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; Blekman 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 organogenetic mechanisms that specify lateral organs and meristems on the periphery (Bennett and Leyser, 2006; Sablowski, 2007; Barton, 2009; Blekman and Simon, 2009; Dodsworth, 2009).

Specific members of the KNOTTED1-like HOMEBOX (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 BELL-like HOMEODOMAIN (BLH) proteins (Hake et al., 2004). In *Arabidopsis*, BLH proteins regulate developmental pathways that control plant architecture, organ specification, and phase change (Haman 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 HOMEBOX1* (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 (Penuel 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 (*API*) 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, API maintains *LFY* expression, creating a positive feedback 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 *API* (Ruiz-Garcia 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 *API* 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 genetic expression studies, it is proposed that STM functions with FT-FD and AGL24-SOCl 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* and *fd-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* 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* was crossed to *f1* and *F2* seed was collected from *F1* plants derived from this cross. Because *FT* and *STM* are linked, the *F3* seed from all *F2* *ft-2* plants was collected individually. Subsequently, the *F3* *ft-2* plants were screened for the *stm* phenotype. 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. F1 plants were self-pollinated and the resulting F2 seed was planted out. 298 F2 plants were scored: ~1/16 ft-3 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 F2 fd-3 plants were screened for the fd-3 stm-10 plants, which segregated 25% of the time. The progeny derived from the F1 ft-3 drm-1 STM/stm-10 plants were used to characterize the ft-3 drm-1 STM 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 F1 plants flowered early and displayed the 35S:FT or 35S:FD phenotypes. The F1 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 F2 plants were screened for: (i) 35S:FT like plants that flowered early and (ii) plants resistant to the herbicide basta. To characterize the 35S:FT stm-10 and 35S:FD stm-10 inflorescences further, F3, 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 API, LFY, AGL24, and SOCI transcripts were examined in wild-type, ft-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 API cDNA lacking the MADS domain was a gift provided by Dr Xuemei Chen. The API 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 (Wegel et al., 1992). For localization of AGL24 and SOCI transcripts, primers were designed and used to PCR amplify gene-specific sequences for these MADS-box genes. Primer sequences for SOCI were SOCI-F (CTTATGAAATTCCGACGTC) and SOCI-R (GAAATATCGACTCATAGAGAGCTGAGGCG-AAGTGTAGAGACATAG). AGL24 primer sequences were AGL24-F (CTCCAGCAGGAAATGAGGAGAC) and AGL24-R (GAAATATCGACTCATAGAGGAGCATTCCCAAGA-GAGGGCCGAG). The T7 RNA polymerase was used to synthesize the UTP-digoxigenin anti-sense SOCI 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; Chua 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.
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-Garcia 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 (2.5) | 2 CLs (0.47) | 3 FLs (3.6) | 4 %FL |
|------------------|------------|--------------|-------------|-------|
| 1. Wild type     | 42.1       | 3.2          | 40.5        | 96%   |
| 2. ft-2          | 44.1       | 9.9          | 39.8        | 90%   |
| 3. ft-3          | 42.7       | 7.0          | 40.1        | 93%   |
| 4. ft-2 stm-10   | 9.4        | 6.9          | 2.3         | 24%   |
| 5. ft-3 stm-10   | 17.7       | 15.5         | 2.1         | 11%   |
| 6. ft-3 stm-10   | 22.5       | 20.5         | 1.9         | 8%    |
| 7. 35S:FT        | 8.1        | 1.3          | 6.6         | 81%   |
| 8. 35S:FD        | 9.7        | 4.4          | 5.1         | 52%   |
| 9. 35S:FD        | 32.3       | 2.6          | 29.5        | 91%   |
| 10. 35S:FD stm-10| 10.4      | 7.2          | 3.1         | 29%   |

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

| Phenotypes | 1 ft 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 reproductive 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-Garcia 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 axis 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. 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,
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.

### 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 average 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 (%CCLs). In our analysis, SCLs were quantified in Class I shoots of rt-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 %CCL |
|------------------|--------|---------|-------|---------|--------|--------|
| 1. Wild type     | 0      | 0       | 0     | 51      | 0%     | 100%   |
| 2. rt-2          | 0      | 0       | 0     | 101     | 0%     | 100%   |
| 3. fd-3          | 0      | 0       | 0     | 101     | 0%     | 100%   |
| 4. stm-10        | 0.69   | 0–2     | 0.54  | 51      | 16%    | 84%    |
| 5. rt-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     | 101     | 0%     | 100%   |
| 8. 35S:FT stm-10 | 0.69   | 0–2     | 0.54  | 51      | 16%    | 84%    |
| 9. 35S:FD        | 0      | 0       | 0     | 101     | 0%     | 100%   |
| 10. 35S:FD stm-10| 1.5    | 0–3     | 0.87  | 51      | 21%    | 79%    |

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.
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, 2009). In tomato, F1 progeny derived from crossing sft mutants with different tomato varieties, 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.

**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. AP1 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; Shalit et al., 2009). 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; Scafield 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–PNF 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, F1 progeny derived from crossing sft mutants with different tomato varieties, 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). SOCI 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 SOCI–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 SOCI localize to the SAM in the stm-10 ft-3 non-flower producing inflorescence shoots. However, LFY is not expressed in these ft-3 stm-10 inflorescence shoots. Therefore, the SOCI–AGL24 complexes are partially dependent upon STM for the activation of LFY. In Fig. 6, it is proposed that SOCI–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
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 meristem identity by activating the late flower meristem identity genes, including AP1 (Percy 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-Garcia 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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