Sequential action of *FRUITFULL* as modulator of the activity of the floral regulators *SVP* and *SOC1*

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Abstract

The role in flowering time of the MADS-box transcription factor *FRUITFULL* (*FUL*) has been proposed in many works. *FUL* has been connected to several flowering pathways as target of the photoperiod, ambient temperature and age pathways, and it is has been shown to promote flowering in a partially redundant manner with *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). However, *FUL* position in these genetic networks as well as the functional output of FUL activity during floral transition remains unclear. In this work, we have undertaken a genetic approach to better understand the functional hierarchies involving *FUL* and other MADS-box factors with well established roles as floral integrators such as *SOC1, SHORT VEGETATIVE PHASE (SVP)* or *FLOWERING LOCUS C (FLC)*. Our results suggest a prominent role of FUL in promoting reproductive transition when the photoinductive signaling is suppressed by short-day conditions or by high levels of *FLC* expression, as in non vernalized winter ecotypes. We propose a model where the sequential formation of FUL-SVP and FUL-SOC1 heterodimers may mediate the vegetative and meristem identity transitions, counteracting the repressive effect of FLC and SVP on flowering.

INTRODUCTION

Arabidopsis thaliana adult life cycle comprises three major phase transitions that are mainly characterized by the identity of the lateral structures produced by the shoot apical meristem (SAM). The vegetative phase transition marks the change from production of juvenile leaves to adult leaves. Both types of leaves form a rosette through the period of vegetative growth of the plant, and then, triggered by both environmental and endogenous cues, the SAM undergoes two subsequent phase transitions leading to reproductive development: the reproductive transition, that causes the bolting of the primary inflorescence and the production of cauline leaves subtending secondary inflorescences, and the meristem identity transition, after which the SAM will produce floral meristems directly (Araki, 2001; Huijser and Schmid, 2011; Yamaguchi *et al.*, 2009).

Both reproductive and meristem identity transitions, that we will collectively name as floral transition, are highly controlled by developmental and environmental signals. Six promoting pathways have been proposed to regulate this process (reviewed in Fornara *et al.*, 2010; Srikanth and Schmid, 2011): the photoperiod, vernalization, ambient temperature, age, autonomous and gibberellin pathways. The first three pathways respond to environmental signals, as day length and seasonal or day growth temperature, while the age and autonomous patways respond to endogenous signals, and the gibberelling pathway to both environmental and endogenous clues. All these pathways converge at the level of a few genes, named floral transition integrators.

Within this group of floral transition integrators, several members of the MADS-box family have major roles: the expression of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) is activated by the photoperiod, age and gibberellin pathways to promote floral transition (Borner *et al.*, 2000; Lee *et al.*, 2000; Lee and Lee, 2010; Samach *et al.*, 2000), which in part is mediated by the activation of the floral identity gene *LEAFY (LFY)* (Lee *et al.*, 2008; Liu *et al.*, 2008). Conversely, *FLOWERING LOCUS C (FLC)* and *SHORT VEGETATIVE PHASE (SVP)* act as floral transition repressors (Hartmann *et al.*, 2000; Michaels and Amasino, 1999; Sheldon *et al.*, 1999). High levels of *FLC* expression compete the inductive floral signals at the SAM*,* and thus, flowering is promoted when the vernalization and autonomous pathways repress *FLC* expression (Hepworth *et al.*, 2002; Kim *et al.*, 2009; Lee *et al.*, 2000; Michaels and Amasino, 1999; Michaels *et al.*, 2004; Sheldon *et al.*, 1999; Sheldon *et al.*, 2000). Likewise, the expression of the flowering repressor *SVP* is controlled by the autonomous, thermosensory and gibberellin pathways (Lee *et al.*, 2007; Li *et al.*, 2008).

FLC and SVP are able to form heterodimers that directly bind to *SOC1* promoter to downregulate *SOC1* expression, as well as to other floral transition integrators such as *FLOWERING LOCUS T (FT)* (Fujiwara *et al.*, 2008; Lee *et al.*, 2007; Li *et al.*, 2008).

The MADS-box transcription factor *FRUITFULL* (*FUL*), a closely related gene to the flower meristem identity genes *APETALA1* and *CAULIFLOWER*, has been associated with several developmental processes. In addition to its well known function during fruit development, *FUL* roles in floral meristem identity specification, shoot maturation and the control of floral transition have also been described (Ferrándiz *et al.*, 2000a; Ferrándiz *et al.*, 2000b; Gu *et al.*, 1998; Hempel *et al.*, 1997; Melzer *et al.*, 2008; Shikata *et al.*, 2009; Wang *et al.*, 2009)

FUL is partially redundant with SOC1 in flowering promotion. Although the *ful* mutants are only slightly late flowering under long day growth conditions (Ferrándiz *et al.*, 2000a), the double *ful soc1* mutants show a strong delay in floral transition (Melzer *et al.*, 2008). As *SOC1*, *FUL* is one of the earliest responsive genes to photoinductive signals (Hempel *et al.*, 1997; Schmid *et al.*, 2003) being target of the FT-FD dimer (Schmid *et al.*, 2003; Teper-Bamnolker and Samach, 2005; Torti *et al.*, 2012). *FUL* also responds to signals derived from the age pathway, being one of the most responsive genes to the SQUAMOSA PROMOTER BINDING LIKE (SPL) proteins (Shikata *et al.*, 2009; Wang *et al.*, 2009; Yamaguchi *et al.*, 2009). A recent work also places *FUL* in the promotion of flowering in response to ambient temperature through the action of miR156/SPL3 and FT (Kim *et al.*, 2012).

In spite of mounting evidence linking *FUL* to the main flowering pathways, the importance of FUL in controlling these processes, as well as its position, downstream effectors and mode of action in these pathways are still unclear. In this work, we have used genetic analyses to better understand the regulatory hierarchies involving *FUL* and other floral integrators of the MADS-box family such as *SOC1*, *SVP* and *FLC* in the control of floral transition in Arabidopsis. Our results show that FUL is able to act both upstream and cooperatively with SOC1, forming a heterodimer and binding directly to the *LFY* promoter. In addition, we show that the promotive effect of FUL on floral transition depends of the presence of a functional allele of *SVP* and that FUL is able to counteract the repressive effect of FLC on flowering both affecting *FLC* expression levels and likely competing with FLC for common targets. Taking all these data together, we propose a dynamic model for the role of FUL during the floral transition, where the progressive formation of different heterodimers of FUL and other MADS transcription factors may act as a molecular switch between vegetative and reproductive state.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana plants were grown in cabinets at 21°C under LD (16 h light) or SD (8 h light) conditions, illuminated by cool-white fluorescent lamps (150 µE m^{-2} s^{-1}), in a 1:1:1 mixture of sphagnum:perlite:vermiculite. To promote germination, seeds were stratified on soil at 4°C for 3 d in the dark. The Arabidopsis plants used in this work were in the Col-0 background, except *ful-1* and 35S::SOC1, that were in L*er*. Mutant alleles and transgenic lines have been previously described: *soc1-2* (Lee *et al.*, 2000), *ful-1* (Gu *et al.*, 1998), *ful-2* (Ferrándiz *et al.*, 2000a), *svp-32* (Lee *et al.*, 2007), FRI FLC (Lee and Amasino, 1995), 35S::SOC1, (Lee *et al.*, 2000), 35S::FUL (Ferrándiz *et al.*, 2000b), 35S::SVP (Masiero *et al.*, 2004), 35S::FLC (Michaels and Amasino, 1999), LFY:GUS (Blázquez *et al.*, 1997) and FLC:GUS (Sheldon *et al.*, 2002).

35S::FUL::GFP was generated by cloning of the FUL CDS into the pEarley103 vector (Earley *et al.*, 2006). Agrobacterium strain C58 pM090 was used to transform Arabidopsis using the floral dip protocol (Clough and Bent, 1998), and transgenic lines carrying a single transgene insertion and with similar phenotypes to the reference 35S::FUL line were selected.

Flowering Time Measurements

Flowering time was scored as number of leaves at bolting. The number of rosette and cauline leaves was counted when the bolting shoot had produced the first open flower. At least fifteen 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.

Chromatin Immunoprecipitation (ChIP)

35S::FUL and 35S::FUL::GFP seeds were grown for 15 d in soil, and inflorescences were collected for analysis. The ChIP experiments were performed as previously described (Sorefan *et al.*, 2009) with minor modifications using an anti-GFP antibody (Abcam, Ab290). Q-PCR was performed using SYBR®Green PCR Master Mix (Applied Biosystems) in a ABIPRISM 7700 sequence detection system (Applied Biosystems). The values correspond to the ratios between the pull-down DNA with GFP antibody from 35S::FUL and 35S::FUL:GFP lines and between a 10% fraction of the input genomic

DNA from both samples, all of them initially normalized by ACT7 or UBQ10 genomic region. The primers used for this work are described in Supplementary table 1.

Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from whole plants with the RNeasy Plant Mini kit (Qiagen). 2 µg of total RNA were used for cDNA synthesis performed with the First-Strand cDNA Synthesis kit (Invitrogen) and the qPCR master mix was prepared using the iQTM SYBR Green Supermix (Bio-rad). Results were normalized to the expression of the *TIP41-like* reference gene. The PCR reactions were run and analyzed using the ABI PRISM 7700 Sequence detection system (Applied Biosystems). Three technical and two biological replicates were performed for each sample. See Supplementary table 1 for primers sequences.

ß-Glucuronidase (GUS) staining and activity measurements.

For GUS histochemical detection, samples were treated for 15 min in 90% ice-cold acetone and then washed for 5 min with washing buffer (25 mM sodium phosphate, 5 mM ferrocyanide, 5 mM ferricyanide, and 1% Triton X-100) and incubated from 4 to 16 h at 37°C with staining buffer (washing buffer + 1 mM X-Gluc). Following staining, plant material was fixed, cleared in chloralhydrate, and mounted to be viewed under bright-field microscopy.

For quantitative measurements, the protocol described in Blazquez et al, 1997, was followed. Briefly, apices were incubated at 37°C for 16 hours in 1 mM MUG assay solution (1 mM 4-methyl umbelliferyl glucuronide, 50 mM sodium phosphate buffer pH 7, 10 mM EDTA, 0.1% SDS, 0.1% Triton X-100), in individual wells of a microtiter plate. After the reaction had been stopped by the addition of 0.3 M Na2CO3, fluorescence at 430 nm was measured on a luminescence spectrophotometer equipped with an ELISA plate reader (Perkin Elmer, model LS50B).

Bimolecular Fluorescence Complementation (BiFC)

Open reading frames of full-length *FUL*, *SOC1*, and *SVP* CDS were cloned into vectors pYFPN43 and pYFPC43 (http://www.ibmcp.upv.es/FerrandoLabVectors.php), and BiFC was performed as previously described (Belda-Palazon *et al.*, 2012).

Confocal Microscopy

Confocal microscopy was performed using a Leica TCS SL (Leica Microsystems Heidelberg GmbH,Heidelberg, Alemania) equipped with an Argon krypton laser (Leica).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: FUL (AT5G60910), SOC1 (AT2G45660), SVP (AT2G22540), FLC (AT5G10140), FRI (AT4G00650), LFY (AT5G61850), UBQ10 (AT4G05320), ACT7 (AT5G09810), TIP41-like (At4G34270).

RESULTS

Genetic interactions of *FUL* and *SOC1*

We have compared the timing of both reproductive and meristem phase transitions by the quantification of rosette and cauline leaves of wildtype, *ful* and 35S::FUL plants. As previously reported, we observed that the loss of *FUL* function caused a small delay in flowering time both in long day (LD) and short day (SD) conditions, while the overexpression of FUL caused a strong early flowering phenotype (Table 1) (Ferrándiz *et al.*, 2000a; Melzer *et al.*, 2008). The late flowering phenotype of *ful* mutants mainly affected the onset of the meristem identity transition, since the number of rosette leaves did not significantly differ from wild type, while the number of cauline leaves was increased both in LD and SD conditions (Table 1). In addition, when grown in SD, the axillary meristems of cauline leaves of single *ful-2* mutants formed aerial rosettes (Supp. fig. S1), and flowers were subtended by bracts (Sup. fig. S1).

It has been described that *FUL* and *SOC1* have similar roles and probably promote flowering redundantly (Melzer *et al.*, 2008). However, it is still unclear how precisely these two factors interact genetically and how each of them contributes to the reproductive or the meristem identity transitions. To better understand the genetic relationship of *FUL* and *SOC1*, we compared the effect on flowering time of different combinations of *FUL* and *SOC1* loss- and gain-of-function alleles.

In LD conditions, the *ful-2 soc1-2* double mutant showed a synergistic late flowering phenotype, in agreement with previously reported data (Melzer *et al.*, 2008), producing more rosette leaves than the *soc1-2* single mutant and more cauline leaves than both *ful-2* and *soc1-2* single mutants (Table 1). Additional phenotypes were observed such as the production of small leaves subtending flowers, the development of aerial rosettes at the cauline leaf axils and frequent SAM reversion (Supp. fig. S1B), similar to what was

observed in *ful-2* single mutants grown in SD and in other previous studies (Torti *et al.*, 2012).

The *soc1-2* mutant grown in SD showed a dramatic increase in rosette leaf number, and also a delay in meristem identity transition, although not as important as the delay produced by *ful-2* (Table 1). The *ful-2 soc1-2* double mutants grown in SD produced a similar number of rosette leaves than the *soc1-2* mutant, indicating that in the absence of photoperiodic stimulus the promoting role of *FUL* on the reproductive transition could depend on the presence of SOC1. On the other hand, the number of cauline leaves produced by *ful-2 soc1-2* was only moderately higher than in *ful-2* single mutants, suggesting that FUL would have a predominant effect in the control of meristem identity transition (Table 1).

35S::FUL *soc1-2* plants flowered earlier than wildtype, but significantly later than 35S::FUL lines (Table 1) supporting the idea that the flowering promoting role of FUL was partially dependent on the presence of an active allele of SOC1. In contrast, 35S::SOC1 *ful-1* plants were identical to 35S::SOC1 plants in rosette leaf number, while the absence of *FUL* only slightly increased the number of cauline leaves produced in 35S::SOC1 background (Table 1). Finally, lines that overexpressed both genes simultaneously flowered extremely early, producing only two rosette leaves before the SAM directly differentiated into one or two flowers, although occasionally one cauline leaf with an axillary flower was formed (Table 1, Fig 1A, B). Moreover, the axillary meristems from rosette leaves were also converted into flowers (Fig. 1A). This strong synergistic effect, together with the partial dependence of FUL on the presence of SOC1 to promote flowering, was compatible with FUL acting in part as an upstream regulator of *SOC1*, together with a subsequent cooperative action of both proteins in the regulation of putative common targets, although it did not exclude other possible scenarios.

SOC1 and _LFY_ are FUL direct targets

It has been described that FUL and SOC1 are able to interact in yeast two-hybrid experiments as homo- and heterodimers (de Folter *et al.*, 2005; Immink *et al.*, 2012). To further confirm this interaction *in planta,* we performed a Bimolecular Fluorescence Complementation (BiFC) experiment through transient expression on *Nicotiana benthamiana* leaves, observing FUL-SOC1 dimerization in the nuclei of the cells (Fig. 1C).

The floral identity gene *LFY* has been identified as a *bona fide* SOC1 direct target (Lee

et al., 2008). In addition, *FUL* has been also suggested to upregulate *LFY* (Ferrándiz *et al.*, 2000a). To confirm this suggestion, we analyzed the expression of a LFY::GUS reporter line in the *ful-2* and 35S::FUL backgrounds, and observed that *LFY* level of expression was dependent on *FUL*, being lower in the *ful-2* mutant and higher in the 35S::FUL line than in WT plants (Fig. 2A-C). These relative levels of expression were also confirmed by quantitative RT-PCR of *LFY* expression in apices at days 7, 10 and 12 after germination (Fig. 2D). In addition, GUS activity was also quantitatively determined in individual dissected apices, using the substrate 4-methyl umbelliferyl glucuronide (MUG), which is converted by GUS into the fluorescent product 4-MU. We performed a time-course per-apex quantification on the three genetic backgrounds, observing that LFY::GUS activity was consistently higher in 35S::FUL plants and lower in *ful-2* plants than in WT (Fig 2E). Chromatin immunoprecipitations (ChIP) experiments using a 35S::FUL::GFP line (Supp. fig. S2) revealed that FUL was able to bind a region 2.2 kb upstream to the ATG codon of the *LFY* gene (Fig. 2F), overlapping with a previously identified region also bound by SOC1 (Lee *et al.*, 2008).

Moreover, we found FUL-GFP to also bind the *SOC1* promoter, around 800 bp upstream to the ATG codon (Fig. 2G). Again, this region bound by FUL overlaps with a region bound by SOC1 itself, which confirms *in planta* the Y1H experiment reported previously, which shows a FUL-SOC1 heterodimer binding to this fragment of SOC1 promoter (Immink *et al.*, 2012). Taken together, these results strongly support the hypothesis of SOC1 and FUL binding as heterodimers to the promoters of their target genes and could explain the genetic interactions observed.

Genetic interactions of *FUL* and *SVP*

SVP has been shown to directly repress *SOC1*, in part by binding to the *SOC1* promoter as a heterodimer with FLC, a potent repressor of flowering involved in the vernalization and autonomous pathway (Helliwell *et al.*, 2006; Michaels and Amasino, 1999; Sheldon *et al.*, 2002). Our results indicated that FUL could also act as an upstream regulator of *SOC1* binding directly the *SOC1* promoter. To explore whether FUL could interact with SVP to regulate *SOC1*, we characterized the effect on flowering time of different combinations of *FUL* and *SVP* loss- and gain-of-function alleles.

The *svp-32* mutant showed a clear early flowering phenotype both in LD and SD conditions, reducing the number of rosette leaves produced when compared to the WT control, as it was previously described (Lee *et al.*, 2007) (Table 2). *ful-2 svp-32* flowered with a similar number of leaves as the *svp-32* single mutant (Table 2) (Torti *et al.*, 2012), suggesting that SVP represses additional targets that can promote flowering in the

absence of *FUL*, as it has already been proposed (Torti *et al.*, 2012). If this was true, we could expect plants overexpressing *FUL* in a *svp* background to flower earlier or at least like 35S::FUL plants. However, 35S::FUL *svp-32* plants also flowered similarly to *svp-32*, both in LD and SD, (Table 2) suggesting an alternative scenario, where *FUL* overexpression was not able to promote flower transition in the absence of an active SVP protein. Thus, the epistatic effect of *svp* mutation on both *FUL* loss- or gain-of-function may suggest that FUL required SVP to regulate its targets, and this could be mediated by physical interaction of both factors.

Interaction of FUL and SVP proteins has already been reported in yeast-two-hybrid experiments (de Folter *et al.*, 2005; Immink *et al.*, 2012). To test if this heterodimer also occurred *in planta*, we performed a BiFC experiment that confirmed such interaction (Fig 3A). If FUL required interaction with SVP to promote floral transition, we could expect that simultaneous overexpression of FUL and SVP would result in early flowering, overcoming the late flowering phenotype caused by SVP overexpression. We then generated a 35S::SVP 35S::FUL line and quantified flowering time in these double transgenic line. As described above, 35S::FUL flowered early, while 35S::SVP flowered very late, as expected for a potent repressor of flowering transition (Table 2, Fig 3B). The line harboring both the 35S::FUL and the 35S::SVP transgenes flowered early, similarly to 35S::FUL or 35S::FUL *svp* plants (Fig. 3B, Table 2). This phenotype indicated that SVP was not able to repress floral transition when both high levels of SVP and FUL were present, suggesting that the FUL-SVP dimer could suppress the repressor effect of SVP on flowering or even act as a flowering promoting factor.

Genetic interactions of *FUL* and *FLC*

Because the repressor effect of SVP in flowering transition is partially mediated by the formation of a heterodimer with FLC (Fujiwara *et al.*, 2008; Lee *et al.*, 2007; Li *et al.*, 2008), we decided to explore the genetic relationship of *FUL* and FLC.

Much of the natural variation in flowering time in *Arabidopsis* depends on the allelic variation on *FLC* and its positive regulator *FRI* (Amasino, 2010). Late-flowering accessions usually bear functional alleles of both *FLC* and *FRI*, while most rapid-cycling accessions typically possess loss-of-function alleles of either gene. *ful-2* mutants are in the Col-0 genetic background, which has a *fri*;*FLC* genotype and therefore an early flowering habit (Johanson *et al.*, 2000; Michaels, 2009; Sheldon *et al.*, 1999). To study the effect of *ful* mutations in the presence of *FLC*, we introduced the *ful-2* allele in a *FRI;FLC* genetic background derived from the introgression of a *FRI* functional allele

into Col-0 (Lee and Amasino, 1995). *FRI;FLC* plants flower very late in all growing conditions, and are strongly responsive to vernalization treatment to induce flowering (Lee and Amasino, 1995). In LD conditions and without vernalization, the *ful-2* mutation greatly enhanced the late flowering phenotype of *FRI;FLC* plants, as *FRI;FLC ful-2* produced many more rosette and cauline leaves than *FRI;FLC* plants (Table 3, Fig. 4A). Vernalization of both *FRI;FLC* and *FRI;FLC ful-2* significantly accelerated the reproductive transition, and both lines flowered with a similar number of rosette leaves although *FRI;FLC ful-2* still produced more cauline leaves (Table 3, Fig 4A). Thus, vernalization significantly suppressed the effect of *ful-2* on the floral transition of *FRI;FLC* plants, suggesting that, in the presence of high levels of *FLC* (such as in non-vernalized *FRI;FLC* plants), *FUL* was required to promote flowering and that this promotion could either be mediated by negative regulation of *FLC* or by counteracting the repressor effect of *FLC* on flowering.

We also analyzed flowering time in plants resulting from crossing 35S::FUL to *FRI;FLC* and to 35S::FLC lines, thus generating F1 plants heterozygous for the *FRI* allele and hemizygous for the 35S::FUL transgene or hemyzygous for both the 35S::FLC and the 35S::FUL transgenes. and compared the results with the flowering time of the corresponding F1s from crosses between *FRI;FLC* or 35S::FLC to Col-0 wildtype. Constitutive expression of *FUL* caused early flowering in *FRI;FLC* plants and was also able to promote flowering in the 35S::FLC background, although to a lesser extent than when *FLC* expression was controlled by its own regulatory sequences (Table 4). We checked the activity of a FLC::GUS reporter in rosettes of 35S::FUL *FRI;FLC* plants, and found it to be lower than in *FRI;FLC* background (Fig 4B,C,E,F). Quantitative RT-PCR showed that this reduction was modest, but significant (Fig 4H), supporting that FUL could at least partially repress *FLC* expression. Moreover, while *FRI;FLC* plants only flowered when *FLC* levels were almost undetectable in the inflorescence, the 35S::FUL *FRI;FLC* plants flowered when *FLC* was still detected, indicating that FUL could also overcome FLC repressive effect on flowering (Fig 4D,G). Taking all these data together, it appeared that FUL was both repressing *FLC* expression and counteracting the negative effect of *FLC* on flowering, since plants were able to flower even in the presence of significant levels of FLC.

DISCUSSION

The results presented in this work show that *FUL* participates in both reproductive and meristem identity transitions modulating the activity of MADS box factors with major

regulatory roles in these phase changes. The role of FUL in promoting meristem identity transition is cooperative and partly dependent on SOC1, while FUL role in reproductive transition may be mediated both by interfering with the FLC-SVP dimer and/or changing the activity of SVP from repressor to activator of flowering. Taking together our genetic analyses and the results from BiFC dimerization experiments, we propose that these regulatory interactions are likely mediated by the sequential participation of FUL in heterodimers with SVP and SOC1 (Fig 5).

FUL promotes flower initiation together with *SOC1*

Previous studies indicate that *FUL* and SOC1 are able to act redundantly to promote floral transition. FUL and SOC1 share common upstream regulators, as they are both activated by the FT-FD complex and repressed by SVP (Lee *et al.*, 2007; Li *et al.*, 2008; Torti and Fornara, 2012). However, they also respond differently to other flowering pathways, being *FUL* more responsive to the age pathway and *SOC1* to the gibberellin pathway (Porri *et al.*, 2012; Wang *et al.*, 2009; Yamaguchi *et al.*, 2009). Moreover, recent work has also shown how *SOC1* and *FUL* respond differently to the signals from the photoperiodic pathway, where the maintenance of *SOC1* expression in the SAM depends more strongly from continuous photoperiodic stimulus than that of *FUL* (Torti *et al.*, 2012). These differences in regulation could partly explain the phenotypic effects that we observed in *ful* and *soc1* mutants. When grown in SD, *ful* mutants show little effect in reproductive transition, while strongly delay flower production, indicating that when other photoperiod responsive genes like *SOC1* are downregulated, *FUL* plays an important role in promoting floral meristem initiation. Moreover, the presence of binding sites for FUL in the *SOC1* promoter, the similar timing of reproductive transition in *soc1* and *ful soc1* mutants grown in SD, and the significant suppression of the early flowering phenotype of 35S::FUL lines in the *soc1* background, likely places *FUL* upstream of *SOC1*, suggesting that in the absence of a photoperiodic stimulus, *FUL* could directly mediate the activation of *SOC1*. Moreover, previous reports on SOC1 binding to its own promoter (Immink *et al.*, 2012) and our experiments showing binding of FUL to the same region of *SOC1* promoter also suggest that once both factors are present, they could act in a positive feedback loop to maintain high levels of *SOC1* expression. This positive feedback loop could also explain why a *ful* mutant grown in SD, where SOC1 expression is downregulated, shows meristem reversion and bracts subtending flowers. On the other hand, no binding sites for SOC1 on *FUL* promoter have been identified in a recent ChIP-seq experiment (Tao *et al.*, 2012), and loss of *FUL* function does not modify the 35S::SOC1 early flowering phenotype, suggesting that FUL is not a target of SOC1 regulation and therefore of this feedback loop.

Our results also show that FUL and SOC1 appear to act cooperatively in promoting a sharp meristem identity transition through the activation of *LFY*. A similar model has been proposed for the interaction of SOC1 and AGL24, another MADS factor with a flowering promoting role (Michaels *et al.*, 2003). SOC1 has been described as a cytoplasmic protein able to dimerize with AGL24, and translocate to the nucleus to upregulate *LFY* expression (Lee *et al.*, 2008; Li *et al.*, 2008). A similar mechanism appears to be working for FUL and SOC1, as we have observed that FUL and SOC1 are able to dimerize in the nucleus, and that both SOC1 and FUL bind to the same region of the *LFY* promoter. Thus SOC1, AGL24 and FUL could be forming redundant dimers or a higher order molecular complex to ensure the initiation of floral meristems through *LFY* activation.

SVP behavior as a repressor of flowering is likely suppressed by its interaction with FUL.

Because *svp* mutations largely suppress the late flowering phenotype of *soc1* and *ful* mutants, it has been proposed that SVP represses additional flowering promoting factors that would act in parallel to *FUL* and *SOC1* and therefore, even in the absence of FUL and SOC1 functions, the derepression of these factors would still cause early flowering (Torti *et al.*, 2012). Our results, showing that *FUL* overexpression suppresses the strong late flowering phenotype of *SVP* overexpression and that SVP and FUL are able to dimerize, may suggest a different interpretation. A possibility would be that *FUL* overexpression could overcome the downregulation of these additional flowering promoting factors repressed by SVP. However, this is in contradiction with our data showing that *soc1* mutations only partially suppress 35S::FUL early flowering phenotypes and by the phenotype of 35S::SVP 35S::SOC1 plants, which flower earlier than 35S::SVP plants but later than 35S::SVP 35S::FUL plants (Li *et al.*, 2008). We can then speculate about the role of the SVP-FUL putative dimers. Our data are compatible with a model where SVP is inactivated as a flowering repressor upon interaction with FUL. This situation would parallel the switch in SVP activity triggered by SVP dimerization with different MADS transcription factors. Thus, it has been proposed that SVP represses flowering during vegetative development, but upon upregulation of the flowering promoting factor *AGL24* in the SAM, a SVP-AGL24 dimer is formed which is able to activate the expression of *AP1* in early stages of flower development. This model also proposes that once AP1 is present, SVP would be displaced from the interaction with AGL24 to form a complex with AP1, which in turn represses the expression of floral organ identity genes, thus ensuring the proper development of floral meristems (Gregis *et al.*, 2006, 2008; Gregis *et al.*, 2009)

We then propose that SVP would be repressing flowering until other pathways allow the accumulation of SVP interactors such as AGL24 or FUL, which in turn would form protein complexes with SVP to switch off SVP activity as a flowering repressor.

The interaction of *FUL* and *FLC* appears to take place at two levels

Our work suggests a major role of *FUL* in promoting flowering on winter ecotypes, as revealed by the enhanced late flowering phenotype produced by the *ful-2* mutation in the *FRI;FLC* background. Again, this effect is different from that caused by mutations in *SOC1*, since it has been described that *soc1* does not affect the number of rosette leaves of *FRI;FLC* plants or other mutants in the autonomous pathway (Moon *et al.*, 2005). These different effects of *ful* and *soc1* mutations in the *FRI;FLC* background are consistent with the described role of *FLC* in the repression of the photoperiodic stimuli, and the prominent role of *FUL* on flowering promotion under short days. Accordingly, *FUL* loss-of-function delays flowering in *soc1* and *FRI;FLC* backgrounds. While *FT* and *SOC1* are *bona fide* targets of FLC negative regulation, we have not found evidence in the literature of FLC regulating *FUL*, and in agreement with that, no binding of FLC on the *FUL* promoter has been detected in ChIP-seq experiments (Deng *et al.*, 2011). Thus, in non-vernalized winter ecotypes, the expression of *FT* and *SOC1* should be repressed by FLC, but *FUL* expression would be regulated independently of FLC, most likely through signals from the age pathway mediated by miR156-targets of the SPL family (Wang *et al.*, 2009; Wu *et al.*, 2009; Yamaguchi *et al.*, 2009).

We have also observed that *FUL* overexpression was able to both reduce *FLC* expression in the *FRI;FLC* background as well as to counteract the FLC repressive effect on flowering independently of *FLC* regulation, as revealed by the partial suppression of 35S::FLC extreme late flowering phenotype by *FUL* overexpression. These results indicate that FUL could be antagonizing *FLC* at two different levels: by repressing its expression and by competing FLC activity on its targets. *FLC* repression by FUL might not be direct, as we could not detect FUL binding on the CArG boxes of *FLC* promoter in ChIP experiments, but it is evidenced by the observed reduction of FLC::GUS reporter activity in the vegetative tissues of 35S::FUL lines. On the other hand, FUL could be also competing with FLC for SVP dimerization, and thus reduce the repressive effect of FLC-SVP on targets such as *FT* or *SOC1*.

A model for FUL activity as a modulator of reproductive and meristem identity transitions

With our results on the observed protein-protein interactions as well as the genetic analyses of the *FUL/SVP/SOC1* relationship we can speculate on a possible mechanism of FUL action to regulate flowering transition in *Arabidopsis* (Fig 5). During the vegetative phase, both FLC and SVP are able to repress *SOC1* by binding as a heterodimer to the *SOC1* promoter. When FLC and SVP levels are high, as for example in the *FRI;FLC* unvernalized plants, the photoperiodic pathway would be repressed even under long day conditions. *FUL* expression would increase gradually responding to signaling from the age pathway. FUL accumulation could then interfere with the FLC-SVP dimer activity, perhaps by displacing SVP from the complex to form an alternative SVP-FUL heterodimer, and thus releasing *SOC1* repression, and/or leading to *SOC1* activation. Upon subsequent SOC1 accumulation, a FUL-SOC1 dimer would form, driving SOC1 protein to the nucleus to maintain its own expression and to activate *LFY* expression and flower initiation, in a likely redundant manner with AGL24-SOC1 heterodimers.

SUPPLEMENTARY DATA

Supplementary Figure 1: Inflorescence phenotypes of *ful*, *soc1* and *ful soc1* double mutant.

Supplementary Figure 2: Plants used in the ChIP experiments.

Supplementary Figure 3: Negative controls for BiFC experiments

Supplementary Table 1. Primers used in this study.

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TABLES

TABLE 1. Genetic interaction of FUL and SOC1. Effect in flowering

	Long Day		Short Day	
	Rosette Leaves	Cauline Leaves	Rosette Leaves	Cauline Leaves
Columbia-0	10.2±1.0	3.2±0.4	55.1±3.4	9.3±0.7
ful-2	10.7±0.8	4.4±0.5 [a]	59.9±3.8 [a]	23.7±3.2 [a]
soc1-2	19.3±0.9 [a]	4.2±0.5 [a]	75.0±4.2 [a]	15.2±0.5 [a]
ful-2 soc1-2	24.5±0.8 [a,b,c]	9.7±1.9 [a,b,c]	75.1±3.5 [a,b,]	28.1±1.7 [a,b,c]
35S::FUL	3.5±0.5 [a]	1.7±0.7 [a]	10.6±0.9 [a]	3.6±0.7 [a]
35S::FUL *soc1-2*	9.0±1.1 [d]	2.2±0.7 [d]	44.6±12.8 [d]	7.2±4.5 [d]
Landsberg *er*	7.3±0.5	1.8±0.4	nd	nd
ful-1	8.4±0.5 [e]	2.5±0.5 [e]	nd	nd
35S::SOC1	4.0±0.0 [e]	0.4±0.5 [e]	nd	nd
35S::SOC1 *ful-1*	4.0±0.0 [f]	0.7±0.5 [f,g]	nd	nd
35S::FUL 35S::SOC1	2.0±0.0 [g]	0.2±0.4 [g]	nd	nd

Flowering time expressed as the mean of rosette and cauline leaves produced in long and short day conditions. Errors are represented as the standart deviation. [a], [b], [c], [d], [e], [f], [g] indicates significantly different ($P < 0.05$) from Col, *ful-2*, *soc1-2*, 35S::FUL, L.*er*, *ful-1* and 35S::SOC1 controls respectively according to a t-test. nd= not determined.

TABLE 2. Genetic interaction of FUL and SVP. Effect in flowering

	Long Day		Short Day	
	Rosette Leaves	Cauline Leaves	Rosette Leaves	Cauline Leaves
Columbia-0	12.4±1.7	2.5±0.4	64.4±6.0	8.6±0.8
ful-2	12.9±0.9	3.8±0.6[a]	70.2±7.0[a]	20.8±3.8[a]
svp-32	5.6±0.5[a]	2.8±0.4	16.4±2.1	4.6±1.0
ful-2 svp-32	5.3±0.5[b]	3.3±0.5	16.1±2.5	7.1±1.6
35S::FUL	4.0±0.0[a]	1.4±0.5[a]	8.3±1.8[a]	3.5±0.8[a]
35S::FUL *svp-32*	5.8±0.4	2.5±0.5	14.9±2.1[c,d]	3.4±1.2[c]
35S::SVP	27.5±1.7[a]	7.3±1.0[a]	nd	nd
35S::FUL 35S::SVP	5.8±1.2[e]	2.7±0.8[d,e]	nd	nd

Flowering time expressed as the mean of rosette and cauline leaves produced in long and short day conditions. Errors are represented as the standart deviation. [a], [b], [c], [d], [e] indicates significantly different ($P < 0.05$) from Col, *ful-2*, *svp-32*, 35S::FUL and 35S::SVP controls respectively according to a t-test. nd= not determined.

TABLE 3. Effect of vernalization in flowering time of *ful* mutants

	Long Day			
	-Vernalization		+ Vernalization	
	Rosette Leaves	Cauline Leaves	Rosette Leaves	Cauline Leaves
FRI FLC	57.6±8.0	9.5±2.2	24.4±2.1	5.9±1.0
FRI FLC *ful-2*	73.9±6.2**	19.8±0.9**	23.2±2.9	8.6±0.8

Flowering time expressed as the mean of rosette and cauline leaves produced in long day conditions. Errors are represented as the standart deviation. Asterisk (*) indicates significantly different ($P < 0.05$) from FRI FLC control according to a t-test.

TABLE 4. Genetic interaction of FUL and FLC. Effect on flowering,

	Long Day	
	Rosette Leaves	Cauline Leaves
FRI/+	56.5±1.7	12.0±1.4
35S::FUL/+	7.0±2.3	2.2±0.4
35S::FLC/+	> 80	nd
35S::FUL/+ FRI/+	9.7±1.1 [a,b]	2.3±0.8 [a]
35S::FUL/+ 35S::FLC/+	34.3±7.7 [b,c]	13.8±1.9 [b]

Flowering time expressed as the mean of rosette and cauline leaves produced in long day conditions. Errors are represented as the standart deviation. [a], [b], [c] indicates significantly different ($P < 0.05$) from FRI/+, 35S::FUL/+ and 35S::FLC/+ controls respectively according to a t-test. nd= not determined.

FIGURE LEGENDS

Figure 1. Interaction of FUL with SOC1

A-B. Phenotypes of 35S::FUL 35S::SOC1 double overexpression lines. Only two rosette leaves are produced (arrows in A) and occasionally one cauline leaf (arrow head in B). All axillary meristems are determinate, directly producing flowers. Asterisks mark the cotyledons in A. C. Bimolecular Fluorescence Complementation in tobacco epidermal leaf cells between transiently expressed FUL and SOC1 fusions to the C- and N-terminal fragments of YFP, respectively. Left panel shows reconstituted YFP fluorescence (green) and the right panel is an overlay with a bright field image of the same sector where chlorophyll shows in red. Negative controls for BiFC experiments are shown on Supp. Fig S3. Scale bars: 500 mm (A,B), 40 µm (C).

Figure 2. FUL regulates key genes in the floral transition process binding directly to SOC1 and LFY promoters.

A-C. Histochemical detection of LFY::GUS activity in the apices of 6 day old wild type (A), *ful-2* (B) or 35S::FUL (C) plants. Scale bars, 250 µm.

D. Relative expression of LFY analyzed by qRT-PCR in WT, *ful-2* and 35S::FUL plants at days 7, 10 and 12 after germination. The error bars depict the s.e. based on two biological replicates. Asterisk (*) indicates significantly different ($P < 0.05$) from WT control according to a t-test.

E. Quantification of LFY:GUS activity in WT, ful-2 and 35S::FUL backgrounds. Plants were grown on plates under long days (LD). At each time point, GUS activity was measured in at least twelve individual apices, and the means ± s.e are given.

F. (Top) Schematic diagram of the *LFY* upstream promoter region. First exon is represented by a black box, while the upstream genomic region is represented by a black line. The red stars indicate the sites containing either single mismatch or perfect match with the consensus binding sequence (CArG box) of MADS-domain proteins. Amplicons spanning these sites used in the ChIP analyses are represented by grey lines and marked by roman numbers. (Bottom) ChIP enrichment tests showing the binding of FUL-GFP to the LFY-I region. Bars represent the ratio of amplified DNA (35S::FUL:GFP/35S::FUL) in the starting genomic DNA (input) or in the immunoprecipitated DNA with the GFP antibody (Ab).

G. (Top) Schematic diagram of the *SOC1* genomic region, including upstream promoter, exons 1 and 2 and the first intron. Exons are represented by black boxes, upstream genomic region and intron by a black line. The red stars mark CArG boxes. Amplicons spanning these sites used in the ChIP analyses are represented by grey lines and marked by roman numbers. (Bottom) ChIP enrichment tests showing the binding of FUL-GFP to the SOC1-III region. Bars represent the ratio of amplified DNA (35S::FUL:GFP/35S::FUL) in the starting genomic DNA (input) or in the immunoprecipitated DNA with the GFP antibody (Ab).

Figure 3. Interaction of FUL with SVP

A. BiFC experiments in tobacco leaf cells between transiently expressed FUL and SOC1 fusions to the C- and N-terminal fragments of YFP, respectively. Left panel shows YFP reconstituted fluorescence (green) and the right panel is an overlay with a bright field image of the same sector where chlorophyll shows in red. Negative controls for BiFC experiments are shown on Supp. Fig S3. Scale bars: 40 µm

B. Phenotypes of the 35S::FUL, 35S::SVP and 35S::FUL 35S::SVP double overexpression lines. *FUL* overexpression reverts the late flowering phenotype of 35S::SVP, although inflorescence development is partially restored respect to the 35S::FUL plants.

Figure 4. FUL overexpression suppresses the effects of high levels of *FLC*.

A. Vernalization response of FRI;FLC and FRI;FLC *ful-2* in LD. The *ful-2* mutation greatly enhances the late flowering phenotype of FRI;FLC unvernalized plants (left), while a vernalization treatment causes both genotypes to flower similarly earlier (right). B-G. Histochemical detection of FLC::GUS activity in FRI;FLC (B-D) and FRI;FLC 35S::FUL (E-G) plants. Apices of 10 day-old plants are compared in B,E; first rosette leaf in C, F; and inflorescence apices of plants at bolting in D, G. All plants were heterozygous for the FLC::GUS reporter and for the wildtype dominant alleles of FRI or FLC. 35S::FUL in E-G was also heterozygous. Scale bars: 500 µm (B,C,E,F) or 100 µm (D,G). H. Relative expression of FLC analyzed by qRT-PCR in FRI;FLC and FRI;FLC 35S::FUL plants 10 days after germination. The error bars depict the s.e. based on two biological replicates. Asterisk (*) indicates significantly different ($P < 0.05$) from WT control according to a t-test.

Figure 5. A proposed mechanistic model for the role of FUL during floral transition through interaction with SVP and SOC1 factors.

During vegetative growth FLC and SVP repress the expression of *SOC1* and other flowering promoting factors. Upon FUL accumulation, likely mediated by the age SPL-dependent pathway, FUL-SVP dimerization occurs. The FUL-SVP dimer could compete with the FLC-SVP dimer for binding sites in the *SOC1* promoter and/or directly interfering with the FLC-SVP dimer formation. Lower repressive activity of the FLC-SVP dimer on *SOC1* or even direct activation of SOC1 by FUL-SVP would lead to SOC1 accumulation, the dimerization of FUL-SOC1 and the activation of both *SOC1* and *LFY* promoters, thus triggering flower initiation.

FIGURES

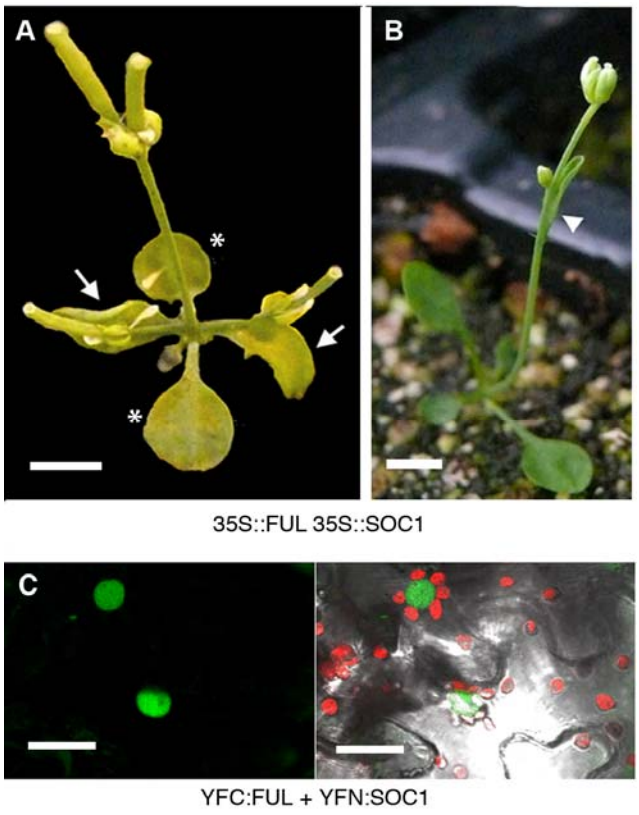

Figure 1. Interaction of FUL with SOC1

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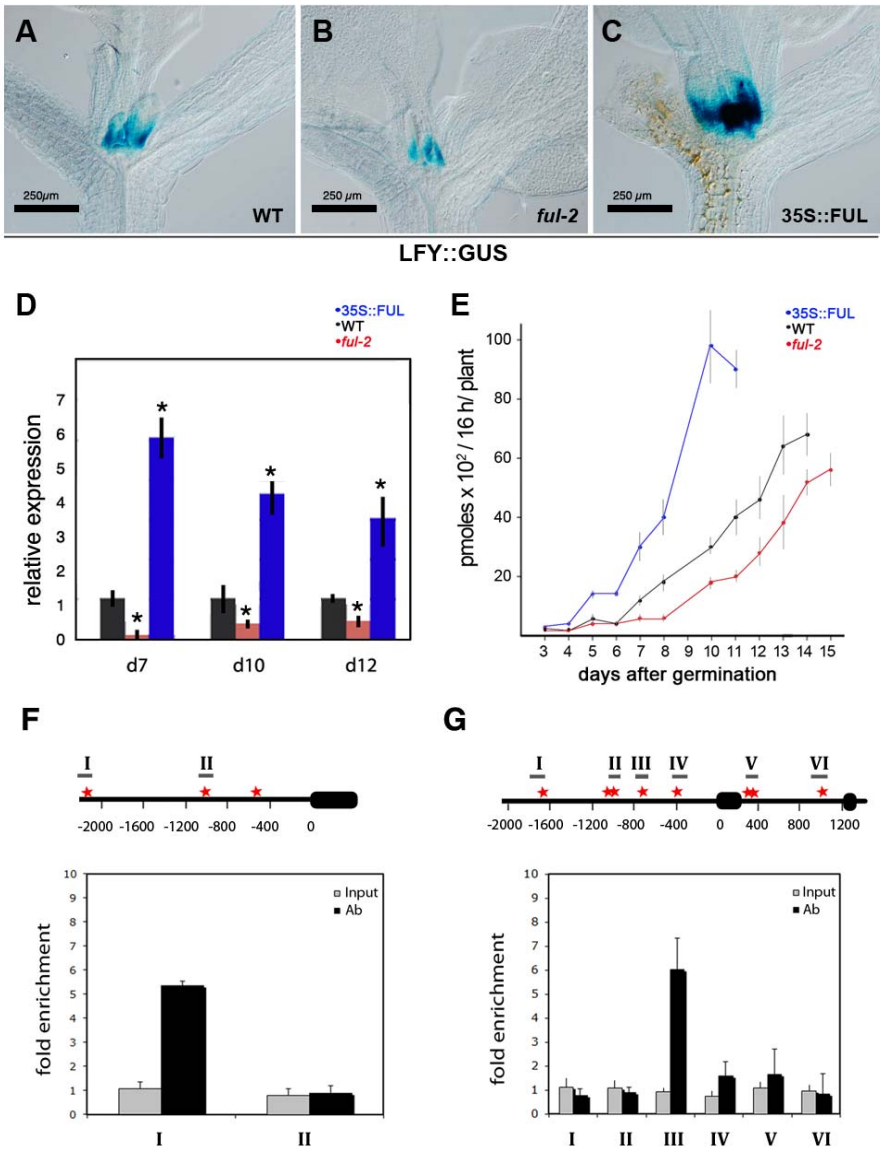

Figure 2. FUL regulates key genes in the floral transition process binding directly to SOC1 and LFY promoters.

A-C. Histochemical detection of LFY::GUS activity in the apices of 6 day old wild type (A), *ful-2* (B) or 35S::FUL (C) plants. Scale bars, 250 µm. D. Relative expression of LFY analyzed by qRT-PCR in WT, *ful-2* and 35S::FUL plants at days 7, 10 and 12 after germination. The error bars depict the s.e. based on two biological replicates. Asterisk (*) indicates significantly different ($P < 0.05$) from WT control according to a t-test. E. Quantification of LFY:GUS activity in WT, ful-2 and 35S::FUL backgrounds. Plants were grown on plates under long days (LD). At each time point, GUS activity was measured in

at least twelve individual apices, and the means ± s.e are given. F. (Top) Schematic diagram of the *LFY* upstream promoter region. First exon is represented by a black box, while the upstream genomic region is represented by a black line. The red stars indicate the sites containing either single mismatch or perfect match with the consensus binding sequence (CArG box) of MADS-domain proteins. Amplicons spanning these sites used in the ChIP analyses are represented by grey lines and marked by roman numbers. (Bottom) ChIP enrichment tests showing the binding of FUL-GFP to the LFY-I region. Bars represent the ratio of amplified DNA (35S::FUL:GFP/35S::FUL) in the starting genomic DNA (input) or in the immunoprecipitated DNA with the GFP antibody (Ab). G. (Top) Schematic diagram of the *SOC1* genomic region, including upstream promoter, exons 1 and 2 and the first intron. Exons are represented by black boxes, upstream genomic region and intron by a black line. The red stars mark CArG boxes. Amplicons spanning these sites used in the ChIP analyses are represented by grey lines and marked by roman numbers. (Bottom) ChIP enrichment tests showing the binding of FUL-GFP to the SOC1-III region. Bars represent the ratio of amplified DNA (35S::FUL:GFP/35S::FUL) in the starting genomic DNA (input) or in the immunoprecipitated DNA with the GFP antibody (Ab).

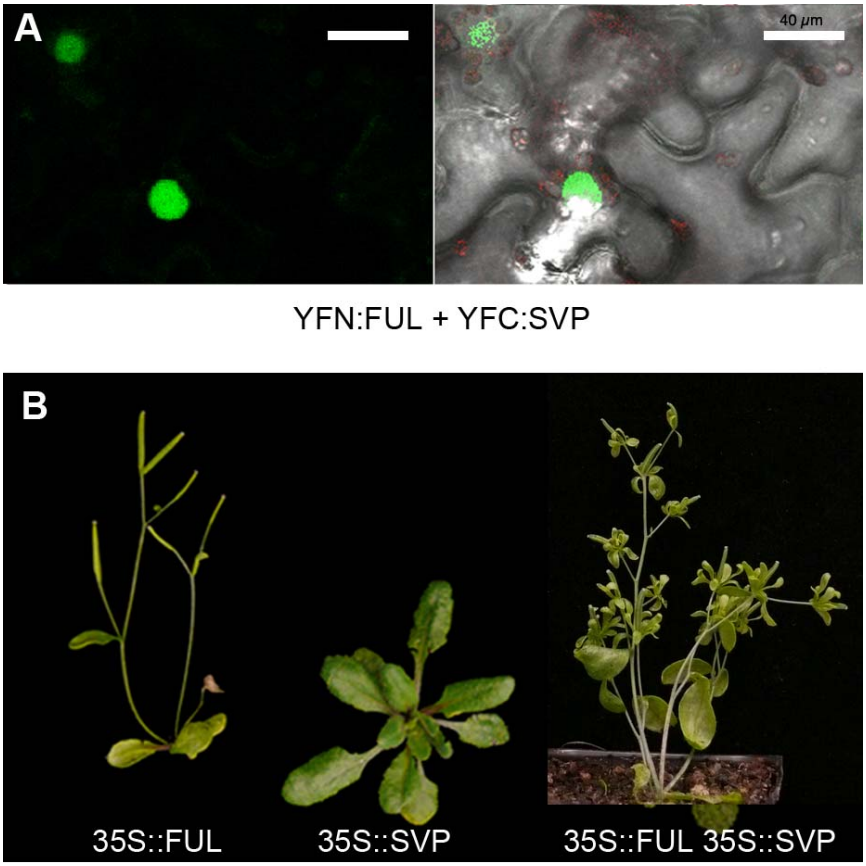

Figure 3. Interaction of FUL with SVP

A. BiFC experiments in tobacco leaf cells between transiently expressed FUL and SOC1 fusions to the C- and N-terminal fragments of YFP, respectively. Left panel shows YFP reconstituted fluorescence (green) and the right panel is an overlay with a bright field image of the same sector where chlorophyll shows in red. Negative controls for BiFC experiments are shown on Supp. Fig S3. Scale bars: 40 µm. B. Phenotypes of the 35S::FUL, 35S::SVP and 35S::FUL 35S::SVP double overexpression lines. FUL overexpression reverts the late flowering phenotype of 35S::SVP, although inflorescence development is partially restored respect to the 35S::FUL plants.

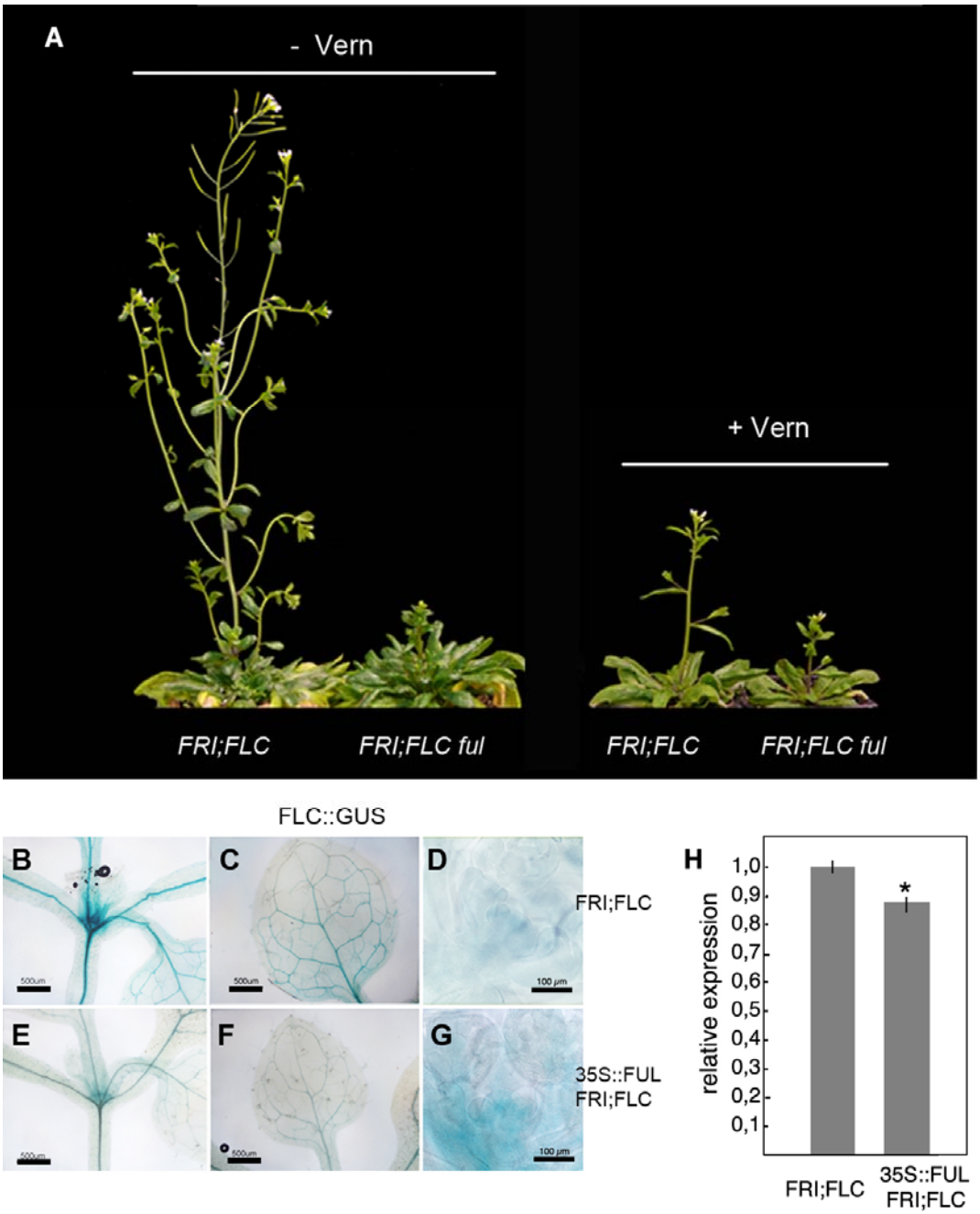

Figure 4. FUL overexpression suppresses the effects of high levels of *FLC*.

A. Vernalization response of FRI;FLC and FRI;FLC *ful-2* in LD. The *ful-2* mutation greatly enhances the late flowering phenotype of FRI;FLC unvernalized plants (left), while a vernalization treatment causes both genotypes to flower similarly earlier (right). B-G. Histochemical detection of FLC::GUS activity in FRI;FLC (B-D) and FRI;FLC

35S::FUL (E-G) plants. Apices of 10 day-old plants are compared in B,E; first rosette leaf in C, F; and inflorescence apices of plants at bolting in D, G. All plants were heterozygous for the FLC::GUS reporter and for the wildtype dominant alleles of *FRI* or *FLC*. 35S::FUL in E-G was also heterozygous. Scale bars: 500 µm (B,C,E,F) or 100 µm (D,G). H. Relative expression of FLC analyzed by qRT-PCR in FRI;FLC and FRI;FLC 35S::FUL plants 10 days after germination. The error bars depict the s.e. based on two biological replicates. Asterisk (*) indicates significantly different ($P < 0.05$) from WT control according to a t-test.

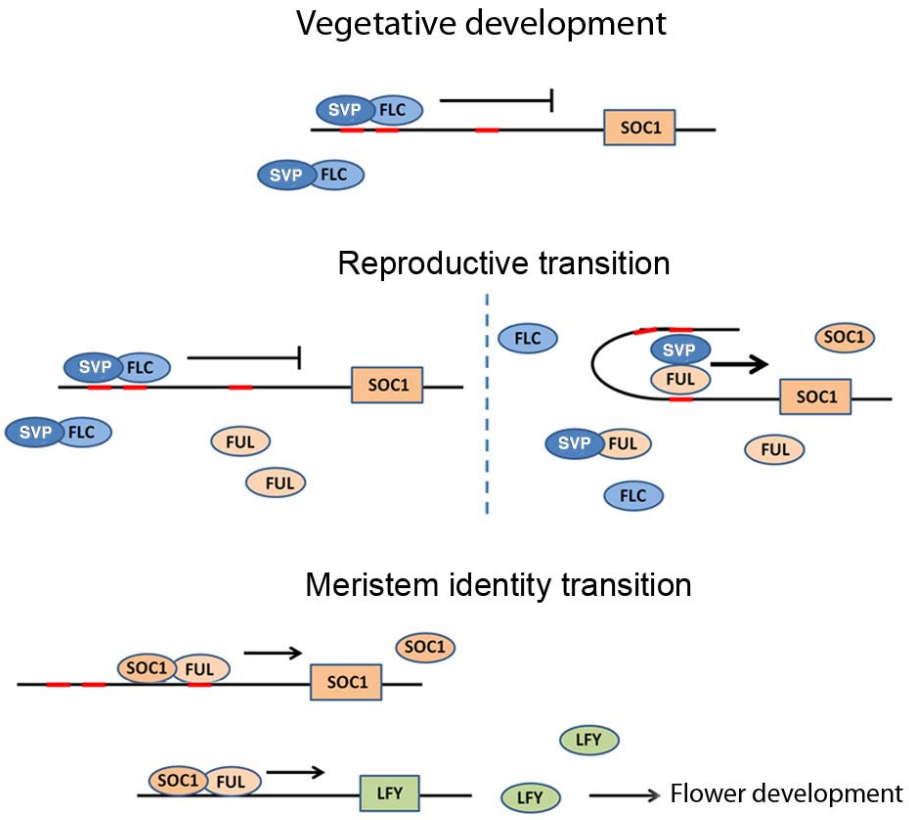

Figure 5. A proposed mechanistic model for the role of FUL during floral transition through interaction with SVP and SOC1 factors.

During vegetative growth FLC and SVP repress the expression of *SOC1* and other flowering promoting factors. Upon FUL accumulation, likely mediated by the age SPL-dependent pathway, FUL-SVP dimerization occurs. The FUL-SVP dimer could compete with the FLC-SVP dimer for binding sites in the *SOC1* promoter and/or directly interfering with the FLC-SVP dimer formation. Lower repressive activity of the FLC-SVP dimer on *SOC1* or even direct activation of *SOC1* by FUL-SVP would lead to SOC1 accumulation, the dimerization of FUL-SOC1 and the activation of both *SOC1* and *LFY* promoters, thus triggering flower initiation.