Genetic Control of Flower Development by Homeotic Genes in *Antirrhinum majus*

ZSUZSANNA SCHWARZ-SOMMER, PETER HUIJSER, WOLFGANG NACKEN, HEINZ SAEDLER, HANS SOMMER

Homeotic mutants have been useful for the study of animal development. Such mutants are also known in plants. The isolation and molecular analysis of several homeotic genes in *Antirrhinum majus* provide insights into the underlying molecular regulatory mechanisms of flower development. A model is presented of how the characteristic sequential pattern of developing organs, comprising the flower, is established in the process of morphogenesis.

FLOWER FORMATION IN HIGHER PLANTS IS A COMPLEX process controlled by genetic as well as environmental factors (*1–5*). Although it is an integrated process, two major phases can be recognized: floral evocation and development. The term evocation designates the transition of the vegetative apical meristem to a floral meristem, that is, a meristem capable of generating a flower primordium after stimulation by internal or external signals. After evocation, floral development starts with the sequential appearance of flower and floral organ primordia and ends with the mature flower composed of functionally and structurally distinct organs. During this process the type, number, and position of the organs constituting the flower are strictly regulated.

In animals, organogenesis occurs mainly during embryo development. In contrast, organ development in plants is not restricted to the embryonic stage (*2–4*). Differentiation and organogenesis occur throughout the lifetime of the plant organism, sometimes over a span of decades. Morphogenetic processes in plants, therefore, unlike in animals, cannot easily be related to maternally determined positional information or, as plant cells do not move relative to each other, to cell migration. Thus, the question is, what are the mechanisms by which meristematic cells in the primordium sense and interpret their position with respect to other cells and differentiate reproducibly and precisely into the correct organs?

Development of the wrong organ at the wrong place (homeosis) is the consequence of a mutation in a gene that affects differentiation. Such homeotic mutations thus identify genes that direct normal development and are useful for the dissection of mechanisms that direct floral morphogenesis (*3, 6, 7*). The objectives of this article are to introduce *Antirrhinum majus* (snapdragon) and its morphogenetic mutants as an experimental system for the study of flower development and to discuss implications of molecular analysis of the mutants for elucidation of the molecular mechanisms that underlie plant developmental processes. Because similar floral homeotic mutants and molecular analyses exist for *Arabidopsis thaliana* (*8*), flower development in the two systems is compared.

The authors are at the Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, Federal Republic of Germany.

Floral Morphogenetic Mutants

Observations of abnormal flowers have a long tradition (*6, 9*), perhaps because monstrous deviations on such graceful and regular structures are eye-catching. In many instances, however, there is no report on the heritability of the phenotypes, hence limiting the use of the information for current analysis.

Antirrhinum was a main object of classical genetic analysis at the beginning of this century (*10*). The plant has many large, colored flowers with zygomorphic (mirror-image) symmetry. The flowers develop in the axils of lateral bracts on a long inflorescence (Fig. 1) and was thus predestined to be scored for mutant floral phenotypes with altered morphogenetic and color features. Several different classes of morphogenetic mutants are known in *Antirrhinum* that could be useful for analysis of the molecular processes underlying floral development (*10, 11*). Because not all of these mutants have been completely characterized by genetic, morphological, and molecular analyses, the scheme in Fig. 2 is only an approximate presentation of the position and function of these genes in the complex morphogenetic process that begins with floral evocation and ends with the mature flower.

Mutations that affect development of the flower primordium (class I). The rather complex phenotypes of the first type of class I mutants, *sterilis* (*10*) and *steriloides* (*12*), seem to indicate that the gene products may interfere with the hormonal control of initiation and formation of the floral primordium on the flanks of the apical meristem after floral evocation. The mutant *sterilis* displays an abnormal inflorescence carrying only bracts, but no flowers in the axils of the bracts. A phenotypically very similar mutant, *steriloides*, was recently isolated in a transposon mutagenesis program (*12*). The mutant *steriloides* also has bracts only on the inflorescence, but occasionally produces a few (sometimes deformed) flowers, probably due to leakiness or somatic instability of the recessive mutation.

A second type of class I mutants, represented by *squamata* and *squamosa* (*10*), interfere with formation of floral primordia after evocation. The phenotype of both mutants suggests that the function of the wild-type genes is to establish the identity of the floral primordium. Initiation of the primordium seems to be normal in the mutants; but, instead of flowers, "shoots" that resemble inflorescences grow in the axils of the bracts (Fig. 1). In addition, leaves of *squamata* plants display altered morphology that indicates this gene

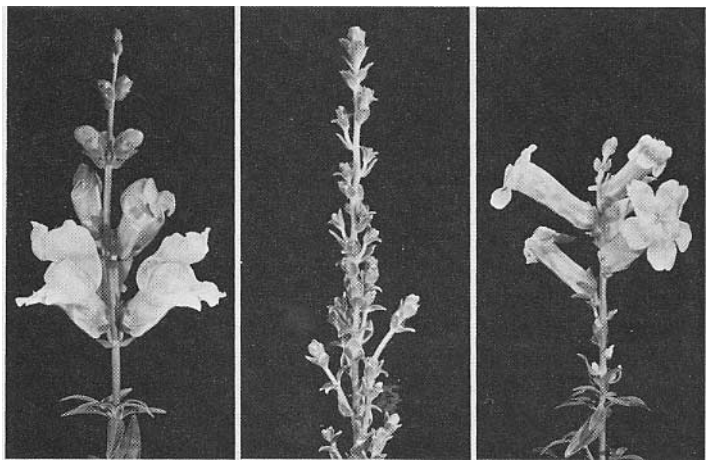
influences both vegetative and reproductive development. Like *steriloides*, *squamosa* occasionally produces a few flowers, probably due to leakiness or instability of the recessive mutation. A phenotypically similar and genetically unstable mutation, *floricaula*, has been recently obtained by transposon tagging (*11*).

Mutations that alter the symmetry of the flower (class II). The symmetry of the flower of various plant species seems to be determined by its position with respect to the shoot or inflorescence axis. For example, terminal flowers, like that of a tulip, are usually radially symmetric (actinomorphic) and lateral flowers, like that of snapdragon, are zygomorphic (*1*). Peloria are unexpectedly occurring actinomorphic flowers on an inflorescence that normally carries zygomorphic flowers. Radial symmetry of lateral flowers may be caused by a malfunction of genes that interpret the position of the floral primordium (type 1). Further, a mechanism (genetic defect or environmental factor) that produces a flower at a terminal position will condition radial symmetry of this flower (type 2). Both contribute to either conditional or heritable pelorism in nature. Because *Antirrhinum* displays an open inflorescence with lateral flowers only (Fig. 1), both types of peloric alterations have been observed.

In the mutant *cycloidea* (Fig. 1), the flowers have a nearly radial symmetric shape and all organs in the respective whorls are arranged in a radial symmetric fashion. Two independent loci are known that, when mutated, confer this phenotype on the flower (*9–11*). Interestingly, mutations in these genes may concomitantly affect the number of organs in several whorls. Several germinally unstable mutant alleles of these genes have been isolated in transposon mutagenesis programs (*10–12*). In a second type of class II mutations, represented by the *centroradialis* mutant (*12*), the inflorescence carries a terminal flower displaying radial symmetry. It has been suggested (*10*) that these class II genes interact with class III genes (see below) to determine the fate of the primordium.

Mutations of homeotic genes that specify organ identity (class III). In *Antirrhinum majus*, homeotic mutations affecting floral organ formation (*10–12*) can be assigned to three different categories: type 1, in which the first and second whorl organs (perianth) are affected; type 2, in which the third and fourth whorl organs (reproductive organs) are transformed with concomitant increase of the number of whorls and organs; and type 3, in which the second and third whorl organs are altered.

Comparison of the phenotypes of these mutants (Figs. 3 and 4) reveals features common to genes that interfere with the determination of floral organ identity. First, in all three types of mutants



Wild type **squamosa** **cycloidea**

Fig. 1. The inflorescence and the flower of *Antirrhinum majus* plants. Genotypes are indicated below the photographs.

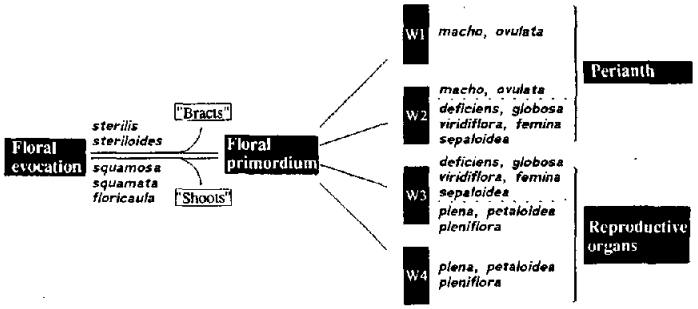


Fig. 2. Spatial and temporal activity of some morphogenic mutants in *Antirrhinum majus*. Quotation marks indicate the change of floral primordium development in the corresponding mutant. W, whorl.

two adjacent whorls are affected simultaneously by mutation of a single gene. This perhaps indicates that the genes are involved in sensing positional information and in interpreting the genesis of specific organs in respective whorls. Second, several independent type 2 and type 3 loci exist that, upon mutation, confer similar homeotic phenotypes on the corresponding types of mutants (for example, the type 3 mutants *deficiens*, *globosa*, *femina*, *sepaloidea*, and *viridiflora*). No other types of single gene mutants are known that show other combinations of simultaneous transformations. For example, carpel-like development of stamens is accompanied by sepal-like development (sepalody) of petals, but never by a stamen-like development (stamenody), although petals have the potential to undergo this type of homeotic change. Thus, the kinds of concomitant organ transformations seem to be limited. Third, it is remarkable that, at least in *Antirrhinum*, no class III mutants have yet been isolated with homeotic alteration of organs in one whorl only. This may be of general significance, because heritable homeotic abnormalities in other plant species also do not indicate the existence of homeotic genes altering the organ identity of either petals or stamina independently (*9*).

Class III mutants of *Antirrhinum*, as well as homeotic mutants of *Arabidopsis* (Fig. 3), are sensitive to environmental signals (*12, 13*), because their phenotype can be systematically influenced by environmental conditions (*14, 15*). Variations of the mutant phenotype along the *Antirrhinum* inflorescence axis also indicate the involvement of endogenous signals. Yet, the dependence of flower morphogenesis on external and internal conditions is not well understood (*5*).

The similarity of the three basic types of homeotic mutants in *Antirrhinum* and *Arabidopsis* (Fig. 3) could indicate that at least some of the genes may have homologous functions in the determination of organ identity. Genes of homologous function may also be conserved in structure. This conservation, however, might not be taken as evidence that the overall mechanisms directing organogenesis are identical in the two species. Homeotic alterations, such as phyllody (transformation of floral organs to leaf-like structures), are typical for some alleles of *apetala-2* in *Arabidopsis* (*14, 15*) and have not yet been observed in *Antirrhinum*. This may be because of the difference in architecture of the inflorescences and the flowers of *Antirrhinum* and *Arabidopsis*.

Instability of Homeotic Mutants

Previous molecular and genetic analyses have shown that mobile insertion elements (*16*) cause the high mutation rate at many loci in *Antirrhinum*. These transposable elements can be used to generate new mutations with high frequency by transposon mutagenesis (*11–13*), as well as to identify and isolate homeotic genes (*13*).

Excision of transposable elements is revealed phenotypically by sectorial reversion of the mutant to wild type. Such somatic excision events are heritable if the progenies of the revertant cell become germinal cells. Excisions can occur at virtually any time during somatic cell proliferation and result in expression of the wild-type gene that can be followed in subsequent cell generations. Thus, "mosaic" structures are generated that may give some insight into the temporal and spatial activity of a particular gene during development.

The morphological analysis of somatic reversion events of *deficiens*^{globifera} (Figs. 2 and 3), for example, led to the following results. First, excision events that occur very late in development of the sepaloid petals in the second whorl restore petaloid features in a clonal manner, and perhaps indicate that the *deficiens* gene acts cell-autonomously (*11, 13*). Second, because of earlier (but still not germinally heritable) somatic excision, the second whorl may consist of near normal petals that still carry stripes of sepaloid tissue. Furthermore, a single second whorl organ may display a sepaloid and a petaloid sector separated by a sharp boundary extending from the bottom to the tip. These observations may indicate that cell groups within an organ primordium are capable of autonomous differentiation, as has also been suggested by analysis of mosaic organs of stable homeotic *Arabidopsis* mutants (*14*). Third, in the third floral whorl of the *globifera* mutant, reappearance of only stamina or staminoid characters was never observed; this suggesting that late restoration of *deficiens* gene activity is unable to rescue staminal organogenesis and, therefore, that *deficiens* gene function is required early in stamen development.

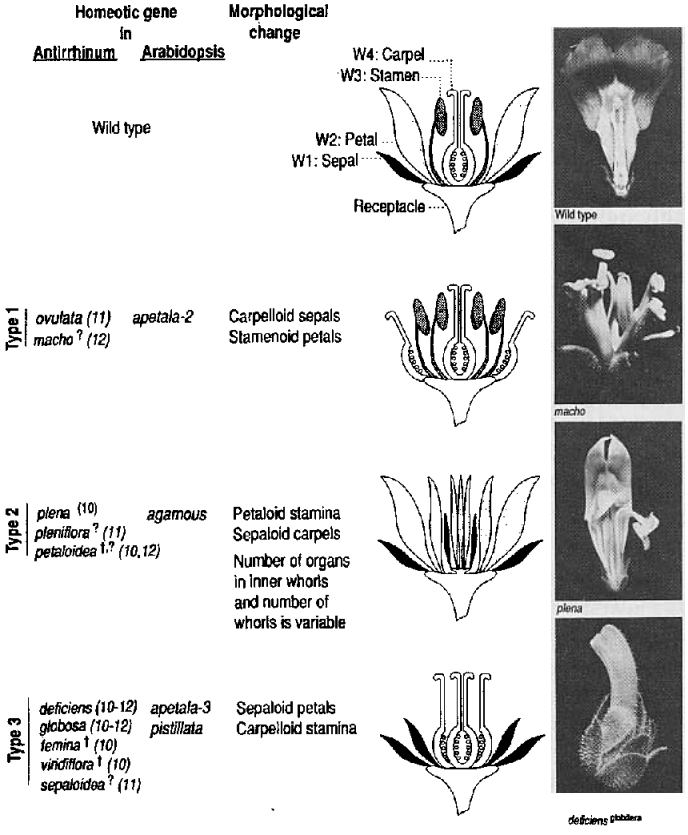


Fig. 3. Compilation of the three types of morphogenic genes (class III) that control floral organ identity in *Antirrhinum* and *Arabidopsis*. The idealized schemes show the direction of transformation of organs. However, in the mutant flowers not all organs in a whorl are equally transformed or transformed in the same direction. The photographs show representatives of each type of mutation in *Antirrhinum*. Except for *deficiens*^{globifera}, the lower lobe of the flowers was removed to reveal the structure of reproductive organs. Mutants of type 1 genes are semidominant in *Antirrhinum* but recessive in *Arabidopsis*. All other mutations are recessive. Symbols: †, mutant isolated but lost; ?, genetic test for allelism with other genes not completed yet. For morphological description of the *Arabidopsis* mutants see (*14, 15, 33*); references for *Antirrhinum* mutants are on the figure.

Homeotic Genes Encode Transcription Factors

The following sections present evidence that the molecular basis of genetic control in plant development in many aspects may be similar, if not identical, to that of animals.

Deficiens *may be a regulatory gene encoding a DNA binding protein.* Recently, the homeotic gene *deficiens* was cloned (*13*). The DEF A protein, encoded by *deficiens*, showed a high degree of homology to the conserved DNA binding and dimerization domains of two known transcription factors in animals and yeast (Fig. 5). In mammals, the serum response factor (SRF) is essential for the serum-inducible transcriptional activation of the c-*fos* nuclear proto-oncogene (*17*) that is involved in the transcriptional regulation of genes controlling cell growth in response to growth factors. In yeast, the MCM1 protein [the product of the minichromosome maintenance gene (*MCM1*) and identical to the general regulator of mating type (GRM) and pheromone receptor transcription factor (PRTF) proteins] participates in the regulation of a- and α-cell–type specific genes (*18, 19*).

That DEF A may be a DNA-binding protein with regulatory functions is substantiated by other evidence as well. For example, a single amino acid exchange in the putative DNA binding domain (*20*) (Fig. 5), which probably decreases the DNA binding affinity, generates the altered phenotype of the *deficiens*^{nicotianoides} allele (Fig.

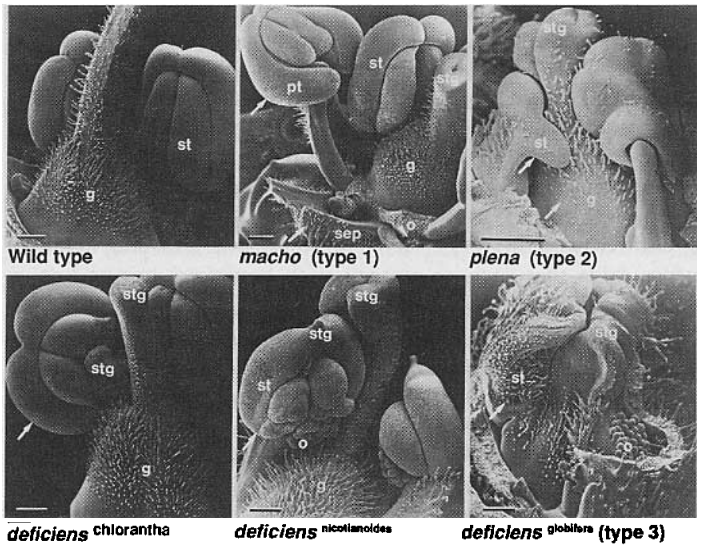


Fig. 4. Homeotic transformation of organs due to mutations of class III genes that control organ identity in *Antirrhinum majus*. Photographs were taken by scanning electron microscopy (*13*) from immature flower buds after removing part of the outer floral organs to visualize organs in the inner whorls (bars, 500 μm). Genotypes are indicated below the photograph, and the phenotype of mature flowers are shown in Fig. 3. Arrows indicate homeotically altered organs, which are designated according to their identity in the corresponding whorl of the wild-type flower. The photographs in the lower panel show an allelic series of mutants of the *deficiens* locus, with feminized stamina. The presence or absence of ovules on the stamina of *deficiens*^{chlorantha} and *deficiens*^{nicotioanoides} flowers depends on the genetic background and on environmental conditions. The sepals and petals are removed, and thus the increasing sepalody of petals (*10*) is not visible [but compare the two whorls of sepals in *deficiens*^{globifera}, Fig. 3; for morphological details see (*13*)]. Sep, sepal; pt, petal; st, stamen; g, gynoeceum; o, ovules; stg, stigmatic tissue.

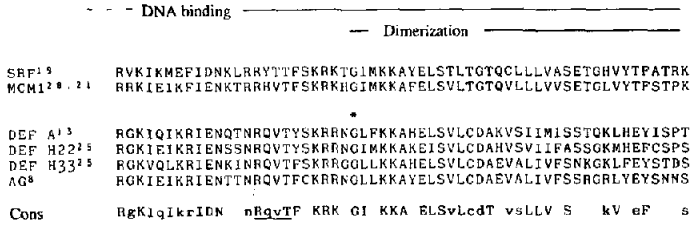


Fig. 5. Conservation of amino acids in the putative DNA binding and dimerization domains of proteins involved in the control of differentiation in mammals, yeast, and plants. Capital letters in the consensus sequence (Cons) indicate homologous amino acids conserved in all six proteins and lower case letters indicate additional positions conserved among plant sequences. Conserved positions are typed in bold letters and homologous exchanges by light letters. The conserved putative phosphorylation site (*29*) is underlined; the asterisk indicates a conserved amino acid that is mutationally altered in the *nicotianoides* allele of the *deficiens* gene. Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, Hys; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

4). Further, preliminary results indicate that DEF A is a phosphorylated nuclear protein (*21*).

The *agamous* gene of *Arabidopsis* is also homologous to the same transcription factors (*8*), although mutation of *agamous* causes a different type of homeotic alteration of floral organs (Fig. 3). Genes regulated by *agamous* or *deficiens* are not identified yet and hence the structure of their binding sites is not known. But the promoter regions of both the *agamous* and the *deficiens* genes contain a sequence motif (*8, 22*) with similarity to the serum response element (SRE), the DNA-sequence motif to which SRF binds, and which is structurally and functionally related to the binding sites of MCM1 (*17, 19*). It is possible, therefore, that the two plant genes are autoregulated, or, alternatively, are regulated by other factors with homology to the conserved domain of the *deficiens* and *agamous* proteins.

Deficiens *belongs to a group of putative transcription factors: The MADS-box.* When the conserved domain is used as hybridization probe to screen a complementary DNA (cDNA) library, eight independent genes are detected (*23*) whose putative protein products are 65 to 90% homologous to the conserved DNA binding domain of DEF A (Fig. 5). Four of these genes are expressed in both vegetative and reproductive organs; expression of the other four is restricted to floral organs. Two of the flower-specific genes can be assigned to known morphogenic mutants of snapdragon: DEF H22 is a protein encoded by the *globosa* gene and DEF H33 is the product of the *squamosa* gene (*23*). The analysis of other homologs is not yet completed, but some of them may represent floral or vegetative morphogenic genes in *Antirrhinum majus.*

Our results indicate that in *Antirrhinum majus*, a distinct family of genes exists that encodes proteins with homology to two known transcription factors, SRF and MCM1. Similar families were found in *Arabidopsis* (*8*), humans, flies, and frogs (*17*). Preliminary evidence suggests that the members of these families may participate in the control of various differentiation processes. In that respect they resemble the homeobox genes known to control differentiation and development in animals (*24*). Since these new families, like the homeobox genes, have a conserved domain in common, we suggest that this domain be called the MADS-box, in reference to the four founding proteins (MCM1, AG, DEF A, and SRF).

Expression Patterns and Post-Transcriptional Modification

Organ-specific regulation of homeotic gene expression. Mutations in the homeotic genes *agamous* (in *Arabidopsis*) and *deficiens* (in *Antirrhinum*) specifically alter organogenesis of floral organs in adjacent whorls. In situ hybridization experiments have revealed that both genes are expressed most strongly in those organs that are homeotically transformed in the respective mutants (Fig. 6) (*8*). More sensitive Northern (RNA) blot analysis of dissected *Antirrhinum* organs shows that *deficiens* is also expressed in low quantities in other floral organs (*13, 25*).

In the *chlorantha* allele of *deficiens*, a small deletion in the promoter region results in decreased gene expression in stamina and petals, but not in the other organs (*22*). This alteration of the spatial expression pattern of the gene confers the homeotically altered phenotype (Fig. 4). The deletion thus seems to affect the *cis*-acting binding site of a transcription factor that upregulates *deficiens* expression specifically in the petals and stamina.

Post-transcriptional modification may modulate specific homeotic gene action. The simultaneous expression of a homeotic gene in two different organs may seem to contradict the appearance of distinct functions in each organ. Hence, we suggest that organ specificity of homeotic genes is the result of a combination of mechanisms that modify their function in different organs.

For SRF and MCM1, which are constantly expressed and yet control the cells response to external and internal factors, modification was invoked as a means to confer specificity. In yeast, for example, MCM1 participates in the regulation of both a- and α-cell–type specific genes, depending on the absence or presence of the α1 repressor and α2 activator proteins in the respective cell types (*19*). In mammals, SRF gene expression is also constitutive (*26*), although it is slightly inducible by growth hormones and external factors. The specific function of SRF in activating the c-*fos* proto-oncogene in response to growth factors is accomplished by post-translational modification [that is, phosphorylation (*27*)] and interaction with other proteins (*28*).

Phosphorylation as a mode to control the function of some plant regulatory proteins is interesting because of its often assumed connection to hormone action and morphogenic processes. A calmodulin-dependent phosphorylation site (*29*) is in fact conserved in the putative DNA binding domains of the DEF A homologous

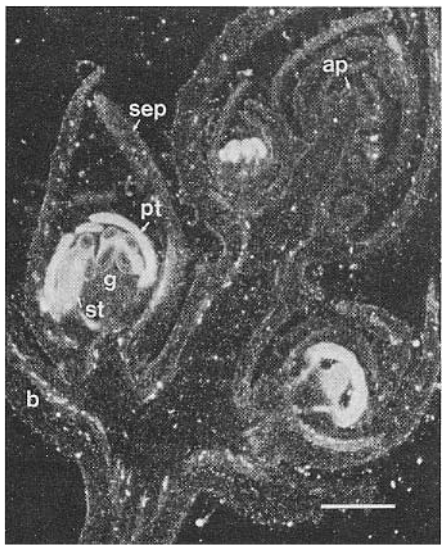


Fig. 6. Spatial expression pattern of the *deficiens* gene of *Antirrhinum majus.* The autoradiograph shows hybridization of the *deficiens* cDNA to a longitudinal section of a young inflorescence with flower buds (bar, 500 μm) developing from the bottom to the tip along the inflorescence axis. Notice the elevated hybridization signals in petals and stamina, the organs homeotically affected when *deficiens* is mutated. The abbreviations used are: b, bract; ap, inflorescence apex; sep, sepal; pt, petal; st, stamen; and g, gynoeceum.

plant proteins (Fig. 4). It is not yet known whether this type of regulation of homeotic gene function could represent the link to the environmental control of development in plants.

Some other structural features of the DEF A protein could indicate the manner in which accessory proteins help to specify its regulatory functions in either petal or stamen development. The partly conserved dimerization domain, essential for SRF function (*17*), is one of these features. Furthermore, the DEF A protein does not contain regions with conserved homology to the regions of other transcription factors that specify functions other than DNA binding (*30*). Thus DEF A may need to be supplemented by other proteins to be fully functional. Such accessory proteins, and combinations thereof, may be different in petals and stamens. The affinity of *cis*-acting binding sites of target genes may also contribute to the specification of spatially different DEF A protein function. In *Drosophila*, such permutations have been postulated for the homeobox proteins, expressed in embryonic cells and perhaps competing for very similar binding sites, to explain their specific regulatory functions in morphogenesis (*31*).

The conserved homology to SRF of a sequence motif in the upstream region of the *deficiens* gene and the conservation of the corresponding DNA binding domain in the *globosa* protein in *Antirrhinum* may indicate involvement of the *globosa* product in the control of expression of the *deficiens* gene. Alternatively, or in addition, these two proteins may form a heterodimer, thereby directing expression of other genes. The *squamosa* gene product could similarly be involved in the interplay of homeotic genes as they control floral organogenesis.

Determination of Floral Organ Identity

Based on genetic and morphological observations of homeotic mutations that interfere with the determination of organ identity, models have been proposed to explain how the actions and interactions of homeotic genes could direct floral organogenesis (*11, 32*). Our scheme (Fig. 7A) incorporates some of the assumptions made in these models. We suggest that after floral evocation, induction of at least two developmental pathways are required for (i) the formation of four different whorls of organs and (ii) the generation of the three basic types of homeotic mutations in *Antirrhinum* and *Arabidopsis* (Fig. 3).

According to the scheme, stamen development, for instance, is initiated and governed by early and concomitant induction of pathways A and B. In fact, staminal carpelody or petalody (because of mutational or conditional changes in either pathway A or B, respectively) is frequently observed in nature (*9*). In contrast, staminal sepalody rarely occurs naturally (*9*), but can be induced by double mutations (*14, 32*). The rarity of rescue of stamina by late restoration of *deficiens* gene function in the unstable *globifera* allele (*13*) also indicates early involvement of *deficiens* in complex events of stamen development.

Mutations in two different pathways allow generation of three types of homeotic alterations. Recessive homeotic mutations of type 2 (such as *agamous*) and type 3 (such as *deficiens*) can easily be generated by loss of function in pathway B or A, respectively (Fig. 7A). In fact, evidence suggests that at least some of the genes in either pathway are regulators of transcription, involved in, but not the sole factors for, regulation of expression of genes essential for the formation of a particular organ.

Type 1 homeotic mutations (such as *macho*) may thus be caused by the mutationally established expression of pathway B in the first and second whorls. Without experimental evidence, the molecular basis of such a "gain of function" is difficult to predict. Variability in expression of type 1 mutant phenotypes could indicate that the corresponding homeotic genes are related to the transmission of external or internal signals (*15*). Expression of pathway B in the incorrect whorl thus could either be because of the loss of a protein that represses expression of genes involved in signal transduction or the consequence of a mutation that alters the function of a signal receptor. Thus, type 1 mutations can be recessive, as in *Arabidopsis*, or dominant, as in *Antirrhinum* (Fig. 3). The frequent occurrence of recessive type 1 mutations in *Arabidopsis*, in contrast to dominant type 1 mutations in *Antirrhinum*, may reflect differences in the mechanisms that establish basically similar processes during floral organogenesis in the two plant species.

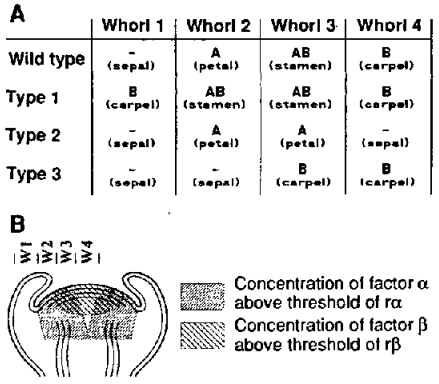


Fig. 7. Theoretical scheme for determination of wild-type floral organ identity by induction of two developmental pathways. (**A**) The developmental pathways A and B are comprised of several homeotic genes whose hierarchical or combinatorial relation is unknown. Pathway A or B is assigned corresponding to the expression pattern of homeotic genes in *Antirrhinum* and *Arabidopsis* (for example *deficiens* belongs to pathway A and *agamous* and *plena* to pathway B). The scheme indicates the pathways induced in the wild-type and homeotic mutants and shows the corresponding altered phenotype of organs in each whorl. The absence of induction of either pathway in the first whorl of the wild type is a consequence of the formalism and is not intended to suggest that sepal development is a continuation of vegetative growth. (**B**) A longitudinal section of a developing flower primordium at the stage of establishment of the second whorl. Shaded areas show the proposed concentric and eccentric distribution of factors α and β, respectively. The abbreviation r is for receptor.

Generation of positional information. Evidence suggests that expression and function of homeotic genes is under genetic control. To direct organogenesis, the functional activity of homeotic genes must be specified, for example, by accessory proteins. In the absence of homeotic gene action (because of mutation) whorls develop and give rise to specific organs, although the organ developed may not be the normal one. Homeotic genes themselves are also subjected to temporal and spatial regulation. The intriguing question of how positional information can be established without additional genetic information remains unanswered. In terms of the scheme outlined above, we can ask what mechanism causes the induction of the two pathways.

Undifferentiated floral meristem cells, destined to give rise to the primordium of a specific organ, have to recognize somehow their position within the developing flower primordium. We propose that different gradients of diffusible factors (for example, hormones or hormone-like compounds) and cellular receptors sensing these factors (Fig. 7B) induce either pathway A or pathway B in the respective whorls. The gradients are apt to change dynamically during development, a process we cannot illustrate in two-dimensional graphics.

To describe induction of pathway A in the second and third whorl, we suggest that a hypothetical factor, alpha, forms an eccentric gradient with the highest concentration about halfway between the center and the edge of the primordium. This type of gradient can perhaps be established by the developing provascular system, transporting nutrients and morphogens from the plant body to cells of the primordium.

The simplest way to describe induction of pathway B in the third

and fourth whorl is to postulate a concentric gradient of another factor, β, that would display the highest concentration in the center of the floral primordium and the lowest at its edge. Concentric gradients may arise from any product of the central undifferentiated floral meristem, because it is maintained the entire time of floral organogenesis and is different from cells differentiating into organs (*4*). Changes in proximodistal information and in its interpretation as basis of flower development have been proposed in a recent model (*33*). Yet, experimental evidence for the existence of gradients of such morphogens is not available.

For induction of stamen development in the wild-type flower, the two gradients must overlap such that simultaneous activation of pathway A and B is allowed in whorl three, and only there. There has to be, hence, a threshold concentration of factor α or β above which the pathways A or B are induced and below which they remain repressed. Pathway B will be actively induced (or its repression abolished) in the first and the second whorl if the receptor of factor β is not functioning or if its sensitivity is mutationally altered. Analysis of type 1 homeotic mutations may prove these assumptions.

In summary, it seems that two hypothetical gradients of factors, formed during differentiation of the flower primordium, suffice to explain differential induction of two developmental pathways. Based on this primary event, floral organogenesis in four whorls can be generally described. Thus the scheme (Fig. 7) may reflect reality, but reality may be more complicated.

Perspectives

At present, morphological, genetic, and molecular information on processes and molecules involved in floral morphogenesis is not sufficient to generate complete models of flower development. Analysis of homeotic genes in two plant species, however, indicates that more knowledge about the regulation of their expression and interactions with other regulatory proteins would help to understand mechanisms controlling determination of floral organ identity. In addition, morphological analysis of double mutants of homeotic genes, for instance, can provide some information about interactions between the developmental pathways (*14, 32*).

Observations like the conservation of protein domains in two homeotic genes and the similarity of organ transformations in three types of homeotic mutants seem to suggest that the principles of floral organogenesis in different plant species may be very similar. Distinctive differences, however, may also exist, such as the number and genetic behavior of genes involved in establishment of developmental pathways, or the absence of certain types of organ transformations. Thus it is possible that the complexity of the processes involved in organ development and flower formation may be different in plants of distantly related genera.

REFERENCES AND NOTES

1. C. W. Wardlaw, *Morphogenesis in Plants* (Methuen, London, 1967), pp. 307–355.
2. V. Walbot, *Trends Genet.* **1**, 165 (1975).
3. G. N. Drews and R. B. Goldberg, *ibid.* **5**, 256 (1989).
4. T. A. Steeves and I. M. Sussex, *Patterns in Plant Development* (Cambridge Univ. Press, New York, 1989), pp. 176–202.
5. G. Bernier, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 175 (1988); J.-M. Kinet, R. M. Sachs, G. Bernier, *The Physiology of Flowering* (CRC Press, Boca Raton, FL, 1985), vol. III.
6. E. M. Meyerowitz, D. R. Smyth, J. L. Bowman, *Development* **106**, 209 (1989).
7. R. Sattler, *Am. J. Bot.* **75**, 1606 (1988).
8. M. F. Yanofsky and E. M. Meyerowitz, *Flowering Newslett.* **9**, 27 (1990); M. F. Yanofsky *et al.*, *Nature* **346**, 35 (1990).
9. V. Meyer, *Bot. Rev.* **32**, 165 (1966); W. Gottschalk, *Die Bedeutung der Genmutationen für die Evolution der Pflanzen* (Fischer, Stuttgart, Federal Republic of Germany, 1971), pp. 139–169.
10. H. Stubbe, *Genetik und Zytologie von Antirrhinum L.* sect. Antirrhinum (Fischer, Jena, Federal Republic of Germany, 1966); E. Baur, *Bibl. Genet.* **4**, 1 (1924).
11. R. Carpenter, D. Luo, S. Doyle, E. S. Coen, *Genes Dev.*, in press.
12. W.-E. Lönnig, H. Saedler, H. Sommer, Z. Schwarz-Sommer, unpublished data.
13. H. Sommer *et al.*, *EMBO J.* **9**, 605 (1990).
14. J. L. Bowman, D. R. Smyth, E. M. Meyerowitz, *Plant Cell* **1**, 37 (1989).
15. M. K. Komaki, K. Okada, E. Nishino, Y. Shimura, *Development* **144**, 195 (1988); L. Kunst, J. E. Klenz, J. Martinez-Zapater, G. Haughn, *Plant Cell* **1**, 1195 (1989).
16. E. S. Coen and R. Carpenter, *Trends Genet.* **2**, 292 (1986); H. Sommer *et al.*, in *Proceedings of the International Symposium Plant Transposable Elements*, O. Nelson, Ed. (Plenum, New York, 1986), p. 227.
17. C. Norman, M. Runswick, R. Pollock, R. Treisman, *Cell* **55**, 989 (1988).
18. E. Dubois, J. Bercy, F. Messenguy, *Mol. Gen. Genet.* **207**, 142 (1987).
19. I. Herskowitz, *Nature* **342**, 749 (1989).
20. R. Hansen and H. Sommer, unpublished data.
21. H. Pape and Z. Schwarz-Sommer, unpublished data.
22. P. Flor and H. Sommer, unpublished data.
23. W. Nacken and H. Sommer, unpublished data.
24. M. Levine and T. Hoey, *Cell* **55**, 537 (1989); C. V. E. Wright, K. W. Y. Cho, G. Oliver, E. M. De Robertis, *Trends Biochem.* **14**, 52 (1989).
25. H. Sommer and J. P. Beltran, unpublished data.
26. P. W. Ingham, *Nature* **335**, 25 (1988); P. Herrlich and H. Ponta, *Trends Genet.* **5**, 112 (1989); P. K. Vogt and T. J. Bos, *Trends Biochem.* **14**, 172 (1989).
27. R. Prywes, A. Dutta, J. A. Cromlish, R. G. Roeder, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7206 (1988).
28. H. Schröter, C. G. F. Mueller, K. Meese, A. Nordheim, *EMBO J.* **9**, 1123 (1990).
29. P. Cohen, *Proc. R. Soc. London Ser. B.* **234**, 115 (1988).
30. P. J. Mitchell and R. Tjian, *Science* **245**, 371 (1989).
31. E. Whitelaw, *J. Cell Sci.* **94**, 169 (1989).
32. G. Haughn and C. R. Sommerville, *Dev. Gen.* **9**, 73 (1988).
33. N. Holder, *J. Theor. Biol.* **77**, 195 (1979).

34. We thank R. Carpenter, E. Coen, and E. Meyerowitz for sending us manuscripts prior to publication. Supported in part by the Deutsche Forschungsgemeinschaft (SFB 274) to Z.S.-S.