AGL24 acts in concert with *SOC1* and *FUL* during Arabidopsis floral transition

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Arabidopsis plants flower in response to long days (LDs). Exposure of leaves to inductive day lengths activates expression of FLOWERING LOCUS T (FT) protein which moves to the shoot apical meristem (SAM) to induce developmental reprogramming. *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FRUITFULL* (*FUL*) are induced by *FT* at the apex. We previously screened the SAM for mRNAs of genes required to promote the floral transition in response to photoperiod, and conducted detailed expression and functional analyses on several putative candidates. Here, we show that expression of *AGAMOUS-LIKE 24* (*AGL24*) is detected at the SAM under SD conditions and increases upon exposure to LDs. Mutations in *AGL24* further delay flowering of a *soc1 ful* double mutant, suggesting that flowering is controlled by *AGL24* partly independently of *SOC1* and *FUL*.

Flowering of Arabidopsis is controlled by endogenous and environmental factors to ensure it occurs at the most appropriate time of year. Day length, or photoperiod, is one of the most reliable indicators of seasonal time and plants have evolved sophisticated networks to monitor its yearly progression. Day length is perceived in the phloem of leaves that produce a mobile signal partly encoded by *FLOWERING LOCUS T* (*FT*). Upon exposure to long days (LDs), the FT protein moves to the shoot apical meristem and activates the signaling cascade that converts the vegetative meristem into a reproductive meristem.[1] Overexpression of *FT* under a viral constitutive promoter (*CaMV 35S*), under a meristem-specific promoter (*KNAT1*) or under a phloem-specific promoter (*SUC2*) activates flowering at the shoot apical meristem (SAM) independently of photoperiodic induction, and induces flower formation also under short day lengths (SDs).[2,3] At the apex, FT activates transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FRUITFULL* (*FUL*).[4-7] Consistently, recent data demonstrated that early flowering caused by ectopic expression of *FT* under a constitutive or phloem-specific promoter is strongly suppressed by a *soc1 ful* double mutant.[8,9] The inductive effects of *SOC1* and *FUL* at the SAM are antagonized by the *SHORT VEGETATIVE PHASE* (*SVP*) floral repressor,[10] because the *soc1-2 ful-2 svp-41* triple mutant can partly restore earlier flowering of the *soc1-2 ful-2* double mutant.[9,11] *SVP* has a dual repressive role in leaves and at the SAM.[11-13] In leaves,

it directly represses *FT* transcription, whereas in the SAM it directly represses *SOC1* transcription.[12,13] The early flowering phenotype observed in *soc1-2 ful-2 svp-41* is likely not caused by increased *FT* transcription in leaves, because overexpression of *FT* from the *SUC2* promoter cannot completely rescue late flowering of the *soc1-2 ful-2* double mutant.[9] These data suggest the existence of additional genes whose expression is required at the SAM to promote flowering in parallel to *SOC1* and *FUL*.

We screened candidate genes that could act with *SOC1* and *FUL* and we focused on *AGAMOUS-LIKE 24* (*AGL24*) for several reasons. First, by screening our data set of genes differentially expressed at the SAM during floral transition, we observed increasing *AGL24* expression upon photoperiodic induction, similarly to *SOC1* and *FUL* expression.[9] However, as opposed to *SOC1* and *FUL*, *AGL24* increase is relatively modest and expression can already be detected in the vegetative meristem prior to induction by LDs. Additionally, *agl24* mutants are late flowering compared with wild-type controls, both under SD and LD conditions, but retain sensitivity to photoperiod.[14,15] We conducted a detailed expression analysis by in situ hybridizations on SAMs grown for 2 weeks under SDs and then shifted to 1, 3 and 5 LDs. The results confirmed that expression of *AGL24* can be detected in non-induced SAMs and progressively increases at the apex and in young leaf primordia upon exposure to inductive LDs (**Fig. 1A**). We also assayed *AGL24* expression on apices of plants returned to SDs after the LD treatments.
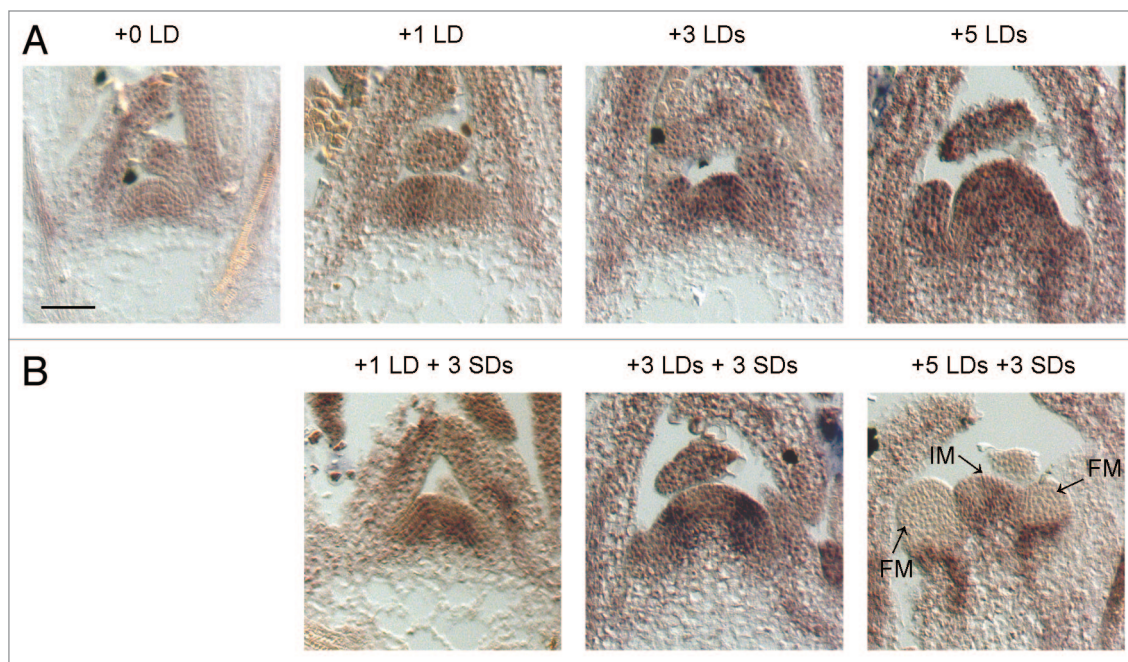
Figure 1. Expression pattern of *AGL24* in response to photoperiod. (A) In situ hybridizations of *AGL24* on apices of wild-type Columbia grown for two weeks in SDs (0 LDs) and then transferred to LDs for one, three or five days. (B) Analysis of *AGL24* expression by in situ hybridizations after transient exposure of SD-grown plants to LDs. Plants were grown for two weeks in SDs, transferred to LDs and then back to SDs as indicated. Samples were harvested at ZT8. The in situ probe spans the 3' end and 3' UTR regions of the *AGL24* transcript, and has been described in.[14] For hybridization methods see.[9] IM, inflorescence meristem; FM, floral meristem. Bar = 50μm.
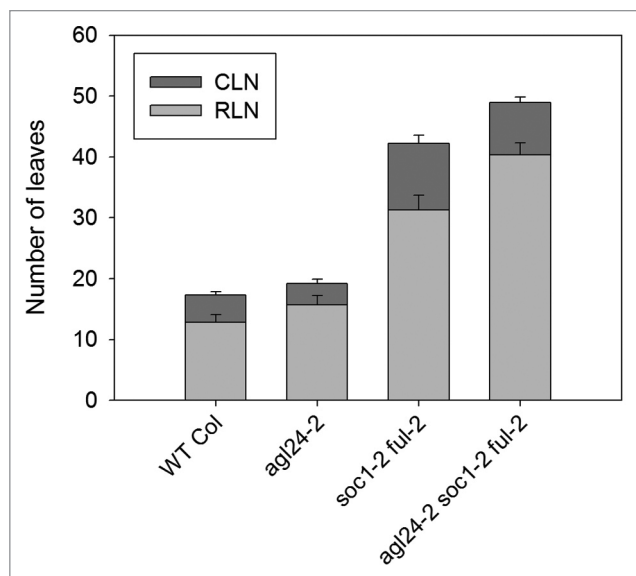
Figure 2. *agl24-2* enhances the late-flowering phenotype of *soc1-2 ful-2* double mutants. Flowering time of plants grown under LDs. CLN, cauline leaf number; RLN, rosette leaf number. Error bars represent standard deviation. At least 8 plants were used to score flowering time of each genotype.

After 3 additional days of growth under SD conditions we still detected *AGL24* expression in the SAM (**Fig. 1B**), suggesting that its expression is maintained independently of day length. A similar behavior was observed for *FUL* expression, particularly in samples committed to flowering (after 5 LDs), but not for *SOC1* expression.[9] Apices harboring floral meristems after 5 d of induction under LDs + 3 SDs showed *AGL24* expression in the undifferentiated inflorescence meristem and at the base of developing floral meristems (**Fig. 1B**). *FT* mRNA expression is induced in leaves of plants transiently exposed to LDs, and is downregulated as soon as plants are returned to SD conditions.[16] Therefore, *AGL24* expression does not follow the pattern of *FT* expression across a SD-LD-SD double shift.

To assess if *AGL24* could genetically act in parallel to *SOC1* and *FUL* we crossed the *soc1-2 ful-2* double mutant with *agl24-2* to generate a triple mutant. We scored flowering time under inductive LDs and observed that mutations in *agl24* can delay flowering of a *soc1-2 ful-2* mutant by around 9 rosette leaves (**Fig. 2**). Taken together, these data suggest that *AGL24* is required to promote the floral transition, in parallel to *SOC1* and *FUL*. Interestingly, removing a functional *AGL24* gene from the *soc1 ful* background has a similar effect to removing *FLOR1*, a gene identified by transcriptomic analyses.[9]

Upon floral induction, the SAM receives inductive signals from several pathways and diverse inputs have to converge into flower development programs. *SOC1*, *FUL*, *SVP* and *AGL24* can act as floral integrator genes that respond to several environmental and endogenous cues[12,17,18] (**Fig. 3**). Notably, all genes belong to the MADS-box family of transcription factors, a group of regulators that play important roles during floral transition and subsequent flower development. Tight regulation of their expression by the environment and cross regulation

between them ensures stable progression of the floral transition.[12,19] The photoperiodic pathway mediates information from day length to floral promoters such as *FT* and its close paralog *TWIN SISTER OF FT* (*TSF*).[20] However, FT and TSF might not be the only signals produced upon exposure to LDs. Exposure to LDs leads to increased expression of *AGL24* at the apex, possibly indicating that *AGL24* is a target of FT or TSF. However, promotion of flowering by ectopic expression of *FT* is strongly suppressed in a *soc1-2 ful-2* double mutant, bearing a functional *AGL24* gene. We speculate that *AGL24* might not be acting downstream of *FT* at the apex, because in that case it would be expected that *FT* ectopic expression in *soc1-2 ful-2* could activate *AGL24* transcription and promote flowering, bypassing the requirement for *SOC1* and *FUL*. Since both *FT*-dependent and *FT*-independent pathways can lead to activation of gene expression at the apex and influence flowering,[9] *AGL24* expression could be enhanced by LDs but be insensitive to induction by FT (**Fig. 3**). Alternatively, upregulation of *AGL24* by *FT* could be mediated by *SOC1*, that can directly promote *AGL24* mRNA expression.[15,19] However, consistent with an *FT*- and *SOC1*-independent regulation of *AGL24* is (i) the fact that *AGL24* expression is activated at the apex also under SDs, when *FT* expression in leaves is very low or absent and *SOC1* is not expressed at the apex, and (ii) expression is stably maintained after returning plants to SD conditions after LD induction.

SOC1, AGL24 and FUL proteins were shown to physically interact with each other in a yeast-two-hybrid screen.[21] Functional data suggest that interaction of AGL24 with SOC1 is required to carry SOC1 protein to the nucleus,[22] indicating that the molecular function of AGL24 depends, at least partly, on SOC1. No functional data about the interaction of AGL24 with FUL have been presented to date. Our genetic analyses
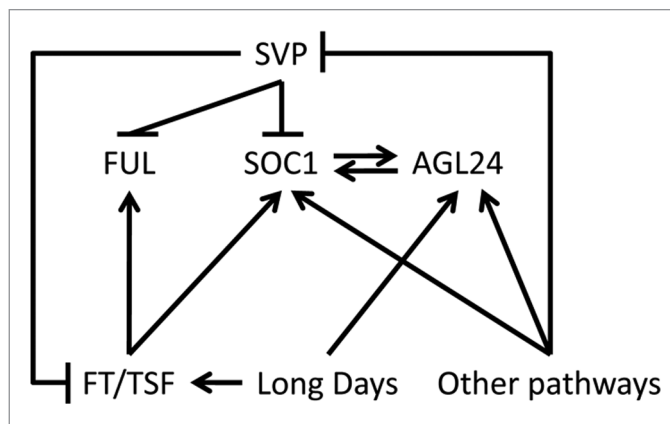
Figure 3. Genetic interactions occurring at the SAM during floral induction. Arrows represent transcriptional activation. Perpendicular lines indicate transcriptional repression.

indicate that the function of AGL24 does not completely depend on SOC1 and FUL, because in that case a *soc1-2 ful-2 agl24-2* mutant should not flower later than *soc1-2 ful-2*. The existence of multiple complexes containing MADS-box proteins at different developmental stages and cell types represents a further layer of complexity overlaid on the genetic pathways that lead to flowering. Elucidating this layer of regulation will be required to improve our understanding of flowering.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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