From floral induction to floral shape
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The initial emphasis in molecular–genetic studies of flower development was on homeotic genes that control organ identity, which is rather invariant between different species. Studies in flower development during the past three years have dealt with more diverse aspects of flower development, including floral induction and floral shape. Genes identified in the respective pathways might hold clues to the diversity of modern angiosperms.

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Abbreviation
PHYA   phytochrome A

Introduction
The transition from vegetative to reproductive development is caused by a still mysterious process called floral induction; although it is common to all angiosperms, the underlying phenomenology is very diverse. Indeed, the chapters that detail the effects of various environmental conditions or hormones on floral induction in different species are invariably the most confusing sections of any plant physiology textbook. A second extraordinarily diverse aspect of flower development is floral form and shape, which can vary substantially even between closely related species. It seems, therefore, that understanding the genetic basis of floral induction and of floral form and shape may hold the key to understanding important elements of angiosperm evolution. A first step in determining how diversity is achieved is to identify common mechanisms that underlie these processes in all plants. One way to do so is by isolating key regulatory genes from a few model species, and then to go on and study these genes in other species. In this review, I discuss recent results obtained mostly with two species, *Arabidopsis thaliana* and *Antirrhinum majus*.

Floral induction
The onset of flowering is under both endogenous and environmental control, thereby ensuring that flowers form during the appropriate season, and that the production of flowers is co-ordinated among members of the same species, which is particularly important for outcrossing plants. The differences in flowering behavior of different species are rather extreme, ranging from plants that can flower several times within the same year, to others that

flower for the first time only after many years despite having been exposed to the right environmental cues repeatedly.

Among environmental signals, the most thoroughly investigated are transient exposure to cold, called vernalization, and changes in day length, called photoperiod. The classic studies by Zeevaart [1] established some forty years ago that a signal promoting flower induction is produced in leaves, and that this signal must travel through the stem to the shoot apex, where flowers are formed. Despite these pioneering studies, the biochemical nature of this signal has remained elusive.

Due to the lack of substantial progress made with a purely physiological approach, several groups have begun to use genetics to dissect the control of flowering time, and this has been most thoroughly done using *A. thaliana* and garden pea, *Pisum sativum*. The latter has the advantage that grafts between plants of different genotypes can be made, which has allowed us to establish where the gene products that are defective in different mutants act. Using such techniques, it has been possible to identify genes controlling the production of a flowering signal in leaves, transmission of the signal through the stem, and its perception at the shoot apex [2]. Unfortunately, the cloning of genes identified only by their mutant phenotype is an arduous task in pea because of the large genome, and none of the flowering genes have been isolated. In contrast, cloning of genes identified only by mutant phenotype is becoming routine in *Arabidopsis*, and the cloning of several flowering-time genes has already been reported in the literature. Two of these genes, *CONSTANS (CO)* and *FCA*, act as genetic switches, with loss-of-function and overexpression having opposite effects on flowering time [3••,4••].

Knockout of either *CO* or *FCA* causes late flowering, although the two genes seem to act in different pathways controlling flowering time. *CO* is an essential component of the pathway that promotes flowering in *Arabidopsis* in response to long days, and flowering of *co* mutants is delayed only under long days. In contrast, *fca* mutants are late under both long and short days, and are thought to act in an environmentally independent, autonomous pathway. These differential effects correlate with their expression patterns. While the levels of *CO* mRNA are much higher in long than in short days, mRNA levels of *FCA*, as well those of another gene in the autonomous pathway, *LUMINIDEPENDENS (LD)*, are unaffected by day length [4••,5,6]. All three genes are expressed at the shoot apex, suggesting that they act relatively far downstream in floral induction. *CO* and *LD* appear to encode transcription factors, while *FCA* encodes an RNA-binding protein,

raising the possibility that all three directly regulate target genes that control the initiation of individual flowers.

The role of *CO* RNA levels in controlling flowering time has been further investigated with transgenic plants in which an inducible version of *CO* is expressed from a strong, constitutive promoter. Simon and colleagues [3••] found that high-level expression of functional *CO* under short days causes transgenic plants to flower even earlier than long-day-grown wild-type plants, indicating that *CO* is not only required for the induction of flowering in long days, but that *CO* itself is the limiting component, and that regulation of *CO* levels is an essential aspect of the determination of flowering time. How this is exactly played out in wild-type is not quite clear yet, but at least two alternatives are possible. *CO* RNA levels might increase continuously during the life cycle of the plant, until they effect flowering. Alternatively, *CO* transcription levels might be controlled by day length but independently of plant age, and flowering would thus be determined by a combination of *CO* levels and competence of the plant to respond to *CO*. That competence plays at least some role in the *CO* response can be deduced from the observation that *CO* overexpressers are not entirely unaffected by day length, but still flower slightly later when exposed to short rather than long days.

One of the immediate consequences of *CO* action is the activation of genes that control the identity of the main shoot apical meristem and of lateral meristems [3••] (Figure 1). The meristem-identity genes come in two 'flavors', either promoting or repressing floral identity (*LFY* and *TFL1*, respectively, are examples for genes in either class). A floral repressor is encoded by the *TERMINAL FLOWER 1 (TFL1)* gene, which is expressed in the shoot apex in a small group of subapical cells [7••] and which is rapidly upregulated upon induction of *CO* activity [3••]. *TFL1* function is, however, not limited to the reproductive phase, but is also required during the vegetative phase to delay precocious flowering [8]. Interestingly, the *TFL1* ortholog in *Antirrhinum* is not expressed during the vegetative phase, and the corresponding mutation has no effect on flowering time [9••]. Moreover, this differential expression of *TFL1* in *Arabidopsis* and *Antirrhinum* is paralleled by the expression pattern of the flower-meristem-identity gene *LEAFY (LFY)*, whose expression is negatively regulated by *TFL1*. Only the *Arabidopsis LFY* gene, but not the *Antirrhinum* ortholog, is extensively expressed during the vegetative phase [10••,11••].

Although it is still unknown how changes in day length are translated into increased *CO* RNA accumulation, at least two possible upstream components have been identified, including the gene encoded by *ELF3*, and phytochrome A (PHYA) photoreceptor (Figure 1). In both *Arabidopsis* and pea, phytochrome A is required to detect extensions of short days by low-fluence light, which is almost as

Figure 1
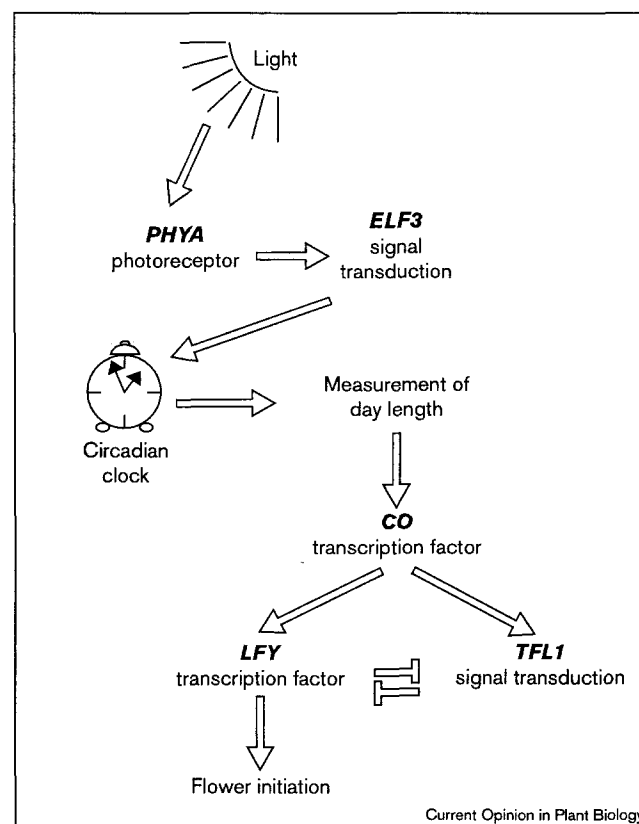
Genetic pathway from light perception to flower initiation in *Arabidopsis*. Identified genes are indicated in bold and italics, along with their putative biochemical functions. This scheme is not meant to imply that the components shown are the only ones acting at a particular step. For example, *PHYA* probably has a smaller effect on *ELF3* activity than other photoreceptors have.

effective in inducing rapid flowering as are long days of high-fluence light. The analysis of *phyA* mutants has also revealed differences between these two species, as the function of PHYA is partially redundant in *Arabidopsis*, but not in pea. In contrast to *Arabidopsis*, pea *phyA* mutants do not respond at all to high-fluence long days and look just like short-day-grown wild-type plants [12,13,14••].

In order for a plant to measure the length of day or night, it has to integrate the environmental input perceived through the photoreceptors with an endogenous circadian rhythm. The first genetic link in this signal transduction chain has been identified with the *early-flowering 3 (elf3)* mutation, which not only affects flowering time, but also eliminates rhythmicity in two circadian responses, leaf movement and activity period of a circadian-regulated promoter *CAB2* [15••]. Importantly, the circadian defect is only observed in constant light, but not in constant dark or in light-to-dark transitions or other regimens that include alternating light and dark periods. This conditional phenotype suggests that *elf3* mutations do not simply inactivate the circadian clock itself, but rather interfere

with signal transduction from photoreceptors to the clock. The link between circadian rhythm and photoreceptor response in *elf3* mutants is further supported by the *elf3* long-hypocotyl phenotype, which resembles the seedling phenotype of photoreceptor mutants [15••].
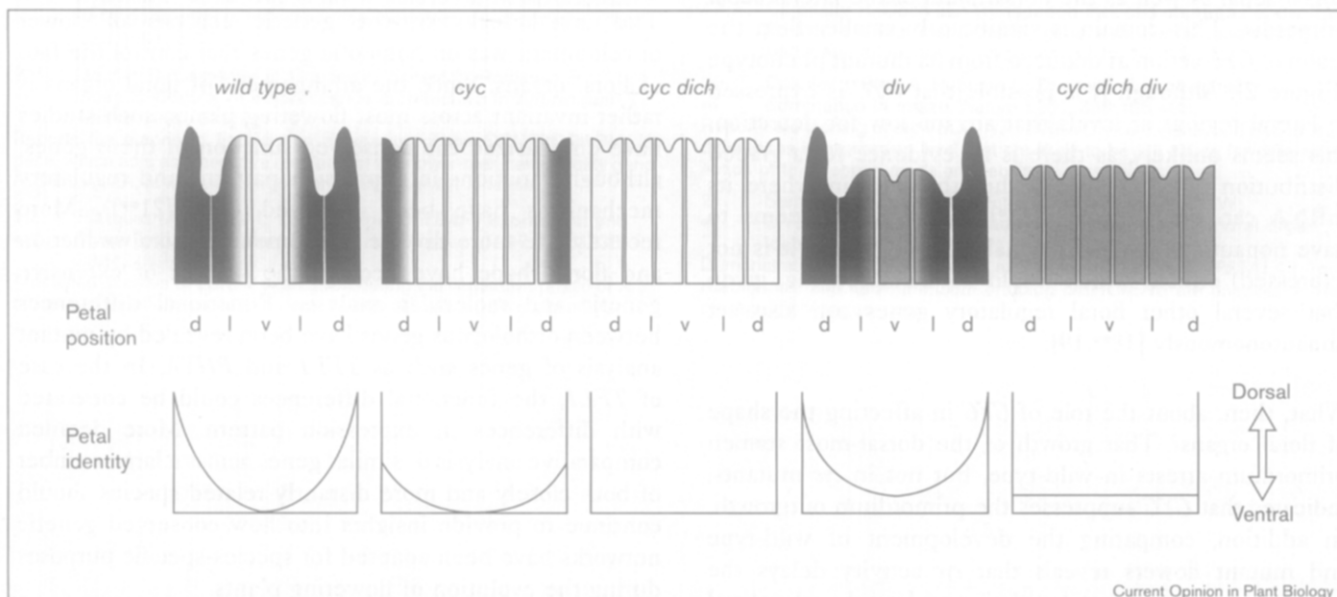
Floral shape

Compared to the molecular analysis of floral induction, the genetic basis of the diversity of floral form and shape is even more sketchy, but a potential solution to this problem might come from the study of genes that specify differences between organs of the same type within a species. Such regulators of intraspecific differences in organ shape would appear to be good candidates for genes that account also for interspecific differences. Intraspecific differences between organs of the same type are found in irregular flowers, which have only a single plane of symmetry, in contrast to regular flowers, in which all organs of one type are identical, resulting in two or more planes of symmetry. One species with irregular flowers and well-characterized genetics is snapdragon, *Antirrhinum majus*, and several mutations that affect floral asymmetry have been identified [16••,17••].

As is typical for dicot flowers, those of *Antirrhinum* have four types of major organs that are arranged in four concentric rings or whorls. The first, outermost whorl is occupied by five sepals, the second whorl by five petals, the third whorl by four stamens, and the central whorl by two carpels. The single axis of symmetry defines

a dorsoventral axis, and along this axis differences are particularly obvious among petals and stamen. The five petals adopt three different identities, with a single ventral petal, two lateral ones and two dorsal ones (Figure 2, [16••,17••]). The ventral petal straddles the single plane of symmetry and is therefore bilaterally symmetric, whereas the lateral and dorsal petals do not straddle the plane of symmetry and, therefore, are individually asymmetric along the dorsoventral axis of the flower. Similar to the petals, the five stamens adopt three different identities. Because stamens arise in alternate positions with the petals, there is a single, bilaterally symmetric dorsal stamen primordium as well as two lateral and two ventral stamens, which again are individually asymmetric along the dorsoventral axis. The single dorsal stamen primordium normally does not fully develop, and becomes a reduced stamen called a staminode instead. Because of the dorsoventral axis defined by petals and stamens, the five sepals can also be grouped into two lateral and two ventral sepals, and a single dorsal sepal, although the sepals themselves do not show any pronounced asymmetries.

At least four loci have been found to be involved in dorsoventral patterning. Mutations at three of these, *CYCLOIDEA (CYC)*, *RADIALIS (RAD)*, and *DICHOTOMA (DICH)*, cause ventralization of the flower, while mutations at the fourth, *DIVARICATA (DIV)*, cause dorsalization. One explanation for there being several loci in the first group is that at least two of them act redundantly. In

Figure 2

Genetic control of floral organ shape in *Antirrhinum*. The five petals are schematically diagrammed, with the ventral petal (v) in the center, the dorsal petals (d) on the outside, and the lateral petals (l) in between. Positional identities are indicated by graded shading, and the identity gradients are redrawn on the bottom. Note that dorsal, ventral and lateral is used in two ways: first, it indicates organ identity according to the position found within the wild-type flower; second, it indicates organ position within both wild-type and mutant flowers. Thus, a dorsal petal in a mutant may have ventral identity. After [17••].

cyc single mutants, the number of sepals, petals and stamens is increased to six each, while the number of carpels is unchanged (Figure 2). In addition, three to four petals are symmetrical and resemble the single ventral petal, with the remaining petals having mixed lateral and dorsal character. Of the stamens, four or five have ventral identity and the remaining one or two, which arise in dorsal positions, have lateral character [16••]. Thus, there is a graded effect of the *cyc* mutation, with lateral organs adopting ventral identity, and dorsal organs completely or partially adopting lateral identity. The progressive ventralization is further enhanced in a *cyc dich* double mutant, such that all six petals and six stamens resemble ventral organs of wild-type (Figure 2). The *dich* mutant on its own shows only a relatively mild defect, with the dorsal petals being slightly more symmetric than those of wild-type [16••,17••]. Since *DICH* has not been cloned, it is unclear whether the mild defect in this mutant reflects that this gene plays a less important role in determining floral shape than *CYC*, or whether it merely reflects that this particular allele is not a null allele. *CYC* has been cloned, however, and it has been shown that even plants carrying a null allele do not have completely ventralized flowers [16••].

While the cloning of *CYC* has not provided any strong clues to its biochemical function, the cloning has allowed the study of its expression pattern in detail [16••]. *CYC* is only expressed in young flower primordia, as soon as these arise. Within the flower primordium *CYC* mRNA is restricted to a small region that includes the primordia of the dorsal stamen, of the dorsal petals and of the dorsal sepal as well as the dorsal parts of the lateral sepal primordia. This domain is significantly smaller than the realm of *CYC* action as deduced from its mutant phenotype (Figure 2). Although it is possible that *CYC* is expressed in lateral regions at levels that are too low for detection, this seems unlikely, as there is no evidence for a graded distribution of *CYC* RNA within the domain where its mRNA can be detected [16••]. Rather, *CYC* seems to have nonautonomous (i.e. signals to cells where it is not expressed) effects, which would not be surprising, given that several other floral regulatory genes can also act nonautonomously [18••,19].

What, then, about the role of *CYC* in affecting the shape of floral organs? That growth of the dorsal-most stamen primordium arrests in wild-type, but not in *cyc* mutants, indicates that *CYC* suppresses the primordium outgrowth. In addition, comparing the development of wild-type and mutant flowers reveals that *cyc* activity delays the outgrowth of dorsal petals relative to lateral and ventral ones [16••]. Although *CYC* does not have a simple effect on organ growth — the final size of dorsal petals in wild-type exceeds that of dorsal petals in *cyc* mutants — one might speculate that members of the *CYC* gene family have

general roles in controlling organ outgrowth. This assertion is supported by the recent discovery of a maize gene that shares strong sequence similarity with *CYC*. Not only does this gene, *TEOSINTE BRANCHED 1 (TB1)*, suppress the outgrowth of axillary organs, but it is differentially active in maize and its wild ancestor, teosinte, and thus accounts for major morphological changes that occurred during the selection of modern maize from teosinte [20••]. Thus, members of the *CYC/TB1* family are indeed excellent candidates for genes that are responsible for interspecific differences in floral organ shape.

How exactly *CYC* affects organ shape is not known, but one putative target gene has already been identified. Mutations in *DIV* cause a phenotype opposite to that of *cyc* mutations, and in *div* homozygotes, the ventral petal is transformed into a lateral petal, although its bilateral symmetry is retained (Figure 2). Lateral petals are also affected, such that their asymmetry along the dorsoventral axis is reduced and the most ventral region identity is eliminated [17••]. The regulatory relationship between *div* and the ventralizing mutations has been examined with double and triple mutants. As with *cyc dich* mutant flowers, *cyc dich div* flowers are radially symmetric. While all petals of *cyc dich* flowers resemble ventral petals of wild-type, those of triply mutant flowers exhibit the ventral-most identity found in *div* mutants, which resembles a lateral petal of wild-type. The ventral requirement for *DIV* function along with the epistasis of *div* regarding regional identity suggest that *DIV* activity is repressed by *CYC/DICH* in the dorsal region of the flower.

Conclusions

The initial focus in the genetic analysis of flower development was on homeotic genes that control the fate of floral organs. Since the arrangement of floral organs is rather invariant across most flowering plants, such studies have emphasized the conserved function of these genes, although variations in expression patterns and regulatory mechanisms have been observed (e.g. [21••]). More recently, the more diverse phenomena of floral induction and floral shape have become the subject of extensive genetic and molecular analysis. Functional differences between orthologous genes have been revealed by mutant analysis of genes such as *TFL1* and *PHYA*. In the case of *TFL1*, the functional differences could be correlated with differences in expression pattern. More detailed comparative analysis of similar genes across a large number of both closely and more distantly related species should continue to provide insights into how conserved genetic networks have been adapted for species-specific purposes during the evolution of flowering plants.

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