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SHORT COMMUNICATION

Regulatory interplay between *LEAFY, APETALA1/CAULIFLOWER* and *TERMINAL FLOWER1*: New insights into an old relationship

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ABSTRACT

The gene regulatory network comprised of *LEAFY (LFY), APETALA1 (AP1)*, the *AP1* paralog *CAULIFLOWER (CAL)*, and *TERMINAL FLOWER1 (TFL1)* is a major determinant of the flowering process in *Arabidopsis thaliana*. TFL1 activity in the shoot apical meristem provides inflorescence identity while the transcription factors LFY and AP1/CAL confer floral identity to emerging floral primordia. It has been thought that LFY and AP1/CAL control the onset of flowering in part by repressing *TFL1* expression in flowers. However, in the June issue of *Plant Physiology*, we reported that LFY and AP1 act antagonistically in the regulation of several key flowering regulators, including *TFL1*. Specifically, *TFL1* transcription was suppressed by AP1 but promoted by LFY. Here, we present additional evidence for the role of LFY as an activator of *TFL1* and propose that this regulatory activity is pivotal for the indeterminate growth of the SAM during the reproductive phase of development.

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Angiosperms integrate a multitude of endogenous and environmental signals to determine a time for flowering that ensures reproductive success. Research conducted over the past 25 years, especially in *Arabidopsis thaliana* (Arabidopsis), has revealed many of the genes that orchestrate the flowering process.[1-3] These include inflorescence meristem identity genes, such as *TERMINAL FLOWER1* (*TFL1*), and floral meristem identity genes, including the transcription factor-coding genes *LEAFY* (*LFY*), *APETALA1* (*AP1*) and the *AP1*-paralog *CAULIFLOWER* (*CAL*). Because these genes together determine when and where flowers will be formed, it has been proposed that differences in their expression patterns or in the functions of the corresponding proteins can explain much of the diversity of inflorescence architectures observed among the angiosperms.[4]

The inflorescence of Arabidopsis is characterized by a main axis (shoot) with an indeterminate shoot apical meristem (SAM) that laterally produces flowers. A number of elegant studies showed that *TFL1* and *LFY/AP1* are essential for building the Arabidopsis inflorescence. *TFL1* is expressed in the central region of the SAM, providing inflorescence identity and allowing the indeterminate growth of the shoot apex, while *LFY* and *AP1/CAL* are expressed in the flanks of the SAM, providing floral identity to emerging primordia.[5-7] In *tfl1* mutants, *LFY/AP1* expression expands into the SAM that, consequently, acquires floral identity and abruptly terminates with the formation of a flower-like structure.[7-9] Conversely, in *lfy* and *ap1* mutants, flowers are substituted by shoot-like structures.[7,10-11] An even more dramatic conversion of flowers into inflorescence-like meristems is observed in *ap1 cal* double-mutant plants.[12] In recent years, several studies have provided a molecular basis for the antagonism between *TFL1* and *LFY/AP1*: both LFY and AP1 proteins were shown to bind to essential *cis*-regulatory elements in the *TFL1* promoter.[13-15]

LFY and AP1 regulate floral development in a partially redundant manner and share many target genes.[13-17] However, it is not known whether LFY and AP1 act together or provide independent inputs to these targets. We addressed this question by analyzing the transcriptional activity of LFY in the absence of AP1/CAL function.[18] To this end, we used a *p35S:LFY-GR* line introgressed into an *ap1 cal* double-mutant background to determine the gene expression changes caused by LFY activation in the inflorescence. We found that LFY can regulate some of its known target genes independently of AP1/CAL activity. In contrast, other LFY targets, including the floral homeotic genes *APETALA3* and *AGAMOUS*, appear to require functional AP1/CAL. In agreement with the results of a previous meta-analysis of published data sets,[18,19] we further found that LFY and AP1/CAL regulate certain targets antagonistically. These included regulators of floral initiation such as *FLOWERING LOCUS D, TEMPRANILLO1, APETALA2* and, notably, *TFL1*. *TFL1* was upregulated in response to LFY-GR activation but downregulated by AP1-GR in *ap1 cal* inflorescences. In agreement with the transcriptional response of *TFL1*, activation of LFY-GR in *ap1 cal* plants led to a significant inhibition of flower formation while activation of AP1-GR

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caused an immediate and synchronized onset of flowering, as previously reported.[17]

These results, as well as a set of previous observations, led us to reconsider the nature of the relationship between LFY and TFL1. Firstly, the expression domains of *TFL1* and *LFY* overlap in the inflorescence-like meristems of *ap1 cal* plants.[20-21] Moreover, weak *LFY* expression has been detected in the stem of wild-type inflorescences,[22] where *TFL1* is also expressed.[23] The reanalysis of published transcriptomics data sets further showed that activation of LFY-GR in seedlings leads to upregulation of *TFL1* expression.[3] Taken together, these results imply that *TFL1* and *LFY* are not necessarily antagonists and that LFY may be able to activate *TFL1*, at least, in the absence of AP1/CAL activity. In line with this idea, LFY was shown to bind to a region approximately 2.8 kilobases (kb) downstream of *TFL1*, which is essential for the maintenance of *TFL1* expression in the inflorescence meristem and, consequently, for SAM indeterminacy.[14,23] Although *LFY* itself does not appear to be expressed in the SAM, it has been demonstrated that LFY protein is mobile and can travel to the inflorescence meristem.[24] Thus, in addition to its role in flower development, LFY might be needed for indeterminate growth of the SAM. This would not be the first described function of LFY in a shoot meristem, as it has been shown previously that LFY stimulates axillary meristem growth.[25]

To test the putative role of LFY in SAM identity via activation of *TFL1*, we made use of a previously generated set of *pTFL1:GUS* reporter lines[23] and monitored *TFL1* expression in genetic backgrounds with modified LFY activity (Fig. 1). First, we tested whether the LFY binding sites located in the 3′ region of the *TFL1* promoter[14] were essential for the transcriptional response of *TFL1* to LFY. To this end, we analyzed the activity of two *pTFL1:GUS* reporters – one containing the LFY binding sites, the other one lacking them – in plants that express a fusion protein between LFY and the VP16 transcriptional activator under the control of a heat-shock inducible promoter (*pHS:LFY-VP16*).[26,27] As described in our recent publication,[18] the activity of the *pTFL1:GUS* reporter containing the full

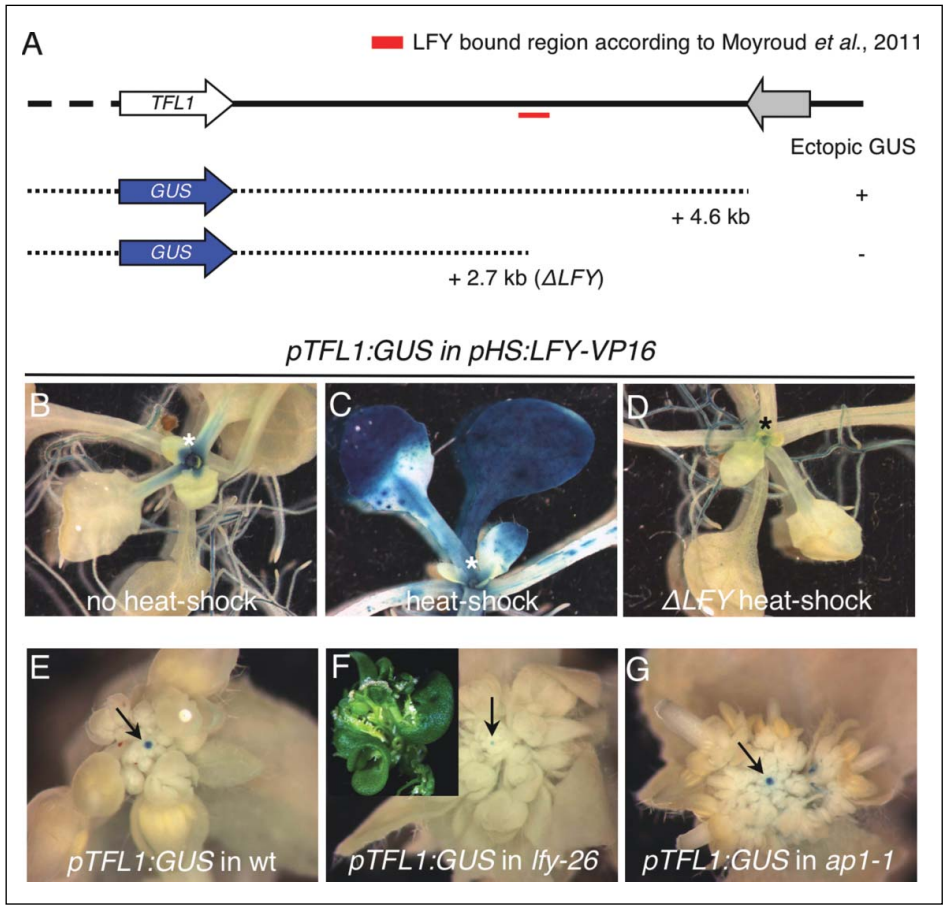

Figure 1. Analysis of *pTFL1:GUS* reporter lines in genetic backgrounds with modified LFY activity. (A) Summary of the experiments performed to test the functional relevance of the LFY binding sites in the 3′ region of the *TFL1* promoter. The activity of 2 *pTFL1:GUS* constructs was assayed in *pHS:LFY-VP16* seedlings: one with the full length *TFL1* promoter (2.2 kb of the 5′ region plus 4.6 kb of the 3′) and one with a truncated version of the promoter lacking the LFY binding region (2.2 kb of the 5′ region plus 2.7 kb of the 3′, *ΔLFY*). 'Ectopic GUS (+)' denotes staining in roots, cotyledons and developing leaves after a heat-shock treatment. Growth conditions, heat-shock treatments (incubation for 3 h at 37°C during 3 consecutive days) and GUS staining were conducted as described previously.[18] (B-D) Representative images of *pHS:LFY-VP16* seedlings containing a *pTFL1:GUS* reporter and stained for GUS: (B) reporter activity of the full length *TFL1* promoter in a *pHS:LFY-VP16* seedling grown under control conditions without heat-shock, (C) reporter activity of the full length *TFL1* promoter in a *pHS:LFY-VP16* seedling after heat-shock, (D) reporter activity of the truncated version of the *TFL1* promoter (*ΔLFY*) in a *pHS:LFY-VP16* seedling after heat-shock. Asterisks point to the position of the SAM. (E-F) Reporter activity of the full length *TFL1* promoter in representative inflorescence apices of wild type (accession Landsberg *erecta*) (E), *lfy-26* (F) and *ap1–1* (G) plants. The inset in (F) shows the terminal carpelloid structure in *lfy-26* shoots. Arrows in the main panels point to the position of the SAM. GUS staining was conducted as described previously.[23]

length promoter of *TFL1* was broadly activated in wild-type seedlings after the heat-shock treatment but restricted to the shoot apex in plants grown under normal conditions (Fig. 1A-C). In contrast, plants carrying a truncated version of *pTFL1:GUS* without the LFY binding sites (*ΔLFY* in Fig. 1) did not exhibit ectopic GUS activity after induction of LFY-VP16 expression (Fig. 1D). Therefore, the LFY binding sites in the 3′ region of the *TFL1* promoter appear to be necessary for the response of *pTFL1:GUS* to LFY-VP16.

Next, we asked whether a loss of LFY function affects *TFL1* transcription in the SAM. To test this, we monitored the activity of *pTFL1:GUS* in the strong *lfy-26* allele.[27] Compared to the wild type, the intensity of the GUS signal significantly decreased in the center of *lfy-26* inflorescences (Fig. 1E-F). This result is in agreement with the absence of *TFL1* expression in the SAM of *lfy-7* mutant plants.[20] In contrast, *pTFL1:GUS* activity was not apparently affected in the inflorescence apex of *ap1–1* mutants (Fig. 1E, G), which also exhibit impaired floral meristem identity.[10] As described previously,[7] we observed that the inflorescences of *lfy-26* mutants terminated in carpelloid structures (Fig. 1F inset). This determinacy phenotype of *lfy-26* plants may be caused by the low levels of *TFL1* expression in the inflorescence apex we detected with the *pTFL1:GUS* reporter. Taken together, these results suggest that LFY promotes *TFL1* expression in the SAM to ensure indeterminate growth.

LFY may also activate *TFL1* in flowers, at least under conditions where AP1/CAL are non-functional. In fact, the results presented in our *Plant Physiology* paper suggest that LFY, AP1/CAL and TFL1 may be part of an incoherent feedback loop[28] during early establishment flower development, where LFY activates both *TFL1* and the repressors of *TFL1*, AP1/CAL.[18] This regulatory loop might ensure that flower formation commences only when AP1/CAL levels are sufficiently high to repress *TFL1* expression and to trigger the genetic program required for flower development. A characterization of the protein complexes that regulate inflorescence and floral development may be required to explain the antagonistic activities of LFY and AP1 in the control of *TFL1* and other flowering regulators.

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