Divergence in the Role of MADS Box Genes in the Determination of Floral Organ Identity

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Studies of homeotic mutants affecting development in both animals and plants have shown that families of related genes interact to specify the identity of different parts of an organism. In animals, homeotic mutants have been identified in a limited number of species, mainly insects. However, a few comparative genetic and molecular studies of mutants between species, for example, between the fruit fly *Drosophila* and the red flour beetle *Tribolium*[1-3], have shown a high degree of functional conservation of the homeobox genes controlling segment identity during evolution. In contrast, homeotic mutants affecting flower development are known in many different plant species[4]. This availability of mutants, coupled to the relative ease with which several plant species can be genetically transformed, provides an ideal opportunity to study the degree of conservation in related developmental processes between diverse species.

Recent genetic analysis of three classes of floral homeotic mutants affecting organ identity in *Antirrhinum* and *Arabidopsis* has led to a combinatorial model to explain the roles of homeotic genes in controlling the fate of organ primordia in the flower[5] (Fig. 1). A typical angiosperm flower is composed of four types of organs, sepals, petals, stamens and carpels, which are arranged in four concentric whorls with the carpels in the centre. All three classes of mutations identified alter the identity of organs in two adjacent whorls. The first class of mutants includes *apetala 2* (*ap2*) in *Arabidopsis* and the semi-dominant *ovulata* in *Antirrhinum* in which carpels and stamens are formed in whorls 1 and 2 respectively, instead of sepals and petals. The second class of the homeotic mutants, in which petals are changed into sepals and stamens into carpels, includes *pistillata* (*pi*) and *apetala 3* (*ap3*) in *Arabidopsis* and *deficiens* (*def*) and *globosa* (*glo*) in *Antirrhinum*. The third class of homeotic mutants, which includes *agamous* (*ag*) of *Arabidopsis* and *plena* (*ple*) of *Antirrhinum*, have petals in whorl 3 and an indeterminate proliferation of various types of floral structures in the centre of the flower. The model proposes that each of the three mutant classes corresponds to one of three homeotic functions called A, B and C. Each function is encoded by one or more genes which are active in two adjacent whorls: A in whorls 1 and 2, B in whorls 2 and 3, and C in whorls 3 and 4. Genes required for the A and C functions are thought to be antagonistic. The combination of functions acting in each whorl has been postulated to specify the organ identity. Thus, in a wild type flower, the combinations in whorls 1, 2, 3 and 4 are A, AB, BC and C, specifying sepals, petals, stamens, and carpels, respectively. This model successfully predicts the phenotype of double and triple mutants in *Antirrhinum* and *Arabidopsis* and explains why, when one of the genes involved in a given function is inactive, effects are seen in the organ identity of two adjacent whorls.

Molecular analysis of homeotic genes involved in the B (*DEF*[6], *GLO*[7] and *AP3*[8]) and C (*AG*[9] and *PLE*[10]) functions have identified a gene family sharing a conserved putative DNA-binding domain, called the MADS box, also found in the MCM1[11] and SRF[12] transcriptional factors from yeast and human respectively. This indicates that the plant genes may function as transcription factors involved in the regulation of downstream genes that determine the identity of floral organs. This conclusion has been confirmed by DNA binding studies[13]. Expression studies by RNA *in situ* hybridization of the B[6-8] and C[9-10] function genes in wild type floral meristems from *Antirrhinum* and *Arabidopsis* have shown that they are expressed in the whorls which the model predicts. Expression and *in vitro* DNA binding studies[6-7,13] on two B function genes, *DEF* and *GLO*, in *Antirrhinum* have provided evidence that the two genes bind as heterodimers resulting in up-regulation and maintenance of their expression in whorls 2 and 3. There is some evidence that the two corresponding B function genes, *AP3* and *PI*, in *Arabidopsis* may interact in a similar way[8].

The overall conclusion from these genetic and molecular studies is that a general mechanism of floral organ identity determination is conserved between the distantly related *Antirrhinum* (Scrophulariaceae) and *Arabidopsis* (Cruciferae) which are estimated to have diverged at least 70 million years ago[8]. However, analysis of floral homeotic mutants in other plant species, for example, the *green petals* (*gp*) mutant in *Petunia hybrida*[14], seems to suggest a departure from this general model. The *gp* mutant has sepals instead of petals in whorl 2 but shows no change of organ identity in the other three whorls of the flower. This is surprising because the taxonomic distance between *Petunia* and *Antirrhinum* is much less than that between *Antirrhinum* and *Arabidopsis*. The estimated divergence time between *Petunia* and *Antirrhinum* is about 42 million years (R. G. Olmstead and J. Nugent, pers. comm.). This observation appears to provide a marked contrast to the situation as it is understood in *Antirrhinum* and *Arabidopsis* where homeotic mutants usually show changes of organ identity in two adjacent whorls rather than just one.

In a recent paper[15], van der Krol *et al.* show that *GREEN PETALS (GP)* corresponds to a *Petunia* MADS box gene called *pMADS1* which is a newly isolated member of MADS box gene family (*pMADS1-pMADS4*) from *Petunia*. Southern analysis of a *gp* mutant produced by gamma irradiation mutagenesis revealed that *pMADS1* is completely deleted in its genome although all the other isolated *Petunia* MADS box genes are present. Confirmation that *pMADS1* corresponds to *GP* was gained by introducing a constitutively expressed *pMADS1* construct into *gp* mutant plants, which resulted in the restoration of petals in whorl 2. Further support was obtained by introducing the same construct into wild type plants to produce a phenocopy of the *gp* mutant

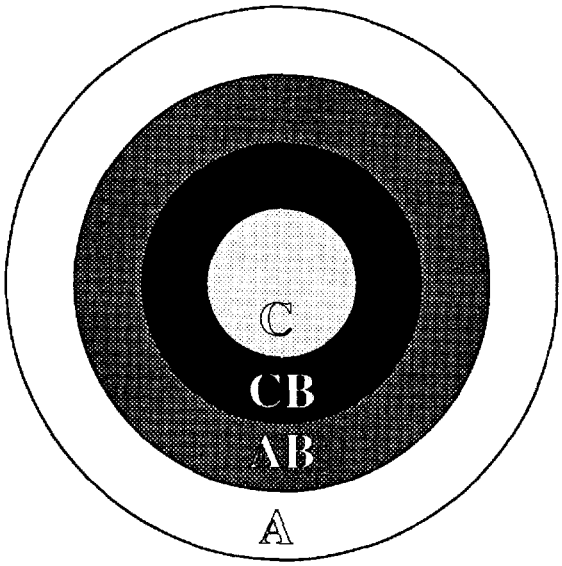

Fig. 1. Schematic illustration of the 4 whorls (1-4) and 3 overlapping regions of homeotic gene functions (A,B,C) of a floral meristem

through co-suppression, the as yet poorly understood phenomenon in which an introduced gene inhibits the activity of the endogenous copies.

The DNA sequence of *GP* is most homologous to the *Antirrhinum* B function gene *DEF* (93% identity at amino acid level between their MADS boxes and 77% identity outside the MADS box)[15]. Expression analysis of *GP* in wild type plants[15] shows that it is expressed in whorls 2 and 3, similar to the B function genes of *Antirrhinum* and *Arabidopsis*[6-7,13]. These results suggest that *GP* is a *DEF*-like gene. However, their mutant phenotypes differ because in *def* mutants both whorls 2 and 3 are affected whereas in the *gp* mutant only whorl 2 is changed. In wild-type *Antirrhinum* and *Arabidopsis* plants, expression of the B function genes increases in whorls 2 and 3 as they develop into mature organs[6-8]. The same is true for *GP* expression in whorl 2 of wild type *Petunia* plants. But, *GP* expression in whorl 3 hardly changes during floral development, suggesting that *GP* does not function in the same way as the B function genes in *Antirrhinum* and *Arabidopsis* during whorl 3 development. This may explain why the loss of *GP* has no effect on stamen development.

Genes similar to *GLO*, the gene known to interact with *DEF* in whorls 2 and 3 of *Antirrhinum*, have also been identified from *Petunia*. The DNA sequences and expression patterns of *pMADS2*[15] and the previously isolated *FBP1* (floral binding protein 1)[16-17] are both very similar to the *Antirrhinum GLO* gene. Both *pMADS2* and *FBP1* are found in *Petunia hybrida* and its putative ancestors[15] and this suggests that there are two *GLO*-like genes in *Petunia*. Their expression is restricted to whorls 2 and 3, and their temporal expression is similar to that found for *GLO*[15-17]. Further evidence to indicate that these genes are functionally homologous to *GLO* has recently been obtained by Angenet *et al.* who demonstrated that a phenocopy of the loss-of-B-function mutant was generated by co-suppression using *FBP1*[17].

Studies of the *GLO*-like gene expression in wild type and *gp* mutant plants suggest that *GP* is required for upregulation of these genes in whorl 2 but not in whorl 3[15-16]. The expression of the two *GLO*-like genes in whorl 2 is greatly reduced and restricted to early stages of flower development in the *gp* mutant. In contrast, their expression in whorl 3 is slightly enhanced. These results suggest that *GP* interacts with the *GLO*-like genes in whorl 2 of *Petunia* flower in a manner similar to that found for *Antirrhinum DEF* and *GLO* genes. However, the expression of the *Petunia GLO*-like genes is independent of *GP* expression in whorl 3. This differs from the situation in *Antirrhinum* in which expression of *DEF* and *GLO* both in whorls 2 and 3 are mutually dependent[13].

The apparent lack of a function for *GP* in whorl 3 in *Petunia* could be explained by the presence of another, as yet unidentified, MADS box protein in the system, or by postulating that the MADS box proteins in whorls 2 and 3 of *Petunia* undergo different types of interactions from those seen in *Antirrhinum* and *Arabidopsis*. The presence of multiple copies of *DEF*-like genes could be due to the hybrid nature of *Petunia hybrida*, which provides a clue as to how such a gene duplication may have arisen. Alternatively, the two *GLO*-like genes in *Petunia hybrida* may not require a *DEF*-like gene such as *GP* and could act as homodimers or interact with each other to specify stamen development. The generation and analysis of floral mutants in the wild species of *Petunia*, thought to have contributed to the genetic background of *Petunia hybrida*, could provide useful information to distinguish these possibilities.

Analysis of the *gp* mutant appears to provide evidence of some divergence in the function of MADS box genes between *Antirrhinum* and *Petunia* even though these species are quite closely related. The further analysis of genes involved in the floral development of *Petunia* and the concomitant analysis of homeotic mutants from a wider range of plant species will no doubt provide additional valuable data about the evolutionary divergence of gene function in flower development.

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References

1 Beeman, R.W. (1987). A homoeotic gene cluster in the red flour beetle. *Nature* **327**, 247-249.

2 Beeman, R.W., Stuart, J.J., Haas, M.S. and Denell, R.E. (1989). Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. *Dev. Biol.* **133**, 196-209.

3 Stuart, J.J., Brown, S.J., Beeman, R.W. and Denell, R.E. (1993). The *Tribolium* homeotic gene *Abdominal* is homologous to *abdominal-A* of the *Drosophila* bithorax complex. *Development* **117**, 233-243.

4 Coen, E.S. (1991). The role of homeotic genes in flower development and evolution. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 241-279.

5 Coen, E.S. and Meyerowitz, E.M. (1991). The war of the whorls: Genetic interactions controlling flower development. *Nature* **353**, 31-37.

6 Sommer, H., Beltran, J.-P., Huijser, P., Pape, H., Lonnig, W.-E., Saedler, H. and

Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: The protein shows homology to transcription factors. *EMBO J.* **9**, 605-613.
7 Trobner, W., Ramirez, L., Motte, P., Hue, I., Huijser, P., Lonnig, W.-E., Saedler, H., Sommer, H. and Schwarz-Sommer, Z. (1992). *GLOBOSA*: A homeotic gene which interacts with *DEFICIENS* in the control of *Antirrhinum* floral organogenesis. *EMBO J.* **11**, 4693-4703.
8 Jack, T., Brockman, L.L. and Meyerowitz, E.M. (1992). The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. *Cell* **68**, 683-697.
9 Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A. and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. *Nature* **346**, 35-39.
10 Bradley, D., Carpenter, R., Sommer, H., Hartley, N. and Coen, E. (1993). Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the *plena* locus of *Antirrhinum*. *Cell* **72**, 85-95.
11 Passmore, S., Maine, G.T., Elble, R., Christ, C. and Tye, B.-K. (1988). A *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of *MATa* cells. *J. Mol. Biol.* **204**, 593-606.
12 Norman, C., Runswick, M., Pollock, R. and Treisman, R. (1988). Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the *c-fos* serum response element. *Cell* **55**, 989-1003.
13 Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P.J., Hansen, R., Tetens, F., Lonnig, W.-E., Saedler, H. and Sommer, H. (1992). Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: Evidence for DNA binding and autoregulation of its persistent expression throughout flower development. *EMBO J.* **11**, 251-263.
14 de Vlaming, P., Gerats, A.G.M., Wiering, H. and Wijsman, H.J.W. (1984). *Petunia hybrida*: A short description of the action of 91 genes, their origin and their map location. *Plant Mol. Biol. Reporter* **2**, 21-42.
15 van der Krol, A.R., Brunelle, A., Tsuchimoto, S. and Chua, N.-H. (1993). Functional analysis of petunia floral homeotic MADS box gene *pMADS1*. *Genes Dev* **7**, 1214-1228.
16 Angenent, G.C., Busscher, M., Franken, J., Mol, J.N.M. and van Tunen, A.J. (1992). Differential expression of two MADS box genes in wild-type and mutant petunia flowers. *Plant Cell* **4**, 983-993.
17 Angenent, G.C., Franken, J., Busscher, M., Colombo, L. and van Tunen, A.J. (1993). Petal and stamen formation in petunia is regulated by the homeotic gene *fbp1*. *Plant J.* **4**, 101-112.

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