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Gene regulation of flowering

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SUMMARY

A major change in the development of plants occurs upon floral induction. Meristems in certain positions become organized to form flowers. We are studying this process using a combination of genetic, molecular and physiological approaches in *Antirrhinum*. In particular, we are exploiting transposon-induced mutations in genes controlling early switches in floral development. These mutations cause homeotic and heterochronic phenotypes and three categories of genes have been identified. The first includes *floricaula* (*flo*), which is required to switch inflorescence meristems to a floral state. This gene has been isolated and shown to be expressed transiently in bract, sepal, petal and carpel primordia. The second group of genes controls the identity (and sometimes the number) of organs in a whorl. These genes affect overlapping whorls and their mutant phenotypes suggest a combinatorial model for gene action in determining the fate of floral primordia. Some of the regulatory interactions between these genes have been revealed by studying *cis*- or *trans*-acting mutations which have resulted in ectopic gene expression. Genes of the third category determine the identity of organs within one whorl and thus affect the symmetry of the flower. We propose that the interactions of these homeotic genes control the basic patterns of inflorescence and flower development not only in *Antirrhinum*, but also in a diverse range of plant species.

1. INTRODUCTION

Plants develop by the sequential addition of structures to the main body. These structures originate from lateral meristems initiated on the flanks of the main apical meristem. The apical meristem is an undifferentiated tissue and throughout development it integrates signals to determine the identity and positioning of these lateral meristems. Typically, plants initially undergo a vegetative phase of development whereby leaves and shoots originate from these meristems. At a later stage, floral induction may occur whereby these lateral meristems are induced to form completely different structures, flowers rather than leafy shoots.

A number of genes affecting the identity of these different meristems have now been isolated from a variety of plant species (Coen & Meyerowitz 1991). Our own study focuses on three classes of gene in *Antirrhinum* that upon mutation give homeotic and, in some cases, heterochronic phenotypes. These genes can affect the switch in the type of meristems produced, the identity of primordia arising in floral meristems, or the switching of a meristem from indeterminate to determinate growth.

Antirrhinum majus has many genetical and physiological attributes that make it amenable to study: its large flowers are easily emasculated, crossed and scored, the different phases of vegetative and reproductive development are easily seen and can be modified environmentally, and its well-characterized transposable elements allow genes to be isolated and clonal sectors of wild-type or mutant tissue to be studied through development (Carpenter & Coen 1990). This paper will focus on *Antirrhinum*, although reference will be made to the other well-analysed species *Arabidopsis*, allowing the conservation or divergence of molecular mechanisms to be compared between two taxonomically distant species.

2. DESCRIPTION OF WILD-TYPE PLANT DEVELOPMENT AND MUTAGENESIS STRATEGY

The initial phase of *Antirrhinum* growth involves a vegetative apical meristem that produces pairs of leaves opposite to each other at each node in a decussate phyllotaxis (i.e. each pair is at a right-angle to the previous). After a period of vegetative growth the apex changes to an inflorescence meristem, producing smaller leaves (bracts) in a spiral arrangement, generally with a single bract at each node (figure 1*a*). In the axils of these bracts, floral meristems are initiated which produce four whorls of organs separated by very short internodes, so that consecutive whorls appear adjacent to each other.

Antirrhinum flowers are zygomorphic: they can be divided into mirror-image halves by a single plane. The flower consists of four whorls of organs, numbered from the outside of the flower; thus whorl 1 is

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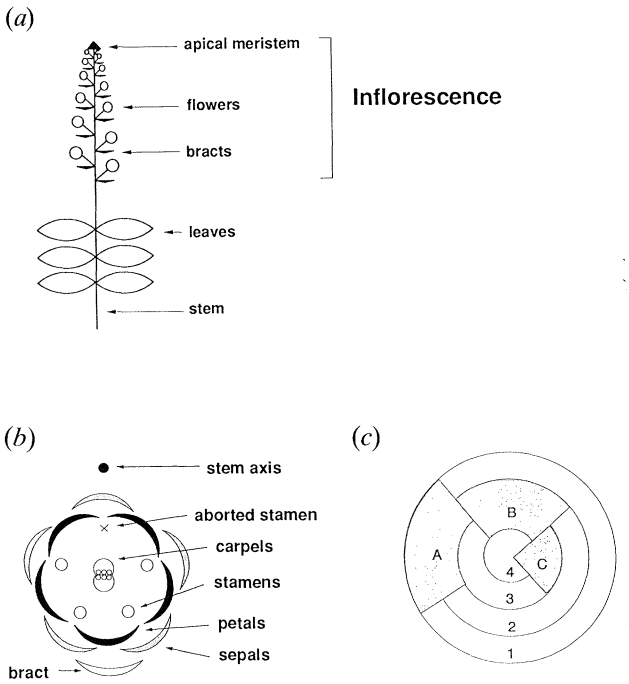

Figure 1. Flowering in *Antirrhinum majus*. (*a*) Schematic illustration of wild-type *Antirrhinum majus* development. (*b*) Floral diagram showing sepal, petal, stamen and carpel organs arranged in four consecutive whorls in the axil of a bract. (*c*) Schematic drawing of the four whorls (1–4) and three regions (A, B and C) of a flower.

outermost and whorl 4 is central (figure 1). The whorl primordia appear sequentially: whorl 1 first, 2 and 3 almost simultaneously and finally whorl 4 (Awasthi *et al.* 1984). Organs within a whorl are upper, if closer to the stem (adaxial), or lower if nearer the bract (abaxial). Whorl 1 consists of five sepals and whorl 2, the corolla, contains five petals which are fused for part of their length, but end in five separate lobes. In whorl 3, five stamen primordia are initiated, though the uppermost fails to develop fully. Whorl 4 is occupied by two united carpels forming a gynoecium with a bilocular ovary (figure 1*b*).

To isolate and study homeotic genes in *Antirrhinum* we have carried out an extensive transposon mutagenesis experiment (Carpenter & Coen 1990). Plants carrying active transposons were grown at 15°C, a temperature inducing transposition in *Antirrhinum* (Carpenter *et al.* 1987), and self-pollinated to give 26 000 progeny, many of which were expected to carry recessive mutations in a heterozygous condition. The homozygous phenotypes were revealed by self-pollination and growing 80 000 plants. Over 15 independent homeotic mutations were obtained from this screen and here we present our conclusions derived from a study of some of the genes involved.

3. GENETIC CONTROL OF THE FIRST STEPS IN FLORAL DEVELOPMENT

The transition from inflorescence to floral meristems represents the first step specific to the floral developmental pathway. Mutants that are unable to carry out this transition might be expected to produce proliferating inflorescence shoots in place of flowers. One mutant obtained with this phenotype is *floricaula* (*flo*) (Carpenter & Coen 1990). The *flo* mutant initiates vegetative growth and the transition to inflorescence meristem in a similar manner to wild-type. However, instead of flowers being produced in the axils of bracts, indeterminate shoots bearing further bracts are produced, each shoot having two opposite bracts at the base followed by a spiral of single bracts (figure 2). Each of these shoots in turn produces further shoots in the axils of their bracts and this sequence is perpetuated. The wild-type *flo* product is therefore necessary for the transition between inflorescence and floral meristems so that in its absence the inflorescence programme is continually reiterated. The *flo* mutant can be considered to be homeotic because it results in one structure (the flower) being replaced by a homologous structure (indeterminate shoot). This mutant can also be viewed as heterochronic (changing the relative timing of events) because it results in an early developmental programme (inflorescence meristem) being continually reiterated.

Other mutants have been described in *Antirrhinum*, such as *squamosa*, which produce inflorescence-like shoots in the axils of bracts, although many of these shoots eventually produce flowers (Stubbe 1966; Huijser *et al.* 1992). Interestingly, although plants with stable *floricaula* alleles never produce flowers, under certain conditions the inflorescence meristem may eventually terminate in carpel-like structures. A similar mutant, *leafy*, has been found in *Arabidopsis* (Haughn & Sommerville 1988; Wiegel *et al.* 1992).

A second class of mutant has almost the opposite effect of those described above because it promotes the conversion of inflorescence to floral meristems in positions where this would not normally occur. Plants with indeterminate inflorescences, such as *Antirrhinum*, do not produce terminal flowers. Thus, the apical inflorescence meristem does not itself undergo the transition to floral. The *centroradialis* mutant in *Antirrhinum* produces terminal flowers, suggesting that the wild-type gene product inhibits this apical conversion (Kuckuck & Schick 1930).

The *flo* gene of *Antirrhinum* has been isolated and extensively characterized (Coen *et al.* 1990). It produces a transcript of about 1.6 kb which has the potential to encode a protein whose sequence shows no extensive homologies with any other proteins in available data-banks, though it contains some motifs found in transcription factors. This suggests that FLO may be a transcriptional activator, although other roles cannot be excluded. RNA analysis reveals that *flo* is induced within two days when plants are shifted to daylength conditions that promote flowering. *In situ* hybridization shows that *flo* is expressed from a very early stage in wild-type inflorescences in a very specific temporal and spatial sequence. The earliest expression seen is in bract primordia and is followed by expression in sepal, petal and carpel primordia, but no expression is seen in stamen primordia. Expression in each organ is transient and is not observed in later stages of development. Taken together, these results

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Figure 2. Part of the inflorescence of a *flo* mutant (left) and wild-type (right). All but one of the structures axillary to the bracts have been removed from each plant for clarity.

suggest that *flo* acts not only as a switch between inflorescence and floral meristems, but is also involved in directing specific patterns of gene expression in the early floral meristem.

4. GENETIC CONTROL OF ORGAN IDENTITY

Homeotic mutations were also isolated that act after the inflorescence to floral transtion has taken place. Many of the genes involved affect the identities of organs in two adjacent whorls (Carpenter & Coen 1990; Sommer *et al.* 1990) and for simplicity three overlapping regions (as illustrated in figure 1*c*) can be defined for wild-type: A (whorls 1 and 2); B (whorls 2 and 3); C (whorls 3 and 4). One class of mutant, *ovulata*, affects region A and gives carpels instead of sepals in whorl 1 and stamens in place of petals in whorl 2, giving the overall phenotype carpel, stamen, stamen, carpel.

The second class affects region B and gives sepals instead of petals in whorl 2 and carpels instead of stamens in whorl 3, giving the phenotype sepal, sepal, carpel, carpel (though whorl 4 does not always develop). This class includes the *deficiens* (*def*), *globosa* (*glo*) and possibly some *sepaloidea* (*sep*) mutants. A third class (e.g. *plena*) affects region C and gives petals in place of stamens in whorl 3 and variable structures in whorl 4. Similar classes of whorl identity genes have been found in *Arabidopsis* (Bowman *et al.* 1989; Haughn & Sommerville 1988) suggesting that the mechanisms controlling whorl identity have been highly conserved in evolution. Furthermore, *def* (affecting region B) and *agamous* (an *Arabidopsis* gene affecting region C) share a highly conserved sequence of about 50 amino acids that is also found in yeast and mammalian transcription factors (Schwarz–Sommer *et al.* 1990).

The action of many gene functions in overlapping regions could give each whorl a unique combination of functions. This might provide sufficient information to specify each whorl's identity. However, in order to model these gene interactions it is necessary to introduce some constraints as to how these gene functions are established. The simplest model allows functions active in region B to be established independently of the others, while gene functions of regions A and C are antagonistic (Carpenter & Coen 1990). Consequences of this antagonism have been revealed at the molecular level in *Arabidopsis* and *Antirrhinum*. In wild-type *Arabidopsis* flowers, the gene required for the C function, *agamous*, is expressed only in whorls 3 and 4. However, if the A function is removed, by mutation of *apetala*-2, then *agamous* extends its domain of expression to all whorls (Drews *et al.* 1991). A complementary test for the model is revealed through mutations of *plena*, the *Antirrhinum* homolog of *agamous*, that result in a gain of C function. In this case, certain *cis*-mutations cause ectopic *plena* expression that allows the C function to become established in all whorls, generating a phenotype similar to loss of the A function.

5. REGULATION OF GENES THAT SPECIFY WHORL IDENTITY

Two general types of model can be imagined to explain the expression patterns of genes that deter-

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mine whorl identity. In a purely spatial model, concentric fields could be set up in the early floral meristem, independently of the sequence of primordium initiation. The gene functions restricted to the defined regions A, B and C could be activated in the appropriate field and hence specify the fate of primordia growing out from the various regions of the meristem. This type of model is formally similar to models proposed for the control of segment identity in *Drosophila* (Ingham 1988). In the sequential type of model, which shares some features of early models of flower development (Heslop-Harrison 1964), the consecutive growth of the primordia would be essential for the establishment of the domains of activity. The various gene functions would be activated in a manner that reflected the sequence of primordium initiation.

Some supporting evidence for the latter sequential model has come from studies on the *flo* gene of *Antirrhinum*. As described above, *flo* is expressed sequentially and appears to be activated in regions where primordia are being initiated. A further feature of *flo* expression is that it occurs transiently in bract, sepal, petal and carpel but not stamen primordia. This pattern suggests that *flo* not only acts to switch inflorescence to floral meristems, but also interacts in a sequential manner with the whorl identity genes that pattern the floral meristem (Coen *et al.* 1990).

6. GENETIC CONTROL OF WHORL NUMBER

Some of the whorl identity mutants also affect whorl number. Extreme mutants in genes affecting region B often give fewer whorls than wild-type. Mutants in genes acting in region C usually have an increase in the number of whorls and a more-or-less indeterminate growth pattern. These findings reveal two functions of the whorl identity genes, namely the control of organ fate and the control of whorl number. The extent to which these separate roles are mediated by the homeotic genes activating particular pathways or target genes is not fully understood.

7. GENETIC CONTROL OF DIFFERENCES WITHIN A SINGLE WHORL

In radially symmetric flowers (actinomorphic), all organs in the same whorl have very similar or identical morphologies, while in zygomorphic flowers (e.g. *Antirrhinum*), one or more whorls have organs which are distinct. The *cycloidea* (*cyc*) mutations in *Antirrhinum* give flowers with a more radially symmetric appearance than wild-type (Stubbe 1966) and four such mutations were isolated in our transposon mutagenesis screen. Extreme *cyc* mutations give flowers with petals all resembling the lowest petal of wild-type. Therefore, *cyc* is a homeotic mutation since organs of one type are replaced by another type. Furthermore, *cyc* appears to affect several whorls, since all stamens develop in *cyc* mutants compared to the abortion of the upper stamen in wild-type.

Does *cyc* affect organs in the same way irrespective of the whorl they occupy? In wild-type, the abortion of the uppermost stamen depends upon *cyc* activity. When stamens are found in whorl 2, in the case of *ovu* mutants, the upper stamens are again aborted, suggesting that *cyc* interacts with primordia in a similar way, irrespective of the whorl they occupy. Thus the fate of a primordium depends on a combination of interactions that determine whorl identity and relative upper or lower position.

8. INFLORESCENCE EVOLUTION

Antirrhinum is among the species with zygomorphic flowers that also have indeterminate inflorescences and so do not normally terminate with a flower. Nevertheless, exceptional plants which produce terminal flowers have been found among these species and they develop terminal flowers with radial symmetry (Peyritsch 1872). This phenotype has been studied in *Antirrhinum* and shown to be due to a recessive allele of the locus *centroradialis*. A few normal flowers are produced laterally in this homeotic mutant, but the apical inflorescence soon produces a radially symmetric terminal flower, with all petals resembling that of the lower petal of wild-type plants, similar to the conversion seen in *cyc*. An explanation for the symmetry of this terminal flower can be found in a polar-coordinate model for zygomorphy (Carpenter & Coen 1990). Floral meristems in axillary positions are in an asymmetrical environment with the main apex above and a bract below them, subjecting them, therefore, to a gradient of *cyc* activity. In contrast, a terminal flower is already in a radially symmetric environment and so may either experience no *cyc* gradient, or no *cyc* activity itself may occur in such an environment.

Mutations such as *centroradialis* also have implications for inflorescence evolution. Some plants may produce a large single flower at the end of their shoots, while in most species flowers are clustered to form inflorescences. In plants with determinate inflorescences, the apical meristem must convert into a floral meristem, with other flowers found in axillary positions or terminating side-branches. An indeterminate inflorescence can produce a quite different array of flowers, as each shoot only produces flowers in axillary positions. The isolation of genes such as *flo*, that regulate the transition from inflorescence to floral meristems, will allow us to address the question of inflorescence evolution at the molecular level. Furthermore, the interactions of *flo* and the organ identity genes should add insight as to how plants overcome the constraints imposed by meristems when trying to pattern and control sequential organ development.

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