Cell

miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in *Arabidopsis thaliana*

Jia-Wei Wang,[1] Benjamin Czech,[1,2] and Detlef Weigel[1,*]
[1]Department of Molecular Biology, Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany
[2]Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
*Correspondence: weigel@weigelworld.org
DOI 10.1016/j.cell.2009.06.014

SUMMARY

The *FT* gene integrates several external and endogenous cues controlling flowering, including information on day length. A complex of the mobile FT protein and the bZIP transcription factor FD in turn has a central role in activating genes that execute the switch from vegetative to reproductive development. Here we reveal that microRNA156-targeted *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes not only act downstream of FT/FD, but also define a separate endogenous flowering pathway. High levels of miR156 in young plants prevent precocious flowering. A subsequent day length-independent decline in miR156 abundance provides a permissive environment for flowering and is paralleled by a rise in *SPL* levels. At the shoot apex, FT/FD and SPLs converge on an overlapping set of targets, with SPLs directly activating flower-promoting MADS box genes, providing a molecular substrate for both the redundant activities and the feed-forward action of the miR156/SPL and FT/FD modules in flowering control.

INTRODUCTION

The onset of flowering is a major developmental transition that is critical to the reproductive success of plants. In *Arabidopsis thaliana*, the MADS domain protein APETALA1 (AP1) and the plant-specific transcription factor LEAFY (LFY) are master regulators that control the fate of flowers produced at the shoot apex. In the absence of AP1 and LFY, flowers are replaced by shoots or by flowers that have shoot-like characteristics (reviewed in Lohmann and Weigel, 2002). The activation of *LFY* and *AP1* in response to floral inductive signals involves several MADS domain proteins, including SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), FRUITFULL (FUL), and AGAMOUS-LIKE 24 (AGL24) (Lee et al., 2008; Li et al., 2008; Liu et al., 2007, 2008; Michaels et al., 2003; Yu et al., 2002). Genetic and expression studies have shown that *SOC1*, *FUL*, and *AGL24* all function downstream of CONSTANS (CO), the output of the photoperiod pathway, which accelerates flowering of *A. thaliana* in long days (Borner et al., 2000; Lee et al., 2000; Melzer et al., 2008; Samach et al., 2000; Schmid et al., 2003; Yu et al., 2002). CO is connected to the MADS box genes through activation of *FT*, which integrates information from several positively and negatively acting signaling pathways. An overwhelming amount of circumstantial evidence indicates that the small FT protein is a long-distance signal that moves directly from leaves to the shoot apex (reviewed in Bäurle and Dean, 2006; Kobayashi and Weigel, 2007; Turck et al., 2008). FT executes its role through interaction with the bZIP transcription factor FD, which is expressed specifically at the shoot apex, where the FT/FD complex activates several MADS box genes (Abe et al., 2005; Searle et al., 2006; Wigge et al., 2005). Like *FT* in leaves, *FD* at the shoot apex is a direct target of the potent repressor FLOWERING LOCUS C (FLC), which mediates the effects of winter-like exposure to cold temperature, vernalization (Searle et al., 2006). FLC forms a complex with another MADS domain protein, SHORT VEGETATIVE PHASE (SVP), and together they repress not only *FT* but also the *FT* target *SOC1* (Lee et al., 2007; Li et al., 2008).

Another set of transcriptional regulators that is expressed before *LFY* and *AP1* are activated at the shoot apex comprises several miRNA156-regulated SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) factors (Cardon et al., 1997; Schmid et al., 2003). This class of proteins was discovered in snapdragon, from which the founding member was isolated based on its ability to bind a conserved motif in the promoter of the *AP1* ortholog *SQUAMOSA* (Klein et al., 1996). In *A. thaliana*, there are two broad classes of miR156 targets: *SPL3*, *SPL4*, and *SPL5* encode small proteins that consist mostly of the SBP DNA binding domain, whereas the other eight miR156 targets in *A. thaliana* encode much larger proteins (Cardon et al., 1999; Yang et al., 2008). *SPL3*, *SPL4*, and *SPL5* appear to function mostly in the control of flowering time and phase change, whereas *SPL9* and its paralog *SPL15*, which are members of the second group, also have strong effects on leaf initiation rate. Consistent with both groups of SPL factors being important for flowering, overexpression of SPL3 and SPL9 accelerates flowering, whereas reduction in SPL activity through miR156 overexpression delays the onset of flowering (Cardon et al., 1997; Gandikota et al., 2007; Schwab et al., 2005; Schwarz et al., 2008; Wang et al., 2008; Wu and Poethig, 2006).

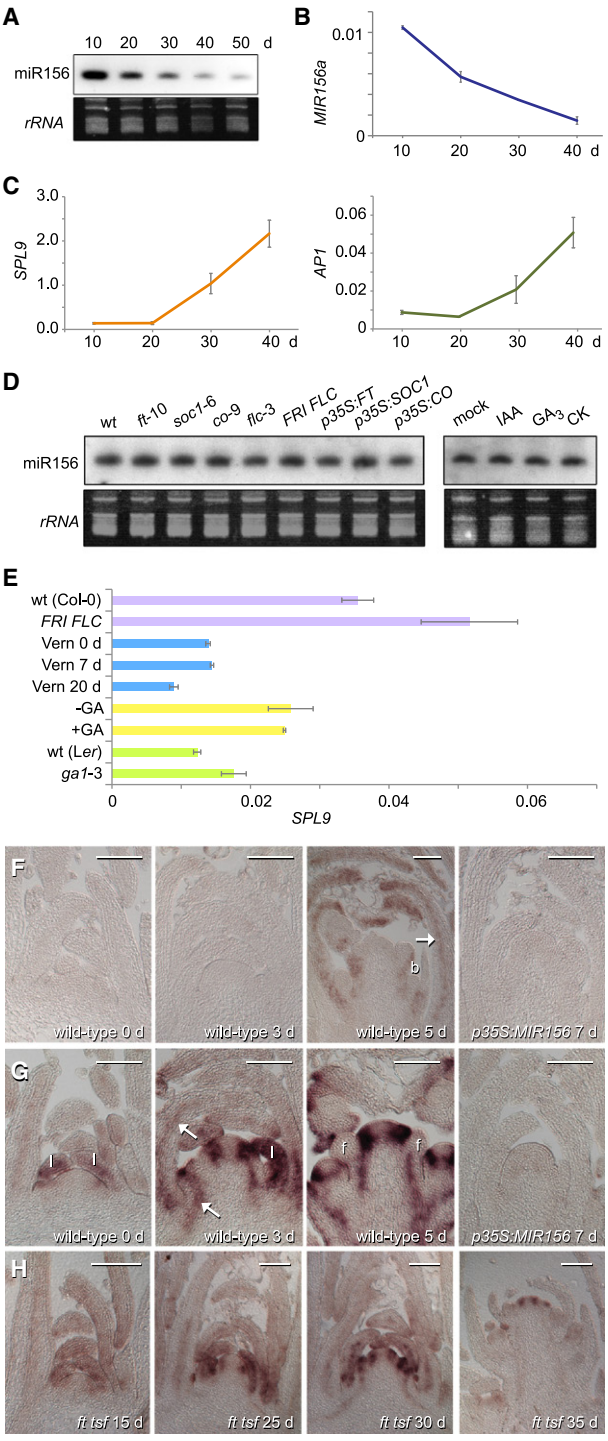

Figure 1. Expression of miR156, *SPL3*, and *SPL9*

(A) RNA blot analysis of mature miR156 in short-day-grown plants.

(B) qRT-PCR analysis of *MIR156A* primary transcripts, normalized to that of *β-TUBULIN-2*. Error bars indicate range of replicates.

(C) qRT-PCR analysis of *SPL9* expression in short days, with floral marker *AP1* as control.

(D) RNA blot analysis of miR156 in different genetic backgrounds and in response to 6 hr treatments with 100 μM GA_3, IAA, or the cytokinin t-zeatin (CK). Seedlings were 1 week old.

(E) qRT-PCR analysis of *SPL9* expression in different genetic backgrounds and in response to vernalization or GA_3 treatment. Nonvernalized seedlings were 2 weeks old. For vernalization, 1-week-old seedlings were transferred to 4°C for the indicated time. For GA treatment, seedlings were sprayed with 100 μM GA_3 at 1 and 2 weeks of age.

(F and G) In situ hybridization to detect *SPL3* (F) and *SPL9* (G) in shoot apices. Days refer to time after transfer of 30-day-old plants from short to long days. b, cryptic bract; f, flower primordium; l, leaf primordium. Arrows point to provascular strands.

(H) *SPL9* expression in apices of *ft*-10 *tsf*-1 double mutants. Plants were grown in long days and harvested continuously to obtain apices during the vegetative phase, at the beginning, during, and after the transition to flowering (from left to right). Scale bars represent 50 μm.

Previous expression studies have suggested that the *SPL* genes affect flowering as downstream targets of *CO* and *FT* at the shoot apex (Schmid et al., 2003). Here, we show that *SPL* genes have an important *FT*-independent role in regulating flowering. SPLs and the FT/FD complex share several direct targets, including *AP1*, that act in multiple feed-forward loops. We conclude that the miR156/SPL module represents a major regulatory axis for the promotion of flowering in the absence of photoperiodic cues.

RESULTS

Expression Pattern of miR156 and *SPL* Genes

We used small RNA blots to complement previous studies on the expression pattern of miR156 during the *A. thaliana* life cycle (Gandikota et al., 2007; Wang et al., 2008; Wu and Poethig, 2006). In short days, miR156 levels were highest in young seedlings and subsequently declined over several weeks (Figure 1A; Wu and Poethig, 2006). We observed a similar pattern for *pri-MIR156a* by using reverse transcription followed by quantitative real-time PCR (qRT-PCR) (Figure 1B), suggesting that the primary regulation of miR156 is at the transcriptional level. The temporal expression pattern of *SPL3* is the inverse of miR156, with low levels in short-day grown seedlings and a gradual increase during vegetative development (Wu and Poethig, 2006). We observed a similar profile for *SPL9* (Figure 1C). The AtGenExpress expression atlas (Schmid et al., 2005) indicated similar trends for several additional miR156 targets (Figure S1 available online).

Inactivation or overexpression of the flowering regulators *FLC*, *CO*, *FT*, or *SOC1* had no obvious effects on miR156 levels in whole seedlings. This was also true for plants treated with plant hormones, including the gibberellin GA_3, which accelerates flowering (Langridge, 1957; Michaels and Amasino, 1999; Sheldon et al., 1999; Wilson et al., 1992), auxin (IAA), or cytokinin (Figure 1D). Similarly, *SPL9* transcript levels in seedlings were not affected by increased expression of the floral repressor *FLC* in plants that have a functional copy of the *FLC* activator *FRIGIDA* (*FRI*) (Michaels and Amasino, 1999), nor did they respond to vernalization, to GA_3 treatment, or to elimination of endogenous gibberellins in the *ga1*-3 mutant (Figure 1E). Vernalization or GA treatment accelerated the flowering of *p35S:MIR156* plants, but less than that of wild-type (Table S1). The flowering responses together with the absence of major effects of vernalization and GA treatment on miR156 and *SPL* levels suggest that the

miR156/SPL module promotes flowering in parallel with, or upstream of, these two pathways, which was further supported by the finding that *SPL3* overexpression from a *p35S:rSPL3* construct (see below) could accelerate the flowering of *FRI FLC* or *ga1*-3 plants (Table S1).

At the shoot apex, upregulation of *SPL3* and *SPL9* is readily detectable by microarrays within 3 days after transfer of vegetative plants from short days to inductive long days, and this induction is much reduced in *co* and *ft* mutants (Schmid et al., 2003). Much higher levels of expression in florally induced apices compared to vegetative apices were observed for most miR156 targets (Figure S1). Transfer to long days did not cause an obvious change in miR156 levels at the shoot apex (Figure S2), suggesting that photoperiod-dependent upregulation of *SPL*s is not due to a release of miR156 inhibition.

In situ hybridization did not reveal a distinct pattern of *SPL3* expression in vegetative apices, whereas *SPL9* transcripts were strongest in leaf primordia and the provascular strands of young leaves (Figures 1F and 1G; Wang et al., 2008). After transfer to long days, *SPL3* and *SPL9* levels increased in both existing leaves and newly formed primordia, with *SPL9* being also expressed in provascular strands below the shoot apical meristem. *SPL9* was transiently upregulated in floral anlagen and very early floral primordia, but declined again by stage 2 of flower development. Expression of *SPL3* in newly formed primordia was strongest in cryptic bracts subtending flowers (Long and Barton, 2000). miR156 overexpression reduced *SPL3* and *SPL9* transcripts to background levels (Figures 1F and 1G).

p35S:MIR156 plants had a reduced response to transient exposure to long days, with 5 instead of 2 days required for quantitative induction of flowering (Schwarz et al., 2008; data not shown). This was not caused by attenuated *FT* induction, as determined by transiently exposing short-day-grown *p35S:MIR156* plants to 2 long days (Figure S3A), nor was it due to reduced expression of the *FT* partner *FD* before photoperiodic induction (Figure S3B).

Finally, in *ft*-10 *tsf*-1 plants, which lack activity of both *FT* and its paralog *TWIN SISTER OF FT* (*TSF*) (Michaels et al., 2005; Yamaguchi et al., 2005), *SPL9* expression in shoot apices increased from the vegetative phase until and through the transition of flowering (Figure 1H), confirming a photoperiod-independent but age-dependent rise in *SPL9* expression. Taken together, these data indicate that *SPL* levels increase throughout the plant with increasing age, but that in addition, acute induction of flowering through the photoperiod pathway can cause an immediate and strong upregulation of *SPL* genes at the shoot apex.

Tissue-Specific Effects of *SPL*s on Flowering Time

The effects of miR156 and its *SPL* targets on leaf initiation rate, which is accelerated in plants with attenuated *SPL* activity but reduced in plants with increased *SPL* activity (Schwarz et al., 2008; Wang et al., 2008), confounds the measurement of flowering time, which is often expressed as number of leaves produced before the first flower is made. Nevertheless, when taking both leaf number and chronological time into account, it is clear that overexpression of miR156, which reduces *SPL* expression, moderately delays flowering, whereas inhibition of miR156 activity, either by mutating its target site in *SPL3* (*p35S:rSPL3*) or through a miR156 target mimic (*p35S:MIM156*), accelerates flowering (Franco-Zorrilla et al., 2007; Gandikota et al., 2007; Schwab et al., 2005; Wang et al., 2008; Wu and Poethig, 2006). In general, the flowering time effects were more pronounced in short than in long days (Table 1).

To explore the tissue requirement of *SPL*s, we made use of plants that misexpress miR156 from the *SUC2* promoter in phloem companion cells, the site of *FT* action (An et al., 2004; Takada and Goto, 2003), or in *FD*-expressing cells at the shoot apex. *pFD:MIR156* plants (in which plastochron length is unaffected; Wang et al., 2008) flowered even later than *p35S:MIR156* plants. This was slightly enhanced when we introduced in addition a *pSUC2:MIR156* transgene, which on its own had a lesser effect on flowering (Table 1). Consistent with *SPL*s being able to act both in the phloem and at the shoot apex, expression of the nontargeted version of *SPL3*, *rSPL3*, from either the *SUC2* and *FD* promoter led to early flowering (Table 1; Wang et al., 2008). Thus, although the shoot apex has a higher requirement for *SPL* activity than does the phloem, *SPL* genes act at both sites, similar to *SOC1* and *FLC* (Searle et al., 2006). *pFD:rSPL3 ft*-10 plants were intermediate between the parents, but the *ft*-10 mutation suppressed the effects of *rSPL3* expression in the phloem (Table 1). Similar results were obtained with misexpression of *rSPL9* (Wang et al., 2008). Thus, *SPLs* may act through *FT* in the phloem.

Identification of MADS Box Genes as SPL Targets

To identify *SPL* targets, we analyzed the transcriptome of *argonaute1* (*ago1*-27) and *serrate* (*se*-1) plants, which both overexpress *SPL* genes resulting from reduced miR156 accumulation (Ronemus et al., 2006; Wang et al., 2008). We compared these two genotypes to *p35S:MIR156 se*-1, in which *SPL* transcripts are reduced, and to wild-type. Several MADS box genes, including *FUL* and *SOC1*, which have redundant roles in promoting flowering (Melzer et al., 2008), and the *SOC1* paralog *AGL42* showed a profile across the four genotypes similar to that of the *SPL*s (Figure S4A). qRT-PCR confirmed that irrespective of photoperiod, *FUL*, *SOC1*, and *AGL42* levels were increased in *p35S:MIM156* seedlings, which have elevated *SPL* levels because of expression of a miR156 target mimic (Franco-Zorrilla et al., 2007), but decreased in *p35S:MIR156* plants, which have reduced *SPL* levels (Figure 2A; Figure S4B). In contrast to *FUL*, *SOC1*, and *AGL42*, the expression levels of the *SOC1* partner *AGL24* or of the *AGL24* homolog *SVP* do not substantially increase at the shoot apex in conjunction with the transition to flowering (Figure S1; Hartmann et al., 2000; Michaels et al., 2003; Yu et al., 2002). *AGL24* was unaffected in *p35S:MIR156* or *p35S:MIM156* plants (data not shown). There appears to be some differentiation in SPL function, as indicated by the fact that *p35S:rSPL3* strongly induced *FUL* but had much weaker or no effects on *SOC1* and *AGL42*, whereas *pSPL9:rSPL9* had similar effects on all three targets, especially in short days (Figure 2A; Figure S4B).

We used in situ hybridization to compare the expression patterns of *SOC1* and *FUL* as well as the floral marker *AP1* at the shoot apex with those of *SPL3* and *SPL9*. No distinct patterns of *SOC1* and *FUL* RNA were detected in vegetative shoot apices, but discrete sites of expression became detectable

Table 1. Effects of Reduced and Increased *SPL* Activity on Flowering

	Leaves		Flowering Time (Days)	
Genotype	Average	Range	Average	Range
Experiment 1: Long Day				
Wild-type	13.4 ± 0.5	13–14	16.6 ± 0.9	15–18
p35S:MIR156	23.4 ± 0.5	19–21	20.4 ± 0.9	19–22
p35S:MIM156	5.0 ± 0.6	4–6	15.0 ± 1.2	13–17
p35S:rSPL3	6.7 ± 0.5	6–7	13.9 ± 0.9	13–15
Experiment 2: Short Day				
Wild-type	54.4 ± 1.3	53–57	58.4 ± 2.1	55–62
p35S:MIR156	91.4 ± 3.6	84–97	75.6 ± 4.5	70–85
p35S:MIM156	32.9 ± 2.1	30–36	57.1 ± 3.3	50–62
p35S:rSPL3	29.1 ± 0.8	27–30	33.9 ± 2.0	31–38
Experiment 3: Long Day				
Wild-type	13.6 ± 0.5	13–14	17.1 ± 0.9	15–18
pFD:MIR156[a]	21.1 ± 1.2	19–23	23.9 ± 1.0	23–25
pSUC2:MIR156	20.6 ± 0.7	20–22	18.8 ± 1.1	18–21
pFD:MIR156 pSUC2:MIR156	26.9 ± 1.1	26–29	25.3 ± 0.9	24–27
pFD:rSPL3	9.57 ± 0.5	9–10	14.8 ± 1.0	13–16
pSUC2:rSPL3	9.36 ± 0.5	9–10	13.9 ± 0.6	13–15
pFD:rSPL3 pSUC2:rSPL3	8.43 ± 0.7	7–9	13.2 ± 0.7	12–14
ft-10	40.1 ± 0.5	39–41	35.3 ± 1.5	33–38
pFD:rSPL3 ft-10	24.8 ± 0.9	24–26	27.4 ± 1.1	26–30
pSUC2:rSPL3 ft-10	39.9 ± 0.6	39–41	35.1 ± 137	33–38

Standard deviation is given. n = 14 for all genotypes.
[a] Note that plastochron length is unaffected by *pFD:MIR156* (Wang et al., 2008) and that leaf number is therefore a good indicator of flowering time.

within a day after transfer of plants from short to long days, which was 2 days earlier than for *AP1* (Figures 3A, 3C, and 3E; Borner et al., 2000; Hempel et al., 1997; Samach et al., 2000; Schmid et al., 2003). Similar to *SPL3* and *SPL9* (Figures 1F and 1G), strong expression of *SOC1* and *FUL* in floral anlagen was transient, and both were also upregulated in the provascular strains of existing leaf primordia. Activation of *SOC1* and *FUL* at the shoot apex was delayed in *p35S:MIR156* plants, in a manner similar to that in *fd*-2 plants (Figures 3B and 3D).

To confirm that MADS box genes are early targets of SPLs, we expressed a translational fusion of the hormone-binding domain of rat glucocorticoid receptor (GR) to SPL9 under the control of *SPL9* genomic sequences. Treatment with the GR ligand dexamethasone (DEX) induced phenotypes mimicking those of *pSPL9:rSPL9* plants (Wang et al., 2008). *FUL* RNA levels were increased 4-fold after 6 hr in DEX-treated plants and up to 10-fold after 12 hr (Figure 2B). We also generated a translational fusion of β-glucuronidase (GUS) that included 2.5 kb promoter and the first intron of *FUL*. In wild-type seedlings, GUS staining was mostly in vascular tissue of young leaves. GUS activity was reduced in *p35S:MIR156* plants and strongly increased in *p35S:rSPL3* plants (Figure 2C). These results indicate that *FUL* is transcriptionally regulated by SPLs in both leaves and at the shoot apex.

Next, we performed chromatin immunoprecipitation (ChIP) assays with hemagglutinin (HA) antibodies on chromatin extracted from *p35S:rSPL3-HA* plants, which were indistinguishable from *p35S:rSPL3* plants. We focused on four regions of the *FUL* promoter and first intron that contained GTAC boxes, which have been identified as the core binding motif of SPLs in vitro (Figure 2D; Birkenbihl et al., 2005; Klein et al., 1996; Kropat et al., 2005; Liang et al., 2008). A fifth region, in the last exon and lacking a GTAC motif, was included as a control. Chromatin extracted from 1-week-old seedlings was treated with either HA or Myc antibody, and the precipitated DNA was subjected to qPCR. There was no apparent enrichment of any of the fragments in wild-type samples, while regions I to IV were readily amplified from *p35S:rSPL3-HA* samples after immunoprecipitation with HA, but not with Myc, antibody (Figures 2E and 2F).

We used *pSPL9:GFP-rSPL9* plants, which express a translational fusion of SPL9 to green fluorescent protein (GFP) and have phenotypes similar to those of *pSPL9:rSPL9*, to determine whether SPL9 might bind *SOC1* and *AGL42*, since both were strongly induced in short-day-grown *pSPL9:rSPL9* plants (Figure 2A; Figure S4B). The genomic regions of *SOC1* and *AGL42* also contain several GTAC boxes in their promoters and first introns (Figure 2D; Figure S4C), and several fragments spanning the GTAC motifs were efficiently enriched after ChIP with GFP antibodies, indicating that *SOC1* and *AGL42* are direct SPL9 targets (Figure 2G; Figure S4D).

Tissue-Specific Effects of *FUL* on Flowering Time

Because *SPL3* affected primarily *FUL* expression, we examined the genetic interaction of *SPL3* with *FUL*. *p35S:rSPL3 ful*-7

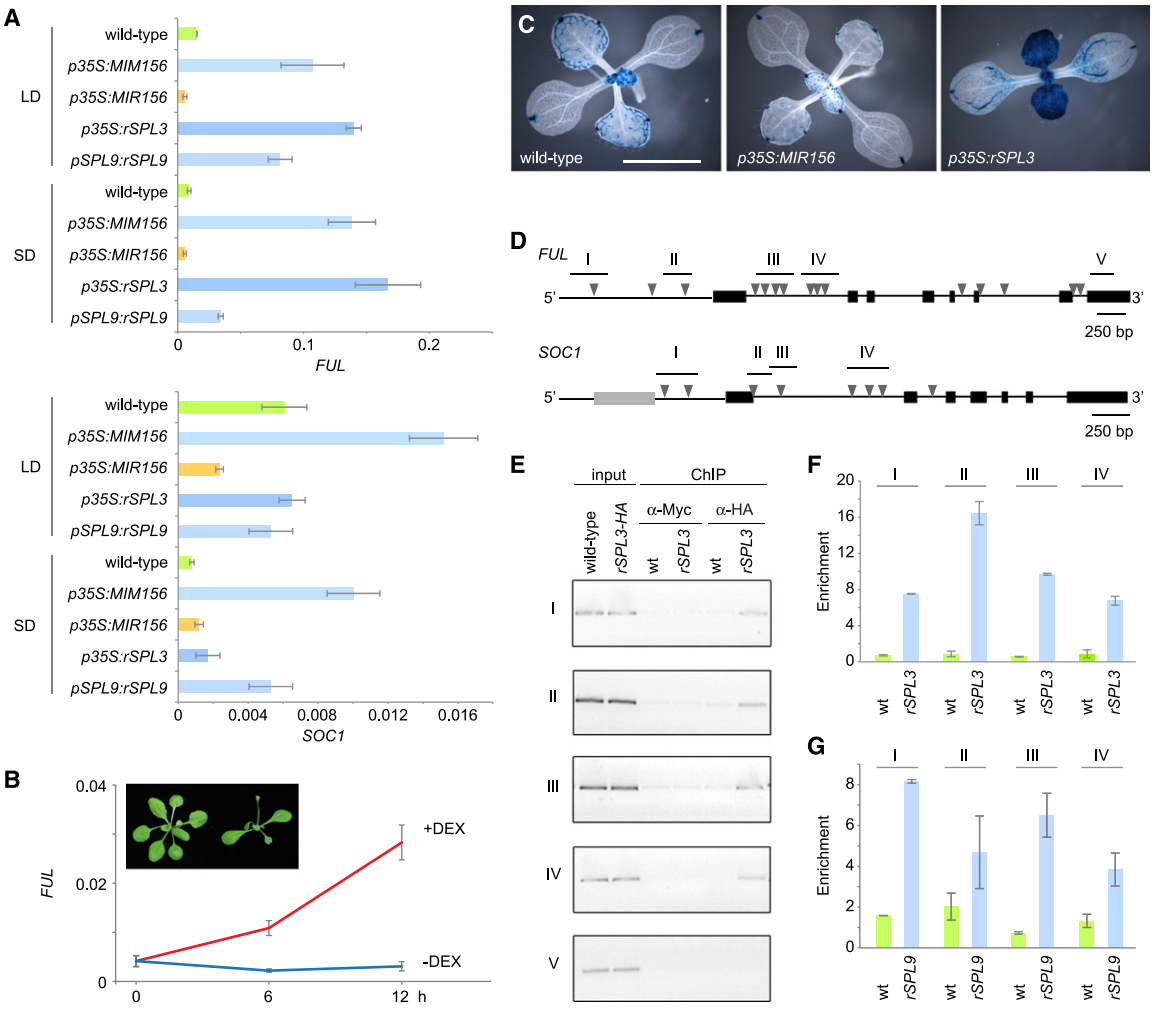

Figure 2. Identification of MADS Box Genes as Direct SPL Targets

(A) qRT-PCR analysis of *FUL* and *SOC1* expression in 7-day-old long-day-grown and 10-day-old short-day-grown seedlings.

(B) qRT-PCR analysis of *FUL* expression in *pSPL9:rSPL9-GR* seedlings after DEX induction. Inset shows mock-treated (left) and DEX-treated *pSPL9:rSPL9-GR* seedlings.

(C) *pFUL:GUS* activity in 1-week-old seedlings. Scale bar represents 1 cm.

(D) Diagram of *FUL* and *SOC1* genomic regions. Triangles indicate GTAC boxes in promoter and introns. The first exon of *SOC1*, indicated in light gray, is entirely noncoding and is not included in all transcripts from this locus.

(E) ChIP analysis of *FUL* genomic fragments in wild-type and *p35S:rSPL3-HA* seedlings.

(F) ChIP followed by qPCR of *FUL* genomic fragments in wild-type and *p35S:rSPL3-HA* seedlings. Relative enrichment of fragments was calculated by comparing samples treated with HA and Myc antibodies.

(G) ChIP followed by qPCR of *SOC1* genomic fragments in wild-type and *pSPL9:GFP-rSPL9* seedlings, with Myc antibodies as control.

plants were phenotypically intermediate between the parents, and both flowering time in days and total leaf number were similar to that of wild-type (Table 2; Figure S5A). Conversely, in *p35S:FUL* plants, which flowered early (Ferrándiz et al., 2000b) and were similar to *p35S:rSPL3* plants in both leaf number and days to flowering, the *p35S:MIR156* phenotype was strongly suppressed (Table 2; Figure S5B).

Since *FUL* is expressed in both leaves and at the shoot apex, and since reducing activity of the SPL upstream regulators in either tissue affects flowering time, we determined whether *FUL* could promote flowering in both tissues as well. In long days, misexpression of *FUL* from either the phloem-specific *SUC2* promoter or from the shoot-apex-specific *FD* promoter caused early flowering (Table 2), similar to what has been reported for *SOC1* (Searle et al., 2006). The *ft*-10 mutation completely suppressed the early-flowering phenotype of *pSUC2:FUL*, suggesting that *FUL* can accelerate flowering through *FT*, again similar to *SOC1* (Searle et al., 2006) and to *SPL3*. In contrast, *pFD:FUL ft*-10 plants flowered earlier than nontransgenic *ft*-10 plants, indicating that *FUL* acts at the shoot apex downstream of or in parallel with the FT/FD complex.

In a complementary set of experiments, we tested the requirement for *FUL* activity in leaves and at the shoot apex by reducing its expression with an artificial miRNA (amiR-FUL) (Schwab et al.,

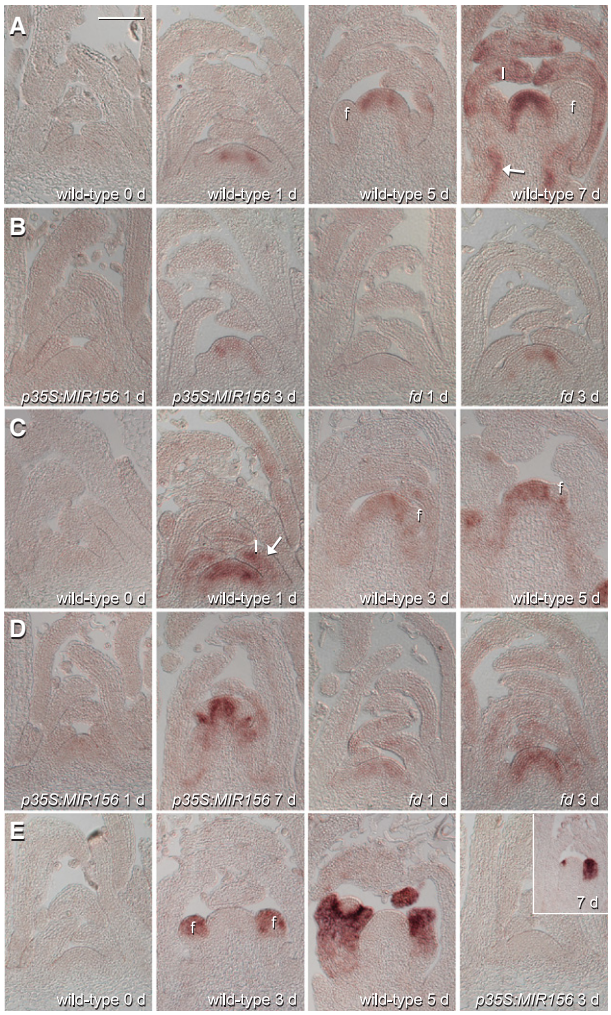

Figure 3. Expression of *SPL* Targets at the Shoot Apex

In situ hybridization to detect *FUL* (A, B), *SOC1* (C, D), and *AP1* (E) in 30-day-old short-day-grown plants shifted to long days. The second time point shown for *p35S:MIR156* and *fd-2* is the one where expression became first apparent. f, flower primordium; l, leaf primordium. Arrows point to provascular strands. Scale bar represents 50 μm, except 100 μm for inset in (E).

2006). Inactivation of *FUL* causes only a small delay in flowering, but it substantially enhances the late-flowering defects of *soc1* mutants in both long and short days (Ferrándiz et al., 2000a; Melzer et al., 2008), and double mutants are similar to *ft*-10 null mutants (Table S2). In addition, double mutants showed a disproportionate increase in the number of cauline leaves (Table S3), indicating a partial uncoupling of stem elongation, one of the responses to floral induction, and specification of floral meristem identity. Because a *ful* mutation on its own does not strongly delay flowering, we introduced *pSUC2:amiR-FUL* and *pFD:amiR-FUL* constructs into the *soc1*-6 strain. *pSUC2:amiR-FUL* had only a minor effect on the flowering of *soc1*-6, but *pFD:amiR-FUL soc1*-6 plants flowered almost as late as the *soc1-6 ful-7* double mutants (Table 2). We conclude that, at least in long days, there is a higher requirement of *FUL* at the shoot apex than in the phloem, but that similar to *SPL*s and *SOC1*, *FUL* functions in both tissues.

Requirement of *FD* and *SPL*s for Floral Meristem Identity

Several previous reports have indicated that MADS box genes controlling the onset of flowering and flower initiation, such as *SOC1*, *FUL*, and *AP1*, act downstream of *FD* (Abe et al., 2005; Searle et al., 2006; Teper-Bamnolker and Samach, 2005; Wigge et al., 2005). We confirmed that induction of *FUL* and *SOC1* at the shoot apex in response to long days was delayed in *fd*-2 mutants (Figures 3B and 3D). Conversely, *FUL* and *SOC1* were elevated in *p35S:FD* plants (Figure S6). The hypothesis that *FD* and *SPL*s act in parallel is consistent with the observation that *FD* overexpression could accelerate the flowering of *p35S:MIR156* plants and that *p35S:rSPL3 fd-2* plants flowered earlier than *fd-2* plants (Table 2). Similarly, abrogating *SPL* expression in the *FD* domain through *pFD:MIR156* further considerably increased the leaf number of *p35S:amiR-FT/TSF* plants (Mathieu et al., 2007), which mimic the phenotype of *ft tsf* double mutants (Table 2; Mathieu et al., 2007).

Surprisingly, *pFD:MIR156 fd-2* plants had a novel inflorescence phenotype, reminiscent of *ft lfy*, *fd lfy*, and *lfy ap1* double mutants (Figure 4; Abe et al., 2005; Ruiz-García et al., 1997; Wigge et al., 2005). Wild-type plants normally have two or three cauline leaves, in the axils of which side shoots develop, whereas flowers of *A. thaliana*, in contrast to many other species, are not subtended by leaf-like bracts. The first few flowers of *pFD:MIR156* plants were associated with bracts, but the overall number of cauline leaves with side shoots was not substantially increased in *fd-2* or *pFD:MIR156*. In contrast, *pFD:MIR156 fd-2* inflorescences produced a large number of cauline leaves from which side shoots arose that repeated the pattern of the main inflorescence with many leaves (Figures 4A and 4B; Figures S7A and S7B). The few flowers that eventually appeared on the inflorescence were subtended by bracts, indicating that complete floral identity was never attained in these plants (Figure S7B).

Because of the similarity of the *pFD:MIR156 fd-2* and *fd-2 lfy*-12 phenotypes (Figure 4B), we tested whether the miR156-regulated SPLs act primarily through *LFY*. Shoot-apex-specific reduction of SPL activity with *pFD:MIR156* enhanced the *lfy*-12 phenotype, and the inflorescence defects were similar to those of *fd-2 lfy*-12 (Figure 4B). In addition, we found that some of the severe floral defects in *fd-2 lfy*-12 plants were rescued after introduction of *pSPL9:rSPL9* into these plants (Figure 4B), further confirming that miR156-regulated SPLs do not act merely through *LFY*.

To understand the origin of the severe inflorescence defects in *pFD:MIR156 fd-2* plants, we analyzed the expression of *LFY* and *AP1* as well as *TERMINAL FLOWER1* (*TFL1*), which suppresses floral identity in the inflorescence shoot and has a mutually antagonistic relationship with *LFY* and *AP1* (Liljegren et al., 1999; Parcy et al., 2002; Ratcliffe et al., 1999). *LFY* and *AP1* levels in *pFD:MIR156 fd-2* inflorescences were much lower than in wild-type inflorescences and resembled those in vegetative apices of wild-type (Figure 4C). Consistent with the shoot character of the lateral primordia produced by *pFD:MIR156 fd-2* inflorescences, these strongly expressed *TFL1*, whereas

Table 2. Genetic Interaction of *SPL*s with *FT*, *FD*, *SOC1*, and *FUL*

	Leaves		Flowering Time (Days)	
Genotype	Average	Range	Average	Range
Experiment 1: Long Day				
Wild-type	13.3 ± 0.5	13–14	16.9 ± 0.7	16–18
ful-7	15.8 ± 0.7	14–17	18.1 ± 0.9	16–19
p35S:rSPL3	6.6 ± 0.5	6–7	13.7 ± 0.8	13–15
p35S:rSPL3 ful-7	12.5 ± 0.8	11–14	15.5 ± 0.9	14–17
p35S:FUL	6.21 ± 0.6	5–7	12.7 ± 1.0	11–15
p35S:MIR156	23.1 ± 0.6	22–24	19.6 ± 1.0	18–21
p35S:FUL p35S:MIR156	11.5 ± 0.8	10–13	15.4 ± 0.9	14–17
Experiment 2: Long Day				
Wild-type	13.8 ± 0.4	13–14	16.6 ± 0.6	16–18
p35S:FUL	7.1 ± 0.5	6–8	13.5 ± 0.8	12–15
pFD:FUL	8.9 ± 0.5	8–10	14.8 ± 1.0	13–17
pSUC2:FUL	7.4 ± 0.5	7–8	13.9 ± 0.6	13–15
ft-10	40.1 ± 0.5	39–41	35.7 ± 1.0	35–38
pFD:FUL ft-10	20.4 ± 0.7	19–21	24.9 ± 1.8	23–28
pSUC2:FUL ft-10	39.9 ± 0.9	38–42	34.4 ± 1.2	33–37
Experiment 3: Long Day				
Wild-type	14.0 ± 1.1	13–16	17.7 ± 0.7	17–19
soc1-6	23.2 ± 0.9	22–25	25.1 ± 0.8	24–26
ful-7 soc1-6	37.0 ± 1.0	36–39	29.8 ± 0.8	29–31
pFD:amiR-FUL soc1-6	34.3 ± 1.0	32–35	28.4 ± 1.0	26–30
pSUC2:amiR-FUL soc1-6	25.1 ± 1.3	23–27	26.1 ± 0.7	25–27
Experiment 4: Long Day				
Wild-type	12.6 ± 0.6	11–13	16.4 ± 0.5	16–17
fd-2	23.6 ± 0.9	23–26	23.4 ± 1.1	22–25
p35S:rSPL3	6.4 ± 0.5	6–7	12.9 ± 0.7	12–14
p35S:rSPL3 fd-2	13.1 ± 0.8	9–11	16.8 ± 1.3	15–19
pFD:MIR156	22.1 ± 0.5	19–21	21.5 ± 0.9	20–23
pFD:MIR156 fd-2[a]	54.4 ± 3.2	50–59	39.8 ± 1.5	38–42
p35S:FD	9.6 ± 0.5	9–10	11.9 ± 0.5	11–13
p35S:MIR156	22.6 ± 0.5	22–23	19.0 ± 0.7	18–20
p35S:FD p35S:MIR156	13.4 ± 1.0	11–14	14.7 ± 0.6	14–16
Experiment 5: Long Day				
p35S:amiR-FT/TSF	54.9 ± 1.3	54–57	40.2 ± 2.2	38–44
p35S:amiR-FT/TSF pFD:MIR156	>90	n/a	80.2 ± 11.8	70–110

Standard deviation is given. n = 14 for all genotypes except *pSUC2:amiR-FUL soc1-6* (n = 12) and *p35S:amiR-FT/TSF* (n = 9). n/a, not available.
[a] Only rosette leaves were counted.

TFL1 was not detected in the shoot apical meristem, similar to the situation in vegetative wild-type plants (Figure 4C; Figure S8).

Two fragments in the promoter of *AP1*, including one that includes a binding site for *LFY* (Parcy et al., 1998) and overlaps a region that strongly responds to *FD* activity (Wigge et al., 2005), were enriched in chromatin immunoprecipitated with GFP antibodies from *pSPL9:GFP-rSPL9* inflorescence tissue (Figures S4C and S4E), suggesting that SPLs directly regulate not only upstream-acting MADS box genes such as *SOC1* and *FUL* but also *AP1* itself.

DISCUSSION

In this work, we have revealed a molecular pathway that can induce flowering in the absence of the FT/FD complex, thought to be the major integrator of positive and negative signals controlling the onset of flowering. The importance of the miR156/SPL pathway in flowering at least partially explains why inactivation of *FT* and its close paralog *TWIN SISTER OF FT* (*TSF*) does not prevent flowering of *A. thaliana* (Michaels et al., 2005; Yamaguchi et al., 2005). Conversely, that FT/FD activity can largely compensate for a lack of SPL function

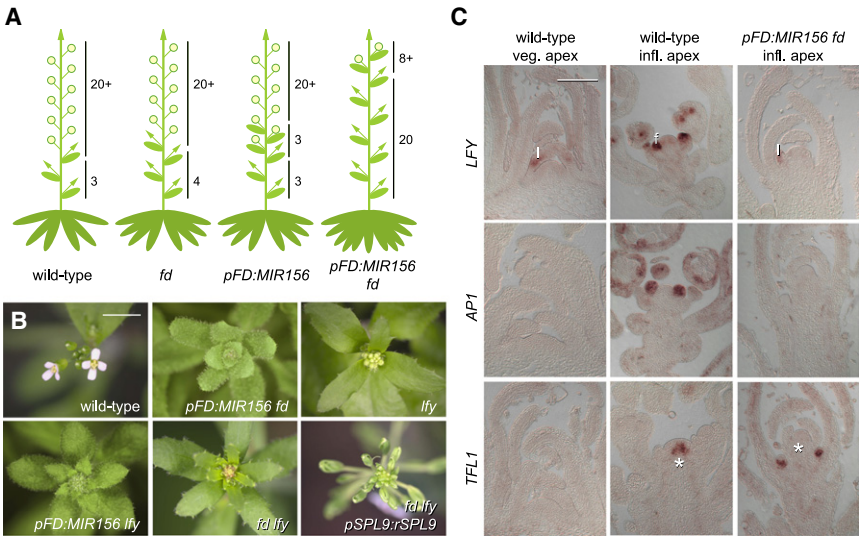

Figure 4. Effect of *SPL*s and *FD* on Meristem Identity

(A) Plant cartoons. Arrows indicate shoots and circles flowers. Numbers indicate average number of side shoots, flowers subtended by bracts, and normal flowers.

(B) Top view of inflorescences. Not shown are *fd*-2, *pFD:MIR156*, and *pSPL9:rSPL9*, all of which have normal, wild-type-appearing flowers. Late-arising flowers of *lfy*-12 mutants, shown here, are replaced by structures with mixed floral and shoot character (Huala and Sussex, 1992; Schultz and Haughn, 1991; Weigel et al., 1992). Like *fd*-2 *lfy*-12 plants (Abe et al., 2005; Wigge et al., 2005), *pFD:MIR156 lfy*-12 never produced flower-like structures. *pFD:MIR156 fd*-2 inflorescences eventually formed a few flowers with a normal complement of floral organs, but subtended by bracts (Figure S7). *pSPL9:rSPL9 lfy*-12 *fd*-2 inflorescences are similar to those of *lfy*-12 single mutants. Scale bar represents 0.5 cm.

(C) In situ hybridization to detect *LFY*, *AP1*, and *TFL1* in wild-type and *pFD:MIR156 fd*-2. l, leaf; f, flower primordium. Asterisks indicate shoot apical meristem. Scale bar represents 50 μm.

explains that despite their discovery more than 10 years ago (Klein et al., 1996), the exact role of SPLs in flowering control has been unclear until now.

In young seedlings, miR156 levels are high and *SPL* levels are low. Even without photoperiodic induction of flowering, miR156 levels decline over several weeks, with a concomitant rise in *SPL* levels (this work; Schwarz et al., 2008; Wu and Poethig, 2006). This change in miR156/*SPL* levels appears to be independent of several known floral regulators, such as flower-promoting gibberellins, which are probably produced in leaves and transported to the shoot apex (Eriksson et al., 2006), or *FLC*, which is the target of vernalization and can repress flowering in both the phloem and at the shoot apex (Searle et al., 2006). miRNAs were first discovered through their role in controlling developmental timing in *C. elegans* (Pasquinelli and Ruvkun, 2002). Similar to miR156, *C. elegans* miRNAs *lin-4* and *let-7* change in abundance as development progresses. An important outcome of their study during the last decade has been that there is not a simple mechanism that controls the expression levels of these miRNAs, but that likely a series of switches of feedback loops together shape their temporal profile (Moss, 2007). How the expression of miR156 and the closely related miR157, which are encoded in the *A. thaliana* genome by at least a dozen precursors, is regulated is an important question.

A large body of work during the past few years has taught us the details of photoperiodic promotion of flowering dependent on the CO-FT/FD axis. In response to long days, CO protein is stabilized in the phloem companion cells of *A. thaliana*, where it induces transcription of *FT* and its paralog *TSF*. The protein products are conveyed through the sieve elements to the shoot apex, where a complex of the bZIP transcription factor FD and FT complex activates expression of genes that execute the switch from the formation of leaves to the production of flowers (reviewed in Bäurle and Dean, 2006; Kobayashi and Weigel, 2007; Turck et al., 2008). It is intriguing that the *SPL*s, in addition to being posttranscriptionally regulated by miR156, are also targets of the FT/FD transcription factor complex. We propose that during early development, high miR156 levels reduce the ability of FT/FD to induce flowering by repressing SPL activity. With decreasing miR156 activity, flowering can be promoted through the photoperiod-dependent FT/FD pathway, and eventually plants can flower without FT/FD activity, as SPL levels continue to rise.

Our findings add to the growing evidence for an intricate web of feed-forward and feedback loops that ensure a sharp and rapid transition from vegetative to reproductive development (Figure 5). In the early part of the flowering network, the FLC/SVP complex represses not only *FT* and *SOC1* in phloem companion cells of leaves, but also *SOC1* and *FD* at the shoot apex (Hepworth et al., 2002; Lee et al., 2007; Li et al., 2008; Searle et al., 2006), similar to the dual action of SPLs in both tissues. Although the roles of SOC1 and FUL in leaves are not fully understood, one possibility is that they compete with the FLC/SVP complex for access to the *FT* promoter and thus counteract the repressive activity of FLC/SVP (Hepworth et al., 2002; Li et al., 2008; Liu et al., 2008). Since both SOC1 and FUL proteins can interact with SVP, this might even involve action in a common, large complex of MADS domain proteins (de Folter et al., 2005).

At the shoot apex, both *FUL* and *SOC1* are targets of SPLs and the FT/FD complex. A heterodimeric complex of SOC1 and AGL24 in turn promotes flowering by activating *LFY* and *AP1*, with the latter being directly regulated by SPLs and FT/FD as well (this work; Lee et al., 2008; Liu et al., 2008; Wigge et al., 2005). These factors thus form an interlocking set of feed-forward loops (Figure 5). Like SOC1 and AGL24, LFY and AP1 enhance each other's expression, further stabilizing the switch from vegetative to reproductive development (Bowman et al., 1993; Liljegren et al., 1999; Parcy et al., 2002; Wagner et al., 1999). Subsequent stages also involve negative feedback loops, such as repression of *AGL24* by *AP1* and *LFY* or repression of

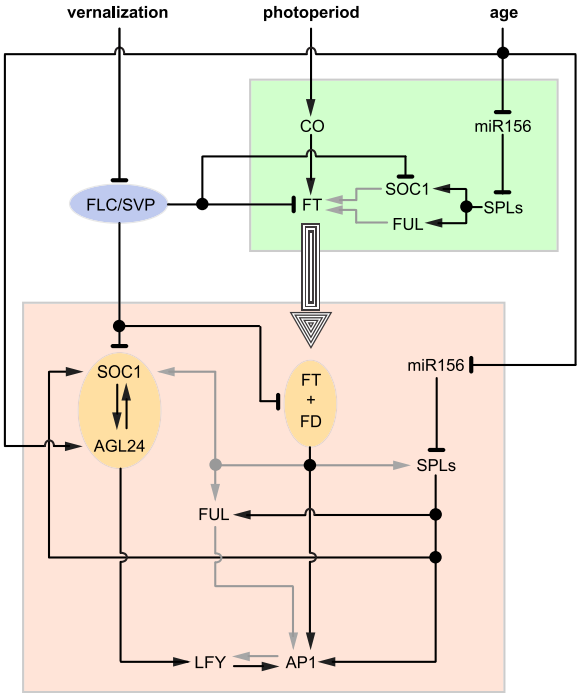

Figure 5. Summary of Regulatory Interactions in the Phloem of Leaves and at the Shoot Apex

Green box indicates phloem of leaves; pink box indicates shoot apex. Known direct interactions are shown as black lines. Ellipses indicate protein complexes. Not shown is the gibberellin pathway, which affects several flowering regulators, but for which the primary targets are unclear.

FUL by *AP1* (Ferrándiz et al., 2000a; Liu et al., 2007). These findings are not unique to *A. thaliana*. For example, in wheat and barley, the *FUL/AP1* homolog *VRN1* appears to be both an activator and a target of an *FT* homolog (Hemming et al., 2008; Li and Dubcovsky, 2008; Shimada et al., 2009).

In conclusion, we have demonstrated that the miR156-regulated *SPLs* are major factors that allow *A. thaliana* plants to flower in an FT/FD-independent manner. Previous investigations have emphasized the importance of the GA pathway in the absence of photoperiodic input (e.g., Blázquez et al., 1998; Eriksson et al., 2006; Hisamatsu and King, 2008; Reeves and Coupland, 2001); however, we have not found any evidence for GA levels having a major effect on miR156 or *SPL*s. Where the GA and miR156/SPL pathways converge is one important topic for future investigation. Finally, many aspects of the FT/FD pathway are conserved in cereals (Izawa, 2007; Kobayashi and Weigel, 2007; Trevaskis et al., 2007). It will therefore be interesting to learn which contribution miR156 targets, which are also present in grasses (Chuck et al., 2007; Wang et al., 2005; Yang et al., 2008), make to flowering control in this group of plants.

EXPERIMENTAL PROCEDURES

Sequences of oligonucleotide primers are given in Table S4.

Plant Material

Plants were grown at 23°C in long days (16 hr light/8 hr dark) or short days (8 hr light/16 hr dark). *p35S:MIR156*, *pSUC2:MIR156*, *pFD:MIR156*, *p35S:FD*, *p35S:rSPL3*, *p35S:amiR-FT/TSF*, *co*-9, *ft*-10, *ft*-10 *tsf*-1, *fd*-2, *lfy*-12, *lfy*-12 *fd*-2, *flc*-3, and *FRI FLC* have been described (Balasubramanian et al., 2006; Mathieu et al., 2007; Michaels and Amasino, 1999; Wang et al., 2008; Wigge et al., 2005; Yoo et al., 2005). *ful*-7 (SALK_033647) and *soc1*-6 (SALK_138131) are T-DNA insertion lines (Alonso et al., 2003). Double mutants were identified in the F_2 generation by PCR-based genotyping. Wild-type was Columbia (Col-0) except for *ga1*-3, which is in the L*er* background (Wilson et al., 1992).

Transgenic Plants

Genomic fragments of *SPL3* and *FUL* were amplified by PCR using Pfusion DNA polymerase (New England Biolabs, Ipswich, MA), cloned into a Gateway entry vector, and subsequently recombined into destination vectors that harbor *35S*, *SUC2*, or *FD* promoters, using Gateway LR clonase II Enzyme mix (Invitrogen, Carlsbad, CA). For *pSPL9:rSPL9-GR*, an *rSPL9* genomic fragment (Wang et al., 2008) was PCR amplified and fused to a fragment encoding the rat glucocorticoid receptor binding domain (Lloyd et al., 1994). At least 50 T_1 seedlings were analyzed for each construct.

RNA Analyses

Total RNA was extracted from seedlings or vegetative shoot apices with the Plant RNeasy Mini kit (QIAGEN, Hilden, Germany) or with Trizol reagent (Invitrogen).

For qRT-PCR, 1 μg of total RNA was DNase I treated and used for cDNA synthesis with oligo(dt) primer and Superscript reverse transcriptase (Invitrogen). PCR was performed with SYBR-Green PCR Mastermix (Invitrogen) and amplification was monitored on an MJR Opticon Continuous Fluorescence Detection System (Bio-Rad, Hercules, CA). Expression was normalized against *β-TUBULIN-2*. An amplification efficiency of 1.8 per cycle was assumed. At least two biological replicates were performed, with two technical replicates for each. Results from one biological replicate are shown. For small RNA blots, locked nucleic acid (LNA; Exiqon, Vedbaek, Denmark) oligonucleotide probes were used. GUS staining was performed as described (Blázquez et al., 1997). Probe synthesis and hybridization for analyses with Affymetrix (Santa Clara, CA) ATH1 arrays were performed as described (Schmid et al., 2003). Array data were normalized using gcRMA implemented in GeneSpring 5.1 (Agilent Technologies, Santa Clara, CA). In situ hybridization was carried out as described (Wang et al., 2008).

Chromatin Immunoprecipitation

ChIP was performed as described (Wigge et al., 2005). Crude chromatin extract was split into three parts. One part was saved for the input control. The other two were used for immunoprecipitation with Myc, HA, or GFP antibodies (Santa Cruz). After several washes, chromatin crosslinking was reversed, and DNA was purified with the PCR purification kit (QIAGEN). 1 μl of DNA solution was used for quantitative PCR analyses as described above for the RNA analyses. Relative enrichment of each fragment was calculated by determining the difference in amplification between experimental and control antibody reactions (HA or GFP versus Myc). qPCR was also used to confirm similar amounts of input for each reaction.

ACCESSION NUMBERS

Arabidopsis Genome Initiative gene identifiers are as follows: *SPL3* (At2g33810); *SPL9* (At2g42200); *SUC2* (At1g22710); *FD* (At4g35900); *FLC* (At5g10140); *CO* (At5g15840); *BETA-TUBULIN-2* (At5g62690); *LFY* (At5g61850); *FT* (At1g65480); *SOC1* (At2g45660); *FUL* (At5g60910); *AGL42* (At5g62165); *TFL1* (At5g03840); *AP1* (At1g69120); *MIR156a* (At2g25095). NCBI-GEO accession numbers for microarrays are GSM402170 to GSM402177, series GSE16061.

SUPPLEMENTAL DATA

Supplemental Data include eight figures and four tables and can be found with this article online at http://www.cell.com/cell/supplemental/S0092-8674(09)00713-2.

ACKNOWLEDGMENTS

We thank the European Arabidopsis Stock Centre for seeds; Eunyoung Chae, Yasushi Kobayashi, François Parcy, Patrice Salomé, Markus Schmid, the members of Weigelworld Team MiRNA, and Gang Wu and Scott Poethig for discussion; and Ayako Yamaguchi and Doris Wagner for the exchange of unpublished information. This work was supported by an EMBO Long-Term Fellowship to J.-W.W. (ALTF 274-2006), by a DFG-SFB 446 grant, and by European Community FP6 IP SIROCCO (contract LSHG-CT-2006-037900).

Received: February 6, 2009
Revised: April 24, 2009
Accepted: June 2, 2009
Published: August 20, 2009

REFERENCES

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K., and Araki, T. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science *309*, 1052–1056.

Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., Stevenson, D.K., Zimmerman, J., Barajas, P., Cheuk, R., et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science *301*, 653–657.

An, H., Roussot, C., Suárez-López, P., Corbesier, L., Vincent, C., Piñeiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C., et al. (2004). *CONSTANS* acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. Development *131*, 3615–3626.

Balasubramanian, S., Sureshkumar, S., Agrawal, M., Michael, T.P., Wessinger, C., Maloof, J.N., Clark, R., Warthmann, N., Chory, J., and Weigel, D. (2006). The *PHYTOCHROME C* photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. Nat. Genet. *38*, 711–715.

Bäurle, I., and Dean, C. (2006). The timing of developmental transitions in plants. Cell *125*, 655–664.

Birkenbihl, R.P., Jach, G., Saedler, H., and Huijser, P. (2005). Functional dissection of the plant-specific SBP-domain: overlap of the DNA-binding and nuclear localization domains. J. Mol. Biol. *352*, 585–596.

Blázquez, M.A., Soowal, L., Lee, I., and Weigel, D. (1997). *LEAFY* expression and flower initiation in *Arabidopsis*. Development *124*, 3835–3844.

Blázquez, M.A., Green, R., Nilsson, O., Sussman, M.R., and Weigel, D. (1998). Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter. Plant Cell *10*, 791–800.

Borner, R., Kampmann, G., Chandler, J., Gleissner, R., Wisman, E., Apel, K., and Melzer, S. (2000). A MADS domain gene involved in the transition to flowering in *Arabidopsis*. Plant J. *24*, 591–599.

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M., and Smyth, D.R. (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. Development *119*, 721–743.

Cardon, G.H., Hohmann, S., Nettesheim, K., Saedler, H., and Huijser, P. (1997). Functional analysis of the *Arabidopsis thaliana* SBP-box gene *SPL3*: a novel gene involved in the floral transition. Plant J. *12*, 367–377.

Cardon, G., Hohmann, S., Klein, J., Nettesheim, K., Saedler, H., and Huijser, P. (1999). Molecular characterisation of the *Arabidopsis* SBP-box genes. Gene *237*, 91–104.

Chuck, G., Cigan, A.M., Saeteurn, K., and Hake, S. (2007). The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. Nat. Genet. *39*, 544–549.

de Folter, S., Immink, R.G.H., Kieffer, M., Parenicova, L., Henz, S.R., Weigel, D., Busscher, M., Kooiker, M., Colombo, L., Kater, M.M., et al. (2005). Comprehensive interaction map of the *Arabidopsis* MADS box transcription factors. Plant Cell *17*, 1424–1433.

Eriksson, S., Bohlenius, H., Moritz, T., and Nilsson, O. (2006). GA_4 is the active gibberellin in the regulation of *LEAFY* transcription and *Arabidopsis* floral initiation. Plant Cell *18*, 2172–2181.

Ferrándiz, C., Gu, Q., Martienssen, R., and Yanofsky, M.F. (2000a). Redundant regulation of meristem identity and plant architecture by *FRUITFULL, APETALA1* and *CAULIFLOWER*. Development *127*, 725–734.

Ferrándiz, C., Liljegren, S.J., and Yanofsky, M.F. (2000b). Negative regulation of the *SHATTERPROOF* genes by *FRUITFULL* during *Arabidopsis* fruit development. Science *289*, 436–438.

Franco-Zorrilla, J.M., Valli, A., Todesco, M., Mateos, I., Puga, M.I., Rubio-Somoza, I., Leyva, A., Weigel, D., García, J.A., and Paz-Ares, J. (2007). Target mimicry provides a new mechanism for regulation of microRNA activity. Nat. Genet. *39*, 1033–1037.

Gandikota, M., Birkenbihl, R.P., Hohmann, S., Cardon, G.H., Saedler, H., and Huijser, P. (2007). The miRNA156/157 recognition element in the 3′ UTR of the *Arabidopsis* SBP box gene *SPL3* prevents early flowering by translational inhibition in seedlings. Plant J. *49*, 683–693.

Hartmann, U., Hohmann, S., Nettesheim, K., Wisman, E., Saedler, H., and Huijser, P. (2000). Molecular cloning of *SVP*: a negative regulator of the floral transition in *Arabidopsis*. Plant J. *21*, 351–360.

Hemming, M.N., Peacock, W.J., Dennis, E.S., and Trevaskis, B. (2008). Low-temperature and daylength cues are integrated to regulate *FLOWERING LOCUS T* in barley. Plant Physiol. *147*, 355–366.

Hempel, F.D., Weigel, D., Mandel, M.A., Ditta, G., Zambryski, P., Feldman, L.J., and Yanofsky, M.F. (1997). Floral determination and expression of floral regulatory genes in *Arabidopsis*. Development *124*, 3845–3853.

Hepworth, S.R., Valverde, F., Ravenscroft, D., Mouradov, A., and Coupland, G. (2002). Antagonistic regulation of flowering-time gene *SOC1* by CONSTANS and FLC via separate promoter motifs. EMBO J. *21*, 4327–4337.

Hisamatsu, T., and King, R.W. (2008). The nature of floral signals in *Arabidopsis*. II. Roles for *FLOWERING LOCUS T* (*FT*) and gibberellin. J. Exp. Bot. *59*, 3821–3829.

Huala, E., and Sussex, I.M. (1992). *LEAFY* interacts with floral homeotic genes to regulate *Arabidopsis* floral development. Plant Cell *4*, 901–913.

Izawa, T. (2007). Adaptation of flowering-time by natural and artificial selection in *Arabidopsis* and rice. J. Exp. Bot. *58*, 3091–3097.

Klein, J., Saedler, H., and Huijser, P. (1996). A new family of DNA binding proteins includes putative transcriptional regulators of the *Antirrhinum majus* floral meristem identity gene *SQUAMOSA*. Mol. Gen. Genet. *250*, 7–16.

Kobayashi, Y., and Weigel, D. (2007). Move on up, it's time for change mobile signals controlling photoperiod-dependent flowering. Genes Dev. *21*, 2371–2384.

Kropat, J., Tottey, S., Birkenbihl, R.P., Depege, N., Huijser, P., and Merchant, S. (2005). A regulator of nutritional copper signaling in *Chlamydomonas* is an SBP domain protein that recognizes the GTAC core of copper response element. Proc. Natl. Acad. Sci. USA *102*, 18730–18735.

Langridge, J. (1957). Effect of day-length and gibberellic acid on the flowering of *Arabidopsis*. Nature *180*, 36–37.

Lee, H., Suh, S.S., Park, E., Cho, E., Ahn, J.H., Kim, S.G., Lee, J.S., Kwon, Y.M., and Lee, I. (2000). The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev. *14*, 2366–2376.

Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., and Ahn, J.H. (2007). Role of *SVP* in the control of flowering time by ambient temperature in *Arabidopsis*. Genes Dev. *21*, 397–402.

Lee, J., Oh, M., Park, H., and Lee, I. (2008). SOC1 translocated to the nucleus by interaction with AGL24 directly regulates LEAFY. Plant J. *55*, 832–843.

Li, C., and Dubcovsky, J. (2008). Wheat FT protein regulates *VRN1* transcription through interactions with FDL2. Plant J. *55*, 543–554.

Li, D., Liu, C., Shen, L., Wu, Y., Chen, H., Robertson, M., Helliwell, C.A., Ito, T., Meyerowitz, E., and Yu, H. (2008). A repressor complex governs the integration of flowering signals in *Arabidopsis*. Dev. Cell *15*, 110–120.

Liang, X., Nazarenus, T.J., and Stone, J.M. (2008). Identification of a consensus DNA-binding site for the *Arabidopsis thaliana* SBP domain transcription factor, AtSPL14, and binding kinetics by surface plasmon resonance. Biochemistry *47*, 3645–3653.

Liljegren, S.J., Gustafson-Brown, C., Pinyopich, A., Ditta, G.S., and Yanofsky, M.F. (1999). Interactions among *APETALA1, LEAFY*, and *TERMINAL FLOWER1* specify meristem fate. Plant Cell *11*, 1007–1018.

Liu, C., Zhou, J., Bracha-Drori, K., Yalovsky, S., Ito, T., and Yu, H. (2007). Specification of *Arabidopsis* floral meristem identity by repression of flowering time genes. Development *134*, 1901–1910.

Liu, C., Chen, H., Er, H.L., Soo, H.M., Kumar, P.P., Han, J.H., Liou, Y.C., and Yu, H. (2008). Direct interaction of AGL24 and SOC1 integrates flowering signals in *Arabidopsis*. Development *135*, 1481–1491.

Lloyd, A.M., Schena, M., Walbot, V., and Davis, R.W. (1994). Epidermal cell fate determination in *Arabidopsis*: patterns defined by a steroid-inducible regulator. Science *266*, 436–439.

Lohmann, J.U., and Weigel, D. (2002). Building beauty: the genetic control of floral patterning. Dev. Cell *2*, 135–142.

Long, J., and Barton, M.K. (2000). Initiation of axillary and floral meristems in *Arabidopsis*. Dev. Biol. *218*, 341–353.

Mathieu, J., Warthmann, N., Küttner, F., and Schmid, M. (2007). Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis*. Curr. Biol. *17*, 1055–1060.

Melzer, S., Lens, F., Gennen, J., Vanneste, S., Rohde, A., and Beeckman, T. (2008). Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. Nat. Genet. *40*, 1489–1492.

Michaels, S.D., and Amasino, R.M. (1999). *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell *11*, 949–956.

Michaels, S.D., Ditta, G., Gustafson-Brown, C., Pelaz, S., Yanofsky, M., and Amasino, R.M. (2003). *AGL24* acts as a promoter of flowering in *Arabidopsis* and is positively regulated by vernalization. Plant J. *33*, 867–874.

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

Moss, E.G. (2007). Heterochronic genes and the nature of developmental time. Curr. Biol. *17*, R425–R434.

Parcy, F., Nilsson, O., Busch, M.A., Lee, I., and Weigel, D. (1998). A genetic framework for floral patterning. Nature *395*, 561–566.

Parcy, F., Bomblies, K., and Weigel, D. (2002). Interaction of *LEAFY, AGAMOUS* and *TERMINAL FLOWER1* in maintaining floral meristem identity in *Arabidopsis*. Development *129*, 2519–2527.

Pasquinelli, A.E., and Ruvkun, G. (2002). Control of developmental timing by micrornas and their targets. Annu. Rev. Cell Dev. Biol. *18*, 495–513.

Ratcliffe, O.J., Bradley, D.J., and Coen, E.S. (1999). Separation of shoot and floral identity in *Arabidopsis*. Development *126*, 1109–1120.

Reeves, P.H., and Coupland, G. (2001). Analysis of flowering time control in *Arabidopsis* by comparison of double and triple mutants. Plant Physiol. *126*, 1085–1091.

Ronemus, M., Vaughn, M.W., and Martienssen, R.A. (2006). MicroRNA-targeted and small interfering RNA-mediated mRNA degradation is regulated by argonaute, dicer, and RNA-dependent RNA polymerase in *Arabidopsis*. Plant Cell *18*, 1559–1574.

Ruiz-García, L., Madueño, F., Wilkinson, M., Haughn, G., Salinas, J., and Martínez-Zapater, J.M. (1997). Different roles of flowering time genes in the activation of floral initiation genes in *Arabidopsis*. Plant Cell *9*, 1921–1934.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science *288*, 1613–1616.

Schmid, M., Uhlenhaut, N.H., Godard, F., Demar, M., Bressan, R., Weigel, D., and Lohmann, J.U. (2003). Dissection of floral induction pathways using global expression analysis. Development *130*, 6001–6012.

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., and Lohmann, J.U. (2005). A gene expression map of *Arabidopsis thaliana* development. Nat. Genet. *37*, 501–506.

Schultz, E.A., and Haughn, G.W. (1991). *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. Plant Cell *3*, 771–781.

Schwab, R., Palatnik, J.F., Riester, M., Schommer, C., Schmid, M., and Weigel, D. (2005). Specific effects of microRNAs on the plant transcriptome. Dev. Cell *8*, 517–527.

Schwab, R., Ossowski, S., Riester, M., Warthmann, N., and Weigel, D. (2006). Highly specific gene silencing by artificial microRNAs in *Arabidopsis*. Plant Cell *18*, 1121–1133.

Schwarz, S., Grande, A.V., Bujdoso, N., Saedler, H., and Huijser, P. (2008). The microRNA regulated SBP-box genes *SPL9* and *SPL15* control shoot maturation in *Arabidopsis*. Plant Mol. Biol. *67*, 183–195.

Searle, I., He, Y., Turck, F., Vincent, C., Fornara, F., Kröber, S., Amasino, R.A., and Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. Genes Dev. *20*, 898–912.

Sheldon, C.C., Burn, J.E., Perez, P.P., Metzger, J., Edwards, J.A., Peacock, W.J., and Dennis, E.S. (1999). The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell *11*, 445–458.

Shimada, S., Ogawa, T., Kitagawa, S., Suzuki, T., Ikari, C., Shitsukawa, N., Abe, T., Kawahigashi, H., Kikuchi, R., Handa, H., et al. (2009). A genetic network of flowering time genes in wheat leaves, in which an *APETALA1*/*FRUITFULL*-like gene, *VRN1*, is upstream of *FLOWERING LOCUS T*. Plant J. *58*, 668–681.

Takada, S., and Goto, K. (2003). TERMINAL FLOWER2, an *Arabidopsis* homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of *FLOWERING LOCUS T* by *CONSTANS* in the vascular tissues of leaves to regulate flowering time. Plant Cell *15*, 2856–2865.

Teper-Bamnolker, P., and Samach, A. (2005). The flowering integrator *FT* regulates *SEPALLATA3* and *FRUITFULL* accumulation in *Arabidopsis* leaves. Plant Cell *17*, 2661–2675.

Trevaskis, B., Hemming, M.N., Dennis, E.S., and Peacock, W.J. (2007). The molecular basis of vernalization-induced flowering in cereals. Trends Plant Sci. *12*, 352–357.

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu. Rev. Plant Biol. *59*, 573–594.

Wagner, D., Sablowski, R.W., and Meyerowitz, E.M. (1999). Transcriptional activation of *APETALA1* by LEAFY. Science *285*, 582–584.

Wang, H., Nussbaum-Wagler, T., Li, B., Zhao, Q., Vigouroux, Y., Faller, M., Bomblies, K., Lukens, L., and Doebley, J.F. (2005). The origin of the naked grains of maize. Nature *436*, 714–719.

Wang, J.W., Schwab, R., Czech, B., Mica, E., and Weigel, D. (2008). Dual effects of miR156-targeted *SPL* genes and *CYP78A5*/*KLUH* on plastochron length and organ size in *Arabidopsis thaliana*. Plant Cell *20*, 1231–1243.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., and Meyerowitz, E.M. (1992). *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell *69*, 843–859.

Wigge, P.A., Kim, M.C., Jaeger, K.E., Busch, W., Schmid, M., Lohmann, J.U., and Weigel, D. (2005). Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science *309*, 1056–1059.

Wilson, R.N., Heckman, J.W., and Somerville, C.R. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol. *100*, 403–408.

Wu, G., and Poethig, R.S. (2006). Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. Development *133*, 3539–3547.

Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M., and Araki, T. (2005). *TWIN SISTER OF FT* (*TSF*) acts as a floral pathway integrator redundantly with *FT*. Plant Cell Physiol. *46*, 1175–1189.

Yang, Z., Wang, X., Gu, S., Hu, Z., Xu, H., and Xu, C. (2008). Comparative study of SBP-box gene family in *Arabidopsis* and rice. Gene *407*, 1–11.

Yoo, S.K., Chung, K.S., Kim, J., Lee, J.H., Hong, S.M., Yoo, S.J., Yoo, S.Y., Lee, J.S., and Ahn, J.H. (2005). *CONSTANS* activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in *Arabidopsis*. Plant Physiol. *139*, 770–778.

Yu, H., Xu, Y., Tan, E.L., and Kumar, P.P. (2002). AGAMOUS-LIKE 24, a dosage-dependent mediator of the flowering signals. Proc. Natl. Acad. Sci. USA *99*, 16336–16341.