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Analysis of the *Arabidopsis* Shoot Meristem Transcriptome during Floral Transition Identifies Distinct Regulatory Patterns and a Leucine-Rich Repeat Protein That Promotes Flowering [C][W][OA]

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Flowering of *Arabidopsis thaliana* is induced by exposure to long days (LDs). During this process, the shoot apical meristem is converted to an inflorescence meristem that forms flowers, and this transition is maintained even if plants are returned to short days (SDs). We show that exposure to five LDs is sufficient to commit the meristem of SD-grown plants to flower as if they were exposed to continuous LDs. The MADS box proteins SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) and FRUITFULL (FUL) play essential roles in this commitment process and in the induction of flowering downstream of the transmissible FLOWERING LOCUS T (FT) signal. We exploited laser microdissection and Solexa sequencing to identify 202 genes whose transcripts increase in the meristem during floral commitment. Expression of six of these transcripts was tested in different mutants, allowing them to be assigned to FT-dependent or FT-independent pathways. Most, but not all, of those dependent on FT and its paralog TWIN SISTER OF FT (TSF) also relied on SOC1 and FUL. However, this dependency on FT and TSF or SOC1 and FUL was often bypassed in the presence of the *short vegetative phase* mutation. *FLOR1*, which encodes a leucine-rich repeat protein, was induced in the early inflorescence meristem, and *flor1* mutations delayed flowering. Our data contribute to the definition of LD-dependent pathways downstream and in parallel to FT.

INTRODUCTION

In annual plants, such as *Arabidopsis thaliana*, floral transition initiates reproduction and the end of vegetative development. The timing of this transition is crucial for reproductive success and is therefore stringently controlled by developmental and environmental response pathways. In *Arabidopsis*, six genetic pathways contribute to the regulation of flowering (Fornara et al., 2010). Here, we focus on the photoperiod pathway that strongly promotes flowering under long days (LDs), while under short days (SDs), flowering is delayed. Photoperiod is perceived in the leaves, whereas flowers develop at the shoot apical meristem.

FLOWERING LOCUS T (*FT*) and its close homolog *TWIN SISTER OF FT* (*TSF*) are transcribed in the vascular tissue in response to LDs (Kardailsky et al., 1999; Kobayashi et al., 1999; Yamaguchi et al., 2005; Jang et al., 2009). Activation of these genes in response to LDs requires the CONSTANS (CO) transcription factor, which is stabilized specifically under these conditions (Turck et al., 2008). FT and TSF are small proteins related to phosphatidyl ethanolamine binding proteins. FT, and probably TSF, is a mobile signal that can move from the leaf to the meristem where it activates the floral transition (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007; Tamaki et al., 2007). FT and TSF interact with FD, a bZIP transcription factor (Abe et al., 2005; Wigge et al., 2005). *FD* is strongly and broadly expressed in the shoot apical meristem before the floral transition, and mutations in *FD* reduce the early flowering phenotype of plants overexpressing FT. Comparison of gene expression patterns in wild-type, *fd*, and *ft* mutants identified genes that are proposed to be activated in response to FT arrival at the meristem (Schmid et al., 2003; Searle et al., 2006; Wang et al., 2009). *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), which encodes a MADS box transcription factor, is of particular interest as this is the earliest activated gene in the meristem that has so far been identified after transferring plants from SDs to LDs (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000). Early activation of *SOC1* depends on *FT* and *TSF* and is delayed by mutations in *FD* (Searle et al., 2006). After activation, *SOC1* plays an important role as a floral promoter at the meristem. *FRUITFULL* (*FUL*), another MADS box gene closely related to *SOC1*, is also activated in response to FT (Schmid et al., 2003; Teper-Bamnolker and Samach, 2005). Although loss-of-function mutations of this gene only weakly

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delay flowering (Ferrándiz et al., 2000), *ful* mutations enhance the late-flowering phenotype of *soc1* mutants, and *soc1-3 ful-2* double mutants are strongly attenuated in their response to *FT* overexpression from the viral *35S* promoter (Melzer et al., 2008).

Several members of the *SQUAMOSA PROMOTER BINDING LIKE* (*SPL*) gene family, particularly *SPL3*, *SPL4*, *SPL5*, *SPL9*, and *SPL15*, also play important roles in the floral transition (Cardon et al., 1997; Schwarz et al., 2008; Wang et al., 2009; Yamaguchi et al., 2009). Most of these genes are increased in expression in the shoot apex in response to LDs based on Affymetrix microarray analysis (Schmid et al., 2003). Additionally, the temporal and spatial patterns of *SPL3*, *SPL4*, *SPL5*, and *SPL9* mRNA expression have been described by in situ hybridization during or after the floral transition (Cardon et al., 1999, 1997; Wang et al., 2009). These SPL factors directly bind to and activate transcription of *SOC1*, *APETALA1* (*AP1*), *LEAFY* (*LFY*), and *FUL* (Wang et al., 2009; Yamaguchi et al., 2009). The *SPL* genes proposed to have roles in floral transition belong to those negatively regulated by microRNA156 and microRNA157 (Schwab et al., 2005).

Repressors of flowering are also present in the meristem prior to floral transition. Of these, the MADS box proteins encoded by *FLOWERING LOCUS C* (*FLC*) and *SHORT VEGETATIVE PHASE* (*SVP*) strongly delay flowering (Michaels and Amasino, 1999; Sheldon et al., 1999; Hartmann et al., 2000). These proteins interact to form a heterodimer proposed to repress *SOC1* transcription (Fujiwara et al., 2008; Li et al., 2008). However, genetic analysis demonstrated that *flc svp* double mutants are earlier flowering than either single mutant, suggesting that these proteins likely have distinct as well as overlapping functions (Fujiwara et al., 2008; Li et al., 2008). Both proteins act in the leaf and meristem to delay flowering by repressing *FT* and *TSF* or *SOC1* transcription, respectively (Searle et al., 2006; Lee et al., 2007; Jang et al., 2009).

The process of floral induction ends with the initiation of flower development. Expression of *AP1* defines this stage, and by the time *AP1* is expressed, floral determination has occurred, so that plants continue to flower independently of environmental cues, such as light quality and daylength (Hempel et al., 1997). *AP1* encodes a MADS box transcription factor that together with LFY specifies floral identity, and during the early stages of floral development, its expression is restricted to the floral primordium.

Present genetic data as well as temporal and spatial expression patterns are consistent with a largely linear process during the early stages of floral induction by photoperiod. According to this representation, FT/TSF acting through FD activates *SOC1* and *FUL* transcription in the meristem, leading to downstream events, such as the activation of *SPL* genes. SPLs would then activate *LFY* and *AP1* transcription. However, arguing against such a simple representation, the *soc1-3 ful-2* double mutant retains a response to photoperiod; indeed, those plants are only moderately late flowering in LDs compared with wild-type plants and much earlier flowering than *ft-10 tsf-1* double mutants (Melzer et al., 2008). Furthermore, downregulation of expression of several *SPL* genes by *35S*:*miR156* does not strongly delay flowering under LDs (Schwab et al., 2005). Such observations suggest that additional genes promote the floral transition in response to photoperiod through FT and TSF. Consistent with this proposal, expression profiling of apices during floral induction identified several hundred genes that changed in expression within 7 d of exposure to LDs and a few hundred prior to *AP1* induction (Schmid et al., 2003). Such data suggest a complex transcriptional reprogramming of the apex during floral induction.

In this study, we employed a highly tissue-specific approach using laser microdissection to collect specifically shoot apical meristems of plants shifted from SDs to LDs and studied global gene expression changes in this material by sequencing cDNA. We identified ~200 genes that are upregulated in the shoot meristem prior to induction of *AP1* and placed them in hierarchies relative to FT/TSF, SOC1/FUL, and SVP. Inactivation of one of the newly identified genes encoding a leucine-rich repeat (LRR) protein caused later flowering and enhanced the late flowering of *soc1 ful* double mutants.

RESULTS

Contribution of *SOC1*, *FUL*, and *SVP* to Floral Commitment in Response to Long Photoperiods

Arabidopsis plants are induced to flower by exposure to LDs and when exposed to sufficient LDs can maintain the floral transition even if returned to SDs, a process called commitment. To better characterize this process, the minimum number of LDs required to stably commit *Arabidopsis* Columbia (Col) to flowering was determined. Two-week-old SD-grown plants were transferred to LDs for various lengths of time and then returned to SDs. After exposure to five LDs, 80 to 100% of plants flowered with a similar number of leaves to plants transferred to continuous LDs (Figure 1A). Therefore, five LDs were sufficient to commit the plants to flower. In agreement with this conclusion *AP1* mRNA was already expressed in floral primordia after exposure to five LDs (see Supplemental Figure 1A online). Exposure to only one LD did not influence flowering time, whereas three LDs accelerated flowering, but not to the same extent as plants exposed to continuous LDs.

The effect of varying the duration of SD exposure prior to transfer to LDs was also tested. In addition to 2 weeks in SD conditions, plants were grown for 1, 3, or 4 weeks in SDs before transfer to LDs (see Supplemental Figure 1B online). Generally, plants grown for longer in SDs became more sensitive to LD exposure. Two weeks in SDs was chosen as the period of vegetative growth prior to transfer to LDs for all of the subsequent experiments because under these conditions, the floral transition takes place within a relatively extended time window of five LDs, allowing processes to be temporally separated at the meristem during the transition.

The MADS box proteins SOC1 and FUL are both required to maintain the transition to flowering when plants are exposed continuously to LDs (Melzer et al., 2008). To test the contributions of SOC1 and FUL to floral commitment, 2-week-old SD-grown *soc1-2* and *ful-2* mutants, as well as the *soc1-2 ful-2* double mutant, were transiently exposed to LDs. The *soc1-2* and *ful-2* single mutants required nine and seven LDs, respectively, to become fully committed to flowering, and unlike wild-type plants

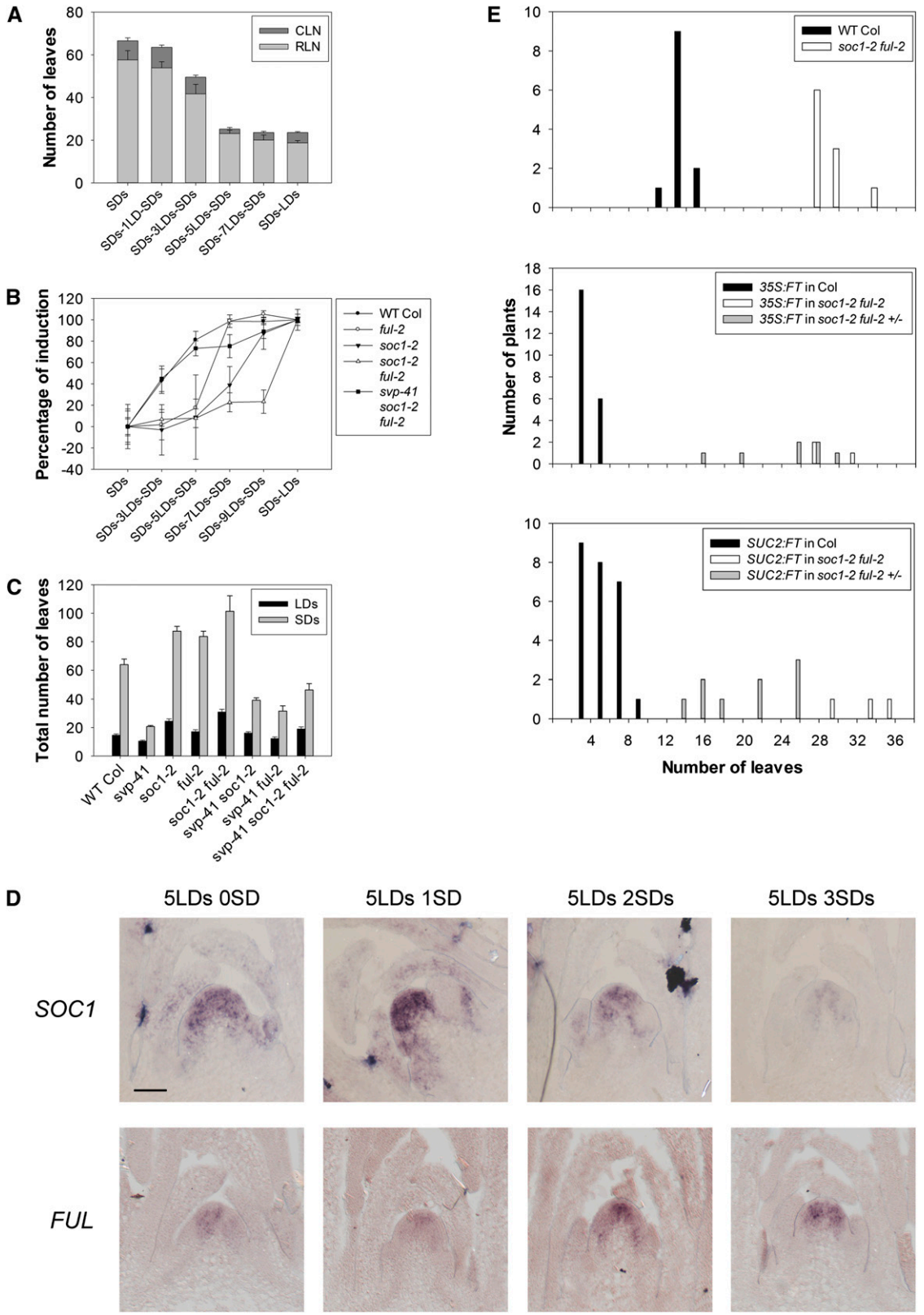

Figure 1. The Influence of *SOC1* and *FUL* on the Commitment to Flower.

were almost insensitive to shorter exposures of three and five LDs (Figure 1B). Therefore, *soc1-2* and *ful-2* mutants required significantly longer exposure to LDs than Col to become committed to flowering, although when grown continuously under LDs, the *ful-2* mutant flowered only after forming two to three more leaves than Col (Figure 1C). The double mutant *soc1-2 ful-2* required longer exposure to LDs than either single mutant to become committed, and even after 9 d exposure only 20% of plants were committed, supporting the idea that *SOC1* and *FUL* are partially redundant (Figure 1B; see Supplemental Figure 1C online). Despite this dramatic increase in LD exposure required for commitment, *soc1-2 ful-2* plants were not dramatically late flowering under continuous LDs, as previously reported (Melzer et al., 2008), and flowered after forming around 12 leaves more than the wild type (Figure 1C).

In response to LDs, *FT* and *TSF* expression in the leaves is necessary for activation of *SOC1* and *FUL* transcription in the meristem. However, *FT* transcription is rapidly repressed after return of plants from LDs to SDs (Corbesier et al., 2007). Therefore, *SOC1* and *FUL* might maintain the floral state because after transfer to SDs their mRNA expression persists in the meristem even after reduction of *FT* transcription in the leaves or because after exposure to several LDs, commitment becomes independent of *SOC1* and *FUL* expression. To test these possibilities, in situ hybridizations were performed on apices of plants exposed to one, three, or five LDs and then transferred back to SDs (Figure 1D; see Supplemental Figure 2 online). Both *SOC1* and *FUL* were reduced in expression on return to SDs when plants were exposed to three or fewer LDs (see Supplemental Figure 2 online), consistent with these plants not being committed to flower. *SOC1* expression was also gradually reduced in the shoot apical meristem after return to SDs when plants were exposed to five LDs. By contrast, *FUL* expression was maintained under SDs after exposure to five LDs (Figure 1D). Consistent with the commitment step, *AP1* mRNA was visible by in situ hybridization at the floral meristem after five LDs and remained expressed after the committed plants were moved to SDs (see Supplemental Figure 2 online). These results suggest that maintenance of high levels of *SOC1* mRNA requires continued exposure to LDs and is not required for commitment to flowering. By contrast, *FUL* becomes independent of LDs once plants are committed to flower, and its expression does not subside after return to SDs, suggesting that mechanisms independent of *FT* expression or continued exposure to LDs maintain *FUL* expression.

Previously, SVP was shown to repress *FT* and *TSF* expression in the leaves and *SOC1* expression at the meristem (Lee et al., 2007; Li et al., 2008; Jang et al., 2009). To better characterize the function of SVP in the meristem, *svp-41 soc1-2 ful-2* triple mutants were constructed and compared with *soc1-2 ful-2*. When exposed continuously to LDs or SDs, the *soc1-2 ful-2* double mutant flowered later than either single mutant, as described previously (Melzer et al., 2008), and was extremely late flowering under SDs. By contrast, the triple mutant *svp-41 soc1-2 ful-2* flowered only slightly later than Col under LDs and much earlier than Col under SDs, although the triple mutant was later flowering than *svp-41* mutants under both conditions (Figure 1C). In addition, *soc1-2 ful-2* double mutants underwent reversion to vegetative growth after transition to flowering, as previously observed for *soc1-3 ful-2* (Melzer et al., 2008), and the *svp-41* mutation also partially suppressed this aspect of the phenotype. The *svp-41 soc1-2 ful-2* triple mutant still formed an additional set of leaves growing on the secondary inflorescences that does not occur in wild-type plants (see Supplemental Figure 3 online), but did not produce the typical aerial rosette leaves on the senescent inflorescences of the *soc1-2 ful-2* double mutant. Therefore, *svp-41* can almost completely suppress the effect of *soc1-2 ful-2* on flowering time and inflorescence development. These data suggest that additional targets of SVP at the apex can promote flowering in the absence of *SOC1* and *FUL*. Alternatively, *FT* and *TSF* overexpression in leaves of the *svp-41* mutant (Jang et al., 2009) might be responsible for activating floral promoters other than *SOC1* and *FUL*.

These possibilities were first tested by exposing SD-grown *soc1-2 ful-2* and *svp-41 soc1-2 ful-2* plants to LDs and then returning them to SDs (Figure 1C; see Supplemental Figure 1C online). The *svp-41* mutant grown for 2 weeks in SDs was fully committed to flower after exposure to one LD, while if grown for only 1 week in SDs was fully committed after three LDs and is therefore more sensitive to LDs than Col. Furthermore, the

Figure 1. (continued).

(A) Commitment of wild-type Col to flower after exposure to LDs. Flowering time expressed as leaf number. Plants were grown for 2 weeks in SDs and then transferred to different numbers of LDs, as indicated in the axis legend, before returning to SDs. CLN, cauline leaf number; RLN, rosette leaf number. Error bars represent SD. Sixteen plants were used to score flowering time under each condition.

(B) Commitment of *soc1-2 ful-2* and *svp-41 soc1-2 ful-2* as well as control genotypes after SD-grown plants were exposed to various numbers of LDs. Percentage of induction is calculated according to the formula indicated in Methods. Error bars represent SD. At least eight plants were used to score flowering time under each condition and genotype. WT, wild type.

(C) Flowering time of *soc1-2 ful-2* and *svp-41 soc1-2 ful-2* as well as control genotypes grown continuously under LDs (black columns) or SDs (gray columns). The number of leaves is total leaves (rosette plus cauline). Error bars represent SD. At least eight plants were used to score flowering time under each genotype.

(D) Analysis of *SOC1* and *FUL* expression after transient exposure of SD-grown plants to LDs. Plants were grown for 2 weeks in SDs, transferred to LDs for 5 d, and transferred back to SDs as indicated above each panel. Bar = 50 μm.

(E) Effects of Pro*35S:FT* and Pro*SUC2:FT* on flowering time in Col and *soc1-2 ful-2* genotypes. Number of leaves is rosette leaf number in individual T1 plants. The *ful-2* homozygote is difficult to transform so that *soc1-2* homozygous *ful-2* heterozygous plants were transformed. T1 plants are therefore all *soc1-2* homozygous but segregate for the *ful-2* mutation. Only *soc1-2 ful-2* and *soc1-2 ful-2*/+ individuals are shown.

[See online article for color version of this figure.]

reduction of sensitivity to LDs in the *soc1-2 ful-2* double mutant was almost completely overcome in the *svp-41 soc1-2 ful-2* triple mutant. These results indicate that in addition to repressing *SOC1*, SVP likely represses genes that can bypass the effect of *soc1-2 ful-2* on floral commitment and flowering.

The strong early flowering phenotype conferred by *FT* in a single Pro*35S*:*FT* transformant was suppressed in the *soc1-3 ful-2* double mutant (Melzer et al., 2008), suggesting that increased expression of *FT* in *svp-41 soc1-2 ful-2* plants is unlikely to be the cause of suppression of the *soc1-2 ful-2* phenotype. To test this observation further in a wider range of Pro*35S*:*FT* transformants and in Pro*SUC2:FT* plants, in which *FT* is overexpressed directly in the companion cells where *FT* is normally expressed, Pro*35S*:*FT* and Pro*SUC2*:*FT* transgenes were introduced into *soc1-2 ful-2* and Col plants by *Agrobacterium tumefaciens*–mediated transformation (see Methods). The effect of overexpressing *FT* from Pro*35S*:*FT* or Pro*SUC2*:*FT* was strongly suppressed in *soc1-2 ful-2* plants and did not result in a significant acceleration of flowering of the double mutants (Figure 1E). Therefore, the suppression of *soc1-2 ful-2* by *svp-41* is unlikely to be due to increased *FT* expression and suggests that SVP represses additional genes at the meristem that are capable of suppressing *soc1-2 ful-2* when increased in expression.

Taken together, the transient exposure of plants to LDs reveals a role for SOC1 and FUL in committing the plant to flower in response to LDs, and this effect is markedly stronger than the delay in flowering observed under continuous LDs. SOC1 and FUL are functionally important downstream of FT, and LD induction of *SOC1* is not maintained when plants are returned to SDs, suggesting that *SOC1* is highly responsive to *FT*/*TSF* expression levels and that maintenance of a high level of *SOC1* expression is not required for commitment. The requirement for SOC1 and FUL to commit plants to flowering can be overcome in an *svp* mutant background, suggesting that SVP represses further redundant genes.

RNA-Seq Analysis of Laser-Dissected Meristems to Identify Further Genes Expressed during the Floral Transition

The analysis of *svp-41 soc1-2 ful-2* triple mutants suggested that additional genes are likely to be active in the meristem and that some of these can overcome the requirement for *SOC1* and *FUL* during floral induction. To identify a wider range of genes increased in expression in the meristem during the transition to flowering, RNA-seq was performed on laser-dissected meristems. Use of this approach excluded young leaves that are present in hand-dissected apical samples and therefore further enriched for meristem-expressed mRNAs. Meristems were dissected from plants 8 h after dawn (Zeitgeber time [ZT] 8) after 2 weeks in SDs, and 1 and 3 d after transfer to LDs (see Supplemental Figure 4 online). This procedure was repeated in triplicate. RNA was extracted from these meristems, and the resulting cDNA from the nine samples was sequenced using Solexa/Illumina technology. The sequences obtained were then aligned to cDNAs of *Arabidopsis* using Megablast (see Methods). The number of sequence reads obtained in all nine samples that aligned to each *Arabidopsis* cDNA are shown in Supplemental Data Set 1 online.

Genes that increased in expression during the floral transition after exposure to LDs were then identified from the sequence reads by screening for cDNAs to which more sequence reads aligned from the 2 weeks of SDs plus LDs sample than the 2 weeks of SDs sample (see Methods). The initial comparison was done using the 2 weeks of SDs plus three LDs sample because LD-induced genes were often more highly expressed as the exposure to LDs was extended. Only cDNAs that aligned to more sequence reads from the 2 weeks of SDs plus three LDs sample in all three experiments with a P value of ≤ 0.05 were initially considered. Although genes that exhibited reduced expression during this period were present in the reads, these were not studied in detail. In total, 202 upregulated genes were identified using the criteria described, and these genes are listed in Supplemental Data Set 2 online. The list included previously characterized flowering time genes that increase in expression in the meristem during floral transition, such as *SOC1*, *FUL*, and *SPL9*. The number of reads obtained for each of these genes at different time points is illustrated graphically in Figure 2A. No reads were detected for the *AP1* or *LFY* genes in any of the samples, consistent with the idea that the mRNA was extracted early in the flowering process before these genes are expressed (Figure 2B). The mRNAs of other flowering genes known to be expressed in the shoot apical meristem, such as *AGL24*, *SVP*, and *FD*, were detected in the samples (Figure 2B). These genes are expressed in the SDs sample, as observed previously (Yu et al., 2002; Wigge et al., 2005; Jang et al., 2009) and therefore were not consistently detected as increased in abundance in the 2 weeks of SDs plus three LDs samples and thus do not appear in Supplemental Data Set 2 online.

A Gene Ontology (GO) enrichment analysis was performed on the 202 upregulated genes compared with the whole genome (Figure 2D; see Supplemental Data Set 3 online; see Methods). Some terms expected to be enriched were indeed overrepresented, such as genes involved in postembryonic development, reproductive development, and flower development. In addition, enzymes involved in oxidation/reduction reactions were also unexpectedly overrepresented.

The Genesis program was used to cluster and identify coregulated genes among the 202 candidate genes proposed to be upregulated in the meristem during the floral transition (see Methods; Figure 2E; see Supplemental Figure 5 online). This approach identified classes of genes for further analysis, some of which are studied in detail in the following section.

Spatial and Temporal Patterns of Expression of Selected Genes during the Transition to Flowering

The temporal and spatial patterns of expression of a subset of the candidate genes were characterized in detail by in situ hybridization. These genes increased in expression during the time course and were initially named *FLORAL TRANSITION AT THE MERISTEM1* (*FTM1*) to *FTM6* (Figure 2C; see Supplemental Figure 6A online). Three of the candidate genes were chosen because they belonged to different groups in the cluster analysis (*FTM1*, *FTM2*, and *FTM5* [*FAF2*]; illustrated in Figure 2E and described in Table 1). In addition, *FTM4* was chosen because, although it was excluded by the stringent criteria applied to

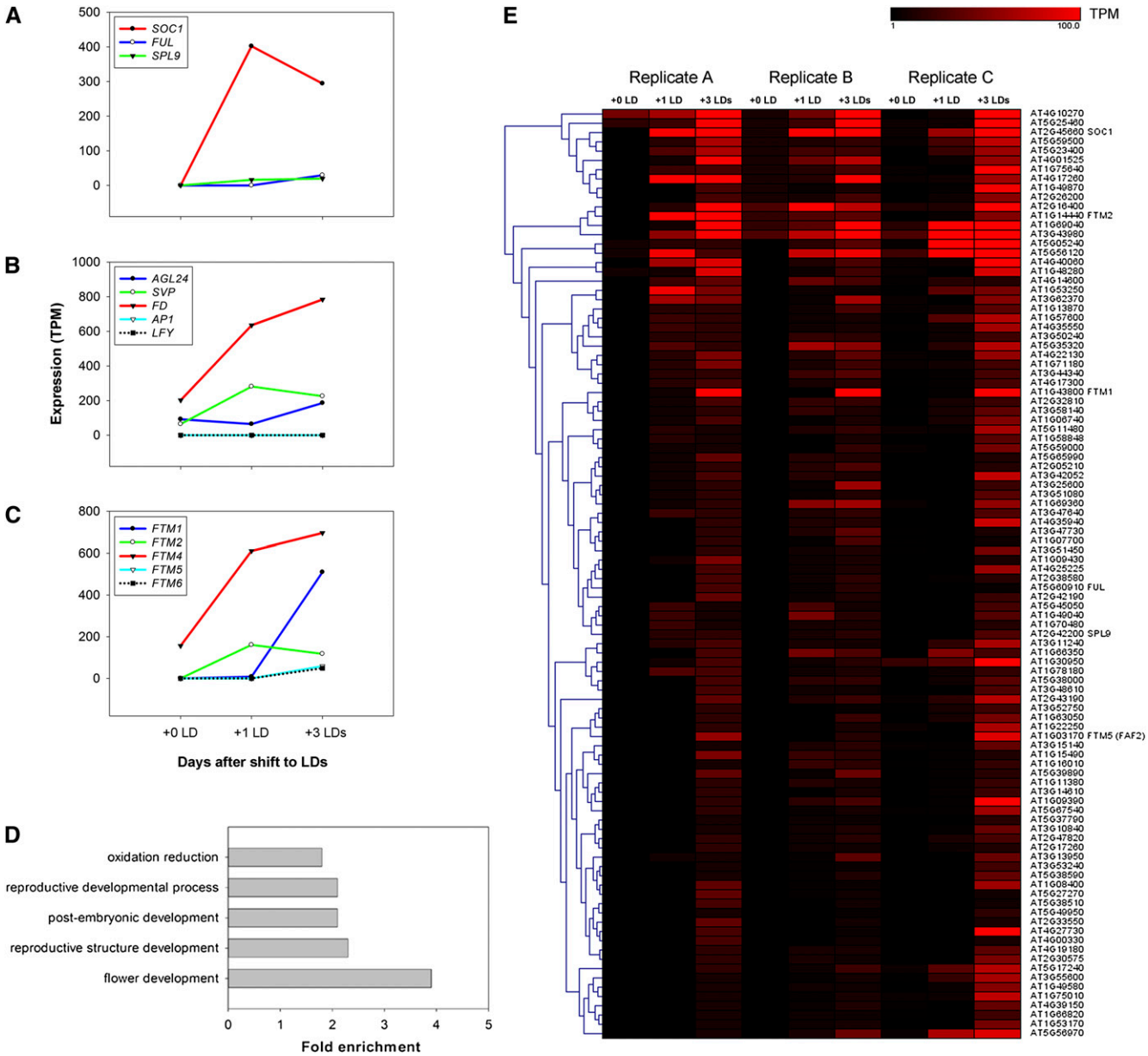

Figure 2. Transcriptomics of Shoot Apical Meristems Undergoing the Floral Transition.

(A) to **(C)** Analysis of expression of specific genes in the Solexa sequence reads (for replicate A). Expression level is indicated on the *y* axis as transcripts per million (TPM) reads. Time points are represented on the *x* axis: 2 weeks in SDs (+0 LDs); 2 weeks in SDs plus 1 LDs (+1 LDs); 2 weeks in SDs plus 3 LDs (+ 3 LDs).

(A) Known flowering time genes not expressed in the meristem prior to LD exposure.

(B) Floral meristem identity genes and flowering time genes (*FD* and *AGL24*) expressed prior to exposure to LDs.

(C) *FTM* genes identified and studied here in detail. Data for *FTM3* are shown in Supplemental Figure 5 online.

(D) GO terms of classes of genes enriched among the 202 upregulated genes shown in Supplemental Data Set 3 online. These classes are enriched in the 202 genes compared with the whole genome.

(E) Heat map showing a clustering of genes among the 202 upregulated genes. *FTM* genes and flowering time genes are indicated on the right side along with all gene codes.

assemble Supplemental Data Set 2 online, it appeared to be expressed in the meristem of plants exposed to three LDs, was clearly upregulated in two experiments, and little data on its expression during floral induction is available because it is not present on the ATH1 Affymetrix array. *FTM6* was upregulated after three LDs in the Illumina sequence data. Also, because it is a member of the *SPL* family of transcription factor–encoding genes that are important in the floral transition, it was important to define its temporal expression pattern during floral induction. Finally, *FTM3* was included because it is upregulated in the

Table 1. Summary of Genes Identified as Being Upregulated and Examined by in Situ Hybridization in Different Genetic Backgrounds

At Gene No.	Gene Isolation Nos.	Other Names	Predicted Gene Product
AT1G43800	*FTM1*	*S-ACP-DES6*	Stearoyl-ACP-desaturase
AT1G14440	*FTM2*	*ATHB31*	Zinc finger-homeodomain protein.
AT2G18160	*FTM3*	*GBF5, BZIP2*	bZIP transcription factor
AT3G12145	*FTM4*	*FLOR1*	LRR protein
AT1G03170	*FTM5*	*FAF2*	Unknown protein
AT1G53160	*FTM6*	*SPL4*	SQUAMOSA PROMOTER BINDING LIKE transcription factor

sequence data, but it was not included in Supplemental Data Set 2 online because its complex annotation led to an ambiguous assignment of reads to transcripts and therefore prevented its detection in the Megablast pipeline (see Methods; see Supplemental Methods 1, Supplemental References 1, Supplemental Data Set 4, and Supplemental Figure 6B online). These six genes also encode proteins with diverse biochemical functions, including enzymes (FTM1), transcription factors (FTM2, FTM3, and FTM6), and novel proteins of unknown functions (FTM4 and FTM5). Some of these genes have been studied previously (see below), but their temporal and spatial expression patterns in the early stages of floral transition have not been described. In situ hybridizations were performed on the meristems of wild-type plants after growth for 2 weeks under SDs and then 1, 3, and 5 d after transfer to LDs (Figure 3). *SOC1* was used as a control.

SOC1 (Figure 3) was not expressed after 2 weeks in SDs, and expression of this gene increased progressively during exposure from one to five LDs, as described previously (Borner et al., 2000; Samach et al., 2000; Searle et al., 2006).

FTM1 encodes the enzyme stearoyl-ACP desaturase, and expression of this member of the gene family could not be detected previously by RT-PCR (Kachroo et al., 2007). However, *FTM1* mRNA is present in the epidermis of young leaves of SD-grown plants by in situ hybridization (Figure 3). As the floral transition proceeds, *FTM1* mRNA also becomes expressed in the apex, in the region of the rib meristem, of plants exposed to three and five LDs.

FTM2 encodes the zinc finger homeodomain transcription factor ATHB31 (Tan and Irish, 2006). The mRNA of *FTM2* was not detected in the meristem prior to exposure to LDs, but after exposure to three LDs, it was present on the flanks of the meristem adjacent to floral primordia (Figure 3). The mRNA persisted in a similar pattern after five LDs.

FTM3 encodes the bZIP transcription factor AtbZIP2, related to ATB2, which is involved in Suc signaling (Rook et al., 1998; Jakoby et al., 2002). The mRNA was expressed in SD apices, specifically in a region in the center of the SAM; however, after three LDs, its abundance increased strongly. After five LDs, *FTM3* mRNA was also detected in regions extending into the vascular tissue adjacent to the shoot apical meristem.

FTM4 is *FLOR1*, which encodes an intracellular LRR protein described to interact with the MADS box transcription factor AGAMOUS (Gamboa et al., 2001). It was weakly expressed prior to floral induction but increased strongly in expression in the meristem after exposure to three LDs, as the meristem changes shape, becoming more domed (Figure 3). After five LDs, this gene was strongly expressed in the meristem but appeared to be excluded from the developing floral primordia (see also below).

FTM5 is *FANTASTIC FOUR2* (*FAF2*), which encodes a protein of unknown function and was previously shown to be increased in expression during the floral transition by analysis of Affymetrix array data (Wahl et al., 2010), although its spatial expression was not followed through an inductive time course as shown in Figure 3. *FAF2* mRNA was only faintly detected in the center of the meristem in 2-week-old SD-grown plants but increased in the meristem after exposure to three and five LDs.

FTM6 is *SPL4* and its mRNA was not detected prior to LD induction. However, it was detected by in situ hybridization after exposure to five LDs in the rib meristem, in a distinct spatial pattern to that described at a similar stage for *SPL3* and *SPL9* (Wang et al., 2009). This pattern of *SPL4* mRNA expression likely persists after floral induction into the mature inflorescence meristem (Cardon et al., 1999).

These data demonstrate that the mRNAs of genes identified in the expression profiling are increased in abundance in the meristem early during the floral transition, within three to five LDs after transfer from SDs and that they accumulate in different spatial patterns within the meristem and shoot apex. Once expressed, their mRNAs persist throughout the period that the meristem undergoes commitment to flowering.

Roles of Known Floral Regulators in Induction of *FTM* Genes during the Floral Transition

Several mutations that impair early changes in gene expression at the shoot apical meristem during photoperiodic floral induction have been characterized (Turck et al., 2008). To place the induced genes within this regulatory hierarchy, in situ hybridizations were performed for the subset of induced genes described above on apices of *ft-10 tsf-1*, *soc1-2 ful-2*, *svp-41*, *svp-41 ft-10 tsf-1*, and *svp-41 soc1-2 ful-2* plants transferred from SDs to LDs. The results are shown in Figure 4 for plants exposed to five LDs, and the complete time course of in situ hybridizations is shown in Supplemental Figure 7 online.

Flowering of the *ft-10 tsf-1* double mutant is insensitive to daylength due to loss of photoperiodic signaling between the leaf and the meristem; therefore, in this double mutant, the expression of many genes induced in the meristem on transfer to LDs is expected to be impaired (Yamaguchi et al., 2005; Jang et al., 2009). As previously shown, *SOC1* expression is delayed in *ft-10 tsf-1* double mutants (Jang et al., 2009), and it is not induced in those mutants after transferring plants to five LDs (see Supplemental Figure 8 online). Similarly, *FLOR1*, *FTM2*, *FAF2*, and *SPL4*

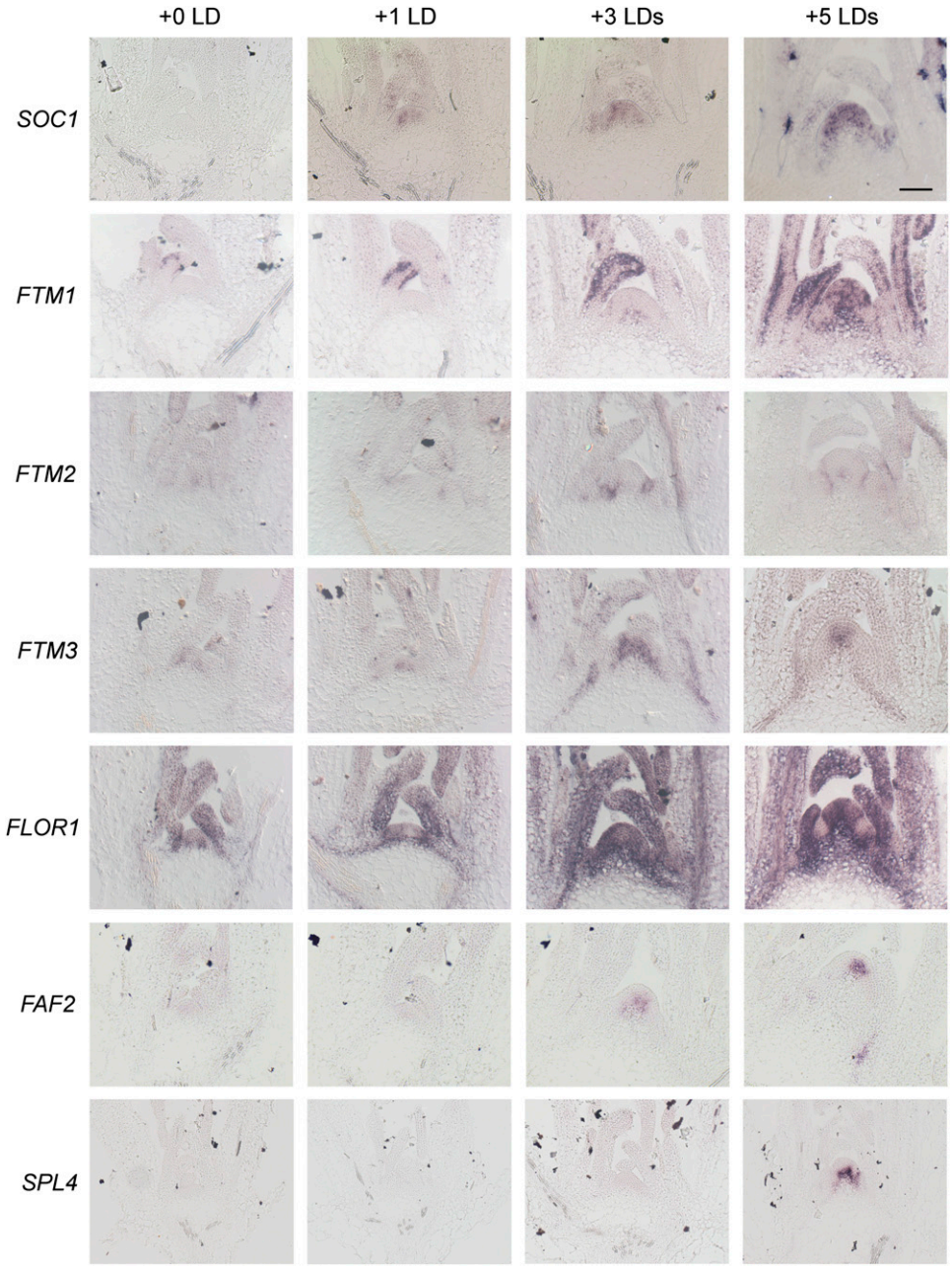

Figure 3. Spatial Patterns of Expression in the Shoot Apex of Candidate Genes during Floral Transition Induced by Transfer from SDs to LDs.

In situ hybridization on apices of wild-type Col grown for 2 weeks in SDs (0 LD) and then transferred to LDs for one LD, three LDs, or five LDs. The genes used as probe are shown on the left of each time course. Samples were harvested at ZT8. *SOC1* was used as a control. The remaining six genes were extracted from the Solexa reads, as described in the text. Bar = 50 μm.
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were not induced in *ft-10 tsf-1* double mutants after exposure to five LDs (Figure 4). These experiments confirm that FT and TSF ultimately regulate the expression of a wide range of genes expressed in diverse spatial patterns in the meristem in response to photoperiod, as previously proposed based on Affymetrix array analysis of apical RNA samples extracted from *ft* mutants (Schmid et al., 2003). However, surprisingly, some genes were induced in the meristem in response to photoperiod even in the absence of FT and TSF. In *ft-10 tsf-1* double mutants, an increase in expression of *FTM3* still occurs in the meristem and vascular tissue after transfer to five LDs, although this increase is reduced compared with Col (Figure 4). Similarly, *FTM1* is induced in the

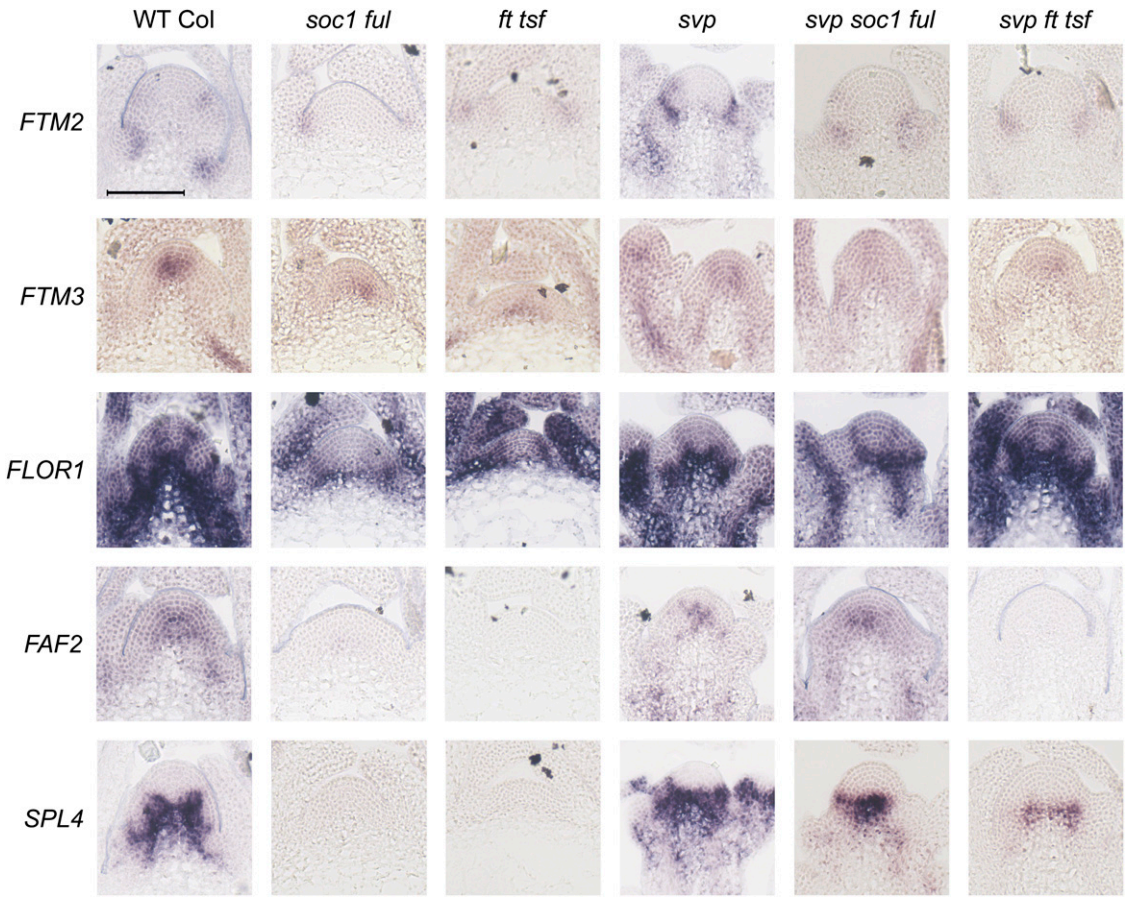

Figure 4. Expression of Candidate Genes in the Shoot Apices of Mutants Impaired in Various Genes of the Photoperiodic Pathway after Exposure to Five LDs.

In situ hybridization on apices of wild-type Col and illustrated mutant genotypes grown for 2 weeks in SDs and then transferred to five LDs. The genes used as probes are shown on the left. The entire time course after transfer is shown in Supplemental Figure 7 online. The *svp* mutant flowers early under SDs; therefore, these plants were grown for only 10 SDs before transfer. Samples were harvested at ZT8. *FTM1* is not shown as its expression did not change significantly in these genotypes, and it is shown in Figure 5. Bar = 50 μm.

center of the meristem of *ft-10 tsf-1* double mutants around three LDs after transfer in a similar way as it is in Col plants (Figure 5A). These data demonstrate that some genes are induced in the meristem in response to LDs at least partly independently of FT and TSF.

FT and TSF are proposed to activate flowering through the rapid induction of *SOC1* in the shoot apical meristem. Therefore, we tested whether the expression of the analyzed genes in the meristem in response to LDs depends on *SOC1* and the partially redundant gene *FUL*. The abundance of *FLOR1*, *FTM2*, and *SPL4* mRNA was not increased in response to five LDs in *soc1-2 ful-2* plants (Figure 4); therefore, the strong response of these genes to five LDs in Col plants depends on *SOC1* and *FUL*. By contrast, *FAF2* expression was increased in the *soc1-2 ful-2* double mutant 5 d after transfer to LDs, but to a lesser extent than in Col (Figure 4; see Supplemental Figure 7 online). Furthermore, in agreement with what was observed in *ft-10 tsf-1* double mutants, *FTM3* was induced in *soc1-2 ful-2* double mutants after transfer to LDs (Figure 4).

The *svp-41* mutation strongly suppressed the effect of *ft-10 tsf-1* and *soc1-2 ful-2* on flowering time (Jang et al., 2009) (Figure 1). Therefore, to test whether the effect of *ft-10 tsf-1* and *soc1-2 ful-1* on induction of gene expression could be overcome by the *svp-41* mutation, in situ hybridizations were performed on *svp-41 soc1-2 ful-2* and *svp-41 ft-10 tsf-1* plants transferred from SDs to LDs. *SPL4* was induced in *svp-41 soc1-2 ful-2* and, to a lesser extent, in *svp-41 ft-10 tsf-1* after five LDs (Figure 4), whereas no *SPL4* mRNA was detected in *soc1-2 ful-2* or *ft-10 tsf-1* meristems (Figure 4). Therefore, in *svp-41* mutants, SOC1 and FUL are not required for *SPL4* activation in response to LDs, and the requirement for FT and TSF is reduced. By contrast, *FAF2* mRNA

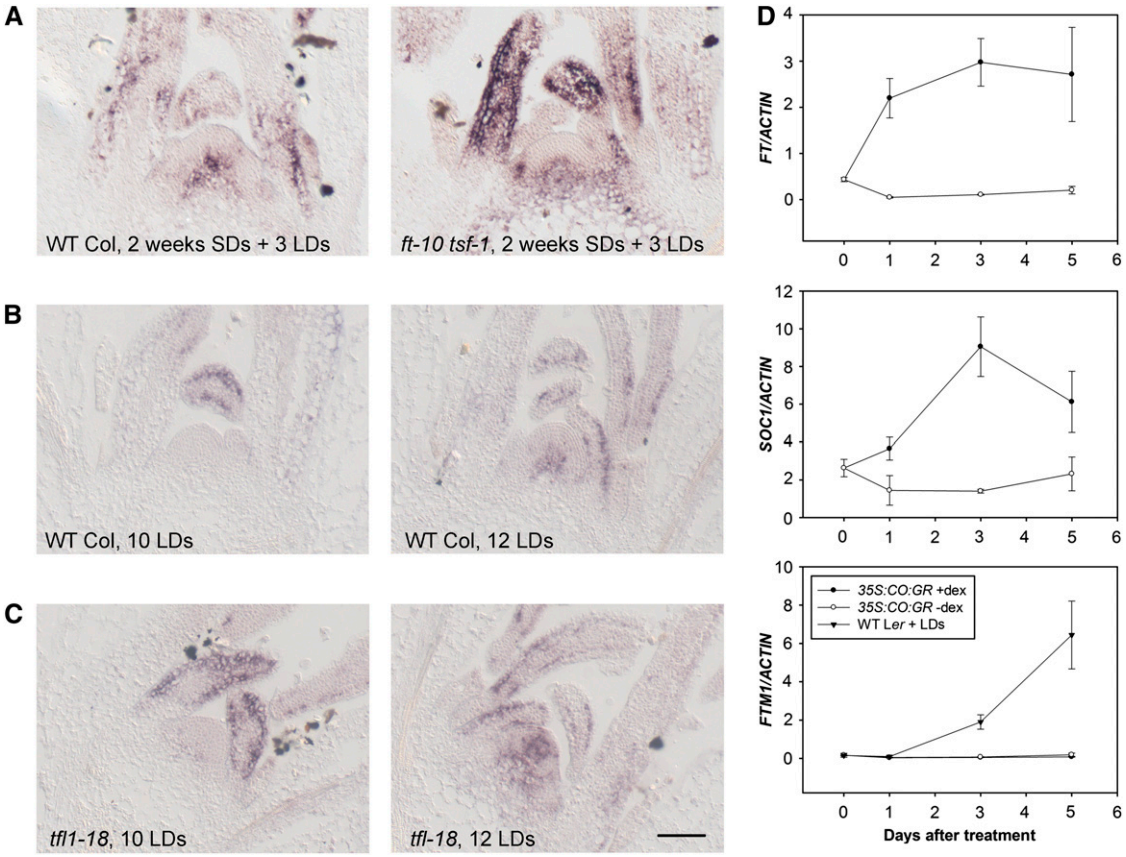

Figure 5. Relationship between Induction of *FTM1* and Activity of *FT*.

(A) In situ hybridization analysis of *FTM1* expression in apices of Col (left panel) and *ft-10 tsf-1* (right panel) grown for 2 weeks in SDs and then induced for three LDs. WT, wild type.

(B) In situ hybridization analysis of *FTM1* in apices of Col grown from germination under LDs for 10 (left panel) or 12 (right panel) d.

(C) In situ hybridization analysis of *FTM1* in apices of *tfl1-18* mutants grown from germination under LDs for 10 (left panel) or 12 (right panel) d. Bar = 50 μm.

(D) Activation of *FT* by CO does not lead to *FTM1* activation under SDs. Pro*35S*:*CO*:*GR* plants were grown under SDs and exposed to dex. L*er* plants were transferred from SDs to LDs. RNA was extracted from apices. Top panel: Analysis of *FT* mRNA by RT-PCR in Pro*35S*:*CO*:*GR* plants treated with dex or a mock solution . Middle panel: Analysis of *SOC1* mRNA by RT-PCR in Pro*35S*:*CO*:*GR* plants treated with dex or a mock solution. Bottom panel: Analysis of *FTM1* mRNA by RT-PCR in Pro*35S*:*CO*:*GR* plants treated with dex or a mock solution. L*er* plants transferred from SDs to LDs act as a control. Two biological replicates of the experiment were performed, and for each of them three technical replicates were compared in the PCR reaction. Error bars represent SD in one experiment. In all panels, *x* axis is days after treatment. Closed circles, Pro*35S*:*CO*:*GR* treated with dex; open circles, Pro*35S*:*CO*:*GR* treated with mock. In the bottom panel, closed triangles indicate Col transferred from SDs to LDs.

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expression was dependent on *FT* and *TSF*, even in *svp-41* mutants. In *svp-41 ft-10 tsf-1* triple mutant plants, *FAF2* mRNA was not detected in the meristem after exposure to five LDs, but in *svp-41 soc1-2 ful-2* plants, *FAF2* mRNA was expressed in the meristem (Figure 4). This result indicates that *svp-41* can overcome the requirement for SOC1 and FUL for activation of *FAF2* in response to LDs but not the requirement for FT and TSF. *FLOR1*, on the other hand, seemed to show a higher dependency on SOC1 and FUL than FT and TSF in an *svp-41* mutant background. In *svp-41 ft-10 tsf-1* plants, *FLOR1* mRNA was expressed after exposure to five LDs and in a similar spatial pattern to wild-type plants (Figure 4). However, in *svp-41 soc1-2 ful-2* plants, *FLOR1* mRNA level was not as highly or broadly expressed after five LDs as in wild-type or *svp-41 ft-10 tsf-1* plants. These results suggest that *svp-41* can overcome the requirement for *FT* and *TSF* in *FLOR1* induction but can only partially do so for *SOC1* and *FUL*.

Taken together, these results suggest that early induction of gene expression in the meristem in response to LDs is regulated to a large extent by FT and TSF as well as by SOC1 and FUL. Generally, *FTM2*, *FLOR1*, and *SPL4* expression in the meristem correlates with flowering in all genotypes. However, exposure to LDs does lead to the activation of some genes in the meristem independently of flowering and of SOC1 and FUL or FT and TSF. Also, the capacity of loss of SVP to overcome the impairment of SOC1 and FUL or FT and TSF function differs depending on the downstream gene, so that *FAF2* does not respond to *svp-41* in the absence of FT and TSF, whereas *SPL4* responds more

strongly to *svp-41* in the absence of SOC1 and FUL than in the absence of FT and TSF.

FTM1 Is Induced in the Shoot Meristem during Floral Induction but Is Not Activated by the Photoperiodic Flowering Pathway

FTM1 expression was increased in the meristem of Col and *ft-10 tsf-1* plants transferred from SDs to LDs (Figure 5A). To test whether this induction is associated with floral induction or with another process related to transfer from SDs to LDs, the pattern of *FTM1* mRNA was followed in plants of different ages continuously grown in LDs. *FTM1* mRNA was not detected in the shoot meristem of 10-d-old wild-type plants, which are still vegetative, but was present in the meristems of 12-d-old plants, which are in the early stages of floral induction (Figure 5B). Therefore, in these plants, *FTM1* expression in the meristem correlates with floral induction. The expression pattern of *FTM1* was also tested in *tfl1-18* mutants grown under LDs, which flower earlier than the wild type (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). *FTM1* mRNA was already weakly detected in 10-d-old *tfl1-18* plants and was strongly detected in the meristem of 12-d-old plants (Figure 5C) and so is expressed earlier than in wild-type plants, again correlating with floral induction. These experiments indicate that *FTM1* expression in the meristem is associated with the floral transition even in plants grown continuously in LDs.

FT and *TSF* were not required to induce *FTM1* expression in the meristem of plants transferred from SDs to LDs. However, FT and TSF might still activate *FTM1*, but do so redundantly with another LD-regulated signal. To test whether induction of *FT* mRNA is able to activate *FTM1* expression, transgenic plants in which *FT* expression can be induced in SDs due to activation of CO function were used (Simon et al., 1996). Pro*35S*:*CO*:*GR* plants grown in SDs were treated with dexamethasone (dex) to induce CO activity. RT-PCR analysis demonstrated that expression of *FT* and *SOC1* mRNA was induced rapidly after treatment with dex (Figure 5D). However, no increase in abundance of *FTM1* mRNA was detected in these plants. By contrast, RT-PCR analysis did detect an increase in *FTM1* expression after transferring Landsberg *erecta* (L*er*) plants from SDs to LDs, as shown above by in situ hybridization for wild-type Col. Therefore, activation of CO and FT activities is not sufficient to induce expression of *FTM1* under SDs, and FT is neither required nor sufficient to induce *FTM1* expression. Thus, the induction of *FTM1* in the shoot apical meristem during floral induction in LDs is independent of CO, FT, and TSF.

FLOR1 Contributes to Early Flowering

FLOR1 is weakly expressed in the rib meristem before floral transition in plants grown under SDs and strongly increased in expression after transfer to LDs (Figure 3). The strong expression in the inflorescence rib meristem persists after floral induction and is excluded from floral primordia that express *LFY* and *AP1* mRNA (Figures 6A and 6B). To test whether *FLOR1* contributes to floral induction, the gene was overexpressed and insertion mutations were identified. Overexpression of *FLOR1* from the cauliflower mosaic virus *35S* promoter had no consistent effect on flowering, indicating that *FLOR1* expression is not limiting on flowering. In addition, two T-DNA insertion mutations were identified, *flor1-1* and *flor1-2*, both of which carry a T-DNA insertion in the single intron of *FLOR1*. No expression of the second exon was detected by RT-PCR in either mutant (see Supplemental Figure 9A online). Furthermore, in the *flor1-1* mutant, no *FLOR1* mRNA was detected in the meristem region by in situ hybridization (Figure 6C). These results suggest that both mutant alleles reduce *FLOR1* activity.

The flowering time of *flor1-1* and *flor1-2* mutants was measured under LDs and SDs. Both mutants flowered slightly late under LDs, producing two to three more leaves than wild-type plants (Figure 6D). Under SDs, no effect on flowering time was detected. To test whether *flor1* mutations enhance the effect of *soc1-2* and *ful1-2* on flowering time, the double and triple mutant combinations were constructed. The *flor1-1 soc1-2* and *flor1-1 ful-2* double mutants flowered at similar times to *soc1-2* and *ful-2* single mutants (see Supplemental Figure 9B online). However, the triple mutant *flor1-1 soc1-2 ful-2* flowered significantly later than the *soc1-2 ful-2* control, producing ~10 more leaves than *soc1-2 ful-2* double mutants (Figure 6E). Consistent with this conclusion, quantitative RT-PCR analysis of *LFY* and *AP1* mRNAs in *flor1-1* mutants transferred from SDs to LDs showed that these genes are more rapidly induced in Col plants than *flor1-1* mutants (Figure 6F). Thus, *FLOR1* promotes flowering and its role is partially redundant with *SOC1* and *FUL*.

DISCUSSION

Analysis of the meristem during the five LDs required to commit the plant to flowering demonstrated the complexity of the inductive pathways involved in the early stages of floral induction within the shoot apical meristem. Pathways downstream of FT and TSF were found to act in a nonlinear way, and pathways acting in parallel to FT and TSF were identified. Mutations in one of the genes strongly induced in the inflorescence meristem, *FLOR1*, that encodes a LRR protein enhanced the late-flowering phenotype of *soc1-2 ful-2* plants. Our conclusions are summarized in the diagram shown in Figure 7.

Transient Exposure to LDs and Commitment of the Meristem to Flowering

Commitment to flowering after transient exposure to LDs indicates that the inflorescence meristem can stably maintain its identity in noninductive conditions. Expression of *AP1*, which only occurs in floral primordia, was detected after five LDs and defines floral commitment. *AP1* expression was previously shown to mark the time of commitment, or determination, after exposure to different wavelengths of light (Hempel et al., 1997). The duration of this commitment period likely depends on the age of the plants and the precise environmental conditions, such as light intensity, wavelength of light, and temperature (Corbesier et al., 1996; Hempel et al., 1997; King et al., 2008).

The related MADS box transcription factors SOC1 and FUL have partially redundant roles in flowering time and maintenance

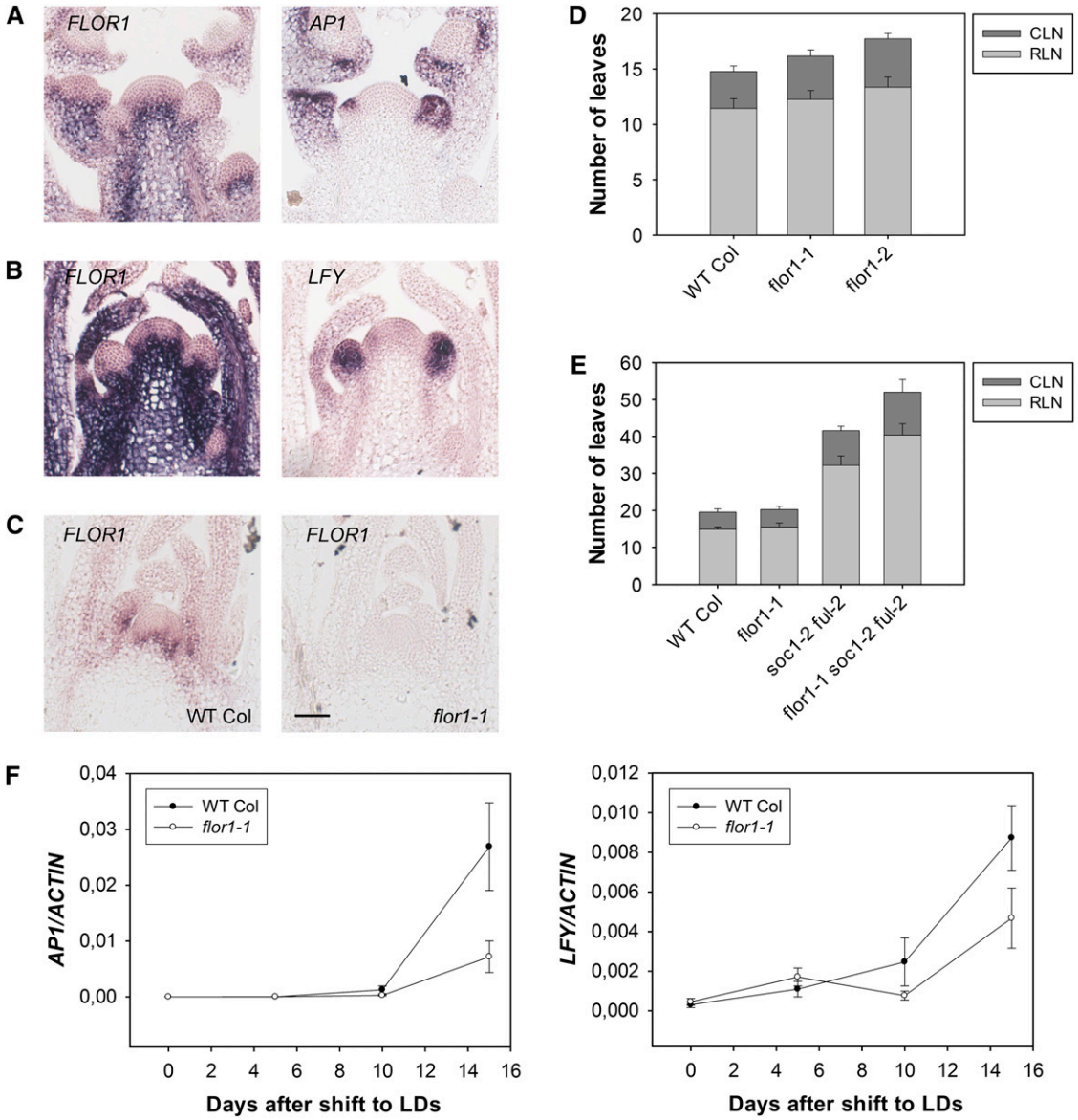

Figure 6. Genetic Analysis of Role of Candidate Genes in the Floral Transition.

(A) In situ hybridization of *FLOR1* and *AP1* probes on consecutive sections of apices of 22-d-old Col plants grown under LDs.

(B) In situ hybridization of *FLOR1* and *LFY* probes on consecutive sections of apices of 22-d-old Col plants grown under LDs.

(C) In situ hybridization of *FLOR1* probe on apices of Col and *flor1-1* mutant plants grown for 12 d under LDs. WT, wild type. Bar = 50 μm.

(D) Flowering time in LD of *flor1-1* and *flor1-2* plants compared with wild-type Col. The *flor1-2* mutant was later flowering than Col ($P < 0.05$ in two experiments). *flor1-1* was significantly late flowering ($P < 0.05$) in terms of total leaf number in only two of five experiments. CLN, cauline leaf number; RLN, rosette leaf number. Error bars represent SD. At least eight plants were used to score flowering time of each genotype in each experiment.

(E) *flor1-1* enhances the late-flowering phenotype of *soc1-2 ful-2* double mutants ($P < 0.05$ in two experiments). Plants were grown under LDs. CLN, cauline leaf number; RLN, rosette leaf number. Error bars represent SD. At least eight plants were used to score flowering time of each genotype in each experiment.

(F) The *flor1-1* mutation delays activation of *AP1* and *LFY*. RNA was extracted from apices of plants grown under SDs for 2 weeks and transferred to LDs. Left panel: *AP1* expression, as determined by qRT-PCR. Right panel: *LFY* expression. *x* axis: days after transfer from SDs to LDs. Two biological replicates of the experiment were performed, and for each of them three technical replicates were compared in the PCR. Error bars represent SD in one experiment, and the other biological replicate produced similar results.

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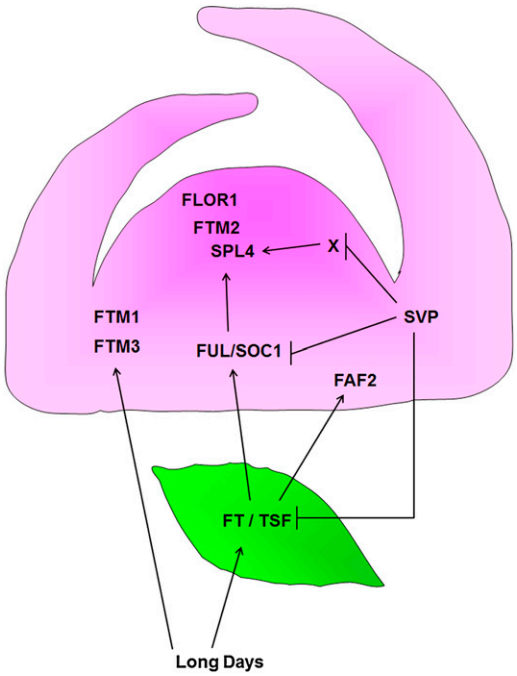

Figure 7. Summary of the Major Gene Interactions during Floral Induction Identified by Genetic and Experimental Analyses Described in the Text.

Exposure of plants to LDs activates *FT* and *TSF* transcription in leaves. *FT* and *TSF* are required for activation of *SOC1* and *FUL* in the shoot apical meristem. Expression of *FLOR1*, *FTM2*, and *SPL4* in the meristem of wild-type plants during floral induction depends on *FT* and *TSF* as well as *SOC1* and *FUL*. *FAF2* can be activated independently of *SOC1* and *FUL* but depends on *FT* and *TSF*. *FTM1* and *FTM3* are activated independently of *FT* and *TSF* and *SOC1* and *FUL*. Mutation of *SVP* increases expression of *FLOR1*, *FTM2*, and *SPL4* even in the absence of FUL and SOC1 or FT and TSF. Leaf shown in green; meristem shown in pink.

of the inflorescence meristem in plants continuously exposed to LDs (Melzer et al., 2008). The expression of both genes is induced in the meristem after exposure of SD-grown plants to one to three LDs (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000). Failure of *soc1-2 ful-2* to commit to flowering in response to five LDs is probably due to a severe delay in activation in the meristem of many flowering genes. The failure of the *soc1-2 ful-2* double mutant to maintain the inflorescence under continuous LDs (Melzer et al., 2008) may be related to the commitment to flower phenotype described here; however, the maintenance phenotype was observed in plants that had undergone the transition to flowering and then reverted to vegetative growth, which cannot easily be explained by delayed flowering. The inflorescence maintenance phenotype may be a consequence of impairment of a later function of SOC1 in the inflorescence meristem or floral primordia (Lee and Lee, 2010).

Commitment of the meristem to continue forming flowers after return to SDs is not due to continued expression of *FT* in the leaves (Corbesier et al., 2007). *SOC1* mRNA level in the meristem fell substantially when plants exposed to five LDs were returned to SDs, suggesting that *SOC1* mRNA level is highly responsive to FT. The reduction in *SOC1* mRNA after return of plants to SDs suggests that its contribution to floral commitment has already occurred during the first five LDs. By contrast, *FUL* mRNA level was maintained in the meristem after return to SDs and indeed appeared to continue to increase, perhaps allowing it to continue to contribute to floral commitment. This expression pattern suggests the presence of a positive feedback loop that maintains *FUL* expression even after *FT* and *SOC1* mRNA levels have declined. Such feedback loops mark developmental shifts in other systems (Adrian et al., 2009). SPL transcription factors, whose mRNAs rise in expression after induction of *SOC1* and *FUL*, might be involved in maintaining *FUL* expression because SPL3, for example, has been shown to directly activate *FUL* (Wang et al., 2009; Yamaguchi et al., 2009). Furthermore, we found that *SOC1* and *FUL* are required for activation of *SPL4*. These observations support the existence of a loop in which FUL is involved in activation of *SPL* genes, which then maintain *FUL* expression.

The *svp-41* mutation can overcome the strong impairment of floral commitment and delay of flowering time in *soc1-2 ful-2* double mutants. SVP directly represses *SOC1* expression in the meristem, but the observation that *svp-41* can overcome the phenotype of *soc1-2 ful-2* argues that SVP has additional targets in the meristem. If it represses expression of such genes, then when derepressed in an *svp-41* mutant their increased expression might overcome the late flowering phenotype of the *soc1-2 ful-2* double mutant. These additional genes are unlikely to be the known targets of SVP in the leaves, *FT* and *TSF*, because overexpression of *FT* from the phloem-specific *SUC2* promoter had little effect on the flowering time of *soc1-2 ful-2* plants, as shown previously for the *35S* promoter (Melzer et al., 2008). Understanding the multiple roles of SVP in the meristem in delaying flowering time would contribute to unraveling the layers of genetic redundancy that exist during the early stages of floral induction.

Transcriptional Reprogramming of the Shoot Apical Meristem during Floral Induction

Laser microdissection combined with next-generation sequencing or microarrays was applied to plant tissues previously, for example, the shoot apical meristem and leaves of maize (*Zea mays*) or the female gametophyte of *Arabidopsis* (Jones-Rhoades et al., 2007; Li et al., 2010; Matias-Hernandez et al., 2010). The spatial resolution of the method allowed us to focus specifically on meristem tissue, excluding the leaf primordia, young leaves, and vascular tissue that are present in hand-dissected apical samples. Such enrichment of meristem tissue likely also increases the sensitivity with which meristem transcripts are detected. GO analysis demonstrated that genes involved in processes such as reproductive development were indeed enriched in upregulated genes compared with the whole transcriptome. However, enzymes involved in oxidation-reduction reactions were also unexpectedly highly overrepresented. The function of most of these enzymes is unknown, but the expression pattern of one of them, *FTM1*, was confirmed by in situ hybridization.

Previously, Affymetrix ATH1 microarray hybridizations were used to analyze gene expression changes in apical samples during floral induction (Schmid et al., 2003). Of the 202 genes

identified here as being upregulated in the shoot apical meristem, only 174 were present on the ATH1 microarray. Of these 174, three were detected in the Affymetrix analysis as being upregulated by at least twofold in the apical samples after 3 d and nine after 7 d. Of the six genes analyzed in detail in this study, one, *FLOR1*, was not annotated on the ATH1 array. Only *FAF2* of the remaining five was upregulated by at least twofold in the three LD apical sample. At seven LD, *SPL4* and *FTM1* were also upregulated twofold. *FTM3* and *FTM2* never reached twofold upregulation in the apical samples, even after exposure to seven LDs. Therefore, there is an overlap between genes upregulated in the laser microdissection Solexa data and the Affymetrix apical samples. However, genes were also identified in the laser microdissection data that were not present in the apical Affymetrix data, partly because of the limited annotation of the ATH1 array and presumably also due to the higher sensitivity conferred by enriching for meristems with the laser microdissection method. Furthermore, in our experiments, plants were grown for a shorter time in SD conditions prior to transfer to LDs, and this may have helped to reduce the expression level in the SD control samples of genes associated with flowering.

Induced Genes Differentially Respond to Known Floral Regulators Active in the Meristem

A prediction of the current model of photoperiod response is that many or all of the genes induced in wild-type meristems by exposure to LDs would not be induced in *ft-10 tsf-1* double mutants. Indeed, this was the case for *SOC1*, *FLOR1*, *FTM2*, *FAF2*, and *SPL4*. Under SDs, some of these genes might respond to other inputs as has been shown for *SOC1* (Moon et al., 2003). Complex mechanisms must be required to interpret the FT signal transmitted from the leaves to ultimately activate distinct pathways leading to expression of genes in such diverse spatial patterns.

Surprisingly, meristem induction of *FTM1* and *FTM3* still responded to daylength in *ft-10 tsf-1* double mutants with similar kinetics and amplitude observed in wild-type plants. *FTM3* encodes a bZIP transcription factor in Group S (Jakoby et al., 2002). The best-characterized member of this group, ATBZIP11, is expressed in sink tissues and responds to Suc levels (Rook et al., 1998). During the transition to flowering, the shoot apical meristem becomes a stronger sink tissue and levels of Suc increase at the meristem (Bernier et al., 1993). Therefore, the increase in *FTM3* mRNA observed in the meristem might be a response to increasing carbohydrate levels. If so, this would represent an FT- and TSF-independent mechanism by which expression of regulatory genes can increase at the meristem during floral induction. *FTM1* is also involved in metabolism, encoding a putative stearoyl-acyl carrier protein-desaturase (S-ACP-DES) enzyme. This gene family is composed in *Arabidopsis* of seven members (Kachroo et al., 2007). The best characterized member is *SUPPRESSOR OF SA INSENSITIVITY2*, and mutants for this gene contain low levels of the monosaturated fatty acid and are impaired in salicylic acid as well as jasmonic acid signaling (Kachroo et al., 2001; Shah et al., 2001). Although other members of the S-ACP-DES gene family also seem to regulate lipid profiles, it is not clear to what extent they regulate SA or JA signaling (Kachroo et al., 2007). The specific expression pattern of *FTM1* during floral induction could indicate involvement in the meristem during floral induction of SA signaling, which has been reported to alter flowering time (Martínez et al., 2004). *FTM1* is not regulated by FT, and the mechanism by which it is induced in the meristem during floral induction is not known.

Genes that require FT and TSF for their induction in the meristem generally also require SOC1 and FUL. Thus, *FLOR1*, *SPL4*, and *FTM2* expression patterns are similarly affected in *ft-10 tsf-1* and *soc1-2 ful-2* backgrounds. However, the spatial patterns of SPL4 and FTM2 do not overlap, indicating that SOC1 and FUL somehow activate spatially restricted pathways within the meristem. Also, *ft-10 tsf-1* and *soc1-2 ful-2* double mutants are not identical with respect to meristem gene expression patterns. *FAF2* mRNA is faintly detected in *soc1-2 ful-2*, but absent from *ft-10 tsf-1*, suggesting a stronger requirement for *FT* and *TSF*. Also, these genes can be divided into distinct regulatory hierarchies based upon their regulation by SVP, as described below. In addition, the late flowering phenotype of *ft-10 tsf-1* is more extreme than that of *soc1-2 ful-2*, excluding the possibility that FT and TSF act only through *SOC1* and *FUL*.

SPL4 expression is restored in the meristems of *svp-41 soc1-2 ful-2* or *svp-41 ft-10 tsf-1* plants exposed to five LDs. Therefore, genes deregulated in the meristems of *svp-41* mutants are able to induce *SPL4* expression in *ft-10 tsf-1* or *soc1-2 ful-2*. This expression of *SPL4* correlates with earlier flowering of *svp-41 ft-10 tsf-1* and *svp-41 soc1-2 ful-2* compared with *ft-10 tsf-1* or *soc1-2 ful-2*. By contrast, induction of *FAF2* during floral induction showed an absolute requirement for FT and TSF even in the presence of *svp-41*. *FAF2* is a member of a gene family comprising four related genes (Wahl et al., 2010). Based on analysis of Pro*35S*:*FAF* plants, the *FAF* genes were proposed to regulate meristem function by modulating the *WUSCHEL* (*WUS*)-*CLAVATA3* (*CLV3*) feedback loop that maintains the meristem. These results of Wahl et al. (2010) together with our observations that *FAF2* expression depends on FT and TSF suggest a link between FT (and TSF) signaling and the *WUS-CLV3* feedback loop that maintains meristem function.

The *PENNYWISE* (*PNY*) and *POUNDFOOLISH* (*PNF*) homeobox genes are also strongly required for expression of *SPL4* and *SPL5* at the apex and suppress the early flowering caused by *FT* overexpression (Kanrar et al., 2008; Lal et al., 2011). These effects are similar to those described above for *soc1-2 ful-2*. However, the mechanism by which *PNY* and *PNF* affect *SPL4* and *SPL5* mRNA levels is unlikely to be through *SOC1*, as this gene is expressed at almost normal levels in *pny pnf* plants (Smith et al., 2004). How the function of the *PNY* and *PNF* genes intersects with that of *FT* and *TSF* as well as *SOC1* and *FUL* remains unclear.

Role of FLOR1 in Regulating Flowering Time

FLOR1 is a member of the plant-specific LRR protein subfamily (Gamboa et al., 2001; Acevedo et al., 2004). Unlike most plant-specific LRR proteins that have been characterized, FLOR1 has an intracellular location in the cytoplasm and nucleus (Acevedo et al., 2004). FLOR1 was initially isolated because it interacts in

vitro and in yeast with AGAMOUS, a MADS box transcription factor involved in the development of stamens and carpels. In situ hybridizations demonstrated that *FLOR1* mRNA is present in mature stamens and carpels and therefore overlaps with *AG* expression (Acevedo et al., 2004). Our in situ analyses showed that *FLOR1* is weakly expressed in the vegetative shoot meristem and then upregulated in the inflorescence meristem within 3 d of induction. Subsequently, it is excluded from young floral primordia marked with *AP1* and *LFY* mRNAs. This inflorescence pattern is similar to that of *SOC1* mRNA, which is excluded from the domain in which *AP1* is expressed but is expressed later in the center of the flower. As observed for AG, FLOR1 might interact with other MADS box proteins expressed in the inflorescence meristem during floral transition. Such proteins are unlikely to be SOC1 or FUL because *flor1* mutations delayed the flowering phenotype of *soc1-2 ful-2* double mutants. However, several other MADS box proteins are expressed in the inflorescence meristem (Michaels et al., 2003; Dorca-Fornell et al., 2011). The biochemical significance of the interaction between FLOR1 and MADS box proteins has not been established but is likely to represent a function not previously implicated in floral induction.

METHODS

Plant Material and Growth Conditions

Plants used in this study were *Arabidopsis thaliana* accession Col or L*er*. Mutant alleles in the Col background were previously described: *soc1-2* (Lee et al., 2000), *ft-10 tsf-1* (Jang et al., 2009), *ful-2* (Ferrándiz et al., 2000), *svp-41* (Hartmann et al., 2000), and *tfl1-18* (Conti and Bradley, 2007). Lines carrying T-DNA insertions in the *FLOR1* locus were SALK_093764 for *flor1-1* and SALK_120575 for *flor1-2*.

The Pro*35S:CO:GR* line is in the *co-2* background in L*er* (Simon et al., 1996).

Plants were genotyped using specific primers listed in Supplemental Table 1 online.

To promote germination, seeds were stratified on soil at 4°C for 3 d in the dark. Plants for the experiments were grown in growth cabinets. LDs were 16 h of light and 8 h of dark, and SDs were 8 h of light and 16 h of dark. The temperature in the growth cabinet was 18°C.

Flowering Time Measurements and Percentage of Induction

Flowering time was scored as number of leaves at bolting. The number of rosette leaves was counted until the bolting shoot reached around 1 cm in length. Cauline leaves were counted when they were all visible on the shoot. At least eight genetically identical plants were used to score flowering time of each genotype. The Student's *t* test was used to test the significance of flowering time differences.

For each population X (a distinct genotype or a population with a certain time of vegetative growth in SDs), the following formula was used to calculate the degree of induction in the double shift experiments:

$$\text{Percentage of induction } (X) = \frac{\text{NSD} - \text{NX}}{\text{NSD} - \text{NLD}} \times 100,$$

where NSD is the number of leaves at flowering for the plants in SDs, NLD is the number of leaves at flowering for the plants shifted from SDs to LDs, and NX is the number of leaves at flowering of the plants for which the percentage of induction is calculated. NSD, NLD, and NX are total leaf numbers (rosette plus cauline leaves), calculated as an average for the population exposed to a particular condition. SD was calculated according to the classical propagation of errors for a division.

Plant Transformation

Plants were transformed with *Agrobacterium tumefaciens* strain GV3101 pMP90 RK by floral dipping (Clough and Bent, 1998). Plasmids containing Pro*SUC2:FT* and Pro*35S:FT* in *Agrobacterium* were used to stably transform wild-type Col and *soc1-2 ful-2* double mutants and they were described (An et al., 2004).

Plants carrying the transgene were selected by spraying with glufosinate (BASTA) at 100 mg/L, and then the insertion of the transgene was checked with specific primers by PCR on genomic DNA extracted from single leaves from the independent lines. Plants at the T1 generation were scored for flowering time. To make transgenic lines of *soc1-2 ful-2* double mutants, plants homozygous for the *soc1* mutation and heterozygous for the *ful-2* mutation were transformed with *Agrobacterium*. The *ful-2* mutation at the T1 generation was followed by sequencing the PCR product with specific primers (see Supplemental Table 1 online) for the heterozygotes and by the silique phenotype for the *ful* homozygotes.

In Situ Hybridizations

The methods used for digoxigenin labeling of mRNA probes, tissue preparation, and in situ hybridization were previously described (Bradley et al., 1993) with small modifications. Protease treatment was performed with Proteinase K (1 μg/mL in 100 mM Tris, pH 8, and 50 mM EDTA) at 37°C for 30 min, instead of Pronase. The posthybridization washes were performed in 0.1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate).

For *AP1*, a previously described plasmid (Mandel et al., 1992) was used to synthesize a probe of 720 bp.

For all the other genes, templates for probes to detect their transcripts were PCR amplified from cDNA using specific primer pairs (see Supplemental Table 1 online) that amplify part of the cDNA, with T7 RNA polymerase binding sites attached to the reverse primers. Hybridization experiments for each gene were generally repeated three times on each set of samples using at least two biological replicates.

Sample Collection and Preparation for Laser Microdissection

The procedure used to prepare the samples followed the one used for the in situ hybridization with specific modifications.

Seedlings were collected and fixed with ethanol:acetic acid in a 3:1 ratio and continuously kept on ice during the harvesting to preserve the RNA. To allow penetration of the fixative, the tissue was vacuum infiltrated using a pump, fixative was replaced, and the samples left at 4°C on ice overnight. The following day, the fixative was replaced with a stepwise ethanol:water series at 4°C (85% ethanol, 4 h; 95% ethanol, 4 h; 100% ethanol, overnight; 100% ethanol, fresh). The samples were stored at 4°C in 100% ethanol until embedding.

Samples were stained with eosin (0.1% Eosin Y in 100% ethanol) prior to embedding in paraffin. Embedding in Paraplast Plus (McCormick) paraffin was performed with the automated system ASP300 tissue processor (Leica). Wax blocks with eosin-stained samples were stored at 4°C until sectioning.

The slides used for laser microdissection were PALM MembraneSlides (PEN-membrane, 1 mm) from P.A.L.M. Microlaser Technologies. They were treated to remove possible RNase contamination with dry heat at 180°C for 4 h. This was followed by UV treatment by irradiation with UV light at 254 nm for 30 min using a cross-linker UV Stratalinker 1800 (Stratagene). This provides further sterilization and allows the membranes to become more hydrophilic.

Embedded plants were sectioned using a rotary microtome (Leitz 1512) at 10-μm thickness and collected on the laser microdissection slides.

Laser Microdissection

To dissolve the paraffin, the slides were exposed to Histo-clear solvent (National Diagnostics) and then to a series of ethanol/water solutions with increasing concentrations of water (100% Histo-clear, 2 min; 100% Histo-clear, 2 min; 100% ethanol, 1 min; 96% ethanol, 1 min; 70% ethanol, 1 min; 50% ethanol, 1 min; water, 1 min) and allowed to dry.

Laser microdissection optionally coupled to laser pressure catapulting was performed with the HAL 100 model (230 VZ) from P.A.L.M., equipped with an Axiovert 200 M from Zeiss. After microdissection of the tissue, the sample was collected into PALM AdhesiveCaps (from P.A.L.M.).

RNA Extraction and Amplification after Laser Microdissection

The samples were dissolved from the caps of the collection tubes, to extract the RNA, with 100 μL RLT buffer (from the RNeasy kit; Qiagen) + β-mercapto-ethanol (10 μL for 1 mL of buffer).

After 10 min at room temperature, tubes were vortexed for 10 min and spun in a bench-top centrifuge at 13,400 relative centrifugal force for 5 min. Samples were stored at −80°C to avoid RNA degradation. Total RNA was extracted using a PicoPure extraction kit (Arcturus).

RNA amplification was performed using a RiboAmp HS amplification kit (Arcturus). The procedure was followed according to the manufacturer's manual.

The RNA quality tests were performed with the Agilent 2100 bioanalyzer (Agilent Technologies) using an RNA 6000 Pico assay kit (Agilent Technologies).

RT-PCR Methods

Total RNA was extracted using an RNAeasy kit (Qiagen). Any DNA contamination was removed by DNaseI treatment (Ambion). cDNA synthesis was performed with SuperScript II (Invitrogen) using oligo(dT) primers. Three micrograms of RNA per sample was used to synthesize cDNA for quantitative real-time PCR. The synthesized cDNA was diluted to a final volume of 150 μL with water, and 3 μL was used for the PCR amplification.

For real-time PCR, three replicates were used for each sample. Amplified products were detected using SyBR green I in an IQ5 (Bio-Rad) thermal cycler. *ACTIN2* was used as a housekeeping gene to normalize the expression of the genes investigated.

Illumina-Solexa Sequencing

To evaluate gene expression in the samples collected by laser microdissection, the RNA was converted into double-stranded cDNA with two methods depending on the samples. For replicates A and B, the cDNA synthesis was done using the RiboAmp kit (Arcturus), and the synthesis was initiated using polyT-based primers. Samples were then sequenced as "genomic sample preparation." For the samples of replicate C, the mRNA-Seq sequencing protocol derived from Illumina was followed and random primers were used to initiate the cDNA synthesis. The sequencing was performed by FASTERIS Life Sciences. Short sequence tags of 35 bp were obtained as single-end for replicates A and B and 36 bp were obtained as paired-end for replicate C.

Analysis of Short-Sequence Reads from Illumina-Solexa Sequencing

The reads can be accessed from the National Center for Biotechnology Information (NCBI), as described below under Accession Numbers.

Trimming and Filtering

The data were initially filtered using Seqclean (release dated August 18, 2005). This program trims matches against user-specified target sequences, which here were the primer sequences (5′-GACGGCCAGTGAATTGTAATACGACTCACTATAGGGAGATCTGTATGCTGG-3′ and 5′-CCAGCATACAGATCTCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTC-3′), as well as the UniVec_Core database (dated October 8, 2008), poly(A) tails, and ends rich in undetermined bases. After trimming, a read may be removed entirely for one of three reasons: (1) the sequence is shorter than the minimum length specified via the "−l" parameter (here, 30), (2) the percentage of undetermined bases is >3%, and (3) <40 nucleotides of the sequence is left unmasked by the "dust" low-complexity filter. Further details on analysis of sequences is provided in the Supplemental Methods 1 online.

Mapping the Reads

Each data set of reads was converted into a blast database using "formatdb." To identify matches to known genes, the TAIR8 cDNA collection (TAIR8_cdna_20080325) was then compared with the read databases using Megablast (settings: -v 2000 -b 500 -a 4 -W16 -p 0.6 -e 1 -D3).

The initial runs were performed using the Megablast version BLASTN 2.2.13 (November 27, 2005); the last runs were done with BLASTN 2.2.21 (June 14, 2009).

Determining Raw Expression Counts of Genes (Loci)

The Megablast output was converted to an expression count by the following four steps, in this order: (1) Discard a match if its bit score is 5 or more below the best bit score that is reached by the respective read. (2) Discard a match if it is shorter than 20 bp or if its edit distance (number of gaps + number of mismatches) is 4 or more, or if the match does not start within the first three bases of the read (rationale for the last condition: sequencing quality is best at the 5′ end; therefore, a true match should cover the 5′ end). (3) Discard all matches involving reads that map to more than a single locus (note that a locus can encompass more than a single transcript [cDNA]). (4) For each locus, count the number of different reads that map to it (a single read can map multiple times to a locus if the locus has multiple transcripts; yet, the read will be counted only once at this step). The count of step (4) is output as a raw expression count.

Identifying Differentially Expressed Genes

The count data were normalized with edgeR (Robinson et al., 2010), and the package's exact test for the negative binomial distribution was used to compute P and false discovery rate values.

Clustering and Heat Map

Clustering and heat map of the normalized count data were performed using Genesis software (Sturn et al., 2002). Genes were clustered by hierarchical clustering (parameters: distance = mismatch distance; agglomeration rule = average linkage clustering).

Gene Functional Classification

The DAVID gene functional annotation tool (Huang et al., 2009) was used for the gene-GO term enrichment analysis. The 202 upregulated genes were classified into the different GO FAT terms (Biological Process). The following thresholds were used: minimum number of counts (2) and EASE score (0.05).

ATH1 Microarray Comparison

Archives corresponding to the processed data (ID: E-GEOD-576) were downloaded from the Array Express repository (http://www.ebi.ac.uk/arrayexpress/).

Accession Numbers

The Solexa sequence reads are available from NCBI with the series entry GSE34476. Access to these sequences is provided at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34476.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Floral Commitment in *Arabidopsis* Wild Type and Mutants.

Supplemental Figure 2. In Situ Hybridization on Wild-Type Col Apices in Double Shift Experiments Using Probes for *SOC1*, *FUL*, *SVP*, and *AP1*.

Supplemental Figure 3. Comparison of Inflorescences of *soc1-2 ful-2* and *svp-41 soc1-2 ful-2* Plants.

Supplemental Figure 4. Laser Microdissection Performed on Wild-Type Col Apices during the Floral Transition.

Supplemental Figure 5. K-Means Clustering of the Upregulated Genes.

Supplemental Figure 6. Annotation and Sequence Reads of *FTM3* (At2G18160).

Supplemental Figure 7. Time Course of in Situ Hybridizations in Different Genotypes for Genes Identified as Being Upregulated during Floral Induction.

Supplemental Figure 8. Time-Course Analysis of *SOC1* Expression in Col and *ft-10 tsf-1*.

Supplemental Figure 9. Genetic and Molecular Analysis of *flor1* Mutations.

Supplemental Table 1. List of Primers Used in This Study.

Supplemental Data Set 1. Number of Reads Aligned to Each *Arabidopsis* Gene in Each Experiment.

Supplemental Data Set 2. List of 202 Genes Upregulated in the Shoot Apical Meristem during Floral Induction According to the Criteria Described in the Text.

Supplemental Data Set 3. Gene Ontology Analysis.

Supplemental Data Set 4. Number of Reads Aligned to Each *Arabidopsis* Gene in Each Experiment Taking into Account the Promiscuous Counts.

Supplemental Methods 1. Methods for the Supplemental Data.

Supplemental References 1. References for the Supplemental Data.

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AUTHOR CONTRIBUTIONS

S.T., F.F., and G.C. designed the experiments. S.T., F.F., C.V., and F.A. performed the experiments. K.N., U.G., D.K., and H.S. performed the bioinformatics analysis. S.T., F.F., C.V., F.A., and G.C. analyzed the data. S.T. and G.C. wrote the article.

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