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RESEARCH PAPER

Specification of reproductive meristems requires the combined function of SHOOT MERISTEMLESS and floral integrators FLOWERING LOCUS T and FD during *Arabidopsis* inflorescence development

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Abstract

In *Arabidopsis*floral meristems are specified on the periphery of the inflorescence meristem by the combined activities of the FLOWERING LOCUS T (FT)–FD complex and the flower meristem identity gene *LEAFY*. The floral specification activity of FT is dependent upon two related BELL1-like homeobox (BLH) genes *PENNYWISE* (*PNY*) and *POUND-FOOLISH* (*PNF*) which are required for floral evocation. PNY and PNF interact with a subset of KNOTTED1-LIKE homeobox proteins including SHOOT MERISTEMLESS (STM). Genetic analyses show that these BLH proteins function with STM to specify flowers and internodes during inflorescence development. In this study, experimental evidence demonstrates that the specification of flower and coflorescence meristems requires the combined activities of FT–FD and STM. FT and FD also regulate meristem maintenance during inflorescence development. In plants with reduced STM function, ectopic FT and FD promote the formation of axillary meristems during inflorescence development. Lastly, gene expression studies indicate that STM functions with FT–FD and AGAMOUS-LIKE 24 (AGL24)–SUPPRESSOR OF OVEREXPRESSION OF CONTANS1 (SOC1) complexes to up-regulate flower meristem identity genes during inflorescence development

Key words: Coflorescence, development, floral transition, flower specification, homeobox, inflorescence, shoot apical meristem.

Introduction

The shoot apical meristem (SAM) is the site at which organs, meristems, and structures are produced such as leaves, axillary meristems (AMs), and internodes (Sablowski, 2007; Barton, 2009; Bleckmann and Simon, 2009; Dodsworth, 2009). The continuous growth and development displayed by shoots is dependent upon the ability of the meristem to maintain an intricate balance between the perpetuation of stem cells in the central apical zone and the organogenic mechanisms that specify lateral organs and meristems on the periphery (Bennett and Leyser, 2006; Sablowski, 2007; Barton, 2009; Bleckmann and Simon, 2009; Dodsworth, 2009).

Specific members of the KNOTTED1-like HOMEOBOX (KNOX) family of transcription factors regulate SAM function during plant development (Hake *et al.*, 2004; Scofield and Murray, 2006; Hay and Tsiantis, 2009). In addition, Class I KNOX proteins regulate leaf dissection in a subset of plants (Champagne and Sinha, 2004; Barkoulas *et al.*, 2008). Null alleles of *knotted1* (*kn1*) and *shoot meristemless* (*stm*) produce terminal shoots comprised of cotyledons and, in some cases, a leaf or two, in maize and *Arabidopsis*, respectively (Barton and Poethig, 1993; Vollbrecht *et al.*, 2000). In *Cardamine hirsuta*, RNAi lines directed against the orthologue of *STM* also produce

a terminal shoot phenotype (Hay and Tsiantis, 2006). Interestingly, an allele of *stm* called *gorgon* causes an increase in the size of the SAM indicating that STM regulates stem cell homeostasis (Takano *et al.*, 2010). *STM* and *kn1* also act to regulate reproductive patterning events as plants with decreased levels of these *KNOX* genes alter flower patterning, branching, as well as internode growth (Clark *et al.*, 1996; Endrizzi *et al.*, 1996; Kerstetter *et al.*, 1997; Kanrar *et al.*, 2006; Scofield *et al.*, 2007; Yu *et al.*, 2009; Takano *et al.*, 2010).

KNOX proteins interact with members of the BELL1-like HOMEODOMAIN (BLH) proteins (Hake *et al.*, 2004). In *Arabidopsis*, BLH proteins regulate developmental pathways that control plant architecture, organ specification, and phase change (Hamant and Pautot, 2010). For example, two paralogous BLH proteins, PENNYWISE (PNY: also known as BLH9, BELLRINGER, REPLUMLESS, and VAAMANA) and POUND-FOOLISH (PNF), are essential for floral evocation, internode patterning, and specification of AMs during inflorescence development (Byrne *et al.*, 2003; Roeder *et al.*, 2003; Smith and Hake, 2003; Bao *et al.*, 2004; Bhatt *et al.*, 2004; Smith *et al.*, 2004; Cole *et al.*, 2006; Rutjens *et al.*, 2009). In addition, genetic studies indicate that BLH proteins ARABIDOPSIS THALIANA HOMEOBOX1 (ATH1), PNY, and PNF function with STM to maintain meristem maintenance patterning events during shoot development (Byrne *et al.*, 2003; Bhatt *et al.*, 2004; Kanrar *et al.*, 2006; Rutjens *et al.*, 2009). Therefore, Class I KNOX function is modulated through the interaction with specific BLH proteins, which co-ordinate meristem maintenance and shoot patterning events throughout development.

Shoot and organ architecture are modified and altered as plants transition through each phase of development (Poethig, 2003). The floral transition is a major developmental phase change in which flower inductive cues produced in the leaves converge at the SAM to mediate the transition from vegetative to inflorescence development (Kobayashi and Weigel, 2007; Turck *et al.*, 2008; Zeevaart, 2008). FLOWERING LOCUS T (FT) functions as a mobile photoperiodic signal that moves from the leaves to the SAM to promote flowering (Kobayashi and Weigel, 2007; Turck *et al.*, 2008; Zeevaart, 2008). In the SAM, FT associates with the b-ZIP transcription factor, FD, to promote floral evocation and flower meristem specification (Pnueli *et al.*, 2001; Abe *et al.*, 2005; Wigge *et al.*, 2005). Moreover, recent studies in tomato not only demonstrate the mobile flowering function of FT, but also show that FT modifies leaf morphology and meristem activity in conjunction with auxin and TERMINAL FLOWER1 (TFL1), respectively (Shalit *et al.*, 2009).

In *Arabidopsis*, flower specification involves the activation of flower meristem identity genes during AM development (Liu *et al.*, 2009). Flower specification is controlled in part by *LEAFY* (*LFY*), which is induced by multiple flowering time pathways (Nilsson *et al.*, 1998; Blazquez and Weigel, 2000; Schmid *et al.*, 2003; Eriksson *et al.*, 2006). Two MADS box transcription factors, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) and AGAMOUS-LIKE 24 (AGL24) function together to activate *LFY* directly in response to floral inductive cues (Lee *et al.*, 2008; Liu *et al.*, 2008). In turn, LFY positively regulates APETALA1 (*AP1*) directly and through a cascade of late flower meristem identity genes (Bowman *et al.*, 1993; Schultz and Haughn, 1993; Parcy *et al.*, 1998; Liljegren *et al.*, 1999; Wagner *et al.*, 1999; William *et al.*, 2004; Saddic *et al.*, 2006). Once activated, AP1 maintains *LFY* expression, creating a positive feed-back loop, which functions to maintain flower meristem identity (Bowman *et al.*, 1993; Schultz and Haughn, 1993; Liljegren *et al.*, 1999). The FT–FD complex also functions to specify flower meristem identity by directly activating *AP1* (Ruiz-García *et al.*, 1997; Abe *et al.*, 2005; Wigge *et al.*, 2005). In addition, the FT–FD complex indirectly regulates *LFY* by positively regulating *SOC1* (Abe *et al.*, 2005; Moon *et al.*, 2005; Wigge *et al.*, 2005; Yoo *et al.*, 2005; Searle *et al.*, 2006).

Recent studies showed that the expression of *LFY* and *AP1* requires PNY and PNF (Smith *et al.*, 2004; Kanrar *et al.*, 2008). Moreover, the flower specification function of FT is dependent upon PNY and PNF (Kanrar *et al.*, 2008). Lastly, the interplay between PNY/PNF and the floral specification integrators LFY and FT is not only crucial for floral determination but this network also regulates the formation of coflorescence meristems. Given the interplay between PNY/PNF and FT together with the genetic and biochemical studies showing that STM–PNY and STM–PNF act to specify floral meristems, the relationship between STM and FT–FD was examined during inflorescence development. In this study, genetic analyses showed that the specification of coflorescence and floral meristems requires both STM and FT/FD function during inflorescence development. Surprisingly, a role for FT in meristem maintenance and carpel development was identified. Based on gene expression studies, it is proposed that STM functions with FT–FD and AGL24–SOC1 for the activation of flower meristem identity genes.

Materials and methods

Genetic analyses

The *Arabidopsis* plants used in this study were grown under long-day growth conditions: 16/8 h light/dark cycle at 22 °C. Genetic studies were performed to analyse the inflorescence phenotypes resulting from combining *stm-10* with *ft-2* and *fd-3* mutants, in the Columbia ecotype (Koornneef *et al.*, 1991; Abe *et al.*, 2005; Wigge *et al.*, 2005; Kanrar *et al.*, 2006). The *ft-2* and *fd-3* mutants are likely null alleles, while *stm-10* is a weak allele in which a stop codon is located in the first helix of the homeodomain (Koornneef *et al.*, 1991; Kardailsky *et al.*, 1999; Abe *et al.*, 2005; Wigge *et al.*, 2005; Kanrar *et al.*, 2006).

In order to determine the genetic relationship between *FT* and *STM*, *ft-2* was backcrossed into the Columbia ecotype three times (Kanrar *et al.*, 2008). *FT* and *STM* are located on chromosome one, separated by approximately 12.6 Mb. Pollen from *stm-10* was crossed to *ft-2* and F_2 seed was collected from F_1 plants derived from this cross. Because *FT* and *STM* are linked, the F_3 seed from all F_2 *ft-2* plants was collected individually. Subsequently, the F3 *ft-2* plants were screened for the *stm-10*-like phenotypes. Seed

derived from *ft-2 STM/stm-10* parental plants were used to characterize the inflorescence phenotypes of *ft-2 stm-10* plants, which segregated 25% of the time.

The genetic relationship between *STM* and *FD* was determined by transferring pollen from *stm-10* to the carpels of *fd-3* mutants. F_1 plants were self-pollinated and the resulting F_2 seed was planted out. 298 F_2 plants were scored: ~1/16 *fd-3 stm-10* plants, ~3/16 *fd-3* plants, ~3/16 *stm-10* plants, and ~9/16 wild-type plants. Genotype determination via PCR was used to verify *fd-3* homozygous plants. Seed collected from F_2 *fd-3* plants were screened for the *fd-3 stm-10* plants, which segregated 25% of the time. The progeny derived from the F_3 *fd-3 STM/stm-10* plants were used to characterize the *fd-3 stm-10* inflorescence phenotypes.

To determine how ectopic FT or FD alter reproductive patterning events in *stm-10*, *35S:FT* and *35S:FD* was crossed with *stm-10*. All F_1 plants flowered early and displayed the *35S:FT* or *35S:FD* phenotypes. The F_1 plants were self-pollinated and seed from these crosses were planted. Because *35S:FT* and *35S:FD* plants are resistant to the herbicide, glufosinate, the resulting F_2 plants were screened for: (i) *stm-10* like plants that flowered early and (ii) were resistant to the herbicide basta. To characterize the *35S:FT stm-10* and *35S:FD stm-10* inflorescences further, F_3 *35S:FT* and *35S:FD* plants that segregated for the *stm-10* phenotype were identified.

To determine the fold change in number of cauline leaves/coflorescences produced in *ft-2* and *fd-3*, the average number of cauline leaves produced by *ft-2* or *fd-3* was divided by the average number of cauline leaves initiated in wild-type (note: in wild-type, *ft-2* and *ft-3* plants, all cauline leaves contained a coflorescence shoot in its leaf axil). The fold change in the number of cauline leaves produced by *ft-2 stm-10* and *fd-3 stm-10* was calculated by dividing the average number of cauline leaves initiated in *ft-2 stm-10* and *fd-3 stm-10* by the average number of cauline leaves formed in *stm-10* inflorescence shoots.

In situ *hybridization*

The expression patterns of *AP1, LFY, AGL24*, and *SOC1* transcripts were examined in wild-type, *fd-3* and *stm-10* inflorescence apices as well as in the non-flower producing shoot tips of *fd-3 stm-10*. The *pKY89* vector containing the *AP1* cDNA lacking the MADS domain was a gift provided by Dr Xuemei Chen. The *AP1* UTP-digoxigenin anti-sense probe was synthesized using the SP6 RNA polymerase (Promega, Madison). Using the T7 RNA polymerase (Promega, Madison), the *LFY* UTP-digoxigenin anti-sense probe was synthesized from the *pDW122* vector (Weigel *et al.*, 1992). For localization of *AGL24* and *SOC1* transcripts, primers were designed and used to PCR amplify gene-specific sequences for these MADS-box genes. Primer sequences for SOC1 were SOC1-F (CTTATGAATTCGCCAGCTCC) and SOC1-R (GAAATAATACGACTCACTATAGGGACTCTAGAGAGGCAAGTGTAAGAACATAG). AGL24 primer sequences were AGL24-F (CTCCAGCTCAAGAATGAGAGAC) and AGL24-R (GAAATAATACGACTCACTATAGGGACTCATTCCCAAGATGGAAGCCCAAGC). The T7 RNA polymerase was used to synthesize the UTP-digoxigenin anti-sense *SOC1* and *AGL24* probes [note: the reverse (R) primer contains the T7 promoter (underlined)]. Plant fixation, sectioning, and mRNA *in situ* hybridization were performed as described previously (Jackson, 1991; Chuck *et al.*, 2002).

Results

The combined functions of FT, FD, and STM are crucial for flower formation

In *Arabidopsis*, inflorescence architecture is, in part, dependent upon developmental patterning events that regulate the formation and identities of AMs (Benlloch *et al.*, 2007; Prusinkiewicz *et al.*, 2007). In wild-type plants, the SAM initiated 2–4 (average=3.2) coflorescence meristems subtended by cauline leaves during the initial stages of inflorescence development (Fig. 1A) (Table 1: row 1, column 2). After the SAM completed the vegetative to inflorescence transition, the SAM initiated flowers (Fig. 1A), which are subtended by cryptic bracts (Long and Barton, 2000). To determine the interplay between STM and FT or FD, the inflorescence phenotypes of single and double mutant combinations were characterized. In these analyses, loss of function alleles of *ft-2* or *fd-3* was combined with a weak allele of *stm*, *stm-10*, to determine the role of these gene products in floral specification.

In *ft-2* and *fd-3* mutants, the SAM initiated 3.1-fold and 2.2-fold more coflorescence meristems subtended by cauline leaves than wild-type plants, respectively, indicating that the FT–FD complex plays a role in the specification of floral meristems (Fig. 1B, G; Table 1: rows 2 and 3, column 2) (Ruiz-García *et al.*, 1997; Abe *et al.*, 2005; Wigge *et al.*, 2005). Overall, the inflorescences of *ft-2* and *fd-3* produced similar numbers of flowers as the wild type (Table 1: rows 1–3, column 3). In *stm-10*, an inflorescence shoot typically produced 4–11 (average=6.9) cauline leaves and 0–4 (average=2.3) floral nodes before shoot growth ceased with the formation of a terminal flower (Fig. 1C, inset; Table 1: row 4, columns 2 and 3). The shoots of *stm-10* also displayed an internode patterning defect (data not shown). In *stm-10* plants, the terminal growth habit of the primary reproductive shoots resulted in the outgrowth of secondary coflorescences shoots, which also produced terminal flowers. This pattern of growth, arrest, and the initiation of higher order coflorescences, repeated with each successive shoot resulting in a bushy phenotype (data not shown).

Genetic studies showed that three classes of inflorescence phenotypes were produced when *ft-2* and *fd-3* were combined with *stm-10* (Fig. 1D–F and H–J, respectively; Table 2: columns 1–3). The first phenotypic class had an overall morphology similar to *stm-10* inflorescences; however, this class of *ft-2 stm-10* and *fd-3 stm-10* reproductive shoots produced, on average, 2.2-fold and 2.9-fold more cauline leaves than the *stm-10* plants, respectively, before the formation of the terminal flower (Fig. 1D, H; Table 2: column 1). The second inflorescence phenotypic class displayed a non-flower-producing phenotype in which these inflorescences initiated cauline leaves for 90–200 d before the plants senesced without producing a single flower (Fig. 1E, I, K, L; Table 2: column 3). These inflorescences produced up to 100 cauline leaves with shoots growing up to 78 cm in length (data not shown). Internode patterning defects observed in *ft-2 stm-10* and *fd-3 stm-10* were probably due to the decrease in STM function, since the inflorescences of *stm-10* often produced shortened internodes. Taken together, the fact that *ft-2 stm-10* and *fd-3 stm-10* produced significantly more cauline leaves before the SAM was converted into a terminal flower showed that the combined functions of STM and FT/FD are crucial for the specification of flower meristems.

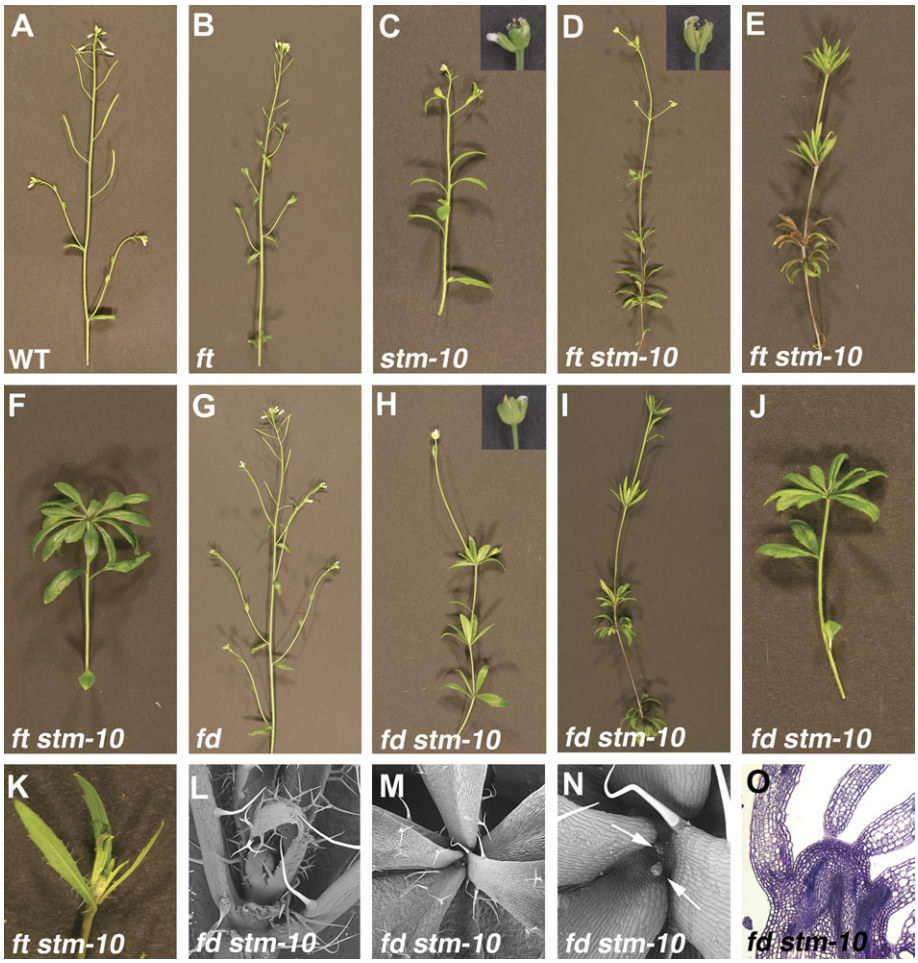

Fig. 1. Interplay between STM and FT/FD is crucial for inflorescence development. (A) Wild-type, (B) *ft*, (C) *stm-10*, (D–F) *ft stm-10,* (G) *fd-3*, and (H–J) *fd stm-10* inflorescences. The Class I phenotype for (D) *ft stm-10* and (H) *fd stm-10* initiated inflorescences that were morphologically similar to *stm-10*; however, these shoots initiate approximately 2-fold more cauline leaves than *stm-10* before terminating with flowers (inset: Table 2). The (E) *ft stm-10* and (I) *fd-3 stm-10* non-flowering Class II phenotype produced inflorescences that initiated cauline leaves indefinitely. In the Class III inflorescence shoots, (F) *ft-2 stm-10* and (J) *fd-3 stm-10* terminated without producing a single flower generating an 'umbrella' phenotype. Close up of the Class II non-flowering producing (K) *ft-2 stm-10* and (L) *fd-3 stm-10* apices. Bar=0.5 mm. (M–N) Scanning Electron Microscopy images of *fd-3 stm-10* umbrella like apices. Arrows point at stipules. (M) Bar=1 mm and (N) Bar=0.25 mm. (O) Histological longitudinal cross section through an *fd-3 stm-10* umbrella-like apex. Bar=0.3 mm.

In the third phenotypic class of inflorescences produced by *ft-2 stm-10* and *fd-3 stm-10*, the SAM terminated with a compact cluster of cauline leaves, resembling an umbrella (Fig. 1F, J; Table 2: column 2). The umbrella-like inflorescence shoots initiated 8–18 (average=12.7) cauline leaves before growth terminated, without producing a single flower. Examination of the shoot apices of these plants showed that mature leaves directly emanated from the centre of the inflorescence apex (Fig. 1M). Scanning electron microscopy (SEM) demonstrated that the umbrella apex of *fd-3 stm-10* lacked a meristem (Fig. 1N) (note: stipules were detected at the base of the cauline leaves). Histological examination of these umbrella apices showed that meristems were not evident in shoot apex of *fd-3 stm-10* (Fig. 1O). Similar results were obtained with *ft-2 stm-10* umbrella-like shoots (data not shown). These results indicate that FT and FD function with STM to maintain meristem integrity during inflorescence development.

Specification of coflorescence meristems requires STM and FT–FD

Since FT plays a role in coflorescence specification (Ruiz-García *et al.*, 1997; Kanrar *et al.*, 2008), the role of STM and FD/FT in the formation of coflorescence meristems was investigated. Coflorescences develop in the axils of cauline leaves in wild-type, *ft-2*, and *fd-3* (Fig. 2A, B, C, respectively). In *stm-10* plants, coflorescences developed in the axils of cauline leaves 55% of the time (Fig. 2D, H; Table 3: row 4, column 6). However, 45% of the cauline leaves produced by *stm-10* inflorescence were devoid of

Table 1. Floral specification

The average number of nodes (Ns), cauline leaves (CLs), and flowers (FLs) was determined for each genotype. Standard deviation was determined and displayed in parentheses. In our analysis, Ns, CLs, and FLs were quantified in the Class I *ft-2 stm-10* and *fd-3 stm-10*. The percentage of flowering was determined by dividing the average number of flowers by the average number organs produced by the inflorescence shoot. *Note: Student's *t* test was performed (P <0.0001).

Genotype	1 Ns	2 CLs	3 FLs	4 %FL
1. Wild type	42.1 (2.5)	3.2 (0.47)	40.5 (3.6)	96%
2. *ft-2*	44.1 (4.1)	9.9 (1.2)	39.8 (3.1)	90%
3. *fd-3*	42.7 (3.6)	7.0 (0.8)	40.1 (4.2)	93%
4. *stm-10*	9.4 (2.4)	6.9 (1.8)	2.3 (1.2)	24%
5. *ft-2 stm-10*	17.7 (7.2)	15.5 (6.9)	2.1 (1.5)	11%
6. *fd-3 stm-10*	22.5 (9.4)	20.5 (9.6)	1.9 (1.3)	8%
7. *35S:FT*	8.1 (1.7)	1.3 (0.44)	6.6 (2.1)	81%
8. *35S:FT stm-10*	9.7 (4.3)	4.4 (1.0)	5.1 (4.1)	52%
9. *35S:FD*	32.3 (2.7)	2.6 (0.49)	29.5 (3.2)	91%
10. *35S:FD stm-10*	10.4 (2.4)	7.2 (1.7)	3.1 (2.7)	29%

Table 2. Penetrance of the classes of inflorescence phenotypes produced in *ft stm-10* and *fd stm-10*

Phenotypes	1 *stm-10*-like	2 Umbrella	3 Non-flower producing
1. *ft stm-10*	72%	10%	18%
2. *fd stm-10*	46%	23%	31%

coflorescence development and referred to as the solitary cauline leaf phenotype (Fig. 2E, I; Table 3: row 4, columns 1 and 5). Histological analyses of *stm-10* inflorescences showed that coflorescence meristems were not specified in all cauline leaf axils (Fig. 2L). During the later stages of growth, coflorescence meristems developed into reproduction shoots (Fig. 2M). After termination of the primary *stm-10* shoot, AMs failed to develop in the axils of the solitary cauline leaves (Fig. 2O).

Inflorescence shoots of *ft-2 stm-10* and *fd-3 stm-10* showed a marked decrease in the development of coflorescences such that 74% and 81% of the inflorescences initiated solitary cauline leaves, respectively (Fig. 2F, G; Table 3: rows 5 and 6, columns 1 and 5). SEM analysis of the *ft-2 stm-10* and *fd-3 stm-10* showed that coflorescence meristems were not detected in the axils of the solitary cauline leaves (Fig. 2J, K). Further, histological analysis showed that AM formation was not apparent during the early stages of cauline leaf development in *fd-3 stm-10* (Fig. 2P, arrows). Similar results were obtained with *ft-2 stm-10* (data not shown). The substantial decrease in the specification of coflorescence meristems in *ft-2 stm-10* and *fd-3 stm-10* resulted in plants that were less bushy than *stm-10* (data not shown). Taken together, these results showed that the combined functions of STM and FT or FD are crucial for the specification of coflorescence meristems.

Ectopic FT partially restores reproductive meristems and structures in stm-10 *plants*

Previous studies indicate that FT activity is partially required for coflorescence specification (Ruiz-García *et al.*, 1997; Kanrar *et al.*, 2008). However, it has not been demonstrated that FT can promote the formation of AMs during inflorescence development. To determine if ectopic *FT* can induce AM formation, the development of coflorescence shoots in *35S:FT stm-10* plants was examined. Results showed that ectopic expression of *FT* in *stm-10* increased the specification of coflorescence shoots in the axils of cauline leaves from 55% to 84% (Fig. 3C, F; Table 3: row 8, columns 1, 5, and 6). Likewise, *35S:FD stm-10* displayed a 24% increase in coflorescence specification compared to *stm-10* (Table 3; row 10, columns 1, 5, and 6). The restoration of coflorescence specification was also apparent in high order coflorescence shoots of *35S:FT stm-10* (Fig. 3G) and *35S:FD stm-10* plants (data not shown). The increase in coflorescence specification in the reproductive shoots of *35S:FT stm-10* and *35S:FD stm-10* resulted in plants that were extremely bushy compared to *stm-10* (data not shown). Taken together, these results showed that both FT and FD function is not only required for coflorescence formation there but that these floral integrators also promote the specification of AMs. Moreover, increased levels of FT and FD partially compensate for STM, when the function of this homeodomain protein is reduced during reproductive development.

In *Arabidopsis*, the inflorescences of *35S:FT* plants transition rapidly to flower production, initiating fewer coflorescence shoots subtended by cauline leaves than wild-type plants (Fig. 3A; Table 1: row 7, column 2). *35S:FT* inflorescences produced 5–8 (average=6.6) floral nodes before the SAM was transformed into a floral meristem (Fig. 3H; Table 1: row 7, column 3). If STM is a crucial component, which functions with FT and FD to specify flowers, then an increase in the levels of FT and/or FD in *stm-10* may augment the floral specification potential in these shoots. Results showed that, *35S:FT stm-10* initiated flowers earlier in inflorescence development than *stm-10* (Table 1: row 8, column 2). Moreover, *35S:FT stm-10* plants, on average, produced twice as many flowers as *stm-10* (Fig. 3J, N; Table 1: row 8, column 3). Unlike FT, ectopic expression of FD in *stm-10* had little effect on the timing of flower specification (Table 1: row 10, column 2). Taken together, these results indicate that increased levels of FT can partially restore meristem activity and floral specification potential when STM levels are limiting.

STM function is required for carpel formation and development (Clark *et al.*, 1996; Endrizzi *et al.*, 1996; Scofield *et al.*, 2007; Yu *et al.*, 2009). Carpels specified during flower patterning developed into the fruits or siliques in wild-type plants (data not shown) and *35S:FT* (Fig. 3K). Unlike wild-type and *35S:FT* flowers, *stm-10* displayed a marked reduction in the specification of carpels (Fig. 3L, M) (Clark *et al.*, 1996; Endrizzi *et al.*, 1996; Scofield *et al.*, 2007; Yu *et al.*, 2009). Interestingly, 4% of the *35S:FT stm-10* plants initiated fused carpels

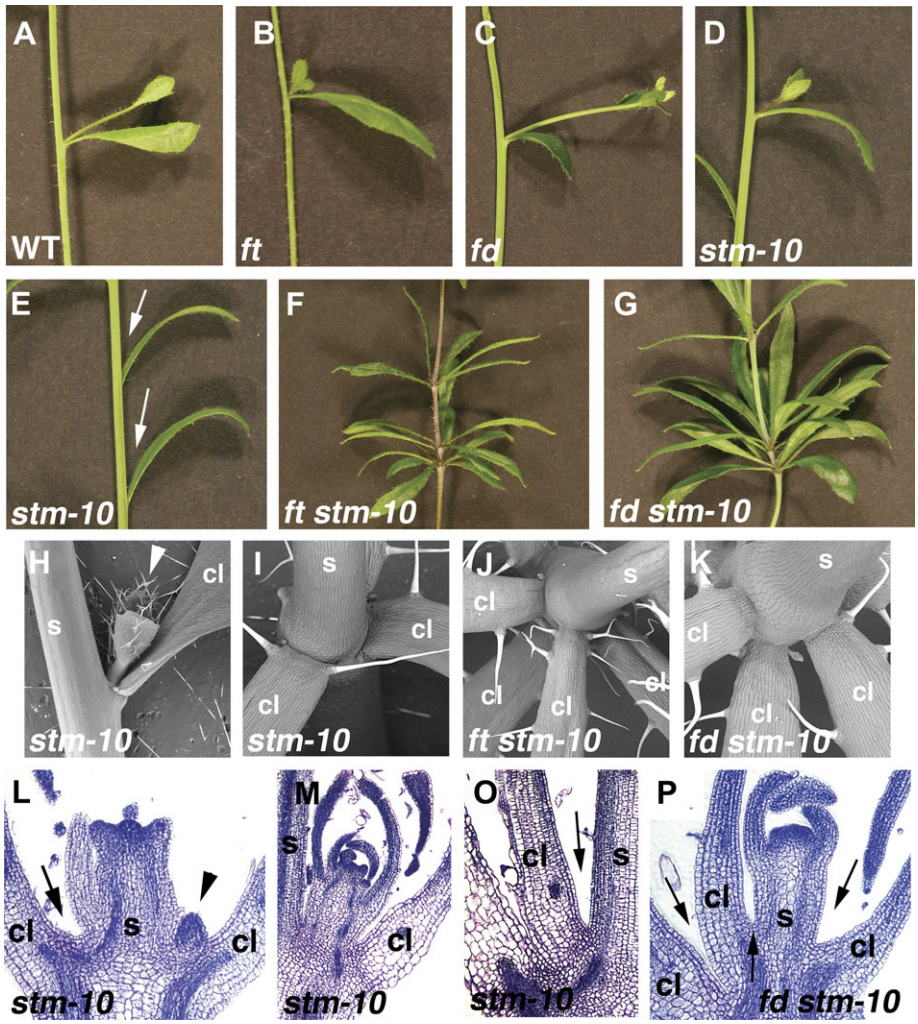

Fig. 2. Coflorescence meristem specification. Coflorescences develop in the axils of cauline leaves in (A) wild-type, (B) *ft-2*, and (C) *fd-3* plants. In *stm-10*, (D) coflorescence shoots develop in the axils of some cauline leaves, (E) while the remaining leaves displayed an empty axil phenotype, which were devoid of coflorescence development (arrows). (F) *ft-2 stm-10* and (G) *fd-3 stm-10* inflorescence stems displayed an increased number of empty leaf axils. (H) Scanning electron microscopy image of a coflorescence shoot that developed in the axil of a cauline leaf in *stm-10* (arrowhead points to coflorescence shoot). Scanning electron image of empty leaf axils in (I) *stm-10*, (J) *ft-2 stm-10*, and (K) *fd-3 stm-10*. (L) Longitudinal cross-section through an *stm-10* inflorescence apex (arrow points to empty leaf axil and arrow-head points to a coflorescence meristem). (M) Transverse section through an *stm-10* cauline leaf with a young coflorescence that developed in the axil. (O) After termination of shoot development, coflorescence meristems failed to develop in the axils of solitary cauline leaves in *stm-10*. (P) Longitudinal section through an *fd-3 stm-10* non-flowering apex. Arrows point at the empty cauline leaf axils. (H, I) Bar=0.5 mm. (J, K) Bar=0.25 mm. (L–P) Bar=0.5 μm. cl, cauline leaf; s, stem.

(Fig. 3N), which produced 3–5 seeds. When germinated, these seeds gave rise to plants with the *35SFT stm-10* phenotype (data not shown). Taken together, these experiments show that increased levels of FT can partially compensate for a reduction in STM activity during carpel development.

Expression of flower meristem identity genes in fd-3 stm-10 *non-flower producing shoots*

During inflorescence development, flowers are specified on the flanks of the SAM by the activity of flower meristem identity genes (Liu *et al.*, 2009). To determine if STM acts with FT/FD to specify flower meristem identity, the expression patterns of *AP1* and *LFY* were examined in wild-type, *fd-3*, *stm-10*, and the non-flower producing *fd-3 stm-10* inflorescence apices. *In situ* hybridization was performed in *fd-3 stm-10*, since the non-flowering phenotype was less penetrant in *ft-2 stm-10* (Table 2, column 3).

In the wild type, *AP1* transcripts accumulate in floral meristems and eventually become restricted to whorls 1 and 2 during the later stages of flower development (Mandel *et al.*, 1992) (Fig. 4A). In *fd-3*, the onset of *AP1* expression in AMs is delayed because flower meristems are converted to coflorescence meristems during the early stages of inflorescence development (Wigge *et al.*, 2005). However,

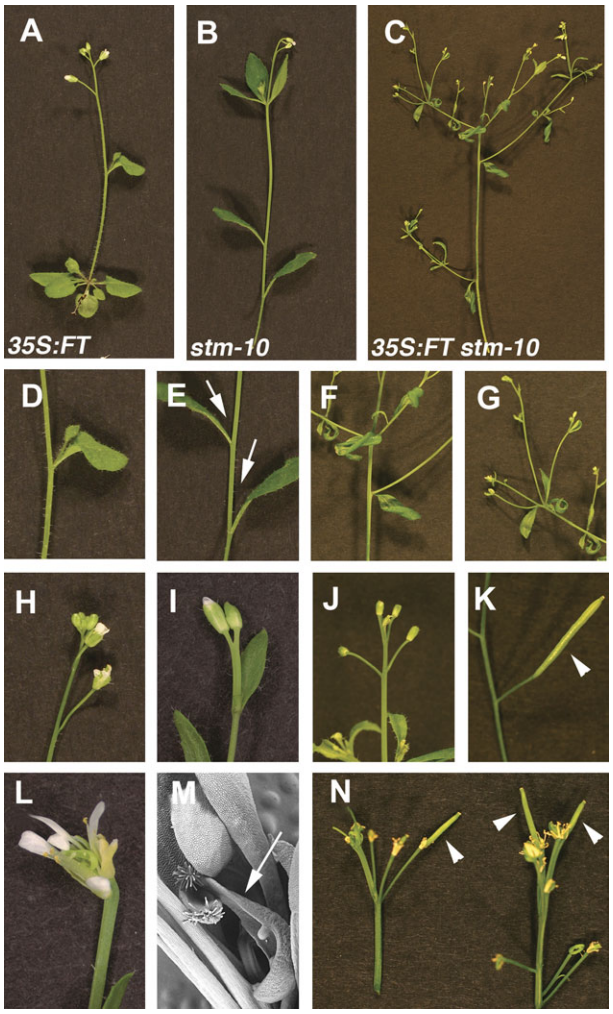

Fig. 3. Ectopic *FT* partially rescues the reproductive defects displayed in *stm-10*. (A) *35S:FT* plant. Inflorescence shoots of (B) *stm-10* and (C) *35S:FT stm-10*. (D–F) Close up of cauline leaf on the inflorescence shoot of (D) *35S:FT*, (E) *stm-10*, and (F) *35S:FT stm-10*. (G) *35S:FT stm-10* secondary coflorescence shoot initiated multiple cauline leaves and axillary shoots. (E) Arrows point to the empty leaf axils in the *stm-10* inflorescence. (H) Terminal flower in (H) *35S:FT* and (I) *stm-10*. (J) Close up of an inflorescence shoot initiating mutiple flowers in *35S:FT stm-10*. (K) *35S:FT* silique. Arrow-head points at the silique. (L, M) Carpel-like organs developed in an *stm-10* flower. Bar=0.5 mm. (N) At a low penetrance, siliques develop in *35S:FT stm-10* plants. Arrow-heads point to the siliques.

Table 3. Production of the 'solitary' cauline leaves displayed on the main shoot

The average number of solitary cauline leaves (SCLs) produced by the inflorescences was determined for each genotype. The standard deviation (SD) was determined and the number (*n*) of shoots examined is also displayed in the table. The % of SCLs produced was calculated by dividing the averages number of SCLs by the average number of total cauline leaves with and without a coflorescence shoot in its axil (Table 2). The percentage of cauline leaves containing a coflorescence shoot in the leaf axil is also displayed (%CLs). In our analysis, SCLs were quantified in Class I shoots of *ft-2 stm-10* and *fd-3 stm-10*. Student's *t* test was performed ($P < 0.0001$).

Genotype	1 SCLs	2 Range	3 SD	4 *n*	5 %SCL	6 %CL
1. Wild type	0	0	0	51	0%	100%
2. *ft-2*	0	0	0	51	0%	100%
3. *fd-3*	0	0	0	51	0%	100%
4. *stm-10*	3.1	1–7	1.4	51	45%	55%
5. *ft-2 stm-10*	11.5	3–32	6.73	51	74%	26%
6. *fd-3 stm-10*	16.6	5–44	9.1	51	81%	19%
7. *35S:FT*	0	0	0	51	0%	100%
8. *35S:FT stm-10*	0.69	0–2	0.54	51	16%	84%
9. *35S:FD*	0	0	0	51	0%	100%
10. *35S:FD stm-10*	1.5	0–3	0.87	51	21%	79%

once *fd-3* shoots complete the transition from coflorescence to flower production, the expression pattern for *AP1* was similar to the wild type (Wigge *et al.*, 2005) (Fig. 4B). In *stm-10*, *AP1* transcripts were detected in the SAM, possibly during the formation of the terminal flower (Fig. 4C). *AP1* transcripts were also detected in developing sepal and petal primordia (data not shown). Consistent with the non-flower producing phenotype, *AP1* was not detected in the *fd-3 stm-10* inflorescence apices (Fig. 4D). Because LFY controls *AP1* expression in parallel with FT–FD, the expression patterns of *LFY* in *fd-3 stm-10* were examined. In wild-type and *fd-3*, *LFY* transcripts were visualized in cells on the flanks of the SAM and in developing flower meristems (Weigel *et al.*, 1992) (Fig. 4E, F). *LFY* transcripts were also detected in the terminal flower meristems of *stm-10* (Fig. 4G). However, in *fd-3 stm-10*, *LFY* expression was dramatically reduced in the shoot apices of these non-flower-producing inflorescences (Fig. 4H). Taken together, the *in situ* hybridization results show that the combined functions of STM and FT–FD are crucial for activating *LFY* and *AP1*.

MADS-box floral integrator genes are expressed in the SAM of fd-3 stm-10 *non-flower-producing shoots*

The floral integrator genes *SOC1* and *AGL24* are expressed in the inflorescence meristem and function together to activate *LFY* during reproductive development (Lee *et al.*, 2008; Liu *et al.*, 2008). Because LFY is not expressed in the non-flower-producing shoots of *fd-3 stm-10*, the expression pattern of *AGL24* and *SOC1* was examined during inflorescence development. In the wild type, *AGL24* and *SOC1* transcripts were detected in the inflorescence meristem (Borner *et al.*, 2000; Samach *et al.*, 2000; Yu *et al.*, 2002; Michaels *et al.*, 2003) (Fig. 5A, E, respectively). During inflorescence development, *AGL24* and *SOC1* mRNAs localized to the SAM in *fd-3* and *stm-10* reproductive shoots (Fig. 5B, C, F, G). Interestingly, in the non-flower-producing inflorescence shoots of *fd-3 stm-10*, both *AGL24* and *SOC1* transcripts were visualized in the SAM (Fig. 5D, H, respectively). Thus, in the absence of FD, SOC1 and AGL24 partially depend on the function of STM in order to activate *LFY*.

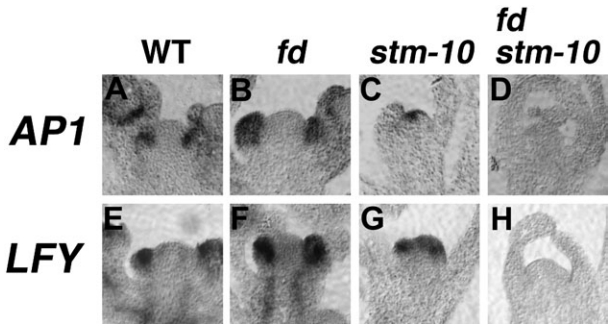

Fig. 4. Expression analysis of flower meristem identity. Localization of *AP1* transcripts in (A) wild-type, (B) *fd-3*, (C) *stm-10*, and (D) *fd-3 stm-10* reproductive apices. *LFY* mRNA was localized in (E) wild-type, (F) *fd-3*, (G) *stm-10*, and (H) *fd-3 stm-10* Class II inflorescence shoot tips. Bar=50 μm.

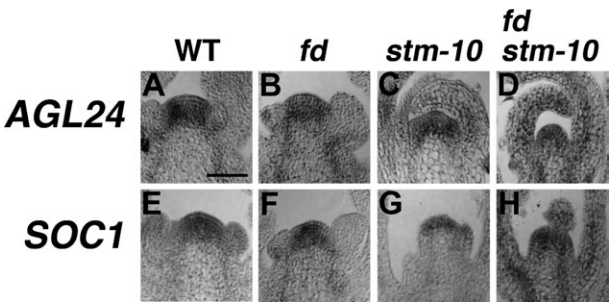

Fig. 5. Expression of floral integrator genes. Expression patterns for *AGL24* were determined in (A) wild-type, (B) *fd-3*, (C) *stm-10*, and (D) *fd-3 stm-10* Class II inflorescence apices. Localization of *SOC1* mRNA in (E) wild-type, (F) *fd-3*, (G) *stm-10*, and (H) *fd-3 stm-10* reproductive shoot apices. Bar=50 μm.

Discussion

The floral transition is a pivotal phase change event, which establishes reproductive growth patterns that are often distinct from vegetative modes of development (Benlloch *et al.*, 2007). It is proposed that, in response to flowering time signals, floral integrators must somehow act with the proteins that control meristem maintenance and function in order to establish and maintain inflorescence patterns of growth. FT is the universal florigen signal that promotes flowering and regulates meristem activity (Kobayashi and Weigel, 2007; Turck *et al.*, 2008; Zeevaart, 2008; Shalit *et al.*, 2009). In the SAM, FT associates with FD and together these proteins mediate the floral transition as well as flower meristem specification (Kobayashi and Weigel, 2007; Turck *et al.*, 2008; Zeevaart, 2008). At the molecular level, how FT regulates meristem activity is not well understood. STM and related KNOX proteins regulate meristem maintenance and reproductive pattern events during shoot maturation (Hake *et al.*, 2004; Scofield and Murray, 2006; Hay and Tsiantis, 2009). In this paper, a reduction in FT or FD enhances the floral and coflorescence specification phenotypes displayed in *stm-10*. At the same time, ectopic FT or FD restores coflorescence specification in *stm-10* plants. An increase in the levels of FT augments the floral specification potential of *stm-10* inflorescence meristems. Thus, STM, FT, and FD play a fundamental role in the specification of axillary meristems during reproductive development. It is proposed that STM–PNY and STM–PNF complexes act with FT and FD to promote the formation of coflorescence meristems as well as specify flower meristem identity (Figure 6).

Studies in tomato indicate that the homologue of FT called SINGLE FLOWER TRUSS (SFT) acts as a general plant growth regulator, which functions to promote meristem determinacy (Shalit *et al.*, 2009). The inhibitor of SFT, SELF-PRUNING (SP), which is a homologue of TFL1, acts to control the terminal growth effect of SFT in shoot meristems (Shalit *et al.*, 2009). Previous studies show that increased levels of *FT* convert the indeterminate inflorescence meristem into a determinate meristem with floral identity in *Arabidopsis* (Kardailsky *et al.*, 1999; Kobayashi and Weigel, 2007). In tomato, F_1 progeny derived from crossing *sft* mutants with different tomato varities, which are homozygous for *SFT* produce significantly more fruit than the parental lines (Krieger *et al.*, 2010). The heterozygous effect of *SFT* produces shoots that display a decrease in meristem determinacy, indicating that the levels of SFT is crucial for shoot architecture and productivity. In this study, ectopic FT promotes the formation of coflorescence and floral meristems as well as carpels during inflorescence and flower development, respectively, in *stm-10* plants. The fact that ectopic FT partially suppresses some of the reproductive phenotypes of STM indicates that these factors act to regulate inflorescence and floral development.

Networks controlling flower meristem identity

In *Arabidopsis*, flower meristem identity is specified on the flanks of the SAM by the activity of *LFY*. The promoter of *LFY* integrates floral inductive cues mediated by long-day photoperiod and gibberellin (Blazquez and Weigel, 2000). *SOC1* and *AGL24* encode MADS-box proteins that are induced by multiple flowering time pathways in the SAM (Borner *et al.*, 2000; Samach *et al.*, 2000; Yu *et al.*, 2002; Michaels *et al.*, 2003; Moon *et al.*, 2003). Recent studies indicate that the SOC1–AGL24 complex positively regulates *LFY* transcription in response to floral inductive cues (Yu *et al.*, 2002; Moon *et al.*, 2003; Lee *et al.*, 2008). Studies in this paper show that flower meristem specification is reduced and often completely impaired in *stm-10 fd-3* and *stm-10 ft-2* plants. Transcripts for *AGL24* and *SOC1* localize to the SAM in the *stm-10 fd-3* non-flower producing inflorescence shoots. However, *LFY* is not expressed in these *fd-3 stm-10* inflorescence shoots. Therefore, the SOC1–AGL24 complexes are partially dependent upon STM for the activation of *LFY*. In Fig. 6, it is proposed that SOC1–AGL24 complexes require STM–PNY/STM–PNF for the activation of *LFY*.

In yeast, specific mating cell types are specified by the co-operative interaction between the MADS-box protein minichromosome maintenance protein 1 (MCM1) and the

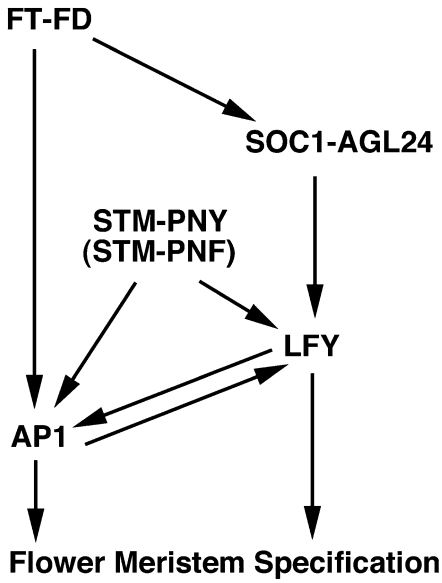

Fig. 6. A model for flower specification during *Arabidopsis* inflorescence development. It is proposed that STM-PNY/PNF dimers and the floral integrator complexes FT–FD and AGL24-SOC1 function to specify flower meristem identity. In this model, STM-PNY/PNF function with AGL24-SOC1 to regulate *LFY*. The late flower meristem identity gene *AP1* requires the combined functions of FT–FD, STM-PNY/PNF, and LFY-UFO complexes as well as SPLs.

homeodomain proteins Mating type-a (MATa) or MATalpha (Johnson, 1995). In plants, BELL1 (BEL1), the founding member of the BLH class of transcription factors, associates with the AGAMOUS and SEPALATA3 MADS-box dimer, possibly forming a trimeric complex, which acts to specify integument cell identity during ovule development (Brambilla *et al.*, 2007). Therefore, it may be possible that PNY/PNF–STM complexes directly associate with AGL24–SOC1 dimers and/or tetramers to specify flower meristem identity by activating *LFY*.

LFY functions to specify flower meristem identity by activating the late flower meristem identity genes, including *AP1* (Parcy *et al.*, 1998; Wagner *et al.*, 1999). LFY interacts with the F-box protein UNUSUAL FLORAL ORGANS (UFO), which is an orthologue of the *Petunia* DOUBLE TOP (DOT) protein (Hepworth *et al.*, 2006; Chae *et al.*, 2008; Souer *et al.*, 2008). Recent studies showed that UFO and DOT function in specifying flower meristem identity (Hepworth *et al.*, 2006; Souer *et al.*, 2008). Taken together, the LFY–UFO complex directly regulates *AP1* in a pathway parallel with FT–FD (Fig. 6) (Ruiz-García *et al.*, 1997; Abe *et al.*, 2005; Wigge *et al.*, 2005). Previous studies showed that FT–FD requires PNY and PNF for flower formation and the activation of *AP1* (Kanrar *et al.*, 2008). Because *AP1* expression is not detected in the non-flower producing *fd-3 stm-10* shoots, it is proposed that STM–PNY/PNF functions with the FT–FD and LFY–UFO complexes co-operatively to regulate *AP1* during the later stages of flower meristem specification (Fig. 6).

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