Development 139, 4072-4082 (2012) doi:10.1242/dev.080879

Spatial control of flowering by DELLA proteins in *Arabidopsis thaliana*

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SUMMARY

The transition from vegetative to reproductive development is a central event in the plant life cycle. To time the induction of flowering correctly, plants integrate environmental and endogenous signals such as photoperiod, temperature and hormonal status. The hormone gibberellic acid (GA) has long been known to regulate flowering. However, the spatial contribution of GA signaling in flowering time control is poorly understood. Here we have analyzed the effect of tissue-specific misexpression of wild-type and GA-insensitive (*della*Δ*17*) DELLA proteins on the floral transition in *Arabidopsis thaliana*. We demonstrate that under long days, GA affects the floral transition by promoting the expression of flowering time integrator genes such as *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) in leaves independently of *CONSTANS* (*CO*) and *GIGANTEA* (*GI*). In addition, GA signaling promotes flowering independently of photoperiod through the regulation of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes in both the leaves and at the shoot meristem. Our data suggest that GA regulates flowering by controlling the spatial expression of floral regulatory genes throughout the plant in a day-length-specific manner.

KEY WORDS: Gibberellic acid, Flowering, DELLA, *Arabidopsis thaliana*

INTRODUCTION

Since its discovery in the 1930s, giberellic acid (GA) has been shown to affect such diverse biological processes as seed germination, root development, cell elongation, flower development and flowering time (Davies, 2004). However, only recently have we begun to understand the molecular mechanisms that underlie GA signaling. GA is perceived by its receptor, GID1, which undergoes conformational changes after binding to bioactive GA. These changes facilitate the interaction between GID1 and DELLA proteins, which ultimately results in their degradation (Fu et al., 2004; Griffiths et al., 2006; Willige et al., 2007; Murase et al., 2008). The DELLA proteins have been named after a conserved motif of five amino acids in their N-terminal region (Peng et al., 1997; Silverstone et al., 1998; Dill et al., 2001), which were later shown to be required for interaction with GID1 (Griffiths et al., 2006; Willige et al., 2007; Murase et al., 2008). Deletion of the DELLA motif confers dwarfism and dark green color, similar to mutants with impaired GA biosynthesis, such as *ga1-3*. However, in contrast to *ga1-3*, deletion of the DELLA domain cannot be fully rescued by exogenous GA (Koornneef and van der Veen, 1980; Koornneef et al., 1985; Peng et al., 1997).

The *Arabidopsis thaliana* genome contains five DELLA genes, *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR OF ga1-3* (*RGA*), *RGA-LIKE1* (*RGL1*), *RGL2* and *RGL3*, that exhibit partial functional redundancy (Dill and Sun, 2001; Lee et al., 2002; Bolle, 2004; Gallego-Bartolome et al., 2010). Gene expression analysis has demonstrated that hundreds of genes are differentially expressed in response to GA and that this response is DELLA-dependent (Ogawa et al., 2003; Willige et al., 2007). However, DELLA proteins exert their function mainly by regulating transcription factor activity through protein-protein interactions (Daviere et al., 2008; de Lucas et al., 2008; Feng et al., 2008).

The role of GA in regulating flowering was first studied by the application of GA to plants (Lang, 1957; Langridge, 1957). Only later, after the isolation of GA biosynthesis and signaling mutants, such as *ga1-3*, could the GA-mediated control of flowering be investigated in detail (Koornneef and van der Veen, 1980; Sun et al., 1992; Wilson et al., 1992). *ga1-3* mutants completely failed to flower when grown under short-day (SD) conditions, whereas flowering was only moderately delayed under long-day (LD) conditions (Wilson et al., 1992), suggesting that GA was not required to induce flowering under inductive photoperiod. However, more recent analyses strongly indicate that GA contributes to the regulation of flowering time in *A. thaliana* in response to LD conditions after all (Griffiths et al., 2006; Willige et al., 2007; Hisamatsu and King, 2008; Osnato et al., 2012; Porri et al., 2012).

The role of *FLOWERING LOCUS T* (*FT*) in mediating flowering in response to inductive photoperiod has well been documented. It is now widely accepted that the FT protein acts as a florigen and conveys the information to induce flowering from the leaves to the shoot meristem (Wigge et al., 2005; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007; Tamaki et al., 2007; Liu et al., 2012). At the shoot meristem, FT interacts with 14-3-3 proteins and the bZIP transcription factor FD to form a heterotrimeric complex that is thought to bind to the regulatory regions of target genes to trigger the transition to the reproductive phase (Abe et al., 2005; Wigge et al., 2005; Taoka et al., 2011).

Besides FT, the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors have been shown to regulate flowering (Cardon et al., 1997; Wang et al., 2009; Yamaguchi et al., 2009). The *A. thaliana* genome contains 17 *SPL*-like genes, 11 of which are targets of microRNA156 (miR156) (Rhoades et al., 2002; Guo et al., 2008). The levels of mature

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Accepted 7 August 2012

miR156 decrease as a plant ages. As a consequence, *SPL* transcripts become more abundant, which ultimately induces flowering (Wang et al., 2009). The regulation of flowering by *SPLs* is in part due to the induction of miR172 (Wu et al., 2009). miR172 targets mRNAs of *APETALA2*-like (*AP2*-like) genes, which regulate flowering by directly binding to and repressing genes such as *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) (Aukerman and Sakai, 2003; Schmid et al., 2003; Chen, 2004; Schwab et al., 2005; Mathieu et al., 2009; Yant et al., 2010).

In contrast to this detailed picture of the regulation of flowering by photoperiod and age, little is known about how the floral transition is regulated by GA. To address this question we carried out a comprehensive analysis of the regulation of flowering by DELLA proteins under both SD and LD conditions. Our results indicate that under LD conditions the DELLA proteins regulate the expression of flowering time genes in leaves and at the shoot meristem. By contrast, the effects of DELLA proteins on flowering under SD conditions seem to be limited to the shoot meristem.

MATERIALS AND METHODS

Plant material

Wild-type plants used in this work are of the Columbia (Col-0) and Landsberg erecta (L*er*) accessions. The mutants *ga1-3*, *rga-24*, *gai-t6*, *rga-t2*, *rgl1-1*, *rgl2-1*, *gai-1* and *sly1-10* are in L*er* background and have been described (Koornneef et al., 1985; Sun et al., 1992; Peng and Harberd, 1993; Peng et al., 1997; Silverstone et al., 1998; Lee et al., 2002; McGinnis et al., 2003; Achard et al., 2007). The triple *gid1a-c* mutant, *ft-10*, *tsf-1*, *pFT:GUS* and *p35S:MIM172* are in Col-0 background (Takada and Goto, 2003; Michaels et al., 2005; Yoo et al., 2005; Willige et al., 2007; Todesco et al., 2010). Genotypes were confirmed by PCR using published oligonucleotides (supplementary material Table S1).

Growth conditions and plant transformation

All plants were grown in chambers in controlled photoperiod at 16°C or 23°C, 65% humidity and a mixture of Cool White and Gro-Lux Wide Spectrum fluorescent lights, with a fluence rate of 125 to 175 μmol m^{-2} s^{-1}. LD conditions are defined as 16 hours light/8 hours dark and SD conditions as 8 hours light/16 hours dark.

Plant transformation was carried out as previously described (Clough and Bent, 1998). Transgenic T1 plants were raised on soil or MS medium supplemented with 0.1% glufosinate (BASTA) or 50 μg/ml kanamycin, respectively, after stratification for 4 days at 4°C in darkness. For germination of *gid1a-1 gid1b-1 gid1c-2* triple mutant, the seed coat was manually removed. *ga1-3* plants were germinated by treatment with 50 μM GA_3 in 0.1% agarose. GA_3 stock solutions were prepared in pure ethanol and working solutions containing 0.01% (v/v) Tween-20 (Sigma-Aldrich) were prepared in distilled water. After 3 days of incubation in darkness at 4°C, the seeds were washed at least ten times with distilled water to remove excess GA_3. Treatment of plants was performed by spraying with 50 μM GA_3.

Molecular cloning

All nucleotides and constructs used in this work are listed in supplementary material Tables S1 and S2. All constructs were confirmed by Sanger sequencing. For misexpression of *GA2ox8*, the open reading frame (ORF) was amplified from cDNA using Phusion High-Fidelity DNA Polymerase (New England Biolabs) and oligonucleotides G-31688 and G-31689. The fragment was purified and ligated into the Gateway-compatible vector pJLSmart to create pVG-412, and subsequently used for recombination into pGREEN-IIS destination vector (Mathieu et al., 2007) containing the *SUC2* promoter to create the construct pVG-417.

The complete ORFs of the five DELLA genes (*RGA*, *GAI*, *RGL1*, *RGL2*, *RGL3*) were amplified directly from *A. thaliana* genomic DNA with specific oligonucleotides. The amplified PCR products were cloned into Gateway-compatible vector pJLSmart using T4 DNA ligase (Fermentas) to create the entry vectors pVG-156, pVG-157, pVG-158, pVG-159 and pVG-160. The 17-amino-acid deletion in RGL1, RGL2 and RGL3 to create GA-insensitive DELLA was created by overlapping PCR. First, the two halves of the ORFs were amplified separately using the oligonucleotides G-25736/G-25731 and G-25732/G-25735 (*RGL1*), G-25739/G-25737 and G-25738/G-25740 (*RGL2*), and G-25743/G-25746 and G-25744/G-25745 (*RGL3*). The two fragments were fused in a second PCR using forward and reverse oligonucleotides G-25733/G-25734 (*RGL1*), G-25741/G-25742 (*RGL2*) and G-25747/G-25748 (*RGL3*). *GAI* and *RGA* deletions were amplified directly from genomic DNA of *rgaΔ17* and *gai-1*. The amplified fragments were ligated into pJLSmart using T4 DNA ligase to create the entry vectors pVG-104, pVG-105, pVG-118, pVG-119 and pVG-120. Expression vectors suitable for plant transformation were created by recombination into pGREEN-IIS plant binary destination vectors (Mathieu et al., 2007) containing the *SUC2*, *FD* and *CLV3* promoters, respectively (supplementary material Table S2).

Expression analysis

Total RNA was extracted using either the RNeasy Kit (Qiagen) or TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. At least 600 ng total RNA was treated with DNase I and used for cDNA synthesis using oligo (dT) and the RevertAid First Strand cDNA Synthesis Kit (Fermentas). Quantitative real-time PCR (qPCR) was performed using the Platinum SYBR Green qPCR Supermix-UDG (Invitrogen) and specific oligonucleotides (supplementary material Table S1) on an MJR Opticon Continuous Fluorescence Detection System. Expression was normalized against *A. thaliana β-TUBULIN* or *ACTIN 2*, and expression differences were calculated using the ΔΔCT method. For each sample, material from a minimum of 15 seedlings was pooled per replicate and at least two biological and two technical replicates were used for the analysis. A minimum of 40 apical meristems was dissected for each biological replicate for RNA extraction.

Small RNA northern blots were performed using 2 μg total RNA resolved on a 17% polyacrylamide gel in denaturing conditions (7 M urea). The RNA was transferred to HyBond-N^+ membranes and hybridized with digoxigenin-labeled oligonucleotides (supplementary material Table S1). Probe labeling was carried out using the DIG Oligonucleotide 3′-End Labeling Kit, 2nd generation (Roche). microRNA quantitative PCR was performed as previously described (Chen et al., 2005).

GUS staining was performed as described (Blazquez et al., 1997) and pictures obtained using the Leica MZ FLIII microscope. Transcriptome analysis was performed using publicly available data downloaded from AtGenExpress (Schmid et al., 2005).

RESULTS

DELLA proteins repress flowering under LD photoperiod

Genetic analyses have shown that *DELLA* genes have partially overlapping function in controlling various aspects of plant development (Dill and Sun, 2001; Lee et al., 2002; Cheng et al., 2004; de Lucas et al., 2008; Feng et al., 2008); however, their relative contribution to the regulation of flowering under inductive photoperiod is still unclear. To address this question we first analyzed the effect of *della* gain- and loss-of-function mutations on flowering time. We observed that under LD conditions, the loss-of-function mutants *gai-t6* and *rga-24* flowered early with 9.9±0.8 and 9.9±0.5 leaves, respectively, compared with wild type, which produced 11.3±0.6 leaves ($P<0.00001$, unpaired *t*-test; Table 1). However, these single mutants still flowered later than wild-type plants treated with 50 μM GA_3, which produced 7.8±0.9 leaves. In agreement with the notion of functional redundancy among the *DELLA* genes, early flowering was enhanced in a *gai-t6 rga-24* double mutant and a *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* pentuple mutant, which produced 8.6±0.7 and 7.6±0.9 leaves, respectively ($P<0.00001$; Table 1 and supplementary material Fig. S1A,B). By contrast, the semi-dominant GA-insensitive *gai-1* allele flowered considerably late with about 16.8±1.0 leaves ($P<0.00001$; Table 1

Table 1. Flowering time of plants used in this study

Genotype	RL	CL	Total	Deviation	Range	*n*
Experiment 1						
Ler-1	8.3	3.0	11.3	0.6	10-12	17
ga1-3	10.9	2.9	13.7	0.8	13-15	7
gai-t6	7.0	2.9	9.9	0.8	8-11	25
rga-24	6.9	3.0	9.9	0.5	9-11	25
gai-t6 rga-24	5.7	2.9	8.6	0.7	7-10	25
ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1	4.5	3.1	7.6	0.9	6-9	20
Ler-1 (GA_3 50 μM)	5.2	2.6	7.8	0.9	7-9	10
gai-1	14.5	2.3	16.8	1.0	16-19	16
Experiment 2						
Col-0	11.1	3.0	14.1	1.0	12-16	22
Col-0 (GA_3 50 μM)	8.1	3.4	11.5	1.1	8-13	20
gid1b-1 gid1c-2	13.5	2.8	16.3	1.1	14-18	19
gid1a-1 gid1b-1 gid1c-2	n.a.	n.a.	n.a.	n.a.	n.a.	8
Experiment 3						
Col-0	10.1	2.7	12.8	1.8	9-16	29
pSUC2:RGA (T1)	15.1	3.2	18.3	3.3	10-23	26
pSUC2:rgaΔ17 (T1)	15.6	4.4	20.0	3.4	14-28	18
pSUC2:GAI (T1)	14.2	3.2	17.4	3.3	12-23	26
pSUC2:gaiΔ17 (T1)	12.3	2.8	15.1	1.8	13-18	25
pSUC2:RGL1 (T1)	15.1	4.0	19.1	2.9	12-23	27
pSUC2:rgl1Δ17 (T1)	14.5	3.8	18.3	3.2	12-23	18
pSUC2:RGL2 (T1)	14.1	3.8	17.9	3.9	12-23	28
pSUC2:rgl2Δ17 (T1)	17.2	5.1	22.3	1.8	12-33	30
pSUC2:RGL3 (T1)	15.0	3.9	18.9	4.0	13-23	33
pSUC2:rgl3Δ17 (T1)	18.1	4.2	22.3	4.5	14-30	27
Experiment 4						
Col-0	10.7	2.8	13.5	1.3	11-16	24
pFD:RGA (T1)	11.0	1.9	12.9	1.9	9-19	52
pFD:rgaΔ17 (T1)	29.4	0.3	29.7	8.0	14-52	*21*
pFD:GAI (T1)	11.6	2.0	13.6	2.5	9-20	*51*
pFD:gaiΔ17 (T1)	25.0	0.4	25.4	10.4	13-52	*49*
pFD:RGL1 (T1)	11.2	1.8	13.0	2.4	9-22	*51*
pFD:rgl1Δ17 (T1)	20.3	0.8	21.1	4.6	10-35	*55*
pFD:RGL2 (T1)	11.1	2.0	13.1	1.8	9-17	*37*
pFD:rgl2Δ17 (T1)	21.0	0.5	21.5	8.5	7-40	*46*
pFD:RGL3 (T1)	10.9	2.2	13.1	2.6	9-22	*39*
pFD:rgl3Δ17 (T1)	11.7	1.7	13.4	2.2	10-19	*26*
pCLV3:RGA (T1)	10.0	2.8	12.8	1.4	10-15	35
pCLV3:rgaΔ17 (T1)	19.7	8.1	27.8	10.1	11-46	44
pCLV3:GAI (T1)	11.1	2.8	13.9	1.5	9-17	48
pCLV3:gaiΔ17 (T1)	17.5	5.6	23.1	8.0	12-42	45
pCLV3:RGL1 (T1)	11.3	2.8	14.1	1.7	11-18	42
pCLV3:rgl1Δ17 (T1)	17.6	5.6	23.2	7.4	13-41	48
pCLV3:RGL2 (T1)	10.9	2.5	13.4	0.9	12-15	18
pCLV3:rgl2Δ17 (T1)	17.5	5.5	23.0	7.5	13-45	54
pCLV3:RGL3 (T1)	11.6	2.6	14.2	1.6	11-18	52
pCLV3:rgl3Δ17 (T1)	11.4	2.9	14.3	2.1	11-20	50
Experiment 5						
p35S:MIM172 (GA_3 50 μM; 23°C)	13.7	6.9	20.5	2.1	15-25	31
p35S:empty (GA_3 50 μM; 23°C)	8.4	2.9	11.3	0.9	9-13	33
p35S:MIM172 (mock; 23°C)	23.7	4.0	27.7	1.9	23-31	32
p35S:empty (mock; 23°C)	10.8	2.8	13.6	1.4	10-16	36
p35S:MIM172 (GA_3 50 μM; 16°C)	26.8	8.7	35.5	3.1	31-40	22
p35S:empty (GA_3 50 μM; 16°C)	13.0	7.3	20.3	2.3	17-24	24
p35S:MIM172 (mock; 16°C)	45.1	9.4	54.6	3.2	50-60	9
p35S:empty (mock; 16°C)	20.4	4.8	25.2	1.9	22-29	24

RL, rosette leaves; CL, cauline leaves; n.a., plants did not flower in the course of the experiment.

and supplementary material Fig. S1A,B). Similarly, and in agreement with a previous report (Willige et al., 2007), the *gid1a-c* triple mutant did not flower at all under our LD conditions. Presumably due to high functional redundancy among the GID1 receptors, flowering time was almost, but not completely, recovered ($P<0.00001$) in the *gid1b-1 gid1c-2* double mutant, which flowered with 16.3±1.1 leaves compared with 14.1±1.0 in wild-type plants (Table 1 and supplementary material Fig. S1A,C). Together, our results confirm that DELLA proteins act as repressors of flowering and that their GID1-mediated, GA-dependent degradation contributes to induction of flowering under LD conditions.

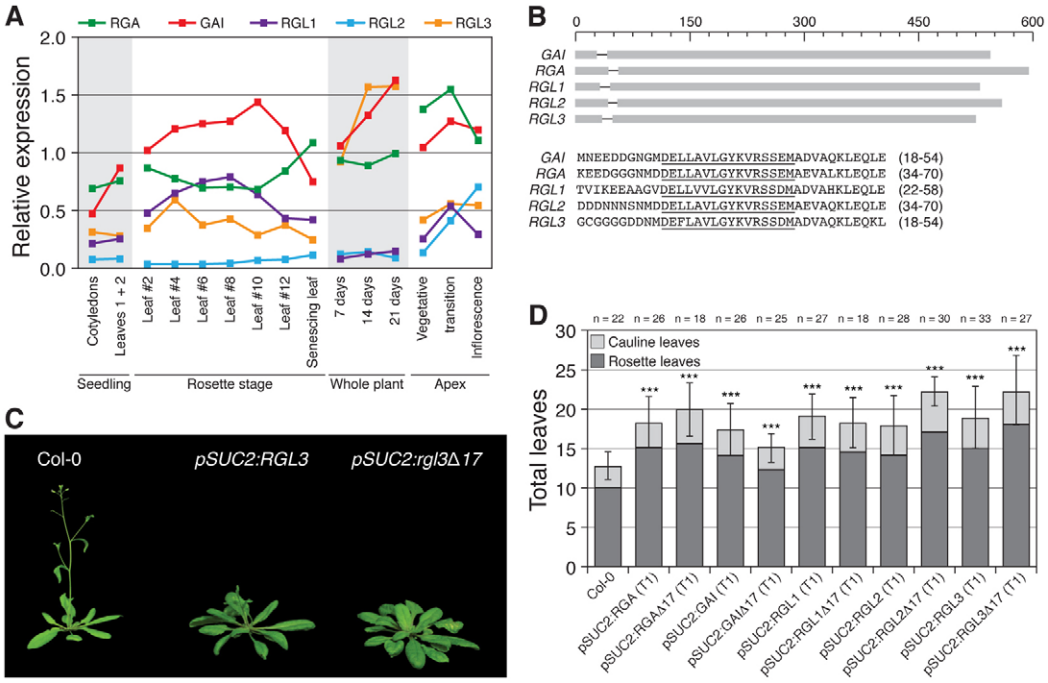

Fig. 1. Accumulation of DELLA proteins in vasculature delays flowering under LD conditions. (**A**) *DELLA* genes are expressed in *A. thaliana* leaves and at the shoot meristem throughout development [data from AtGenExpress atlas (Schmid et al., 2005)]. (**B**) GA-insensitive DELLA proteins were created by deleting 17 amino acids at the N-terminal region, corresponding to the deletion originally identified in the dominant *gai-1* allele. Underlined amino acids correspond to deleted residues in *della*Δ*17* mutants. (**C**) Expression of *RGL3* and *rgl3*Δ*17* in phloem companion cells delays flowering in LD conditions at 23°C. Shown are 30-day-old plants. (**D**) Flowering time of *pSUC2:DELLA* and *pSUC2:della*Δ*17* (T1) lines under LD conditions at 23°C. Transgenic plants (C,D) are in Col-0 background. Error bars indicate the standard deviation (s.d.) of total leaf number; n indicates the number of T1 plants analyzed. Significance was calculated using the unpaired Student's *t*-test: **P*<0.05, ***P*<0.01, ****P*<0.001.

DELLA proteins regulate flowering under LD conditions in the leaf vasculature

The control of flowering can be spatially divided into processes that occur in leaves, such as perception of photoperiod, and those that occur at the shoot meristem (Kobayashi and Weigel, 2007). The analysis of publicly available microarrays (Schmid et al., 2005) revealed a dynamic regulation of the five *DELLA* genes in different plant tissues, including the leaves and the shoot meristem (Fig. 1A), indicating that the DELLA proteins could affect flowering in either of those two tissues. To investigate their spatial contribution to the regulation of flowering we employed tissue-specific expression of wild-type (*GAI*, *RGA*, *RGL1*, *RGL2* and *RGL3*) and GA-insensitive versions (*gai*Δ*17*, *rga*Δ*17*, *rgl1*Δ*17*, *rgl2*Δ*17*, *rgl3*Δ*17*) of the *DELLA* cDNAs. The latter were created by introducing a 17-amino-acid deletion into the *DELLA* cDNAs, analogous to the one originally identified in the *gai-1* mutant (Fig. 1B) (Peng et al., 1997).

Transgenic T1 plants expressing *della*Δ*17* from the phloem companion cell (PCC)-specific *SUC2* promoter (Stadler and Sauer, 1996) exhibited the dark green color typically observed in GA-deficient mutants. We found that *pSUC2:rga*Δ*17*, *pSUC2:rgl1*Δ*17*, *pSUC2:rgl2*Δ*17* and *pSUC2:rgl3*Δ*17* delayed flowering more strongly than *pSUC2:GAI*Δ*17*, although late-flowering individuals were occasionally observed among the latter ($P<0.00001$; Fig. 1C,D; supplementary material Figs S2, S3). Furthermore, transgenic plants expressing full-length *DELLA* ORFs also displayed an intermediate dark green color and late-flowering phenotype ($P<0.00001$; Fig. 1C,D). In particular, *pSUC2:RGA* and *pSUC2:RGL1* flowered almost at the same time as *pSUC2:rga*Δ*17* and *pSUC2:rgl1*Δ*17* (Fig. 1C,D; supplementary material Figs S2, S3).

To ensure that also the endogenous DELLA proteins regulate flowering in the leaf PCCs, we expressed the GA catabolic enzyme GA2ox8 under control of the *SUC2* promoter (Stadler and Sauer, 1996; Olszewski et al., 2002; Rieu et al., 2008). The reasoning for this is that it would reduce the pool of bioactive GA, resulting in higher DELLA protein levels specifically in the PCCs. Indeed, transgenic T1 plants expressing *pSUC2:GA2ox8* displayed a dark green color and flowered later (14.1±1.5 rosette leaves) than control plants (10.4±0.8; $P<0.00001$; supplementary material Fig. S4). Taken together, these observations suggest that the DELLA proteins regulate flowering in response to GA under LD conditions in the leaf PCCs.

CO- and GI-independent regulation of *FT* by DELLA proteins in the vasculature

The *FT* gene has been shown to be specifically expressed in leaf vasculature in response to inductive photoperiod (Kobayashi and Weigel, 2007; Turck et al., 2008). To test if the late flowering observed in the *pSUC2:della*Δ*17* lines (Fig. 1C,D; supplementary material Figs S2, S3) was due to a reduction in *FT* expression, we introduced *pSUC2:rgl3*Δ*17* into a *pFT:GUS* reporter line (Takada and Goto, 2003). T2 plants derived from seven independent T1 lines that varied in their flowering time from wild-type-like to late flowering were analyzed and a clear anti-correlation between flowering time and expression of the endogenous *FT* gene was observed (Fig. 2A). *FT* expression was strongly reduced in late-flowering *pSUC2:rgl3*Δ*17* T2 lines, whereas lines flowering at the same time as the control plants had almost wild-type-like *FT* expression (Fig. 2A). Similarly, the

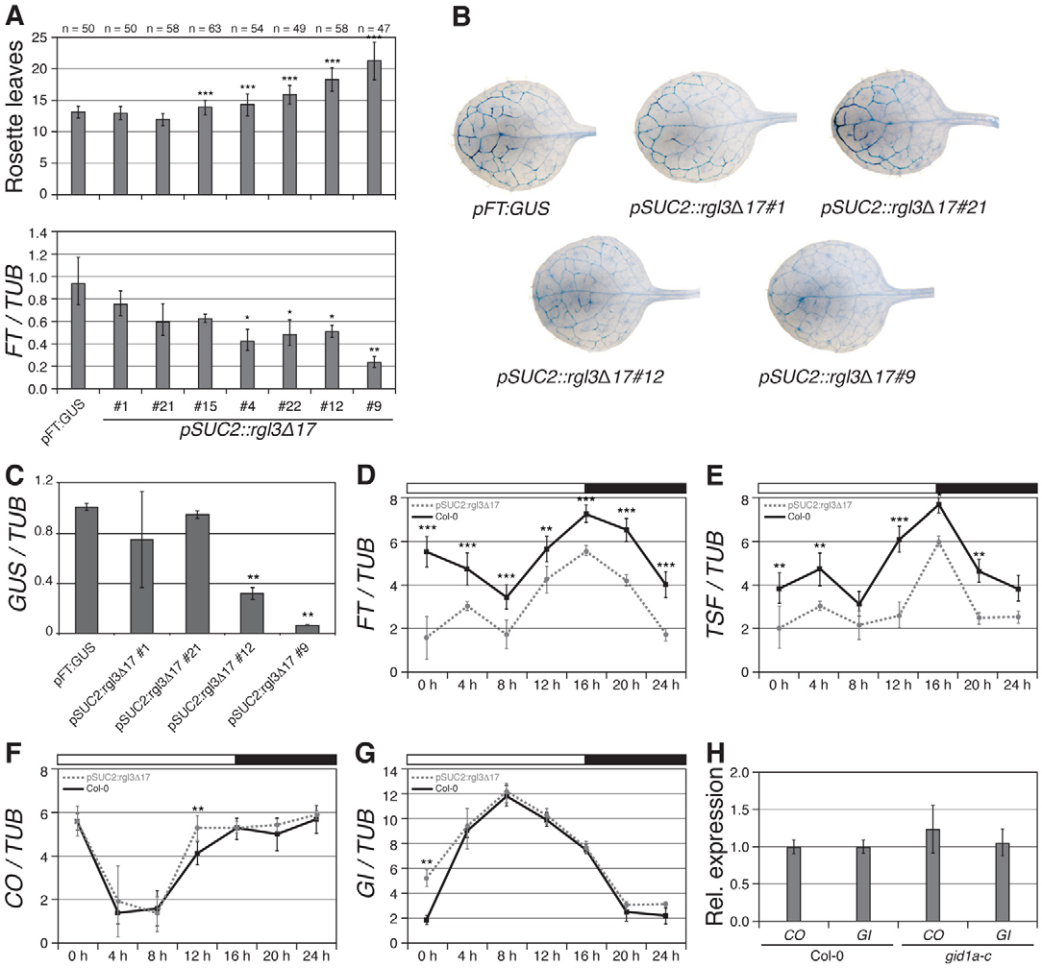

Fig. 2. DELLA proteins regulate *FT* and *TSF* expression under LD conditions. (**A-C**) Repression of *FT* by RGL3 was confirmed in *pSUC2:rgl3Δ17 pFT:GUS* (T2) plants by (A, bottom) quantitative RT-PCR of *FT*, (B) GUS staining, and (C) *GUS* quantitative RT-PCR. GUS staining represents the third leaf of 10-day-old transgenic plants at *zeitgeber* (ZT) 16 grown under LD conditions at 23°C. (**D-G**) Diurnal expression profile of *FT*, *TSF, CO* and *GI* in *pSUC2:rgl3Δ17* (T2). Plants were grown under SD conditions for 30 days and shifted to LD conditions for 5 days to induce flowering. Transgenic plants (A-G) are in Col-0 background. The aerial part of the plants was collected every 4 hours for 24 hours. Bars on the top indicate day (white) and night (black) phases. (**H**) Expression of *CO* and *GI* in 3-week-old triple *gid1a-c* mutant plants growing at 23°C under LD conditions. The error bars indicate the s.d. of rosette leaf number (A, top) and quantitative expression of at least two biological and two technical replicates each (A, bottom; C-H); n indicates the number of plants analyzed. Significance was calculated using the unpaired Student's *t*-test: **P*<0.05, ***P*<0.01, ****P*<0.001.

pFT:GUS reporter showed a much decreased expression and staining in the vasculature of late-flowering plants (Fig. 2B,C).

As *FT*, as well as its closest paralog *TWIN SISTER OF FT* (*TSF*), are under the control of the circadian clock, we analyzed the diurnal expression of these two genes in the late-flowering *pSUC2:rgl3Δ17* line. Quantitative analysis showed that both *FT* and *TSF* maintained their diurnal expression but at a reduced level (Fig. 2D,E). By contrast, expression of *GIGANTEA* (*GI*) and *CONSTANS* (*CO*), which act upstream of *FT*, was unchanged in *pSUC2:rgl3Δ17* and in the strong *gid1a-c* mutant (Fig. 2F,G,H). Together these results suggest that the DELLA proteins participate in the regulation of *FT* and *TSF* expression in PCCs and contribute to their regulation under LD conditions independently of *CO* and *GI*.

Regulation of *FT* and *TSF* by GA

To confirm that *FT* and *TSF* are regulated by GA, and to ensure that the effects we had observed in the *pSUC2:rgl3Δ17* line reflected normal DELLA function, we analyzed their expression in GA biosynthesis and signaling mutants. Results obtained in the strong GA biosynthesis mutant *ga1-3* had suggested that GA does not substantially contribute to the regulation of flowering time under LD conditions (Wilson et al., 1992). Consistent with this, *FT* and *TSF* were expressed normally in *ga1-3* under LD conditions (Fig. 3A). By contrast, expression of *FT* and *TSF* was reduced approximately twofold in the partially GA-insensitive *sly1-10* mutant, which accumulates higher levels of DELLA proteins (McGinnis et al., 2003) compared with wild type (Fig. 3A). Similarly, *FT* and *TSF* expression was reduced to ~30% in the non-flowering *gid1a-c* triple mutant compared with control plants (Fig. 3B).

In agreement with GA regulating *FT* independently of the photoperiod pathway, we also observed increased levels of *FT* in a diurnal timecourse in the early-flowering *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2*-1 mutant compared with wild-type plants (Fig. 3C). Furthermore, *FT* was precociously expressed in leaves of the *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* mutant compared with L*er*-1. Expression of *FT* was comparable between the two genotypes 3

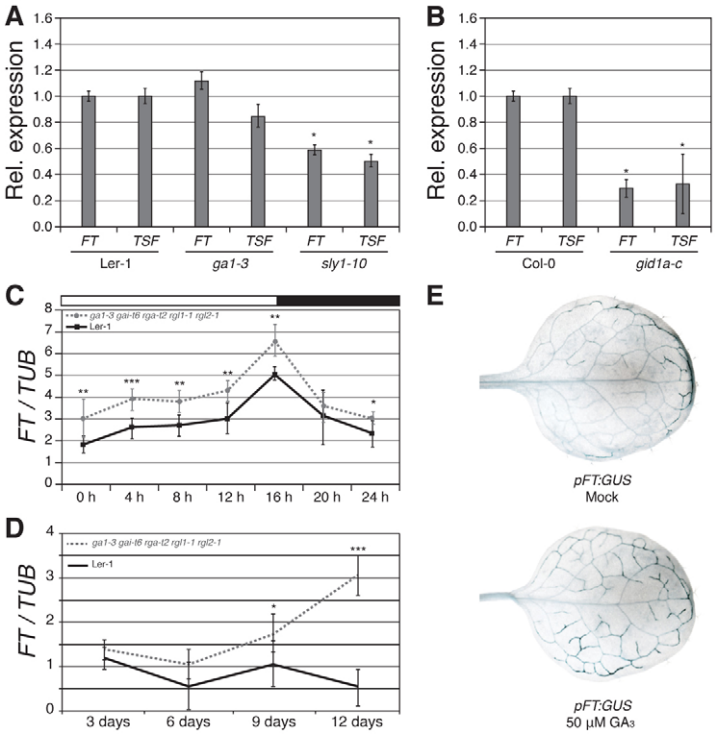

Fig. 3. GA regulates *FT* expression in the leaf vasculature. (**A**) Relative expression of *FT* and *TSF* at ZT 16 in seedlings grown for 14 days under LD conditions at 23°C. (**B**) Relative expression of *FT* and *TSF* in the triple *gid1a-c* mutant compared with wild-type plants. Plant material was collected 3 weeks after germination at ZT 16. (**C**) *FT* diurnal expression in leaves of 8- to 9-day-old *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* and Ler-1 plants grown under LD conditions at 23°C. (**D**) *FT* expression in leaves of *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* and Ler-1 plants 3, 6, 9 and 12 days after germination. Plants were grown under LD conditions at 23°C and cotyledons (day 3) and rosette leaves (days 6, 9 and 12) were harvested at ZT 15. (**E**) Increased GUS staining of *pFT:GUS* in response to exogenous GA_3. GUS staining represents the third rosette leaf of 12-day-old plants at ZT 16 grown under LD conditions at 23°C. Transgenic plants are in Col-0 background. Error bars for quantitative RT-PCR indicate s.d. of two biological and two technical replicates each. Significance was calculated using the unpaired Student's *t*-test: $*P<0.05$, $**P<0.01$, $***P<0.001$.

days after germination but gradually increased in the *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* mutant (Fig. 3D). To confirm that GA can promote *FT* expression even under LD conditions, plants containing the *pFT:GUS* reporter were treated with GA_3 or mock-treated every other day for 12 days. In contrast to mock-treated plants, in which the GUS staining was mostly restricted to the peripheral veins, GA_3-treated plants displayed a stronger and more dispersed GUS signal (Fig. 3E). This finding was corroborated by quantitative RT-PCR, which revealed a 2.5-fold increase in GUS expression in the GA_3-treated samples (supplementary material Fig. S5). Taken together, these results suggest that GA substantially promotes the expression of *FT* and *TSF* in PCCs and thus the induction of flowering even under LD conditions.

DELLA proteins repress flowering under LD conditions at the shoot meristem

Even though plants expressing *della*Δ*17* and *DELLA* cDNAs in the PCCs were clearly late flowering, these plants nevertheless flowered earlier than the triple *gid1a-c* mutant, suggesting that GA signaling in tissues other than the leaf vasculature contributes to the regulation of flowering. To investigate the contribution of DELLA proteins to flowering-time regulation at the shoot apex, we expressed the *della*Δ*17* and *DELLA* cDNAs under control of the meristem-specific *FD* (*pFD*) and the shoot stem cell niche-specific *CLAVATA3* (*pCLV3*) promoters (Fig. 4). Expression of *rga*Δ*17*, *gai*Δ*17*, *rgl1*Δ*17* and *rgl2*Δ*17* ($P<0.00001$), but not *rgl3*Δ*17* ($P>0.05$), at the shoot apex from either *pFD* or *pCLV3* delayed flowering even more strongly than observed in the *pSUC2* lines (Table 1; Fig. 4; supplementary material Figs S2, S3). In general, the delay in flowering was stronger in the *pFD:della*Δ*17* lines compared with the *CLV3* promoter lines, which is probably a consequence of the larger *FD* expression domain. By contrast, expression of the wild-type *DELLA* did not significantly affect flowering time ($P>0.05$; Table 1; Fig. 4B,C; supplementary material Figs S2, S3), suggesting that endogenous GA levels at the meristem are sufficiently high to target misexpressed DELLA proteins for degradation. Taken together, these results highlight the importance of DELLA degradation in promoting flowering at the shoot meristem downstream of the photoperiodic signal produced in leaves.

*della*Δ*17* delay flowering at the shoot meristem under SD conditions

To better understand the contribution of DELLA proteins in controlling the transition to flowering under non-inductive photoperiod, we scored flowering time in transgenic plants expressing *della*Δ*17* and wild-type *DELLA* in the PCCs (*pSUC2*) and at the shoot meristem (*pFD; pCLV3*) in SD conditions. We observed that expression of *rga*Δ*17*, *gai*Δ*17*, *rgl1*Δ*17* and *rgl2*Δ*17* at the shoot meristem caused plants to flower extremely late or not to flower at all even after 6 months of vegetative growth (supplementary material Figs S6, S7). As observed in LD conditions, expression of *rgl3*Δ*17* at the shoot meristem did not affect flowering. However, in contrast to what we had observed in LD conditions, misexpression of *della*Δ*17* and *DELLA* in the phloem companion cells just had a minor effect on flowering time under SD conditions (supplementary material Fig. S6).

DELLA proteins regulate *SPL* expression at the shoot meristem

SPL genes constitute a class of transcription factors that regulates diverse aspects of plant development at the shoot meristem, including the transition to flowering (Cardon et al., 1997; Wang et al., 2009; Jung et al., 2011; Kim et al., 2012). Interestingly, we observed a significant reduction of *SPL3*, *SPL4* and *SPL5* mRNA levels in dissected apices of LD-grown late-flowering *pFD:rgl2*Δ*17* plants compared with Col-0 (Fig. 5A). By contrast, *SPL9* and *SPL15* transcripts were downregulated only twofold, and expression of *SPL10* and *SPL11* remained nearly unchanged. Supporting the idea that *SPL3*, *SPL4*, *SPL5* and *SPL9*, but not *SPL11*, are targets of GA signaling, we observed reduced expression of these genes in the

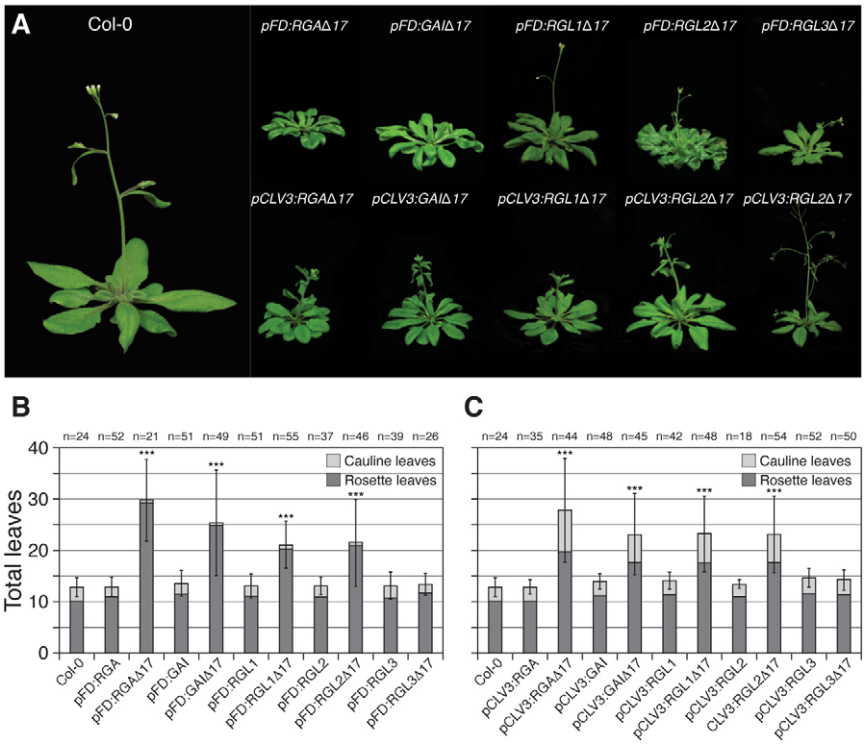

Fig. 4. Expression of *della*Δ*17* at the shoot meristem delays flowering under LD conditions. (**A**) Phenotypes and (**B**,**C**) flowering time of transgenic T1 plants expressing *della*Δ*17* and *DELLA* genes from the *FD* and *CLV3* promoters under LD conditions at 23°C. Transgenic plants are in Col-0 background. Shown are 28-day-old plants (Col-0 and *RGL3* lines) and 40-day-old plants (*GAI, RGA, RGL1, 2* lines). Error bars indicate the s.d. of total leaf number; n indicates the number of T1 plants analyzed. Significance was calculated using the unpaired Student's *t*-test: **P*<0.05, ***P*<0.01, ****P*<0.001.

gid1a-c triple mutant grown under LD conditions (Fig. 5B). In addition, *SPL3*, *SPL4* and *SPL5* were precociously expressed in dissected apices of the early-flowering *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* pentuple mutant compared with wild type (Fig. 5C,D,E). By contrast, expression of these genes remained at low levels in apices of the late-flowering *gai-1* mutant (Fig. 5C,D,E). Together these findings indicate that GA transcriptionally regulates these three important *SPL* genes at the shoot meristem.

A gene that has been shown to respond strongly to GA under SD conditions is the MADS-domain transcription factor SOC1 (Bonhomme et al., 2000; Moon et al., 2003; Jung et al., 2011). By contrast, *SOC1* expression was only moderately increased in apices of the *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* mutant compared with L*er*-1 plants (supplementary material Fig. S8). In addition, application of GA_3 in the strong photoperiod pathway mutant *ft-10 tsf-1* resulted in only very mild induction of *SOC1*. Together, these results indicate that *SOC1* is only a minor target of GA signaling at the shoot meristem under inductive photoperiod.

DELLA proteins regulate *SPL3* expression in leaves

SPL3 and FT have recently been shown to regulate each other's expression in a feedback loop in which SPL3 directly binds to and regulates *FT* in leaves, whereas FT seems to feed back onto *SPL3* expression (Jung et al., 2011; Kim et al., 2012). Interestingly, we observed elevated levels of *SPL3* in leaves of LD-grown *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* plants compared with L*er*-1 and *gai-1* mutant (Fig. 5F). This result suggests that, in addition to the shoot meristem, GA also controls *SPL3* expression in leaves.

p35S:MIM172 partially suppress acceleration of flowering in LD and SD conditions

It has recently been shown that at least one of the *MIR172* genes, *MIR172b*, is a direct target of SPL proteins (Wang et al., 2009; Wu et al., 2009; Yamaguchi et al., 2009). miR172 and its targets, a clade of six *AP2*-like transcription factors, are known regulators of flowering in both leaves and at the shoot meristem (Rhoades et al., 2002; Aukerman and Sakai, 2003; Schmid et al., 2003; Schwab et al., 2005; Mathieu et al., 2009; Yant et al., 2010). To test the possibility that the miR172/*AP2*-like module participates in the GA-mediated regulation of flowering, we analyzed the response of a late-flowering *p35S:MIM172* line, which displays artificially reduced levels of mature miR172 (Franco-Zorrilla et al., 2007; Todesco et al., 2010), to exogenous GA_3. We observed that the late flowering of *p35S:MIM172* could be overcome only partially by GA_3 treatment under LD conditions at both 16°C and 23°C (Fig. 6A,B; Table 1; supplementary material Fig. S9). At 16°C GA_3-treated control plants flowered with only 20.3±2.3 leaves, compared with 25.2±1.9 leaves produced by untreated plants. By contrast, GA_3-treated *p35S:MIM172* flowered much later with 35.5±3.1 compared with 54.6±3.2 leaves of untreated plants (Fig. 6A,B). A similar but weaker effect was observed in plants grown at 23°C (Fig. 6B; supplementary material Fig. S9A). In addition, *p35S:MIM172* also partially blocked the flower-promoting effect of GA in non-inductive SD conditions (supplementary material Fig. S9A,B). Taken together, these results suggest that GA regulates flowering, in part through the miR172/*AP2*-like module, or that the miR172/*AP2*-like genes and the GA pathway converge on the same targets.

Expression of *della*Δ*17* represses miR172

The partial suppression of the GA-mediated induction of flowering observed in the *p35S:MIM172* line suggested that *MIR172* itself could be regulated by GA. To test this possibility we analyzed miR172 levels by small RNA northern blot. Under SD conditions, we observed an increase in mature miR172 levels in the pentuple *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2*-1 relative to *ga1-3*, indicating that DELLA proteins repress *MIR172* (Fig. 6C). By contrast, and in agreement with a previous report (Jung et al., 2011), the levels of mature miR156, which is genetically upstream of MIR172, were unchanged in *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* (Fig. 6D).

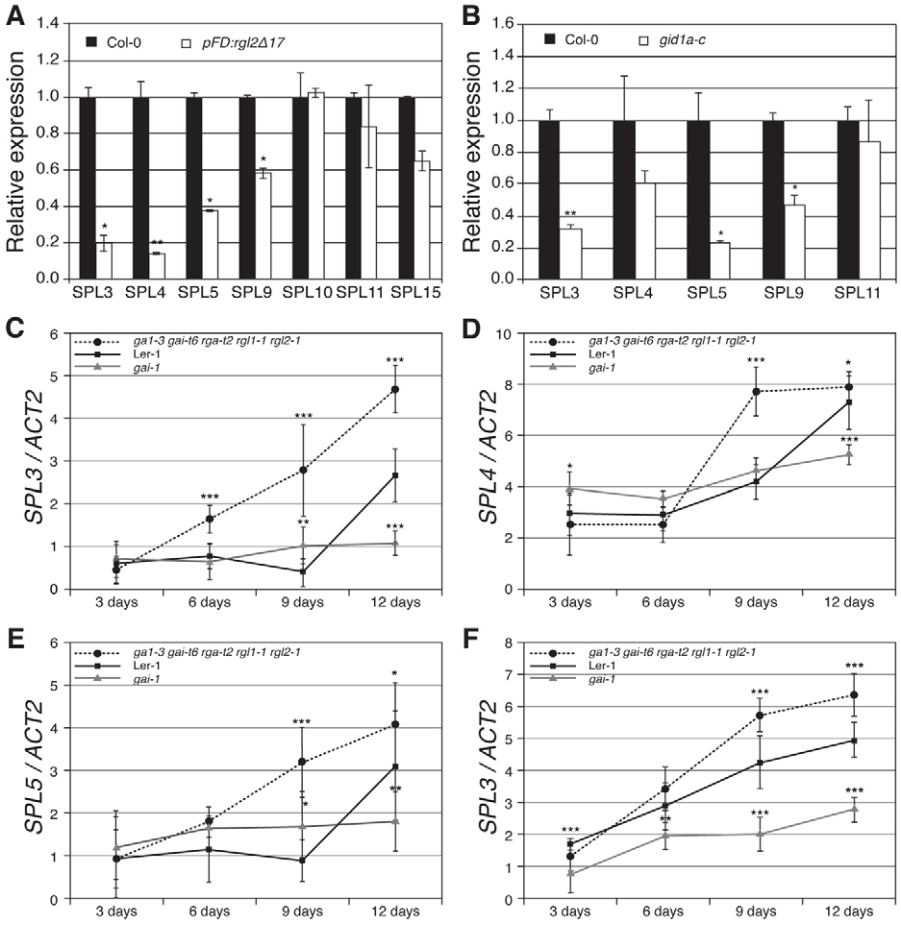

Fig. 5. GA regulates *SPL* expression at the shoot meristem and in leaves.
(**A**) Expression of *SPL* transcripts at the shoot meristem of *pFD:rgl2Δ17* plants. Apices of 12-day-old plants grown under LD 23°C were dissected at ZT 12-16. (**B**) Quantitative analysis of *SPL* gene expression in triple *gid1a-c* mutant grown under LD conditions compared with wild-type plants (ZT 16). (**C-E**) Expression of (C) *SPL3*, (D) *SPL4* and (E) *SPL5* in shoot meristem of L*er*-1, *gai-1,* and *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1*. Apices (C-E) were dissected at ZT 12-16 3, 6, 9, and 12 days after germination from plants grown under LD conditions at 23°C. (**F**) *SPL3* expression in cotyledons (day 3) and true leaves (days 6, 9 and 12) of L*er*-1, *gai-1* and *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* harvested 3, 6, 9 and 12 days after germination at ZT 15. Error bars represent the s.d. of two biological and two technical replicates each. Significance was calculated using the unpaired Student's *t*-test: **P*<0.05, ***P*<0.01, ****P*<0.001.

Similar results were obtained in the late-flowering *pSUC2:rgl3Δ17* and *pFD:rgl2Δ17* lines. Quantitative analysis showed that the mature miR172 was moderately more abundant throughout the day in Col-0 plants grown under SD conditions for 30 days and shifted to LD conditions for 5 days to induce flowering when compared with *pSUC2:rgl3Δ17* plants (Fig. 6E). By contrast, the levels of miR156 were comparable between the two genotypes (Fig. 6F). Similarly, the level of miR172 was reduced in apices of *pFD:rgl2Δ17* compared with LD-grown Col-0 (Fig. 6G). Together, these results indicate that DELLA proteins regulate *MIR172* expression, which could therefore contribute to the GA-mediated control of flowering in both SD and LD conditions.

DISCUSSION

Arabidopsis thaliana controls the transition to reproductive development through a complex regulatory network that integrates environmental and endogenous signals to ensure the correct timing of flowering. The hormone GA has been shown to be essential for flowering under SD photoperiod (Wilson et al., 1992). However, its role in regulating flowering under LD conditions is less well understood. Here we demonstrate that the DELLA proteins, which are key components of GA signaling, contribute substantially to the regulation of flowering under LD conditions. In agreement with previous reports (Silverstone et al., 1997; Dill and Sun, 2001; Dill et al., 2004) we found that the loss of individual *DELLA* genes resulted in only a minor acceleration in flowering. By contrast, flowering was induced much earlier in higher order mutants. These results not only confirm the importance of the DELLA proteins during flowering in LD conditions but also suggest a certain degree of functional redundancy between the individual proteins. The extreme delay in flowering observed in LD-grown triple *gid1a-c* mutants, which is due to an increase in DELLA protein (Griffiths et al., 2006; Willige et al., 2007), further strengthens the notion that the accumulation of DELLA proteins contributes substantially to the regulation of flowering under inductive LD conditions.

In addition, expression of GA-insensitive DELLA proteins (*della*Δ*17*) in leaves and at the shoot apex consistently demonstrated that these proteins can act as floral repressors in different tissues throughout the plant. However, there are clear differences in the effectiveness of individual DELLA proteins in regulating flowering in different tissues. For example, we observed that RGL3 reproducibly delayed flowering only when expressed in leaves, but not at the shoot apex. This observation was not completely unexpected, as genetic and molecular analysis of DELLA mutants had previously demonstrated some functional specificity of DELLA proteins, despite their generally high functional redundancy (Dill and Sun, 2001; King et al., 2001; Lee et al., 2002; Piskurewicz et al., 2009; Gallego-Bartolome et al., 2010).

Interestingly, the delay in flowering observed in *pSUC2:rgl3Δ17* plants was clearly correlated with a reduction in *FT* expression in the PCCs in the leaves, suggesting that at least part of the effect of DELLA proteins on flowering time in LD conditions is through the regulation of *FT*. In agreement with this we observed increased *pFT:GUS* expression in response to GA_3 application specifically in the leaf vasculature and not in other tissues. In addition, the reduction of *FT* expression most likely accounts at least in part for

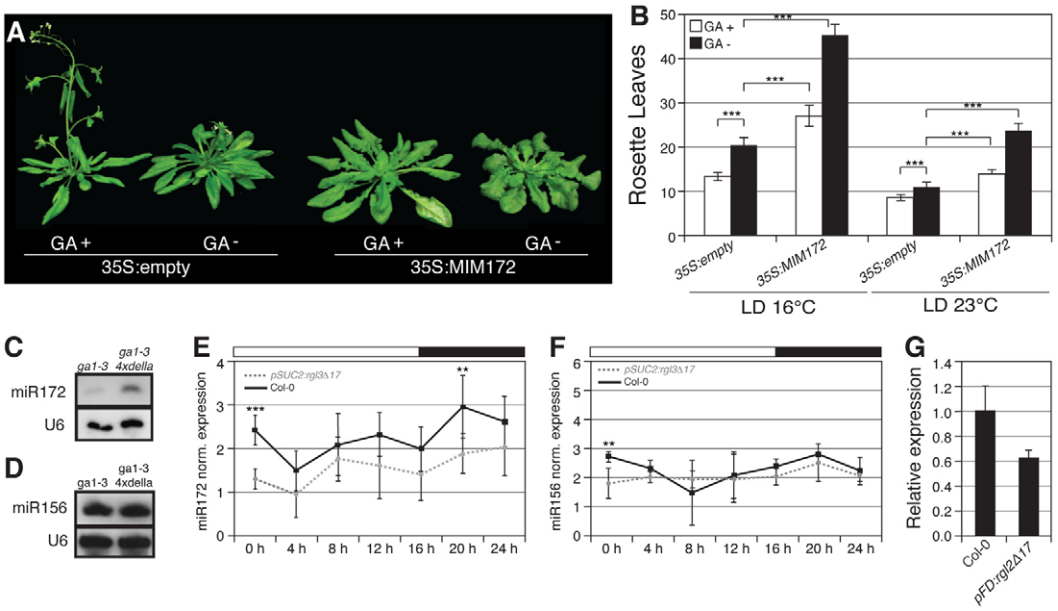

Fig. 6. GA controls flowering at least partially through miR172. (**A**,**B**) *p35S:MIM172* overexpression partially suppresses the inductive effect of exogenously applied GA on flowering under LD conditions at 16°C (A,B) or 23°C (B). GA_3 treatments were performed every third day throughout vegetative growth until the plants had started to flower. 35-day-old plants are shown. (**C**,**D**) Small RNA northern blot of miR172 (C) and miR156 (D) in *ga1-3* and *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* (labeled *ga1-3 4xdella*) mutants grown under SD conditions (ZT 8). Samples were collected 25 days after germination. (**E**,**F**) Diurnal expression of mature miR172 (E) and miR156 (F) in *pSUC2:rgl3Δ17* (T2). Plants were grown at 23°C for 30 days under SD conditions and shifted to 23°C LD conditions to induce flowering. Samples were harvested 5 days after the shift from SD to LD conditions every 4 hours for 24 hours. (**G**) Quantification of mature miR172 in dissected apices of 12-day-old Col-0 and *pFD:rgl2Δ17* plants harvested at ZT 12-16. Transgenic plants are in Col-0 background. Error bars indicate s.d. of rosette leaf number (B) and of two biological and two technical replicates each for quantitative PCR of small RNAs (E-G). Significance was calculated using the unpaired Student's *t*-test: **P*<0.05, ***P*<0.01, ****P*<0.001.

the late flowering of the *gid1a-c* mutant, which displays elevated levels of the DELLA proteins. Further evidence that the DELLA proteins repress *FT* comes from the observation that the early flowering *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* mutant exhibits increased *FT* expression. By contrast, the targeted reduction of bioactive GAs in the PCCs by the misexpression of the catabolic enzyme GA2ox8 significantly delayed flowering. Taken together, our data strongly indicate that DELLA protein accumulation contributes to the regulation of *FT* in the PCCs under LD conditions. However, DELLA-mediated GA signaling is only one of several inputs that converge on *FT*, which probably explains why mutations in the *DELLA* genes result in only a minor delay in flowering under LD conditions.

Although the delay in flowering we observed in response to misexpression of GA-insensitive DELLA proteins in the PCCs was to be expected based on the phenotypes of dominant DELLA mutants such as *gai-1*, it was surprising to see that transgenic plants expressing full-length DELLA proteins were also late-flowering. One possible explanation for this finding is that in the misexpression lines, DELLA proteins accumulate to such high levels that they can no longer be efficiently degraded even in the presence of GA, as has been previously demonstrated for GAI (Fleck and Harberd, 2002).

By contrast, when expressed at the shoot meristem only the GA-insensitive *della*Δ*17*, and not the full-length DELLA proteins, delayed flowering efficiently. It has been previously shown that bioactive GA accumulates at the shoot meristem before the transition to flowering (Eriksson et al., 2006). Assuming that other factors, such as the GID1 receptors or downstream components, are not limiting at the shoot meristem, this would result in a locally increased capability to degrade DELLA proteins, which might explain why meristem-specific expression of DELLA proteins at the meristem has little effect on flowering. Alternatively, the promoters used in this study (*pFD*, *pCLV3*) might be too weak to drive the expression of DELLA proteins beyond the capacity of the endogenous GA-signaling machinery to degrade (Lee et al., 2002).

It has previously been shown that GA signaling controls flowering at the shoot meristem specifically under SD conditions (Blazquez et al., 1998; Blazquez and Weigel, 2000; Moon et al., 2003; Achard et al., 2004). By contrast, the finding that *pFD:della*Δ*17* and *pCLV3:della*Δ*17* lines displayed pronounced late flowering, as well as a recent report describing the effects of *GA2ox7* misexpression on flowering (Porri et al., 2012), indicate that the accumulation of DELLA proteins at the shoot meristem contributes to the induction of flowering under LD conditions after all. GA positively regulates *SOC1* expression through DELLA proteins under non-inductive SD conditions (Moon et al., 2003). However, we and others (Porri et al., 2012) have observed only a mild effect of GA on *SOC1* expression under LD conditions. This is in stark contrast to the strong effect of GA under SD conditions and suggests that under LD conditions GA signaling controls flowering at the shoot meristem predominantly downstream of the photoperiodic pathway and *SOC1*.

Recently, Wang and colleagues proposed the existence of an endogenous microRNA-regulated pathway that ensures that plants eventually make the transition to flowering even under a non-inductive photoperiod (Wang et al., 2009). This pathway relies on the gradual increase of *SPL* transcripts in response to the decrease of miR156 level during *A. thaliana* development. The increase in SPL protein level would ultimately lead to the activation of floral

regulators and transition to flowering (Wang et al., 2009; Yamaguchi et al., 2009). The observation that *SPL9* and miR156 level remains unchanged in the *ga1-3* mutant when treated with exogenous GA leads to the conclusion that the *SPL*/miR156 module constitutes a pathway that regulates flowering under SD conditions independently of GA (Wang et al., 2009). Indeed, in our experiments and in agreement with previous work (Jung et al., 2011) miR156 levels remained unchanged in response to GA. However, the expression of the miR156-targets *SPL3*, *SPL4* and *SPL5* is significantly altered at the shoot meristem in response to GA, indicating that GA contributes to the regulation of the floral transition by modulating *SPL* gene expression independently of miR156 under both SD and LD conditions.

In contrast to miR156, there is at least circumstantial evidence for a role of yet another microRNA, miR172, in GA-mediated control of flowering. Plants with artificially reduced miR172 levels were still responsive to treatment with exogenous GA but did not completely recover the early flowering phenotype observed in control plants. One explanation for this behavior could be that the miR172 targets, a clade of *AP2*-like transcription factors that function as floral repressors (Aukerman and Sakai, 2003; Mathieu et al., 2009; Yant et al., 2010), were expressed too highly in the MIM172 lines for exogenous GA to compensate. In this scenario GA and miR172 would act in parallel signaling pathways that converge on the same targets. However, the observation that miR172 levels were elevated in *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* and reduced in *pSUC2:rgl3Δ17* suggests that DELLA proteins act at least partially through the miR172/*AP2*-like module.

In contrast to the results observed in LD conditions, regulation of flowering under SD photoperiod seems to be mostly restricted to the shoot meristem. Plants expressing *della*Δ*17* proteins from the *FD* or *CLV3* promoters under SD conditions in many cases completely failed to flower, whereas the expression of these proteins in leaves of SD-grown plants seems to have little or no effect. Interestingly, although GAI, RGA, RGL1 and RGL2 seem to be able to repress flowering in SD conditions when ectopically expressed at the shoot meristem, the *gai-t6 rga-24* double mutant has been reported to rescue the non-flowering phenotype of *ga1-3* in SD (Dill and Sun, 2001), suggesting that these two DELLA proteins are crucial for repressing flowering at the shoot meristem under a non-inductive photoperiod. Taken together, our results demonstrate that under LD conditions GA promotes flowering through the degradation of DELLA proteins in different parts of the plant, whereas its effect under a non-inductive photoperiod seems to be mostly restricted to the shoot meristem.

Acknowledgements
We thank the European Stock Centre for seeds, Dr Nicholas Harberd for *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* mutant, Dr Claus Schwechheimer for *gid1a-c* mutant, Dr Marco Todesco and Dr Ignacio Rubio-Somoza for *p35S:MIM172* and *p35S:empty* lines, Dr Koji Goto for *pFT:GUS* line, Dr Rüdiger Simon for a plasmid with *CLV3* regulatory sequences, Johanna Weirich for technical support, and Dr Levi Yant for critical comments on the manuscript.

Funding
Work in the Schmid laboratory on the regulation on flowering time is supported by the Max Planck Institute for Developmental Biology and grants from the Deutsche Forschungsgemeinschaft (DFG) [SCHM 1560/5-1].

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.080879/-/DC1

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.
Achard, P., Herr, A., Baulcombe, D. C. and Harberd, N. P. (2004). Modulation of floral development by a gibberellin-regulated microRNA. *Development* **131**, 3357-3365.
Achard, P., Liao, L. L., Jiang, C. F., Desnos, T., Bartlett, J., Fu, X. D. and Harberd, N. P. (2007). DELLAs contribute to plant photomorphogenesis. *Plant Physiol.* **143**, 1163-1172.
Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G. T. S. and Genschik, P. (2009). Gibberellin signaling controls cell proliferation rate in Arabidopsis. *Curr. Biol.* **19**, 1188-1193.
Aukerman, M. J. and Sakai, H. (2003). Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. *Plant Cell* **15**, 2730-2741.
Blazquez, M. and Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. *Nature* **404**, 889-892.
Blazquez, 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.
Blazquez, M. A., Soowal, L. N., Lee, I. and Weigel, D. (1997). LEAFY expression and flower initiation in Arabidopsis. *Development* **124**, 3835-3844.
Bolle, C. (2004). The role of GRAS proteins in plant signal transduction and development. *Planta* **218**, 683-692.
Bonhomme, F., Kurz, B., Melzer, S., Bernier, G. and Jacqmard, A. (2000). Cytokinin and gibberellin activate SaMADS A, a gene apparently involved in regulation of the floral transition in Sinapis alba. *Plant J.* **24**, 103-111.
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.
Chen, C., Ridzon, D. A., Broomer, A. J., Zhou, Z., Lee, D. H., Nguyen, J. T., Barbisin, M., Xu, N. L., Mahuvakar, V. R., Andersen, M. R. et al. (2005). Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res.* **33**, e179.
Chen, X. M. (2004). A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science* **303**, 2022-2025.
Cheng, H., Qin, L., Lee, S., Fu, X., Richards, D. E., Cao, D., Luo, D., Harberd, N. P. and Peng, J. (2004). Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. *Development* **131**, 1055-1064.
Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J.* **16**, 735-743.
Corbesier, L., Vincent, C., Jang, S. H., Fornara, F., Fan, Q. Z., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C. et al. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316**, 1030-1033.
Daviere, J. M., de Lucas, M. and Prat, S. (2008). Transcriptional factor interaction: a central step in DELLA function. *Curr. Opin. Genet. Dev.* **18**, 295-303.
Davies, P. J. (2004). *Plant Hormones: Biosynthesis, Signal Transduction, Action!* (3rd edn). Dordrecht, The Netherlands: Springer.
de Lucas, M., Daviere, J. M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J. M., Lorrain, S., Fankhauser, C., Blazquez, M. A., Titarenko, E. and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. *Nature* **451**, 480-484.
Dill, A. and Sun, T. (2001). Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. *Genetics* **159**, 777-785.
Dill, A., Jung, H. S. and Sun, T. P. (2001). The DELLA motif is essential for gibberellin-induced degradation of RGA. *Proc. Natl. Acad. Sci. USA* **98**, 14162-14167.
Dill, A., Thomas, S. G., Hu, J., Steber, C. M. and Sun, T. P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell* **16**, 1392-1405.
Eriksson, S., Bohlenius, H., Moritz, T. and Nilsson, O. (2006). GA4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. *Plant Cell* **18**, 2172-2181.
Feng, S., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J., Wang, F., Chen, L., Yu, L., Iglesias-Pedraz, J. M., Kircher, S. et al. (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. *Nature* **451**, 475-479.
Fleck, B. and Harberd, N. P. (2002). Evidence that the Arabidopsis nuclear gibberellin signalling protein GAI is not destabilised by gibberellin. *Plant J.* **32**, 935-947.
Franco-Zorrilla, J. M., Valli, A., Todesco, M., Mateos, I., Puga, M. I., Rubio-Somoza, I., Leyva, A., Weigel, D., Garcia, J. A. and Paz-Ares, J. (2007). Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* **39**, 1033-1037.

Fu, X. D., Richards, D. E., Fleck, B., Xie, D. X., Burton, N. and Harberd, N. P. (2004). The Arabidopsis mutant sleepy1(gar2-1) protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. *Plant Cell* **16**, 1406-1418.
Gallego-Bartolome, J., Minguet, E. G., Marin, J. A., Prat, S., Blazquez, M. A. and Alabadi, D. (2010). Transcriptional diversification and functional conservation between DELLA proteins in Arabidopsis. *Mol. Biol. Evol.* **27**, 1247-1256.
Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z. L., Powers, S. J., Gong, F., Phillips, A. L., Hedden, P., Sun, T. P. et al. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. *Plant Cell* **18**, 3399-3414.
Guo, A. Y., Zhu, Q. H., Gu, X. C., Ge, S., Yang, J. and Luo, J. C. (2008). Genome-wide identification and evolutionary analysis of the plant specific SBP-box transcription factor family. *Gene* **418**, 1-8.
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**, 3821-3819.
Jaeger, K. E. and Wigge, P. A. (2007). FT protein acts as a long-range signal in Arabidopsis. *Curr. Biol.* **17**, 1050-1054.
Jung, J. H., Ju, Y., Seo, P. J., Lee, J. H. and Park, C. M. (2011). The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. *Plant J.* **69**, 577-588.
Kim, J. J., Lee, J. H., Kim, W., Jung, H. S., Huijser, P. and Ahn, J. H. (2012). The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 module regulates ambient temperature-responsive flowering via FLOWERING LOCUS T in Arabidopsis. *Plant Physiol.* **159**, 461-478.
King, K. E., Moritz, T. and Harberd, N. P. (2001). Gibberellins are not required for normal stem growth in Arabidopsis thaliana in the absence of GAI and RGA. *Genetics* **159**, 767-776.
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.
Koornneef, M. and van der Veen, J. (1980). Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L) Heynh. *Theor. Appl. Genet.* **58**, 257-263.
Koornneef, M., Elgersma, A., Hanhart, C. J., Vanloenenmartinet, E. P., Vanrijn, L. and Zeevaart, J. A. D. (1985). A gibberellin insensitive mutant of Arabidopsis thaliana. *Physiologia Plantarum* **65**, 33-39.
Lang, A. (1957). The effect of gibberellin upon flower formation. *Proc. Natl. Acad. Sci. USA* **43**, 709-717.
Langridge, J. (1957). Effect of day-length and gibberellic acid on the flowering of Arabidopsis. *Nature* **180**, 36-37.
Lee, S., Cheng, H., King, K. E., Wang, W., He, Y., Hussain, A., Lo, J., Harberd, N. P. and Peng, J. (2002). Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. *Genes Dev.* **16**, 646-658.
Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y. and Yu, H. (2012). FTIP1 is an essential regulator required for florigen transport. *PLoS Biol.* **10**, e1001313.
Mathieu, J., Warthmann, N., Kuttner, 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.
Mathieu, J., Yant, L. J., Murdter, F., Kuttner, F. and Schmid, M. (2009). Repression of flowering by the miR172 target SMZ. *PLoS Biol.* **7**, e1000148.
McGinnis, K. M., Thomas, S. G., Soule, J. D., Strader, L. C., Zale, J. M., Sun, T. P. and Steber, C. M. (2003). The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *Plant Cell* **15**, 1120-1130.
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.
Moon, J., Suh, S.-S., Lee, H., Choi, K.-R., Hong, C. B., Paek, N.-C., Kim, S.-G. and Lee, I. (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. *Plant J.* **35**, 613-623.
Murase, K., Hirano, Y., Sun, T. P. and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* **456**, 459-463.
Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y. and Yamaguchi, S. (2003). Gibberellin biosynthesis and response during Arabidopsis seed germination. *Plant Cell* **15**, 1591-1604.
Olszewski, N., Sun, T. P. and Gubler, F. (2002). Gibberellin signaling: biosynthesis, catabolism, and response pathways. *Plant Cell* **14 Suppl.**, S61-S80.
Osnato, M., Castillejo, C., Matias-Hernandez, L. and Pelaz, S. (2012). TEMPRANILLO genes link photoperiod and gibberellin pathways to control flowering in Arabidopsis. *Nat. Commun.* **3**, 808.
Peng, J. R. and Harberd, N. P. (1993). Derivative alleles of the Arabidopsis gibberellin-insensitive (Gai) mutation confer a wild-type phenotype. *Plant Cell* **5**, 351-360.
Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P. and Harberd, N. P. (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11**, 3194-3205.
Piskurewicz, U., Tureckova, V., Lacombe, E. and Lopez-Molina, L. (2009). Far-red light inhibits germination through DELLA-dependent stimulation of ABA synthesis and ABI3 activity. *EMBO J.* **28**, 2259-2271.
Porri, A., Torti, S., Romera-Branchat, M. and Coupland, G. (2012). Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. *Development* **139**, 2198-2209.
Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B. and Bartel, D. P. (2002). Prediction of plant microRNA targets. *Cell* **110**, 513-520.
Rieu, I., Eriksson, S., Powers, S. J., Gong, F., Griffiths, J., Woolley, L., Benlloch, R., Nilsson, O., Thomas, S. G., Hedden, P. et al. (2008). Genetic analysis reveals that C(19)-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. *Plant Cell* **20**, 2420-2436.
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., Scholkopf, B., Weigel, D. and Lohmann, J. U. (2005). A gene expression map of *Arabidopsis thaliana* development. *Nat. Genet.* **37**, 501-506.
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.
Silverstone, A. L., Mak, P. Y., Martinez, E. C. and Sun, T. P. (1997). The new RGA locus encodes a negative regulator of gibberellin response in Arabidopsis thaliana. *Genetics* **146**, 1087-1099.
Silverstone, A. L., Ciampaglio, C. N. and Sun, T. P. (1998). The Arabidopsis RGA gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* **10**, 155-169.
Stadler, R. and Sauer, N. (1996). The Arabidopsis thaliana AtSUC2 gene is specifically expressed in companion cells. *Botanica Acta* **109**, 299-306.
Sun, T. P., Goodman, H. M. and Ausubel, F. M. (1992). Cloning the Arabidopsis Ga1 locus by genomic subtraction. *Plant Cell* **4**, 119-128.
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.
Tamaki, S., Matsuo, S., Wong, H. L., Yokoi, S. and Shimamoto, K. (2007). Hd3a protein is a mobile flowering signal in rice. *Science* **316**, 1033-1036.
Taoka, K., Ohki, I., Tsuji, H., Furuita, K., Hayashi, K., Yanase, T., Yamaguchi, M., Nakashima, C., Purwestri, Y. A., Tamaki, S. et al. (2011). 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* **476**, 332-335.
Todesco, M., Rubio-Somoza, I., Paz-Ares, J. and Weigel, D. (2010). A collection of target mimics for comprehensive analysis of microRNA function in Arabidopsis thaliana. *PLoS Genet.* **6**, e1001031.
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.
Wang, J. W., Czech, B. and Weigel, D. (2009). miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell* **138**, 738-749.
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.
Willige, B. C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E. M., Maier, A. and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. *Plant Cell* **19**, 1209-1220.
Wilson, R. N., Heckman, J. W. and Sommerville, C. R. (1992). Gibberellin is required for flowering in Arabidopsis thaliana under short days. *Plant Physiol.* **100**, 403-408.
Wu, G., Park, M. Y., Conway, S. R., Wang, J. W., Weigel, D. and Poethig, R. S. (2009). The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. *Cell* **138**, 750-759.
Yamaguchi, A., Wu, M. F., Yang, L., Wu, G., Poethig, R. S. and Wagner, D. (2009). The MicroRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. *Dev. Cell* **17**, 268-278.
Yant, L., Mathieu, J., Dinh, T. T., Ott, F., Lanz, C., Wollmann, H., Chen, X. and Schmid, M. (2010). Orchestration of the floral transition and floral development in Arabidopsis by the bifunctional transcription factor APETALA2. *Plant Cell* **22**, 2156-2170.
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.