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Phloem transport of flowering signals

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Seasonal variability in environmental parameters such as day length regulates many aspects of plant development. The transition from vegetative growth to flowering in *Arabidopsis* is regulated by seasonal changes in day length through a genetically defined molecular cascade known as the photoperiod pathway. Recent advances were made in understanding the tissues in which different components of the photoperiod pathway act to regulate floral induction. These studies highlighted the key role of the FT protein, which is produced in the leaves in response to inductive day lengths and traffics through the phloem to initiate flowering at the shoot apex. Unveiling the cellular and molecular details of this systemic signaling process will be required for a complete understanding of flowering regulation and other photoperiodic processes.

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Introduction

In many plants flowering is induced only on exposure to appropriate environments. Day length and temperature are the most important of these environmental signals, providing seasonal cues that enable varieties of a species to become adapted to life at particular latitudes or altitudes. Flower development occurs at the shoot apical meristem or in lateral meristems, but these are often covered in growing leaves so that their exposure to light is limited. Thus, it is, perhaps, not surprising that day length should be perceived in the leaves, as these evolved to maximize light perception for photosynthesis. The spatial separation between the organs that perceive light and those in which floral development occurs raises issues of the mechanisms by which the floral signal is communicated between organs of the plant. Recently, work in *Arabidopsis* provided evidence that the small FLOWERING LOCUS T (FT) protein is at least a component of this signal, and work in other species strengthened this conclusion. These results were reviewed at length during the last year [1,2]. Here, we briefly summarize the molecular-genetic data and give emphasis to papers that appeared most recently, as well as to the questions of cell biology that arise from current models.

Pathways that regulate flowering in the leaf

Arabidopsis flowers early under long days characteristic of summer and late under short winter days. Isolation of late-flowering mutants of *Arabidopsis* that are insensitive to day length, flowering at similar times under long and short days, provided access to genes that regulate flowering in response to photoperiod [1,2]. These genes comprise a regulatory pathway often referred to as the photoperiodic flowering pathway or long-day pathway. The mRNA of two of the genes in this pathway, *FT* and its close homolog *TWIN SISTER OF FT* (*TSF*), are expressed under long days but not short days [3–5]. The FT and TSF proteins belong to the CETS family, named after the three founding members, CENTRORADIALIS, TERMINAL FLOWER, and SELF PRUNING [6], and strongly promote flowering [3,7,5]. The mechanisms that lead to *FT* activation specifically under long days involve transcriptional and post-transcriptional regulation of the zinc finger protein CONSTANS (CO) so that CO-mediated activation of *FT* transcription only occurs under LDs [8•].

Extrapolation from the physiological data suggests that the machinery required for day-length perception should be expressed in the leaves, and this was found to be the case for several of the genes in the photoperiodic pathway [9–11]. Fusions of the promoters of *CO* and *FT* to marker genes were expressed in the vascular tissue, whereas the *CDF1* and *FKF1* genes that encode regulators of *CO* are also expressed mainly in the vascular tissue. Expression of *CO* and *FT* from heterologous promoters demonstrated that their expression in the phloem companion cells was sufficient to induce flowering and complement the corresponding mutations [9,12,13]. Similarly, a synthetic microRNA designed against *FT* and expressed in the phloem companion cells delayed flowering, demonstrating the requirement for *FT* expression in these cells in wild-type plants [14••]. Finally, grafting plants expressing *CO* to *co* mutants caused earlier flowering of the *co* mutant, demonstrating that CO controls a graft transmissible signal [9,12,13]. Similar results were obtained for *FT* [15••]. CO activity is dependent on FT function [16],

suggesting that FT and CO control the same graft transmissible signal. Taken together these data demonstrate that a graft transmissible signal produced in response to activation of the *Arabidopsis* photoperiod pathway occurs downstream of *FT* mRNA.

Activation of transcription of *FT*-like genes in leaves has been observed in other species, and appears to be a highly conserved aspect of floral induction by photoperiod. Expression of such genes has been shown to be highly regulated by day length in rice [17], barley [18], poplar [19,20], and Japanese Morning Glory [21], and to occur independently of photoperiod in tomato [22]. Genetic evidence for the involvement of *FT*-like genes with the promotion of flowering has been obtained in rice [17], wheat [23], barley [23], and tomato [22], whereas analysis of transgenic plants supports such a role in Japanese Morning Glory and poplar. In rice, flowering is abolished in double mutants in which activity of two *FT*-like genes is impaired [17]. In most photoperiodic systems studied in detail, transcription of *FT*-like genes is under the control of day length so that they are only expressed in photoperiods that induce flowering. An interesting exception is *Cucurbita moschata* where FT activity appears to be regulated at the post-transcriptional level so that day length determines its capacity to move between cells (see below).

In *Arabidopsis*, apart from being activated by the photoperiod pathway through CO activity, *FT* transcription is modulated by several other transcription factors, some of which mediate environmental cues. The MADS box transcription factors FLC and SVP directly bind to *FT* and repress its transcription [24,25]. Repression of *FT* transcription by FLC is important in the activation of flowering by low temperatures during winter (termed vernalization) because FLC blocks *FT* transcription until the plant is exposed to low temperatures that repress *FLC* transcription, allowing induction of *FT* the following spring as the photoperiod lengthens. The chromatin-associated proteins, TERMINAL FLOWER 2 (TFL2) and EARLY BOLTING IN SHORT DAYS (EBS), repress *FT* transcription [11,26], and TFL2 binds directly to *FT* chromatin [27]. Mutations in these genes cause early flowering, because *FT* mRNA levels are increased, particularly under short days, indicating that chromatin regulation is important in ensuring stable repression of *FT*, and therefore late flowering, under short days.

Linking leaves to the shoot apical meristem: the role of the FT protein

The observation that *FT* mRNA is expressed in the vascular tissue and acts there to promote flowering suggested that either FT itself or an FT-target mediates the promotion of flowering of *Arabidopsis* in response to long days. Evidence described below that FT interacts with a transcription factor expressed at the meristem first strengthened the idea that a product of *FT* is transmitted. Grafting experiments strongly argued against movement of *FT* mRNA. When tomato plants overexpressing *SINGLE FLOWER TRUSS* (*SFT*, a tomato homologue of *FT*) from the *35S* promoter were grafted to *sft* mutants, all phenotypic effects of the *sft* mutation were corrected by graft transmission of a signal from the *35S:SFT* plant [22]. *SFT* mRNA derived from the transgene was stringently tested for in the recipient mutant and was found to be undetectable, strongly suggesting that the transmitted signal was not the *SFT* mRNA. Later, a similar experiment was performed in *Arabidopsis* with the same result [15••].

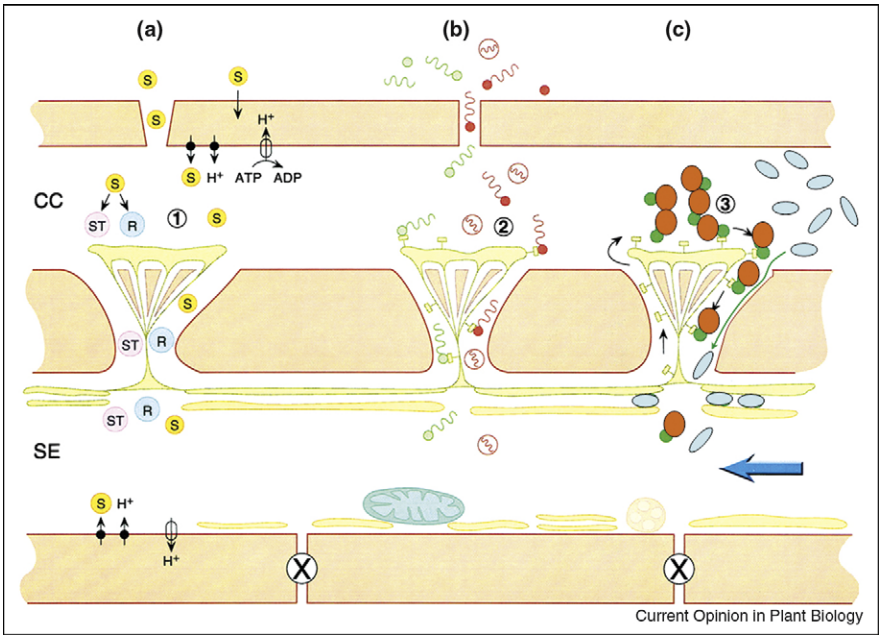
The small size of FT protein (19.8 kDa), indirectly supported an argument for movement of the protein. Direct evidence for FT protein movement from the vascular tissue to the meristem came from several experiments performed in different species. FT::GFP fusion proteins expressed in phloem companion cells of *Arabidopsis* or rice were detected at the meristem, demonstrating their capacity for long-distance movement [15••,28••]. In *Arabidopsis*, FT::GFP expressed from a heterologous promoter in the phloem companion cells was shown to cross graft junctions [15••]. Furthermore, in nontransgenic plants, FT-like proteins were detected by mass spectrometry in the phloem sap of *Brassica*, *Cucurbita maxima*, and rice plants [29,30••,31], suggesting that they can move in the phloem stream, as classically suggested for the floral stimulus. In *C. moschata*, the appearance of FT-like proteins in the phloem stream occurred only in day lengths that induce flowering, and these proteins could cross graft junctions from *C. maxima* to *C. moshata* [30••].

Demonstration that FT protein moves through the phloem is consistent with it being a flowering signal but does not prove the hypothesis. Alternative models proposed that FT could act in the leaves to generate an intermediate signal, and that movement of FT protein may not be critical for floral induction. These issues are addressed in the following section where we describe the mechanisms by which proteins may move from the companion cells into the phloem stream.

The cell biology of FT protein movement

The mechanisms by which FT proteins enter the phloem sieve elements from the companion cells, move long distances and are downloaded into the meristem remain to be fully elucidated. The sieve elements are the conducting tissue of the phloem, and are made up of enucleate cells that are connected to each other at their end walls (Figure 1). On becoming mature, these sieve elements act as a tube that connects the photosynthetically active leaves to the growing points of the plant at the shoot and root apices [32,33]. The sieve elements are closely connected to companion cells, which are nucleate. Several promoters active in and largely specific to

Figure 1



Modes of macromolecule entry into the phloem network of plants. At least three different types of macromolecules namely sugars, proteins, and RNA traffic **(a)** *Sugar trafficking*. Sucrose (S) enters the companion cells (CC) either symplastically through plasmodesmata or apoplastically. In the companion cells sucrose is polymerized in raffinose (R) and stachyose (ST) both of which enter the sieve elements (SE) through the modified plasmodesmata of the CC–SE junctions. **(b)** *RNA trafficking*. Both endogenous (green) and viral (red) transcripts enter the CCs symplastically from the mesophyll. Intact viruses (red circles) can also enter symplastically. **(c)** *Protein trafficking*. Entry of proteins into the CC–SE plasmodesmata can be either selective or passive, via diffusion in a size-depended manner. Selective trafficking of high molecular weight targets (>30 kDa, orange circles) often depends on the presence of certain interactors (green circles) [53]. Passive entry is possible for targets up to 30 kDa (blue circles) [35]. Inside the SE proteins can traffic together with photoassimilates. Whether FT traffics passively or by a targeting mechanism is not known. (Modified from [54].)

companion cells have been described [34,35]. Expression of *CO* or *FT* in the companion cells is sufficient to complement the corresponding mutations, and reduction of *FT* expression in companion cells delays flowering, suggesting that these are the cells in which *FT* mRNA is expressed and required to promote flowering. The companion cells are connected to the sieve elements by specialized branched plasmodesmata [33]. Plasmodesmata facilitate entry of macromolecules such as sugars, RNA, and proteins into the sieve elements (Figure 1). These plasmodesmata have a large size exclusion limit that allows movement of proteins between the companion cells and the sieve elements. This exclusion limit is certainly large enough to allow movement of GFP into the sieve elements when expressed from a promoter specific to companion cells [35]. The movement of proteins into sieve elements can apparently occur either by diffusion, as with GFP, or by selective trafficking, a specific mechanism requiring protein–protein interaction [33,36]. FT is smaller than the size exclusion limit of these plasmodesmata, and therefore could move into the sieve elements by diffusion. However, the protein is expressed at extremely low level in wild-type plants, and therefore a specific trafficking mechanism allowing efficient movement of FT into the sieve elements may be required. The observation that in *C. moschata* movement of FT-like proteins into the sieve elements seems to be regulated by photoperiod suggests involvement of a specific mechanism that can be influenced by photoperiod rather than diffusion [30••].

The idea that proteins larger than the exclusion limit of companion cells could be used to anchor proteins in the companion cell and thereby prevent their movement was used to address whether FT movement is required for flowering. Fusion of FT to GFP more than doubles the size of the FT protein, and therefore might be expected to change its movement characteristics. Expression of FT::GFP from the *GAS1* promoter, which is specific for the phloem companion cells of minor veins in leaves, did not complement the *ft* mutation, while expression of the smaller FT protein from the same promoter did complement [15••]. Although expression of *GAS1:FT::GFP* failed to complement the late-flowering phenotype, expression of FT-responsive genes in the leaves of *GAS1:FT::GFP* plants, such as *FRUITFUL*, were increased in expression in a similar way to previously reported for *35S:FT* plants [37]. This experiment suggested that FT movement from the minor veins is reduced through fusion to GFP so that FT::GFP could

still activate gene expression in the leaves, but could not promote flowering because it could not move to the meristem from the minor veins [15••]. Similarly, double GFP fusions to FT were made to anchor FT in companion cells and prevent its movement into the phloem stream. FT:GFP::GFP did not complement the *ft* mutation when expressed from the *SUC2* promoter in companion cells of minor and major veins, but did so when expressed throughout the plant from the *35S* promoter [14••]. Therefore anchoring FT in the companion cells with a double GFP prevented its ability to promote flowering, but when expressed more widely throughout the plant, including the meristem, it would complement the *ft* mutation. That movement of FT from the phloem is required for its floral-inducing activity was further supported by showing that coexpressing in the companion cells a protease that released FT from FT:GFP::GFP facilitated complementation of the *ft* mutation [14••]. This experiment strongly suggested that releasing a smaller, mobile FT was required to induce flowering. Similarly, targeting FT protein to the nucleus in the companion cells prevented complementation of the *ft* mutant, suggesting that targeting FT to the nucleus prevents long-distance movement to the meristem [38•]. Taken together these experiments support the idea that FT protein movement is required for FT to promote flowering.

Classical grafting experiments and comparison of the velocities of movement of florigen and labeled photosynthate indicated that florigen moves along with photosynthate through the sieve elements [39]. This movement is assumed to be driven by a pressure gradient between source and sink tissues. If FT moves passively by such a mechanism, then presumably it is transported to other sink tissues as well as shoot meristems. The observation that grafting additional sink leaves delays the arrival of florigen at the apex would support such a model [40]. Additionally, *Arabidopsis* genes upregulated in the shoot apical meristem during flowering are often also upregulated in young leaves, and this might be due to FT protein being uploaded generally into sink tissues, which would include young leaves as well as the meristem.

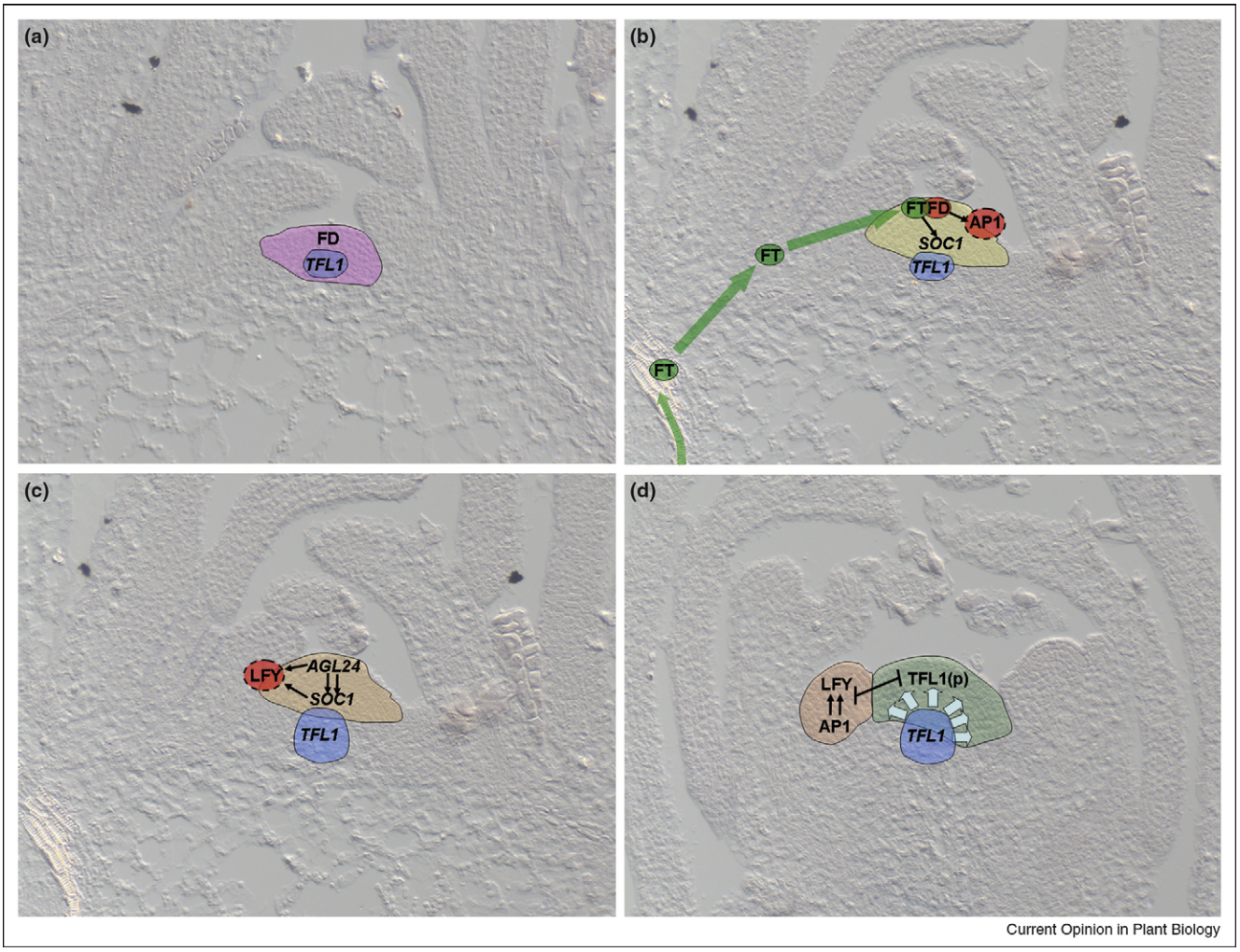
Phloem unloading at sink tissues is assumed to occur symplastically [33]. FT protein may be unloaded directly from the sieve elements into companion cells of the sink tissue. Further movement from the end of the phloem system through the meristem region could then occur through plasmodesmata. Specific evidence for such movement also comes from the analysis of the FT-like protein TERMINAL FLOWER 1 (TFL1). The *TFL1* mRNA is expressed in a localized region at the base of the meristem, but the protein is found much more broadly through the whole of the meristem ([41•]; Figure 2d). Regulation of the size exclusion limit of plasmodesmata in the meristem might influence the rate at which these proteins spread through the meristem or the developmental time at which the proteins move. Differences in the movement of fluorescent dyes into the meristem before and after flowering demonstrated that passive movement through plasmodesmata is under developmental control [42].

Role of FT in the meristem

In the meristem, FT is believed to activate transcription of specific target genes by interacting with the bZIP transcription factor FD [43,13]. The *FD* mRNA is present throughout the shoot apical meristem of short-day grown plants (Figure 2a), but presumably does not activate transcription of genes associated with flowering until plants are exposed to long days leading to the expression and transport of FT protein. Both FT and FD are required for the upregulation of *SOC1*, which encodes a MADS box transcription factor, and is upregulated in the shoot apical meristem early after the shift from short days to long days ([24]; Figure 2b). SOC1 binds to the promoter of *AGL24* and activates its transcription, while AGL24 similarly directly activates *SOC1* [44]. Subsequent to FT activation of *SOC1*, the SOC1 and AGL24 proteins may, therefore, mutually activate each other's expression to promote the floral transition in the shoot apical meristem (Figure 2c). Indeed, AGL24 and SOC1 were recently shown to colocalize to the nucleus [45•], suggesting that physical interaction between these two proteins allows them to activate gene expression in the nucleus. One of the genes proposed to be activated by SOC1 and AGL24 is the floral meristem identity gene *LEAFY*, as this is expressed only in those tissues where both *SOC1* and *AGL24* are expressed ([45•]; Figure 2c). The FT/FD complex also upregulates *AP1* expression in young floral primordia ([43,13]; Figure 2b). Chromatin immunoprecipitation experiments demonstrated that FD recruits FT directly to a region of the *AP1* promoter [43]. AP1 promotes floral fate in the floral primordium by directly repressing additional shoot promoter genes such as *AGL24*, *SOC1*, and *SVP* [46•,47]. LFY and AP1 repress TFL1 protein in the developing floral primordia and TFL1 in turn represses both LFY and AP1 expression in the inflorescence meristem ([41•]; Figure 2d). Although the details are still not precisely clear, these data begin to outline a series of physical interactions from arrival of the FT protein to activation of transcription factors associated with floral meristem identity in the developing primordia.

Recently, this transcription-based model was complicated by a proposed vacuolar function for TFL1 [48]. The FT and TFL1 proteins have very similar structures, and are assumed to have similar biochemical functions [49]. TFL1 also interacts with FD in yeast, and one model proposes that the TFL1/FD heterodimer represses transcription of FD target genes and thereby directly

Figure 2



Spatial pattern of expression and molecular cascades associated with reprogramming of the floral meristem upon floral induction. **(a)** Vegetative meristem (no floral induction). *FD* mRNA (purple) is present at low abundance throughout the meristem while *TFL1* mRNA (blue) is expressed at low abundance in the center of the meristem. **(b)** Transition meristem (floral induction has occurred but no floral primordia are visible). Upon arrival from the phloem to the apex the FT protein (green) interacts with the FD protein (red). This results in the direct upregulation of *SOC1* mRNA (yellow), one of the earliest known molecular markers of floral induction in the meristem. The FT–FD protein complex also upregulates, with a small delay compared to *SOC1*, *AP1* mRNA expression in the flanks of the meristem, in a region which will develop into a floral primordium (dashed red circle). **(c)** Floral committed meristem, early stage (floral commitment occurred but no floral primordia are visible). *SOC1* and a gene called *AGL24* encoding another MADs box protein, participate in a positive feedback loop which eventually upregulates *LFY* expression in the flanks of the IM [45•] with a small delay compared to AP1. **(d)** Floral committed meristem (floral primordia are visible) *TFL1* mRNA (blue) is now strongly expressed in the center of the IM while the protein (green) traffics intercellularly (blue arrows) throughout the whole IM and represses *LFY* and *AP1* transcription in the IM. At the same time both AP1 and LFY proteins (orange) ensure TFL1 does not accumulate in the floral primordia, separating these two different features of the apical meristem. LFY and AP1 maintain their expression in the developing floral primordium through reciprocal upregulation. IM: inflorescence meristem.

antagonizes activation of transcription by the FT/FD heterodimer [48]. In apparent conflict with this model, *tfl1* mutants were recently reported to impair trafficking of proteins to protein storage vacuoles, and the TFL1 protein was found localized to endomembrane compartments rather than to the nucleus. This work led to the proposal that the role of TFL1, and by analogy FT, is to regulate the release of regulatory proteins from the protein storage vacuole. At present, this model has not been reconciled with the one that proposes FT and TFL1 are recruited to the promoters of FD target genes, and further work is required to distinguish between these models or to demonstrate how FT/TFL1 carry out such apparently different functions.

Conclusion

The analyses described above strongly argue that FT protein is transported from the companion cells to the

meristem through the phloem sieve elements, and that this transport is required for floral induction. Furthermore, *FT* expression from heterologous promoters in the phloem dramatically accelerates flowering under noninductive conditions, arguing that *FT* expression is sufficient to promote flowering. Nevertheless, FT might still move through the phloem along with other signal components, so that florigen may represent a mixture of substances of which FT is one. Many outstanding questions regarding the route and mechanisms associated with FT movement, as well as the biological function of FT protein, were highlighted in the text. The response to photoperiod also shows extensive phenotypic variation within species creating quantitative differences that apparently allow adaptation of varieties to life at different latitudes, and variation between species generating life history changes such as juvenility, in which plants do not respond to photoperiod until they reach a certain age, or polycarpy, in which only some of the meristems of a plant respond to the floral signal. Analysis of natural-genetic variation affecting photoperiodic flowering in *Arabidopsis* [50–52] and of the control of bud dormancy by photoperiod in trees [20] has initiated the process of understanding how the diversity in these responses is generated, but much more extensive analysis will be required to understand the wide range of photoperiodic phenotypes we see around us.

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- •• of outstanding interest

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