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The Metamorphosis of Flowers

Enrico S. Coen[1] and Rosemary Carpenter

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

INTRODUCTION

One of the unifying theories of plant biology is that the variety of plant forms are simply different modifications of a common growth plan. Different permutations of a few key features of plant growth can generate a bewildering array of seemingly distinct forms. There is perhaps no better illustration of this than the comparison of a flower and a shoot. The idea that these two apparently different structures might be fundamentally equivalent goes back to Goethe's treatise on metamorphosis, published in 1790. He concluded, "Flowers which develop from lateral buds are to be regarded as entire plants, which are set in the mother plant, as the mother plant is set in the earth" (Goethe, 1790). In equating flowers and shoots, four key assertions need to be made.

First, the different parts of the flower (sepals, petals, stamens, and carpels) are equivalent to the leaves of a shoot. Second, the organs of both shoot and flower are separated by internodes, but in the case of the flower these are so short as to be barely visible. Third, the organs of shoot and flower usually have a distinct phyllotaxy, or arrangement around the central axis. Finally, the indeterminate growth that so characterizes a shoot is suppressed in the case of a flower, both apically, because it eventually stops producing organs around the central axis, and laterally, because branches do not normally arise in the axils of floral organs.

The comparison of flower and shoot therefore highlights four key variables: organ identity, internode length, phyllotaxy, and determinacy. The numerous forms and habits of plants simply reflect different variations and permutations of these four fundamental aspects of growth. What is their developmental basis?

The development of shoots and flowers depends on the behavior of meristems. On the periphery of meristems, groups of cells are partitioned off to form either secondary meristems or organ primordia. Phyllotaxy depends on the precise pattern in which partitioning occurs. Determinacy also reflects partitioning, most importantly whether it favors meristems or primordia. Organ identity depends on the developmental characteristics of primordia. Finally, the growth of regions between primordia determines internode length. In the last few years, the systematic analysis of flower development has allowed genetic control of these key aspects of plant development to be dissected and studied in detail. Some of the findings have been reviewed elsewhere (Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991). The aim of this review is to give a general overview of the field, highlighting some of the more recent problems and results obtained from studies of Antirrhinum and Arabidopsis.

Genetic studies have defined two main types of gene as being involved in flower development, meristem and organ identity genes. Meristem identity genes generally affect all four of the key aspects of plant growth and, therefore, the properties of meristems as a whole. Organ identity genes more specifically affect the fate of primordia and, hence, the types of organ that are made. However, there is considerable overlap between these categories. Meristem identity genes may sometimes have more restrictive effects. Conversely, organ identity genes can have more extensive effects and influence aspects of growth other than just the fate of primordia.

CONTROL OF MERISTEM IDENTITY

During early plant growth, leaf primordia are produced on the periphery of a vegetative meristem at the shoot apex. When plants are induced to flower, two new types of meristem are usually produced. First, the apical meristem switches to become an inflorescence meristem. Second, floral meristems are produced, often arising as small bulges on the periphery of the inflorescence meristem. The induction of these new meristem types depends on many environmental and developmental signals (Bernier, 1988). The process of induction and the developmental behavior of meristems are generally treated as completely separate problems. Furthermore, the production of floral meristems is often treated as a secondary event, dependent on the prior induction of inflorescence meristems. These views are likely to be oversimplistic.

The various signals that promote or inhibit flowering need to be combined or integrated by the plant so that a final "decision" whether or not to make flowers can be made (see Bernier et al., 1993, this issue). It is likely that this integration occurs at a variety of levels and locations in the plant. For example, the leaves may need to combine factors such as light intensity and daylength before sending a signal to the apex. The apex in turn may receive various types of signals that are

[1] To whom correspondence should be addressed.

further integrated, leading to the initiation of inflorescence and floral meristems. The induction of these two meristem types may be at least partially separable. For example, in Antirrhinum, it is possible to devise environmental conditions in which flowers are induced but most of the characteristics of the inflorescence, such as small bracts, short internodes, and hairy stem, have not been induced (D. Bradley, R. Carpenter, and E. S. Coen, unpublished results). Conversely, several mutants have been described in which inflorescence-like shoots proliferate without producing flowers. This suggests that some signal integration may occur separately for floral and inflorescence meristems. It is possible that this signal integration directly involves genes controlling inflorescence or floral meristem identity.

A pair of key genes involved in the establishment of floral meristem identity has been isolated from both Antirrhinum and Arabidopsis. Mutations in these genes result in floral meristems being replaced by meristems that have some or all of the characteristics of inflorescences. Therefore, in these cases, there is a failure, or delay, in the production of flowers and a proliferation of inflorescence-like structures in their place. However, there are significant differences between mutant phenotypes observed in these species, which to some extent reflect differences in their wild-type development.

Wild-type Antirrhinum flowers consist of concentric whorls of four different types of organs: whorl 1 (outermost) contains sepals, whorl 2 consists of petals, whorl 3 contains stamens, and whorl 4 (innermost) contains carpels. Flowers are borne in the axils of small leaflike organs called bracts. The bracts are arranged in a spiral and are separated by shorter internodes than those separating leaves of the vegetative shoot. In *floricaula* (*flo*) mutants of Antirrhinum, shoots with the characteristics of an inflorescence grow in place of flowers (Carpenter and Coen, 1990; Coen et al. 1990). These shoots differ from flowers in four key aspects: organ identity (bracts rather than floral organs), internode length (short rather than undetectable), phyllotaxis (spiral rather than whorled), and determinacy (indeterminate rather than determinate). However, if mutant plants are kept for a long enough time in small pots, terminal structures bearing carpel-like organs are very occasionally produced on one or two branches (E.S. Coen and R. Carpenter, unpublished observations). Thus, some floral aspects can eventually be restored to *flo* mutants, whereas others, such as the production of petals or stamens, have not been observed so far.

Wild-type Arabidopsis flowers are also composed of four concentric whorls, but, unlike those of Antirrhinum, they are not subtended by bracts. Flowers are separated by long internodes and borne in a spiral. In *leafy* (*lfy*) mutants, the lower flowers of the inflorescence are replaced by shoots in the axils of small leaflike organs (cauline leaves). However, the phenotype changes gradually as one moves up the plant, with the size of the cauline leaves decreasing and the lateral shoots aquiring more floral features, eventually producing "flowers" with sepals and carpels having an almost spiral phyllotaxis (Schultz and Haughn, 1991; Weigel et al., 1992). The *lfy* mutant in Arabidopsis therefore confers a similar phenotype to *flo* but activates some aspects of floral development much more readily. The *flo* and *lfy* genes encode proteins that are 70% identical to each other, although their precise biochemical role remains to be elucidated. The expression pattern of *lfy* is very similar to that of *flo*, which is diagrammed in Figure 1. Both *flo* and *lfy* RNA are detected by in situ hybridization in very young floral meristems and transiently in flower organ primordia. However, there is one important difference, which has given some clues as to how meristems may develop and evolve.

This difference arises from the fact that Antirrhinum flowers are subtended by bracts but those of Arabidopsis are not. The Antirrhinum *flo* gene is expressed in the bract primordia as well as in floral meristems. By contrast, in situ hybridization against wild-type Arabidopsis has shown that there is no expression of *lfy* in regions subjacent to the flower meristems, which might have been thought to correspond to the bract-forming regions in Antirrhinum. In the *lfy* mutant, shoots or "flowers" with subtending cauline leaves appear where bractless flowers would normally arise. These cauline leaves may be equated with bracts, suggesting that the wild-type product of *lfy* normally suppresses bract formation or initiation. Interestingly, the subtending bract primordia of *lfy* mutants do accumulate *lfy* RNA. To reconcile these observations, it has been proposed that *lfy* is expressed in a common group of cells, or anlage, that in the wild type gives rise to a flower but in the *lfy* mutant gives rise to both a "flower" and a subtending bract (Weigel et al., 1992). This suggests that the *lfy* gene affects the partitioning of a group of cells on the periphery of the inflorescence meristem; in wild type, all or most of the cells form the floral meristem, whereas in *lfy* mutants, the cells are

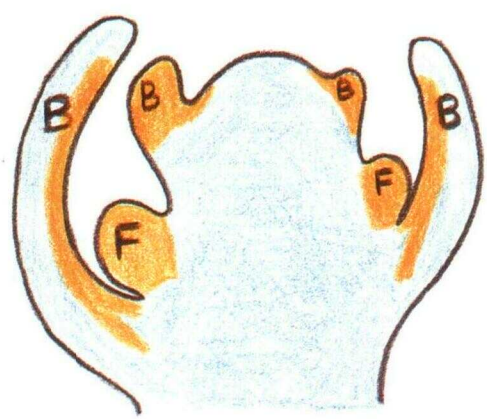

Figure 1. Typical RNA Expression Patterns for a Meristem Identity Gene.

Inflorescence apex with bracts (B) and floral meristems (F) initiating on its periphery. Lower meristems represent progressively older stages of development. The regions expressing the meristem identity gene *flo* are shown in orange. In the case of Arabidopsis, the floral meristems are not subtended by bracts.

partitioned into a meristem and a bract primordium. In this respect, the *lfy* mutant is similar to the wild type of species such as Antirrhinum that also partition their cells into a meristem and a bract. Thus, the way that cells are partitioned into meristems and primordia is not a fixed character but can change in evolution. How might this arise?

The presence of bracts is generally considered to be an ancestral condition, and their absence in Arabidopsis and other members of the Cruciferae is a derived condition (Saunders, 1923). The role of *lfy* in meristem partitioning may therefore have been acquired quite recently in evolution. Originally, *lfy* was presumably expressed in flower meristems and bract primordia, as in Antirrhinum. Subsequently, in a common ancestor of the Cruciferae, it may have acquired a role in recruiting cells that would normally form the bract to become part of the floral meristem. One possible advantage of recruiting all cells to form a flower without the attending bract is that flower development is accelerated. In *lfy* mutants, for example, the development of a meristem is retarded when subtended by a bract (Weigel et al., 1992), possibly because it starts off with fewer cells.

Another gene involved in floral meristem identity is *squamosa* (*squa*) in Antirrhinum and its counterpart *apetala1* (*ap1*) in Arabidopsis (Irish and Sussex, 1990; Huijser et al., 1992; Mandel et al., 1992b). In *squa* mutants, instead of flowers, inflorescence-like shoots are produced that may eventually give rise to malformed flowers. These flowers often appear to be deformed, but they can have all of the normal floral organs, although their number and arrangement are usually aberrant. In *ap1* mutants, flowers are formed in their normal positions, but they lack petals and in the first whorl they have leaflike organs bearing flowers in their axils. There is therefore a loss of lateral determinacy even though apical determinacy appears normal. One interpretation of the *ap1* phenotype is that the cells that normally give rise to the outer two whorls are partitioned in a new way to produce bracts with subtending floral meristems rather than alternating sepal and petal primordia. Both *squa* and *ap1* products belong to the MADS family of transcription factors, which includes mammalian SRF and yeast MCM1. Members of this family share a region of homology covering ~ 50 amino acids (the MADS box), but *squa* and *ap1* are more similar to each other over their entire length (68% identity) than to any other member of this family. Their expression patterns are also very similar; in both cases, RNA is detected in very young floral meristems and in sepal and petal primordia, although *squa* is also expressed in carpel primordia and *ap1* is not.

The interactions between each pair of floral meristem identity genes, *flo* and *squa* in Antirrhinum and *lfy* and *ap1* in Arabidopsis, have also been studied. In *flo* mutants, the *squa* gene is expressed, and in *squa* mutants, *flo* is expressed, showing that *flo* and *squa* are activated independently (Huijser et al., 1992). Similarly, *lfy* is expressed in *ap1* mutants (Weigel et al., 1992). This independence is confirmed by the analysis of double mutants. Plants mutant for both *ap1* and *lfy* are more extreme in phenotype than plants carrying only one of the mutations: the double mutants do not produce flowers or carpel-like organs but continue to proliferate inflorescence-like shoots. The increased severity of the double mutants as compared to either single mutant indicates that these genes are activated independently and that they act synergistically to promote floral development (Huala and Sussex, 1992; Weigel et al., 1992). Similarly, weak alleles of *flo* appear to greatly enhance the *squa* phenotype, sometimes resulting in a failure to produce any flowers (R. Carpenter, S. Doyle, and E.S. Coen, unpublished results). Thus, in each species, at least two meristem identity genes (*flo* and *squa* or *lfy* and *ap1*) act together to promote flower development.

The general picture that emerges from these studies is of an overall concordance in the structure and function of meristem identity genes between Antirrhinum and Arabidopsis, but with important differences in some aspects of expression or function. The extent of these differences often depends on the developmental age or environment of the plant. For example, the production of carpel-like structures on *lfy* mutants occurs much more readily at later stages of inflorescence growth and in plants grown in long days rather than short days (Huala and Sussex, 1992). Similarly, the rare carpels seen on *flo* mutants are observed only on very old plants (R. Carpenter and E.S. Coen, unpublished results). This suggests that the balance in the action of meristem identity genes and their interactions with other gene products could vary with species, age, or environmental circumstance, accounting for the phenotypic variation observed.

One area of future research will be to identify and isolate further genes involved in the control of floral meristem identity. Good candidates are *centroradialis* (*cen*) in Antirrhinum and *terminal flower* (*tfl*) in Arabidopsis (Coen, 1991; Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). The inflorescences of both Antirrhinum and Arabidopsis are normally indeterminate: they continue to produce flowers in lateral positions but never at the apex, which would terminate their growth. This correlates with the observed expression of *flo*, *lfy*, *squa*, and *ap1* in flanking meristems but not in the apical dome of the inflorescence (Figure 1). However, flowers are produced at the apex of *cen* and *tfl* mutants and, as expected, genes such as *lfy* are expressed in the apices of these mutants (Weigel et al., 1992). Furthermore, environmental conditions, such as short daylength, that exacerbate the *lfy* phenotype have the complementary effect of attenuating the *tfl* phenotype. Thus, *cen* and *tfl* appear to be antagonistic to genes promoting floral development and may prevent their expression in the inflorescence apex.

CONTROL OF ORGAN IDENTITY

Several mutations affect the identity of floral organs. The phenotypes conferred by most of these affect two adjacent whorls

and can be divided into three classes: (1) mutants that have carpels growing in place of sepals and stamens in place of petals, (2) mutants that have sepals instead of petals and carpels instead of stamens, and (3) mutants that have petals instead of stamens and sepals or petaloid organs instead of carpels. Each class of mutation corresponds to a loss in one of the three homeotic functions *a*, *b*, or *c*. These homeotic functions act in combination to specify organ type, so that in wild-type floral meristems, the combinations in whorls 1 to 4 are *a*, *ab*, *bc*, and *c*, corresponding to sepals, petals, stamens, and carpels, respectively (Coen and Meyerowitz, 1991). To account for single and double mutant phenotypes, it has been proposed that *a* and *c* are antagonistic to each other and act in mutually exclusive domains but that *b* is established independently of *a* or *c*. It is important to distinguish between genes and homeotic functions because several genes may be involved in establishing each function. Significant progress has been made in understanding the action and regulation of *b* and the basis of the antagonism between *a* and *c*.

Two genes, *deficiens* (*def*) and *globosa* (*glo*), required for the *b* function in Antirrhinum have been studied in detail (Schwarz-Sommer et al., 1992; Trobner et al., 1992). Both of these genes belong to the MADS family of transcription factors. Homology between these genes is very low outside the MADS box, but they show some homology in a region termed the K box, which is located downstream of the MADS box. The K box plays an important role, because amino acid substitutions in this region create temperature-sensitive mutations in both the Antirrhinum genes and their Arabidopsis homologs (Schwarz-Sommer et al., 1992; Trobner et al., 1992). In vitro translation of *def* and *glo* cDNAs indicates that the encoded proteins, DEF and GLO, can bind to DNA as heterodimers but not as homodimers. The core consensus DNA sequence recognized by the DEF/GLO heterodimer is closely related to that recognized by yeast MCM1 and mammalian SRF. These in vitro studies readily explain why mutations in either *def* or *glo* confer the same class *b* phenotype: both gene products are partners necessary for the same transcriptional activity.

Two genes in Arabidopsis, *apetala3* (*ap3*) and *pistillata* (*pi*), also confer the class *b* phenotype. The *ap3* gene encodes a product that is 58% identical to that of *def*, and it seems likely that *pi* will turn out to be the counterpart of *glo* (Jack et al., 1992).

The expression patterns of *ap3*, *def*, and *glo* have been determined by RNA in situ hybridizations (Jack et al., 1992; Schwarz-Sommer et al., 1992; Trobner et al., 1992). In each case, expression is first detected at a developmental stage when sepal primordia (whorl 1) start to appear as small bulges on the perimeter of the floral meristem. As illustrated in Figure 2, expression becomes strongest in the regions destined to form petal and stamen primordia (whorls 2 and 3). Thus, even though Arabidopsis meristems are much smaller than those of Antirrhinum, activation of *b* function genes occurs at a very similar morphological stage of development. This stage, when the sepal primordia are visible but the petal, stamen, and carpel primordia are not, has been called the floritypic stage because it may represent an evolutionarily conserved

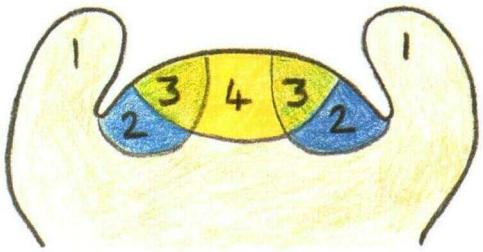

Figure 2. Floral Meristem at the Floritypic Stage.

Whorls 1 to 4 are indicated. The region expressing *b* function genes is blue, the region expressing *c* function genes is yellow, and the region expressing both *b* and *c* function genes is green. In Antirrhinum, it is not clear whether whorl 4 is defined during or just after the floritypic stage.

stage at which many of the key homeotic genes have been activated (Bradley et al., 1993). After the floritypic stage, expression of the *b* genes continues to be high in whorls 2 and 3 as their primordia appear and develop into mature organs. This expression pattern correlates very well with the proposed action of the *b* function in whorls 2 and 3, indicating that transcriptional regulation of these genes plays an important role in determining the domain of *b* activity.

Several experiments have addressed the question of how the expression of these genes is established and maintained. At early developmental stages, in situ hybridizations using a *def* probe on *glo* mutants or a *glo* probe on *def* mutants give similar results to hybridizations on wild type, showing that the establishment of *def* and *glo* expression patterns is mutually independent (Trobner et al., 1992). Similarly, early *ap3* expression is unaffected by *pi* mutations (Jack et al., 1992). However, at later developmental stages, similar hybridizations reveal a marked reduction of expression in mutants relative to wild type, indicating that each gene is involved in the upregulation of its partner. To account for this, it has been proposed that the DEF/GLO heterodimer is directly involved in the upregulation of *def* and *glo* during late stages of development; possible binding sites for DEF/GLO in the promoters of these genes have been identified (Trobner et al., 1992).

One gene that appears to be involved in the establishment of the *b* expression pattern, called *flo10* or *superman* (*sup*), has been identified in Arabidopsis (Schultz et al., 1991; Bowman et al., 1992). Plants carrying mutations in this gene have flowers with extra whorls of stamens in their center instead of carpels. Genetic analysis indicates that the *b* function is ectopically expressed in whorl 4 of these flowers so that the combination of homeotic functions is *a*, *ab*, *bc*, *bc* instead of *a*, *ab*, *bc*, *c*. This is confirmed by in situ hybridizations, which show that expression of *ap3* extends into whorl 4 in young floral meristems of *sup* mutants (Bowman et al., 1992). Thus, one role of *sup* in wild-type plants is to prevent *ap3* expression in whorl 4.

A second area of intensive research has been analysis of the *a* and *c* functions. It has been proposed that *a* and *c* are antagonistic so that they act in mutually exclusive domains:

a in whorls 1 and 2, which contain sterile organs (sepals and petals), and *c* in whorls 3 and 4, which contain sexual organs (stamens and carpels). Each of these functions can become active in all four whorls when its antagonist is inactivated by mutation. This is reflected in their complementary mutant phenotypes: flowers with mutations affecting *a* have sex organs in the outer whorls in place of sterile organs, whereas flowers lacking *c* have sterile organs in the central whorls instead of sex organs. The *agamous* (*ag*) gene is required for the c function in Arabidopsis and encodes a protein belonging to the MADS family (Yanofsky et al., 1990). Its counterpart required for the *c* function in Antirrhinum, *plena* (*ple*), encodes a protein that is 64% identical to that encoded by *ag* (Bradley et al., 1993). In situ hybridization shows that *ag* and *ple* RNAs are first detected during the floritypic stage in a region destined to form stamen and carpel primordia (whorls 3 and 4; Figure 2), and expression continues in these whorls as their primordia emerge and develop into mature organs (Bowman et al., 1991; Drews et al., 1991; Bradley et al., 1993). This correlates with the proposed region of *c* activity in whorls 3 and 4, indicating that transcriptional regulation of *ag* and *ple* plays a role in determining the domain of *c* action.

According to this view, mutants showing a gain of *c* activity in whorls 1 and 2 should show a corresponding gain in *ag* or *ple* RNA. This was tested in Arabidopsis by in situ hybridization using an *ag* probe against recessive *apetala2* (*ap2*) mutants, which lack the *a* function. As predicted, high levels of *ag* RNA were detected in whorls 1 and 2 (Drews et al., 1991). Thus, one role of the *a* function in Arabidopsis is to prevent *ag* RNA accumulation in the outer two whorls of the flower. Bradley et al. (1993) came to a similar conclusion from studies in Antirrhinum through the analysis of a very different type of mutation. Unlike the other mutations described so far, all of which are recessive, mutations affecting the *a* function in Antirrhinum are semidominant. This suggests that the action of *a* is actively prevented by these mutations, even when they are heterozygous with wild type (Carpenter and Coen, 1990). The mutations are caused by transposon insertions in an intron of the *c* function gene, *ple*, resulting in ectopic expression of *ple* in whorls 1 and 2. One interpretation is that the intron of *ple* plays a role in preventing *ple* expression in whorls 1 and 2 of wild-type flowers, and this process can be blocked by insertion of a transposon. These observations also suggest that expression of *ple* in whorls 1 and 2 is sufficient to promote the *c* function and hence sex organ development. A similar conclusion has been derived from studying the effects of ectopically expressing *ag* in transgenic plants: sex organs are produced in the outer whorls of the flower (Mandel et al., 1992a; Mizukami and Ma, 1992). Unlike in Arabidopsis, no recessive, loss-of-function mutants in the *a* function have been described in Antirrhinum. Whether this points to a fundamental difference between the two species or to a more trivial one (e.g., that there is more redundancy in Antirrhinum) remains to be shown.

In addition to affecting organ type, many of the organ identity genes also influence meristem determinacy. Mutations giving loss of *c* function result in flowers containing a proliferation of whorls and hence an indeterminate apical growth pattern (lateral determinacy is still preserved). Conversely, a gain of *c* expression caused by loss of *a* can, in some cases, result in fewer whorls than in wild type (Drews et al., 1991). Mutations that give a loss of *b* function can sometimes result in flowers with only three whorls, suggesting that determinacy has been activated prematurely (Coen, 1991). Conversely, an increase in *b* function activity in inner whorls, as seen in *flo10lsup* mutants in Arabidopsis, reduces determinacy and gives additional whorls (Schultz et al., 1991; Bowman et al., 1992). Another way to describe these results is that organ identity genes can determine whether groups of cells become primordia or continue as meristems. In wild-type floral meristems, for example, the cells in the central region at the floritypic stage are destined to become carpel primordia, whereas in mutants that have lost the *c* function, these cells retain their meristematic behavior and continue to generate primordia indeterminately.

INTERACTIONS BETWEEN MERISTEM AND ORGAN IDENTITY GENES

Although the details of how meristem and organ identity genes interact are still unknown, some principles are starting to emerge. It is convenient to consider each of the homeotic functions in turn.

Mutations in the Arabidopsis *a* function gene *ap2* can sometimes enhance the phenotype of mutations in the meristem identity genes *ap1* and *lfy*, suggesting that *ap2* may also play a role in establishing meristem identity (Irish and Sussex, 1990; Huala and Sussex, 1992). Conversely, the strong early expression of the meristem identity gene *flo* in the outer two whorls of the Antirrhinum flower has led to the suggestion that it may be involved in promoting the *a* function (Coen et al., 1990). Perhaps meristem identity genes and organ identity genes such as *ap2* collaborate to establish the *a* function. However, if this is true, how do meristem identity mutants eventually manage to produce "flowers" with an apparently active *a* function (i.e., with sepals or petals)? The simplest explanation is that there is some genetic redundancy so that a mutation in a meristem identity gene may eventually be overcome by some other route. The isolation and analysis of *ap2* should help to clarify these interactions (see Okamuro et al., 1993, this issue).

Activation of the *b* function seems to require *flo* or *lfy* expression because even when "flowers" are produced in *flo* or *lfy* mutants, they consist of only sepals and carpels. This has led to the suggestion that *lfy* enhances transcription of *b* function genes such as *ap3* in Arabidopsis (Weigel et al., 1992). In Antirrhinum, transcripts of the *b* function gene *def* are not normally detected in the meristems of *flo* mutants (S. Hantke, R. Carpenter, and E.S. Coen, unpublished results). However, it seems likely that the activation of *def* by *flo* is indirect, because *flo* expression is very weak in the third whorl by the time

that *def* transcripts begin to be detected there. The activation may be mediated by other genes such as *sepaloidea*, which can give a class *b* mutant phenotype in Antirrhinum (Carpenter and Coen, 1990).

Transcripts of the *c* function gene *ple* are not normally detected in the meristems of *flo* mutants, although they are present in the rare carpelloid "flowers" (D. Bradley, R. Carpenter, and E.S. Coen, unpublished results). This indicates that *flo* is involved in activating *ple* transcription but that this requirement can eventually be circumvented. As with the activation of *def*, it seems unlikely that *ple* activation is direct, because *flo* is expressed very weakly in the central dome of the meristem by the time *ple* RNA is detected. Analysis of double mutants in Arabidopsis indicates that the *ag* gene is active in the "flowers" of *lfy* mutants (Huala and Sussex, 1992; Weigel et al., 1992). It is not known if *ag* is expressed in the inflorescence shoot meristems that replace lower flowers in *lfy* mutants.

The general conclusion from these studies is that the meristem identity genes may activate and/or act in parallel with organ identity genes. However, a further complication is that the organ identity genes may in turn regulate the expression of meristem identity genes. For example, *flo* is not expressed in whorl 3, possibly because the *b* and *c* functions negatively regulate its expression (Coen et al., 1990). In agreement with this view, mutants lacking *b* or *c* express *flo* in whorl 3 (S. Hantke, R. Carpenter, and E.S. Coen, unpublished results). Similarly, the absence of *ap1* expression in whorls 3 and 4 has been proposed to be due to negative regulation by the *c* function gene *ag* (Mandel et al., 1992b). The functional significance of these interactions should be revealed by studies in which the meristem identity genes are expressed ectopically.

FUTURE PROSPECTS

The equivalence of flowers and shoots claimed by Goethe has been fully vindicated by molecular genetic analysis. Genes that affect one or more of the key aspects of flowers have been isolated and studied in detail. However, many new problems have been raised by these investigations. How are environmental and developmental signals connected to the activation of meristem identity genes? How do meristem identity genes interact with organ identity genes to establish specific patterns of expression? How do these genes affect the way that cells are recruited or partitioned? How do meristem or organ identity genes affect target genes involved in organ morphogenesis?

A variety of approaches will be needed to solve these problems. Many more genes that interact with the ones so far described need to be isolated and characterized. Physiological, morphological, and biochemical studies of gene action are still in their infancy. The use of mosaics and chimeras also needs to be exploited to assess whether genes are acting autonomously or whether cell–cell signaling is involved (for some recent examples, see Carpenter and Coen, 1990; Szymkowiak and Sussex, 1992). The combination of these different approaches should provide us with a much deeper understanding of how flowers develop.

A great advantage of studying plants is the large number of genetically well-characterized species. In addition to Antirrhinum and Arabidopsis, the molecular genetics of flower development is now being extended to other species such as tomato (Pneuli et al., 1991), petunia (see van der Krol and Chua, 1993, this issue), and maize (see Veit et al., 1993, this issue). So far, the conclusion to emerge from all of these studies is that many of the basic mechanisms controlling flower development have been conserved in evolution but that important differences in the balance and interactions of genes also exist. It should be possible to further investigate the molecular basis of these differences further by introducing genes from one species into another. For example, does the *flo* gene of Antirrhinum complement the *lfy* mutant of Arabidopsis? It will also be particularly interesting to study genes for which there are no obvious counterparts in other species. The *cycloidea* gene is involved in the control of dorsoventral asymmetry (zygomorphy) in Antirrhinum (Carpenter and Coen, 1990). Does *cycloidea* have a counterpart in species such as tomato or Arabidopsis, which have radially symmetrical flowers? There can be little doubt that the answers to many of these questions will provide new insights into the mechanism and evolution of flower development.

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