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Regulation of Flowering Time: Arabidopsis as a Model System to Study Genes that Promote or Delay Flowering

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Regulation of flowering time: *Arabidopsis* as a model system to study genes that promote or delay flowering

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SUMMARY

The time that plants flower is often tightly regulated and adapted to the locations in which they grow. The basis of this regulation has been analysed using genetic and physiological approaches since the early decades of this century. The study of flowering time in the model plant species *Arabidopsis thaliana* has allowed many genes involved in regulating flowering time to be identified as mutations, and for the genetic interactions between these mutations to have been studied. Furthermore, two genes required to promote flowering of *Arabidopsis* have recently been isolated, and their sequences have provided some insight into the identity of proteins involved in regulating flowering time.

1. INTRODUCTION

The flowering times of different varieties of the same plant species often vary dramatically depending on the geographical locations in which they are found. Optimization of the number and quality of seeds that are formed is probably the major selective advantage in regulating flowering time: typically, early flowering varieties form fewer flowers, but prolonged delay of flowering can mean that seed development is prematurely terminated by extremely hot summer temperatures or by the onset of winter (Worland *et al.* 1988). The regulation of flowering time by environmental conditions allows optimization of the duration of vegetative growth for the particular conditions in which the plant is growing.

The most important environmental signals that are used to regulate flowering time are temperature and the duration of daylight, although light intensity and quality can also be important. The major division of daylength-sensitive plants is between those responding to short days and long days: in the former a 24 h day must contain a long dark-period (and therefore a photoperiod below a critical daylength), whereas in the latter short dark-periods and daylengths longer than the critical daylength are promotive. Extended periods of low temperatures also promote flowering in many plant species, particularly in varieties from northern latitudes.

Sensitivity to environmental stimuli, particularly daylength, often increases with the age of the plant, indicating that in addition to environmental stimuli, internal factors that change with plant development affect flowering time (e.g. Mozley & Thomas 1995). In plant varieties in which flowering is largely unaffected by environmental stimuli, this developmental control represents the primary regulation of flowering time. The growth of annual and biennial plants is usually terminated by flowering, whereas in perennials vegetative growth is often restored after flowering.

2. PHYSIOLOGICAL APPROACHES TO ANALYSING THE CONTROL OF FLOWERING TIME

The regulation of flowering time by environmental stimuli requires a method of detecting and measuring the severity or duration of the stimulus, a means of transmitting this information to the cells in the apex of the plant where flower development occurs, and then in response to this signal terminating the development of vegetative structures and initiating floral development. For the control of flowering by daylength, these steps have been analysed using a variety of physiological approaches including making grafts between plants growing in inductive and non-inductive conditions (e.g. Lang *et al.* 1977), analysing the concentrations of substances before and after exposure to inductive conditions (e.g. Lejeune *et al.* 1988; Ishioka *et al.* 1990), measuring the effect that varying the duration of the light and dark periods or disrupting the dark period with night breaks has on flowering time (Hamner & Bonner 1938; Goto *et al.* 1991), microscopic analysis of the morphology of the apex in inductive and non-inductive conditions (e.g. Vaughn 1955), and isolating cDNAs expressed at different levels in plants induced to flower (Kelly *et al.* 1990; Melzer *et al.* 1990). These physiological approaches (excluding genetics, which is discussed below) have suggested general concepts that are widely believed to be important in regulating flowering time in response to daylength, but have not definitively identified genes or substances or gene products that are important in the regulation of flowering time (O'Neill 1992).

Perhaps the most enduring observation from these

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experiments is that in response to inductive daylengths floral induction occurs in the leaves, and a graft transmissible substance is then transported to the apex where it triggers flowering (Lang *et al.* 1977). Similarly, there is evidence that in the leaves of plants grown in non-inductive conditions, substances are formed that inhibit flowering (Lang *et al.* 1977). This has led to many attempts to purify these substances, and the failure to identify an individual substance that might be involved led to the suggestion that a complex mixture of substances that were shown to change in concentration during floral induction might be responsible (Bernier 1988). However, it has been difficult to demonstrate a causal relation between the appearance of these substances and the onset of flowering.

Before the inductive events in the leaf, exposure to the appropriate daylength must be recognized by the plant which requires both a time-keeping mechanism and a light receptor. This was analysed first for short-day plants, notably *Xanthium* species, in which it was recognized that exposure to long dark periods was crucial for flowering to occur, and therefore that it was the length of the dark period that was measured (Hamner & Bonner 1938; Vince-Prue 1975; King 1984). The effectiveness of the dark period could be reduced by disrupting it with flashes of red light, and this could be reversed by subsequent treatment with far red light. The wavelengths of the light used and the reversibility, indicated that phytochrome was the important light receptor in the photoperiodic control of flowering (Borthwick *et al.* 1952).

An endogenous circadian rhythm acts as the timekeeper and interacts with the light receptor (reviewed by King 1984 and Lumsden 1991). Evidence for such a rhythm came from the demonstration that if the short-day plant *Chenopodium rubrum* is kept in constant light and exposed to dark periods of different durations at varying times the capacity of the plants to flower in response to the dark period fluctuates rhythmically (Cumming *et al.* 1965). However, the biochemical basis of the endogenous circadian rhythm, how this is affected by the input from the light receptor phytochrome and how the outputs from the clock affect the flowering process are still unknown (Lumsden 1991; Evans 1993). Possibly, novel genetic approaches being used to study rhythmicity in chlorophyll a/b gene expression in *Arabidopsis* (Millar *et al.* 1995), will assist in identifying components of the clock that affect flowering time and how these interact with genes involved in the flowering process.

3. GENETIC APPROACHES TO UNDERSTANDING FLOWERING TIME

Genetic approaches have been used to study the flowering-time differences between varieties of the same species since the early decades of the century. Typically this was done by making hybrids between two varieties showing different flowering times and then following the segregation of flowering time among the progeny of the hybrid. Between 1910–1920, this approach was used to analyse flowering time in peas (Keeble & Peelew 1910), rice (Hoshino 1915), cotton (Leake 1911), wheat (Thompson 1918) and tobacco (Allard 1919). In certain cases, the difference between a pair of varieties was shown to be due to a single genetic locus that regulated flowering time (Allard 1919), but more often it proved to be the result of interactions between multiple loci (Goodwin 1944).

Induced mutations can also be useful in studying flowering time, because the difference between a particular mutant and its progenitor can be more easily shown to be caused by a single gene difference, and not to be a complex interaction between multiple differences. Among early examples of altering flowering behaviour with induced mutations was the alteration in the vernalization response of barley, creating a line with the flowering behaviour of a winter variety from a spring one (Stubbe 1959), and several mutations of *Arabidopsis* that each delayed flowering (Redei 1962).

4. LATE-FLOWERING MUTANTS OF *ARABIDOPSIS*

The genetic analyses described above, illustrated the importance of genes and combinations of genes in determining the distinct flowering times of varieties of the same species. However, in no case did the identification of these segregating loci help to identify biochemical products important in regulating flowering time, because none of the genes could be isolated or the function of their products addressed. In this respect, flowering time mutations identified in *Arabidopsis* are important because of the possibility to use molecular-genetic approaches such as T-DNA mutagenesis and chromosome walking to isolate the affected genes (Dean 1993).

Flowering time of *Arabidopsis* is regulated by environmental stimuli. Commonly used laboratory varieties flower early. Normally the first open flower will appear after approximately 3 weeks, if exposed to light for 16 h in each day. However, if exposed to short days of 8–10 h the first open flower will appear much later, after at least 6 weeks (Redei 1962; Koornneef *et al.* 1991). Short days increase the duration of all phases of development, so that an increased number of rosette leaves, cauline (stem) leaves and flowers are formed (Schultz & Haughn 1993). In addition, flowering is accelerated by extended exposure (for 3–6 weeks) to low temperatures after germination (Koornneef *et al.* 1991; Clarke & Dean 1993). No mutations that abolish the transition to flowering have been identified, but mutations that disrupt flowering responses in distinct ways have been described. These can be classified broadly into mutations that cause a delay in flowering and those that cause early flowering.

Late-flowering mutants were the first group to be identified and analysed in detail (Redei 1962; Koornneef *et al.* 1991). They have a dramatic phenotype when growing under standard long-day conditions: the mutants flower at least one week and often two or three weeks later than wild type and produce many more rosette leaves, cauline leaves and flowers

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Figure 1. Comparison of a wild-type *Arabidopsis* plant and the late-flowering mutant *constans* (*co*). Both plants are 21 days old and growing under long day conditions. The plant on the left is wild type and the one on the right is homozygous for the *co* mutation. The mutant is still in the vegetative phase and forming rosette leaves, while wild type has flowered. The increased number of vegetative leaves formed by the mutant is clearly visible. The wild-type plant exhibits the morphological features, such as the rosette, elongated stem, cauline (stem) leaves and flowers, that are typical of a mature *Arabidopsis* plant. Each pot measures 3.5 cm wide.

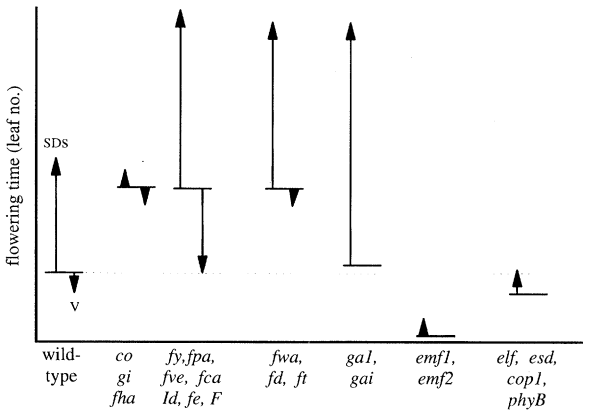

Figure 2. Effects of environmental stimuli on the flowering time of mutants and wild-type plants. Flowering time, represented as leaf number, is plotted on the vertical axis. Wild type and mutants showing flowering time phenotypes are grouped along the horizontal axis. The relative flowering time of each group under long days is represented by a horizontal line, and the dotted line is the flowering time of wild type for comparison. For each group, the arrow on the left represents the effect of growing the plants in short days (SDs), whereas the arrow on the right shows the effect of vernalization (V). Absence of an arrow indicates that data for this treatment are unavailable, an arrow resting on the horizontal line means that this treatment has almost no effect on flowering time. This diagram is adapted and extended from a similar one of Koornneef *et al.* (1991).

(Martinez-Zapater *et al.* 1995; see figures 1 and 2). The late-flowering mutants can be divided into three groups according to their responses to daylength and vernalization (see figure 2). All of these phenotypes are the result of mutations in single genes, and in many cases they have been located on the *Arabidopsis* genetic map (Koornneef *et al.* 1991). Most of the mutations are recessive, although *FRI* (previously called *F*) and *fwa* are dominant. Eleven of these mutations were identified and classified in the Landsberg *erecta* variety. In addition, the mutations *ld* and *FRI* cause strong late-flowering phenotypes in several *Arabidopsis* varieties, but have never been recovered in Landsberg *erecta*. This was recently shown to be due to a gene, *FLC*, whose Landsberg *erecta* allele suppresses the effect of mutations in both *ld* and *FRI*, but apparently *FLC* alleles present in other ecotypes do not act as suppressors (Koornneef *et al.* 1994; Lee *et al.* 1994).

Mutations first identified as affecting synthesis or responses to the plant hormone gibberellic acid (GA) also cause late flowering. In general, these have slight effects on flowering time under long days, but severe effects under short days (see figure 2), suggesting that the hormone is more important for flowering under short days (Wilson *et al.* 1992). Unlike the *ga* mutants, the genes affected in most of the late-flowering mutants are probably required to promote flowering under both long and short days. However, the *co*, *gi* and *fha* mutants are exceptions, in that they show delayed flowering only under long days (see figure 2) and the gene products of these genes are therefore probably required to promote flowering only under these conditions.

The genes affected in two late-flowering mutants, *ld* and *co*, that fall in different classes with respect to their responses to environmental conditions (see figure 2), were recently cloned. An *ld* allele caused by insertion of

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the T-DNA of *Agrobacterium tumefaciens* was used to isolate the gene (Lee *et al.* 1994). The *LD* protein is predicted to contain 953 residues, a bipartite nuclear localization signal and a high proportion of glutamine residues towards the carboxy-terminus that is reminiscent of some mammalian transcriptional activators. Interestingly, the T-DNA induced *ld*-3 allele is very likely to be a null, because the T-DNA insertion is towards the amino terminus of the protein and the mutant contains no detectable *LD* mRNA (Lee *et al.* 1994). That these mutants do flower supports the proposal that loss of function of *LD*, and possibly of genes affected in other late-flowering mutants, can be partially compensated for by other genes. Similarly, because the late-flowering phenotype of *ld*-3 can be corrected by vernalization, the product of *LD* is not required for early flowering when plants are vernalized.

The *ld* mutation also seems to prevent the recognition of day extensions of low-intensity light. Wild-type plants exhibit a long-day phenotype when grown under conditions of 8 h high-intensity light and a 12 h extension of low-intensity white light, but exhibit a short day phenotype if the 12 h extension is omitted. *ld* mutants, however, show a short-day phenotype even when exposed to the 8 h high-intensity light followed by the day extension. How the *ld* mutation prevents the response to day extensions is unknown, but strikingly mutations that affect the gene encoding phytochrome A have a similar effect and prevent the mutants from flowering as early as wild type when exposed to day extensions (Johnson *et al.* 1994). The promotive effect of the extension might require a high irradiance response mediated by phytochrome A in response to far-red light, which would explain why the *phyA* mutation causes late-flowering under these conditions (Thomas 1991; Johnson *et al.* 1994). The similar effect of *ld* mutations, suggests that the *LD* product might act downstream of phytochrome A to promote flowering in response to day extensions. This suggests that at least under these conditions phytochrome A, probably in response to relatively high ratios of far red light, is one of the light receptors that is required to promote flowering of *Arabidopsis*. This is in agreement with previous observations that far red and blue light have promotive and additive effects on flowering time (Brown & Klein 1971; Eskins 1992). The identity of the blue light receptor that acts to promote flowering of *Arabidopsis* is still not known, although the first such receptor was recently cloned from *Arabidopsis* (Ahmad & Cashmore 1993).

Mutation of the *CO* gene also results in late flowering of *Arabidopsis*, and the gene was cloned by chromosome walking. It encodes a protein predicted to contain 373 residues and two zinc fingers of the $C\text{-}X_2\text{-}C\text{-}X_{16}\text{-}C\text{-}X_2\text{-}C$ class (Putterill *et al.* 1995). That the zinc fingers are important for *CO* activity was supported by showing that the first two alleles to be analysed both contained changes within the region of the protein containing the zinc fingers; one is a nine base deletion that precisely removes three codons, the other is a missense mutation. All of the available *co* alleles have been sequenced and none of the mutations certainly abolish *CO* function, so it is not clear whether the eventual flowering of these mutants requires residual *CO* activity. The *CO* zinc fingers are characteristic of proteins that bind to DNA, and show a similar spacing of cysteines, but little direct homology, to those found in GATA transcription factors. *CO* mRNA is present at very low abundance but was detected by RT-PCR in total RNA extracted from leaves, and stems from which the leaves had been removed. That *CO* is required to promote flowering was supported by demonstrating that transgenic plants containing extra transgenic copies of *CO* flowered earlier than wild-type plants. The transcript is present at higher abundance in RNA extracted from young seedlings grown under long days than under short days. The reduced abundance of *CO* transcript under short days might be important in determining that *Arabidopsis* flowers later under short days (Putterill *et al.* 1995).

5. EARLY-FLOWERING MUTANTS OF *ARABIDOPSIS*

Early-flowering mutations were screened for directly in tissue culture conditions or by growing plants under short days. All of the mutations are recessive, and therefore the early flowering phenotype is probably due to the inactivation of genes required to repress flowering. The most severe of these mutants, *embryonic flower* (*emf1* and *emf2*), do not produce any vegetative rosette leaves, but flower directly after germination (Sung *et al.* 1992; Yang *et al.* 1995). These mutants are insensitive to daylength, flowering with no rosette leaves under both long and short days (see figure 2). Less severe early flowering mutants, *early flowering* (*elf*; see Zagotta *et al.* 1992) and *early short days* (*esd*; see Coupland *et al.* 1993) have a recognizable vegetative phase and form a rosette similar to that of wild type (see figure 3). They flower much earlier than wild type under short days, and in some cases slightly earlier under long days.

Mutants that were first identified on the basis of other phenotypes were subsequently shown to be early flowering. Two of these affect photomorphogenic responses: mutations in the genes encoding the photoreceptor phytochrome B or in the *CONSTITUTIVE PHOTOMORPHOGENIC 1* (*cop1*) gene, which is thought to encode a protein involved in a signal transduction chain downstream of phytochrome, both cause early flowering (Goto *et al.* 1991; McNellis *et al.* 1994). A third gene, *terminal flower* (*tfl*), is required to prevent a flower developing at the very apex of the shoot by preventing the expression of floral meristem identity genes in this region, and *tfl* mutations also cause early flowering (Shannon & Meeks-Wagner 1991).

None of the genes affected in mutants identified by screening for early flowering mutants have been isolated. However, the *PHYB* and *COP1* genes have both been cloned, and mutations affecting them both disrupt the regulation of photomorphogenesis and cause early flowering. *phyB* mutations affect the gene encoding a light stable member of the phytochrome family of photoreceptors (Reed *et al.* 1993). As well as

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causing early flowering under short days, *phyB* causes mutants to show in the light some of the features of dark grown plants: for example the hypocotyls are extended and the shoot is elongated. *cop1* mutations have the reverse effect on plant morphology, mutants grown in the dark show many of the features of light grown plants. For example, dark grown *cop1* mutants have a short hypocotyl and genes, such as those encoding chlorophyll a/b binding protein, which are normally only expressed in light grown plants are expressed in the mutants in the dark. *COP1* contains a zinc finger motif, WD-40 repeats and shows homology to the β subunits of trimeric G proteins (Deng *et al.* 1992). This combination of motifs suggests that the *COP1* protein binds DNA and interacts with other proteins; it is also located in the nucleus of dark-grown plants and has been proposed to act as a repressor of transcription (Deng *et al.* 1992; von Arnim & Deng 1994). *COP1* is thought to act downstream of phytochrome in the same pathway, because double mutants containing *cop1* and *phyB* show the *cop1* hypocotyl phenotype. The early flowering phenotype of these two mutants suggests that the products of these genes might also be involved in one pathway, activated by phytochrome B, that is required to repress flowering.

6. INTERACTIONS BETWEEN FLOWERING TIME MUTATIONS OF *ARABIDOPSIS*

The flowering times of double mutants carrying two mutations, each of which causes late flowering, have been measured. In general, these indicate that when two mutations that have similar effects on environmental responses are combined, the double mutant simply flowers as late as the later of the two single mutants. However, combining mutations from different groups produces a double mutant that flowers much later than either parent. This led to the proposal that each class of mutations represents one pathway leading to flowering, and that these pathways are partially redundant (Koornneef *et al.* 1991). Those combinations which do not lead to an enhancement in phenotype would therefore be the result of blocking a single pathway in two places, whereas the stronger phenotype would be caused by inactivating two distinct pathways. The latter conclusion, however, assumes that the alleles used to construct the double mutants were nulls, and because in most cases the affected genes are not cloned, the data to demonstrate this are often not available.

Mutations causing early and late flowering have also been combined. *emf1* and *emf2*, which cause flowering of plants with no rosette leaves, are epistatic to *co* and *gi*, which cause late flowering (Yang *et al.* 1995). Similarly, the most severe of the *esd* mutations, *esd4*, completely suppresses the phenotype of *co* under long days such that the double mutant *co esd4* has a flowering time similar to that of wild type (see figure 3). The epistasis of the mutations causing early flowering is consistent with the genes affected in these mutants acting as repressors of flowering that are downstream in the flowering pathway of the genes affected in late-flowering mutants.

7. INTERACTIONS BETWEEN FLOWERING TIME MUTATIONS AND MUTATIONS AFFECTING OTHER PROCESSES

LFY is one of the earliest acting genes in the development of *Arabidopsis* flowers, plants homozygous for *lfy* null alleles and growing under long days, form cauline leaves and inflorescences at many positions on the shoot where flowers would normally develop (Weigel *et al.* 1992). The *LFY* gene interacts with other floral meristem identity genes such as *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*) and *APETALA2* (*AP2*) and several of the double mutant combinations of these mutations suggest that their gene products have partially overlapping functions: for example, *ap1 lfy* double mutants show a more severe phenotype than either single mutant as do *ap1 cal* double mutants (Weigel *et al.* 1992; Bowman *et al.* 1993; Schultz & Haughn 1993; see table 1). The phenotypes of mutants in the meristem identity genes are also strongly influenced by daylength, as *lfy* mutants show a much stronger phenotype under short than long days. This

Table 1. *Summary of interactions between* co *and other* Arabidopsis *mutations*

mutant	phenotype	phenotype enhanced by growth under short days	interacting mutations that enhance phenotype	interacting mutations that supressed or partially suppress phenotype	references
co	late flowering	no	some other mutations causing late flowering e.g. *fca*, *fve*, *fwa*.	*emf1*, *emf2*, *esd4*	Putterill *et al.* 1995 Sung *et al.* 1992 Yang *et al.* 1995
lfy	inflorescences replace some flowers; petal and stamen development reduced	yes	*ap1*, *ap2*, *co*	–	Weigel *et al.* 1992 Schultz & Haughn 1993 Putterill *et al.* 1995
ap1	no petals, ectopic flowers develop near positions normally occupied by petals	yes	*cal*, *lfy*,*ap2*, *co*	–	Bowman *et al.* 1993 Simon, unpublished data
gai	reduced height, dark green leaves, late flowering	yes	*co*	–	Putterill *et al.* 1995

Figure 3. The *early short day 4* (*esd4*) mutation causes early flowering and can suppress the late-flowering phenotype caused by *constans* (*co*). The four plants are the same age and growing under long days. Their genotypes are (from left to right): wild type, *esd4*, *co* and *co esd4*. The wild-type plant is flowering, whereas the *co* mutant is still growing vegetatively, as shown in figure 1. The *esd4* plant flowered earlier than wild type, and this is apparent because many of its flowers have already self-fertilized and formed seed pods while this has not yet occurred on the wild-type plant. The co esd4 double mutant flowered at a time very similar to that of wild type, and therefore intermediate between esd4 and co. Each pot measures 3.5 cm wide.

latter result indicated that environmental conditions that influence flowering time affect the interactions between the meristem identity genes. Furthermore, *co lfy* double mutants growing under long days have a similar phenotype to *lfy* mutants growing under short days, suggesting that the enhancement of *lfy* seen under short days is due to a reduction in the function of genes affected in late-flowering mutants (Putterill *et al.* 1995).

Similarly, the phenotypes of *ga* mutants have a more severe effect on flowering time under short than long days, whereas *co* has the reverse effect, delaying flowering only under short days. Double mutants containing *co* and the *gibberellic acid insensitive* (*gai*) mutation show a dramatic enhancement of the *gai* phenotype under long days, suggesting that the enhancement of the *gai* phenotype that is normally seen under short days is caused by a lack of *CO* activity (Putterill *et al.* 1995; see table 1). The analysis of the double mutant is consistent with the *CO* and *GAI* genes having overlapping functions, such that mutations in one gene can be partially compensated for by activity of the other one. This implicates the *CO* gene in some responses to the plant hormone GA.

8. FUTURE PERSPECTIVES

The isolation of two genes required to promote flowering of *Arabidopsis* is an exciting development that provides for the first time an indication of the identity of some of the proteins specifically involved in regulating flowering time. However, the genetic and physiological experiments described above, clearly indicate the involvement of many genes in the control of flowering time and these could act in principle in any of the organs of the plant and at any level in the regulatory system from the perception of environmental signals to the activation of floral meristem identity genes in the apex. It will be necessary to isolate more of the genes required to regulate flowering time to analyse in detail how the gene products interact, and how the pathways they represent act in concert to regulate flowering. It will also be important to try to determine how the genes that have been cloned relate to the processes identified by the physiological experiments as being important in regulating flowering time. At present it is not clear how the *LD* and *CO* genes relate to processes such as timekeeping, inductive events in the leaf or evocative events at the apex, nor is it known in which tissues their activity is required to promote flowering. Furthermore, their likely biochemical function as transcription factors does not identify the biochemical processes in which they are involved, especially in the absence of knowledge of the identity of their target genes.

How far can the flowering time work on *Arabidopsis* be generalized to other species? The *LD* and *CO* genes are clearly present in other species, as based on the results of Southern hybridization experiments (Lee *et al.* 1994; M. Igeno, L. Robert & G. Coupland, unpublished data), however it is less clear whether they retain their significance in regulating flowering time in other species. The observation that *co esd4* double mutants have a flowering time similar to that of wild type (see figure 3), although each of the single mutants has widely different flowering times, suggests that there

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are multiple genetic routes to the same flowering time. This is also suggested by the large number of mutations that can influence flowering time. It is therefore not at all clear that the same genes will be critical in determining the flowering times of different species, or different varieties of the same species. However, this can now be tested for the *LD* and *CO* genes, and for other genes as they are cloned from *Arabidopsis*.

REFERENCES

Ahmad, M. & Cashmore, A. R. 1993 *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue light receptor. *Nature, Lond.* **366**, 162–166.

Allard, H. A. 1919 Gigantism in *Nicotiana tabacum* and its alternative inheritance. *Am. Nat.* **53**, 218–233.

Bernier, G. 1988 The control of floral evocation and morphogenesis. *A. Rev. Pl. Physiol. Pl. molec. Biol.* **39**, 175–219.

Borthwick, H. A., Hendricks, S. B. & Parker, M. W. 1952 The reaction controlling floral initiation. *Proc. natn Acad. Sci. U.S.A.* **38**, 929–934.

Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. M. & Smyth, D. R. 1993 Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* **119**, 721–743.

Brown, J. A. M. & Klein, W. H. 1971 Photomorphogenesis in *Arabidopsis thaliana* (L.) Heynh. *Pl. Physiol.* **47**, 393–399.

Clarke, J. H. & Dean, C. 1994 Mapping *FRI*, a locus controlling flowering time and vernalization response. *Molec. gen. Genet* **242**, 81–89.

Coupland, G., Dash, S., Goodrich, J., Lee, K., Long, D., Martin, M., Puangsomlee, P., Putterill, J., Robson, F., Sundberg, E. & Wilson, K. 1993 Molecular and genetic analysis of the control of flowering time in response to daylength in *Arabidopsis thaliana*. *Flowering Newslett.* **16**, 27–32

Cumming, B. G., Hendricks, S. B. & Borthwick, H. A. 1965 Rhythmic flowering responses and phytochrome changes in a selection of *Chenopodium rubrum*. *Can. J. Bot.* **43**, 825–853.

Dean, C. 1993 Adavantages of *Arabidopsis* for cloning plant genes. *Phil. Trans. R. Soc. Lond.* B **342**, 189–195.

Deng, X.- W., Matsui, M., Wei, N., Wagner, D., Chu, A. M., Feldmann, K. A. & Quail, P. H. 1992 COP1, an *Arabidopsis* regulatory gene, encodes a protein with both a zinc-binding motif and a G_b homologous domain. *Cell* **71**, 791–801

Eskins, K. 1992 Light quality effects on *Arabidopsis* development. Red, blue and far-red regulation on flowering and morphology. *Physiologia Pl.* **86**, 439–444

Evans, L. T. 1993 The physiology of flower induction – Paradigms lost and paradigms regained. *Aust. J. Pl. Physiol.* **20**, 655–660.

Goto, N., Kumagai, T. & Koornneef, M. 1991 Flowering responses to night breaks in photomorphogenic mutants of *Arabidopsis*. *Physiologia Pl.* **83**, 209–215.

Goodwin, R. H. 1944 The inheritance of flowering time in a short-day species, *Solidago sempervirens L. Genetics* **29**, 503–519.

Hamner, K. C. & Bonner, J. 1938 Photoperiodism in relation to hormones as factors in floral initiation and development. *Bot. Gaz.* **100**, 388–431.

Ishioaka, N., Tanimoto, S. & Harada, H. 1991 Flower inducing activity of phloem exudates from *Pharbitis* cotyledons exposed to various photoperiods. *Pl. Cell Physiol.* **32**, 921–924.

Johnson, E., Bradley, M., Harberd, N. P. & Whitelam, G. C. 1994 Photoresponses of light grown *phyA* mutants of *Arabidopsis*. *Pl. Physiol.* **105**, 141–149

Keeble, F. & Pellew, C. 1910 The mode of inheritance of stature and time of flowering in peas (*Pisum sativum*) *J. Genet.* **1**, 47–56.

Kelly, A. J., Zagotta, M. T., White, R. A., Chang, C. & Meeks-Wagner, D. R. 1990 Identification of genes expressed in the tobacco shoot apex during the floral transition. *Pl. Cell* **2**, 963–972.

King, R. W. 1984 Light and photoperiodic timing. In *Light and the flowering process* (ed. D. Vince-Prue, B. Thomas & K. E. Cockshull), pp. 91–105. London: Academic Press.

Koornneef, M., Blankestijn-de Vries, H., Hanhart, C., Soppe, W. & Peters, T. 1994 The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg *erecta* wild-type. *Pl. J.* **6**, 911–919.

Koornneef, M., Hanhart, C. J. & van der Veen, J. H. 1991 A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Molec. gen. Genet.* **229**, 57–66.

Leake, H. M. 1911 Studies in Indian cotton. *J. Genet.* **1**, 205–272.

Lee, I., Aukerman, M. J., Gore, S. L., Lohman, K. N., Michaels, S. D., Weaver, L. M., John, M. C., Feldmann, K. A. & Amasino, R. M. 1994 Isolation of *LUMINIDEPENDENS*: A gene involved in the control of flowering time in *Arabidopsis*. *Pl. Cell* **6**, 75–83.

Lejeune, P., Kinet, J.-M. & Bernier, G. 1988 Cytokinin fluxes during floral induction in the long-day plant *Sinapis alba L. Pl. Physiol.* **86**, 1095–1098.

Lumsden, P. J. 1991 Circadian rhythms and phytochrome. *A. Rev. Physiol. Pl. molec. Biol.* **42**, 351–371.

Melzer, S., Majewski, D. M. & Apel, K. 1990 Early changes in gene expression during the transition from vegetative to generative growth in the long-day plant *Sinapis alba*. *Pl. Cell* **2**, 953–961.

Martinez-Zapater, J. M., Jarillo, J. A., Cruz-Alvarez, M., Roldçn, M. & Salinas, J. 1995 *Arabidopsis* late flowering *fve* mutants are affected in both vegetative and reproductive development. *Pl. J.* **7**, 543–552.

Martinez-Zapater, J. M., Coupland, G., Dean, C. & Koornneef, M. 1994 *The transition to flowering in* Arabidopsis (ed. C. R. Somerville & E. M. Meyerowitz). Cold Spring Harbor: Cold Spring Harbor Laboratory Press. pp. 403–434.

McNellis, T. W., von Arnim, A. G., Araki, T., Komeda, Y., Miséra, S. & Deng, X.-W. 1994 Genetic and molecular analysis of an allelic series of *cop1* mutants suggests functional roles for the multiple protein domains. *Pl. Cell* **6**, 487–500

Millar, A. J., Carré, I. A., Strayer, C. A., Chua, N.-H. & Kay, S. A. 1995 Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. *Science, Wash.* **267**, 1161–1163.

Mozley, D. & Thomas, B. 1995 Developmental and photobiological factors affecting photoperiodic in *Arabidopsis thaliana* Heynh. Landsberg *erecta*. *J. expl Bot.* **46**,

O'Neill, S. 1992 The photoperiodic control of flowering: progress toward understanding the mechanism of induction. *Phytochem. Photobiol.* **56**, 789–801.

Parks, B. & Quail, P. H. 1991 Phytochrome-deficient *hy1* and *hy2* long hypocotyl mutants of *Arabidopsis* are defective in phytochrome chromophore biosynthesis. *Pl. Cell* **3**, 1177–1186.

Putterill, J., Robson, F., Lee, K., Simon, R. & Coupland, G. 1995 The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* **80**, 847–858

Redei, G. P. 1962 Supervital mutants of *Arabidopsis*. *Genetics* **47**, 443–460.

Reed, J. W., Nagpal, P., Poole, D. S., Furuya, M. & Chory, J. 1993 Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *Pl. Cell* **5**, 147–157.

Schultz, E. A. & Haughn, G. W. 1993 Genetic analysis of the floral initiation process (FLIP) in *Arabidopsis*. *Development* **119**, 745–765.

Shannon, S. & Meeks-Wagner, D. R. 1993 Genetic interactions that regulate inflorescence development in *Arabidopsis*. *Pl. Cell* **5**, 639–655.

Stubbe, H. 1959 Considerations on the genetic and evolutionary aspects of some mutants of *Antirrhinum*, *Hordeum* and *Lycopersicon*. *Cold Spring Harb. Symp. quant. Biol.* **24**, 31–40.

Sung, R., Belachew, A., Shunong, B. & Bertrand-Garcia, R. 1992 *EMF*, an *Arabidopsis* gene required for vegetative shoot development. *Science, Wash.* **258**, 1645–1647.

Thomas, B. 1991 Phytochrome and photoperiodic induction. *Physiol. Plant.* **81**, 571–577.

Thompson, W. P. 1918 The inheritance of the length of the flowering and ripening periods in wheat. *Trans. R. Soc. Can.* **12,** 69–87.

Vaughn, J. G. 1955 The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursapastoris* (L.) Medic. & *Anagallis arvensis*. *J. Linn. Soc. Bot.* **55**, 279–301.

Vince-Prue, D. 1975 *Photoperiodism in plants*. London: McGraw-Hill.

von Arnim A. G. & Deng, X.-W. 1994 Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* **79**, 1035–1045

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. & Meyerowitz, E. M. 1992 *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* **69**, 843–859.

Wilson, R. N., Heckman, J. W. & Somerville, C. R. 1992 Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Pl. Physiol.* **100**, 403–408.

Worland, A. J., Petrovic, S. & Law, C. N. 1988 Genetic analysis of chromosome 2D of wheat II. The importance of this chromosome to Yugoslavian varieties. *Pl. Breed.* **100**, 247–259.

Yang, C.-H., Chen, L.-J. & Sung, Z. R. 1995 *Devl biol.* (In the press.)

Zagotta, M. T., Shannon, S., Jacobs, C. & Meeks-Wagner, D. R. 1992 Early flowering mutants of *Arabidopsis thaliana*. *Aust. J. Pl. Physiol.* **19**, 411–418.

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gure 1. Comparison of a wild-type *Arabidopsis* plant and the late-flowering mutant *constans* (*co*). Both plants are 21 ıys old and growing under long day conditions. The plant on the left is wild type and the one on the right is omozygous for the *co* mutation. The mutant is still in the vegetative phase and forming rosette leaves, while wild type ıs flowered. The increased number of vegetative leaves formed by the mutant is clearly visible. The wild-type plant hibits the morphological features, such as the rosette, elongated stem, cauline (stem) leaves and flowers, that are pical of a mature *Arabidopsis* plant. Each pot measures 3.5 cm wide.

gure 3. The *early short day 4* (*esd4*) mutation causes early flowering and can suppress the late-flowering phenotype used by *constans* (*co*). The four plants are the same age and growing under long days. Their genotypes are (from t to right): wild type, *esd4*, *co* and *co esd4*. The wild-type plant is flowering, whereas the *co* mutant is still growing getatively, as shown in figure 1. The *esd4* plant flowered earlier than wild type, and this is apparent because many its flowers have already self-fertilized and formed seed pods while this has not yet occurred on the wild-type plant. ne co esd4 double mutant flowered at a time very similar to that of wild type, and therefore intermediate between d4 and co. Each pot measures 3.5 cm wide.