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Universal florigenic signals triggered by *FT* homologues regulate growth and flowering cycles in perennial day-neutral tomato

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Abstract

The transition from vegetative to floral meristems in higher plants is programmed by the coincidence of internal and environmental signals. Classic grafting experiments have shown that leaves, in response to changing photoperiods, emit systemic signals, dubbed 'florigen', which induce flowering at the shoot apex. The florigen paradigm was conceived in photoperiod-sensitive plants: nevertheless it implies that although activated by different stimuli in different flowering systems, the signal is common to all plants. Tomato is a day-neutral, perennial plant, with sympodial and modular organization of its shoots and thus with reiterative regular vegetative/reproductive transitions. *SINGLE FLOWER TRUSS* a regulator of flowering-time and shoot architecture encodes the tomato orthologue of *FT*, a major flowering integrator gene in *Arabidopsis*. *SFT* generates graft-transmissible signals which complement the morphogenetic defects in *sft* plants, substitute for light dose stimulus in tomato and for contrasting day-length requirements in *Arabidopsis* and *MARYLAND MAMMOTH* tobacco. It is discussed how systemic signals initiated by *SFT* interact with the *SELF PRUNING* gene to regulate vegetative to reproductive (V/R) transitions in the context of two flowering systems, one for primary apices and the other for sympodial shoots.

Key words: Florigen, sympodial programme, tomato flowering, universal signals.

Considerations of floral termination and plant architecture

Growth habit in plants is described by two fundamental models, monopodial and sympodial. Both growth habits are found in families of the most primitive plants, such as liverworts, mosses, and cycads (Bell, 1992). In annuals with a simple monopodial shoot, a single vegetative phase is replaced by a single reproductive phase, signalling the completion of the life cycle. However, growth habits of many other plants, particularly perennial trees, require the cohabitation of vegetative and floral buds along their shoots and thus the constant regulation of vegetative–reproductive transitions at the whole-plant level.

Classical grafting experiments have shown that leaves, in response to changing photoperiods, emit systemic signals, dubbed 'florigen', which induce flowering in vegetative shoot apical meristems (SAMs) (Chailakhyan, 1936; Zeevaart, 1976). Genetic studies in *Arabidopsis* and rice defined major pathways that transduce environmental signals to integrators, predominantly *FLOWERING LOCUS T* (*FT*), which induce flowering in long-day and short-day plants (reviewed in Mouradov *et al*., 2002; Boss *et al*., 2004). However, the genetic components of 'florigen' and their link to characterized flowering pathways remain elusive.

Tomato plants are photoperiod-insensitive perennials in their native habitat, and exhibit perennial characteristics of growth, even during one short seasonal cycle. The developmental versatility and architectural flexibility of tomato are reflected in a plethora of gene mutations, affecting single growth modules such as the primary shoot, or the whole plant constitution. Several examples are shown in Fig. 2 and Table 1. Advantage has been taken of the

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Table 1. *Tomato mutants impaired in general or specific components of the compound shoot*

Shoot component Mutant or variant	No. of extra before primary termination[a]	Inflorescence architecture	Sympodial unit length	Molecular nature	References
Primary termination					
terminating flower (*tmf*)	None	First with single flower, later, normal	3	Unknown	Hareven *et al*., 1994
leafless (*lfs*)	–10	Normal but abnormal flowers	No	Unknown	Menda *et al*., 2004
Sympodial flowering					
self pruning (*sp*)	None	Normal, but epistatic to many vegetative inflorescences	Gradual decrease from 3 to 0	CETS	Pnueli *et al*., 1998
delayed sympodial termination (*dst*)	1–2	Normal	5–15	Unknown	Present report
Solanum pennellii	2–4	Branched with bracts subtending flowers	2		Present report
Inflorescence architecture					
compound inflorescence (*s*)	0–2	Highly branched	3	Unknown	Quinet *et al*., 2006
jointless (*j*)	0–1	Vegetative reversion	3	MADS	Szymkowiak and Irish, 2005
macrocalyx (*mc*)	0–1	Large sepals, vegetative reversion	3	MADS	Verbalov *et al*., 2002
annantha (*an*)	1–2	Proliferative meristems	3	Unknown	Pnueli *et al*., 1998
Primary, sympodial and inflorescence shoots					
falsiflora (*fa*)	1–5	Leafy, excessively branched	3–4	LEAFY	Molinero-Rosales *et al*., 2004
uniflora (*uf*)	1–50	'Pseudo shoot'	5–10[b]	Unknown	Dielen *et al*., 2004
single flower truss (*sft*)	5–7	'Vegetative inflorescence'	8–10[b]	CETS	Lifschitz *et al*., 2006
blind (*bl*)	1–3	1–2 flowers	5–10[b]	MYB	Schmitz *et al*., 2002

[a] Number of leaves relative to wild-type siblings or common tomato lines.
[b] In these cases, sympodial buds are inhibited, and sympodial shoots might acquire a basal lateral shoot programme.

complex, but otherwise regular and predictable, developmental pattern of the tomato shoot to investigate how the meristems' response to cycling vegetative and reproductive messages is regulated by specific flowering genes. A broader perspective, however, is provided by the model *Arabidopsis* in which extensive analyses of the shoot constituents have been carried out in the context of an annual monopodial shoot.

Growth and termination/flowering cycles in the compound shoot of tomato

The primary shoot of wild-type tomato is terminated by the first inflorescence (Fig. 1A, primary termination), after 8–12 leaves. Although the time to primary termination is determined genetically, it is also responsive to environmental conditions, particularly total daily light integrals (Atherton and Harris, 1986). Subsequently, the apparent main shoot consists of an upright array of reiterated lateral branches called sympodial units (SUs), each with three vegetative nodes and a terminal inflorescence. The first SU (Fig. 1B, SU1) is subtended by the leaf just below the first terminal inflorescence. It unites with the basal part of the host leaf (HL), and due to vigorous growth extends the leaf above the inflorescence and displaces the inflorescence sideways. All subsequent SUs are also laterals, each arising from the most proximal (third) axillary bud of the preceding unit (Fig. 1B). The continuous aerial growth of the wild-type tomato is therefore carried out by compound shoots made up of SUs, whose inflorescences grow to be positioned between the second and the third leaves of the SUs which they terminate (Fig. 1C).

Further architectural elaboration is achieved by lateral shoots released sequentially from the axils of the two more distal mature leaves of each SU (Fig. 1C, LS). Such laterals do not unite with the bases of their subtending leaves, and thus appear as genuine axillary branches. Most significant, unlike the proximal laterals which are fated to form SUs and terminate regularly after three leaves, the distal laterals, just like the primary shoots, are first terminated after a variable number of leaves before converting to the robust three-leaf sympodial pattern (Fig. 1C).

Floral meristems are always determinate, but in tomato, in contrast to *Arabidopsis*, the sympodial habit dictates that transition to flowering is synonymous with termination of the vegetative apical meristem itself. Termination is used here to describe a *regulated* developmental event in which the vegetative growth of the SAM is replaced by a type of growth committed to form a terminal differentiated organ such as a flower, thorn or leaf. It is distinct, for example, from a temporary arrest of a primordial axillary bud.

The inflorescence shoots and leaves of the tomato plant are also assembled from modular, determinate, morphogenetic units. The tomato inflorescence can be looked upon as a condensed compound shoot, consisting of one-nodal sympodial units (see also Cronquist, 1988;

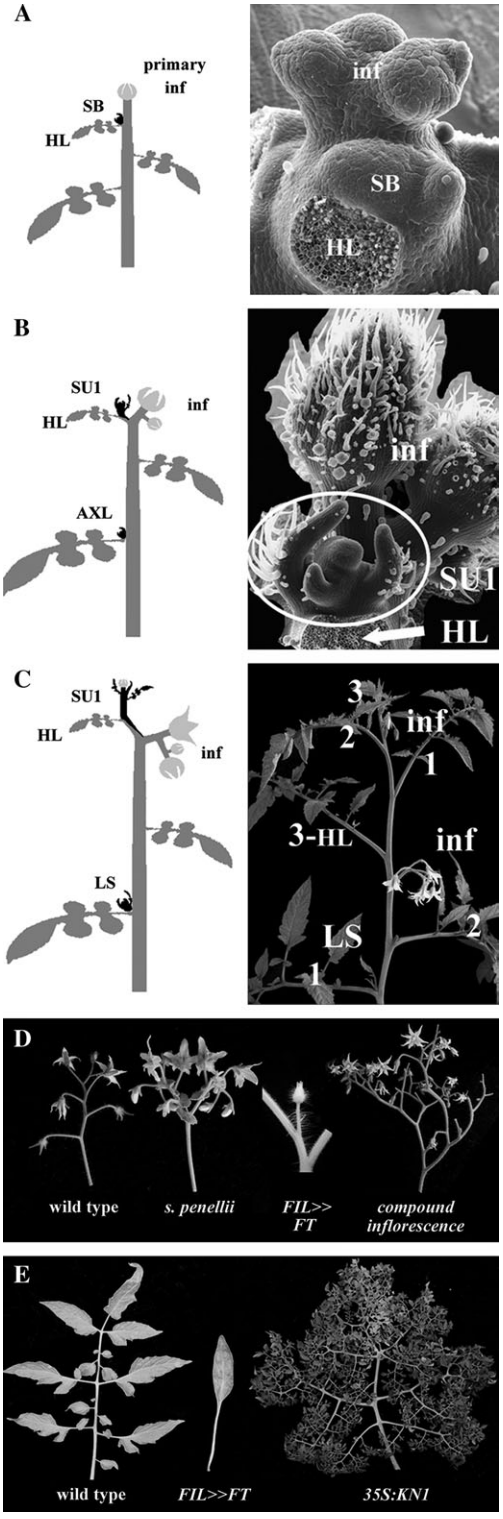

Fig. 1. The compound tomato shoot. (A–C) The gradual development of the tomato compound shoot. (A) Primary termination of the shoot apical meristem by an inflorescence (inf) is accompanied by immediate release of apical dominance over the axillary bud of the uppermost host leaf (HL). This axillary will give rise to the sympodial bud (SB). These three elements, inf, HL, and SB, comprise the sympodial fork. (B, C) The rapid development of the sympodial unit (SU1, circled) positions the host leaf above the inflorescence which is pushed to the side. Basal axillaries later develop into 'juvenile' lateral shoots (LS) that do not displace their host leaf and terminate after a variable number of leaves before resuming a regular sympodial habit. (D) The compound inflorescence shoot of tomato is comprised of condensed sympodial units of a single leaf (bract) and a terminal flower. The single leaf is usually absent in wild-type tomato (left) but present in the sibling species, *S. pennellii*. Modifications of the basic pattern are evident by the abrupt termination of the inflorescence shoot in *FIL≫FT* or elaborate branching in *compound inflorescence* (*s*) backgrounds. (E) The compound tomato leaf (left) can be much simpler, as in *FIL≫FT*, or complex, as in *35S:KN1*, depending on various growth and termination signals.

Szymkowiak and Irish, 2005). Each SU is comprised of a single modified leaf (bract) and is terminated by a single flower. Subsequently, a new lateral of a single flower shoot arises at the axil of the subtending bract. While bracts are not visible in the cultivated tomato, in the sibling species *Solanum pennellii* they are clearly associated with each floral SU of the inflorescence (Fig. 1D). The potential of the inflorescence to form sympodial shoots in which single flowers are separated, or replaced by leaves, is manifested in several mutants such as *falsiflora*, *jointless*, *blind, single flower truss*, *uniflora*, or *macrocalyx* (Table 1; Szymkowiak and Irish, 2005). Thus, these mutants can be exploited to study the consequences of altered vegetative/reproductive (V/R) balance in the inflorescence shoot as well, providing an additional developmental context for the evaluation of 'floral' genes. Since termination is synonymous with flowering, and both the compound shoots and the inflorescence shoots are sympodial, gene mutations that affect flowering time or sympodial patterns also determine shoot and inflorescence architecture. Likewise, the compound tomato leaf is composed of a terminal leaflet and 3–5 pairs of primary lateral leaflets which develop in a basipetal order along the rachis. Leaflets are capable of forming second-and third-order duplications of the basic pattern late into maturation, and secondary intercalary laterals (foliols) may develop in the fully expanded leaf (Fig. 1E; Hareven *et al*., 1996). The compound leaf, like the compound shoot, is therefore a chimera of mature and developing leafy organs.

The most important decisions with respect to the evolving architecture of the compound tomato shoots relate to the interplay between three apical elements: the third leaf and the terminal inflorescence of a given SU, and the auxillary bud destined to form the next sympodial unit. These are referred to, collectively, as the sympodial fork. (Fig. 1A). Modifications in the developmental balance among the three elements will disrupt the regularity of the sympodial pattern and will change the shoot architecture. The most prominent known genes regulating the tomato architecture by their primary effect on the elements of the sympodial fork are *SINGLE FLOWER TRUSS* (*SFT*, Kerr, 1982; Molinero-Rosales *et al*., 2004) and the *SELF PRUNING* (*SP*, Yeager 1927; Pnueli *et al*., 1998). The *SP* gene, a homologue of *TFL1* and *CEN* (Pnueli *et al*., 1998), promotes the indeterminate state of the apical

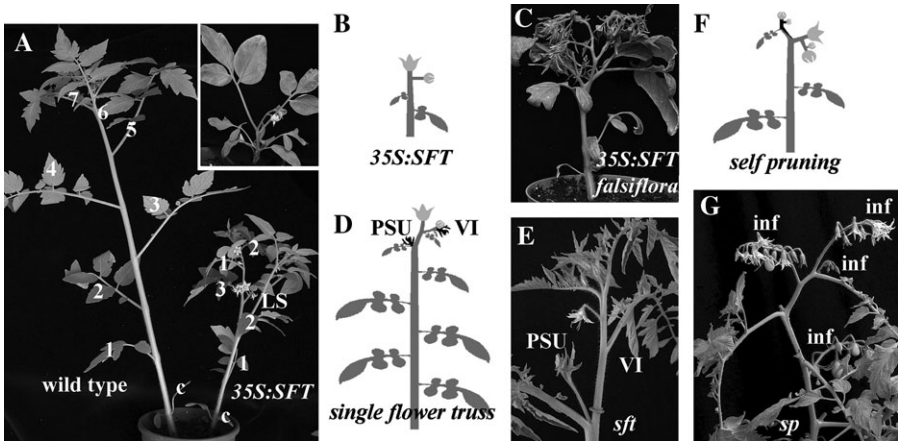

Fig. 2. Tomato shoots with altered SP-to-SFT ratios. (A) Extreme early flowering after three leaves (numbered), short internodes, small and less complex leaves in *35S:SFT* plants. In homozygous lines (inset), primary termination is accompanied by transient arrest of the sympodial bud release, as illustrated in (B). (C) Early flowering stimulated by SFT is independent of FALSIFLORA (LEAFY orthologue) activity or floral identity. (D, E) Late flowering, mixed floral and vegetative indeterminate inflorescence (VI) and delayed release of the prospective sympodial unit (PSU) in *sft* mutants. (F, G) Gradual reduction in length of sympodial units results in the formation of consecutive inflorescences (inf) and arrest of vegetative growth conditioned by a mutation in the *SP* gene.

meristems, whereas *SFT*, a homologue of *FT,* promotes termination/flowering by triggering a signalling pathway that abides by all the tenets of the florigen paradigm (Lifschitz *et al*., 2006).

The tomato *FT* gene, *SINGLE FLOWER TRUSS,* induces early primary flowering in day-neutral tomato and tobacco

Orthologues of the *FT* gene accelerate flowering in the long-day *Arabidopsis* and the short-day rice. In both cases their effect on flowering time is mediated by *CONSTANS* orthologues (Hayama and Coupland, 2004). It has been shown that constitutive expression of the *AtCO* gene, or of two tomato *CO* homologues, fail to enhance flowering-time in day-neutral tomato and tobacco (Ben-Naim *et al*., 2006). Overexpression of *AtCO* in potato caused a graft-transmissible reduction in the response of tuberization to photoperiod, but had no effect on flowering time (Martinez-Garcia *et al*., 2002). Yet, overexpression of *SP* conditions late-flowering in tomato, tobacco, and *Arabidopsis* (Pnueli *et al*., 1998, 2001). The *Arabidopsis FT* gene was expressed in tomato and tobacco and it was found that it induces extremely early flowering in both day-neutral species. A putative orthologue of *FT*, formerly identified as *SP3D* (Carmel-Goren *et al*., 2003), also induced extreme precocious flowering in day-neutral tomato and tobacco (Fig. 2A). Sequence analysis of four mutant alleles, and complementation of *sft* by the constitutive expression of *SP3D*, identified the tomato *FT* orthologue as represented by the *SFT* gene, a late-flowering morphogenetic gene (Lifschitz *et al*., 2006).

The primary shoots of *sft* plants produce an inflorescence after 15–20 leaves, as compared to the 8–12 leaves in their wild-type siblings. The first organ in the terminal inflorescence is usually a flower with an enlarged adaxial sepal but the inflorescence is indeterminate, bearing leaves instead of flowers (Molinero-Rosales *et al*., 2004). Unlike in the wild-type tomato, this vegetative, terminal inflorescence (VI) of *sft* plants exerts partial apical dominance over the presumptive sympodial bud, thus maintaining a pole position (Fig. 2D, E). Moreover, due to its vigorous growth, and the release of internal axillary shoots, the VI itself becomes the main shoot. Occasionally, the VI shoot is again terminated by a new VI, bearing one or two flowers. Significantly, in a large-scale mutant hunt (Menda *et al*., 2004), the three most extreme late-flowering mutant lines were *sft* alleles. The *sft* mutation therefore represents a shift in the vegetative/reproductive equilibrium within the sympodial fork in favour of the vegetative state.

In independent lines of wild-type tomato plants expressing the *35S:SFT* transgene, the first inflorescence arose after three to five leaves, compared with 10–12 leaves of their siblings (Lifschitz *et al*., 2006). Premature flowering induced by *SFT* was associated with modifications in the sympodial pattern. In lines where termination occurred after three leaves, the prospective sympodial bud was temporarily arrested and an inflorescence, with a significantly reduced number of flowers, maintained a pole position (Fig. 2A, B). When the proximal axillary eventually emerged, it did not unite with the host leaf petiole. However, subsequent SUs maintained the regular three-nodal sympodial size.

Overexpression of *SFT* in the background of several mutants which differed in the nature of their terminal inflorescence, showed that all flowered as early as wild-type transgenic plants, but unlike *sft* plants, the developmental fate of the terminating organs was not affected (illustrated

for *falsiflora* in Fig. 2C). Thus, the role of *SFT* is to confer termination of growth and initiation of a terminal inflorescence, but not to determine the identity of the ensuing terminal organ.

In *Arabidopsis* and tobacco, transitions to flowering are associated with elongation of the inflorescence shoots, i.e. bolting. Acceleration of flowering, by *SFT* or other genes, like *sp*, in tomato is associated with shortening of the internodes. In addition to shorter internodes, there was overall growth attenuation: smaller leaves, sometimes with a reduced number of lateral leaflets, much thinner stems, and a faster growth rate. The seasonal life-cycle of *35S:SFT* transgenic tomato plants, may be completed within 9–10 weeks rather than the 15–18 week taken by their progenitors.

Floral-promoting SFT signals are graft-transmissible and complement all developmental defects of *sft* mutant plants

Several indications have implied that the *Arabidopsis FT* gene provides a genetic link between the systemic and the cell-autonomous pathways to flowering. *FT* encodes a signalling CETS factor, it is not expressed in the SAM proper but can be detected, upon induction, in shoot apices (SAP) containing young leaves. Flowering is delayed in *ft* mutant plants and when *FT* is over-expressed, flowering occurs earlier with a determinate inflorescence (reviewed by Jack, 2004). *FT* is regulated by *CONSTANS* in both long and short-day plants and grafting experiments in *Arabidopsis* have shown that systemic induction of flowering by *CONSTANS* is most likely mediated by *FT* (An *et al*., 2004). Huang *et al*. (2005) recently showed that heat-induction of *FT* in a single leaf is sufficient to promote flowering, and that a fraction of the heat-induced FT RNA is found in SAPs, suggesting that the FT mRNA itself may be a florigenic agent.

The universality of the florigen paradigm has been demonstrated by interspecies grafting experiments (Zeevaart, 1976). Grafting results are independent of the validity of promoters, the resolution of *in situ* hybridization, inferences derived from the activation of upstream genes or interpretations of clonal analysis. Due to the ease of grafting, the photoperiod-independent flowering, the compound shoot, and the perennial habit, the premise has been tested that orthologues of the *FT* gene trigger a *universal* florigenic signal in tomato.

In all reciprocal grafts between *sft* receptor and *35S:SFT* donor plants, *sft* receptor shoots produced normal flowers, normal inflorescences, and normal sympodial architecture, 3–5 weeks after grafting (Lifschitz *et al*., 2006). Thus, flowering signals initiated by the *SFT* gene rescue flowering-time and morphogenetic defects in *sft* mutant plants by both endogenous expression and graft transmission. The rescue of receptor *sft* in grafts required the persistent emission of systemic *SFT* signals: formation of normal SUs, inflorescences and flowers in receptor *sft* shoots continued only as long as the *35S:SFT* donor was present. Complementation of *sft* by graft, using *35S:SFT* donor shoots, suggests that transcriptional auto-regulation is not an *obligatory* component of the systemic regulation of flowering by *SFT* (Lifschitz *et al*., 2006).

In contrast to *35S:SFT*, wild-type donors failed to complement the *sft* phenotype. This failure was attributed to the endogenous consumption of scarce SFT signals, coupled with a low efficiency of transmission. Floral suppressors, autonomous or systemic, which like *SP* (see later) balance flowering promoting signals, and regulators of transmission *per se*, may also play a role. The rescue of flowering, under short days, by wild-type donors may be difficult in *Arabidopsis* as well, but in species where efficient auto-regulation or relay-amplification mechanisms evolved, uninduced wild-type donors may work.

SFT generates universal florigenic signals

Variations in responses to florigenic stimulus, presumably due to different components of a mechanism shared by all plants, or to independent 'florigens' operating differentially in different plants (Bernier, 1988), have been recorded. *FT* orthologues induce, via endogenous expression, flowering in mono and dicotyledonous plants, in long-day, short-day, and day-neutral plants and the *SFT* gene triggers systemic signals that rescue its own mutant phenotype in day-length neutral tomato. Therefore, it was asked whether the systemic rescue of *sft* represents a variable component of the florigen mechanism, or one of its core conserved elements. To answer this, the potential of a systemic, not merely an endogenous, SFT signal, to substitute for light requirements in short-day tobacco, long-day *Arabidopsis* as well as light intensity-sensitive tomato background, was explored.

High irradiance requirements in tomato

In a given genetic constitution, the number of leaves to the first inflorescence depends primarily on the total daily light integrals during early growth, less on light-intensities and practically not at all on day-length (Kinet, 1977, for a comprehensive analysis, and reviews by Wittwer and Aung, 1969; Picken *et al*., 1985). In cultivars such as Money Maker, M82, or VFNT-cherry used in our experiments, the number of leaves to primary termination, under constant low or high daily light integrals, may vary between 6 and 16 and the number of leaves per SU between three and six. An extreme case of light-dose-dependent flowering is conditioned by a recessive mutation in the *UNIFLORA* (*UF*) gene. No other tomato gene displays such a differential sensitivity to the light doses (Dielen *et al*., 2004). Under given conditions (70–150 $\mu mol^{-2}\ s^{-1}$ at 18–24 °C) one-third of the *uf* plants never flower, while the rest formed the first, sometimes only flower, after 30–50

Fig. 3. Differential transmission of systemic signals. (A, B) Maryland Mammoth tobacco plants will never flower under long days, but when an *35S:SFT* tomato shoot was grafted on its leaf petiole (arrow in B), flowering was evident three weeks later. (C) No morphological alterations are evident in wild-type tomato receptor grafted on the TKN2-misexpressing dominant mutation *Curl* donor.

leaves. Thus, *uf* plants allow the study of 'flowering time' in a virtually all-or-none situation. The single *uf* flower is borne by a vegetative inflorescence shoot (termed pseudoshoot, PS, in Lifschitz *et al*., 2006) which is positioned like a regular inflorescence, but in which subsequent flowers are replaced by leaves. Strikingly, during the Israeli summer, *uf* plants growing in an open field are barely distinguishable from their wild-type progenitors.

Under light conditions set to suppress flowering, *uf 35S:SFT* plants produced flowering PSs after 3–5 leaves, and many more flowers replaced leaves along the PSs. Likewise, *uf* receptors grown in non-permissive light were stimulated to flower by *35S:SFT* donors, demonstrating that graft-transmissible SFT-borne signals substituted for the high light-dose requirements, and converted leaf-primordia of the *uf* PSs to flowers (Lifschitz *et al*., 2006).

Graft-transmissible signals generated in tomato substitute for short-day stimulus in Maryland Mammoth tobacco

In a seminal discovery, Garner and Allard (1920) identified a recessive mutation which confers a short-day response over a day-neutral background in the Maryland Mammoth (MM) tobacco plants. Under long days, cv. Samsun plants flower after 24–25 leaves while MM plants do not flower at all (Fig. 3A). The tomato *35S:SFT* transgene induced similar early flowering effects in both tobacco strains under long and short days. To see if the SFT systemic pathway itself, not just *SFT* function, is mediated by the same mechanism in tomato and the short-day tobacco, *35S:SFT* tomato donor shoots were grafted onto leaf petioles of long-day-grown MM plants. The recipient MM plants flowered 3–4 weeks later, indicating that *35S:SFT* signals, generated in tomato and transmitted via leaf petioles, induced flowering in MM apices under conditions in which they otherwise never flower (Lifschitz *et al*., 2006; Fig. 3B). The long-day character of MM is conditioned by a single recessive allele. It would be of interest to see whether the requirement for a long-day stimulus by the diploid tobacco species *Nicotiana sylvestris* can also be substituted by systemic SFT, as well as whether alleles of the same gene are involved in these opposite day-length responses in tobacco.

Systemic SFT signals substitute for the long-day stimulus in Arabidopsis

Plants of independent *Arabidopsis* lines expressing *SFT* or *FT* driven by the leaf-specific promoter *BLS* flowered under short days after only five to seven leaves, as compared with 15–19 leaves of wild-type plants. Other leaf promoters used to express *FT* in *Arabidopsis* had similar effects (Abe *et al*., 2005). The *BLS:SFT* transgene induced early-flowering responses and complemented the *sft* mutant; it also induced early flowering in the short-day MM tobacco lines (Lifschitz *et al*., 2006).

Complementation of *sft* by continuous graft-transmissible signals from *35S:SFT* donor shoots, or by endogenous expression of *BLS:SFT* in leaves, indicates that systemic SFT signals played their primary *flowering* role at the apical meristems of the tomato. The dynamic temporal and spatial expression of *FT* or *SFT* in *Arabidopsis* or tomato leaves and the observation that elimination of young tomato leaves enhances flowering (Leopold and Lam, 1960) indicate that the source, mobility, distribution, and targets of SFT signals in tomato are tightly regulated. Evidently, mis-expression of *SFT* is insufficient to promote systemic flowering as illustrated by the failure of leafless donors to enhance flowering (our work) and by the failure of root-specific expression of *FT* to promote flowering in *Arabidopsis* (Abe *et al*., 2005).

SFT is not alone: the *SELF PRUNING* inhibitory function dominates the switch to termination during the sympodial cycles

The pattern of termination in sympodial systems is species-specific. Whereas in wild-type tomato there are three leaves

per SU, there are only two in the sibling species *Solanum pennellii*. In petunia, a close relative of tomato, SUs consist of one leaf and one terminal flower.

A major modification of the robust sympodial regularity is referred to as 'determinate' and 'indeterminate' and describes the growth habits of mutant *sp* and wild-type *SP* plants, respectively. The introduction of the 'determinate' (*sp*) character resulted in plants with a bushy constitution and nearly homogeneous fruit setting which facilitated mechanical harvesting and thus revolutionized the tomato industry (Rick, 1978). In *sp* plants, the number of leaves per SU in the main and lateral shoots decreases progressively with age, from three to two to one until, eventually, the last SU generates only an inflorescence and the shoot system terminates with two consecutive inflorescences (Fig. 2F, G). *SP* therefore maintains the three-nodal system by inhibiting precocious termination of the sympodial apical meristems. Constitutive expression of *SP* delays primary termination, increases the number of leaves per SU and induces indeterminate, partially leafy, inflorescences (Pnueli *et al*., 1998). Environmental conditions such as low-light integrals may also increase the number of leaves per SU, but significantly, no growth conditions in tomato are known to reduce the number of leaves in SUs to a regular one or two.

SP is a functional homologue of *TFL1* and a member of the CETS family of signalling/adaptor factors (Pnueli *et al*., 1998). The range of CETS-interacting proteins and their molecular identity suggest they function as adaptors in signalling or transcription complexes (Yeung *et al*., 1999; Pnueli *et al*., 2001). One class of CETS partners, 14-3-3 adaptors, participate in many, sometimes unrelated cell processes, by interacting with different signalling molecules or with the same factor in different cellular contexts. Similarly, the nature and range of interacting proteins suggest analogous functions for SP and SFT in different signalling pathways. Such roles are reflected in the range of pleiotropic effects which are manifested as changes in the growth/termination balance, but not in organ identity. For example: *sp*, like *35S:SFT*, is epistatic to *falsiflora* (*fa*), the tomato *LEAFY* gene, with respect to termination patterns, while *fa* is epistatic with respect to floral identity. *sp* is also a suppressor of indeterminacy and leaf formation in inflorescences of several late-flowering mutants (E Lifschitz, unpublished data; but see also Pnueli *et al*., 1998; Szymkoviak and Irish, 2005). Moreover, in a context-dependent manner, *sp* is an 'enhancer' of primary flowering time as well. *sft fa* (Molinero-Rosales *et al*., 2004) and *sft uf* (our observation) never flower, but formation of inflorescences is partially rescued in triple mutant combinations with *sp* (E Lifschitz, Y Eshed, unpublished data).

Extreme SFT doses, which induce primary termination after three leaves, do not alter the *regularity* of the growth/termination cycles in the compound shoot. Conversely, the gradual but continuous reduction of leaf number per SU in *sp* mutants is not associated with premature primary termination, suggesting that *SFT* and *SP* are not simply antagonists.

Termination and the SFT/SP ratio in the primary shoot

All *sft* alleles produce a first flower in a VI and all mutant alleles of *FT* eventually flower in *Arabidopsis*. The termination of SUs in *sp* plants is gradual, rather than abrupt, and SAM termination in *tfl1 Arabidopsis* mutants is suppressed under short days. *SFT*/*FT* and *SP/TFL1* are, therefore, not required for determination of the vegetative or reproductive states of the SAMs. How, therefore, do systemic termination signals by *SFT*, and growth-promoting/anti-termination functions of *SP*, regulate the fate of the primary apex?

Flowering in tomato is induced very early and evocation has been estimated to occur in seedlings with two to four expanding leaves (Picken *et al*., 1985). One possibility is that *SFT* is increasingly up-regulated in the primary vegetative shoot relative to *SP* and that *SP* only becomes expressed at high levels in the axillary and sympodial buds. This would predict a result similar to that observed; a primary SAM that is indifferent to *sp* but highly sensitive to SFT and secondary shoots that are relatively refractory to increased levels of SFT but sensitive to a reduction in SP.

SFT to SP ratios regulate termination and apical dominance in the sympodial fork

Termination of the SUs uniquely after three leaves in co-ordination with the discriminatory release of the most proximal bud from apical dominance, requires the renewed balancing of contrasting growth and termination signals by the SAMs in each nodal segment of the SU. The age-dependent termination of SUs by *sp* implies that to maintain regularity, the anti-termination function of *SP*, is constantly upgraded at the whole-plant level. Thus, to ensure the robustness of the sympodial sequence, primary termination establishes a graded SFT to SP threshold ratio, in which the inhibitory effects of *SP* become dominant. How the SP function is annulled after three leaves remains unknown, but since regularity, under extremely high SFT levels can be changed from three to two, additional factors beside SP, must be involved.

The morphogenetic alterations conditioned by *sp* and *sft* are ultimately manifested as changes in local and global apical dominance. In wild-type SUs, *SP* maintains the indeterminate state of the SAM, which in turns suppresses the release of the first two axillary shoots. When the function of *SP* in the SAM is compromised, either locally or by systemic SFT signals, the prospective sympodial bud is released. In the new primordial SU, the SFT to SP ratio is

presumably lower again by an unknown mechanism, and the cycle repeats. As long as *SP* is functional, regularity at any threshold level is maintained. Auxin is the most likely mediator of indeterminacy and apical dominance within SUs by SP (Pnueli *et al*., 2001). SP and SFT belong to the same protein family, they interact with the same classes of factors, and only a few amino acids distinguish their floral-promotion and floral-inhibition functions (Hanzawa *et al*., 2005; Ahn *et al*., 2006). Thus, the floral inhibitory function of SP is probably mediated by systemic inhibitory roles too (Lang *et al*., 1977). However, since *SP* is expressed in the youngest leaf primordia and in the SAMs of sympodial units, its systemic effects may be short-range and confined to a modified source–sink track within the sympodial fork itself.

The two-flowering systems model

Inactivation of *SP* results in the complete collapse of the sympodial regularity but is inconsequential in the timing of termination of primary apices (Fig. 2; Pnueli *et al*., 1998). Although tomato strains differ with respect to their primary termination, all maintain the subsequent robust three-leaf pattern. Under conditions of high irradiance where the number of leaves to the first inflorescence may be reduced by 50%, the sympodial rhythm is still maintained. Genetic factors also distinguish primary meristems. In *terminating flower* (*tmf*) the primary shoot is terminated by a solitary abnormal flower, but subsequent lateral shoots are perfectly normal. Thus primary shoots of tomato are extremely sensitive, while SUs are relatively refractory to changes in genes activity with the exception of SP where the situation is reversed (Fig. 2). The primary apex may be preferentially sensitive to SFT because it is laid down in embryogenesis prior to build-up of inhibiting factors like *SP*. Interestingly, Furr *et al*. (1947), reported on a gap of several years between first and subsequently flowering in perennial trees. Indeed, the responses of *Arabidopsis* plants to *FT* and *TFL1* (Ratcliff *et al*., 1998) and expression of *SFT/FT* under different promoters in *Arabidopsis* clearly distinguishes between the response of primary and lateral shoots (E Lifschitz, unpublished data). Inevitably, flowering in both species and the function of *SFT/SP* (*FT/TFL1*) is regulated, and has to be considered in the framework of two systems, one for primary shoots and the other for sympodial in tomato and laterals in *Arabidopsis*.

The molecular components of the florigen pathway in tomato and *Arabidopsis*

Yeast-two-hybrid screens uncovered four different SP interacting proteins (SIPs): A NIMA-like protein kinase (SPAK) involved in cell division, 14-3-3 adaptor proteins, a bZIP G-box (SPGB) factor, and an SP-specific interactor, SIP4. SPAK also interacts with the 14-3-3s which in turn also interact with SPGB and SIP4. SP and 14-3-3 share a SPAK-interacting site. With the exception of SIP4, other SIPs interact also with TFL1, CEN, and FT (Pnueli *et al*., 2001). Abe *et al*. (2005) and Wigge *et al*. (2005) showed that one *Arabidopsis* homologue of *SPGB* is encoded by the late-flowering gene *FD*, and that *FD* is partially required for the proper function of *FT*. RNA expression data have suggested that *FT* is expressed primarily in mature leaves whereas FD is expressed predominantly in the SAM. This separation of expression territories promoted the hypothesis that to induce flowering, *FT* primary products must travel from leaves to SAMs (Abe *et al*., 2005). Concomitantly, Huang *et al*. (2005) developed an ingenious experimental protocol to induce *FT*, driven by a heat-shock promoter, in a single leaf of *Arabidopsis*. Using this method, a leaf-induced FT RNA was detectable in the SAM proper and several 1-mm leaf primordia, several hours after local heat induction. FT RNA may thus function as a florigenic signal. It becomes important, therefore, to determine whether the moving RNA is functional and being translated in the target SAM proper, is distributed in the other parts of the plant as expected of a florigenic substance, and whether the FT RNA remaining in the induced leaves, is unable to induce flowering if its movement is prevented.

In tomato, we failed to detect *35S:SFT*- or *FIL:FT*-born transcripts beyond graft unions in rescued *sft* shoots (Lifschitz *et al*., 2006). Considerations of universality and differences in the assay sensitivities notwithstanding, other differences between tomato and *Arabidopsis* may be important. Although the two are not extremely divergent, one is perennial and sympodial the other annual and monopodial. The inductive system of *Arabidopsis* may be more permissive to systemic *FT* signals such that a single burst is sufficient for the single switch. Lasting systemic induction in the robust, day-neutral, and cycling flowering system of tomato, requires persistent emission of *SFT*-triggered signals. In the tomato experimental system, the source and target were separated by graft unions, by considerable distance and, significantly, by genotype. Moreover, if movement of florigens abides by the sink–source rules, the organization of the sympodial shoot in quasi-autonomous units may constitute another important difference.

At another level, the tomato genes encoding the SPGB and 14-3-3 SP-interacting proteins are expressed in all leaves and throughout development, potentially making it unnecessary for *SFT* RNA to travel (Lifschitz *et al*., 2006). Two *FD*-like genes have been identified in *Arabidopsis*, but their differential affinity to FT and TFL1 is still debated (Abe *et al*., 2005; Wigge *et al*., 2005). It is possible therefore that our yeast two hybrid screen missed a SPGB homologue, which is expressed solely in the SAM.

Currently it is assumed that the termination versus growth effects of SFT and SP in the vegetative meristems of the leaves, stems, and SAMs, require interactions with the same factors. And since meristematic activity in tomato leaves continues well into maturation, it follows that the FD-like function is required in leaves as well. In addition, FD transcripts have been detected in expanding *Arabidopsis* leaves, and in agreement with this, a curled leaf phenotype, stimulated by 35S:FT or 35S:SFT is largely dependent on FD activity; although this, it may be argued, is due to the expression of FD in leaf primordia only (Teper-Bamnolker and Samach, 2005).

There are additional uncertainties and inconsistencies in our current understanding of the floral-integrator genes and their targets. The partial suppression of *FT*-induced flowering by *fd* mutants led to a model whereby FD and FT complexes co-operately activate floral genes, such as *AP1* (Wigge *et al*., 2005). However, *ap1* or *leafy Arabidopsis* mutants are not late-flowering and *fd* plants misexpressing *FT* flower at least as early as the wild-type, albeit not as early as *35S:FT (*Abe *et al*., 2005).

RNA movement

In tomato, Kim *et al*. (2001) reported that leaf morphology defects by *Mouse ears* (*Me*) and *Curl* (*Cu*), both dominant mutations in the *TKn2* gene are graft transmissible and that such a systemic effect is associated with *Me* transcripts crossing the graft junctions. In more than 120 grafts in tomato and tobacco, no support could be found for the systemic transmission of the *Me*, *Curl*, or *Kn1* morphological defects (Fig. 3C). It was also noted that the *Me* mutation encodes a fusion transcript with almost all of its 5′ end comprised of *PFP*, a gene for a protein involved in fructose metabolism (Parnis *et al*., 1997). The relevance of the translocated chimeric PFP-TKn2Kn1 RNA transcripts for normal development is thus questionable. Similarly, no support for long-distance transmission of microRNA-mediated alterations could be provided by tomato or tobacco grafting (Alvarez *et al*., 2006). Taken together, a model is currently favoured in which a downstream systemic pathway is initiated by cell-autonomous functions of *SFT* RNA. From this perspective, the options of systemic SFT polypeptides, intercellular signal transduction pathway, or a moving secondary metabolite are equally plausible.

Systemic acceleration of flowering and termination are pleiotropic functions of *TFT* to *SP* (*FT* to *TFL1*) ratios

It has been shown that mis-expression of *SFT* attenuates growth of intercalary peripheral and plate meristems and all these features are greatly enhanced in an *sp* background. Apical meristems were induced to form terminal inflorescences, or were temporarily or permanently arrested, by high levels of systemic SFT signals, and these were also enhanced in an *sp* background (Lifschitz E and Eshed Y unpublished data). Thus, all responses stimulated by the SFT to SP balance involve changes in growth, suggesting that floral transition and growth attenuation, instead of being the consequence of one another, are two facets of the same cellular responses. Since all the facets of the SFT/SP ratio are also graft-transmissible, it is speculated that growth and termination are targets for florigen-compatible signals and that boosting flowering is a pleiotropic effect of *FT* orthologues (Lifschitz *et al*., 2006). If growth were the primary target of *SFT*, the systemic signals might involve conditioning of the apical meristem via a finely regulated temporal change in cell-proliferation patterns, providing the context/time required for the vegetative-to-reproductive switch.

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