Florigens and antiflorigens: a molecular genetic understanding

Ianis G. Matsoukas

School of Life Sciences, The University of Warwick, Gibbet Hill Campus, Coventry, CV47AL. United Kingdom.

Abstract

Florigens, the leaf-derived signals that initiate flowering, have been described as ‘mysterious’, ‘elusive’ and the ‘Holy Grail’ of plant biology. They are synthesized in response to appropriate photoperiods and move through the phloem tissue. It has been proposed that their composition is complex. The evidence that FLOWERING LOCUS T (FT) protein and its parologue TWIN SISTER OF FT (TSF) act as florigen, or represent at least part of it, in diverse plant species has attracted considerable attention. In Arabidopsis thaliana, inductive photoperiodic conditions perceived in the leaf lead to stabilization of CONSTANS protein, which induces FT and TSF transcription. When they have been translated in the phloem companion cells, FT and TSF enter the phloem stream and are conveyed to the shoot apical meristem, where they act together with FLOWERING LOCUS D to activate transcription of floral meristem identity genes, resulting in floral initiation. At least part of this model is conserved, with some variations in several species. In addition to florigen(s), a systemic floral inhibitor or antiflorigen contributes to floral initiation. This chapter provides an overview of the different molecules that have been demonstrated to have florigenic or antiflorigenic functions in plants, and suggests possible directions for future research.

Keywords:
Antiflorigen, florigen, flowering, flowering locus T, juvenility, photoperiodism, signal transduction.
Introduction: the florigen mystery

The first historical record of attempts to understand flowering behaviour can be found in the writings of the Greek philosopher Theophrastus (c. 370–285 BC). Many centuries later, Mendel included flowering among the traits that he examined genetically, but his data were never presented in full. Detailed examination and quantification of flowering time variation began between 1910 and 1920, when the effects of low temperatures, nutritional conditions and daylength were first quantified.

The response of plants to the relative length of day and night is known as photoperiodism. Photoperiodism was first described in detail in 1920 by Garner and Allard, who demonstrated that plants flower in response to altered photoperiodic conditions [1]. The photoperiodic response allows plant species to adapt to seasonal changes in their environment [2]. Based on their flowering response, short-day plants (SDPs) flower after the daylength becomes shorter than a critical length (the inductive photoperiod), long-day plants (LDPs) flower when the daylength becomes longer than a critical length, and day-neutral plants flower irrespective of daylength. Both SDPs and LDPs can be either obligate (also termed qualitative) or facultative (also termed quantitative). Obligate plants have an absolute requirement for inductive photoperiods in order to flower, whereas the flowering of facultative plants is only accelerated in the inductive photoperiod [2]. Arabidopsis thaliana is a facultative LDP, as its flowering is promoted by long days (LDs) and delayed by short days (SDs).

Over the years, a number of studies have led to the development of three models to explain the photoperiodic regulation of floral induction. The first model is that of a flowering hormone-like substance, or florigen, which was first postulated by Mikhail Chailakhyan [3]. The florigen concept was based on the transmissibility of floral inductive signals across grafts between reproductive donor stems and juvenile recipients in Nicotiana tabacum. It was proposed that florigen was synthesized in the leaves under inductive photoperiods and transported to the shoot apical meristem (SAM) via the phloem. It was also proposed that florigen has a universal function in plants [4,5]. Many different molecules have been suggested to be components of florigen. The detection of a graft-transmissible floral antagonist also led to the theory of a competing ‘antiflorigen.’ Despite many years of research aimed at identifying the florigen and antiflorigen molecules in the phloem exudates of several plant species, their molecular character has remained elusive until recently.

The difficulty in distinguishing the hypothetical floral hormone-like substance from phloem-transported assimilates led to the development of a second model, known as the nutrient diversion hypothesis. According to this model, floral-promotive conditions result in an increase in the amount of photosynthate that is translocated to the SAM, which in turn promotes floral induction.

The theory that photosynthate translocation is uniquely important in the promotion of floral induction was displaced by the multifactorial control hypothesis proposed by Georges Bernier [6,7]. According to this model, several promoters and/ or inhibitors belonging to the classes of nutrients and hormones are involved in floral induction in the SAM, and genetic variation as well as past and present environmental conditions result in different factor(s) becoming limiting in different genotypes and/or under diverse environmental conditions.
The molecular nature of florigen has long been a key research question in plant developmental biology. Interestingly, elegant molecular genetic approaches have recently pinpointed possible florigen and antiflorigen components. A vast body of literature suggests that FLOWERING LOCUS T (FT) protein [8–11] and its parologue TWIN SISTER OF FT (TSF) [12] act as florigen, or at least that they represent part of it in a range of different plant species. The panoptic theme of floral induction pathways has attracted much attention, and a number of comprehensive review articles have been published [13–15]. However, this chapter will highlight the literature that has increased our understanding of several aspects of florigenic and antiflorigenic signalling in plants.

**Floral signal transduction**

Flowering time has been genetically explored in several plant model systems, and many genes have been cloned through the study of natural variation and induced mutations (Figure 1). This has led to the conclusion that several interdependent genetic pathways control floral induction [13–15]. The photoperiodic and vernalization pathways control time to flowering in response to environmental signals such as daylength, light and temperature, whereas the autonomous and gibberellin (GA)-dependent pathways monitor endogenous indicators of the plant's age and physiological status. Other factors and less well-characterized pathways also play a role in the control of floral initiation. These include microRNAs (miRs), ethylene, brassinosteroids, salicylic acid (SA) and cytokinins (CKs). The photoperiodic pathway is of particular relevance to the focus of this chapter, and is therefore the only pathway that will be described here.

The actions of all flowering time pathways ultimately converge to control the expression of a small number of so-called floral pathway integrators (FPiS), which include FT, TSF, SUPPRESSOR OF CONSTANS 1 (SOC1) and AGAMOUS-LIKE 24 (AGL24). These act on the floral meristem identity (FMI) genes LEAFY (LFY), FRUITFULL (FUL) and APETALA 1 (AP1), resulting in the initiation of flowering.

![Figure 1. The **ft-1** mutant of *Arabidopsis thaliana* flowers significantly later than the Ler-0 wild type under LD conditions.](image)
The photoperiodic pathway: a model for the mode of action of FT

The photoperiodic pathway starts with the perception of light in leaves by the red/far-red light receptors, namely phytochromes (PHYA-E), and the blue/UV-A light receptors, namely cryptochrome (CRY1 and CRY2) [16]. The photoreceptors initiate signals that interact with a circadian clock and entrain the circadian rhythm. In plants, the circadian clock regulates a wide range of biological processes and represents the plant’s endogenous timekeeper. Central to the photoperiod pathway in Arabidopsis is CONSTANS (CO), a zinc finger transcription factor (TF). Cycling of CO mRNA is regulated transcriptionally by the circadian clock through a protein complex formed by GIGANTEA (GI), and FLAVIN BINDING, KELCH REPEAT, F-BOX 1 (FKF1). The GI–FKF1 interaction modulates CO protein stability through degradation of the CO repressor CYCLING DOF FACTOR 1 (CDF1). High CO levels promote the expression of FT and TSF, two key members of the phosphatidylethanolamine-binding protein (PEBP) gene family [17–19].

Based on the temporal and spatial expression patterns of FT and TSF, a model for the photoperiodic induction of Arabidopsis has been proposed (Figure 2) [13–15]. According to this model, FT protein and TSF are part of the long-distance floral stimulus, florigen. Inductive LD conditions perceived in the leaf stabilize CO protein, which induces FT transcription in the
leaf and TSF in the stem. Once they have been translated in the phloem companion cells, FT and TSF are loaded into the phloem and translocated to the SAM. FT-INTERACTING PROTEIN 1 (FTIP1) [20] and PHOSPHOLIPID PHOSPHATIDYLCHOLINE (PC) [21] have been identified as key regulators of FT transport. At the SAM, a series of direct interactions between FPIs, SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) TFs and FMI genes promotes the LD floral induction pathway [18,22–25]. FT and TSF have been shown to physically interact with the locally transcribed bZIP TF FLOWERING LOCUS D (FD). The FT–TSF/FD transcriptional complex activates the expression of SOC1 [18]. After the induction of SOC1, expression of SPL3, SPL4 and SPL5 is rapidly induced in the SAM. These three members of the SPL family are direct targets of SOC1 and FD, whereas their expression also requires FT–TSF and SOC1–FUL activity [23,24]. When the transcription of FMI genes is stabilized, FT and TSF are no longer essential, and the SAM becomes fully committed to floral initiation. Under non-inductive SD conditions, FT expression levels are reduced. However, as plant development proceeds, FT expression levels show a clear increase.

Evidence has been provided for FT mRNA trafficking via the phloem to the SAM, independently of the FT protein [26]. Whether FT mRNA also participates in systemic floral regulation remains controversial [13]. However, under inductive LD conditions there is another florigenic signal involving specific GAs. Thus there may be multicomponent floral signalling in LDs involving FT and GAs, and an additional role for photosynthates has also been proposed (Figure 2) [13].

The role of FT homologous, orthologous and paralogous genes in other species

At least part of the regulatory mechanism described above is conserved, with some variations, in several plant species. Loci orthologous to FT have been identified in several dicotyledonous and monocotyledonous plants. Species that possess FT genes include trees, woody perennials, grasses, legumes and ornamentals. Ectopic overexpression of FT orthologous genes hastens the juvenile-to-adult phase transition, and promotes time to flowering in several transgenic homologous and heterologous plants [13]. In addition, in several species the interaction between FT and FD has been either demonstrated or proposed [27,28].

Recent studies have revealed the persistently elusive florigens in species such as Oryza sativa, Lycopersicon esculentum and Zea mays. The Z. mays CENTRORADIALIS 8 (ZCN8) gene is expressed in the leaf and is able to induce flowering in Arabidopsis ft mutants when expressed under the control of a phloem-specific promoter [29]. In L. esculentum, the SINGLE FLOWER TRUSS (SFT)-dependent graft-transmissible elements complement developmental defects in sft mutants, and substitute for LD conditions in Arabidopsis [8]. As an SDP, O. sativa requires different regulatory mechanisms for the photoperiodic control of flowering [30]. However, as in Arabidopsis, the central pathway in O. sativa consists of the GI–CO–FT regulatory module. The O. sativa homologues of GI, CO and FT are OsGIGANTEA (OsGI), HEADING-DATE 1 (HD1) and HEADING-DATE 3a (HD3a), respectively (Figure 3) [31–34]. At the SAM, HD3a interacts with its intracellular receptor 14-3-3 protein, and binds to the OsFD1 to form a ternary ‘florigen activation complex’ [35], which induces transcription of FMI genes that lead to floral induction.
1 (RFT1), another orthologue of Arabidopsis FT, is a major floral promoter under LD conditions [36]. However, a unique O. sativa pathway with no obvious orthologue in Arabidopsis is also involved in photoperiodic regulation of floral induction. EARLY HEADING DATE 1 (EHD1), a B-type response regulator, functions upstream of HD3a and RFT1. Mutations in EHD1 cause delayed flowering under SD conditions [37]. In conclusion, this conservation, together with the small protein size, renders FT partially capable of fulfilling the requirements of one of the florigen candidates, or one of its important components.

Florigen: beyond floral signal transduction

Photoperiod affects diverse developmental pathways in plants. A vast body of literature suggests that FT has universal effects on plant development. Recent studies have led to the identification of members of the FT gene family as a major component of the tuber-inducing signal, tuberigen [30]. Tuberization is a complex developmental process that initially leads to the formation of horizontally growing underground stems known as stolons. Tuberigen is first synthesized in the leaves under SD conditions, is graft-transmissible, and is transported through the phloem to the stolons. The molecular nature of tuberigen is not yet known, but it might be similar to that of florigen. Interestingly, StSP6A, a Solanum tuberosum FT homologue, has been shown to control tuberization [30]. Overexpression of the O. sativa florigen gene, HD3a, induced StSP6A expression and promoted tuberization as well as floral induction in S. tuberosum lines. This finding supports the hypothesis that florigen functions as tuberigen.

Florigen appears to play a broad pleiotropic role in the regulation of generalized growth of vegetative and reproductive organs. FT homologues are reportedly involved in SD-induced growth cessation and bud set in Populus species [19,38], in the control of leaf morphology and

![Figure 3. A simplified model of the photoperiodic induction pathway in LD and SD plant species.](image-url)
plant architecture in perennial *L. esculentum* [8] and *Z. mays* [39], in stomatal control in *Arabidopsis* [40], in fruit yield in *L. esculentum* [41], and in flowering repression in *Beta vulgaris* [42]. Interestingly, and perhaps understandably, the discovery of pleiotropic effects of FT genes has emerged as a field of research with significant potential to enhance several aspects of crop performance and quality.

**Antiflorigenic signal transduction**

The concept of certain antiflorigen(s) or floral repressor(s) was proposed almost as long ago as that of a floral stimulus [2]. Classical physiological studies have suggested the existence of certain antiflorigen(s) that are synthesized in leaves (Table 1). Interestingly, the PEBP gene family has evolved both activators and repressors of flowering.

Antiflorigenic effects of FT-like genes have been postulated in several plant species. *Arabidopsis thaliana* RELATIVE OF CENTRORADIALIS (*ATC*), an *Arabidopsis* FT parologue, functions as an antiflorigen [28]. ATC probably antagonizes FT activity, because both ATC and FT interact with FD to regulate the same downstream FMI genes, but in an opposite manner [28]. Similarly, the SD plant *Chrysanthemum seticuspe* produce FT-like proteins with antagonistic functions. *C. seticuspe* FT-like 3 (*CsFTL3*) is activated in SDs and promotes floral induction, whereas *C. seticuspe* ANTI-FLORIGENIC FT (*CsAFT*) is activated in non-inductive LDs and represses floral initiation [27]. The antagonism of CsAFT and CsFTL3 occurs through competition for CsFDL1, a *C. seticuspe* FD homologue. In *B. vulgaris*, time to flowering is regulated by the interplay of two paralogues of *Arabidopsis* FT that have evolved antagonistic functions. *B. vulgaris* FT 2 (*BvFT2*), which is functionally conserved with FT, is essential for floral induction, whereas *BvFT1* acts as an antiflorigen [42]. Similarly, the *Helianthus annuus* FT 1 (*HaFT1*) parologue acts as a floral promoter, whereas the frame shift allele *HaFT4* functions as an antiflorigen [43].

Several studies have identified the antiflorogenic functions of LIKE HETEROCHROMATIN PROTEIN 1 (LHP1, also known as TERMINAL FLOWER2) [44], TEMPRANILLO 1 (TEM1) and TEM2 proteins [45]. It has been demonstrated that LHP1, TEM1 and TEM2 function as leaf-based antiflorigenes that might also be able to move to the SAM. The LHP1 represses the expression of FT, but with no effect on the expression of TSF [12] or the other FPI and downstream FMI genes [44]. TEM1 and TEM2 genes play a key role in inhibiting flowering under SDs and LDs by directly repressing FT and GA biosynthesis genes [45].

miRs are short non-translated RNAs that are processed by Dicer-like proteins from large, characteristically folded precursor molecules. The majority of plant miRs target TFs, and therefore regulate several developmental events, including juvenility and floral induction [13,46]. Delicate grafting experiments have shown that the effect of several miRs is transmissible via grafts [47], which suggests that they are transportable. miR156 is an antiflorigen and key regulator of the juvenile-to-adult and vegetative-to-reproductive phase transitions in several plant species [48–50]. Constitutive expression of miR156 prolongs juvenility and time to flowering. It has been demonstrated that the juvenile-to-adult phase transition is accompanied by a decrease in miR156 levels, and a concomitant increase in the levels of miR172 and SPL TFs [51–54]. Expression of miR172 activates florigen in the leaves through repression of AP2-like TFs TARGET OF EAT1-3 (TOE1-3), SCHLAFMÜTZE (SMZ) and SNARCHZAPFEN (SNZ) [55–57], whereas the increase in SPL transcript levels in the SAM leads to the
| Gene name                        | Abbreviation | Species                  | Supporting evidence                                                                 |
|---------------------------------|--------------|--------------------------|-------------------------------------------------------------------------------------|
| *Arabidopsis thaliana* RELATIVE | ATC          | *Arabidopsis thaliana*   | ATC antagonizes FT activity. Both ATC and FT interact with FD                       |
| of CENTRORADIALIS               |              |                          |                                                                                     |
| *Beta vulgaris FT2*             | BvFT1        | *Beta vulgaris FT1*      | BvFT1 is likely to regulate downstream flowering promoters, as part of the vernalization response |
| *Chrysanthemum seticuspe* ANTI  | CsAFT        | *Chrysanthemum seticuspe*| CsAFT antagonizes CsFTL3 activity. CsAFT and CsFTL3 compete for CsFDL1, an FD homologue |
| *FLORENCIA* FT                  |              |                          |                                                                                     |
| *Helianthus annuus FT4*         | HaFTA4       | *Helianthus annuus*      | A potential mechanism for the negative interaction between the HaFT1 and HaFT4 could be interference with binding of HaFT4 to shoot apex proteins required for floral induction |
| *MicroRNA156*                   | miRNA156     | *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* | In *Arabidopsis*, miR156 overexpression results in a prolonged juvenile phase and a delay in flowering. miR156 targets the transcripts of SQUAMOSA PROMOTER BINDING-LIKE (SPL) transcription factors |
| *TEMPRANILLO1* / *TEMPRANILLO2* | TEM1/ TEM2   | *Arabidopsis thaliana*   | TEM1 and TEM2 repress flowering in SDs and LDs, by directly repressing FT and GA biosynthesis genes |
| *TERMINAL FLOWER2 (LIKE  HETEROCHROMATIN PROTEIN1)* | TFL2 (LHP1) | *Arabidopsis thaliana*   | LHP1 (TFL2) represses the expression of FT                                                                 |
transcription of FMI genes [25,54]. Interestingly, miR156a and miR156c, which are the major sources of miR156 in Arabidopsis, are significantly down-regulated by sugars [58,59].

Antiflorogens are of great importance as they prolong the juvenile-to-adult and vegetative-to-reproductive phase transitions. This allows the necessary assimilate reserves to be accumulated, ensuring unimpeded reproductive development.

**Floral induction may involve multiple florigens and antiflorogens**

The existence of long-distance signalling molecules for floral induction has been known for several decades. Some of these molecules have florigenic or antiflorgenic functions, whereas others make only a floral-promoting or floral-repressing contribution (Table 2). Other compounds that have been postulated as graft-transmissible floral regulators are described below.

**Phytohormones in floral signal transduction**

Among the phytohormones, GAs are of special importance because of their ability to induce flowering in LD plants grown under non-inductive SDs [60]. In Lolium temulentum, GA5 and GA6 have been demonstrated to be LD mobile floral signals that traffic to the SAM. GA4 promotes export of assimilates, and in combination with sucrose has a synergistic florigenic effect on the activation of FMI genes [61]. Moreover, GAs have important roles in promoting transcription of FT, TSF and SPL genes during floral induction in response to LDs. It has been shown that these functions are spatially separated between the leaf and the SAM [13].

CKs are another class of phytohormones with reported roles in regulation of flowering time in Sinapis alba and Arabidopsis [7,13]. Phloem sap analyses in Arabidopsis have revealed increased levels of the isopentenyl type of CKs during floral initiation in response to photoperiod. In S. alba, the florigenic effect of CK, which bypasses Sinapis alba FT, acts via its paralogue TSF and SOC1 [62]. CKs and SaFT may therefore be integral parts of the florigen in S. alba.

Abscisic acid (ABA) was proposed as the first identified antiflorigen. Arabidopsis mutants that are defective in or insensitive to ABA flower early, whereas mutants that overproduce ABA flower late. The inhibitory effect of ABA on time to flowering might be explained by sugar repression-related events. This view is supported by the early flowering phenotype of ABA-deficient mutants and their allelism to sugar-insensitive mutants. However, despite its antiflorogenic function in some species, many other species are not affected, and therefore ABA seems to have no general function as a floral transmissible repressor [60].

Polyamines such as putrescine and spermidine might also represent part of the florigen. Photoperiodic induction of S. alba was correlated with a significant increase in the levels of putrescine, the major polyamine in the leaf phloem sap. Auxins have also been detected in phloem sap, and in conjunction with other molecules may have potential florigenic activity. The altered flowering phenotypes of plants with impaired auxin biosynthesis and signalling may indicate that modulation of flowering time by auxins is indirect, occurring via interaction with other phytohormones, such as CKs, ethylene, ABA and GAs [63]. Other molecules, such as ascorbic acid (AA), ethylene, salicylic acid (SA), brassinosteroids and peptides, have also been suggested to act as floral promoters [7,13,60].
Table 2. Summary of putative components of florigen and antiflorigen

| Molecule | Function                      | Supporting evidence                                                                 | Conflicting evidence                                                                 |
|----------|-------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Proteins | Florigenic                    | FT protein, as a florigen, shows universality across daylength response types        | Differences in different proteins present in phloem exudates between induced and non-induced plants |
|          |                               | Transported from leaves to SAM                                                       | Repress flowering time                                                                |
|          | Antiflorigenic                 | Differences in different proteins present in phloem exudates between induced and non-induced plants | Transported from leaves to SAM                                                         |
| miRNAs   | Florigenic                    | Promote flowering time (miRNA172)                                                   | Diferences in different proteins present in phloem exudates between induced and non-induced plants |
|          |                               | Transported from leaves to SAM                                                       | Repress flowering time (miRNA156)                                                   |
| mRNAs (FT)| Floral promoting             | Evidence shows that FT mRNA may act together with FT protein                        | Positive disproof of the mRNA hypothesis by using artificial miRNA against FT mRNA, and a synthetic FT gene with extensive nucleotide substitutions throughout the ORF and foreign UTRs, respectively |
|          |                               |                                                                                      |                                                                                      |
FT mRNA promotes flowering in *Nicotiana tabacum* Maryland Mammoth. Transformed from leaves to SAM.

| Component | Description |
|-----------|-------------|
| Sucrose   | Florigenic  | Promotes flowering time in several plant species | Difficult to distinguish between signalling and metabolic effects |
| Peptides  | Florigenic  | Promote flowering time | The effects of most peptides on flowering have not yet been tested |
| Cytokinins| Florigenic  | Promote flowering time | Not sufficient to induce flowering when applied exogenously |
| Gibberellins| Florigenic | Act as florigens in LD species | May not act as universal florigens across daylength response types |

*Continued*
Table 2. Summary of putative components of florigen and antiflorigen (Continued)

| Molecule       | Function        | Supporting evidence                                                                 | Conflicting evidence                                                                 |
|----------------|-----------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Ethylene       | Florigenic      | Have a floral repressing role in some woody species of horticultural importance      |                                                                                      |
|                |                 | Transported from leaves to SAM.                                                       |                                                                                      |
| Ethylene       | Florigenic      | Induces flowering in plant species belonging to the Bromeliaceae                     | Inhibits flowering in many other plant species                                         |
|                |                 | It is not clear whether ethylene synthesis is induced under inductive photoperiods   |                                                                                      |
| Brassinosteroids| Floral promoting| Have a floral repressing role in some woody species of horticultural importance      |                                                                                      |
| Abscisic acid  | Floral promoting| Promotes flowering when given after or later in the dark period in *Pharbitis nil*  | Inhibits flowering in many plant species                                              |
|                | Floral repressing| Inhibits flowering when given before or early in the dark period in *Pharbitis nil* | Promotes flowering in *Pharbitis nil*                                               |
| Polyamines     | Floral promoting| Putrecine levels are significantly increased in photoinduced leaves of *Sinapis alba*| Polyamines alone do not influence floral induction                                    |
| Salicylic acid | Floral promoting| Promotes flowering in *Arabidopsis* and several species of the Lemnaceae              | Photoperiod-related changes in endogenous levels of salicylic acid have not been detected|
| Ascorbic acid  | Floral promoting| Low levels cause accelerated flowering under LDs                                       | May not act as a universal floral promoter                                           |
|                | Floral repressing| Low levels delay flowering in SDs through GA deficiency events                      | May not act as a universal floral repressor                                         |
Sucrose as a florigen

Sucrose is the most extensively studied compound that may potentially participate in floral signal transduction [7,13,60]. It is the dominant transport metabolite for long-distance carbon transport between source and utilization sinks. In Arabidopsis and S. alba that are exposed to inductive LDs, sucrose levels increase rapidly and transiently in phloem leaf exudates. Defoliation experiments have demonstrated that the increase in sucrose export coincides with the start of mobile floral signal transport, and occurs before the activation of cell division at the SAM [64]. Ample evidence has been provided that sucrose may act dependently and/or independently of FT florigen [13,65]. Arabidopsis plants flower rapidly in SDs after exposure to 8–12 days at a high light integral. It has been shown that this ‘photosynthetic’ response is FT independent. In contrast, the IDD8 locus of Arabidopsis was reported to have a role in FT-dependent induction of flowering by modulating sugar transport and metabolism via the regulation of Sucrose Synthase4 activity [66].

Because sucrose affects many aspects of plant development, it is difficult to demonstrate whether its effects on floral signal transduction are direct or indirect. The broad-spectrum effect of sucrose is clouded by its dyadic function as a nutrient and signalling molecule, and by the interaction between sucrose signalling and hormonal networks [60]. However, trehalose-6-phosphate (Tre6P), a metabolite of emerging significance, acts as a signal of sucrose status in plant tissues. Interestingly, Arabidopsis plants with impaired Tre6P signalling are late flowering. This late-flowering phenotype has been found to be caused by reduced expression levels of FT, elevated levels of miR156, and reduced levels of at least three miR156-regulated transcripts, namely SPL3, SPL4 and SPL5 [67].

Conclusions

A major challenge in modern biotechnology is to develop new elite crop varieties with enhanced agronomic traits, such as optimal flowering timing and plant architecture to meet the growing demand for food, feed and biofuel resources. Decades of research on photoperiodic control of floral induction have greatly expanded our understanding of the molecular mechanisms that initiate and drive juvenility and floral induction in different species. It has been shown that juvenility and floral induction are controlled by long-distance communication via the phloem, which recruits a variety of florigenic and antiflorigenic signals. One of these signals, the FT protein, fulfils all of the criteria that were postulated for the flowering hormone florigen by Mikhail Chailakhyan. However, fundamental questions remain unanswered. The properties of florigen have been insufficiently studied, and the complete composition of florigen has not been established. Under inductive LD conditions, there is another florigenic signal that involves GAs. Thus, apart from the FT protein, there may be a multicomponent floral signal in LDs involving GAs and/or sugars. In addition, further research is needed to determine how the florigenic and antiflorigenic signals leave the phloem in order to reach their target tissues. It has been suggested that FT transport may also depend on other molecules, and that compounds other than FT and TSF might be components of florigen.

FT and TSF are regulated by CO, are phloem mobile, and require other components to be present in the SAM to function as floral promoters. In addition, other proteins, as well as factors such as miRNAs, sugars, GAs and CKs, have roles in the activation of FMI genes that lead
to flowering. Therefore the multifactorial florigen concept proposed by Georges Bernier [6,7] should be accommodated.

**Summary**

- *Arabidopsis* FT protein is a component of florigen, a systemic signal that has been demonstrated to control photoperiodic flowering in diverse plant species.
- FT is synthesized in the leaves and selectively transported to the SAM. In the SAM cells, FT binds 14-3-3 and FD to form a ternary ‘florigen activation complex’, which activates FMI genes.
- Many different molecules have been postulated to be components of florigen, including sucrose, GAs, CKs, other plant hormones, certain amino acids, proteins, miRNAs and SA. Complex interactions between the signaling pathways that control the synthesis of these various florigenic molecules may occur.
- Photoperiodic regulation of floral initiation is also regulated by a systemic floral inhibitor or antiflorigen, which is exported from the non-induced leaves. The antiflorigen production system prevents precocious flowering.
- A more detailed understanding of the molecular mechanisms involved in florigenic and antiflorigenic signal transduction has many biotechnological applications.

Research in the laboratory of I.G. Matsoukas is supported by the University of Bolton, U.K.

**References**

1. Garner, W.W. and Allard, H.A. (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agric. Res. 18, 553–606.
2. Thomas, B. and Vince-Prue, D. (1997) Photoperiodism in Plants, Academic Press, San Diego.
3. Chailakhyan, M.K. (1936) About the mechanism of the photoperiodic response. Dokl. Akad. Nauk SSSR 1, 85–89.
4. Lang, A. (1965) Physiology of flower initiation. In Encyclopedia of Plant Physiology (Ruhland, W., ed.), pp. 1380–1536, Springer, Berlin.
5. Zeevaart, J.A.D. (1976) Physiology of flower formation. Annu. Rev. Plant Physiol. 27, 321–348.
6. Bernier, G. (1988) The control of floral evocation and morphogenesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 175–219.
7. Bernier, G. and Perilleux, C. (2005) A physiological overview of the genetics of flowering time control. Plant Biotechnol. J. 3, 3–16.
8. Lifschitz, E. and Eshed, Y. (2006) Universal florigenic signals triggered by FT homologues regulate growth and flowering cycles in perennial day-neutral tomato. J. Exp. Bot. 57, 3405–3414.
9. Corbesier, L., Vincent, C., Jang, S.H., Fornara, F., Fan, O.Z., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C. and Coupland, G. (2007) FT protein movement
contributes to long-distance signaling in floral induction of Arabidopsis. Science 316, 1030–1033.

10. Lin, M.K., Belanger, H., Lee, Y.J., Varkonyi-Gasic, E., Taoka, K.I., Miura, E., Xoconostle-Cazares, B., Gendler, K., Jorgensen, R.A., Phinney, B. et al. (2007) FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. Plant Cell 19, 1488–1506.

11. Mathieu, J., Warthmann, N., Kuttner, F. and Schmid, M. (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr. Biol. 17, 1055–1060.

12. Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M. and Araki, T. (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant Cell Physiol. 46, 1175–1189.

13. Matsoukas, I.G., Massiah, A.J. and Thomas, B. (2012) Florigenic and antiflorigenic signalling in plants. Plant Cell Physiol. 53, 1827–1842.

14. Srikanth, A. and Schmid, M. (2011) Regulation of flowering time: all roads lead to Rome. Cell Mol. Life Sci. 68, 2013–2037.

15. Andres, F. and Coupland, G. (2012) The genetic basis of flowering responses to seasonal cues. Nat. Rev. Genet. 13, 627–639.

16. Thomas, B. (2006) Light signals and flowering. J. Exp. Bot. 57, 3387–3393.

17. Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. and Coupland, G. (2001) CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. Nature 410, 1116–1120.

18. Wigge, P.A., Kim, M.C., Jaeger, K.E., Busch, W., Schmid, M., Lohmann, J.U. and Weigel, D. (2005) Integration of spatial and temporal information during floral induction in Arabidopsis. Science 309, 1056–1059.

19. Bohlenius, H., Huang, T., Charbonnel-Campaa, L., Brunner, A.M., Jansson, S., Strauss, S.H. and Nilsson, O. (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312, 1040–1043.

20. Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y. and Yu, H. (2012) FTIP1 is an essential regulator required for florigen transport. PLoS Biol. 10, e1001313.

21. Nakamura, Y., Andres, F., Kanehara, K., Liu, Y.C., Dormann, P. and Coupland, G. (2014) Arabidopsis florigen FT binds to diurnally oscillating phospholipids that accelerate flowering. Nat. Commun. 5, 3553.

22. Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K. and Araki, T. (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309, 1052–1056.

23. Torti, S., Fornara, F., Vincent, C., Andres, F., Nordstrom, K., Gobel, U., Knoll, D., Schoof, H. and Coupland, G. (2012) Analysis of the Arabidopsis shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. Plant Cell 24, 444–462.

24. Jung, J.H., Ju, Y., Seo, P.J., Lee, J.H. and Park, C.M. (2012) The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. Plant J. 69, 577–588.

25. Yamaguchi, A., Wu, M.F., Yang, L., Wu, G., Poethig, R.S. and Wagner, D. (2009) The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. Dev. Cell 17, 268–278.

26. Li, C., Gu, M., Shi, N., Zhang, H., Yang, X., Osman, T., Liu, Y., Wang, H., Vatish, M., Jackson, S. and Hong, Y. (2011) Mobile FT mRNA contributes to the systemic florigen signalling in floral induction. Sci. Rep. 1, 73.

27. Higuchi, Y., Narumi, T., Oda, A., Nakano, Y., Sumitomo, K., Fukai, S. and Hisamatsu, T. (2013) The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums. Proc. Natl Acad. Sci. U.S.A. 110, 17137–17142.
28. Huang, N.C., Jane, W.N., Chen, J. and Yu, T.S. (2012) Arabidopsis thaliana CENTRORADIALIS homologue (ATC) acts systemically to inhibit floral initiation in Arabidopsis. Plant J. 72, 175–184.

29. Meng, X., Muszynski, M.G. and Danilevskaya, O.N. (2011) The FT-like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize. Plant Cell 23, 942–960.

30. Navarro, C., Abelenda, J.A., Cruz-Oro, E., Cuellar, C.A., Tamaki, S., Silva, J., Shimamoto, K. and Prat, S. (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature 478, 119–122.

31. Kojima, S., Takahashi, Y., Kobayashi, Y., Monna, L., Sasaki, T., Araki, T. and Yano, M. (2002) Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant Cell Physiol. 43, 1096–1105.

32. Komiya, R., Ikegami, A., Tamaki, S., Yokoi, S. and Shimamoto, K. (2008) Hd3a and RFT1 are essential for flowering in rice. Development 135, 767–774.

33. Hayama, R., Yokoi, S., Tamaki, S., Yano, M. and Shimamoto, K. (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature 422, 719–722.

34. Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Shimamoto, K., Umehara, Y., Nagamura, Y. and Sasaki, T. (2000) Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12, 2473–2484.

35. Taoka, K., Ohki, I., Tsuji, H., Furuita, K., Hayashi, K., Hayashi, K., Yanase, T., Yamaguchi, M., Nakashima, C., Purwastri, Y.A., Tamaki, S. et al. (2011) 14–3–3 proteins act as intracellular receptors for rice Hd3a florigen. Nature 476, 332–335.

36. Komiya, R., Yokoi, S. and Shimamoto, K. (2009) A gene network for long-day flowering activates RFT1 encoding a mobile flowering signal in rice. Development 136, 3443–3450.

37. Doi, K., Izawa, T., Fuse, T., Yamanouchi, U., Kubo, T., Shimatani, Z., Yano, M. and Yoshimura, A. (2004) Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. Genes Dev. 18, 926–936.

38. Hsu, C.Y., Adams, J.P., Kim, H., No, K., Ma, C., Strauss, S.H., Drnevich, J., Vandervelde, L., Ellis, J.D., Rice, B.M. et al. (2011) FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar. Proc. Natl Acad. Sci. U.S.A. 108, 10756–10761.

39. Danilevskaya, O.N., Meng, X., McGonigle, B. and Muszynski, M.G. (2011) Beyond flowering time: pleiotropic function of the maize flowering hormone florigen. Plant Signal. Behav. 6, 1267–1270.

40. Kinoshita, T., Ono, N., Hayashi, Y., Morimoto, S., Nakamura, S., Soda, M., Kato, Y., Ohnishi, M., Nakano, T., Inoue, S. and Shimazaki, K. (2011) FLOWERING LOCUS T regulates stomatal opening. Curr. Biol. 21, 1232–1238.

41. Krieger, U., Lippman, Z.B. and Zamir, D. (2010) The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato. Nat. Genet. 42, 459–463.

42. Pin, P.A., Benlloch, R., Bonnet, D., Wremerth-Weich, E., Kraft, T., Gielen, J.J.L. and Nilsson, O. (2010) An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. Science 330, 1397–1400.

43. Blackman, B.K., Strasburg, J.L., Raduski, A.R., Michaels, S.D. and Rieseberg, L.H. (2010) The role of recently derived FT paralogs in sunflower domestication. Curr. Biol. 20, 629–635.

44. Gaudin, V., Libault, M., Pouteau, S., Juul, T., Zhao, G., Lefebvre, D. and Grandjean, O. (2001) Mutations in LIKE HETEROCHROMATIN PROTEIN 1 affect flowering time and plant architecture in Arabidopsis. Development 128, 4847–4858.

45. Castillejo, C. and Pelaz, S. (2008) The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. Curr. Biol. 18, 1338–1343.

46. Matsoukas, I.G. (2014) Attainment of reproductive competence, phase transition, and quantification of juvenility in mutant genetic screens. Front. Plant Sci. 5, 32.

47. Buhtz, A., Pieritz, J., Springer, F. and Kehr, J. (2010) Phloem small RNAs, nutrient stress responses, and systemic mobility. BMC Plant Biol. 10, 64.
48. Wu, G. and Poethig, R.S. (2006) Temporal regulation of shoot development in Arabidopsis thaliana by miR156 and its target SPL3. Development 133, 3539–3547.

49. Chuck, G., Cigan, A.M., Saetern, K. and Hake, S. (2007) The heterochronic maize mutant Corngrass1 results from overexpression of a tandem microRNA. Nat. Genet. 39, 544–549.

50. Wang, J.W., Park, M.Y., Wang, L.J., Koo, Y., Chen, X.Y., Weigel, D. and Poethig, R.S. (2011) miRNA control of vegetative phase change in trees. PLoS Genet. 7, e1002012.

51. Shikata, M., Koyama, T., Mitsuda, N. and Ohme-Takagi, M. (2009) Arabidopsis SBP-box genes SPL10, SPL11 and SPL2 control morphological change in association with shoot maturation in the reproductive phase. Plant Cell Physiol. 50, 2133–2145.

52. Shikata, M., Yamaguchi, H., Sasaki, K. and Ohtsubo, N. (2012) Overexpression of Arabidopsis miR157b induces bushy architecture and delayed phase transition in Torenia fournieri. Planta 236, 1027–1035.

53. Jung, J.H., Seo, P.J., Kang, S.K. and Park, C.M. (2011) miR172 signals are incorporated into the miR156 signaling pathway at the SPL3/4/5 genes in Arabidopsis developmental transitions. Plant Mol. Biol. 76, 35–45.

54. Wang, J.W., Czech, B. and Weigel, D. (2009) miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. Cell 138, 738–749.

55. Jung, J.H., Seo, Y.H., Seo, P.J., Reyes, J.L., Yun, J., Chua, N.H. and Park, C.M. (2007) The GIGANTEA–regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in Arabidopsis. Plant Cell 19, 2736–2748.

56. Mathieu, J., Yant, L.J., Murdter, F., Kuttner, F. and Schmid, M. (2009) Repression of flowering by the miR172 target SMZ. PLoS Biol. 7, e1000148.

57. Aukerman, M.J. and Sakai, H. (2003) Regulation of flowering time and floral organ identity by a microRNA and its APETALA2–like target genes. Plant Cell 15, 2730–2741.

58. Yang, L., Xu, M., Koo, Y., He, J. and Poethig, R.S. (2013) Sugar promotes vegetative phase change in Arabidopsis thaliana by repressing the expression of MIR156A and MIR156C. eLife 2, e00260.

59. Yu, S., Cao, L., Zhou, C.M., Zhang, T.Q., Lian, H., Sun, Y., Wu, J., Huang, J., Wang, G. and Wang, J.W. (2013) Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. eLife 2, e00269.

60. Matsoukas, I.G. (2014) Interplay between sugar and hormone signaling pathways modulate floral signal transduction. Front. Genet. 5, 218.

61. Eriksson, S., Böhlenius, H., Moritz, T. and Nilsson, O. (2006) GA4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. Plant Cell 18, 2172–2181.

62. D’Aloia, M., Bonhomme, D., Bouche, F., Tamseddak, K., Ormenese, S., Torti, S., Coupland, G. and Perilleux, C. (2011) Cytokinin promotes flowering of Arabidopsis via transcriptional activation of the FT parologue TSF. Plant J. 65, 972–979.

63. Lu, C. and Fedoroff, N. (2000) A mutation in the Arabidopsis HYL1 gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. Plant Cell 12, 2351–2365.

64. Corbesier, L., Lejeune, P. and Bernier, G. (1998) The role of carbohydrates in the induction of flowering in Arabidopsis thaliana: comparison between the wild type and a starchless mutant. Planta 206, 131–137.

65. Matsoukas, I.G., Massiah, A.J. and Thomas, B. (2013) Starch metabolism and antiflorigenic signals modulate the juvenile-to-adult phase transition in Arabidopsis. Plant Cell Environ. 36, 1802–1811.

66. Seo, P.J., Ryu, J., Kang, S.K. and Park, C.M. (2011) Modulation of sugar metabolism by an INDETERMINATE DOMAIN transcription factor contributes to photoperiodic flowering in Arabidopsis. Plant J. 65, 418–429.

67. Wahl, V., Ponnu, J., Schlereth, A., Arrivaull, S., Langenecker, T., Franke, A., Feil, R., Lunn, J.E., Stitt, M. and Schmid, M. (2013) Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science 339, 704–707.
