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Physiological Signals That Induce Flowering

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INTRODUCTION

The timing of the transition from vegetative growth to flowering is of paramount importance in agriculture, horticulture, and plant breeding because flowering is the first step of sexual reproduction. Studies to understand how this transition is controlled have occupied countless physiologists during the past half century and have produced an almost unmanageably large amount of information (Bernier et al., 1981a; Halevy, 1985–1989; Bernier, 1988; Kinet, 1993).

A majority of plants use environmental cues to regulate the transition to flowering because all individuals of a species must flower synchronously for successful outcrossing and because all species must complete their sexual reproduction under favorable external conditions. Any environmental variables exhibiting regular seasonal changes are potential factors that control the transition to flowering. The major factors are photoperiod, temperature, and water availability. Plants that do not require a particular photoperiod or temperature to flower, i.e., the so-called "autonomous-flowering" plants, are usually sensitive to irradiance. The environmental factors are perceived by different parts of the plant. Photoperiod and irradiance are perceived mainly by mature leaves in intact plants. Temperature is perceived by all plant parts, although low temperature (vernalization) is often perceived mainly by the shoot apex. Water availability is perceived by the root system.

There are strong interactions between these different factors, so that each factor can change the threshold value for the effectiveness of the others. Plants, as opportunists, will thus make use of a different critical factor in different environments. *Melilotus officinalis*, for example, is a biennial with a vernalization requirement in temperate zones and an annual long-day (LD) plant with no cold requirement in arctic regions. In photoperiodic species, such as the short-day (SD) plant *Pharbitis nil* and the LD plant *Silene armeria*, flowering in unfavorable photoperiods can be caused by changing temperature, irradiance, or nutrition or by removing the roots. Similarly, in some late-flowering mutants of Arabidopsis, vernalization and an increase in the proportion of far-red light in the light source can substitute for one another in promoting the transition to flowering (Martínez-Zapater and Somerville, 1990; Bagnall, 1992). Clearly, there are alternate pathways to flowering in most, if not all, plants. Because the different flowering-promoting factors are perceived by different parts of the plant, this implies that these parts interact and that the fate of the apical meristem—remaining vegetative or becoming reproductive—is controlled by an array of long-distance signals from the entire plant.

The ability of subsets of plant parts to control flowering is also underscored by the fact that some plants may flower almost normally after complete defoliation (*Hyoscyamus niger*, red *Perilla*, *Chenopodium amaranticolor*) or derooting (*Perilla*, *Lolium temulentum*, *Sinapis alba*). This does not mean that these plant parts, when present, do not participate in the control of flowering. Plants are well adapted to partial destruction, for example by herbivorous animals, and it is known that the remaining parts can often substitute for the transiently missing part in providing the appropriate nutrients and signals.

Evidence that photoperiod leads to the production of transmissible flowering signals has come from grafting experiments. Such experiments have shown that leaves of photoperiodic plants produce promoters and inhibitors of flowering when exposed to favorable and unfavorable daylength regimes, respectively. These signals are generally transported from leaves to the apical meristem in the phloem with the assimilates. On the other hand, signals originating in roots are presumably transmitted in the xylem with the transpiration stream.

The nature of these transmissible signals is still a controversial issue (O'Neill, 1992). Three major theories attempt to explain the chemical control of the transition to flowering. The "florigen/antiflorigen" concept (Lang, 1984) proposes that the floral promoter and inhibitor are each a simple, specific, and universal hormone that remain to be isolated and identified. The "nutrient diversion" hypothesis (Sachs and Hackett, 1983) postulates that floral induction, whatever the nature of the involved environmental factors, is a means of modifying the source/sink relationships within the plant in such a way that the shoot apex receives a better supply of assimilates than under noninductive conditions. Finally, the theory of "multifactorial control" (Bernier et al., 1981b; Bernier, 1988) postulates that several chemicals—assimilates and known phytohormones—participate in floral induction. Genetic variation, as well as past and present growing conditions, result in different factor(s) of the complex becoming the limiting factor(s) in

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different species or genotypes or in a given genotype grown in different environments.

Identification of these signals is of the utmost fundamental and practical importance. Our aim here is to explore recent physiological and genetic approaches to this problem, focusing on some model experimental plants. We shall discuss the results obtained with *S. alba* and Arabidopsis, two mustard species between which we believe knowledge is easily transferable. Our analysis shows that both physiological and genetic results support the theory of a multifactorial control of flowering.

THE *S. ALBA* CASE: A PHYSIOLOGICAL MODEL

In studies on the transition to flowering, physiologists often favor photoperiodic plants that flower in response to a single inductive cycle because these are the only plants in which there is a precise zero time for induction and a high synchrony among the individual plants of a population during the transition (Bernier et al., 1981a). For example, the LD plant *S. alba*, when 2 months old, can be induced to flower by exposure to either a single LD or a single displaced SD, that is, an SD of normal duration (8 hr) delayed by 10 hr within a 24-hr cycle (Bernier et al., 1981a). Induction by one LD involves a photoextension of the SD, that is, a lengthening of the photosynthetic light period and, thus, of the light energy input. By contrast, plants induced by one displaced SD receive exactly the same amount of light energy as noninduced controls kept in standard SD.

The inductive treatments are perceived by the mature leaves, as shown in Figure 1 (Havelange and Bernier, 1991), and defoliation experiments indicate that export of the slowest floral promoter by induced leaves starts at around 16 hr after the beginning of the LD or displaced SD (Bernier et al., 1974). Initiation of the first flower primordia by the apical meristem starts ~2 days later.

One way to identify the endogenous signals that induce *S. alba* flowering in response to a photoperiodic treatment is to compare the composition of exudates collected from induced and noninduced plants. Exudate composition is complex, and the kinds of chemicals that are analyzed are selected based on their ability to mimic some of the normal events observed in the apical meristem after photoperiodic induction. These compounds include carbohydrates and cytokinins. Auxin, polyamines, and Ca^{2+} are also being analyzed because these molecules are known to interact with cytokinins in several other physiological processes. Exudates are collected from roots, mature leaves, and stem top (Lejeune et al., 1988, 1993). The root exudate, collected at the cotyledonary node, is essentially the xylem sap moving from roots to leaf blades; the leaf exudate, collected at the petiole base of mature leaves, is mainly the phloem sap exported by these leaves; and the apical exudate, collected at the top of the stem just below the apical bud, is essentially the phloem sap reaching this bud. The duration of exudation varies with the kind of chemicals analyzed; for example, it is 4 hr for carbohydrates and 16 hr for cytokinins.

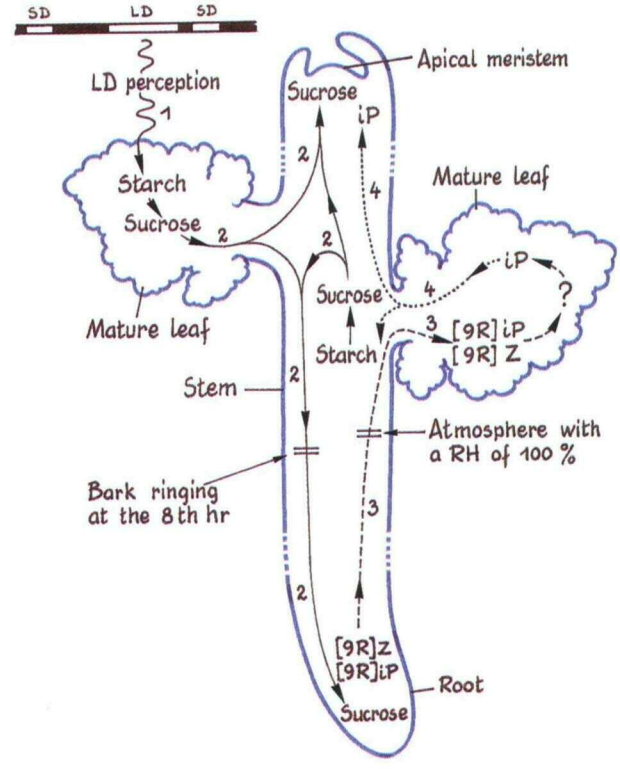

Figure 1. Diagram of a Regulatory Loop Participating in the Control of the Transition to Flowering in *S. alba* and Involving Sucrose and Cytokinins.

Step 1 (wavy arrow): perception of LD induction by mature leaves; step 2 (solid arrow): starch mobilization in leaves and stem followed by transport of sucrose in the phloem to both the apical meristem and roots; step 3 (dashed arrow): transport in the xylem from roots to leaves of zeatin riboside ([9R]Z) and isopentenyladenine riboside ([9R]iP); step 4 (dotted arrow): transport in the phloem from leaves to the apical meristem of isopentenyladenine (iP). RH, relative humidity.

Carbohydrates

S. alba plants exposed to a single SD at an irradiance 2.5 times higher than normal do not flower. However, this treatment causes an increase in sugar levels and acid invertase activity in the apical meristem as well as some ultrastructural changes that are typically observed during the transition to flowering (Pryke and Bernier, 1978; Havelange and Bernier, 1983). These effects, which are due presumably to increased photosynthesis and assimilate availability, emphasize the possible role of carbohydrates in the control of this transition.

Sucrose is the major sugar in both leaf and apical exudates (Lejeune et al., 1991, 1993). Its level increases dramatically, very early and transiently, in both exudates in plants induced by either one LD or one displaced SD. As a result, sucrose accumulates very early in the apical meristem of induced plants (Bodson and Outlaw, 1985). The increased sucrose supply to the meristem precedes the activation of energy-consuming

processes such as mitotic activation and thus does not result from a higher demand by the meristem. This suggests a messagelike role for sucrose.

Work with $^{14}CO_2$ indicates that there is no modification of the supply of the recently synthesized assimilates for the apical bud that can account for the early increase in sucrose in the meristem of induced plants, particularly in plants induced by a displaced SD (Bodson et al., 1977). Strikingly, the early extra sucrose seems thus to arise not from increased photosynthesis but from mobilization of reserve carbohydrates (presumably starch) stored both in the leaves and the stem (Figure 1) (Lejeune et al., 1991, 1993). The challenge now is to determine which degradation enzymes are activated and how this mobilization is so quickly stimulated.

Cytokinins

The application of a single, low dose of a cytokinin to the apical bud of plants grown in SD is another noninductive treatment that causes various events normally observed in the meristem after photoperiodic induction of flowering, such as an increase in the rate and synchronization of cell division, the halving of the size of DNA replication units, and the splitting of vacuoles (Bernier et al., 1977; Havelange et al., 1986; Houssa et al., 1990). Interestingly, these cytokinin-dependent events are different from the sucrose-dependent events.

Floral induction by one LD results in complex changes in cytokinin fluxes, and several possible interactions between cytokinin and sucrose fluxes will be discussed. Figure 1 summarizes the chronology of these changes.

A ring of all living tissues, including phloem, has been surgically removed from the stem of induced plants at a point between the lowest leaf and the root system. This bark-ringing treatment, which interrupts phloem transport from shoot to roots, is inhibitory to flowering when made at the 8th hr of the LD but not when made at the 12th hr or later, as shown in Figure 2. This indicates that exposure to an LD causes the rapid production in mature leaves of a signal that is transported extremely quickly to the root system, presumably in the phloem. The chemical nature of this signal is unknown, but sucrose is a good candidate (Figure 1) because we have observed that its level in roots increases within 1 hr of the photoextension period of the LD (A. Havelange and G. Bernier, unpublished results). The function of this leaf-to-root signal is apparently related to cytokinin export by roots, because the flowering inhibition caused by bark-ringing at the 8th hr is reversed by a cytokinin application on the apical bud at the 16th hr (Figure 2).

The major cytokinin in root exudate (xylem sap) is zeatin riboside ([9R]Z), and a minor component is isopentenyladenine riboside ([9R]iP). The levels of both compounds in the root exudate increase early and transiently in response to the inductive LD (Figure 1) (Bernier et al., 1990; P. Lejeune, G. Bernier, M.-C. Requier, and J.-M. Kinet, manuscript submitted). In some experiments, the increases have been detected already during the exudation period starting at 1 hr within the photoextension period of the LD. These increases might be due to either an enhanced cytokinin biosynthesis by roots or an increased release by roots of preexisting cytokinins. Because the total cytokinin level extracted from root tissues is decreased during the photoextension period of the LD (P. Lejeune, unpublished results), the elevated levels of these hormones in the root exudate are apparently due to activation of cytokinin release rather than biosynthesis.

We have attempted to stop transiently the root-to-shoot flux of cytokinins by raising plants for 24 hr in an atmosphere with a relative humidity of 100% (P. Lejeune and G. Bernier, unpublished results). Although such a treatment may ultimately affect a variety of processes, it is known that its primary effect is to almost stop transpiration and, consequently, the movement of sap in the xylem. When applied during the day before or after the LD, this treatment has no effect on flowering. However, when applied during the LD, it almost completely abolishes the flowering response (Figure 1). This observation supports the idea that the movement of xylem sap and, consequently, the increased root-to-shoot flux of cytokinins are essential for flowering.

The increased cytokinin supply from roots apparently results in elevated levels of these compounds in the mature leaves of induced plants (Bernier et al., 1981b; P. Lejeune, unpublished results). This rise is most marked at 16 hr after the start of the LD, i.e., at 8 hr within the photoextension period. Cytokinins

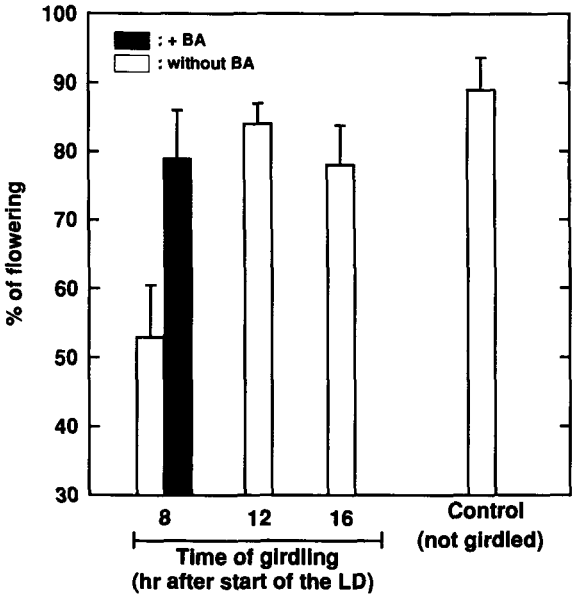

Figure 2. Flowering Response of *S. alba* Plants Exposed to a Single 16-hr LD as a Function of Time of Bark-Ringing (Girdling) between Mature Leaves and the Root System.

Black bar: plants ringed at 8 hr after start of the LD and treated by 5×10^{-5} M benzyladenine (BA) directly on the apical bud at 16 hr after start of the LD. White bars: plants girdled at the indicated times and not treated with BA.

are also released from leaves in the form of isopentenyladenine (iP) (P. Lejeune, G. Bernier, M.-C. Requier, and J.-M. Kinet, manuscript submitted). The iP levels in both leaf and apical exudates start to increase early, as a result of induction. The increase is transient, and there is both direct and indirect evidence indicating that the pulse of iP peaks around 16 hr after the start of the LD (Bernier et al., 1981b, 1990). We do not yet know whether the iP exported by leaves is derived from the [9R]iP imported by them from roots or is produced in the leaves themselves. Roots are known as the major site of cytokinin biosynthesis, but leaves can be additional sites of production in some circumstances (Palni et al., 1990).

The pulse of iP exported by leaves is directed, at least in part, to the apex because the cytokinin content of the apical bud has been found to be elevated at the 16th hr of the LD (Sotta et al., 1992). Another interaction between the increase in sucrose and the increase in cytokinin has been observed at the level of the apical meristem. That is that, as shown in Table 1, the shortenings of the major phases of the cell cycle, G1, S, and G2, that are observed in LD-induced plants are better mimicked by exposing noninduced plants to a combined high-irradiance/cytokinin treatment than by one of these two treatments alone. However, this combined treatment is still not sufficient to cause flowering.

Auxin

Work on auxin fluxes has started recently because we have consistently observed that LD induction results in a decrease of the auxin level at the 16th hr in the apical bud (Sotta et al., 1992). Thus, the auxin-to-cytokinin ratio is decreased in the apical bud of induced plants. This finding is quite exciting given the importance of the balance between these two hormones in the control of many physiological processes (Davies, 1987), including flower formation in cultured tobacco explants (Peeters et al., 1991).

Polyamines

Polyamines are believed to cooperate with cytokinins in the control of several processes, including the cell division cycle (Dumbroff, 1990). In *S. alba* plants induced by one LD, the flowering response is dramatically reduced by an application to leaves of DL-α-difluoromethylornithine, an inhibitor of putrescine biosynthesis (A. Havelange and G. Bernier, unpublished results). This has prompted a polyamine analysis of exudates. Although this work is still in progress, it has already been found that induced leaves export an early pulse of putrescine in the phloem sap (G. Bernier, P. Lejeune, R. Kaur-Sawhney, and A.W. Galston, unpublished observations). The exact function of this chemical in the flowering process is so far unknown.

Calcium

Ca^{2+} has been proposed as a possible second messenger for cytokinins in their effects on the cell division process (Saunders, 1992). The level of Ca^{2+} in the root exudate increases early and transiently in response to induction of *S. alba* plants by one LD or one displaced SD (Havelange and Bernier, 1993). Despite this enhanced supply to the shoot, the levels of this cation in mature leaves and leaf exudate are not altered. However, a pulse of Ca^{2+} reaches the apical bud at 30 to 40 hr after the start of induction. Ca^{2+} is supplied to the bud via the apoplast, not the phloem, and at a time when cell division is activated in the apical meristem and leaf primordia. Thus, the increased supply of Ca^{2+} to the bud appears as a late and secondary effect of induction. Interestingly, unlike Ca^{2+}, Mg^{2+}

Table 1. Duration (hr) of the Cell Cycle (T) and Its Component Phases (G1, S, G2, M) in the Shoot Apical Meristem of *S. alba* Plants Exposed to One LD or to Various Treatments in SD

Daylength Regime	Treatment	Apex Condition	T	G1	S	G2	M
SD	Control	V[a]	66.0	24.0	15.0	25.0	2.1
SD	HI[b]	V	69.0	33.2	12.0	20.2	3.6
SD	BA[c]	V	49.0	24.4	6.0	17.4	1.3
SD	HI + BA	V	40.0	17.9	6.0	13.9	1.2
One LD	–	F[d]	32.0	15.0	5.0	10.0	2.1

[a] V, vegetative.
[b] HI, high irradiance for two consecutive SD.
[c] BA, benzyladenine (4.5×10^{-5}M) applied once directly to the apex.
[d] F, in transition to flowering.
Results for SD control and SD + BA are from Jacqmard et al. (1994). Other results are unpublished.

and K^+ are not supplied in greater amounts to the bud of induced plants (Havelange and Bernier, 1993).

Control of the Transition to Flowering in *S. alba* Is Multifactorial

The photoperiodic induction of flowering causes dramatic and complex alterations of the long-distance signaling system in the entire *S. alba* plant. Not only are the fluxes and levels of nutrients (sucrose, Ca^{2+}) changed, as postulated by the "nutrient diversion" hypothesis (see INTRODUCTION), but the fluxes and levels of other chemicals, including some hormones, are also profoundly altered. The data are thus consistent with the multifactorial model of control of the floral transition (Bernier et al., 1981b; Bernier, 1988). All plant parts participate in the exchange of signals and are very rapidly instructed about the changes of the light/dark regime to which the leaves are exposed.

Although they are complex, the changes in the signaling system appear well ordered in time and space. Signals other than those examined here will doubtless also be found to participate in the control of flowering in other species, even perhaps in *S. alba*, and some of the signals at work in *S. alba* may not be involved in other species. In the LD grass *L. temulentum*, for example, an increase in sucrose does not seem to be part of the floral signaling system (King and Evans, 1991), whereas some gibberellins (GAs) are prominent among the promotive signals produced and exported by their induced leaves (Pharis et al., 1987; Evans and King, 1988). Various GAs, such as GA_{32} and 2,2-dimethyl GA_4, are especially florigenic when applied to noninduced *L. temulentum* plants (Pharis et al., 1987). On the other hand, in *S. alba*, GAs do not seem to be limiting factors in the flowering process, and 2,2-dimethyl GA_4, like other GAs (GA_1, GA_3, GA_4, GA_7, GA_9), has no florigenic activity (Bernier, 1969; G. Bernier and A. Jacqmard, unpublished results). However, sucrose and cytokinins do seem to be involved in the floral signaling system of the SD plant *Xanthium strumarium*, just as in the LD plant *S. alba* (Houssa et al., 1991; J.-M. Kinet, P. Houssa, M.-C. Requier, and G. Bernier, manuscript submitted).

Unfortunately, even the best physiological evidence is only correlative, and definitive conclusions are difficult to reach from the kind of work described above. The inferences drawn from the physiological analysis will need, therefore, to be substantiated by genetic studies. If the control is multifactorial, as proposed here, then many genes will be found to participate in the control of flowering time. In addition, identification of these genes will definitively clarify the exact chemical nature of the factors involved. Unfortunately, the genetics of the model plants used for physiological studies are entirely unknown. Thus, a study of the genetic control of the transition to flowering is possible only in plants such as Arabidopsis or pea, in which many genes affecting flowering time have been identified (Koornneef et al., 1991; Murfet, 1992).

THE ARABIDOPSIS CASE: A GENETIC MODEL

Arabidopsis is a particularly useful genetic model for floral induction because it has many well-characterized metabolic mutants in addition to its numerous flowering mutants and because the physiological control of its flowering exhibits similarities to that of *S. alba*. Both species are facultative LD plants and facultative cold-requiring plants in natural conditions (Bernier, 1969; Napp-Zinn, 1985), and both react better to blue and far-red light than to any other light quality as far as floral induction is concerned (Bernier, 1969; Brown and Klein, 1971; Eskins, 1992). The similarities are such that, when grown side by side in LD in the same growth cabinet, plants of *S. alba* and the Columbia race of Arabidopsis reach anthesis of first flower almost simultaneously. The major difference between the two species is morphological and is apparent only during the vegetative life: vegetative Arabidopsis plants do not elongate their stem internodes and remain as rosettes, whereas vegetative *S. alba* plants have a stem with elongated internodes. At the transition to flowering in Arabidopsis, the last internodes formed before initiation of the first flower elongate to produce a floral stem.

Several research strategies have been devised to use Arabidopsis to investigate the genetic control of floral induction. The first consists of the isolation and analysis of as many mutants in flowering time as possible. Because flowering time is the end result of an array of processes, e.g., perception of vernalization and/or photoperiodic induction, production and transport of signals, meristem sensitivity, and flower initiation and development, it is not known which process is defective in these heterochronic mutants. Such knowledge should, however, follow the molecular identification of these genes.

An alternative strategy is to examine the flowering behavior of well-characterized metabolic and hormone mutants. When such mutants are available, these are the ideal tools for critically testing the inferences of the physiological results in plants such as *S. alba*. Such testing can also be achieved by following an entirely different approach, one that uses Agrobacterium-mediated genetic transformation to engineer transgenic plants in which the level of one hypothetical floral signal is altered.

Mutants of Flowering Time

Koornneef et al. (1991) reported the isolation of 42 single-gene mutants that flower later than their Landsberg *erecta* parental genotype. These mutants represent mutations at 11 different loci. Several additional late-flowering loci have since been identified (Martínez-Zapater et al., 1993). Most of these mutants are recessive, and almost all of them exhibit an altered response to environmental stimuli that induce flowering. Mutants at some *LATE FLOWERING* loci, *FCA* and *FVE*, for example, show a much greater reduction in flowering time than wild-type (WT) plants in response to a given vernalization treatment. Thus, the response of these mutants to vernalization is markedly

increased. In other late-flowering mutants, such as those at the loci *CONSTANS* (*CO*) and *GIGANTEA* (*GI*), the response to both LD and vernalization is almost suppressed. Nonflowering mutants have not yet been obtained. Even double mutants carrying mutations at two different late-flowering loci do ultimately flower (Koornneef et al., 1991). Another set of genes is represented by mutations that cause an early flowering phenotype (Sung et al., 1992; Zagotta et al., 1992). Some of these mutants, such as those at loci *TERMINAL FLOWER* (*TFL*) and *EARLY FLOWERING1* (*ELF1*), are quantitative LD plants like the WT plants, whereas others, such as those at loci *ELF3* and *EMBRYONIC FLOWERING* (*EMF*), do not respond to photoperiod.

Based on their work with the *emf* mutant, which bypasses the rosette vegetative step and produces an inflorescence directly upon germination, Sung et al. (1992) view the transition to flowering as the obligate developmental program of the apical meristem unless the vegetative program is activated at germination by the *EMF* gene product. Once the meristem has started to function vegetatively, its fate, i.e., remaining vegetative or becoming inflorescential, appears to be controlled by several sets of genes and corresponding regulatory pathways. Martínez-Zapater et al. (1993) postulate that there are at least two promotive and two inhibitory pathways. Among the promotive pathways, one, controlled by genes such as *FCA* and *FVE*, is considered to be constitutive because it is only weakly influenced by environmental factors, whereas another, controlled by genes such as *CO* and *GI*, appears to be dependent on environmental stimuli. Similarly, one inhibitory pathway, including genes such as *TFL* and *ELF1*, would be constitutive, whereas another inhibitory pathway, including genes such as *ELF3*, would be SD dependent. Consistent with this hypothesis is the genetic analysis of Koornneef et al. (1991), who showed by double mutant analysis that eight of the loci that mutate to cause late flowering fall into three epistatic groups and thus appear to work in three different pathways.

Efforts aimed at cloning some of the loci responsible for flowering time are under way in several laboratories (Dean et al., 1991; Martínez-Zapater et al., 1993). The next step will be to sequence these genes and to determine their tissue and stage specificity. This will hopefully allow us to understand not only their exact functions but also how they interact, i.e., to tie them in with Koornneef et al.'s (1991) epistatic groups.

Starch Mutants

From the physiological investigations in *S. alba*, it was inferred that starch mobilization could be an early and essential step in floral induction. This idea was tested genetically using the following Arabidopsis mutants (G. Bernier and A. Petitjean, unpublished results): the *phosphoglucomutase* (*pgm* TC75) starchless mutant, which is deficient in plastid phosphoglucomutase (Caspar et al., 1985), and the *starch overproducer* (*sop* TC26T) mutant, which results from a deficiency in an unknown step of starch degradation (Caspar et al., 1991).

When grown in continuous illumination, growth and flowering time of the two mutants are indistinguishable from those of their Columbia parental genotype. However, as the daylength is decreased, their growth is progressively decreased and their flowering delayed relative to that of WT plants. Interestingly, the behavior of the two mutants is almost exactly the same, indicating that their slow-growth, late-flowering phenotype is caused by a common deficiency, which is presumably their inability to mobilize starch.

The late flowering of the mutants might arise as a trivial consequence of their slow growth. However, seed vernalization, a treatment that does not affect the starch levels during further growth in LD or SD of mutant plants, completely suppresses the late-flowering phenotype of the mutants without altering their growth rates. The vernalized *pgm* and *sop* plants flower in LD or in SD at the same time or even earlier than unvernalized WT plants, while keeping a smaller size and weight than the WT plants.

We conclude from these observations that late flowering and slow growth are two independent phenotypic characters in plants with an impaired starch mobilization. Because the promotive effect of vernalization on flowering in Arabidopsis is totally unrelated to starch metabolism, it appears that a normal flowering time can result from two alternate regulatory pathways: (1) the vernalization pathway in *pgm* and *sop* plants or (2) the normal starch mobilization pathway in unvernalized WT plants. This supports the idea that starch mobilization is an essential process in the control of the flowering transition in WT Arabidopsis as it is in WT *S. alba*. It is important to point out that mutations conferring a vernalization requirement may affect genes controlling steps in general metabolic pathways, not necessarily genes controlling flowering specifically (Martínez-Zapater and Somerville, 1990).

Gibberellin Mutants

The *GA deficient* mutant, *ga1-3*, which is severely defective in *ent*-kaurene production (Zeevaart and Talon, 1992), flowers later than the Landsberg *erecta* WT in LD but is totally unable to flower in SD unless treated with exogenous GA_3 (Wilson et al., 1992). Thus, the *ga1-3* mutant, unlike any other known Arabidopsis genotype, is a strict LD plant. This indicates that GA is one of the primary signals for floral induction in Arabidopsis, just as in *L. temulentum*, as described above (see also Okamuro et al., 1993, this issue). A "leaky" allele at the same locus, *ga1-6*, causes less impairment in GA biosynthesis, and *ga1-6* mutants flower at the same time as the WT in LD and somewhat later in SD. A sufficient amount of GA is apparently required if flowering is to occur, and a deficiency in the GA biosynthetic pathway increases the photoperiodic sensitivity. A vernalization treatment is, however, unable to promote flowering of the *ga1-3* mutant in SD.

The *GA insensitive* mutant, *gai*, flowers readily in LD like the WT but has a late-flowering phenotype in SD (Wilson et al.,

1992). Unlike *ga1-3*, the *gai* mutant responds to vernalization by accelerating its flowering in SD.

Although still fragmentary, these results fit well with the physiological body of information concerning GAs. It is well known that the application of GAs to many rosette plants, including WT Arabidopsis grown in unfavorable conditions for flowering, promotes stem elongation and flower formation (Zeevaart, 1983; Napp-Zinn, 1985; Bagnall, 1992). LD and vernalization, which also promote bolting and flowering in rosette plants, have been shown to remove specific blocks in the GA biosynthetic pathway in these plants (Metzger, 1987; Hazebroek and Metzger, 1990; Burn et al., 1993; Zeevaart and Gage, 1993).

Whether GAs primarily control stem elongation and only secondarily control flowering (Zeevaart, 1983; Metzger, 1987) or whether they control both processes independently (Bernier et al., 1981b) is still an unresolved question. However, because 2-chloroethyltrimethylammonium chloride, an inhibitor of GA biosynthesis, delays flowering in several WT Arabidopsis lines (Napp-Zinn, 1985), it seems likely that GAs control flowering directly in this species. More work, using superior GA inhibitors such as tetcyclacis, is needed to confirm this conclusion.

Abscisic Acid and Ethylene Mutants

Both the *abscisic acid* (ABA) *deficient* and *ABA insensitive* mutants have an early flowering phenotype in SD but not in LD (Martínez-Zapater et al., 1993). An *ethylene insensitive* mutant, *etr*, shows a late-flowering phenotype (Bleecker et al., 1988), whereas a *hookless* (*hls1-1*) mutant, which is defective in the modulation of ethylene action, has an early flowering phenotype (Guzmán and Ecker, 1990). Note that *hls1-1* plants also have reduced ethylene production. These preliminary observations suggest that the role of ABA is inhibitory to flowering, in agreement with the literature (Bernier et al., 1981b; Bernier, 1988); the role, if any, of ethylene is not clear.

Transgenic Plants Overproducing Cytokinins

To test whether cytokinins influence flowering, Medford et al. (1989) have transformed Arabidopsis with the *Agrobacterium tumefaciens* isopentenyl transferase (ipt) gene placed under the control of a heat-inducible promoter (maize hsp70). IPT catalyzes the first step in cytokinin biosynthesis (McGaw, 1987), and heat induction causes a huge accumulation of several cytokinins in transgenic plants but has no effect on the time of flowering. At first sight, this result is not in line with the observations reported above in the *S. alba* system. However, the hsp70ipt gene is "leaky" in the transgenic Arabidopsis maintained at normal temperature; therefore, whether heat-induced or noninduced, these plants are enriched in cytokinins everywhere and at all times. This is in complete contrast with what we described in *S. alba*, in which there is a strict spatial and temporal regulation in cytokinin levels at flowering (Figure 1). In further studies of this kind, the ipt or other foreign genes should be placed under the control of more specific promoters, so that they are expressed only in specific tissues or at specific developmental stages.

In addition, even when one signal is increased, as is the case here for cytokinins, other necessary floral signals are not changed. This could also explain the unaltered flowering response of the plants because it is known that cytokinin applications are stimulatory to flowering in most plants only when combined with the application of other regulators or when plants are grown in conditions that cause marginal flowering (Bernier et al., 1981b).

Control of the Transition to Flowering in Arabidopsis Is Multigenic and Multifactorial

The full range of flowering-time and metabolic mutants has not yet been exploited in flowering studies. However, several interesting observations have already been made. First, the control of the transition involves many genes, as is also the case in pea (Murfet, 1992), and several regulatory pathways. This complexity is irreconcilable with the simple florigen/antiflorigen theory (see INTRODUCTION). Among the endogenous factors involved in the control of flowering, both nutrients and hormones are found, in line with the concept that this control is multifactorial and does not simply result from nutrient diversion (Bernier, 1988). In Arabidopsis, starch mobilization—and consequently sucrose availability—seems to be essential, but sucrose does not act in isolation. One or several GAs seem to be included among the other primary signals responsible for the transition. Other factors, such as ABA, might also be involved, but for definitive conclusions to be reached, the kinds of genetic work done so far should be complemented by physiological studies like those performed in *S. alba*. In particular, changes in the levels and fluxes of putative signals during the floral transition should be analyzed comparatively in mutant and WT plants.

In a multifactorial system of control, such as the one being discovered in Arabidopsis, the individual factors are likely to interact at one or another step of the process. Possible interactions between sucrose and cytokinins in *S. alba* were outlined above. GAs and ABA are known to interact with each other in the control of several processes (Davies, 1987), and they might also interact with sucrose because these hormones have been shown to regulate the activities of enzymes of carbohydrate metabolism and assimilate partitioning in various plants (Morris and Arthur, 1985; Brenner, 1987; Cheikh and Brenner, 1992; Cheikh et al., 1992). Whether similar interactions occur during the flowering transition in Arabidopsis await further investigation.

An approach not yet tried in the field of floral induction would consist of producing transgenic Arabidopsis expressing antisense gene constructs. This antisense effect could be targeted to any identified gene that is believed to participate in

the control of induction, and would make it possible to critically assess the function of these genes.

CONCLUSION

The challenge for plants, which are sessile organisms, is to reproduce successfully in an often unpredictable environment. Clearly, we are only now having a first glimpse of the real complexity of the control of their transition to flowering. Further progress will require an integrated combination of approaches: a physiological dissection of the process at the whole-plant level and a genetic and molecular dissection at lower levels of organization. As we learn more about flowering, we shall progressively discover that plants have evolved an incredibly sophisticated machinery for controlling their entry into sexual reproduction.

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