The Control of Flowering Time and Floral Identity in Arabidopsis

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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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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 that have been proposed to represent two of the genetic 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 terminus 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 35S promoter results in a large increase in transcript β accumulation, with smaller increases in transcripts γ and δ. Since the overexpression of

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<tr>
<th>Mutant</th>
<th>Phenotype</th>
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<tr>
<td>FCA</td>
<td>Late flowering; no response to vernalization</td>
<td>Macknight et al. (1997)</td>
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<td>LD</td>
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<td>Late flowering; no response to vernalization</td>
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<td>ga1</td>
<td>Late flowering</td>
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<td>Floral repressor</td>
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<td>efl3</td>
<td>Early flowering; disruption of circadian rhythm</td>
<td>Hicks et al. (1996)</td>
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<td>eml1, eml2</td>
<td>Early flowering</td>
<td>Castle and Sung (1995)</td>
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<td>till</td>
<td>Early flowering; determinate inflorescence</td>
<td>Bradley et al. (1997)</td>
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<td>lhy</td>
<td>Partial transformation of flowers into inflorescence</td>
<td>Weigel et al. (1992)</td>
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<td>ap1</td>
<td>Partial transformation of flowers into inflorescence</td>
<td>Irish and Sussex (1990)</td>
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<td>ap2</td>
<td>Partial transformation of flowers into inflorescence</td>
<td>Jofuku et al. (1994)</td>
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<td>ap1 cal</td>
<td>Partial transformation of flowers into inflorescence</td>
<td>Bowman et al. (1993)</td>
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<td>ag</td>
<td>Floral indeterminate growth; floral reversion (SD)</td>
<td>Mizukami and Ma (1997)</td>
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transcript β did not restore early flowering in fca lines, the slightly early flowering phenotype of 35S::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.

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 conditions, 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 API 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 API expression responds to LD conditions independently of CO (Simon et al., 1996). EMF represses API expression in vegetative tissues (Chen et al., 1997). LFY, AP2, CAL, and API 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).
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 plants that express a chimeric protein of CO fused to the rat GR under the control of the 35S 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. 35S: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, FBA, 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 FBA gene is likely to encode a blue light receptor, because the predicted FBA 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 fba 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 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 fba 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 FBA encodes a blue light receptor and lhy disrupts circadian clock function suggested that FBA 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 35S: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 35S:CO background. 35S:CO fca plants flower slightly later than 35S: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 35S:CO fca compared with 35S: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. Igeno 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
addition, the shoot apical meristem of 35S::AP1 and 35S::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 floral 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 35S::LFY and 35S::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 35S::LFY into ap1 mutants and 35S::API 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 phosphatidyl-ethanolamine-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 carpeloid 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 35S 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. 35S::$LFY$ 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 35S::$CO$ plants flower earlier than 35S::$LFY$ 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 $fl$) 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 $fl/lfy$ 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 $fl$ 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 $fwa$ 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 $ftl$ mutants, so that the double mutants form a terminal flower after producing more lateral flowers than produced in $fwa/lfy$ or $fl/lfy$ plants (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 $fwa$ 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 35S::$CO$ 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 3SS 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 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 35S: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 as d after germination (Araki and Komeda, 1993). Also, the addition of dexamethasone to 35S: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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