

WHEN TO SWITCH TO FLOWERING

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■ **Abstract** At a certain stage in their life cycle, plants switch from vegetative to reproductive development. This transition is regulated by multiple developmental and environmental cues. These ensure that the plant switches to flowering at a time when sufficient internal resources have been accumulated and the environmental conditions are favorable. The use of a molecular genetic approach in Arabidopsis has resulted in the identification and cloning of many of the genes involved in regulating floral transition. The current view on the molecular function of these genes, their division into different genetic pathways, and how the pathways interact in a complex regulatory network are summarized.

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INTRODUCTION

The timing of the transition to flowering is determined by the interaction of the endogenous developmental competence of a plant with environmental cues that signal the onset of conditions favorable for reproductive success. Recent progress in the dissection of these diverse influences and the molecular events that underpin them has been primarily achieved through a molecular genetic approach in *Arabidopsis*. Because the flowering behavior of *Arabidopsis* mirrors that of many other plants, it is likely to be a model of broad utility. Many plants respond to changes in daylength and extended periods of cold temperature, since they are both predictable and reliable indicators of seasonal progression. *Arabidopsis* flowering is accelerated as the daylength increases—an environmental condition that signals the onset of spring and summer in the higher latitudes. This process is known as the photoperiod response. *Arabidopsis* flowering is also accelerated following an extensive period of cold treatment, an environmental condition that signals the passage of winter and the onset of spring. This process is known as the vernalization response. In addition, flowering in *Arabidopsis* is stress responsive; *Arabidopsis* exhibits precocious flowering that enables life-cycle completion under conditions that are unfavorable to survival of the parent. For example, light quality changes that accompany shading by near neighbors (an enrichment in the far-red wavelengths) will promote flowering.

Arabidopsis is an excellent experimental model for a flowering plant: It is of small size, has a short life cycle and a small genome, is amenable to transformation and mutation, exhibits prolific seed production, and significantly, the sequence of its entire genome is close to completion (Meinke et al 1998). Outside of the laboratory, natural *Arabidopsis* populations (ecotypes) adapted to survival in diverse climates provide additional sources of allelic variation to study the regulation of flowering time and enable the investigation of the adaptive mechanisms that have resulted in ecotypes with different reproductive strategies.

This review mainly focuses on the use of molecular genetics to analyze the control of flowering time in *Arabidopsis*. The dissection of the flowering process will add much to our knowledge of the fundamental principles of plant development and will underpin the strategic manipulation of flowering time in a range of crops.

Floral Transition—Some Essentials

Of central importance in the floral transition is a group of stem cells called the shoot apical meristem (SAM) (Figure 1). In plants, organogenesis continues throughout

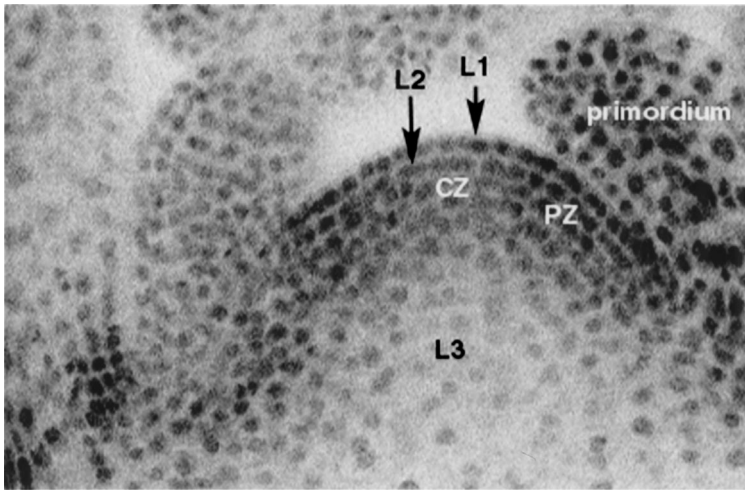


Figure 1 Arabidopsis shoot apical meristem. A single confocal section of a propidium iodide-stained shoot apical meristem illustrating the cellular organization into layers L1, L2, and L3; as well as zones, central zone (CZ) and peripheral zone (PZ), with primordia emerging from the meristem flanks.

development, and the SAM serves as a population of progenitor cells for the aerial parts of the plant: the leaves, stem, and flowers. Changes in organogenesis occur through phase transitions at the meristem, resulting in a change in meristem identity. The SAM is formed in embryogenesis, following which, leaf primordia initiate from the flanks of the vegetative meristem. The vegetative phase is characterized by a juvenile and then a competent adult stage prior to the floral transition. Once the floral transition occurs, floral primordia initiate from the flanks of the SAM and floral organs develop.

Environmental conditions play a major role in the timing of the floral transition. Daylength is perceived in the leaves (first reviewed by Lang 1965); thus, the signal from this environmental cue must be translocated a considerable distance to the SAM. Vernalization, on the other hand, is perceived at the apex, but it is a quantitative response (Napp-Zinn 1987) so there must be a system to measure the length of the cold treatment. Thus an essential feature of the control of flowering is the measurement and transfer of signals to the meristem and the integration of information from the multiple environmental conditions with the endogenous cues. Multiple pathways form a network of floral promotion and repression activities that are integrated in order to initiate the commitment to flower. Genetic and expression analysis indicate that genetic redundancy and quantitative regulation of gene expression are important features of this network.

The change from a vegetative to a floral meristem is executed by floral meristem identity genes and is followed by pattern formation and organogenesis carried out by floral homeotic genes (Weigel & Meyerowitz 1994).

THE SHOOT APICAL MERISTEM AND PHASE TRANSITION

The SAM of higher plants consists of a small number of morphologically undifferentiated dividing cells laid out in an organized manner (Figure 1; reviewed in Evans & Barton 1997). Cells at the summit of the SAM form the central zone (approximately four to six cells wide), which is the source of cells for the peripheral zone, where organ primordia initiate, and for the underlying rib zone, which gives rise to the pith of the stem. Cell division within the central zone is slow and serves to maintain the meristem, while in the peripheral and rib zones, division is rapid. In defined locations of the peripheral zone, the plane of cell divisions is changed, enabling the formation of differentiated primordia. Superimposed on these zones is a partitioning into layers, which in *Arabidopsis* consists of L1, L2, and L3. L1 and L2 remain distinct cell layers as a consequence of the regular anticlinal orientation of their cell divisions, but in the underlying L3 layer, divisions are less ordered (Figure 1). In a mature flower, the epidermis is derived from L1, the subepidermal layers from L2, and the central cells derive from L3. Flower formation and meristem maintenance therefore require coordinated proliferation of cells in all three layers (Laufs et al 1998, Meyerowitz 1997).

Vegetative and Reproductive Phase Change

After its initiation during embryogenesis, the SAM begins a maturation pathway that starts in the juvenile vegetative phase. This is characterized by the production of primordia that develop into leaves with a pattern of differentiation distinct from those produced in the subsequent adult phase (Figure 2). Visual markers of this transition in *Arabidopsis* have been described (Telfer et al 1997): Juvenile leaves produce epidermal hairs (trichomes) only on the upper (adaxial) surfaces, whereas adult leaves produce trichomes on both their upper and lower surfaces. The importance of the juvenile-to-adult vegetative transition to flowering time is that normally, only the adult vegetative meristem is competent to respond to floral induction (Telfer & Poethig 1998, Weigel & Nilsson 1995), and thus regulating this transition affects the following reproductive transition. The early-flowering phenotype of the *Arabidopsis hasty* mutant is the result of a faster transition through the juvenile-to-adult vegetative phase. The vegetative-to-floral transition, however, is unchanged relative to wild-type. *HASTY* promotes a juvenile pattern of vegetative development and inhibits flowering by reducing the competence of the SAM to respond to floral meristem identity genes (Telfer & Poethig 1998). Several late-flowering mutations delay abaxial trichome production (Telfer et al 1997), demonstrating that they function in both the vegetative and reproductive maturation of the shoot. Vegetative phase change and reproductive development are coordinated in *Arabidopsis* and many other plants. However, the mechanism by which they are linked has not yet been established (Telfer et al 1997).

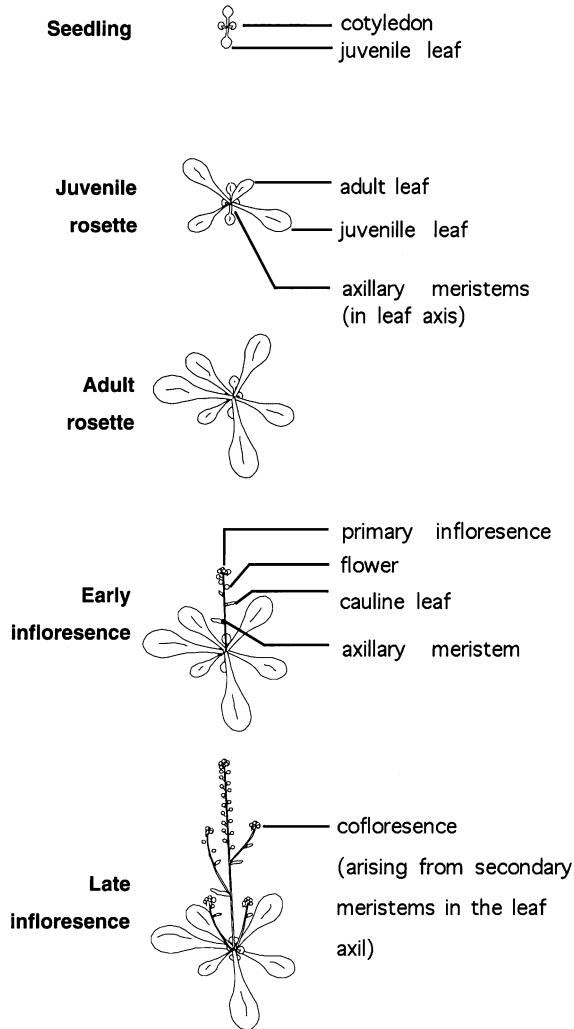


Figure 2 Phase changes during *Arabidopsis* development, as discussed in the text. The cotyledons and the first leaves are preformed in the embryo (seed). Between three and six juvenile leaves are produced, depending on ecotype. Juvenile leaves are distinguished by the presence of trichomes exclusively on the upper adaxial surface. Adult leaves are characterized by the presence of trichomes on both surfaces of the leaf. Following the transition to flowering, a primary inflorescence is produced. Early inflorescence primordia produce cophlorescences subtended by cauline leaves. Late inflorescence primordia give rise exclusively to flowers. Following the transition to flowering, apical dominance decreases, allowing the secondary inflorescences (from the meristems in the axils of the rosette leaves) to develop.

Determinacy of the Apex

The *Arabidopsis* SAM never develops into a flower itself but remains indeterminate, forming flowers in a spiral phyllotaxy until the apex eventually senesces. However, two mutations, *tfl1* and *tfl2*, both result in early flowering and differentiation of the apex into a terminal flower, indicating that the wild-type function of these genes is to delay flowering and suppress flower formation (Shannon & Meeks-Wagner 1991, Sundas Larsson et al 1998). The *tfl1* apical meristem progresses more rapidly through phase transitions such that it enters a terminal phase with a floral identity that is not normally attained before senescence occurs in wild-type plants (Ratcliffe et al 1998). *TFL1* functions to indirectly delay the upregulation of the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*API*), ensuring that the terminal phase is not entered prior to senescence (Ratcliffe et al 1998). The expression of *TFL1* therefore has profound consequences for the life cycle and overall architecture of a plant, modifying its degree of branching and determinacy. *TFL1* was cloned on the basis of its similarity to its *Antirrhinum* orthologue, *CENTRORADIALIS* (Bradley et al 1997), and by T-DNA tagging (Ohshima et al 1997). It encodes a protein showing similarities to phosphatidylethanolamine-binding proteins, which associate with membrane protein complexes and may therefore function in signaling pathways.

Reversion of Phase Transitions

Transient exposure of *Arabidopsis* plants to a sufficient number of long days (LDs) followed by transfer to short days (SDs) will cause them to become irreversibly committed to flowering even though no visible sign of floral development was apparent when they were transferred (Bradley et al 1997). However, in some species and genotypes, under certain environmental conditions, shoot-like structures are formed after flowers in a phenomenon known as floral reversion (Pouteau et al 1997). This observation implies that the genes and processes involved in the transition to flowering are required to both initiate and maintain reproductive development. The genes *LFY* and *AGAMOUS* (*AG*) and the phytochrome and gibberellin signal transduction pathways have been shown to be important in the maintenance of the floral state in *Arabidopsis* (Okamoto et al 1996).

Primordia Identity

The investigation of the floral transition under strongly inductive conditions has begun to shed light on how the process is manifest at the meristem. *Arabidopsis* is induced to flower in LDs, but will flower eventually in SDs. If grown in SD conditions and then transferred to LDs, *Arabidopsis* will flower rapidly with the immediate conversion of the primordia from leaf to flower formation (Hempel & Feldman 1994). Under such conditions, chimeric organs that show characteristics of both leaves and flowers develop at the last node formed prior to the induction of flowering (Hempel & Feldman 1995). This indicates that the floral stimulus acts

directly on the primordium to confer floral identity and that primordia previously destined to become a leaf can change fate and form a flower (Hempel et al 1998).

MULTIPLE PATHWAYS REGULATING THE FLORAL TRANSITION

Genes that affect flowering time in *Arabidopsis* have been identified through two complementary strategies. The first has utilized the variation present in naturally occurring ecotypes of *Arabidopsis* that vary in flowering time. The second approach has utilized a diverse spectrum of induced mutations that result in either early or late flowering. A summary of genes considered to play a role in flowering time control, identified through both approaches, has been recently compiled (Levy & Dean 1998). Taken together, there are currently about 80 genes and loci in *Arabidopsis* known to affect flowering time.

Most of the genes identified by mutagenesis are derived from three rapid-cycling laboratory progenitor ecotypes: Landsberg *erecta* (*Ler*), Wassilewskija (*WS*), and Columbia (*Col*). The analysis of flowering time variation in the naturally late-flowering ecotypes complements the mutagenic approach in the early-flowering ecotypes, particularly in identifying repressors of the floral transition. A number of genes—*FRIGIDA* (*FRI*), *FLC*, *FKR*, *JUVENALIS* (*JUV*), and *KRYOPHILA* (*KRY*)—and quantitative trait loci (QTLs) that are not represented in the mutant collections have been identified by this approach (for a review, see Koornneef et al 1998b). QTLs have also been identified that distinguish an ecotype growing near the equator, in relatively day-neutral conditions (Cape Verde islands) (Alonso-Blanco et al 1998). The differences in the spectrum of genes identified by these two approaches reveals some of the complexity of flowering time and highlights the value of exploiting natural variation. In particular, there are complex interactions between flowering time genes that could not have been predicted without the use of different ecotypes (see *FLC* below).

The study of how flowering time mutants respond to environmental treatments, such as vernalization and photoperiod (Figure 3), combined with the genetic analysis of epistasis, has established the existence of multiple pathways that control flowering time in *Arabidopsis* (Alonso-Blanco et al 1998, Koornneef et al 1998a,b). Two of these pathways clearly mediate signals from the environment. The photoperiod promotion pathway integrates daylength into the flowering decision through a series of genes that sense and respond to the regular day-to-night transition. The vernalization promotion pathway promotes flowering in many late-flowering ecotypes in response to an extended period of cold temperature. This is not simply a stress response but an adaptation to the winter conditions of colder climates to ensure that flowering occurs only after winter, with flower and seed formation occurring in spring. In contrast to these environmentally responsive pathways, there is a collection of genes that promote flowering in a manner independent of environmental signals. Plants carrying mutations in these genes flower late in both



Figure 3 Arabidopsis plants flowering at different times. A typical winter annual Arabidopsis ecotype that has (*right-hand plant*) or has not (*left-hand plant*) been vernalized (6 weeks at 4°C).

LDs and SDs, and these genes have been formed into a separate class known as the autonomous promotion pathway. Members of this epistatic class probably monitor or respond to endogenous cues such as an internal developmental clock.

The phytohormone gibberellic acid (GA) affects multiple aspects of plant development. In Arabidopsis, mutants defective in GA biosynthesis and signal transduction pathways flower early or late. The analysis of the epistatic relationships between GA mutants and other flowering time mutants suggests that they form a promotive pathway distinct from the others. In addition to these established pathways, we have separated another group of late-flowering mutants into a distinct section and refer to them as the *FT* subgroup. Members of this pathway flower late in LDs but show properties that distinguish them from other members of the photoperiod pathway. They may represent a branch of the photoperiod promotion pathway or may be something more distinct. Finally, the fact that so many early-flowering mutants have been identified indicates that the floral transition is normally actively repressed. Multiple genes encoding repressors of the floral transition have been identified, some of which oppose the activity of specific promotive pathways.

Genes Repressing the Floral Transition

The grouping of several Arabidopsis genes together under the heading of repressors is intended to convey only a broad sense of their activity. What unites them as

repressors is their function in repressing the transition to flowering (often independently of environmental signals) and not their action in a unique pathway. Because many of these repressors appear to have functions at other points during development, it is likely that some have a variety of roles, with the repression of flowering being only a component of their spectrum of normal activity. Some repressors can be clearly placed into one of the other pathways, e.g. *LHY*, *CCA1*, and *ELF3* act as repressors that appear to be components of the photoperiod promotion pathway, (Hicks et al 1996, Schaffer et al 1998, Wang & Tobin 1998), whereas for others the distinction is far from clear and will require a detailed molecular analysis to unravel.

Analysis of the natural variation in flowering time in *Arabidopsis* has revealed that the early ecotypes, including *Ler* and *Col*, can be considered as mutants lacking the strong repressor(s) of the floral transition. Crosses between a number of winter (late-flowering, vernalization-responsive) and spring (early-flowering, vernalization-nonresponsive) *Arabidopsis* ecotypes revealed that late flowering and a requirement for vernalization segregated as a dominant monogenic trait, which mapped to the *FRI* locus (Burn et al 1993b, Clarke & Dean 1994, Lee et al 1993, Sanda et al 1997). Dominant alleles at a second locus, *FLC*, interact synergistically with dominant alleles of *FRI* to produce plants that are extremely late flowering (Aukerman & Amasino 1996, Koornneef et al 1994, Lee et al 1994b). The effectiveness of *FRI* and *FLC* in different ecotypes is distinct, and they have been classified as weak or strong alleles on the basis of genetic interactions with each other and with *ld* (Koornneef et al 1994, Lee et al 1994). Although the recent cloning of *FRI* confirms that the weak *Ler* and *Col* *FRI* alleles are loss-of-function alleles (U Johanson & C Dean, unpublished results), the molecular basis of allelic variation of *FLC* is unclear. *FLC* encodes a MADS-box-type transcription factor (Michaels & Amasino 1999, Sheldon et al 1999). Genetic analysis indicates that the C24 ecotype carries a weak *FLC* allele. However, modest upregulation of mRNA from this gene is sufficient to cause late flowering, demonstrating that the allele is not inactive (Sheldon et al 1999). Indeed the deduced amino acid sequence of C24 *FLC* is identical to that of the strong *Col* *FLC* allele (Sheldon et al 1999). Most late-flowering mutants have been isolated in the *Ler* background, and genetic evidence suggests that *Ler* bears a weak *FLC* allele (Koornneef et al 1994, Lee et al 1994). However, the expression pattern and transcript size of *Ler* resembles that of strong *FLC* alleles (Sheldon et al 1999). In view of the molecular relatedness of C24 and *Col* *FLC*, it is possible that the *Ler* *FLC* allele, although less effective, may have some activity that accounts for the late-flowering phenotype of certain *Ler* mutants (see below).

FLC mRNA accumulates in the presence of an active *FRI* allele (Michaels & Amasino 1999, Sheldon et al 1999). When an active *FRI* allele was introgressed into a line bearing an active *FLC* allele, flowering time was delayed (Lee et al 1994b). However, when *FLC* in this introgressed line was disrupted and demonstrated to be null, flowering time was not delayed (Michaels & Amasino 1999). These observations can be explained most simply by stating that *FRI* promotes

FLC mRNA accumulation and that *FLC* but not *FRI* can directly repress the floral transition. These findings provide a molecular explanation for the results of previous genetic experiments in which *FRI* was shown to enhance the late-flowering phenotype of *FLC*. The level of *FLC* mRNA is downregulated by vernalization (Michaels & Amasino 1999, Sheldon et al 1999) even in lines bearing loss-of-function *FRI* alleles.

Allelic variation at *FRI* and *FLC* may have allowed for the spread of *Arabidopsis* from latitudes where the vernalization requirement is easily satisfied by relatively long winters to regions with milder winters that do not receive a sufficiently long or cold period. Interestingly, orthologues of *FRI* and *FLC* are likely to be important in the control of flowering in other species, as the two major QTLs conferring a vernalization requirement in *Brassica* species cosegregate with markers linked to *FRI* and *FLC* (Osborn et al 1997).

The identification of loss-of-function mutations that accelerate flowering in rapid-cycling ecotypes such as *Ler* reveals that even in these early-flowering ecotypes, flowering is ordinarily actively repressed. The products of the corresponding wild-type genes are therefore thought to act in the repression of flowering. However, some genes appear to have pleiotropic effects and may affect other aspects of the flowering network and even processes quite distinct from flowering. Most early-flowering mutants have been categorized by their response to photoperiod; many (e.g. *clf*, *elf1*, *elf2*, *elg*, *esd4*, *pef1*, *pef2*, *pef3*, *phyB*, *speedy*, *tf11*, *tf12*, *wlc*) retain some response to photoperiod, whereas others (*elf3*, *emf1*, *emf2*, *pif*) do not.

The *EMF* genes appear to play a major role in the repression of flowering because both *emf1* and *emf2* mutants flower with essentially no preceding vegetative phase (Sung et al 1992, Yang et al 1995). Upon germination, *emf* mutants immediately enter the reproductive phase, with no juvenile or adult vegetative phase (Sung et al 1992). This suggests that the wild-type *EMF* genes act as strong repressors of flowering in *Arabidopsis*. How the various promotive pathways override or bypass this strong repressive effect is unknown, but there appear to be distinctions between the different pathways on the basis of their interactions with *emf* mutants (Huang & Yang 1998, Yang et al 1995). *EMF* genes may mediate the repression of flowering via their interactions with certain floral meristem identity genes (Chen et al 1997).

Several genes with repressive functions have as yet not been assigned clear roles, but hints of their function are emerging. For example, both *CURLY LEAF (CLF)* and *WLC* act to delay flowering by repressing certain floral meristem identity genes. However, it is not clear whether this repression is a direct effect on the activity of these genes or if this repression feeds through another pathway. The *CLF* gene shares sequence homology with the *Drosophila* polycomb group of genes, which are involved in maintaining the repression of homeotic genes (Goodrich et al 1997). The *wlc* mutant displays hypomethylation of repetitive sequences associated with the centromeres (C Hutchison & C Dean, unpublished results). This suggests that the early-flowering phenotype of *wlc* mutants may be the result of alleviation of repression of a subset of floral meristem identity genes, mediated

by changes in methylation status. In support of this notion is the observation that induced hypomethylation, resulting from constitutive expression of an antisense methyltransferase gene, resulted in the ectopic expression of *AG* and *APETALA3* (*AP3*) and early flowering (Finnegan et al 1996).

Methylation also appears to play a role in the regulation of flowering time by the *FWA* gene. Working with the *ddm1* mutant, which has decreased DNA methylation but unaltered methyltransferase activity (Richards 1997), Kakutani et al (1996) noted late flowering as a frequently appearing phenotype in repeatedly self-pollinated *ddm1* lines. *FTS*, the dominant locus conferring this late-flowering phenotype, was genetically mapped (Kakutani 1997) and localized close to *FWA*, which was previously characterized as a dominant mutation conferring late flowering (Koornneef et al 1991). Subsequent analysis of the methylation status of the genomic region surrounding the *FWA* locus in *ddm1* and in EMS-induced *fwa* alleles showed the region to be hypomethylated (Koornneef et al 1998b). The finding of hypermethylated sites in the *SUPERMAN* (*SUP*) gene in a genetic background showing general hypomethylation means it is difficult to predict whether *FWA* expression will be up- or downregulated in the *fwa* mutant (Jacobsen & Meyerowitz 1997).

Genes Promoting the Floral Transition—the Autonomous Promotion Pathway

The genes *fca*, *fy*, *fpa*, *fve*, *ld*, and *fld* fall into one distinct group; they all flower late under both LDs and SDs, and the late-flowering phenotype can be suppressed by either a vernalization treatment or light conditions with a low red to far-red ratio. These genes function in the autonomous pathway, which in the early ecotypes is functionally redundant with the vernalization pathway. The severity of the late-flowering phenotype of these mutants is affected by the genetic background in which they were isolated: *ld* (Lee et al 1994a) and *fld* (Sanda & Amasino 1996a) appear only late in a background bearing a strong *FLC* allele.

The interrelationship of the genes in this group has been addressed by Koornneef et al (1998a) in an all-combination double-mutant analysis. The results are consistent with the idea that instead of forming a linear pathway, the genes fall into parallel subclasses; the first includes *fca* and *fy*, a second includes *fve* and *fpa*. Combining *fy* and *fpa* is apparently lethal, suggesting that they carry out a redundant but essential function distinct from the regulation of flowering time (Koornneef et al 1998a). Notably, both *fpa* (Telfer et al 1997) and *fy* (S Poethig, personal communication) are particularly delayed in the juvenile-to-vegetative phase transition. The double-mutant data also reveal that *FY* does not need *FCA* for the essential function it performs in *fpa* (because the *fpa*, *fca* double mutant is not lethal), and thus in aspects of development distinct from the floral transition, these genes are genetically separable. Interestingly, *gi*, a mutant in the photoperiod promotion pathway, is epistatic with *fpa*, indicating that the autonomous promotion pathway might interact with the photoperiod promotion pathway (Koornneef

et al 1998a). The late-flowering phenotype of *fca* and *fpa* can be suppressed by vernalization, but suppression of the *fca*, *fpa* double mutant is incomplete (Koorneef et al 1998a). One interpretation of this result is that *FCA* and *FPA* perform redundant roles in the vernalization process; an alternative explanation is that in the double mutant, the lateness conferred is so significant that a more extensive vernalization period is required to suppress it.

The gene encoding *FCA* has been cloned (Macknight et al 1997). It encodes a protein that bears two copies of a well-characterized RNA-binding domain, the RNP motif (Burd & Dreyfuss 1994), and *FCA* has been shown to bind RNA in vitro (Macknight et al 1997). *FCA* also contains a protein-protein interaction domain called the WW domain, which is found in a wide variety of proteins (Sudol 1996). Intriguingly, *FPA*, which was also recently cloned, encodes a protein bearing RNP motifs (R Amasino, personal communication). The presence of RNA-binding domains in these two proteins raises the possibility that post-transcriptional events figure largely in the autonomous promotion pathway. However, RNAs and RNA-binding proteins play rare but well-documented roles in transcriptional regulation (Emerman & Malim 1998, Lucchesi 1998, Lang et al 1999), and therefore such a role for these proteins in the regulation of flowering time cannot be ruled out at this stage. It is also possible that the association of these proteins with RNA is related to sub cellular targeting (Yamashita et al 1998).

The gene encoding LUMINDEPENDENS (*LD*) has also been cloned (Lee et al 1994a). The protein contains a homeodomain at its N terminus and two candidate bipartite nuclear localization signals, and *LD* is targeted to nuclei (Aukerman et al 1999). The protein is otherwise serine-rich, has a repeated motif of unknown function, and has a candidate NTP-binding site. It also has a sequence that matches the consensus derived for the prototypical HIV Rev leucine-rich nuclear export signal (NES) (Lee & Silver 1997). Notably, a severely late allele, *ld-2*, bears a three-amino acid in-frame deletion within this sequence (Lee et al 1994a). Because the consensus for this NES motif is quite loose and other homologies are apparent in this region, its match in *LD* may be coincidental, but in view of the severity of the phenotype associated with this disruption, further studies are warranted. Unusually, the *LD* gene bears a minor-class U12-type intron (Burge et al 1998). It seems reasonable to think of *LD* as a DNA-binding transcription factor because it contains a homeodomain. However, given the fact that at least two other members of this epistatic group encode RNA-binding proteins, it is worth noting that some homeodomain proteins function in RNA processing by binding RNA directly through their homeodomain-encoding regions (Dubnau & Struhl 1996, Lucas et al 1995, Rivera-Pomar et al 1996, Zamore & Lehmann 1996). Because *ld* is late only in a background bearing a strong *FLC* allele (Lee et al 1994b), its interaction with other members of the autonomous class has yet to be determined.

The expression analysis of both *FCA* (Macknight et al 1997) and *LD* (Aukerman & Amasino 1996, Aukerman et al 1999) reveals a pattern that is not restricted to the SAM around the time of floral induction. This might suggest that these proteins have functions in plant development more general than the exclusive regulation of

flowering time. *LD* is expressed primarily in apical proliferative regions of the shoot and root, including the SAM and leaf primordia (Aukerman et al 1999). Sector analysis has shown that *FCA* is not required in the SAM L2 and L3 in order to produce a phenotypically normal plant, demonstrating that *FCA* or its downstream targets can function in a non-cell autonomous manner (Furner et al 1996).

The flowering time behavior of the floral repressors *FRI* and *FLC* resembles the properties of the late-flowering mutants of the autonomous promotion pathway in that they cause later flowering in LDs and SDs that can be overcome by vernalization or low red/far-red light treatments. The molecular characterization of *FRI* (U Johanson & C Dean, unpublished results) and *FLC* (Michaels & Amasino 1999, Sheldon et al 1999) has clarified previous ambiguity surrounding their genetic interactions: *FLC* is a repressor of the floral transition and is itself repressed by the autonomous promotion pathway (see below).

FLC mRNA accumulates in mutants *fca*, *fpa*, *ld*, and *fve* of the autonomous promotion pathway (Michaels & Amasino 1999, Sheldon et al 1999), which suggests that the normal function of these genes is to stably repress *FLC*. The fact that *ld* appears late only in backgrounds bearing a strong *FLC* allele could be explained in quantitative terms: *LD* may function as an enhancer of the autonomous promotion pathway. In a background bearing high *FLC* activity this enhancement of the autonomous promotion pathway's ability to suppress *FLC*-dependent late flowering may be essential. However, in a background bearing a weak *FLC* allele, the activity of the autonomous promotion pathway in the absence of *LD*-dependent enhancement may be sufficient to repress the relatively weak *FLC*-dependent late-flowering phenotype. Alternatively, *LD* may function only in the repression of *FLC*, whereas other members of the pathway may carry out additional functions in the regulation of flowering time. The fact that *FLC* can be upregulated in *Ler* in the presence of a loss-of-function *FRI* allele suggests that the repression of *FLC* by the autonomous promotion pathway is mediated directly by targeting *FLC*, as opposed to operating indirectly through the inactivation of *FRI* (Sheldon et al 1999).

Mutations in the autonomous promotion pathway are epistatic to *tfl1* (Ruiz-Garcia et al 1997). In addition, the late-flowering phenotype of plants expressing *TFL1* with the relatively powerful and constitutive cauliflower mosaic virus 35S RNA promoter (CaMV35S) can be suppressed by a vernalization treatment or by overexpression of *FCA* (Ratcliffe et al 1998; O Ratcliffe, personal communication). These properties are consistent with a connection between *TFL1* and *FLC*. In other words, the repression of meristem identity gene upregulation by *TFL1* (Ratcliffe et al 1999) may be mediated through *FLC*.

A recurring theme is the importance of quantitative interactions in the autonomous promotion pathway and how they appear to correlate with the level of *FLC* mRNA; the effect of *FLC* is gene dosage-dependent (Lee et al 1994, Sanda & Amasino 1996), and the suppression of *FLC* activity by vernalization is a quantitative process (Michaels & Amasino 1999, Sheldon et al 1999). The late-flowering phenotype of lines bearing active *FRI* or mutations in certain members of the

autonomous pathway can be suppressed by overexpression of *FCA* (G Simpson, R Macknight & C Dean, unpublished results). Further investigations into this correlation are likely to follow along with a molecular analysis of the basis of allelic variation at *FLC*.

A downstream function of the autonomous promotion pathway is to relieve the repression of the floral transition mediated by *FLC*. Therefore, it functions upstream of the transcriptional upregulation of the meristem identity genes. However, although overexpression of *AG* (CaMV35S::*AG*) suppresses the late-flowering phenotype of *fca* (Mizukami & Ma 1997), suppression of the late-flowering phenotype of *fca* and *FRI* by CaMV35S::*LFY* is incomplete (Nilsson et al 1998). This suggests that although the autonomous pathway is involved in the regulation of *LFY*, its function cannot lie exclusively upstream of *LFY* transcriptional regulation.

Genes Promoting the Floral Transition—the Vernalization Promotion Pathway

A long cold-temperature treatment, which typifies a winter season, induces or accelerates flowering in many species. This phenomenon, known as vernalization, is observed in naturally occurring late-flowering ecotypes of *Arabidopsis* and in mutants of the autonomous promotion pathway (for reviews, see Napp-Zinn 1985, Wilson & Dean 1996). Vernalization can overcome or bypass the repressive effect of *FRI/FLC* and compensate or substitute for a lack of the autonomous pathway genes. One interpretation of this data is that vernalization may operate through a separate, parallel pathway. There seems to be little influence on vernalization from the circadian clock, as vernalization in complete darkness has an effect similar to vernalization in SDs (Chandler & Dean 1994). However, little else is known about the molecular nature of the vernalization pathway (Chandler et al 1996, Finnegan et al 1998).

One strategy to understand the molecular basis of vernalization has been to identify and analyze mutants impaired in the vernalization response (Chandler et al 1996). The *vrn1* and *vrn2* mutants were isolated on the basis of their reduced vernalization response in the late-flowering vernalization-responsive *fca-1* mutant background. *vrn1* was shown to reduce the vernalization response of other late-flowering vernalization-responsive mutants under photoinductive conditions and of *Ler* under noninductive photoperiods (Chandler et al 1996). Neither *vrn1* nor *vrn2* was impaired in its ability to acclimate to low temperatures, indicating that the defect is specific for the vernalization pathway and not low-temperature responses in general (Chandler et al 1996). *Arabidopsis* mutants and ecotypes that show a strong response to vernalization also show an acceleration of flowering in response to a low ratio of red to far-red light (Bagnall 1993, Martinez-Zapater & Somerville 1990), a response mediated via the phytochromes. Interestingly, both *vrn1* and *vrn2* display additional photomorphogenic phenotypes (Y Levy, A Gendall & C Dean, unpublished results), further linking light quality perception with vernalization. Analysis of the *VRN* genes should identify the molecular

processes important in vernalization and may clarify the connection between vernalization and light quality perception.

Burn et al (1993a) proposed that vernalization causes demethylation, resulting in the activation of a gene involved in an early step of GA biosynthesis, which in turn promotes the floral transition. However, while Arabidopsis plants expressing an antisense transcript to a methyltransferase gene (*MET1*) exhibit substantially reduced levels of cytosine methylation (Finnegan et al 1996), they do not fully recapitulate the effects of vernalization (Finnegan et al 1998).

The level of *FLC* mRNA is downregulated by vernalization even in lines bearing loss-of-function *FRI* alleles (Sheldon et al 1999, Michaels & Amasino 1999), indicating that the vernalization pathway can repress *FLC* via a route that does not involve inactivation of *FRI*. The repression of *FLC* by the autonomous and vernalization pathways may explain, in molecular terms, how these pathways appear genetically parallel in early-flowering ecotypes. The level of *FLC* mRNA accumulates in an *fca*, *vrn2* double mutant, even in the absence of vernalization (Sheldon et al 1999). This suggests that *VRN2* functions to repress *FLC* even in the absence of vernalization.

Genes Promoting the Floral Transition—the Gibberellic Acid Promotion Pathway

In Arabidopsis, signaling mediated by GA appears to play a promotive role in flowering that is particularly apparent under noninductive photoperiods. The results of epistatic analyses with mutants deficient in GA biosynthesis (*gal*), combined with mutants in the photoperiod (Putterill et al 1995) or the autonomous promotion pathways (J Chandler & C Dean, unpublished results), are consistent with GAs functioning in a pathway that is separate from both the photoperiod and autonomous promotion pathways, as well as from the vernalization response (since GA mutants still respond to vernalization).

The application of GA accelerates the flowering time of wild-type plants under SDs (Langridge 1957) and of the late-flowering mutants *gi*, *fca*, *fd*, *fe*, *co*, *fpa*, *ft*, *fve*, and *fwa* under LDs (Chandler & Dean 1994). Under noninductive photoperiods, the *gal* mutant does not flower unless provided with GA (Wilson et al 1992), and the *gibberillin-insensitive* (*gai*) mutant flowers very late. Furthermore, *spy*, a mutant considered to exhibit constitutive GA-mediated signal transduction, flowers early (Jacobsen & Olszewski 1993), as do plants constitutively expressing *FPF1*, a gene that appears to be involved in GA-mediated signal transduction or responsiveness to GAs (Kania et al 1997).

GAs are also likely to be responsible for the acceleration of primordium initiation at the apex, an early manifestation of LD induction (Evans & Blundell 1996). GAs are clearly involved in multiple processes related to flowering, and the interaction of GAs and phytochrome-mediated signaling pathways is complex. Analysis of mutants deficient in both phytochrome and GA responses has shown that a fully functional GA system is necessary for full expression of at least

one aspect of phytochrome deficiency: an elongated hypocotyl (Peng & Harberd 1997). Increased responsiveness of *phyB* mutants to exogenous GAs also suggests an interaction between phytochrome and GA signaling (Reed et al 1996).

The role of GAs in activation of the *LFY* promoter has recently been analyzed (Blázquez et al 1998). The basal level of *LFY* promoter activity is lower in *gal* mutants, and the upregulation by LDs is delayed. In contrast, *LFY* activity is slightly higher in a *spy* mutant grown in SDs, correlating with an acceleration of flowering. A *CaMV35S::LFY* transgene was also found to partly rescue flowering in *gal* mutant plants in SDs. Thus GAs promote flowering in Arabidopsis at least in part by activating *LFY* expression. Blázquez et al (1998) also analyzed the direct effect of GA with and without sucrose on *LFY* promoter activity. GA alone had no effect, sucrose produced a small increase, and when combined they had a synergistic effect. The observation that *CaMV35S::LFY* was epistatic to the late flowering of *gai* but only partly reversed the late flowering of *gal* also shows that constitutive transcriptional activation of *LFY* could bypass the need for GA signaling through *gai* but does not completely bypass the need for GAs (Nilsson et al 1998).

Genes Promoting the Floral Transition—the Photoperiod Promotion Pathway

A collection of mutants flower late in LDs but are not significantly delayed by SDs and do not respond to vernalization. These have been placed in the photoperiod promotion pathway (Koornneef et al 1998a, 1991). This group includes the mutants *co*, *gi*, and *fha*. These genes mediate the promotion of flowering caused by LD photoperiods. Light is perceived by plants through a number of photoreceptors including cryptochromes (CRY 1 and 2) and phytochromes (PHYA through E). The signal transduction pathway after perception of the light signal is poorly understood, but it was recently discovered that phytochromes all have a C terminus with homology to histidine kinases of two-component systems (Fairchild & Quail 1998). Signals from the photoreceptors are then considered to entrain components of the circadian clock, which in turn regulates the expression of effector genes, including *CO*. However, not all photoreceptors regulate flowering time exclusively through the circadian clock (Koornneef et al 1995, Millar et al 1995). It is the combination of inputs from the circadian clock and photoperiod measurement that determine the activity of genes downstream in the photoperiod pathway.

Cryptochromes were definitively shown to be involved in photoperiodic promotion when *CRY2* was found to be the product of the *FHA* gene (Guo et al 1998). Transgenic plants overexpressing *CRY2* flowered earlier than wild-type and had increased levels of *CO* mRNA (Guo et al 1998), suggesting that blue light promotes flowering via *CRY2* and *CO* (see below). Furthermore, the level of *CO* mRNA was found to be reduced in *cry2* mutants grown under LDs but not under SDs (Guo et al 1998), providing a possible explanation for the basis of the original *fha* late-flowering phenotype. Because the levels of both PHYA

(Sharrock & Quail 1989) and CRY2 (Lin et al 1998) proteins drop rapidly and dramatically in the light, it has been proposed that they could provide information to the circadian clock about light-to-dark transitions (Suárez-López & Coupland 1998).

CRY1 was originally identified as the *hy4* mutant, which has a long hypocotyl under blue light, is sensitive to photoperiod, and in a *Ler* background is not delayed in flowering (Ahmad & Cashmore 1993). However, when in non-*Ler* backgrounds, and in blue-enriched light, *hy4* is late flowering and exhibits photoperiod sensitivity (Bagnall et al 1996, Koornneef & Peeters 1997). Clearly *CRY1* is involved in the promotion of flowering, but its interaction with floral promotion pathways is unclear.

Physiological studies on multiple mutant combinations suggest that other light-stable phytochromes in addition to PHYB regulate flowering in response to light quality (Koornneef & Peeters 1997). In contrast, mutations in *PHYA*, which encodes a light-labile photoreceptor, prevent perception of low-fluence-rate, far-red-enriched daylength extensions that promote flowering. These observations suggest that *PHYA* is involved in both daylength perception and the promotion of flowering by inductive photoperiods (Koornneef & Peeters 1997).

ELF3 affects aspects of the function of the circadian clock. The *elf3* mutant is early flowering and perturbs the circadian pattern of expression of several genes (Hicks et al 1996). *ELF3* regulates both the amplitude and periodicity of genes that may themselves be components of the circadian clock.

CCA1 and *LHY* are myb-like proteins that may be components of the circadian clock or may mediate signals to or from it. Both *CCA1* and *LHY* mRNA levels oscillate in a circadian fashion, and constitutive (non-rhythmic) expression of either *CCA1* or *LHY* abolishes or alters the circadian expression of their own transcripts and those of several other circadian-regulated genes (Schaffer et al 1998, Wang & Tobin 1998). Increased and constant *CCA1* or *LHY* expression also leads to late flowering, suggesting that the targets of these genes may encode repressors or that *CCA1* and *LHY* may inhibit transcription of floral promoters. Another likely component of the circadian clock is *TOC1*, which was identified as a semi-dominant mutation that shortened the period of the circadian clock by two to three hours (Somers et al 1998). The *toc1* mutation reduces the sensitivity of plants to photoperiod and causes early flowering under SDs, indicating that quantitative changes in only the period of the circadian clock can alter flowering time.

The signal(s) generated by the circadian clock is believed to affect the expression of downstream genes that operate in the photoperiod promotion pathway, including *GI* and *CO*. *GI*, which has recently been cloned, probably acts upstream of *CO*, because the phenotype of plants that overexpress *CO* is epistatic to the *gi* mutation (Piñeiro & Coupland 1998). *CO* mRNA is expressed throughout the plant and is more abundant in plants grown under LDs than under SDs (Piñeiro & Coupland 1998). It appears likely that other genes in the photoperiod promotion pathways may also be under a similar photoperiod control. Several lines of evidence suggest

that the level of *CO* activity in *Ler* plants is directly correlated with flowering time (reviewed in Piñeiro & Coupland 1998). Using a glucocorticoid receptor-based inducible system, Simon et al (1996) have demonstrated that the induction of *CO* activity was sufficient to rapidly cause flowering under SDs and to initiate transcription of the floral meristem identity genes *LFY* and *TFL1* as rapidly as when these genes were induced by transfer to inductive photoperiods. However, levels of *API* RNA increased more slowly by inducible *CO* activation than by inductive photoperiods (Simon et al 1996), suggesting that *CO* acts in a pathway that is sufficient to activate *LFY* and *TFL1* transcription but that rapid activation of *API* requires an additional pathway.

One of the intriguing features of the photoperiod pathway is the mechanism by which the light signal, perceived in the leaves, is translocated to the shoot apex. The molecular basis by which the floral stimulus is transduced is not known, but it may pass through the phloem (reviewed in Aukerman & Amasino 1998, Ma 1998). The phloem is composed of enucleated sieve tube cells, which serve as a conduit for nutrient delivery throughout the plant. It forms part of a plant-wide cell-cell communication network in which plasmodesmata (cell-cell channels presenting cytoplasmic continuity) also play an important role by integrating cells symplastically (Mezitt & Lucas 1996). It has recently become clear that the processes of systemic acquired resistance, viral movement, and post-transcriptional gene silencing involve the movement of proteins and/or nucleic acids through this network in a manner affected by source-sink transitions (Jorgensen et al 1998, Smyth 1997, Voinnet et al 1998). There is widespread speculation that one native function of this network could be in the regulation of the floral transition and floral development (Aukerman & Amasino 1998, Hake & Char 1997, Ma 1998, Mezitt & Lucas 1996, Smyth 1997, Voinnet et al 1998). Notably, microscopic monitoring of fluorescent tracer movement through plasmodesmata has revealed that the symplastic connections between the phloem and the shoot apex change over developmental time. The apex restricts tracer movement around the time of the floral transition (Gisel et al 1999).

Genes Promoting the Floral Transition—the FT Sub-Group

The late-flowering mutants *ft*, *fd*, *fe*, and *fwa* fall into a class that displays distinct epistatic properties that might be a branch of the photoperiod pathway or something more distinct. All these mutants are late flowering in LDs and somewhat late flowering in SDs, but they are not vernalization responsive (Koornneef et al 1991). Diverse experimental analysis of flowering time mutants has also placed these mutants together as a distinct epistatic class. For example, in analyzing the transcriptional activation of *LFY*, Nilsson et al (1998) found that while almost all the flowering time genes examined promoted the upregulation of *LFY* transcription, *FT*, *FWA*, *FD*, and *FE* did not. Likewise, Roldán et al (1997) found that the late-flowering phenotypes of *co*, *gi*, *fca*, *fpa*, and *fve*, but not *ft* or *fwa*, could be rescued

by growing them on vertical plates containing sucrose. Finally, epistatic analysis by Ruiz-Garcia et al (1997) demonstrated that when *ft* or *fwa* were combined with *lfy*, the severity of meristem identity defects was significantly enhanced, indicating that *ft* and *fwa* function in a parallel pathway to *LFY* to activate other meristem identity genes. Genes of the *FT* subgroup must also function to potentiate the downstream activity of *LFY* (Nilsson et al 1998). *FT* has recently been cloned independently by T-DNA tagging (Araki et al 1998) and activation tagging (D Weigel, personal communication) and found to encode a protein with pronounced similarity to another meristem identity gene, *TFL1* (Bradley et al 1997). Despite their similarity, *TFL1* and *FT* have opposing functions, with one repressing and the other promoting flowering. *ft*, *fd*, and *fe* are all recessive mutations, suggesting that they normally function to promote flowering, whereas *fwa* is a dominant mutation, the wild-type function of which remains to be established. The classification of *FD* in this group is not quite so clear cut because *fd* is not fully epistatic to CaMV35S::*LFY* in contrast to *ft* and *fwa*. In addition, *LFY* expression is downregulated and delayed in *fd* in contrast to *ft*, *fwa*, and wild-type *Arabidopsis* (Nilsson et al 1998). The recent cloning of *FT* and *FWA* (W Soppe & M Koornneef, personal communication) will allow a clearer picture of the role of this group to be determined.

GENES SPECIFYING FLORAL MERISTEMS

The multiple pathways that regulate the timing of the floral transition act directly or indirectly on a group of genes that switch the fate of the meristem from vegetative to floral. These genes are known collectively as floral meristem identity genes and in *Arabidopsis* include *LFY*, *API*, *CAULIFLOWER (CAL)*, *APETALA2 (AP2)*, and *UNUSUAL FLORAL ORGANS (UFO)*. In turn, the floral meristem identity genes control a second set of genes that direct the formation of the various flower parts (termed organ identity genes), which in *Arabidopsis* include *AG*, *AP3*, *PISTILLATA (PI)*, and *SUP*.

Mutations in floral meristem identity genes cause primordia that would normally develop into flowers to form more shoot-like structures. Mutations in the *LFY* gene result in the conversion of first-formed flowers into shoot-like structures subtended by a leaf. Later primordia form more flower-like structures but these lack petals or stamens (Huala & Sussex 1992, Schultz & Haughn 1991, Weigel et al 1992). The *LFY* protein shows no similarity to proteins of known function, localizes to the nucleus, and binds DNA in a sequence-specific manner (Parcy et al 1998, Weigel et al 1992) and so is presumed to act as a transcriptional activator. Overexpression of *LFY* (CaMV35S::*LFY*) causes early formation of determinate floral meristems (Weigel & Nilsson 1995), indicating that *LFY* is sufficient to determine floral fate. *ufo* mutations cause phenotypes that are similar to those caused by partial loss-of-function *lfy* alleles. *UFO* carries a functional F-box,

indicating an involvement in the targeting of other proteins, possibly transcription factors, for ubiquitin-mediated degradation (Lee et al 1997). Overexpression of *UFO* does not rescue *lfy* mutant phenotypes, thus indicating that *UFO* does not act as a simple mediator between meristem and floral organ identity genes (Lee et al 1997).

The *ap1* mutations result in the formation of shoots at the first few positions normally occupied by flowers, with later-forming primordia producing flowers, with no petals or petals that are either leaf-like or stamenoid, and containing secondary flowers in the axils of the outer organs (Bowman et al 1993, Irish & Sussex 1990). *lfy*, *ap1* double mutants show a much more severe phenotype than either *lfy* or *ap1* single mutants, with shoot-like structures formed at almost all the primordia (Bowman et al 1993, Huala & Sussex 1992, Schultz & Haughn 1993, Weigel et al 1992). This demonstrates that *LFY* and *API* have partially redundant functions. Redundancy is also found between *API* and *CAL*, both of which encode transcription factors of the MADS (MCM1-AGAMOUS-DEFICIENS-SRF) class (Kempin et al 1995, Mandel et al 1992). *cal* mutations alone do not confer a mutant phenotype but greatly enhance the *ap1* mutant phenotype that results in inflorescence structures resembling miniature cauliflowers, the result of massive proliferation of arrested floral meristematic tissue (Bowman et al 1993). Overexpression of *API* causes early formation of determinate floral meristems (Mandel & Yanofsky 1995).

The *ap2* mutations enhance both *ap1* and *lfy* mutant phenotypes, showing that *AP2* plays a role in specifying floral meristem identity (Schultz & Haughn 1993, Shannon & Meeks-Wagner 1993). *AP2* encodes a protein thought to act as a transcription factor, which shows homology to ethylene-responsive element-binding proteins (Jofuku et al 1994, Weigel 1995). *AG*, another MADS-type transcription factor, also plays a role in maintaining floral meristem identity (Yanofsky et al 1990), as well as specifying stamens and carpels (Bowman et al 1989). *AG* is also important in the regulation of meristem determinacy, since floral meristems of *ag* mutant plants revert to inflorescence formation in restrictive SD photoperiods (Mizukami & Ma 1997). Overexpression of *AG* results in slightly earlier flowering and conversion of the indeterminate apex into a terminal flower (Mizukami & Ma 1992, 1997).

The floral meristem identity genes are expressed throughout the developing floral primordia, whereas the homeotic genes, determining organ identity, are expressed in distinct domains in the flower. Parcy et al (1998) have investigated how the expression domains of the floral identity genes are established using *LFY* fused to the strong transcriptional activation domain of VP16. Expression of the fusion protein under the endogenous *LFY* promoter led to increased activation of *API* and ectopic and increased activation of *AG*. No effect was observed on *AP3*. Use of the *LFY:VP16* fusion showed that the different functions of *LFY* could be separated and further demonstrated that the specific expression patterns of the floral identity genes are likely to result from the dual action of a general floral regulator overlapping with another regional-specific factor.

Further discussion on the regulation of floral organ identity genes by floral meristem identity genes is limited here by space constraints. The reader is referred to Weigel & Meyerowitz (1994).

ACTIVATION OF FLORAL MERISTEM IDENTITY GENES

How the pathways that regulate the timing of the floral transition activate the floral meristem identity genes is an area of intense current investigation. Models designed to integrate the genetic and molecular data have been proposed and are constantly being updated (Coupland 1995, Koornneef et al 1998b, Levy & Dean 1998, Martinez-Zapater et al 1994, Schultz & Haughn 1993, Yang et al 1995). The promotive pathways are functionally redundant, which explains why no single mutation that prevents flowering has yet been found. The models reflect the multifactorial nature of flowering time control as first proposed by Bernier (1988). The transition to flowering is perhaps not a single switch but a progressively acquired condition resulting in expression of genes regulating meristem identity. Redundancy, potentiation, and additivity have been built into the system to ensure that when it occurs the transition is sharp.

Quantitative increases in *LFY* expression to a threshold level are clearly a major factor in the transition to flowering (Blázquez et al 1998). Expression of *API* is more qualitatively linked to floral determination (Hempel et al 1997). Unlike *LFY* and *AGL-8*, expression of *API* is upregulated after the point of floral determination, but it is found in leaf primordia of strongly induced plants as well as in the developing floral meristem (Hempel et al 1998). The connection between the flowering time genes and *LFY* has been directly addressed (Blázquez et al 1998, Nilsson et al 1998). Indeed, *CO*, *GI*, *FCA*, *FVE*, *GAI*, and *GAI* all play a role in activation of *LFY* and are required to some extent for full expression of *LFY* function.

The inducible activation of *CO* (described above) shows that *CO* is sufficient to induce flowering and to initiate transcription of *LFY* and *TFL1* as rapidly as when these genes are induced by LDs (Simon et al 1996). LDs induced *API* transcription more rapidly than the induced expression of *CO*, suggesting that rapid activation of *API* requires an additional (non-*CO*) pathway. Experiments designed to analyze the molecular basis of *API* activation are ongoing. One gene, *SPL3*, encoding a protein that binds to the promoter region of *API*, has been cloned (by homology to functionally similar genes from *Antirrhinum*) (Cardon et al 1997). Overexpression of *SPL3* produced an early-flowering phenotype and other abnormalities, but surprisingly this phenotype was not dependent on *API* function (Cardon et al 1997). Inducible high-level *CO* expression resulted in plants flowering very early, much earlier than plants overexpressing *LFY*. This result demonstrated that *CO* must also function in the activation of genes that function either in parallel or downstream of *LFY*. A similar conclusion was reached by Page et al (1999) after analyzing the interaction of *FCA* with the meristem identity

genes. *FCA* function was found to promote flowering in multiple pathways, one leading to the activation of *LFY* and *API*, and another acting in parallel with *LFY* and *API*.

THE FLORAL TRANSITION—UNTANGLING THE WEB

Putting the Pathway Components Together

When the known epistatic classes and genetic interactions of genes that regulate *Arabidopsis* flowering time are placed together, the key features of flowering—the perception, transfer, and integration of multiple endogenous and environmental cues—are clearly displayed (Figure 4, see color insert).

The autonomous promotion pathway serves to monitor or respond to the endogenous signals of an internal developmental clock that measures developmental age. Mutations in this pathway cause late flowering in both LDs and SDs. In contrast, a collection of mutants that are late only in LDs identify genes responsive to the inductive photoperiod signal. The vernalization pathway can suppress defects in the autonomous promotion pathway, but not the photoperiod pathway, suggesting that it functions in a manner similar to the autonomous promotion pathway and is thus placed parallel to it. The epistatic analysis of genes that are components of a gibberellin hormone signal transduction pathway indicates that they function to regulate flowering time in a pathway distinct from the others. The severity of late flowering displayed by mutations in this gibberellin-dependent pathway is more pronounced in SDs, indicating that their role can be effectively substituted for or masked by the activity of the inductive photoperiod pathway, thus illustrating the redundancy of these pathways in LDs.

The repression conferred by *FRI* and *FLC* or overexpression of *TFL1* can be overcome by vernalization (the longer the treatment, the more pronounced the effect) or by increasing expression of *FCA* (G Simpson, R Macknight & C Dean, unpublished results). The activity of these repressors and the manner in which they can be suppressed illustrates that these combined pathways are integrated in a quantitative way.

The photoperiod pathway promotes flowering in a manner effectively redundant with the GA and autonomous promotion pathways (see below). The key features of the photoperiod pathway include the integration of light signals transduced through the circadian clock with the measurement of light/dark photoperiods, resulting in a predicted output of a quantitative transcription cascade. It is clear that in the case of the transcription factor *CO*, the most genetically downstream member of this pathway identified so far, its quantitative transcriptional regulation is crucial to the inductive role of this pathway (Putterill et al 1995, Simon et al 1996).

In LDs the key players at the place where flowering-time genes meet meristem identity genes appear to be *LFY* and genes of the *FT* subgroup. The quantitative transcriptional upregulation of the meristem identity gene *LFY* is a main event in the floral transition (Blázquez et al 1997, Nilsson et al 1998, Weigel &

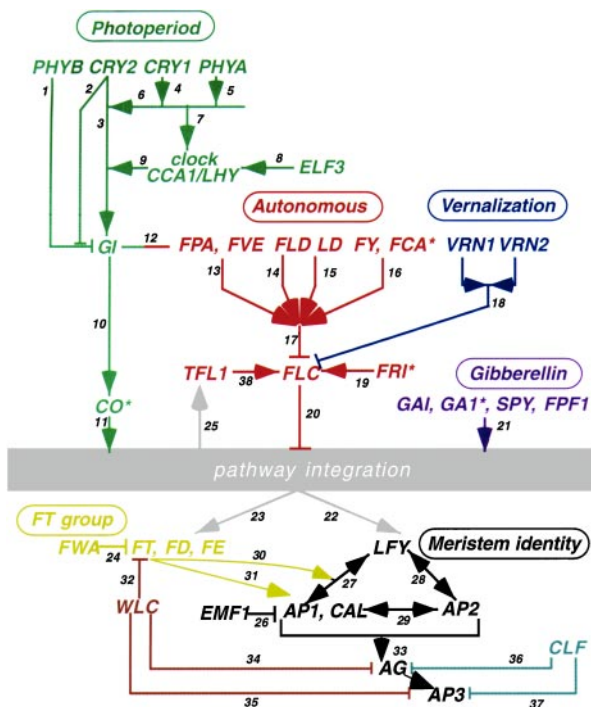


Figure 4 The photoperiod, autonomous, vernalization, and gibberellin promotive pathways are displayed. Each promotive pathway serves ultimately to upregulate the meristem identity genes, but the precise molecular events effecting this are not known, and therefore each is shown to feed into a pathway integration section. An astrisk indicates that there is genetic evidence for an additional role that is downstream of *LFY*. Rationale for the connections made can be found in the text and are annotated as follows: 1, 2: Mockler et al 1999. *PHYB* may also repress the autonomous pathway; see Koornneef et al 1998b; 3–7: photoreceptors serve to entrain the circadian clock and/or measure photoperiod; 8: Hicks et al 1996; 9: Schaffer et al 1998, Wang & Tobin 1998; 10: Piñeiro & Coupland 1998; 11: Simon et al 1996; 12, 13: Koornneef et al 1998a; 14: speculation, *FLD* is a member of the autonomous pathways, but there is on evidence that it regulates *FLC*; 15: Aukerman & Amasino 1996; 16: Koornneef et al 1998a; 17: Michaels & Amasino 1999, Sheldon et al 1999; 18: vernalization pathway is parallel with autonomous promotion pathway, but position of branching or integration is not yet known; 19, 20: Sheldon et al 1999, Michaels & Amasino 1999; 21: Blázquez et al 1998, Nilsson et al 1998; 22, 23: upregulation of *LFY* is important for the activation of meristem identity genes; Nilsson et al 1998, Ruiz-Garcia et al 1997; 24: *FWA* is a repressor of the activity of this epistatic class but its positioning is speculative; 25: Simon et al 1996; 26: Chen et al 1997; 27–29: Bowman et al 1993, Piñeiro & Coupland 1998, Schultz & Haughn 1993; 30: Nilsson et al 1998; 31: Ruiz-Garcia et al 1997; 32: C Hutchison & C Dean, unpublished results; 33: Bowman et al 1993, Schultz & Haughn 1993; 34, 35: C Hutchison & C Dean, unpublished results; 36, 37: Goodrich et al 1997; 38: Ruiz-Garcia et al 1997: speculation, see text on autonomous promotion pathway.

Nilsson 1995). The analysis of the transcriptional activation of *LFY* has revealed that the output of the gibberellin, photoperiod, and autonomous promotion pathways all function to upregulate *LFY* transcription (Nilsson et al 1998, Simon et al 1996). However, this is not the exclusive function of any of these pathways, since all exhibit genetic activities effective downstream of *LFY* (Blázquez et al 1998, Nilsson et al 1998, Page et al 1999, Simon et al 1996). Importantly, members of the *FT* subgroup do not function in the transcriptional activation of *LFY*, but affect *LFY* function and activate other meristem identity genes, notably *API*, redundantly with *LFY*. It is therefore reasonable to expect that the floral transition requires the coordinated activation of *LFY* and genes of the *FT* subgroup. The fact that genes of the *FT* subgroup are late flowering in LDs and, to some extent, in SDs raises the possibility that the photoperiod plus the autonomous and/or GA pathways could be involved in their activation. It is possible that the molecular basis for the genetic activities that *CO*, *GAI*, and *FCA* exhibit downstream of *LFY* (Nilsson et al 1998, Page et al 1999, Simon et al 1996) could be explained, at least in part, by their role in directly or indirectly upregulating genes of the *FT* group. The activation of *LFY* and the *FT* subgroup then promotes the activation and potentiation of other redundant meristem identity genes.

Several mutants defective in carbon metabolism have been shown to affect flowering time (Levy & Dean 1998). Some exhibit properties that suggest they are components of the photoperiod pathway (e.g. *CAMI*), whereas others exhibit properties that suggest they are members of the autonomous promotion pathway (e.g. *PGMI*, *SEX1*). However, a comprehensive analysis of their genetic interactions has not been performed (Levy & Dean 1998).

Mutations in the autonomous promotion pathway and lines delayed in flowering by dominant alleles of *FRI* and *FLC* can be suppressed by growth in light conditions with a low red to far-red ratio (see above). The basis of this suppression has not been characterized, but it suggests the existence of another promotive pathway.

Interaction of the Pathways that Control the Floral Transition

How the different pathways integrate to activate the floral meristem identity genes is not known. In Figure 4, direct connections between the flowering time genes and the meristem identity genes have been avoided, since it is not known how each pathway eventually functions in their upregulation. There are, formally, at least four possible ways in which the divergent pathways could integrate to promote flowering (Figure 5). In the first case (Figure 5a), each of the pathways can be seen as being purely redundant with one another, with each functioning directly in the upregulation of meristem identity genes in an additive way. However, alternative possibilities could allow one to view the pathways as either facilitators or enhancers of the floral transition. For example, in a second scenario (Figure 5b), the autonomous promotion pathway could be viewed as

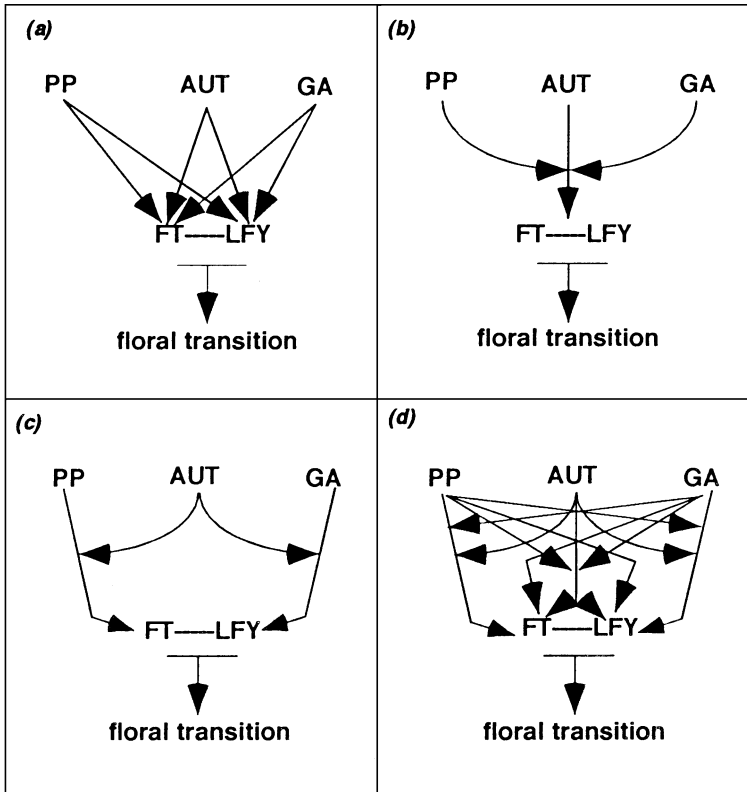


Figure 5 Alternative means of integrating the floral promotion pathways. The scenarios depict how the pathways that promote the floral transition in *Arabidopsis* could functionally integrate to upregulate the meristem identity genes. (a) Each pathway acts separately, redundantly, and additively to upregulate the meristem identity genes. (b) The photoperiod (PP) and gibberellin (GA) pathways enhance the activity of a default autonomous (AUT) promotion pathway. (c) The autonomous promotion pathway acts as a facilitator of the photoperiod and gibberellin pathways. (d) Multiple interdependent interactions form a web of connections through which diverse quantitative responses are integrated and proofread prior to upregulation of meristem identity genes.

the default endogenous floral development pathway, with inductive signals transduced through the photoperiod and GA pathways serving as enhancers of this default development. A third possibility (Figure 5c) is that the photoperiod and GA pathways are the inductive pathways of the floral transition and that the autonomous pathway serves to facilitate their action (in effect, competence). Finally, such representations may oversimplify events, and instead there may be multiple interdependent interactions between the pathways that form a web of connections through which the diverse cues and quantitative responses are

integrated and proofread prior to upregulation of the meristem identity genes (Figure 5*d*). The analysis of the photoperiod and GA pathways in mutants of the autonomous pathway should reveal if and where these pathways meet along the way to the ultimate upregulation of the floral meristem identity genes. For example, it is already clear that since *CaMV35S::CO* significantly suppresses the late-flowering phenotype of *fca* (Piñeiro & Coupland 1998), the possibility displayed in Figure 5*b* cannot accurately represent how the pathways integrate.

The Relative Importance of the Pathways Change in Time and Space

In the natural environment, the movement of cues through the network controlling the floral transition must change with time. For example, in the late *Arabidopsis* ecotypes (germinating in late summer), repression by *FRI* and *FLC* early in development prevents flower formation in the disadvantageous conditions of the following winter. This repression overrides the photoperiod conditions of late summer that would otherwise be considered as favorable. This *FLC*-mediated repression is overcome by the long cold-period of winter, executed and stably maintained by the vernalization and the autonomous pathways. The vernalization and the autonomous promotion pathways thereby facilitate the competence of the meristem to respond to the inductive photoperiod and GA pathways. As time passes into spring, the photoperiod pathway acquires a central role. As daylength increases, the floral transition is accelerated through the coordinated upregulation not only of *LFY* but of the genes of the *FT* group to enhance the upregulation of meristem identity genes that execute the floral transition.

The relative movement of cues through the network must also change in space. The upregulation of *TFL1*, illustrated in Figure 4, occurs only in the center of the meristem. *TFL1* can inhibit the activity of meristem identity genes at the center of the shoot apex by delaying their upregulation and by preventing the meristem from responding to their action. However, at the apex periphery, meristem identity genes such as *LFY*, *API*, and *CAL* prevent *TFL1* transcription (Ratcliffe et al 1999).

A Robust Network Controlling the Floral Transition

The pathways regulating the floral transition exhibit partial redundancy between each other and within each epistatic class (Figure 4). Thus the pathways cannot be described as simply linear. Some components likely activate and/or upregulate multiple targets. Redundancy at the level of individual genes and through parallelism and interlinking of pathways ensures that regulatory networks are more reliable than their parts (McAdam & Arkin 1999).

PERSPECTIVES

The identification and cloning of genes that regulate flowering time allow the move from a purely genetic analysis to a molecular and cellular one. The next phase of research is likely to involve a new generation of genetic screens plus three key experimental approaches. One will be the molecular analysis of the complexes in which these gene products function in order to understand the manner in which they act. Another will use the inducible expression of specific genes followed by an analysis of the gene expression changes they promote as a means of dissecting pathway cascades at the molecular level. A third will involve the use of sector analysis to determine where and when particular genes are required in the floral transition.

It is likely, but remains to be proven, that the same genes identified as controlling flowering in *Arabidopsis* are involved in controlling flowering time in all plant species. Based on the dominance and physiological characteristics conferred by different alleles, a list of possible orthologues from *Arabidopsis*, pea, sugarbeet, barley, and wheat has been compiled (Levy & Dean 1998). Use of either *Arabidopsis* genes or their corresponding orthologues will enable the manipulation of flowering time in crop species, which could have a major impact on agriculture. A two- to three-day acceleration of flowering time in rice could enable two rice crops a year to be produced in regions currently able to produce only one. Prevention of bolting in crops such as sugarbeet, many *Brassica* species, potato, spinach, and lettuce would significantly improve yield. Control of flowering time is clearly an area of biotechnological as well as biological interest.

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