type plants, GUS staining was performed in *pGA20ox2::GA20ox2:GUS* plants (36) growing under LDs, and tissue was harvested prior (8 LDs), during (11 LDs), and after (14 LDs) the transition to flowering (Fig. 5*C*). GUS signal was weakly detected in the center of the SAM of *pGA20ox2::GA20ox2:GUS* plants 8 LDs after germination (Fig. 5*C*). However, at 11 LDs, *GA20ox2:GUS* expression was strongly increased (Fig. 5*C*) at the base of the SAM in the rib meristem region. After the floral transition, 14 LDs after germination, GUS expression was maintained mainly in the elongating region of the rib meristem (Fig. 5*C*). Therefore, *GA20ox2* expression occurs in a specific area of the SAM and correlates with the switch from vegetative growth to flowering. Furthermore, *SVP* and *GA20ox2* have reverse temporal expression patterns at the SAM during flowering in LDs (Fig. 5 *A* and *B*). To assess whether mutation in *SVP* alters the spatial expression pattern of *GA20ox2*, the *pGA20ox2::GA20ox2:GUS* construct was introduced into the *svp-41* mutant by crossing. Similar to *pGA20ox2::GA20ox2:GUS* plants, *svp-41 pGA20ox2::GA20ox2:GUS* plants showed GUS activity in the rib meristem during the transition to flowering at 12 LDs (Fig. S3*A*). These experiments suggest that mutation in *SVP* does not greatly change the spatial pattern of expression of *GA20ox2*, but it does increase *GA20ox2* mRNA levels in the apical region based on the previously described qRT-PCR experiments showing higher levels of *GA20ox2* mRNA in several genetic backgrounds containing the *svp-41* mutation (Fig. 3*B*).

In *A. thaliana*, the photoperiodic response is mediated by increased expression of *FT* and *TSF* in the leaf followed by upregulation of *SOC1* and *FUL* in the meristem (28). During floral induction, SOC1 binds directly to the promoters of several floral integrator genes, including *SVP* (37). Therefore, whether the module *SVP*/*GA20ox2* is controlled by the photoperiod pathway

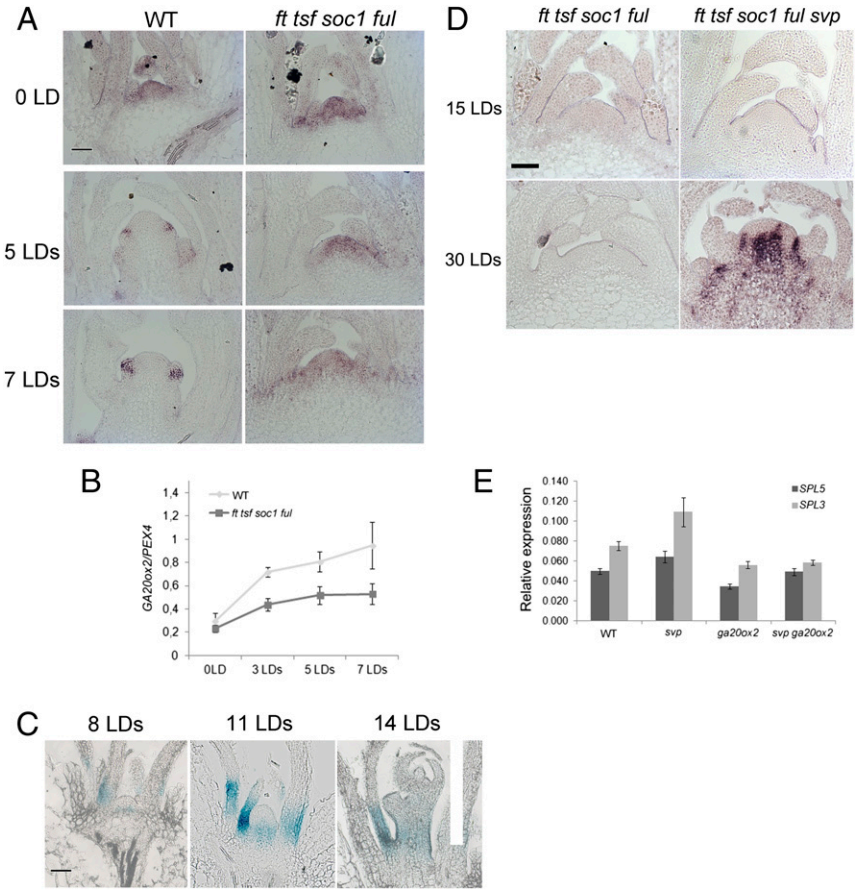

Fig. 5. Photoperiodic regulation of GA biosynthesis and transcriptional activation of *SPLs*. (*A*) Spatial pattern of *SVP* mRNA detected by in situ hybridization during a time course of *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants grown for 3 wk in SDs (0 LD) and then transferred to LDs (3, 5, and 7 LDs). A specific probe was used to detect mRNA of *SVP* at the shoot apex. (*B*) Temporal expression pattern of *GA20ox2* mRNA in apices of wild-type, *ft-10 tsf-1*, and *soc1-2 ful-2* mutant plants grown for 3 wk in SDs (0 LD) and then shifted to LDs (3, 5, and 7 LDs). All samples were harvested 8 h after dawn. (Scale bar: 50 μm.) (*C*) Histochemical localization of GUS activity at SAM of *pGA20ox2::GA20ox2:GUS* seedlings harvested at the beginning (8 LDs), during (11 LDs), and after (14 LDs) the transition to flowering. (Scale bar: 100 μm.) (*D*) Pattern of expression of *SPL4* in *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants grown for 15 (*Upper*) and 30 LDs (*Lower*). (Scale bars: 50 μm.) (*E*) Quantification of the mRNA levels of *SPL5* and *SPL3* in wild-type, *svp-41*, *ga20ox2-1*, and *svp-41 ga20ox2-1* seedlings grown for 2 wk under SDs.

was tested by studying the temporal and spatial expression patterns of *SVP* in meristems of *ft-10 tsf-1 soc1-2 ful-2* mutant plants shifted from SDs to LDs. In contrast to wild-type plants, *SVP* mRNA was still strongly detectable at the center of the meristem of *ft-10 tsf-1 soc1-2 ful-2* plants even after 7 d exposure to LDs, demonstrating that the *FT TSF SOC1 FUL* pathway is required to repress expression of *SVP* during LD induction (Fig. 5*A*). Furthermore, *SVP* transcript persisted at the meristem of the double mutants *soc1-2 ful-2* and *ft-10 tsf-1* for at least 7 d after their transfer from SDs to LDs (Fig. S5). In agreement with these results, the levels of *GA20ox2* mRNA were significantly reduced in the apex of *ft-10 tsf-1 soc1-2 ful-2* plants compared with wild type (Fig. 5*B*). Continued expression of *SVP* in the apices of *ft-10 tsf-1* likely contributes to the reduction of *GA20ox2* mRNA because in apices of *svp-41 ft-10 tsf-1* plants *GA20ox2* mRNA levels were increased (Fig. S3*B*). Interestingly, a slight increase of *GA20ox2* mRNA was still detected in apices of *ft-10 tsf-1 soc1-2 ful-2* plants exposed to LDs (Fig. 5*B*), indicating that *GA20ox2* might also be activated by photoperiod independently of *FT*, *TSF*, *SOC1*, and *FUL*.

***GA20ox2* Is Responsible for the SVP-Mediated Activation of *SPL* Transcription Factors During Floral Induction.** Depletion of GA and reduction of GA signaling in the shoot apical meristem was previously shown to reduce expression of genes encoding SPL transcription factors during floral induction under LDs (38, 39). In addition, the levels of *SPL3*, *-4*, and *-5* transcripts are regulated by *FT*, by *TSF*, and by the downstream acting genes *SOC1* and *FUL* (3, 9). We used the *svp-41* mutation to distinguish the roles of the *FT*, *TSF*, *SOC1*, and *FUL* pathway and GA biosynthesis in the transcriptional activation of *SPL3*, *SPL4*, and *SPL5*. The spatial and temporal expression pattern of *SPL4* was compared by in situ hybridization in shoot apical meristems of *svp-41 ft-10 tsf-1 soc1-2 ful-2* and *ft-10 tsf-1 soc1-2 ful-2* plants grown under LDs. *SPL4* mRNA was strongly detected in the meristem of 30-d-old *svp-41 ft-10 tsf-1 soc1-2 ful-2* plants grown continuously under LDs that were undergoing the transition to flowering whereas the meristem *of ft-10 tsf-1 soc1-2 ful-2* showed no *SPL4* mRNA at the same time (Fig. 5*D* and Fig. S6*B*). A similar experiment was carried out in these genotypes transferred from SDs to LDs. No *SPL4* expression was detected in either genotype under SDs, but in *svp-41 ft-10 tsf-1 soc1-2 ful-2* plants *SPL4* mRNA was detected at the base and on the flanks of the shoot apical meristem after exposure to 7 LDs (Fig. S6*A*) in a similar pattern to 25-d-old *svp-41 ft-10 tsf-1 soc1-2 ful-2* grown continuously in LDs (Fig. S6*B*). By contrast, in the meristem of *ft-10 tsf-1 soc1-2 ful-2*, no *SPL4* mRNA was detectable after similar treatments (Fig. S6 *A* and *B*). Thus, the presence of the

svp-41 mutation accelerates expression of *SPL4* in the absence of *FT*, *TSF*, *SOC1*, and *FUL*, which could be due to the increased GA levels present in the *svp-41* mutant. To test this further, the transcript levels of *SPL3* and *SPL5* were quantified in apices of *svp-41 ga20ox2-1* double mutants and compared with *svp-41*, *ga20ox2-1*, and wild type. The transcript levels of *SPL3* and *SPL5* were higher in *svp-41* apices compared with wild-type and *ga20ox2-1* (Fig. 5*E*). By contrast, in apices of *svp-41 ga20ox2-1*, the abundance of *SPL3* and *SPL5* mRNA was reduced compared with *svp-41* and similar to wild type and *ga20ox2-1*. Therefore, the increased levels of *SPL3* and *SPL5* mRNAs in *svp-41* mutants are dependent on *GA20ox2* activity.

Discussion

In *A. thaliana*, several genetic pathways determine the timing of floral induction (1). These genetically separable pathways mediate responses to seasonal cues such as day length and winter temperatures as well as to endogenous signals including the growth regulator GA. However, whether and how the environmentally regulated pathways controlling floral transition are linked to those regulating GA metabolism is not clear. Here we show that SVP, a MADS box transcription factor with a central role in flowering-time control in response to day length, vernalization, and ambient temperature represses GA biosynthesis. Mutations in *SVP* are associated with higher levels of GA_4, the main bioactive GA in *Arabidopsis*, which was previously shown to promote flowering (40). SVP expression reduces transcription of *GA20ox2*, which encodes a rate-limiting enzyme in synthesis of GA_4 (24, 25, 41). In wild-type plants, *GA20ox2* expression rises in the meristem in response to LDs that induce flowering, and we show that this is mediated by FT TSF. We propose that, in the early stages of the floral transition in response to LDs, FT TSF mediates the repression of *SVP* and that this contributes to an increase in *GA20ox2* expression and GA biosynthesis in the shoot meristem. Such mechanisms might be broadly conserved in other plant species, as overexpression of an *FT* gene in wheat was recently shown to increase GA levels (42).

Regulation of GA Biosynthesis by Day Length. GA contributes to flowering under inductive LDs and noninductive SDs. Under SDs, flowering is delayed and correlates with a gradual increase in bioactive GA at the shoot apex (40). Furthermore, mutations that impair GA biosynthesis prevent flowering under SDs (43). Such observations led to the idea that GA is essential for flowering under SDs, whereas under LDs the requirement for GA is reduced because the photoperiodic flowering pathway acting through CONSTANS (CO) and FT TSF accelerates flowering (43, 44). Nevertheless, genetic analysis also argues for a role for GA in floral induction under LDs. Mutations or transgenic approaches that inactivate the GA receptors, impair GA signaling, or strongly reduce GA biosynthesis delay flowering under LDs (38, 39, 45, 46). GA biosynthesis is also increased by exposure to LDs in rosette species such as *A. thaliana* or spinach, which is associated with increased expression of GA20ox isoforms and is linked to shoot elongation as well as earlier flowering (47, 48). Similarly, the *GIBBERELLIN 3-OXIDASE 1* (*GA3OX1*) and *GA3ox2* genes of *A. thaliana* are coregulated with *FT* by the TEMPRANILLO transcription factors (49). Here, we provide a mechanism by which increased GA levels at the shoot apex are coordinated with floral transition under LDs. Our data demonstrate that under LDs the GA and photoperiodic pathways do not simply act in parallel and converge on integrator genes such as *SOC1*, but that GA biosynthesis is regulated by the photoperiodic pathway at least partially through downregulation of *SVP* and thus increased expression of GA biosynthetic genes.

We monitored the expression pattern of *pGA20ox2::GA20ox2:GUS* (36) in the meristem and found that under LDs *GA20ox2* expression rises in the region of the rib meristem during floral induction. Attempts to support this pattern using in situ hybridization failed, presumably due to the low level of expression of this gene. The expression domains of *SVP* and *GA20ox2* may overlap during the vegetative phase when the *SVP* expression domain encompasses a large part of the SAM (Fig. 5*A*). However, detailed analysis of how much their expression overlaps will require visualizing the patterns of expression of both genes in the same apices during the floral transition, for example, by using fluorescent marker proteins.

This region of the meristem promotes stem elongation (bolting), and floral promoter genes change in expression in this region in *Arabidopsis* after exposure to LDs (9, 50). This indicates that *GA20ox2* expression in this region might have roles in the onset of bolting and floral development and in synchronizing these events during the onset of reproductive development in *Arabidopsis* (50). Furthermore, the spatial expression pattern of *pGA20ox2::GA20ox2:GUS* at the resolution tested was not altered in the *svp* mutant, suggesting that the early flowering caused by increased *GA20ox2* mRNA levels in the *svp* mutant is due to elevated GA20ox2 activity in the rib meristem region. These results are in agreement with previous observations that GA20 oxidases are involved in stem elongation and that mutations in *GA20ox2* delay flowering under LDs (24, 48). The flowering-time defect of the *ga20ox2-1* mutant under LDs is enhanced by mutations in two other paralogues (36), suggesting that these also contribute to GA biosynthesis under these conditions. Nevertheless, in our experiments only *GA20ox2* was negatively regulated by SVP, suggesting that the boost in GA biosynthesis conferred by *svp* mutations and associated with downregulation of *SVP* during floral induction acts predominately through this paralogue. The increase in *GA20ox2* expression observed in the rib meristem under LDs indicates that GA biosynthesis increases specifically in the meristem after downregulation of SVP. This result contrasts with the gradual increase in GA levels under SDs, which could not be correlated with elevated expression in GA biosynthetic genes, suggesting that under these conditions GA is synthesized in other tissues and transported to the meristem (40). The GA synthesized via *GA20ox2* expression in the rib meristem might move locally into other regions of the shoot meristem because GA influences the expression of genes such as *LEAFY* and *SPL9* in more apical regions of the meristem (38, 51). However, it cannot be excluded that non–cell-autonomous factors acting downstream of GA move from the rib meristem into more apical regions.

SVP Mediates Between the Photoperiodic Pathway and GA Regulation. A progressive decrease in *SVP* mRNA in wild-type plants shifted from SDs to LDs is accompanied by a complementary increase in *GA20ox2* mRNA. The reduction of *SVP* mRNA requires the activity of the *FT*, *TSF*, *SOC1*, and *FUL* genes because *SVP* mRNA strongly accumulates at the meristem of the quadruple mutant *ft-10 tsf-1 soc1-2 ful-2* even after several days under LDs. This effect probably occurs mainly at the meristem because mutations of either *FT* or *CO* genes did not result in a significant decrease of *SVP* mRNA level in entire seedlings at early stages of development, as previously shown (17). Therefore, under LDs *FT* and *TSF* and their downstream target genes *SOC1* and *FUL* act to repress *SVP*, which contributes to increases in *GA20ox2* mRNA and GA levels at the SAM. FT and TSF might also act independently of *SVP* repression to increase GA levels.

SOC1 binds directly within an intron of *SVP* (37) where it might contribute to the repression of *SVP* during floral induction. On the other hand, *SOC1* expression is upregulated in *svp-41* mutants (15), and SVP binds directly to the *SOC1* promoter (17, 23), indicating that SVP directly represses *SOC1*. These data demonstrate reciprocal repression of SVP/SOC1, so that SVP represses expression of *SOC1* and vice versa. Consistent with this model, *SVP* and *SOC1* show mutually exclusive temporal expression

patterns at the shoot apical meristem with *SVP* being expressed during the vegetative phase whereas *SOC1* is activated during the transition to flowering (15). Thus, one possibility is that in the vegetative shoot apex *SVP* is activated early during development and acts to repress *SOC1*, whereas during flowering the strong induction of *SOC1* by FT TSF overcomes *SVP* repression and allows SOC1 to repress *SVP* (37) (Fig. 6). In SD, GAs gradually induce *SOC1* expression, which in turn represses *SVP* transcription, and this could explain the repressive effect of the GA pathway upstream of *SVP* observed under these conditions (17) (Fig. 6).

Influence of GA on Shoot Apical Meristem Activity. The influence of GA on meristem activity was demonstrated by the finding that homeobox transcription factors involved in meristem identity and maintenance control GA levels. In the shoot meristem, GA levels are reduced by these factors, preventing differentiation and maintaining meristem activity, whereas on the flanks of the meristem where these transcription factors are not expressed, GA levels rise and contribute to organ differentiation (52, 53). In maize, KNOTTED is expressed in the vegetative meristem and binds directly to a gene encoding GA2ox, an enzyme that reduces bioactive GA levels, to activate its expression (53). Similarly, in *A. thaliana* the SHOOTMERISTEMLESS homeobox transcription factor reduces expression of *GA20ox1* in the shoot meristem (52). This led to models in which homeobox transcription factors repress GA levels in the shoot meristem, preventing differentiation and maintaining meristem activity, whereas, on the flanks of the meristem where these transcription factors are not expressed, GA levels rise and contribute to organ differentiation (52, 53). Our data demonstrate that the MADS domain transcription factor SVP also participates in the control of GA by repressing *GA20ox2* mRNA levels in the vegetative meristem. It remains to be tested whether the action of the homeobox transcription factors and SVP are related or whether they independently repress GA biosynthesis, perhaps by repressing different *GA20ox* paralogues.

During floral induction GA levels rise in the meristem, and our data indicate that this is in part due to repression of *SVP* transcription. It has been shown that the transcription of genes with defined roles in floral transition responds to increasing GA levels (54, 55). Several genes encoding SPL transcription factors, including *SPL3*, *SPL4*, *SPL5*, and *SPL9*, are activated in response to GA (38, 39). In agreement with these data, the expression of *SPL4* is increased in *svp-41* mutants (9) even in the absence of *FT* and *TSF* or *SOC1* and *FUL*, supporting the idea that *SVP* acts downstream of the photoperiod pathway to regulate GA levels and therefore *SPL* gene transcription. The primary mechanism by which GA acts to regulate transcription is likely to be by promoting DELLA protein degradation and thereby releasing transcription factors to regulate transcription of their target genes (56, 57). SPL transcription factors are also targets of GA regulation at this posttranslational level (58). Thus, SPL transcription factors may be targets for activation by GA at different levels of regulation, and these in turn are direct activators of *FUL* and *LFY* (3, 59), perhaps providing one mechanism by which *LFY*, a floral meristem identity gene, is activated by GA (55).

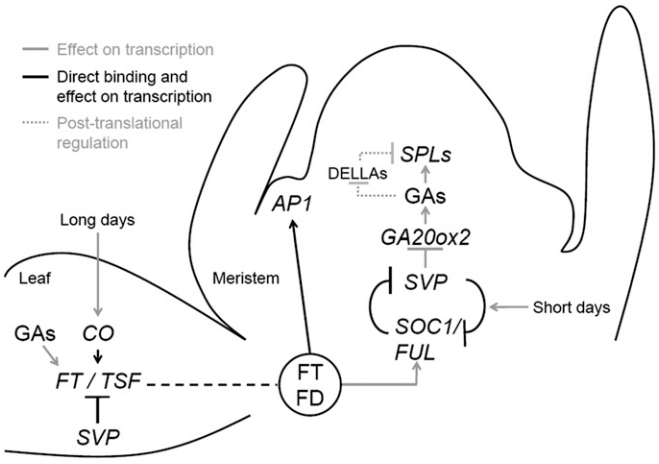

Fig. 6. Proposed model for the activation of GA biosynthesis in the shoot apical meristem during photoperiodic flowering. In plants exposed to LDs, the transcription of *FT* and *TSF* is induced in the leaves. The FT protein moves to the SAM (black dashed line) where *FD* is expressed. The FT FD module is proposed to activate the transcription of downstream floral promoter genes, such as *AP1*, *SOC1*, and *FUL*. SOC1 (and probably also FUL) directly binds to *SVP* and contributes to its repression. Downregulation of *SVP* transcription contributes to increased expression of *GA20ox2* and higher GA content at the shoot apex. Higher GA levels increase transcription of the *SPL* genes and release SPL proteins from DELLA repression during photoperiodic flowering.

Materials and Methods

Growth Conditions and Plant Materials. For all studies *A. thaliana* (L.) ecotype Columbia (Col-0) was used as wild type. Plants were grown on soil under controlled conditions of LDs (16 h light/8 h dark) and SDs (8 h light/16 h dark) at 20 °C. The level of photosynthetic active radiation was 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under both conditions. The *svp-41* mutant and the *35S::SVP* transgenic plants were previously described (14); the double *ft-10 tsf-1* and triple *ft-10 tsf-1 svp-41* mutants were described (15) as was the double mutant *soc1-2 ful-2* (9). These plants were crossed to generate the quadruple *ft-10 tsf-1 soc1-2 ful-2* and the quintuple *ft-10 tsf-1 soc1-2 ful-2 svp-41* mutants. The GA biosynthetic mutants *ga20ox2-1* and *ga20ox1-3* were reported before (24) as well as the *GA20OX2::GA20OX2:GUS* lines (36). The *SVP::SVP:GFP svp-41* transgenic line used for ChIP experiments (*SI Materials and Methods*) has been previously described (60).

GA Treatment. The GA_4 stock (Sigma) was prepared in 100% ethanol with final concentration of 1 mM. GA treatments were performed by spraying 10–12 plants with either a GA solution (GA_4 10 μM, Silwet 77 0.02%) or a mock solution (ethanol 1%, Silwet 77 0.02%).

Quantification of Gibberellins. About 100–200 mg (fresh weight) of frozen material were used to extract and purify the GAs, as described (61). Separated GAs were analyzed by electrospray ionization and targeted selected ion monitoring using a Q-Exactive spectrometer (Orbitrap detector; ThermoFisher Scientific). The [17,17-^{2}H]GAs were added to the extracts as internal standards for quantification, and the concentrations of GAs were determined using embedded calibration curves and the Xcalibur program 2.2 SP1 build 48. The full description of these methods can be found as *SI Materials and Methods*.

Flowering-Time Analysis. Flowering time was determined by counting the number of cauline and rosette leaves of at least 10 individual plants.

In Situ Hybridization and GUS Staining. In situ hybridization was performed according to the method already described (38, 62). Probes used were the following: *SPL3* (3, 63), *SVP* (9), and *SPL4* (38). GUS staining was performed as described (64).

Plasmid Construction, Plant Transformation, and Transformant Selection. Full-length *SVP* cDNAs were amplified by PCR and used to generate an entry clone via BP reaction (Invitrogen). The entry clones were subcloned via the LR reaction into the binary vector *pKNAT1::GW* or *pSUC2::GW* (65) to generate *pKNAT1::SVP svp-41* and *pSUC2::SVP svp-41*, respectively. The plasmids were then introduced into *Agrobacterium* strain GV3101 (pMP90RK) to transform *svp-41* mutant plants by floral dip (66).

Determination of Chlorophyll Concentration, Leaf Radius, and Stem Length. Chlorophyll concentration was estimated by using the SPAD-502 leaf chlorophyll meter (67). Leaf radius and stem length were determined manually by using a ruler.

RNA Extraction and Quantitative Real-Time PCR. Total RNA was isolated from plant tissues by using RNAeasy extraction kit (Qiagen) and treated with

DNA-free DNase (Ambion) to remove residual genomic DNA. One microgram of total RNA was used for reverse transcription (Superscript III, Invitrogen). Transcript levels were quantified by quantitative PCR in a LightCycler 480 instrument (Roche) using the *PEX4* gene (AT5G25760) as a standard. The sequences of the primers to quantify de-expression of *SVP*, *SOC*1, *FUL*, and *SVP* are described in Torti et al. (9) and the ones for *SPL3*, *SPL4*, and *GA20OX1* are described in Porri et al. (38).

Statistical Analysis. All of the statistical analyses were performed by using SigmaStat 3.5 software.

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1. Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. *Nat Rev Genet* 13(9):627–639.
2. Mutasa-Göttgens E, Hedden P (2009) Gibberellin as a factor in floral regulatory networks. *J Exp Bot* 60(7):1979–1989.
3. Wang JW, Czech B, Weigel D (2009) miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell* 138(4):738–749.
4. Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 286(5446):1960–1962.
5. Kardailsky I, et al. (1999) Activation tagging of the floral inducer FT. *Science* 286(5446):1962–1965.
6. Corbesier L, et al. (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* 316(5827):1030–1033.
7. Mathieu J, Warthmann N, Kuttner F, Schmid M (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. *Curr Biol* 17(12):1055–1060.
8. Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. *Curr Biol* 17(12):1050–1054.
9. Torti S, et al. (2012) Analysis of the Arabidopsis shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. *Plant Cell* 24(2):444–462.
10. Schmid M, et al. (2003) Dissection of floral induction pathways using global expression analysis. *Development* 130(24):6001–6012.
11. Samach A, et al. (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. *Science* 288(5471):1613–1616.
12. Borner R, et al. (2000) A MADS domain gene involved in the transition to flowering in Arabidopsis. *Plant J* 24(5):591–599.
13. Lee H, et al. (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. *Genes Dev* 14(18):2366–2376.
14. Hartmann U, et al. (2000) Molecular cloning of SVP: A negative regulator of the floral transition in Arabidopsis. *Plant J* 21(4):351–360.
15. Jang S, Torti S, Coupland G (2009) Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in Arabidopsis. *Plant J* 60(4): 614–625.
16. Lee JH, et al. (2007) Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. *Genes Dev* 21(4):397–402.
17. Li D, et al. (2008) A repressor complex governs the integration of flowering signals in Arabidopsis. *Dev Cell* 15(1):110–120.
18. Fujiwara S, et al. (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in Arabidopsis. *Plant Cell* 20(11):2960–2971.
19. Lee JH, et al. (2013) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. *Science* 342(6158):628–632.
20. Posé D, et al. (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. *Nature* 503(7476):414–417.
21. Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C (2013) The flowering repressor SVP underlies a novel Arabidopsis thaliana QTL interacting with the genetic background. *PLoS Genet* 9(1):e1003289.
22. Tao Z, et al. (2012) Genome-wide identification of SOC1 and SVP targets during the floral transition in Arabidopsis. *Plant J* 70(4):549–561.
23. Gregis V, et al. (2013) Identification of pathways directly regulated by SHORT VEGETATIVE PHASE during vegetative and reproductive development in Arabidopsis. *Genome Biol* 14(6):R56.
24. Rieu I, et al. (2008) The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *Plant J* 53(3):488–504.
25. Coles JP, et al. (1999) Modification of gibberellin production and plant development in Arabidopsis by sense and antisense expression of gibberellin 20-oxidase genes. *Plant J* 17(5):547–556.
26. Huang S, et al. (1998) Overexpression of 20-oxidase confers a gibberellin-overproduction phenotype in Arabidopsis. *Plant Physiol* 118(3):773–781.
27. Fornara F, de Montaigu A, Coupland G (2010) SnapShot: Control of flowering in Arabidopsis. *Cell* 141(3), 550, e1–e2.
28. Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. *Annu Rev Plant Biol* 59:573–594.
29. Melzer S, et al. (2008) Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. *Nat Genet* 40(12):1489–1492.
30. Phillips AL, et al. (1995) Isolation and expression of three gibberellin 20-oxidase cDNA clones from Arabidopsis. *Plant Physiol* 108(3):1049–1057.
31. Xu YL, et al. (1995) The GA5 locus of Arabidopsis thaliana encodes a multifunctional gibberellin 20-oxidase: Molecular cloning and functional expression. *Proc Natl Acad Sci USA* 92(14):6640–6644.
32. Yamaguchi S (2008) Gibberellin metabolism and its regulation. *Annu Rev Plant Biol* 59:225–251.
33. Liu C, et al. (2007) Specification of Arabidopsis floral meristem identity by repression of flowering time genes. *Development* 134(10):1901–1910.
34. Gregis V, Sessa A, Colombo L, Kater MM (2008) AGAMOUS-LIKE24 and SHORT VEGETATIVE PHASE determine floral meristem identity in Arabidopsis. *Plant J* 56(6): 891–902.
35. Liu C, Xi W, Shen L, Tan C, Yu H (2009) Regulation of floral patterning by flowering time genes. *Dev Cell* 16(5):711–722.
36. Plackett AR, et al. (2012) Analysis of the developmental roles of the Arabidopsis gibberellin 20-oxidases demonstrates that GA20ox1, -2, and -3 are the dominant paralogs. *Plant Cell* 24(3):941–960.
37. Immink RG, et al. (2012) Characterization of SOC1's central role in flowering by the identification of its upstream and downstream regulators. *Plant Physiol* 160(1): 433–449.
38. Porri A, Torti S, Romera-Branchat M, Coupland G (2012) Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. *Development* 139(12):2198–2209.
39. Galvão VC, Horrer D, Küttner F, Schmid M (2012) Spatial control of flowering by DELLA proteins in Arabidopsis thaliana. *Development* 139(21):4072–4082.
40. Eriksson S, Böhlenius H, Moritz T, Nilsson O (2006) GA4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. *Plant Cell* 18(9): 2172–2181.
41. Hedden P, Phillips AL (2000) Gibberellin metabolism: New insights revealed by the genes. *Trends Plant Sci* 5(12):523–530.
42. Pearce S, Vanzetti LS, Dubcovsky J (2013) Exogenous gibberellins induce wheat spike development under short days only in the presence of VERNALIZATION1. *Plant Physiol* 163(3):1433–1445.
43. Wilson RN, Heckman JW, Somerville CR (1992) Gibberellin is required for flowering in Arabidopsis thaliana under short days. *Plant Physiol* 100(1):403–408.
44. Reeves PH, Coupland G (2001) Analysis of flowering time control in Arabidopsis by comparison of double and triple mutants. *Plant Physiol* 126(3):1085–1091.
45. Willige BC, et al. (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. *Plant Cell* 19(4):1209–1220.
46. Griffiths J, et al. (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. *Plant Cell* 18(12):3399–3414.
47. Lee DJ, Zeevaart JA (2007) Regulation of gibberellin 20-oxidase1 expression in spinach by photoperiod. *Planta* 226(1):35–44.
48. Xu YL, Gage DA, Zeevaart JAD (1997) Gibberellins and stem growth in Arabidopsis thaliana. Effects of photoperiod on expression of the GA4 and GA5 loci. *Plant Physiol* 114(4):1471–1476.
49. Osnato M, Castillejo C, Matías-Hernández L, Pelaz S (2012) TEMPRANILLO genes link photoperiod and gibberellin pathways to control flowering in Arabidopsis. *Nat Commun* 3:808.
50. Jacqmard A, Gadisseur I, Bernier G (2003) Cell division and morphological changes in the shoot apex of Arabidopsis thaliana during floral transition. *Ann Bot (Lond)* 91(5): 571–576.
51. Blázquez MA, Weigel D (2000) Integration of floral inductive signals in Arabidopsis. *Nature* 404(6780):889–892.
52. Hay A, et al. (2002) The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. *Curr Biol* 12(18):1557–1565.
53. Bolduc N, Hake S (2009) The maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene ga2ox1. *Plant Cell* 21(6):1647–1658.
54. Moon J, et al. (2003) The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. *Plant J* 35(5):613–623.
55. Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. *Plant Cell* 10(5): 791–800.
56. de Lucas M, et al. (2008) A molecular framework for light and gibberellin control of cell elongation. *Nature* 451(7177):480–484.
57. Feng S, et al. (2008) Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. *Nature* 451(7177):475–479.
58. Yu S, et al. (2012) Gibberellin regulates the Arabidopsis floral transition through miR156-targeted SQUAMOSA promoter binding-like transcription factors. *Plant Cell* 24(8):3320–3332.
59. Yamaguchi A, et al. (2009) The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. *Dev Cell* 17(2): 268–278.

60. Gregis V, Sessa A, Dorca-Fornell C, Kater MM (2009) The Arabidopsis floral meristem identity genes AP1, AGL24 and SVP directly repress class B and C floral homeotic genes. *Plant J* 60(4):626–637.
61. Seo M, Jikumaru Y, Kamiya Y (2011) Profiling of hormones and related metabolites in seed dormancy and germination studies. *Methods Mol Biol* 773:99–111.
62. Bradley D, Carpenter R, Sommer H, Hartley N, Coen E (1993) Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the plena locus of *Antirrhinum*. *Cell* 72(1):85–95.
63. Wu G, et al. (2009) The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. *Cell* 138(4):750–759.
64. Adrian J, et al. (2010) cis-Regulatory elements and chromatin state coordinately control temporal and spatial expression of FLOWERING LOCUS T in Arabidopsis. *Plant Cell* 22(5):1425–1440.
65. An H, et al. (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development* 131(15): 3615–3626.
66. Clough SJ, Bent AF (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* 16(6):735–743.
67. Markwell J, Osterman JC, Mitchell JL (1995) Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynth Res* 46(3):467–472.