

Florigen is involved in axillary bud development at multiple stages in *Arabidopsis*

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Abbreviations: BRC1, BRANCHED1; CaMV, cauliflower mosaic virus; Col, Columbia; FT, FLOWERING LOCUS T; *Ler*, Landsberg *erecta*; LFY, LEAFY; *stm*, shoot meristemless; TSF, TWIN SISTER OF FT

The wide variety of plant architectures is largely based on diverse and flexible modes of axillary shoot development. In *Arabidopsis*, floral transition (flowering) stimulates axillary bud development. The mechanism that links flowering and axillary bud development is, however, largely unknown. We recently showed that FLOWERING LOCUS T (FT) protein, which acts as florigen, promotes the phase transition of axillary meristems, whereas BRANCHED1 (BRC1) antagonizes the florigen action in axillary buds. Here, we present evidences for another possible role of florigen in axillary bud development. Ectopic overexpression of FT or another florigen gene TWIN SISTER OF FT (TSF) with LEAFY (LFY) induces ectopic buds at cotyledonary axils, confirming the previous proposal that these genes are involved in formation of axillary buds. Taken together with our previous report that florigen promotes axillary shoot elongation, we propose that florigen regulates axillary bud development at multiple stages to coordinate it with flowering in *Arabidopsis*.

Regulation of axillary bud development is crucial for the plant architecture, which in turn affects assimilate production and flower/seed fecundity. In *Arabidopsis* (*Arabidopsis thaliana*), axillary buds are formed on the base of foliage leaves, and develop into an inflorescence shoot after floral transition of the plant. Our recent work¹ showed that florigen protein, FT, moves from leaves into the subtended axillary bud and promotes the phase transition of the axillary meristem, while BRC1, an axillary bud-specific factor, suppresses it through interaction with FT and another florigen protein TSF. Interaction between BRC1 and FT (and possibly TSF as well) is not mediated by 14-3-3 protein, which is necessary for FT to form a complex with FD.² In the axillary buds of *brc1* mutant, FT-downstream genes are over-induced, and ectopic expression of *BRC1* in the shoot apical meristem causes delay in floral transition. These findings suggest that modulation of florigen signal is conferred by BRC1 through direct interaction with FT or TSF possibly present in the florigen complex (FT/14-3-3/FD or TSF/14-3-3/FD) in axillary buds. Genetic interactions between *BRC1* and FT and other flowering-pathway genes also support the model that BRC1 inhibits the activity of florigen in axillary buds.

It was also demonstrated that the 2 florigen genes are involved in the promotion of axillary shoot elongation.³ In axillary shoots of *ft*, *tsf*, and *ft tsf* mutants, the onset of elongation is delayed and the growth rate is reduced. It was also confirmed that these alterations are independent of the florigen's effect on the floral transition in the primary shoot. These findings imply that

florigen action is not restricted to the promotion of floral transition, but is also involved in coordinated systemic changes of the plant upon flowering. This fits well with a recent notion of multifaceted physiological roles of florigen in diverse plant species (for a review, see ref. 4).

Here, we report another possible role of the 2 florigen genes in axillary bud development revealed by ectopic overexpression analysis. It was previously reported that double overexpression of FT (or TSF) in combination with LEAFY (LFY) under the control of cauliflower mosaic virus (CaMV) 35S promoter drastically accelerates flowering, resulting in formation of the whole shoot system with a few leaves and a single terminal flower.⁵⁻⁹ Closer observation revealed that ectopic overexpression of FT or TSF with LFY induced ectopic axillary buds at the base of cotyledonary petiole in *35S:LFY*-, *35S:FT*-, or *35S:LFY*-, *35S:TSF*- plants, which do not usually appear in the wild-type plants of laboratory accessions such as Columbia (Col) and Landsberg *erecta* (*Ler*) (Fig. 1 and Table 1; see also Figure 3E of ref. 6). LFY or TSF single overexpression also induced ectopic buds at the cotyledonary axils, but at much lower frequency than the double overexpression, which resulted in ectopic bud formation at no less than one-fourth of cotyledonary axils (Table 1). The cotyledonary buds of the double overexpression plants usually consisted of a single floral bud with peduncle, while those of the LFY or TSF single overexpression plants remained without macroscopic floral organs, indicating the single overexpression of either gene is insufficient to support further development (Fig. 1B). These

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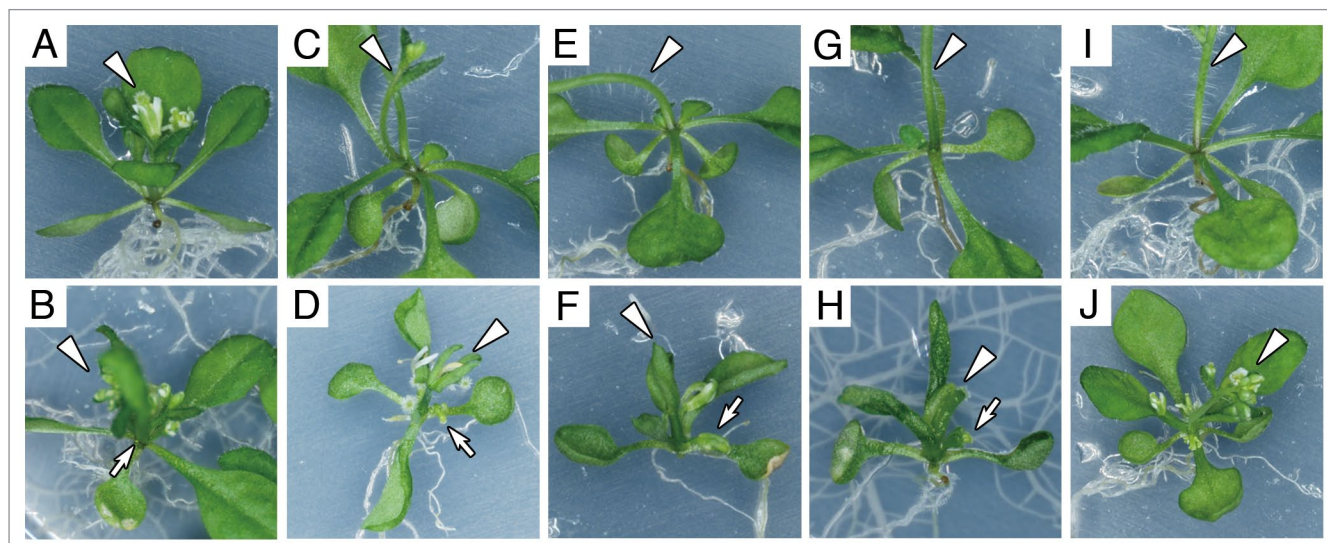


Figure 1. Representative image of transgenic plants. *35S:LFY* (homozygous) or *35S:LFY*^{-/-} (hemizygous) (A, B), *35S:FT* #1 (weak line) (C), *35S:LFY*^{-/-}; *35S:FT* #1/- (D), *35S:FT* #11 (strong line) (E), *35S:LFY*^{-/-}; *35S:FT* #11/- (F), *35S:TSF* #2 (strong line) (G), *35S:LFY*^{-/-}; *35S:TSF* #2/- (H), *35S:TSF* #4 (very weak line) (I), *35S:LFY*^{-/-}; *35S:TSF* #4/- (J) are shown. Plants were grown on 1/2 MS agar plates for 15 d under 16h light/8h dark long day conditions at 22 °C. Arrowheads indicate primary inflorescences or flowers. Arrows indicate ectopic axillary buds at cotyledonary axils. Transgenic lines (all in Col background) were described previously.^{6,8}

Table 1. Frequency of ectopic buds at cotyledonary axils

Genotype	Number of axillary buds at cotyledonary axils	Frequency of axillary bud formation per cotyledonary axil	Number of plants
<i>35S:LFY</i> or <i>35S:LFY</i> ^{-/-}	2	0.04	25
<i>35S:FT</i> #1	0	0	41
<i>35S:FT</i> #11	0	0	42
<i>35S:TSF</i> #2	1	0.01	38
<i>35S:TSF</i> #4	0	0	45
<i>35S:LFY</i> ^{-/-} ; <i>35S:FT</i> #1/-	6	0.38	8
<i>35S:LFY</i> ^{-/-} ; <i>35S:FT</i> #11/-	9	0.25	18
<i>35S:LFY</i> ^{-/-} ; <i>35S:TSF</i> #2/-	11	0.42	13
<i>35S:LFY</i> ^{-/-} ; <i>35S:TSF</i> #4/-	0	0	14

Plants were grown under same conditions as in **Figure 1** for 15 d and the number of axillary buds at cotyledonary axils was counted under a dissecting microscope. Note that the combination between *35S:TSF* #4 (very weak transgene) and *35S:LFY*^{-/-} did not result in axillary bud formation at cotyledonary axils.

observations suggest that FT and TSF, combined with LFY, promote initiation or early development of axillary buds in addition to floral transition and elongation of axillary buds.

Ectopic buds at the cotyledonary axils in these plants are reminiscent of *brc1* mutant, which sometimes develops axillary shoots on the cotyledonary axils.¹⁰ Although how phenotypes of these plants are related in terms of molecular interactions remains to be examined, it is envisaged that BRC1 acts antagonistically to FT and TSF at cotyledonary axils in a similar manner as we previously reported for axillary buds of foliage leaves. Besides *brc1* mutant, some mutants such as *shoot meristemless* (*stm*) also develops ectopic cotyledonary buds, suggesting that *Arabidopsis* has potential to form buds at the cotyledonary axils, although initiation or early development of the buds is suppressed in the

wild-type background of accessions such as Col and *Ler* and/or under normal laboratory growth conditions.¹¹ It was previously suggested that *LFY* stimulates meristematic activity in *Arabidopsis* and other plants.¹²⁻¹⁵ This may explain why *LFY* single overexpression can initiate ectopic buds at the cotyledonary axils although the frequency is very low. That *FT* plays a role in axillary bud initiation was also previously proposed. In certain mutant backgrounds of *Arabidopsis*, such as *stm-10*, further loss of *FT* function causes reduction in the number of axillary buds, implying *FT* has a role in the initiation of axillary meristems redundantly with other factors.^{14,16} Our observation that ectopic overexpression of *FT* alone was not sufficient to induce cotyledonary meristems also indicates that FT requires other factors to promote the formation of axillary buds (Table 1). Thus, our present work provides support for the previously proposed role of *LFY* and *FT* in axillary meristem initiation or development.

In conclusion, florigen (FT and TSF) in *Arabidopsis* regulates meristem initiation or early development of axillary buds. Taken together with our recent findings and previous reports by others,^{1,3,14,16} we propose that florigen is involved in multiple steps of axillary bud development, likely to coordinate axillary shoot development with flowering. In other species, *FT* homologs are also associated with regulation of growth and maturation in various organs such as dormant buds in poplar, compound leaves in tomato, and tubers in potato.¹⁷⁻¹⁹ The modulation of florigen activity by BRC1 suggests the existence of specific modes of action and modulation of the florigen complex depending on organs. It is an important problem to elucidate detailed molecular mechanisms enabling multi-faceted roles of florigen.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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