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Update on Development

The Control of Flowering Time and Floral Identity in Arabidopsis[1]

Manuel Piñeiro and George Coupland*

John Innes Centre, Colney Lane, Norwich, United Kingdom NR4 7UH

The reproductive success of plant varieties is often dependent on their flowering time being adapted to the environment in which they grow. This adaptation involves the regulation of flowering by environmental stimuli such as temperature and day length. Classic grafting experiments performed in several species including perilla and tobacco showed that day length is detected in the leaves and a signal is transmitted from there to the shoot apex (King and Zeevaart, 1973; Lang et al., 1977). Widely used early flowering ecotypes of Arabidopsis such as Columbia and Landsberg *erecta* flower within 3 weeks under LD conditions but not until at least 5 weeks under SD conditions. The shoot apical meristem of Arabidopsis plants grown for 30 d under SD conditions cease producing leaf primordia and start producing flower primordia within a few hours of being shifted to LD conditions (Hempel and Feldmann, 1994). In response to this photoperiodic change, alterations in cell division rates change the shape of the shoot apical meristem, and the primordia produced on the flanks of the meristem form flowers rather than leaves. The rapidity with which the first flowers develop after plants are shifted from SD to LD conditions led Hempel and Feldmann (1995) to propose that in Arabidopsis the signal from the leaves can act directly on existing primordia to alter their identity. The development of chimeric organs showing characteristics of both leaves and flowers at the last node formed prior to the induction of flower primordia also supports the idea that the floral stimulus acts directly on the primordium to confer floral identity (Hempel and Feldmann, 1995).

As well as acting directly to influence primordium development, transient exposure of plants to LD conditions causes them to become irreversibly committed to flowering even after their return to SD conditions. Scanning electron micrographs of shoot apices from plants exposed to 8 d of LD conditions show no visible signs of floral development, but plants shifted back to SD conditions still flower as if grown continuously under LD conditions (Bradley et al., 1997). Therefore, exposure to LD conditions causes either persistent expression of the floral stimulus even after plants are shifted back to SD conditions, or a change in the identity of the shoot meristem such that it is stably committed to form floral primordia. The first possibility is suggested by recent experiments with maize and impatiens, which emphasize the continued requirement of leaves for the meristem to form flowers. Experiments with excised shoot apices of maize plants suggest that the presence of four to six leaves is required for the meristem to become committed to form flowers. Excised apices that retain one or two leaves behave like meristems of very young plants and form tassels only after producing the same number of leaves as plants germinated from seed, whereas excised apices that retain four to six young leaves frequently form tassels after producing fewer new leaves than plants grown from seed (Irish and Nelson, 1991; Irish and Jegla, 1997). In impatiens, continued production of an inductive signal from the leaves is also required to prevent reversion to the vegetative state (Pouteau et al., 1997).

A systematic genetic approach to identifying genes involved in the transition to flowering has been taken with Arabidopsis (Koornneef et al., 1998a) and pea (Weller et al., 1997). Genes that promote the flowering of Arabidopsis were identified as mutations that delay flowering time, and genetic variation causing similar phenotypic effects was recovered by crossing different ecotypes. Alleles causing late flowering extend the duration of vegetative growth and therefore increase the number of leaves formed before the development of flowers. Floral meristem identity genes or floral initiation process genes confer floral identity upon undifferentiated primordia (Schultz and Haughn, 1993; Weigel, 1995a). Mutations in these genes cause primordia that would develop as flowers in wild-type plants to form structures with shoot-like characteristics. One of the roles of floral meristem identity genes is to activate the expression of organ identity genes that act later in flower development (Weigel and Meyerowitz, 1993). The roles of organ identity genes during flower development, and how the spatial pattern of their expression within the developing flower is regulated have been reviewed previously (Ma, 1994; Weigel and Meyerowitz, 1994).

In this *Update* we focus on recent advances in understanding the genetic control of flowering time and floral meristem identity in Arabidopsis and on how genes in-

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* Corresponding author; e-mail couplnd@bbsrc.ac.uk; fax 44–1603–505725.

Abbreviations: GR, glucocorticoid receptor; LD, long-day; SD, short-day.

volved in these processes interact. Studies of their genetics and expression suggest that genetic redundancy and quantitative regulation of gene expression are important to efficiently control flowering time and to define shoot architecture.

GENES THAT PROMOTE FLOWERING IN ARABIDOPSIS

Over 20 late-flowering mutants have been described in Arabidopsis (Martinez-Zapater et al., 1994; Coupland, 1995; Weigel, 1995a; Koornneef et al., 1998b; see Table I). However, mutants that remain in the vegetative phase indefinitely and never undergo the transition to flowering have not been identified. This suggests that some degree of redundancy exists between genes that promote flowering, so that inactivation of a single gene is partially compensated for by other genes. The response of late-flowering mutants to environmental signals and the phenotypes of double mutants divide the genes into at least two groups that have been proposed to represent two of the genetic pathways that promote flowering in Arabidopsis (Fig. 1).

The first group includes the *fca, fpa, ld, fve,* and *fy* mutants. They flower later than wild-type plants under both LD and SD conditions and show a decreased flowering time in response to vernalization treatments. These genes are proposed to act within an autonomous pathway that promotes flowering independent of environmental conditions. A second group of late-flowering mutants, *co*, *fd*, *fe*, *fha*, *ft*, *fwa*, and *gi*, show little or no response to vernalization, and their flowering is delayed under LD conditions but not SD conditions. This group of genes is proposed to act through a pathway that promotes flowering specifically in response to LD conditions (Martinez-Zapater et al., 1994; Weigel, 1995a; Coupland, 1997; Koornneef and Peeters, 1997; Koornneef et al., 1998b). Partial redundancy between these two pathways and the presence of at least one other pathway involving the growth regulator GA probably explains why no single mutation preventing flowering has been isolated. The existence of these three partially redundant pathways is supported by the phenotypes of double mutants: plants carrying two mutations within one group flower at approximately the same time as those carrying a single mutation; however, plants carrying two mutations in different groups flower much later than either parent. Although more complex models have been presented that incorporate other genes affecting flowering time, such as those that mediate the vernalization response and those that repress flowering, the three partially redundant pathways described above form the core of these models (Koornneef et al., 1998b).

Two genes classified as acting in the autonomous flowering pathway have been cloned: *LUMINIDEPENDENS* (*LD*) and *FCA*. *LD* was isolated using an allele caused by a T-DNA insertion. The predicted protein contains 953 residues and includes two bipartite nuclear localization signals, a Gln-rich domain at the carboxy teminus reminiscent of those found in several mammalian transcriptional activators, and a possible homeodomain in the amino terminal region (Lee et al., 1994; Auckerman and Amasino, 1996). Recently, the map-based cloning of *FCA* has also been reported (Macknight et al., 1997). *FCA* encodes a protein that contains two RNA-binding domains and a WW protein interaction domain, suggesting that it may function as a posttranscriptional regulator. The *FCA* transcript itself is alternatively spliced, thereby generating four products with variant abundance (α, β, γ, and δ). *FCA* transcripts are present at low abundance, and both the levels and the ratios relative to each other are constant in all organs and developmental stages analyzed. Transcription of *FCA* from the strong constitutive 35*S* promoter results in a large increase in transcript β accumulation, with smaller increases in transcripts γ and δ. Since the overexpression of

Table I. *Flowering-time and floral-identity genes*

Mutant	Phenotype	Reference
Autonomous pathway		
fca	Late flowering; responds to vernalization	Macknight et al. (1997)
ld	Late flowering; responds to vernalization	Lee et al. (1994)
fpa, fve, fy	Late flowering; responds to vernalization	Martínez-Zapater et al. (1994)
LD pathway		
co	Late flowering; no response to vernalization	Puterill et al. (1995)
fha	Late flowering; no response to vernalization	Lin et al. (1996)
fd, fe, ft, fwa, gi	Late flowering; no response to vernalization.	Martínez-Zapater et al. (1994)
lhy	Late flowering; disruption of circadian rhythm	Coupland (1997)
GA pathway		
ga1	Late flowering	Wilson et al. (1992); Blázquez et al. (1997)
Floral repressor		
elf3	Early flowering; disruption of circadian rhythm	Hicks et al. (1996)
emf1, emf2	Early flowering	Castle and Sung (1995)
tfl	Early flowering; determinate inflorescence	Bradley et al. (1997)
Floral meristem identity		
lfy	Partial transformation of flowers into inflorescence	Weigel et al. (1992)
ap1	Partial transformation of flowers into inflorescence	Irish and Sussex (1990)
ap2	Partial transformation of flowers into inflorescence	Jofuku et al. (1994)
ap1 cal	Partial transformation of flowers into inflorescence	Bowman et al. (1993)
ag	Floral indeterminate growth; floral reversion (SD)	Mizukami and Ma (1997)

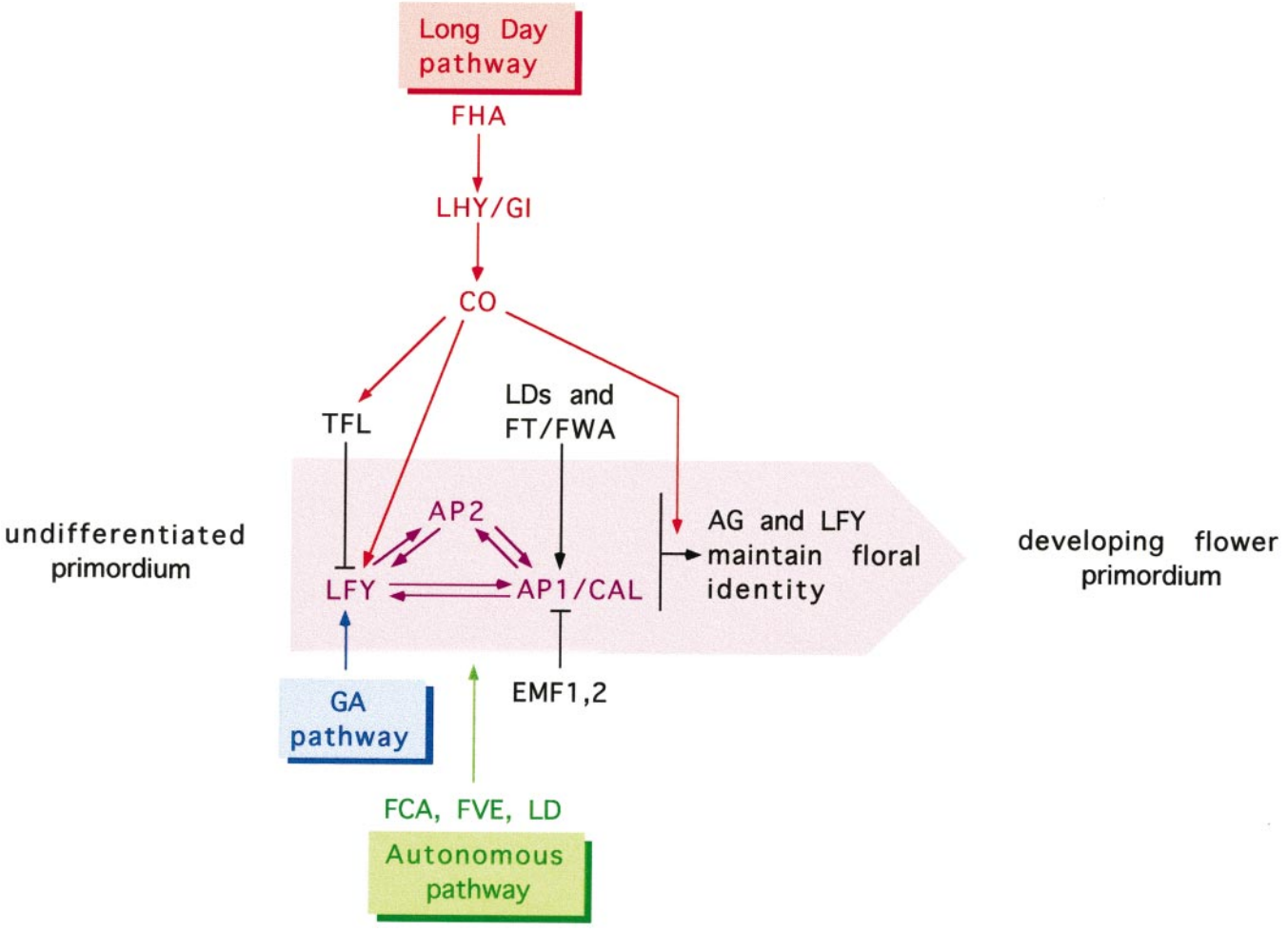

Figure 1. A summary of the genetic and molecular interactions influencing flowering time and floral meristem identity. The central arrow illustrates the process by which floral identity is conferred upon an undifferentiated primordium. The action of the floral meristem identity genes is promoted by flowering-time genes acting within LD conditons, the autonomous, and the GA pathways. *FHA* acts as a light receptor within the LD-promotive pathway and increases *CO* expression under LD conditions (Guo et al., 1998). *CO* acts in the LD-promotive pathway and up-regulates *TFL* and *LFY*, in addition to promoting the response to *LFY* expression (Simon et al., 1996). *TFL* negatively regulates floral meristem identity genes *LFY* and *AP1* in the shoot apical meristem and the inflorescence meristems (Bradley et al., 1997). GA acts to promote *LFY* expression (Blázquez et al., 1997). The autonomous pathway promotes flower development, but it is not yet clear where in the process this pathway acts. *FT* and *FWA* promote flower development by activating floral meristem identity genes other than *LFY* (Ruiz-García et al., 1997), and *AP1* expression responds to LD conditions independently of *CO* (Simon et al., 1996). *EMF* represses *AP1* expression in vegetative tissues (Chen et al., 1997). *LFY*, *AP2*, *CAL*, and *AP1* act additively and cooperatively to confer floral meristem identity (Bowman et al., 1993; Schultz and Haughn, 1993). *AG* and *LFY* maintain floral identity and prevent floral reversion (Okamuro et al., 1996; Mizukami and Ma, 1997).

transcript β did not restore early flowering in *fca* lines, the slightly early flowering phenotype of 35*S*::*FCA* lines may be due to the small increase in the abundance of transcript γ, the only product that encodes the putative full-length *FCA* protein (Macknight et al., 1997). This suggests that splicing of the *FCA* transcripts is regulated and that a factor required for the production of transcript γ limits flowering time.

Analyses of the *LD* and *FCA* genes suggest that both encode regulatory proteins: a transcription factor and an RNA-binding protein. However, how these factors act within the same genetic pathway to regulate flowering time is unclear, and probably requires the isolation of additional genes within the autonomous flowering pathway.

Four genes proposed to act in the LD-responsive pathway have been isolated. The first of these, *CONSTANS* (*CO*), was isolated using a map-based cloning strategy (Putterill et al., 1995). *CO* encodes a protein of 373 residues and contains two putative zinc finger domains reminiscent of those present in members of the GATA-1 family of transcription factors (Putterill et al., 1995). A putative nuclear localization domain is also found in the carboxy terminus of the protein. The levels of *CO* mRNA are extremely low in wild-type plants throughout the developmental stages analyzed, but are reproducibly higher in plants grown under LD rather than SD conditions. This is consistent with the *co* mutant phenotype,

which indicated that *CO* promotes flowering only under LD conditions, and suggests that the promotion of flowering is mediated by up-regulation of *CO* transcription. To examine the effect of regulated *CO* expression on flowering time, Simon et al. (1996) generated transgenic *co-2* plants that express a chimeric protein of CO fused to the rat GR under the control of the 35*S* promoter. The chimeric protein CO/GR is inactive in the absence of the steroid ligand, but the addition of the hormone dexamethasone restored the activity of the protein. 35*S*::CO:GR plants grown under both LD and SD conditions flower rapidly after treatment with dexamethasone, even if treated prior to wild-type flowering time (Simon et al., 1996). The earlier the dexamethasone is provided, the fewer leaves the induced plants produce, suggesting that plants can respond to CO activity at any time from germination until flowering.

Three other genes, *FHA*, *FT,* and *LATE ELONGATED HYPOCOTYL* (*LHY*), which are also involved in the LD-responsive pathway, have been isolated but not yet published in detail. The *FHA* gene is likely to encode a blue light receptor, because the predicted FHA protein is closely related in sequence to cryptochrome and was previously known as *CRY2* (Lin et al., 1996; Guo et al., 1998). Blue and far-red light have previously been shown to promote flowering in Arabidopsis (Brown and Klein, 1971), and the phenotype of the *fha* mutant suggests that blue light acts at least in part through the LD-responsive pathway. The *lhy* mutation is caused by overexpression of a MYB transcription factor closely related to *CCA1*; in addition to causing photoperiod-insensitive late flowering, it also disrupts circadian clock function (Carré, 1996; Coupland, 1997). A similar phenotype is caused by the overexpression of *CCA1* (Wang et al., 1997a, 1997b). This suggests a role for the circadian clock in controlling flowering in response to photoperiod, as had previously been suggested by the phenotype of the *early flowering 3* mutant, which causes both photoperiod-insensitive early flowering and disruption of circadian clock function under LD conditions or continuous light (Hicks et al., 1996; Table I). The *FT* gene is thought to encode a protein that is similar to phosphatidyl ethanolamine binding proteins and to the *TERMINAL FLOWER* gene (Araki et al., 1997; see below).

Molecular analysis of genes within the LD pathway suggests a speculative model for how the pathway acts to regulate flowering time. Physiological experiments suggest that the interaction of light signals with the circadian clock provides a timing mechanism that enables plants to distinguish between LD and SD conditions. The demonstration that *FHA* encodes a blue light receptor and *lhy* disrupts circadian clock function suggested that *FHA* and *LHY* might act within the LD pathway to enable the recognition of LDs. This might result in increased *CO* expression as is observed under LD conditions. The increase in *CO* expression probably results in rapid activation of genes involved in floral development because activation of *CO* in 35*S*::CO:GR plants leads to rapid activation of *LEAFY* (*LFY*) (Simon et al., 1996). Further expression analysis of floral development genes in mutant and transgenic backgrounds, together with the isolation of additional genes acting within the LD-responsive pathway, should test this model.

The proposition that genes affected in late-flowering mutants act within partially redundant pathways suggests that increasing the activity of one of these pathways might partially or entirely compensate for the loss of a parallel pathway. This was tested by introducing the *fca* mutation, which affects the autonomous pathway, into a 35*S*::*CO* background. 35*S*::*CO fca* plants flower slightly later than 35*S*::*CO* plants but much earlier than *fca*, indicating that increasing the activity of the LD-responsive pathway by overexpressing *CO* can compensate almost completely for the delay in flowering caused by the loss of function of the autonomous pathway. However, the slight delay in flowering time of 35*S*::*CO fca* compared with 35*S*::*CO* suggests that these pathways interact and that an intact autonomous pathway is required for the full effect of overexpression of the LD pathway (M.I. Igeño and G. Coupland, unpublished results).

FLORAL MERISTEM IDENTITY GENES

Mutations in floral meristem identity genes cause primordia that develop in the positions occupied by flowers to form organs with some of the characteristics of shoots (Table I). The best characterized of these genes are *LFY*, *APETALA1* (*AP1*), *APETALA2* (*AP2*), and *CAULIFLOWER* (*CAL*). All four genes probably encode transcription factors: *AP1* and *CAL* encode proteins in the MADS (MCM1-Agamous-Deficiens-SRF) box family and are closely related in sequence (Mandel et al., 1992; Kempin et al., 1994); *LFY* is a nuclear product able to bind DNA in vitro, although it is different from any other known transcription factor (Weigel, 1995a); and *AP2* encodes a protein carrying a novel DNA-binding motif with homology to ethylene-responsive element-binding proteins (Jofuku et al., 1994; Weigel, 1995b).

lfy has the most extreme effect of the floral meristem identity mutants. Strong *lfy* mutants form axillary shoots subtended by leaves at the positions occupied by the first flowers of wild-type plants, but later positions are less severely affected and ultimately form flower-like structures. However, these structures do not form petals or stamens and show helical phyllotaxy rather than the typical arrangement of whorls (Schultz and Haughn, 1991, 1993; Huala and Sussex, 1992; Weigel et al., 1992). Mutations in *AP1* also have a stronger effect on flowers that develop at early positions on the shoot. However, *ap1* mutant structures are less affected than the shoots formed in *lfy* mutants; *ap1* flowers are determinate, like those of the wild type, but form secondary flowers in the axils of the outer organs that develop within the primary flower. Therefore, the mature *ap1* flower has a complex, branched structure that contains several individual flowers (Irish and Sussex, 1990; Bowman et al., 1993).

The reduced requirement for *LFY* and *AP1* in later flowers is probably caused by other floral meristem identity genes compensating for their loss of function. For example, the redundancy of *LFY* and *AP1* is revealed in *lfy/ap1* double mutants, which show a more severe phenotype than either single mutant, with flower-like structures observed only very rarely (Huala and Sussex, 1992; Weigel et

al., 1992; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). Redundancy is also evident between *AP1* and *CAL*, so that mutations in the *CAL* gene do not cause a phenotype in otherwise wild-type plants but greatly enhance the effect of *ap1* mutations (Bowman et al., 1993). Also, the effect of *ap2* mutations on floral meristem identity was observed because *ap2* mutations enhance the phenotypes of *ap1* and *lfy* mutants (Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). This redundancy between the four floral meristem identity genes indicates that they have partially overlapping functions. Furthermore, mutations in some of these genes affect the expression of others; for example, in *ap1/cal* double mutants neither *LFY* nor *AP1* is expressed (Bowman et al., 1993). These observations led to the suggestion that in wild-type plants the four genes act collectively, enhancing each others' expression and acting additively on target genes to promote floral meristem identity (Fig. 1). This additive activity might enable plants to make a sharp transition between vegetative and reproductive development (Bowman et al., 1993; Schultz and Haughn, 1993).

In addition to the genes mentioned above, *AGAMOUS* (*AG*) has also been proposed to play a role in floral meristem identity. *AG* encodes a transcription factor of the MADS box family (Yanofsky et al., 1990) that specifies floral organ identity in the third and fourth whorls of the flower and is required for the floral meristem to become determinate after forming the four floral whorls. Furthermore, *AG* seems to have a role in maintaining floral meristem identity, because *ag* mutants grown under SD conditions as well as *ag/co-2* double mutants exhibit floral reversion (Table I). The indeterminate floral meristems of *ag/co-2* plants become transformed to inflorescence meristems and give rise to further floral meristems in a spiral phyllotaxy characteristic of shoots. This suggests that *AG* is required to maintain floral meristem identity during reproductive growth, even in the presence of *LFY* and *AP1* (Mizukami and Ma, 1997).

The redundancy and cooperation between floral meristem identity genes has made the roles of individual genes difficult to study. However, despite the interrelationships between them, ectopic and high-level expression of a single flower meristem identity gene can be sufficient to specify floral development. In Arabidopsis plants ectopically expressing *LFY* or *AP1*, lateral meristems that normally would be shoots are converted into axillary flowers (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). In addition, the shoot apical meristem of 35*S*::*LFY* and 35*S*::*AP1* plants is determinate, forming a terminal flower similar to that of *terminal flower* (*tfl*) mutants (see below). These results demonstrate that both *LFY* and *AP1* are sufficient to convert shoot meristems into flowers. Introduction of 35*S*::*LFY* into *ap1* mutants and 35*S*::*AP1* into *lfy* mutants suggests that *LFY* acts before *AP1* in conferring floral meristem identity (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). In addition to promoting the differentiation of shoots into flowers, overexpression of *LFY* and *AP1* causes early flowering under both LD and SD conditions.

GENES THAT DELAY FLOWERING AND INFLUENCE FLORAL MERISTEM IDENTITY GENE EXPRESSION

The *TFL* gene influences meristem identity, but it has the reverse effect of *LFY*: in *tfl* mutants the apical shoot meristem and axillary shoot meristems become converted to floral meristems in which the *LFY* and *AP1* genes are ectopically expressed (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Bowman et al., 1993). In addition, *tfl* mutants flower early, suggesting a role for *TFL* during vegetative development to influence the timing of the transition to flowering (Table I). The early flowering of *tfl* mutants seems to be the result of an earlier commitment to flowering, since *tfl* mutants are committed to flower after exposure to 5 d of LD conditions, whereas 7 d of LD conditions are required for the wild type (Bradley et al., 1997). The *TFL* gene was recently cloned and shown to encode a protein with similarity to animal phosphatidylethanolamine-binding proteins (Bradley et al., 1997). *TFL* is expressed in a group of cells lying just below the apical dome of the meristem. In wild-type plants *TFL* mRNA is detected from d 2 or 3, but expression is weak up to the point of commitment (d 7), after which it increases (Bradley et al., 1997).

Strong mutant alleles in *EMBRYONIC FLOWER 1* (*EMF1*) cause plants to initiate flowering without forming any rosette leaves (Table I). These mutants form reproductive structures such as stigmatic papillae and ovule-like structures on the surfaces of their cotyledons, and the shoot produces no rosette leaves but often forms carpelloid structures with features of ovules, and terminates in a pistil or flower (Sung et al., 1992; Castle and Sung, 1995). Weak mutant alleles of *emf1* and *emf2* form recognizable leaves, but they are small and sessile. A fusion of the *AP1* promoter to the GUS marker gene was used to monitor expression of *AP1* in *emf* mutants. *AP1* is ectopically expressed in the shoot meristem and leaves of plants carrying weak *emf* alleles, as well as in the shoot apex, hypocotyl, and cotyledons of plants carrying strong *emf* alleles (Chen et al., 1997). Therefore, the *EMF* genes appear to negatively regulate the transition from vegetative to reproductive development, and to negatively regulate the expression of *AP1* in vegetative tissue. The extreme early flowering of *emf* mutants led to the suggestion that *EMF* genes are central repressors of flowering with activities that decline during plant development; when their activity falls below a certain threshold, plants undergo the transition from rosette development to inflorescence development, and from inflorescence development to the formation of single flowers (Chen et al., 1997). Mutations that delay flowering (*co* and *gi*) have no effect on the *emf* phenotype in double mutants, which suggests that the role of these flowering-time genes is to repress the function of the EMF product, and that in the absence of *CO* or *GI*, flowering time is delayed by increased *EMF* function (Martínez-Zapater et al., 1994; Weigel et al., 1995a). Further analysis of the roles of *EMF1* and *EMF2* in the regulation of flowering awaits the isolation of the genes.

RELATIONSHIPS BETWEEN FLOWERING-TIME AND MERISTEM IDENTITY GENES

Flowering-time mutants display their major effects on the duration of vegetative development, whereas mutations in floral meristem identity genes disrupt floral development. Therefore, flowering-time genes are often assumed to act before floral meristem identity genes and, generally, to lead to their activation. The relationships between these two groups of genes have been studied genetically by making double mutants, and to a lesser extent at the molecular level by examining the effect of overexpression of flowering-time genes on meristem identity gene expression. A complex relationship between flowering-time genes and floral meristem identity genes is emerging from these studies.

In general, the effects of *lfy* or *ap1* mutations are enhanced by mutations or conditions that delay flowering. Both mutants possess a stronger phenotype under SD conditions than under LD conditions (Huala and Sussex, 1992; Schultz and Haughn, 1993). Also, the *lfy* mutation is completely recessive under LD conditions, but under SD conditions the heterozygote is impaired in the maintenance of floral meristem identity (Okamuro et al., 1996). This argues for a relationship between LD conditions that promote flowering and *LFY* activity. Furthermore, several mutations causing late flowering broadly enhance the effect of *lfy* or *ap1* (see below), again indicating a close relationship between genes that promote flowering and the action of floral meristem identity genes (Putterill et al., 1995; Ruiz-García et al., 1997).

The promotion of flowering by some treatments seems at least partially to act by causing an increase in the transcription of the *LFY* gene. For example, shifting SD-grown plants to LD conditions or spraying them with GA causes earlier flowering and a rapid increase in *LFY* expression (Blázquez et al., 1997). Similarly, by utilizing transgenic plants in which *CO* activity could be regulated, it has been shown that activation of *CO* causes expression of *LFY* just as rapidly as exposure to LD conditions, and therefore at least one function of the LD-responsive pathway is to activate *LFY* (Simon et al., 1996).

However, the role of the flowering-time genes cannot simply be to activate *LEAFY* expression. Strong expression of *LEAFY* from the cauliflower mosaic virus 35*S* promoter was insufficient to cause flower development without the formation of several vegetative nodes, suggesting that the shoot apical meristem must also become competent to respond to *LEAFY* expression (Weigel and Nilsson, 1995). The shoot meristem's ability to respond to *LEAFY* is also regulated by day length and flowering-time genes. 35*S*::*LFY* plants flower much later under SD conditions than under LD conditions, indicating that a factor required for *LFY* response is regulated by day length (Weigel and Nilsson, 1995). Activation of *CO* has been proposed to enable the shoot meristem to respond more rapidly to *LEAFY* expression since 35*S*::*CO* plants flower earlier than 35*S*::*LFY* plants, particularly under SD conditions (Simon et al., 1996). Exposure to LD conditions and the action of flowering-time genes may activate meristem identity genes that act cooperatively with *LEAFY* to confer floral identity on meristems (see below) or, alternatively, some flowering-time genes might act in the meristem to facilitate the action of the floral meristem identity genes. The homology of *FT* to *TFL* might suggest such a role for *FT*.

Genetic experiments suggest that some flowering-time genes do not act through *LFY* but through other floral meristem identity genes. Two of the late-flowering mutations (*fwa* and *ft*) show a more severe interaction with *lfy* than the others (Ruiz-García et al., 1997). For example, *co*, *fve*, and *fpa* mutations enhance the *lfy* phenotype, but the double mutants formed *lfy*-like flowers late in development (Putterill et al., 1995; Ruiz-García et al., 1997), whereas *fwa/lfy* or *ft/lfy* plants never form flower-like structures and show a phenotype even more severe than *lfy/ap1* double mutants. The *ft* and *fwa* mutations also enhance the *ap1* phenotype, but this enhancement is not as strong as that of *lfy*. On the basis of these interactions, Ruiz-García et al. (1997) proposed that *fwa* and *ft* do not act to promote flower development through *LFY* but through other floral meristem identity genes such as *AP1*. The severe phenotypes of *fwa/lfy* and *ft/lfy* plants can then be explained as the impairment of partially redundant floral meristem identity functions; one involves *LFY* and others may require *FWA* and *FT* to be activated (Ruiz-García et al., 1997).

Flowering-time genes are also likely to be involved in the increased expression of *TFL* that occurs around the time of commitment to flowering, as the activation of *CO* leads to increased expression of *TFL* (Simon et al., 1996; Bradley et al., 1997). Also, the effects of the *tfl* mutation are weakened by environmental conditions such as SD that delay the onset of flowering (Shannon and Meeks-Wagner, 1991). More recently, it was shown that at least some mutations that cause late flowering delay the determinate phenotype of *tfl* mutants, so that the double mutants form a terminal flower after producing more lateral flowers than produced in *tfl* mutants (Ray et al., 1996; Ruiz-García et al., 1997). The double mutants also flower with a similar number of leaves as the late-flowering parents, indicating that the genes affected in the late-flowering mutants are required for the early flowering seen in *tfl* mutants.

IMPORTANCE OF QUANTITATIVE REGULATION OF GENES INVOLVED IN FLOWERING

Expression of both *CO* and *LFY* are tightly regulated so that small changes in their activity affect flowering time or shoot morphology. The promotion of flowering by *CO* in response to LD conditions is probably regulated by transcriptional control of *CO*, because the gene is expressed at higher levels under LD conditions than under SD conditions. Furthermore, *CO* expression seems to be poised at a critical level in LD-grown seedlings: reducing the dosage of the gene in heterozygotes leads to a delay in flowering, whereas increasing *CO* dosage in transgenic plants carrying the wild-type *CO* gene causes an acceleration in flowering time. In addition, its overexpression in 35*S*::*CO* transgenic plants is sufficient to promote very early flowering under SD and LD conditions, and flowering of these plants

is insensitive to day length. Maintaining a balance in expression levels between different flowering-time genes might be important in enabling plants to flower in response to environmental conditions, so that increasing the dosage of the *CO* gene reduces the response to day length, and expression of *CO* from the 35*S* promoter abolishes environmental regulation of flowering time.

Quantitative regulation of *LFY* expression is also important for the proper regulation of flowering time and the node at which flowers are first formed. Under LD conditions *LFY* is expressed throughout plant development, even during the early stages of vegetative development, but its expression increases sharply around the time that flowering occurs (Blázquez et al., 1997). Under SD conditions *LFY* is expressed at initially low levels and increases gradually during the long period of vegetative growth. Increasing the dosage of *LFY* from two in wild-type plants to four in transgenic plants causes an acceleration in flowering time so that two fewer rosette leaves are formed, and the first flower is formed after the formation of two fewer cauline leaves (Blázquez et al., 1997). Tight regulation of *LEAFY* gene expression is therefore important in the regulation of flowering time and in defining shoot morphology.

PERSPECTIVES

The relationships between flowering time and floral meristem identity genes are complex and complicated by functional redundancy. Recently, the functions of individual genes have become clearer through the use of gain-of-function transgenes. Further genetic analyses with such transgenic plants should enable the function of single genes to be studied in the absence of redundant functions. For example, the inactivation of the autonomous and GA flowering-time pathways in a 35*S*::*CO* background should allow the function of the LD-responsive pathway to be studied in the absence of other pathways.

The study of flowering-time genes is also complicated by the lack of knowledge of the timing during plant development or the tissues in which they act. Recent analysis shows that plants are committed to flower within a week of sowing under LD conditions. This suggests that flowering-time genes act early in development. This hypothesis is supported by the phenotype conferred by conditional *gi* alleles, which indicate that *GI* acts 3 d after germination (Araki and Komeda, 1993). Also, the addition of dexamethasone to 35*S*::CO:GR plants at d 7 after sowing produces a phenotype very similar to that of the wild type, which is consistent with *CO* acting around d 7 (Simon et al., 1996).

The tissues in which the flowering-time genes are required to activate flowering have not been studied extensively in Arabidopsis. Grafting experiments with pea have distinguished between genes that act in the leaf and those that act in the meristem (Weller et al., 1997). In maize, reversion of a transposon-induced allele of the flowering-time gene *INDETERMINATE* (*ID*) suggests that it acts in the leaf (Colasanti and Sundaresan, 1997). The expression patterns of the *FCA*, *LD*, and *CO* genes seem to be fairly general, encompassing both the shoot meristem and the leaves. In the case of *FCA*, homozygous mutant sectors were made in a heterozygous background and suggest that the gene product acts non-cell-autonomously to influence flowering time (Furner et al., 1996). Further data on the time of action of these genes and the tissues in which they are required will allow the relationship between the function of flowering-time genes and that of floral meristem identity genes to be established more accurately.

Finally, genetic evidence for the existence of redundant flowering-time pathways is strong and consistent, and molecular relationships between gene products in the same pathway are starting to emerge. For example, *FHA* and *CO* are both in the LD pathway, since *CO* is expressed at lower levels in an *fha* mutant (Guo et al., 1998). The use of gain-of-function transgenes and additional demonstrations that mutations in certain genes influence the activity of other genes in the same pathway should help to determine the order in which flowering-time genes act. How the LD, autonomous, and GA pathways act additively to regulate flowering time also awaits the isolation of common targets for these pathways, and a better understanding of how they interact with the floral meristem identity genes.

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