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 establishment of the regulatory network controlling flowering and illustrate one of the mechanisms by which the levels of growth regulators are synchronized with the floral transition.

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* (*GA2ox2*), 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 SQUAMOUS 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. 1A–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 ft-10 tsf-1* double mutants, respectively (9, 15) (Fig. 1A). 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. 1A 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. 2A), 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 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. S2A–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. 2A and Table S1)."
application of GA₄ 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 GA₄ treatment (Fig. 2C 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 (G₄), 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. 2A). 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 GA₄ (Fig. 2E) (32). The levels of the final GA products of this pathway (GA₉, GA₂₀ₒₓ₂, and GA₄) were significantly increased in svp-41 and reduced in 35S::SVP compared with wild type (Fig. 2F).

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. 3A and Fig. S2E, 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. 2B and Table S1). In addition, GA quantification analyses demonstrated that GA20ox2 was the main contributor to the GA₉, GA₂₀ₒₓ₂, and GA₄ over-accumulation in the svp-41 mutant because the levels of these forms were significantly lower in svp-41 mutants than in wild-type plants, consistent with the mutant containing elevated levels of GA (Fig. 2A). 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 GA₄ (Fig. 2E) (32).
and 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 plants growing under LDs (Fig. S5 A and B).
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. 5A). 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. 5B).

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. 5C). GUS signal was weakly detected in the center of the SAM of pGA20ox2::GA20ox2:GUS plants 8 LDs after germination (Fig. 5C). However, at 11 LDs, GA20ox2::GUS expression was strongly increased (Fig. 5C) 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. 5C). 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. 5A 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. S3A). 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. 3B).

In A. thaliana, the photoperiodic response is mediated by increased expression of FT and TSW 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...
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. 5A). 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. 5B). 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. S3B). 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. 5B), 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 soc1-2 ful-2 showed no SPL4 mRNA at the same time (Fig. 5D and Fig. S6B). 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. S6D) in a similar pattern to 25-d-old svp-41 ft-10 tsf-1 soc1-2 ful-2 grown continuously in LDs (Fig. S6B). By contrast, in the meristem of ft-10 tsf-1 soc1-2 ful-2, no SPL4 mRNA was detectable after similar treatments (Fig. S6A 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. 5E). 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, translational, and ambient temperature represses GA biosynthesis. Mutations in SVP are associated with higher levels of GAs, the main bioactive GA in Arabidopsis, which was previously shown to promote flowering (40). SVP expression represses transcription of GA20ox2, which encodes a rate-limiting enzyme in synthesis of GAs (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. 5A). 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 two GA biosynthetic loci act in concert 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 complementarity 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 GA2ox expression and that this could explain the repressive effect of the GA pathway upstream of SVP observed under these conditions (17) (Fig. 6).

**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 μmol m⁻² 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 *sco1-2 ful-2* (9). These plants were crossed to generate the quadruple ft-10 tsf-1 *sco1-2 ful-2* and the quintuple ft-10 tsf-1 *sco1-2 ful-2* *svp-41* mutants. The GA biosynthetic mutants *ga2ox2-1* and *ga2ox1-3* were reported before (24) as well as the *GA2OX2::GA2OX2: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 GAs 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 (GAs, 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 (ThermoFisher Scientific). The [17,17-D³]GAs were added to the extracts as internal standards for quantification, and the concentration of GAs was 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 RP reaction (Invitrogen). The entry clones were subcloned via the LR reaction into the binary vector pKNAT1::GW or pSU2::GW (65) to generate pKNAT1::SVP *svp-41* and pSU2::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 RAeasy 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 PEKX gene (AT5G27650) as a standard. The sequences of the primers to quantify de-expression of SVP, SOC1, FUL, and VSP are described in Torro et al. (9) and the ones for SPL3, SPL4, and GA200X7 are described in Porri et al. (38).

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


Supporting Information
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SI Materials and Methods

ChIP Experiments and Primers Used in This Work. For ChIP experiments, plants were grown in SD for 2 wk and collected at Zeitgeber 8 (ZT8). A line expressing SVP fused to GFP under its own promoter in the svp-41 mutant [SVP::SVP:GFP svp-41 (1)] was compared with the control line, in our case 35S::GFP. After cross-linking the tissue, the ChIP was performed as in ref. 2 with minor changes. Before proteinase K treatment, samples were treated with RNase for 1 h at 37 °C, purified with MinElute Reaction Cleanup kit (Qiagen) and eluted in 15 μL. Polyclonal antibody against GFP from Abcam (Ab290) was used to immunoprecipitate chromatin. The eluted chromatin was then diluted to a final volume of 100 μL of water, and 3 μL were used for quantitative PCR (qPCR) experiments in a LightCycler 480 Instrument (Roche). Enrichment was evaluated as the percentage of input for each sample with primers expanding the locus of the gene GA20ox2 (Fig. S2 A and B; also see list of primers below). The SEP3 gene was used as a positive control (Fig. S2C).

The following primers were used for ChIP-qPCR:

<table>
<thead>
<tr>
<th>Primer Used for ChIP-qPCR</th>
<th>Primer Sequence (5′ → 3′)</th>
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<tbody>
<tr>
<td>xGA20ox2fwA A</td>
<td>CTTGTCCTTTAGATTGAGACCAAAG</td>
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<td>xGA20ox2reA A</td>
<td>GCAAGCTCCAGTCTAGTGTGTTG</td>
</tr>
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<td>xGA20ox2fwD A</td>
<td>AACCTTCATGGAATTGAGAC</td>
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<tr>
<td>xGA20ox2fwE A</td>
<td>GACCACCTCCTCATCAAGACC</td>
</tr>
<tr>
<td>xGA20ox2reE A</td>
<td>CAATATTGCAACGAAAGCC</td>
</tr>
<tr>
<td>xGA20ox2_fwF A</td>
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</tr>
<tr>
<td>xGA20ox2_reK A</td>
<td>GCTTACATAATTGGTAAATGAAAG</td>
</tr>
<tr>
<td>xSEP3reA A</td>
<td>AGATGAGAAATCGGAC</td>
</tr>
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Quantification of Gibberellins. Aliquots (about 100–200 mg fresh weight) of frozen material were extracted with 80% methanol–1% acetic acid, and the extracts were passed consecutively through hydrophilic–lipophilic Balance (reverse phase), mixed-mode cation exchange (cationic exchange), and mixed-mode weak anion-exchange (ionic exchange) columns (Oasis 30 mg. Waters) to purify the GAs, as described (3). The final residue was dissolved in aqueous 5% (vol/vol) acetonitrile–1% (vol/vol) acetic acid, and the gibberellins (GAs) were separated using an autosampler and reverse-phase UPHL chromatography (2.6 μm Accucore RP-MS column, 50-mm length × 2.1-mm inner diameter; ThermoFisher Scientific) with an aqueous 5–50% (vol/vol) acetonitrile gradient containing 0.05% (vol/vol) acetic acid at 400 μL/min over 14 min. The GAs were analyzed by electrospray ionization (negative mode, spray voltage 3.0 kV, heater temperature 150 °C, sheath gas flow rate 40 μL/min, auxiliary gas flow rate 10 μL/min) and targeted selected ion monitoring (capillary temperature 300 °C, S-lens RF level 70, resolution 70.000) using a Q-Exactive spectrometer (Orbitrap detector; ThermoFisher Scientific). [17,17-2H]GAs (GA4 purchased from L. Mander, Canberra, Australia, and GA12, GA24, GA3, and GA51 from OlChemIm, Olomouc, Czech Republic) were added to the extracts as internal standards for quantification. The concentrations of GAs in the extracts were determined using embedded calibration curves and the Xcalibur program 2.2 SPl build 48.


Fig. S1. Transcriptional control of SVP downstream targets. Expression levels of SOCI (A and C) and FUL (B and D) in different genetic background (A and B) and in a shift experiment (C and D). In A and B, the plants were grown for 2 wk under SDs and the seedlings were harvested at ZT8. In C and D, the wild type and svp-41 plants were grown while they were still at vegetative stage for 14 and 10 SDs, respectively, and then transferred to LDs for 3 and 5 additional days. The apices of these plants were harvested at ZT8. (E) The spatial pattern of FUL mRNA during a time course under LDs in wild type, svp-41, and the ft-10 svp-41 plants grown for 8, 10, 12, and 14 LDs. (Scale bar: 50 μm.)
Fig. S2. ChIP analysis of SVP-GFP at the GA20ox2 locus, response to GA treatments in SVP mutants and overexpressors, and flowering time of svp-41 compared with svp-41 ga20ox2-1 plants in LDs. SVP direct-binding analysis to GA20ox2 by ChIP-PCR. (A) Schematic diagram showing the GA20ox2 genomic region. Exons are represented by black boxes, introns by the black line, and 3′ and 5′ UTR regions are represented white boxes. Consensus-binding sequences (CArG box) of MADS domain proteins are depicted. Gray boxes denote fragments spanning the locus examined by the ChIP enrichment test. (B) ChIP analysis of SVP-GFP binding to different regulatory regions of GA20ox2 described in A. (C) A SEP3 fragment of the promoter was amplified as a positive control for ChIP experiments. Results are represented as the percentage of input. Error bars represent SD. (D) Phenotype of wild type (Top), svp-41 (Middle), and 35S::SVP (Bottom) plants after GA4 treatment under SD conditions. GA4 was applied two times per week at ZT8. (E) Flowering time of svp-41 mutant compared with svp-41 ga20ox2-1 plants grown under LDs. Wild-type and ga20ox2-1 mutant plants were used as control. 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, \( P = 0.022 \)).
Fig. S3. Spatial expression studies of GA20ox2::GUS and effect of misexpression of SVP in the SAM. (A) Histochemical localization of GUS activity at SAM of pGA20ox2::GA20ox2::GUS seedlings harvested at 8, 11, and 14 LDs (see also Fig. 5 legend) and of pGA20ox2::GA20ox2::GUS svp-41 seedlings harvested during the transition to flowering (12 LDs). (Scale bar: 50 μm.) (B) Levels of GA20ox2 mRNA in apices of wild-type, ft-10 tsf-1, and ft-10 tsf-1 svp-41 mutant plants grown for 2 wk in SDs. (C and D) Flowering time of pKNAT1::SVP svp-41 T2 lines: (C) under LDs and (D) under SDs.
Fig. S4. Effect of misexpression of SVP in leaves. Flowering time of transgenic plants misexpressing SVP from the phloem-specific promoter pSUC2 in svp-41 plants under LDs (A) and SDs (B). (C) Expression levels of FT, TSF, and SOC1 in pSUC2::SVP svp-41 plants (lines #21, #24, and #43) grown for 10 d under LDs.
**Fig. S5.** Photoperiodic control of SVP expression involves FT TSF and SOC1 FUL. Temporal and spatial expression patterns of SVP at the meristem of ft-10 tsf-1 and soc1-2 ful-2 double mutants plants grown for 3 wk in SDs (0 LD) and then transferred to LDs (7 LDs). (Scale bar: 50 μm.)

**Fig. S6.** SVP regulates SPL4 expression downstream of the photoperiod pathway. Temporal and spatial expression patterns of SPL4 at the meristem 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) and for 15, 20, 25, and 30 LDs (B). (C) Pattern of SPL4 mRNA expression at the meristem of wild-type control under LDs (9, 13, and 15 LDs). (Scale bar: 50 μm.)
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Chlorophyll content (μmol/m²)</th>
<th>Height (cm)</th>
<th>Radius (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
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<td>45.0 ± 3.4</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>svp</td>
<td>183.6 ± 16.4</td>
<td>51.9 ± 5.0</td>
<td>3.2 ± 0.6</td>
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<tr>
<td>ga2ox2</td>
<td>218.5 ± 15.7⁺</td>
<td>33.8 ± 6.4</td>
<td>1.7 ± 0.2</td>
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<tr>
<td>svp ga2ox2</td>
<td>200.9 ± 11.9⁺ᵇ⁻</td>
<td>39.7 ± 3.4</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

Mean values among the treatment groups show statistical differences (P < 0.001). Mean values among the treatment groups indicated with the same letter do not show statistically significant difference. Leaf radius and chlorophyll content were estimated in 14-old-day plants grown in SDs, and the stem elongation measurement was carried out just before senescence started. n = 10.