

Review

A physiological overview of the genetics of flowering time control

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Summary

Physiological studies on flowering time control have shown that plants integrate several environmental signals. Predictable factors, such as day length and vernalization, are regarded as 'primary', but clearly interfere with, or can even be substituted by, less predictable factors. All plant parts participate in the sensing of these interacting factors. In the case of floral induction by photoperiod, long-distance signalling is known to occur between the leaves and the shoot apical meristem (SAM) via the phloem. In the long-day plant, *Sinapis alba*, this long-distance signalling has also been shown to involve the root system and to include sucrose, nitrate, glutamine and cytokinins, but not gibberellins. In *Arabidopsis thaliana*, a number of genetic pathways controlling flowering time have been identified. Models now extend beyond 'primary' controlling factors and show an ever-increasing number of cross-talks between pathways triggered or influenced by various environmental factors and hormones (mainly gibberellins). Most of the genes involved are preferentially expressed in meristems (the SAM and the root tip), but, surprisingly, only a few are expressed preferentially or exclusively in leaves. However, long-distance signalling from leaves to SAM has been shown to occur in *Arabidopsis* during the induction of flowering by long days. In this review, we propose a model integrating physiological data and genes activated by the photoperiodic pathway controlling flowering time in early-flowering accessions of *Arabidopsis*. This model involves metabolites, hormones and gene products interacting as long- or short-distance signalling molecules.

Keywords: environmental sensing, floral promotion pathways, floral repression pathways, florigenic signals, flowering time control, shoot apical meristem (SAM).

Introduction

A plant is a sessile organism that has to grow and reproduce in nature at the location at which its seed happens to germinate. The success of reproduction thus requires that plants continuously and accurately monitor their local environment to flower at the correct time. This is all the more critical for monocarpic species which have a single chance to sexually reproduce. The mechanisms elaborated to secure this correct timing were first investigated by physiologists, and have essentially been determined in recent years by molecular geneticists. Whilst physiological work explored diversity (various plant species studied in a broad range of environmental conditions), the genetic approach has focused mostly on the

single species *Arabidopsis thaliana*, usually grown in a more restricted set of environmental conditions. It is not surprising, therefore, that these two approaches have yielded a large body of information, but without achieving comprehensive integration at the whole-plant level (Périlleux and Bernier, 2002). Our attempt in this review is to aid such an integration.

Physiological control of flowering time

Environmental factors

In the natural conditions of temperate areas, many factors in the environment influence flowering time (Lang, 1965; Bernier *et al.*, 1981; Thomas and Vince-Prue, 1997). These factors

Table 1 Environmental factors participating in the control of flowering time in temperate areas

| Factor | Main perceiving organ(s) |
|-------------------------------------|--------------------------|
| Highly predictable or primary | |
| Day length (photoperiodism) | Leaves/stem/SAM |
| Winter cold (vernalization) | SAM |
| Moderately predictable or secondary | |
| Ambient temperature | All organs |
| Irradiance | Leaves/stem |
| Water availability | Roots |
| Unpredictable or tertiary | |
| Mineral availability | Roots |
| Light quality | Leaves/stem/SAM |
| Neighbouring vegetation | Leaves/stem/SAM/roots |

SAM, shoot apical meristem.

are either predictable or not predictable, and hence can or cannot be used reliably by plants to time their reproduction. Factors that are highly predictable are considered to be the most specific or 'primary' controlling factors; these include the annual change in day length and the period of winter cold (Table 1). Less predictable climatic factors, such as ambient temperature, light integral (day length \times irradiance) and water availability, are usually viewed as 'secondary' factors that can only modulate the effects of primary factors. Finally, unpredictable or 'tertiary' factors are those that the plant has to face locally, such as mineral availability and neighbours (Table 1). The effects of neighbourhood have sometimes been limited to the response to light quality, although they also involve competition for light, water and minerals.

Although such a classification of controlling factors can account for the predominance of primary factors in many environmental niches, the situation is not so simple: the two primary factors (day length and vernalization) can substitute for each other, and can also be replaced by secondary or tertiary factors (Table 2). The promotion of flowering by a primary factor can also be reduced or even completely suppressed by another factor. Examples include the suppression of flowering, in favourable photoperiodic conditions, by water stress (drought) in the long-day (LD) plant *Lolium temulentum* and the short-day (SD) plants *Xanthium strumarium* and *Pharbitis nil*, or by excess nitrogen input in the LD plant *Sinapis alba* and the SD plant soybean. In other cases, it has been found that a primary factor might be conditional only in certain circumstances. This is true in *Calceolaria*, in which low irradiance creates a requirement for vernalization, although vernalization is not required at high irradiance (Bernier, 1988).

It is important to emphasize here that these interactions have been found in experiments in which only two factors were changed, and each was given at optimal levels. Such situations are probably infrequent in natural environments in which several factors change simultaneously and mutually influence the optimal values of each other. For example, it is known that, in many photoperiodic plants, the critical day length for flowering is affected by the ambient temperature. It is therefore no surprise that, when variations in more than two factors were tested on time to flowering, very complex situations were found. Thus plants, as sessile opportunists, can make use of alternative factors to control their flowering time in natural environments.

Table 2 Examples of factors capable of substituting for a primary factor in the control of flowering time

| Primary factor | Alternative factor acting in conditions in which the primary factor is unfavourable | Examples of plant species |
|----------------|---|---|
| Long days | Vernalization High ambient temperature High irradiance Low nitrogen availability | <i>Silene armeria</i> , <i>Blitum</i> sp., <i>Anagallis arvensis</i> , <i>Sinapis alba</i> <i>Rudbeckia bicolor</i> , <i>Samolus parviflorus</i> , <i>Silene armeria</i> <i>Sinapis alba</i> , <i>Brassica campestris</i> <i>Sinapis alba</i> , late-flowering pea lines |
| Short days | Vernalization High ambient temperature High irradiance Low irradiance Low nitrogen availability | <i>Perilla</i> sp., <i>Pharbitis nil</i> , Maryland Mammoth tobacco <i>Chenopodium polyspermum</i> <i>Pharbitis nil</i> <i>Perilla</i> sp., <i>Salvia occidentalis</i> <i>Perilla</i> sp., <i>Pharbitis nil</i> |
| Vernalization | Long days Short days High ambient temperature High ambient temperature + high irradiance | Late-flowering pea lines, <i>Geum urbanum</i> Some winter cereals and perennial grasses, kohlrabi <i>Scrofularia</i> sp., <i>Festuca arundinacea</i> Shuokan chrysanthemum |

Organs involved in environmental perception

Environmental factors participating in the control of flowering time are not all perceived by the same organ(s) (Lang, 1965; Bernier *et al.*, 1981; Bernier, 1988) (Table 1). Vernalization is generally perceived by the shoot apex [shoot apical meristem (SAM) plus leaf primordia], as shown by the fact that cold treatment applied to excised apices induces their floral transition, provided that they are supplied with nutrients. In pea and a few other species, it has been reported that vernalization is also perceived by leaves.

Day length and light quality are usually believed to be essentially perceived by expanded leaves but, in the absence of leaves, they can also be perceived by the stem. However, excised shoot apices of the SD plants *Perilla frutescens*, *Xanthium* and *Pharbitis* and the LD plant *Anagallis arvensis* respond to day length in the same way as intact plants, suggesting that this plant part is also involved in the response to photoperiod, provided that apices are supplied with sucrose and minerals in the culture medium. Experiments with optical fibres have further demonstrated that the apex of *Pharbitis* seedlings is capable of perceiving light quality treatments controlling flowering time, namely a red night-break or a far-red end-of-day extension.

Thus, all aerial organs participate in the perception of day length and light quality. This is also the case for ambient temperature, which is, of course, sensed by all plant parts, including the roots. For example, high temperature, promoting flowering in *Silene armeria* and inhibiting it in *Brassica pekinensis*, is perceived by the roots. Irradiance is essentially perceived by photosynthetic organs, expanded leaves and stem, whereas water and mineral availability are perceived by the roots. Roots have been found in some studies to promote or inhibit flowering depending on the species and environmental conditions. However, the role of the 'hidden half of the plant' has often been disregarded in physiological studies and genetic work on flowering.

Endogenous cues

In addition to environmental signals, plants are also known to monitor endogenous cues related to their flowering time, such as size, node number or age. Size rather than age was demonstrated to be particularly important in biennials, as well as in long-lived monocarps, in polycarps with long-lived monocarpic ramets and other polycarps (Lacey, 1986). Biennials, for example, generally flower during their second year of growth when they are cultivated in resource-rich conditions, such as experimental growth areas, gardens or agricultural fields.

In natural environments, however, they often flower only during their third or fourth year, or even later (Lacey, 1986; Klinkhamer *et al.*, 1987). They should then be called 'delayed' biennials or, more appropriately, 'monocarpic perennials'. As concluded in many field studies, the best predictor of flowering onset in these plants is the reaching of a threshold size, although this threshold may vary greatly amongst species and ecotypes (Lacey, 1986; Wesselingh *et al.*, 1993). This conclusion is in line with physiological observations showing that partial or complete removal of foliage, i.e. plant trimming, may decrease or even abolish the response of many plants to vernalization or favourable day lengths (Bernier *et al.*, 1981). Size is directly related to the amount of resources accumulated, and thus depends on the ambient temperature, irradiance, water/mineral availability and presence/absence of neighbours. In other words, in natural environments in which many factors are far from optimal, secondary and tertiary factors (Table 1) are often predominant over the primary factors for the control of flowering time. By contrast, flowering in natural populations of annuals is often principally controlled by a primary environmental cue, such as day length, and occurs independently of size or age (Lacey, 1986).

Long-distance signals

Although the fact that most plant parts participate in the sensing of the environmental factors that control flowering time clearly indicates that inter-organ, long-distance signalling must be involved in the triggering of flowering of the SAM, most of the physiological work to date has favoured the study of the unidirectional signalling event linking, in photoperiodic plants, the leaves to the SAM. The leaf-to-SAM signal is called 'florigen' when the leaves are exposed to day lengths favourable to flowering and 'antiflorigen' when the leaves are exposed to day lengths unfavourable to flowering. Numerous grafting experiments have shown the movement of such signals in several plant species (Thomas and Vince-Prue, 1997), but progress in identifying them has been extremely slow. A first success has been recorded recently in the rosette LD plant *Lolium temulentum* (King and Evans, 2003). The exposure of plants to LDs causes an increase in the level of several gibberellins, especially GA₅, in the leaves, and their further transport to the SAM. When applied to leaves at early stages of the floral transition, GA₅ markedly stimulates flowering, and hence may be the '*Lolium* florigen'. At later stages of the transition, other compounds come into play, such as GA₁/GA₄ (King and Evans, 2003) and sucrose (Périlleux and Bernier, 1997). GA₅ is unlikely, however, to be a universal 'florigen', as it is not detected in extracts of florally

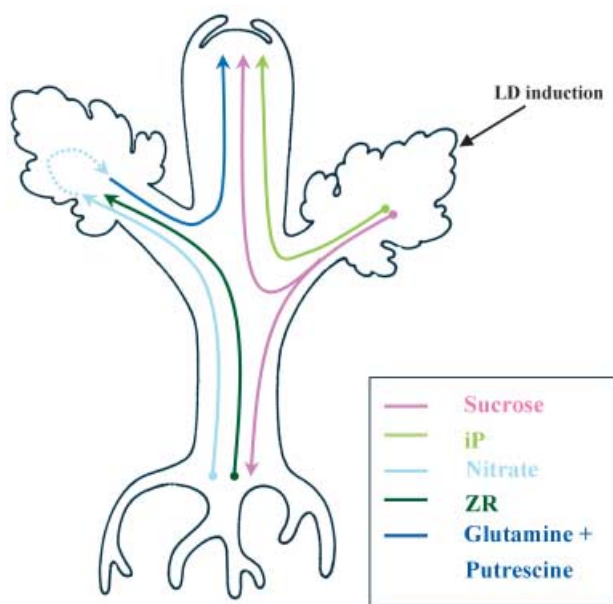


Figure 1 Diagram showing long-distance movements of floral signals in plants of *Sinapis alba* induced to flower by one long day (LD). Early signals, moving very soon within the photoextension period of the LD, are sucrose and isopentenyladenine (iP) in the phloem and zeatin riboside (ZR) and nitrate in the xylem. Other signals, moving several hours later in the phloem, are glutamine and putrescine.

induced *Arabidopsis* and *Sinapis* plants (Xu *et al.*, 1997; Corbesier *et al.*, 2004).

In *Sinapis*, analyses of changes in the contents of phloem and xylem saps during the floral transition have disclosed a complex shoot-to-root-to-shoot signalling loop involving both nutrients and hormones (Havelange *et al.*, 2000; Bernier *et al.*, 2002). As shown in Figure 1, exposure of leaves to an inductive LD results in the rapid export of extra-sucrose (Suc) and extra-cytokinin (CK) of the isopentenyladenine (iP) type in the phloem. Suc was found to move both upwards and downwards. When reaching the roots, Suc causes an increased and early upward export of CKs [mainly zeatin riboside (ZR)] and nitrate in the xylem. Recent experimental evidence has indicated that the major function of root CKs is to bring information to the shoot (essentially the leaves) on nitrogen availability (Schmülling, 2002). Apparently, root CKs are then rapidly metabolized in the leaves (Letham, 1994; Faiss *et al.*, 1997). Extra-nitrate is, on the other hand, presumably converted into glutamine (Gln) and putrescine (Put), as these compounds are later exported in greater amounts in the phloem by induced leaves. GAs do not apparently participate in signal movements in *Sinapis* as their complements and levels remain unchanged in both leaves and shoot tip following floral induction (Corbesier *et al.*, 2004).

All the leaf-exported compounds eventually enter the SAM, and we have shown that Suc and CK cause events there that are specifically related to the induction of flowering. For example, Suc increases invertase activity and, later, energy metabolism; both CK and Suc stimulate cell division (Bernier *et al.*, 1993, 2002). In fact, Suc unloaded from the phloem into the SAM is known to be hydrolysed into hexoses by invertases, which in turn have been found to be activated by sugars and/or CKs (Roitsch and Ehneß, 2000; Koch, 2004). Hence, the co-arrival of extra-amounts of Suc and iP at the *Sinapis* SAM at floral induction provides a mechanism for the amplification of hexose production. Further, the increased input of hexoses may not only stimulate energy-consuming processes in the SAM, but also trigger, together with the CKs, cell divisions via their action on D-type cyclins (Potuschak and Doerner, 2001). An interesting result, in this context, is that over-expression of the *CYCLIN D2* gene of *Arabidopsis* in tobacco plants causes an increase in cell division in the SAM and early flowering (Cockcroft *et al.*, 2000).

The flowering response of SAM to the inductive LD is abolished when the downward movement of Suc is interrupted by girdling, or when the upward movement of ZR and nitrate is prevented by plant exposure to saturating humidity (Havelange *et al.*, 2000), indicating that this long-distance signalling loop is essential for flowering in *Sinapis*. Interestingly, in plants in which flowering is inhibited by the interruption of long-distance signalling, an application of a CK or Gln directly to the apex substantially restores the flowering response (Havelange *et al.*, 2000; A. Havelange and G. Bernier, unpublished data 2002).

As the levels of Suc and CKs in the sap exported by induced leaves change in the SD plant *Xanthium*, as in *Sinapis* (Bernier *et al.*, 1998), it is quite possible that the co-ordinated movement of some nutrients and hormones is part of the controlling system of flowering in this species also.

Long-distance signalling also occurs in so-called 'self-inductive' plants which flower irrespective of vernalization and day length conditions. These plants, when grown continuously in defined environmental conditions, produce a constant number of nodes before starting to initiate flowers. Such is the case in day-neutral tobaccos which, depending on the genotype, may produce from 14 to 60 nodes before flowering. Grafting studies have shown that the specific node numbers in these genotypes are in fact controlled by a combination of two different long-distance signals, one of root and the other of leaf origin, as well as by SAM competence to respond to these signals (McDaniel, 1996). Unfortunately, the chemical nature of the signals and the mechanisms of SAM sensitivity were not determined in these studies.

Genetic control of flowering time

Work in this area has been conducted mainly in *Arabidopsis*, a rosette plant whose flowering is accelerated by LDs, vernalization, a rise in ambient temperature (from about 15 to about 25 °C), a low red/far-red ratio in incoming light and a low mineral availability. The flowering response to these environmental factors involves several signalling pathways that converge towards the regulation of floral meristem identity genes (Mouradov *et al.*, 2002). Two of these genes [*LEAFY* (*LFY*) and *APETALA 1* (*AP1*)] were identified first because their mutation clearly perturbs the fate of the SAM productions. Other genes, called 'integrators', act upstream of *LFY/AP1* and their mutation severely delays flowering in different growing conditions. These genes include *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) (see Figure 2).

As expected, the pathways controlling the flowering time in *Arabidopsis* that were first identified concerned 'primary' promoting factors (LDs and vernalization), as the criteria used to screen and characterize flowering time mutants included these environmental controls (Martínez-Zapater *et al.*, 1994).

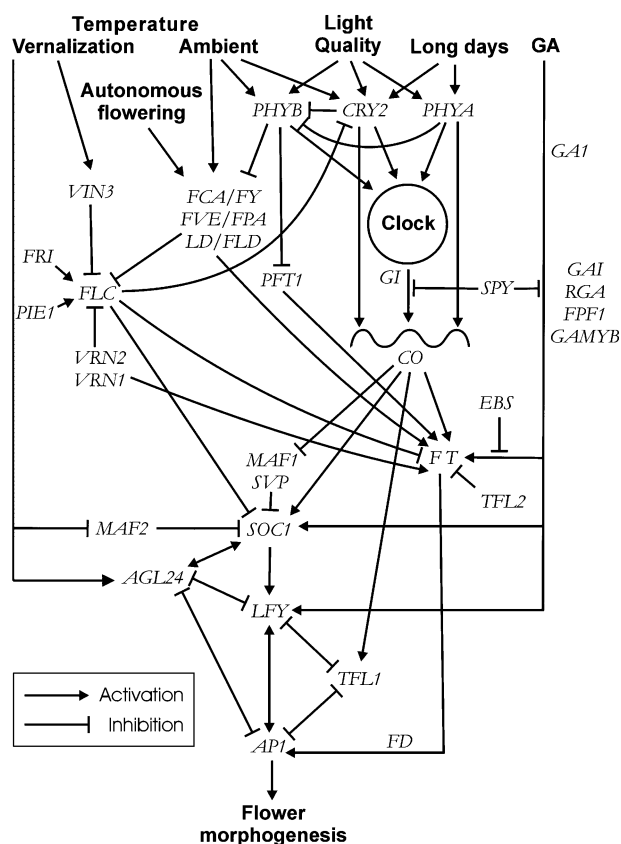


Figure 2 Interactions between genes promoting or repressing flowering in *Arabidopsis thaliana*. Environmental factors which set the different pathways in motion are shown at the top of the figure. The full names of the genes are listed in Table 3. GA, gibberellin.

Late-flowering mutants that were delayed in LDs led to the identification of genes of the 'LD pathway', while mutants which were still responsive to the photoperiod but impaired in the vernalization response were included in a 'vernalization pathway'. Mutants that remained sensitive to both environmental factors were then classified as affected in 'autonomous' flowering. In addition to this trio, a 'GA pathway' was proposed based on the extremely late-flowering phenotype of GA-deficient mutants in SDs.

On the other hand, genes involved in the repression of flowering were identified from early-flowering mutants (Pouteau *et al.*, 2004). These mutants are often very pleiotropic, indicating that the corresponding genes affect many processes, and hence it is difficult to integrate them into specific pathways. Moreover, their sensitivity to environmental factors has not been so well characterized; this is why the corresponding genes are regarded as 'modulating' the primary promoting pathways, which still form the framework of Figure 2, rather than identifying specific repression pathways.

Thus, until recently, most models proposed four promoting pathways (Mouradov *et al.*, 2002). In the last couple of years, models have become more and more complex because of an increased interest in 'secondary' environmental factors, such as ambient temperature (Blázquez *et al.*, 2003). Some of the most recent reviews no longer show the original quartet of pathways, but separate schemes (Boss *et al.*, 2004). Clearly, because the environmental factors controlling flowering time influence each other, as discussed above, cross-talks are continually being discovered between intervening genes, reflecting plant plasticity. For clarity, genes are confined to their main, or best supported, function (Figure 2) and, to limit quotations, we refer to recent reviews (Mouradov *et al.*, 2002; Périlleux and Bernier, 2002; Boss *et al.*, 2004) unless another reference is given. The full names of the genes are listed in Table 3.

Primary promoting pathways

The first signalling pathway promotes flowering in response to LDs. It includes genes encoding the photoreceptors phyA and cry2 and the clock components needed for correct circadian time measurement, several clock-associated genes, such as *GI*, and the downstream gene *CO*. The abundance of *CO* is photoperiod dependent and only in LDs does a large amount of protein coincide with the presence of light, sensed by phyA and cry2 (Valverde *et al.*, 2004). This 'external' coincidence allows *CO* to activate its target *FT*. Other targets of *CO* are *SOC1* and *TFL1*.

A second or 'vernalization' pathway, activated by a period of cold of sufficient duration, acts through repression of the

Table 3 Full names of genes

| | |
|--------------------|--|
| <i>ABI4</i> | <i>ABSCISIC ACID INSENSITIVE 4</i> |
| <i>AGL24</i> | <i>AGAMOUS-LIKE 24</i> |
| <i>AMP1</i> | <i>ALTERED MERISTEM PROGRAM 1</i> |
| <i>AP1</i> | <i>APETALA 1</i> |
| <i>CO</i> | <i>CONSTANS</i> |
| <i>CRY2</i> | <i>CRYPTOCHROME2</i> |
| <i>EBS</i> | <i>EARLY BOLTING IN SHORT DAYS</i> |
| <i>FKF1</i> | <i>FLAVIN BINDING, KELCH REPEAT, F-BOX1</i> |
| <i>FLC</i> | <i>FLOWERING LOCUS C</i> |
| <i>PPF1</i> | <i>FLOWERING PROMOTING FACTOR 1</i> |
| <i>FRI</i> | <i>FRIGIDA</i> |
| <i>FT</i> | <i>FLOWERING LOCUS T</i> |
| <i>GAI</i> | <i>GIBBERELLIC ACID INSENSITIVE</i> |
| <i>GI</i> | <i>GIGANTEA</i> |
| <i>IPT3</i> | <i>ISOPENTENYLTRANSFERASE 3</i> |
| <i>LD</i> | <i>LUMINIDEPENDENS</i> |
| <i>LFY</i> | <i>LEAFY</i> |
| <i>LKP2</i> | <i>LOV KELCH PROTEIN2</i> |
| <i>MAF1,2</i> | <i>MADS AFFECTING FLOWERING 1, 2</i> |
| <i>PGM</i> | <i>PHOSPHOGLUCOMUTASE</i> |
| <i>PHY A, B, E</i> | <i>PHYTOCHROME A, B, E</i> |
| <i>PIE1</i> | <i>PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1</i> |
| <i>PFT1</i> | <i>PHYTOCHROME AND FLOWERING TIME 1</i> |
| <i>RGA</i> | <i>REPRESSOR OF GA1-3</i> |
| <i>SOC1</i> | <i>SUPPRESSOR OF OVEREXPRESSION OF CO1</i> |
| <i>SPY</i> | <i>SPINDLY</i> |
| <i>SVP</i> | <i>SHORT VEGETATIVE PHASE</i> |
| <i>TFL1, 2</i> | <i>TERMINAL FLOWER 1, 2</i> |
| <i>VIN3</i> | <i>VERNALIZATION-INSENSITIVE 3</i> |
| <i>VRN1, 2</i> | <i>VERNALIZATION 1, 2</i> |
| <i>ZTL</i> | <i>ZEITLUPE</i> |

FLC gene, which is itself a strong repressor of flowering. After optimal vernalization, *FLC* expression is abolished and this repression is stable. The vernalization pathway involves the functions of the *VIN3* and *MAF2* genes, which ensure that cold periods of insufficient duration will not cause flowering, and of the *VRN* genes, which are necessary for the maintenance of *FLC* repression after return to warm temperatures (Ratcliffe *et al.*, 2003; Sung and Amasino, 2004a).

A third pathway was initially found to promote flowering independently of environmental factors and hence was called 'autonomous' (Boss *et al.*, 2004). This pathway includes several subsets of independent genes, such as *FCA/FY*, *FVE/FPA*, *LD* and *FLD*. Their promoting effect on flowering is mainly exerted through their common repressive function on *FLC*. Thus, the autonomous and vernalization pathways co-operate to down-regulate *FLC* expression. In contrast, *FLC* is positively regulated by *FRI*: in so-called 'winter' (late-flowering) accessions having an active *FRI* gene, repression of *FLC* by the autonomous pathway is entirely overcome (thus vernalization

is absolutely required), whereas 'summer' (early-flowering) accessions with a loss-of-function *FRI* gene are not dependent on vernalization for flowering. *FLC*-dependent pathways also interact with the photoperiod pathway, as *FLC* has been shown to regulate negatively *CRY2* expression (El Assal *et al.*, 2003). In addition, the *VRN1* gene apparently acts in an *FLC*-independent pathway to positively regulate the downstream gene *FT* (Boss *et al.*, 2004).

A fourth or 'GA promotion' pathway includes genes, such as *GA1*, *GAI*, *RGA*, *PPF1* and *AtMYB33*, with roles in GA biosynthesis or signalling. Applications of physiologically active GAs are known to promote flowering in *Arabidopsis*, especially in SDs, and their amounts increase in the shoot of plants after transfer from SDs to LDs (Bagnall, 1992; Chandler and Dean, 1994; Xu *et al.*, 1997). Curiously, however, mutations impairing the GA pathway are most inhibitory to flowering in plants grown under SDs, indicating that the importance for flowering of these promotive hormones is greater under unfavourable than under favourable day length conditions. However, recent evidence links the GA and LD pathways. Mutations at the *EBS* locus cause early flowering in SDs; this phenotype requires GA biosynthesis (Gómez-Mena *et al.*, 2001) and is due to the fact that *EBS* normally represses the expression of *FT* (Piñeiro *et al.*, 2003). Another example is given by the *SPY* gene, which was first demonstrated to be a negative regulator of GA signalling, and which has now been found to interact with the LD pathway upstream of *CO* (Tseng *et al.*, 2004).

Secondary pathways

Superimposed on these four 'primary' pathways, secondary factors have been investigated recently and have been found to involve the same actors (Figure 2).

Ambient temperature has recently been suggested to act through the autonomous pathway, as the *fca* and *fve* mutants were found to flower as late at 23 °C as at 16 °C (Blázquez *et al.*, 2003). In the promotive effect of higher ambient temperature, night temperature may be more important than day temperature (Thingnaes *et al.*, 2003).

Light quality is obviously perceived by the photoreceptors involved in the LD pathway (*phyA* and *cry2*), which mediate the positive effects of far-red and blue light, respectively, on flowering. Interestingly, the mutant phenotype of *cry2* is strongly accentuated at 16 °C (Blázquez *et al.*, 2003), and hence *CRY2* function is influenced by ambient temperature. On the other hand, *PHYB* acts as a repressor of flowering, as the *phyB* mutant exhibits a strong early-flowering phenotype. The divergent effects of these different photoreceptors

find some explanation in the fact that they exert mutual control on each other (Mockler *et al.*, 2003). *PhyB* is the major contributor to the physiological responses of green seedlings to red light and is involved in shade avoidance (Smith and Whitelam, 1997). Interestingly, the early-flowering phenotype of *phyB* is also temperature sensitive: at 16 °C, the *phyB* phenotype is completely lost; hence, *phyB* is completely inactive and replaced by *phyE* (Halliday *et al.*, 2003). *PHYB* is thus believed to act in 'light quality' and 'ambient temperature' pathways, and it has recently been suggested that *PHYB* does so by regulating *FT* activity via an intermediate gene *PFT1* (Cerdán and Chory, 2003). However, *PHYB* is also believed to interact with the autonomous pathway, as the early-flowering phenotype of *phyB* requires *FCA* function (Koorneef *et al.*, 1998).

Photoreceptors may also affect flowering time indirectly through their effects on the biological clock. Clock entrainment is known to involve different photoreceptors in different light conditions (quality and irradiance), and novel blue light photoreceptors of the *ZTL/FKF1/LKP2* family have recently been shown to regulate clock components and downstream genes acting in the photoperiod pathway (Boss *et al.*, 2004).

Light irradiance may also influence flowering through photosynthesis. The *phyA* and *fca* mutants respond much more to increased irradiance than do wild-type plants (Bagnall, 1992; Bagnall and King, 2001), indicating that the promotive effect of the corresponding wild-type genes may be, at least partly, mediated through photosynthesis. Unfortunately, we still do not know how other late-flowering mutants would react to irradiance.

Integrator and floral meristem identity genes

As shown in Figure 2, the four 'primary' promotion pathways regulate the activity of 'integrator' genes, which are also under the influence of repressors, ensuring fine tuning of the process.

FLC is a repressor whose activity is balanced between the genetic reinforcing effect of *FRI* and the weakening effects of the autonomous and vernalization pathways.

FT is a promoter whose activity is mainly up-regulated by LDs through *CO*, but may also be up-regulated by the interconnected autonomous, light quality and ambient temperature pathways. Genes involved in the vernalization pathway also act on *FT*, which is down-regulated by *FLC*, but up-regulated by *VRN1* independently of *FLC*. *TFL2* is another repressor of *FT* activity (Kotake *et al.*, 2003).

SOC1 is up-regulated by *CO* and the GA pathway and down-regulated by *FLC*. Recently, *SOC1* has also been found

to cross-talk with *AGL24*, a promoter of inflorescence fate, whose activity is induced by vernalization independently of *FLC* (Michaels *et al.*, 2003). Interestingly, *AGL24* is close in sequence to *SVP*, which exerts an antagonizing repression effect on flowering. *SVP* and another gene, *MAF1* (or *FLM*), are thought to be part of an autonomous pathway exerting its function in repressing the floral transition independently of *FLC* and interacting with the photoperiod pathway (Hartmann *et al.*, 2000; Scortecci *et al.*, 2003).

Further downstream, *FT* and *SOC1*, together with the GA pathway, co-operate in the up-regulation of the floral meristem identity genes *LFY/AP1*. These genes up-regulate each other and are essentially expressed in the lateral productions of the SAM where individual flowers form. In the centre of the SAM, *LFY/AP1* activity is antagonized by *TFL1*, whose expression maintains the indeterminacy of the SAM (Ratcliffe *et al.*, 1999). *LFY/AP1* also repress *AGL24*, which promotes inflorescence fate rather than flower formation (Yu *et al.*, 2004). Thus, spatial patterning is finely regulated in the SAM on floral transition to organize the inflorescence architecture.

Localization of pathway activities within the wild-type plant

Most genes controlling flowering time are expressed across a wide range of organs and tissues, but a survey of available data on their spatial expression patterns reveals that many genes show preferential expression in more limited areas. Table 4 shows the result of a careful re-examination of published pictures (including references); some uncertainty remains, however, as localization studies were occasionally inconsistent, possibly because of the different techniques used.

Genes of the photoperiod promotion pathway could have been expected to show preferential expression in expanded leaves as day length is classically believed to be principally perceived by these organs (see above). The situation is in fact different: the genes encoding *PHYA* and *CRY2* are most expressed in the SAM and root apical meristem (RAM) of the vegetative seedling and less expressed in the hypocotyl and cotyledons. The downstream gene *CO* is expressed relatively weakly in SAM and leaf primordia and also, quite strikingly, strongly in the vascular tissues (mainly phloem) of the hypocotyl, expanded leaves and roots.

For most genes of the autonomous and vernalization pathways, including *FLC*, the highest expression was observed in both the SAM and RAM, i.e. in areas in which cell division is mostly active in vegetative plants. These observations are consistent with the fact that vernalization is known to be perceived by the SAM. At the molecular level, events are also

Table 4 Localization of expression of genes controlling flowering time in wild-type plants

| | Gene expression | | |
|---|---|--|--|
| | High in SAM and/or surrounding tissues | High in RAM | In vascular tissues |
| Long day pathway | <i>PHYA</i> ¹ , <i>CRY2</i> ¹ , <i>CO</i> ^{2,3} | <i>PHYA</i> ¹ , <i>CRY2</i> ¹ | <i>CO</i> ^{3,4} |
| Autonomous/vernalization pathway | <i>FCA</i> ⁵ , <i>FY</i> ⁶ , <i>LD</i> ⁷ , <i>FLD</i> ⁸ , <i>VIN3</i> ⁹ , <i>VRN2</i> ⁶ , <i>FLC</i> ⁹ , <i>FRI</i> ⁶ , <i>PIE1</i> ¹⁰ , <i>AGL24</i> ¹¹ | <i>FCA</i> ⁵ , <i>FY</i> ⁶ , <i>LD</i> ⁷ , <i>FLD</i> ⁸ , <i>VIN3</i> ⁹ , <i>VRN2</i> ⁶ , <i>FLC</i> ⁹ , <i>FRI</i> ⁶ , <i>AGL24</i> ¹¹ | <i>FCA</i> ⁵ , <i>AGL24</i> ¹¹ |
| Gibberellin pathway | <i>GA1</i> ¹² , <i>PPF1</i> ¹³ , <i>AtMYB33</i> ¹⁵ | <i>GA1</i> ¹² , <i>SPY</i> ¹⁴ , <i>AtMYB33</i> ¹⁵ | <i>GA1</i> ¹² , <i>AtMYB33</i> ¹⁵ |
| Integrator and floral meristem identity genes | <i>SOC1</i> (<i>AGL20</i>) ^{16,17} , <i>LFY</i> ^{18,19} , <i>AP1</i> ^{18,20} | | <i>SOC1</i> ¹⁷ , <i>FT</i> ⁴ , <i>LFY</i> ^{18,19} |
| Repressors | <i>PHYB</i> ¹ , <i>TFL2</i> ^{4,21} , <i>MAF1</i> (<i>FLM</i>) ²² , <i>SVP</i> ²³ , <i>TFL1</i> ^{2,20} | <i>PHYB</i> ¹ , <i>TFL2</i> ^{4,21} , <i>MAF1</i> (<i>FLM</i>) ²² , <i>SVP</i> ²³ | <i>TFL2</i> ^{4,21} |

RAM, root apical meristem; SAM, shoot apical meristem.

¹Tóth *et al.* (2001); ²Simon *et al.* (1996); ³An *et al.* (2004); ⁴Takada and Goto (2003); ⁵Macknight *et al.* (2002); ⁶C. Dean, personal communication (2004); ⁷Aukerman *et al.* (1999); ⁸He *et al.* (2003); ⁹Sung and Amasino (2004b); ¹⁰Noh and Amasino (2003); ¹¹Michaels *et al.* (2003); ¹²Silverstone *et al.* (1997); ¹³Kania *et al.* (1997); ¹⁴Swain *et al.* (2002); ¹⁵Gocal *et al.* (2001); ¹⁶Samach *et al.* (2000); ¹⁷Borner *et al.* (2000); ¹⁸Bowman *et al.* (1993); ¹⁹Blázquez *et al.* (1997); ²⁰Ratcliffe *et al.* (1999); ²¹Kotake *et al.* (2003); ²²Scortecci *et al.* (2001); ²³Hartmann *et al.* (2000).

well confined, as many genes of these pathways are involved in the stable modification of *FLC* chromatin structure, leading to its mitotically stable repression (Boss *et al.*, 2004; Sung and Amasino, 2004a). We have also seen above that genes of the autonomous pathway seem to be involved in sensing ambient temperature, and it is known that temperature influences the cell division process, and hence meristematic zones.

Work on the expression pattern of genes involved in the GA pathway has revealed that the GA biosynthesis gene *GA1* is essentially expressed in vegetative plants just below the SAM, and also in the veins of expanding and mature leaves and in the RAM. During the transition to flowering, *GA1* is strongly activated in the inflorescence SAM. Expression of the GA sensitivity *PPF1* and *GAMYB*-like gene, *AtMYB33*, also strongly increases in the SAM during this transition. These findings suggest that the amount of GAs and the activity of the GA pathway are tightly restricted in the SAM at the vegetative state, but become unrestricted at the floral transition.

Of the integrator genes, *FT* is active exclusively in the phloem tissues of the leaves and inflorescence stem. *SOC1* and *LFY* are weakly active in the apical tissues of vegetative plants, but their expression is strongly up-regulated during floral transition and occurs in the SAM and surrounding leaf primordia. Later, *LFY* and *AP1*, but not *SOC1*, are expressed in incipient floral meristems; *SOC1* and *LFY* are also expressed in the vasculature of the incipient inflorescence stem.

Some floral repression genes are ubiquitously expressed, whereas others are mostly expressed in the SAM, RAM and leaf primordia (Table 4), as is the case for *PHYB*, *MAF1*, *SVP* and *TFL2*. Contrary to *TFL2*, which is similarly expressed at all

developmental stages, *MAF1* and *SVP* are down-regulated and *TFL1* is up-regulated in response to floral induction. Up-regulation of *TFL1* is early as it precedes *LFY* and *AP1* activation and, remarkably, is restricted to a small area located just below the SAM.

In conclusion, it appears that: (i) of the genes acting in the promotion or repression of flowering, none (except *FT*) is exclusively expressed in expanding and/or expanded leaves before and during the floral transition; (ii) most genes (except *CO* and *FT*) are preferentially expressed inside and/or around the SAM, which thus appears to be fairly autonomous for flowering time control.

Given the large number of genes expressed in the RAM (Table 4), the root system is presumably capable of reacting to some critical environmental changes and, as a result, influences to some extent the flowering process. Alterations in root size, morphology or function are indeed observed in the *fca*, *gai*, *spy* and *tfl2* mutants, but not in *co* (Macknight *et al.*, 2002; Swain *et al.*, 2002; Kotake *et al.*, 2003). Strikingly, the root alterations in *fca* are suppressed by vernalization, just as the delay in flowering time, suggesting that both phenotypes are intimately related, possibly through *FLC* regulation. Expression of *FLC* in the RAM was unexpected, as there is no indication that cold treatment of roots alone is capable of promoting flowering in intact plants. Recently, *PIE1* (a positive regulator of *FLC*) was found to be expressed in the SAM only, shedding some light on a possible discrimination in *FLC* activity in SAM and RAM (Noh and Amasino, 2003). However, more work on the root functions in the flowering process in *Arabidopsis* is warranted.

Of particular interest are the genes active in the vascular tissues (Table 4), especially the phloem, as it is known that florigenic and antiflorigenic signals are transported in these tissues (Lang, 1965; Bernier *et al.*, 1981). This raises the question of whether the products of these genes might be the signals themselves, or precursors. Such an hypothesis makes sense only for early expressed genes, such as *CO*, *FT*, and *GA1*. For others, such as *AGL24*, *SOC1* and *LFY*, which are clearly expressed in the vasculature after completion of the floral transition, the start of this expression pattern has not been determined precisely.

Long-distance signals

At this point, physiological and genetic work have arrived at different conclusions. On the one hand, the physiological studies summarized above have demonstrated the existence of long-distance signals moving up and down the plant in the phloem and xylem saps and participating in the flowering process at the SAM. In *Arabidopsis*, the movement of one or several floral signals from the leaves to SAM was inferred from sequential defoliation experiments (Corbesier *et al.*, 1996). On the other hand, the *Arabidopsis* SAM seems to be fairly autonomous as most genes controlling flowering time are active there. The autonomy of the SAM is further supported by the fact that excised shoot apices of various plants are capable of perceiving several environmental factors controlling flowering, but only if provided with sugar and minerals in the culture medium (see above). Thus, a simplistic model of flowering control would be that the plant only provides nutrients to the SAM. The predominant role attributed classically to expanded leaves in day length perception could then be explained on the basis that they are the major providers of nutrients to the SAM (Périlleux and Bernier, 2002).

As far as minerals are concerned, it has been known for some time that a reduction in their supply promotes flowering in *Arabidopsis* (Lang, 1965). This has been confirmed more recently by the observation that increasing mineral supply to the roots delays flowering in several mutants of the photoperiod and autonomous pathways, as well as in wild-type plants (van Tienderen *et al.*, 1996). An important part of this inhibition is presumably due to nitrogen (Bernier *et al.*, 1981). An explanation for this puzzling effect might come from our own result in *Sinapis*, where the increased export of Suc towards the SAM at the floral transition (see Figure 1) is greatly reduced when plants are grown on high nitrate-supplemented medium (Corbesier *et al.*, manuscript in preparation).

There is plenty of evidence that Suc supply to the SAM is essential for flowering in *Arabidopsis*. First, the Suc level in

the phloem sap exported by leaves increases early and markedly during floral induction and, when this increase is prevented, as in the starch-deficient *pgm* mutant, flowering is inhibited (Corbesier *et al.*, 1998). Second, Suc application to wild-type plants grown in suboptimal conditions for photosynthesis, as well as to the late-flowering *phyA* mutant, promotes flowering (King and Bagnall, 1996; Bagnall and King, 2001). Third, Suc supply to the aerial part, presumably the SAM, of plantlets grown *in vitro* almost completely suppresses the late-flowering phenotype of mutants, such as *gi*, *co* and *fca*, but is unable to rescue *ft* (Roldán *et al.*, 1999). This suggests that Suc is involved somewhere in between the LD and autonomous signalling pathways, but upstream of *FT*. Fourth, Suc is required for the up-regulation of *LFY* by exogenous GA (Blázquez *et al.*, 1998).

However, Suc may not be the only missing signal needed by the SAM to achieve flower initiation, and the fact that the *CO* and *FT* genes are active in the phloem of mature leaves has motivated careful experiments to see whether their products (transcripts or proteins) could be translocated in the plant.

With regard to *CO*, different promoters were fused to the coding region to target *CO* activity in complementation experiments. Remarkably, although *CO* is expressed in the SAM of wild-type plants, misexpression of *CO* in the SAM does not rescue the late-flowering phenotype of the *co* mutant, but its expression in the companion cells of the phloem does (An *et al.*, 2004). The effect of *CO* misexpression in the phloem has been found to involve cell autonomous activation of *FT*. On the other hand, *FT* misexpression, either in the phloem or in the SAM, can correct the late-flowering phenotype of *co* plants, indicating that *CO* promotes flowering by up-regulation of *FT* in the phloem, but that the effectiveness of *FT* in promoting flowering is not restricted to these cells (An *et al.*, 2004). The *FT* protein is only 23 kDa, which is below the size exclusion limit of plasmodesmata (Imlau *et al.*, 1999); hence, it might move freely between cells from the ends of provascular strands towards and inside the SAM (An *et al.*, 2004). *FT* then interacts in the SAM with the product of the *FD* gene (Daimon *et al.*, 2004), and this interaction eventually up-regulates *AP1*. Plants misexpressing *CO* in phloem cells, in a background in which *FT* is inactive, are still capable of flowering, confirming that *CO* can promote flowering through *FT*-independent processes, e.g. through *SOC1* and *LFY* (Figure 2; An *et al.*, 2004).

Although these results suggest that *FT* may be a mobile signal in the LD signalling pathway, micrografting experiments came to a different conclusion. Indeed, it was observed that, although the late-flowering phenotype of a *gi* or *co* receptor was partially rescued by grafting with a wild-type donor shoot,

the response of an *ft* receptor was much less convincing (An *et al.*, 2004; Turnbull and Justin, 2004). Together with the previous observation that Suc corrects the flowering time of *gi* or *co* *in vitro*, but fails to do so for *ft*, these data support the idea that the floral-promoting material provided by the wild-type donor is Suc and not FT. This discrepancy emphasizes that results of misexpression experiments must be interpreted cautiously, especially when misexpression is higher than native expression in the target tissue (Turnbull and Justin, 2004).

The participation of other compounds, particularly GAs, CKs and abscisic acid (ABA), in long-distance signalling in *Arabidopsis* should also be considered. The importance of GAs in the control of flowering is well established (Figure 2), but whether these hormones originate from distant plant parts and/or are synthesized in the SAM is not clear. Indeed, it was found that the GA biosynthetic *GA1* gene is expressed in the veins of expanding and expanded leaves, in the RAM and in tissues below the SAM before the transition to flowering. Thereafter, expression extends to the SAM itself (Silverstone *et al.*, 1997).

CKs are also promoters of flowering in *Arabidopsis*, as shown by the fact that transgenics that are deficient in CKs are late flowering (Werner *et al.*, 2003), whereas plants that are enriched in CKs are early flowering (Chaudhury *et al.*, 1993). Because CKs affect the rate of leaf initiation more than the flowering time, these results have sometimes been overlooked when the flowering time is expressed in terms of the leaf number, but this difficulty must not mask the real promotive effect of these hormones on flowering, as discussed elsewhere (Bernier, 2003). Plants deficient in CKs may even stay vegetative until death, as found with some transgenics over-expressing a CK oxidase/dehydrogenase gene (Werner *et al.*, 2003), or with some triple mutants lacking three histidine kinase CK receptors, whilst single or double mutants have no flowering phenotype (Nishimura *et al.*, 2004). In wild-type plants, CK application accelerates flowering, but only when irradiance is low, indicating that this promotive effect is dependent on sugars (Dennis *et al.*, 1996). The promotive effect of CKs is also more important in LDs, as shown by multiple approaches: (i) the CK-enriched *amp1* mutant rescues the late-flowering phenotype of the *gi* mutant, but not of *fca*, suggesting that these hormones act essentially in relation with the LD pathway downstream of *G1* (Dennis *et al.*, 1996); (ii) the amounts of iP-type CKs increase in LD-induced *Arabidopsis* plants in the leaves, in the phloem sap and in the SAM (Corbesier *et al.*, 2003), where they may activate cell proliferation, a very precocious event of the SAM transition to flowering (Jacqumard *et al.*, 2003). Contrary to

the situation presented above for GAs, CKs detected in the SAM are probably transported from other plant parts, as none of the CK biosynthetic genes encoding ATP/ADP isopentenyltransferases (IPT) are expressed in the SAM itself (Miyawaki *et al.*, 2004). The main source of CKs arriving at the SAM is probably the expanded leaves as the *IPT3* gene is specifically expressed in the phloem cells of these organs.

ABA is regarded as a general inhibitor of flowering (Bernier, 1988), and this is confirmed in *Arabidopsis* where mutants deficient in or insensitive to ABA are early flowering in SDs (Martínez-Zapater *et al.*, 1994). Interestingly, two ABA-deficient mutants, *aba2* and *aba3*, and an ABA-insensitive mutant, *abi4*, are allelic to sugar-insensitive mutants, indicating that signalling pathways mediated by ABA and sugars interact to regulate plant development (Gibson, 2004).

Other substances might be involved in signalling during the transition to flowering. Mutants deficient in salicylic acid or insensitive to ethylene are indeed late flowering (Boss *et al.*, 2004). Brassinosteroids have recently been suggested to be involved in the autonomous pathway, but also to co-operate with GAs in controlling flowering time (Domagalska *et al.*, 2004).

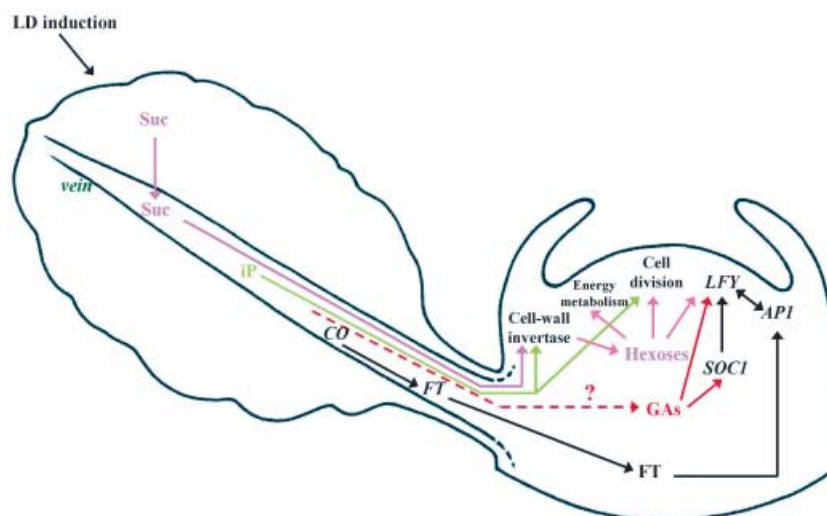
An integrated model of flowering time control

If we summarize the achievements identifying the elusive 'florigen' involved in the floral transition of wild-type *Arabidopsis* in LDs, it appears that it could be formed of both long-distance and short-distance signalling molecules: potential long-distance signalling molecules (Suc and CK) have been identified by the analysis of phloem sap exported by leaves in response to floral induction and by genetic approaches (FT may move in the phloem from leaf veins to SAM); short-distance signalling within the SAM is suggested by the fact that most components of the genetic machinery controlling flowering (except *CO* and *FT*) are highly active in or nearby the SAM (Table 4). As discussed earlier, the question of whether GAs act as a long-distance signal of leaf origin or as a short-distance signal produced locally, or both, is unresolved.

At this stage, we propose a model, shown in Figure 3, based on the data presented above and integrating events occurring in wild-type *Arabidopsis* plants in LDs; this model holds for plants with non-inhibitory *FLC* expression levels (summer early-flowering accessions or winter vernalized plants).

In our model, Suc has a dual role. In addition to the direct role of Suc in the floral transition, we hypothesize that Suc plays a crucial, albeit indirect, role in flowering, as it is the major component of phloem sap in most plants and so controls the mass flow of solutes operating between source

Figure 3 A model of control of the shoot apical meristem (SAM) floral transition in *Arabidopsis thaliana* plants exposed to an inductive long-day (LD) treatment. This transition is supposed to occur in response to arrival at the SAM of long-distance signals of leaf origin and their interactions with short-distance signals produced in the SAM itself. Long-distance signals include sucrose (Suc), the cytokinin isopentenyladenine (iP) and the FT protein produced following activation of the CO gene. In the case of gibberellins (GAs), it is unclear whether they are imported from leaves or produced locally.



leaves and sinks. We believe that Suc loading in leaf phloem and unloading at the SAM are important checkpoints in the control of SAM flowering, and it has previously been reported that the increased export of Suc by *Arabidopsis* leaves in response to LD induction might be due, at least in part, to increased efficiency of Suc loading (Corbesier *et al.*, 1998).

After unloading in the SAM, the long-distance signals entrained in the Suc streaming stimulate a number of cellular and molecular events (Bernier, 1988). Suc is first hydrolysed by local invertases. Although the general belief is that vacuolar invertase plays a prominent role in meristematic areas (Koch, 2004), participation of cell wall invertase activity may have been overlooked. Indeed, it has been observed recently that the flowering of *Arabidopsis* plants in LDs can be accelerated by the over-expression of cell wall invertase in the SAM, while flowering in SDs is not modified (Heyer *et al.*, 2004). On the contrary, transgenics having an increased cytosolic (vacuolar) invertase activity are delayed in flowering, in both LDs and SDs.

CKs activate invertase activity and, together with the products of Suc hydrolysis, increase the rate of cell division (see above). Hexoses also participate with GAs in the up-regulation of *LFY* expression, whilst the other floral meristem identity gene, *API*, is activated by FT, which is itself positively regulated by CO. In Figure 3, FT is speculated to move from leaf to SAM in the phloem, whilst CO is unable to move out of the phloem (An *et al.*, 2004). A difficulty remains, however, in the fact that flowering can be promoted by misexpression of CO in the phloem, independently of FT. This suggests that CO may activate other targets in the phloem. *SOC1* is a candidate but, although expression in the wild-type plant was reported in the vasculature of the nascent stem at

the floral transition (Borner *et al.*, 2000), *SOC1* activity is much higher in the SAM. Activation of *SOC1* in the SAM might thus be due to other signals, possibly a CK or a GA (Bonhomme *et al.*, 2000; Borner *et al.*, 2000).

The model of Figure 3 is obviously not complete. More long-distance signals presumably operate during LD induction – for example Gln (Corbesier *et al.*, 2001), whose function is unknown – as well as more short-distance signals. As several genes expressed in the SAM were found to control its spatial patterning, the short-distance signalling presumably involves some of their products (RNA and protein), as suggested recently (Wu *et al.*, 2003). However, it is clear that we are getting closer and closer to the identification of the elusive multifactorial ‘florigen’, at least in *Arabidopsis*.

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