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MADS Monsters: Controlling Floral Organ Identity

Homeotic genes, which specify the identities of organs or body parts, have been the subject of intensive research in genetics for more than 100 years. William Bateson, in his treatise on genetic variation (Bateson, 1894), coined the term "homeosis" to describe variations in form that resulted in the abnormal patterning or positioning of normal body parts or organs—for example, "modification of the antenna of an insect into a foot, of the eye of a Crustacean into an antenna, of a petal into a stamen, and the like." Floral abnormalities, such as "double" flowers containing more than the normal number of petals, have been recognized and described by naturalists for centuries. Linnaeus and Goethe, in the mid to late 18th century, were among the first to consider that monstrous or abnormal flowers might provide important information about the rules that govern genetic inheritance (reviewed by Meyerowitz et al., 1989). Goethe (1790) studied homeotic variation in flowers and wrote that the study of this "abnormal metamorphosis" would enable us "to unveil the secrets that normal metamorphosis conceals from us, and to see distinctly what, from the regular course of development, we can only infer." (Arber, 1946) Applied to mutant analysis in general, this prophetic statement presaged the course of genetic inquiry for the next 200 years and promises to provide a solid foundation for genetics research well into the future.

Many homeotic genes have been cloned in plants and animals, and most of them have been found to encode transcription factors. In animals, these proteins often carry a specific DNA binding domain called the homeodomain and are encoded by so-called homeobox genes. Plants also contain homeobox genes, such as those that encode the KNOTTED and KNOX classes of homeodomain proteins that regulate cell fate and patterning in leaves. However, most of the floral homeotic genes that have been identified are MADS box genes, which encode a different class of transcription factors containing a MADS DNA binding/dimerization domain (Riechmann and Meyerowitz, 1997; Ng and Yanofsky, 2001). One such floral homeotic gene is petunia *FBP2* (Figure 1). In this issue of *The Plant Cell*, **Ferrario et al. (pages 914–925)** report a functional analysis of petunia *FBP2* that will further our understanding of how MADS domain proteins control floral organ identity.

ABCs OF FLORAL ORGAN IDENTITY

A number of models were devised in the early 1990s to explain the development of floral organ identity (reviewed by Theissen,

Figure 1. Floral Inflorescence of a Petunia *fbp2* Cosuppression Mutant.

The mutant shows a *sepallata*-like (many sepals) phenotype, wherein all three inner whorls (corresponding to petals, stamens, and carpels in the wild type) are converted to sepaloid organs. The mutant is further characterized by an indeterminate floral meristem, and a new mutant inflorescence is seen here growing out of the center of the older inflorescence. (*Figure courtesy of Gerco Angenent.*)

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2001), and the "ABC" model (Coen and Meyerowitz, 1991) gained wide acceptance. This model was developed from the study of homeotic mutants of Arabidopsis and Antirrhinum and states that the expression of A-, B-, and C-class genes, individually or in pairs in each whorl of the floral meristem, specifies the identity of organs in each whorl. A-class genes specify sepals in the outer whorl (whorl 1); the activity of A- and B-class genes together specifies petals in whorl 2; B- and C-class genes act together to specify stamens in whorl 3; and finally, C-class genes operate alone in whorl 4 to specify carpels. Furthermore, A- and C-class genes antagonize each other, inhibiting their simultaneous function in individual whorls, thus helping to maintain these specific patterns of activity. The classification of a floral gene into one of these three categories depends on the mutant versus wild-type phenotype and on patterns of gene expression. A-class genes are expressed mainly in whorls 1 and 2, and mutations in these genes alter the identity of sepals and petals. B-class genes are expressed in whorls 2 and 3, and mutations here alter the identity of petals and stamens. C-class genes are expressed in whorls 3 and 4, and mutations in these genes affect stamen and carpel identity. This model appears to describe flower development reasonably well (with some modifications) in other species, such as petunia, tomato, and maize. Classic A-class genes that follow these rules are *APETALA1* (*AP1*) and *AP2* in Arabidopsis. B-class genes include Arabidopsis *AP3* and *PISTILLATA* (*PI*), Antirrhinum *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*), and petunia *pMADS1*, *pMADS2*/*FBP3*, and *FBP1*. C-class genes are represented by Arabidopsis *AGAMOUS* (*AG*), Antirrhinum *PLENA*, and petunia *pMADS3*.

The symmetry and simplicity of the ABC model led to its widespread use in the classification of new floral homeotic mutants and genes. However, it was recognized early on that some floral homeotic genes did not represent canonical A-, B-, or C-class genes. Gerco Angenent and colleagues isolated the petunia *FBP1* and *FBP2* genes by screening a petal-specific cDNA library for genes containing the MADS box sequence (Angenent et al., 1992). The expression pattern of *FBP1* in whorls 2 and 3 was indicative of a B-class gene, but the expression of *FBP2* (strong expression in whorls 2, 3, and 4) meant that this gene could not be classified so easily. Further investigation of *FBP2* by cosuppression of the gene confirmed that it belonged to a new class of floral identity genes that control organ identity in the inner three whorls and determinacy of the floral meristem (Angenent et al., 1994) (Figure 1). Pelaz et al. (2000) took this notion one step further with their characterization of a triple mutant of three closely related MADS box genes in Arabidopsis, called *SEPALLATA1/2/3* (*SEP1/2/3*), which are highly similar to and considered putative orthologs of petunia *FBP2* and the *TM5* gene in tomato (Pneuli et al., 1994). Pelaz et al. (2000) showed that the function of B- and C-class genes was dependent on the activity of the functionally redundant *SEP* genes. Colombo et al. (1995) previously showed that two other MADS box genes, *FBP7* and *FBP11*, acted to specify ovule identity (the ovule develops within the fused carpels of whorl 4), and these genes were labeled D-class floral identity genes (Angenent and Colombo, 1996). Thus, Theissen (2001) suggested that the *SEP* genes might be referred to as E-class genes.

THE QUARTET MODEL

Theissen (2001) outlined a new "quartet model" of floral organ identity, based on numerous observations in the literature of interactions between the MADS domain proteins. MADS domain proteins interact to form DNA binding dimers, and all of the MADS domain floral homeotic proteins from Arabidopsis can interact in vitro. Interestingly, B-class proteins (DEF and GLO in Antirrhinum and AP3 and PI in Arabidopsis) form dimers only with each other, and not with proteins of other classes, and there is evidence that C-class proteins interact with E-class proteins. Egea-Cortines et al. (1999) were among the first researchers to report that MADS domain proteins form higher order complexes, with the observation that heterodimers of the Antirrhinum B-class proteins DEF and GLO interact with the A-class protein SQUAMOSA. Honma and Goto (2001) presented an elegant series of experiments providing direct evidence that the formation of ternary or quaternary complexes of B- and C-class proteins with A-class and SEP (E-class) proteins controls floral organ identity, and additionally, that the SEP proteins act to restrict the action of the other classes to the flower. This information, together with functional genetic analyses of floral homeotic genes, led Theissen to propose that floral organ identity might be determined by four different combinations of four floral homeotic proteins. For example, in Arabidopsis, heterodimers of the B-class proteins AP3 and PI would complex with heterodimers of class-A AP1 and class-E SEP proteins to specify petals in whorl 2 and with heterodimers of class-C AG and class-E SEP to specify stamens in whorl 3. In whorl 4, carpels would be specified by complexes made up of AG-SEP heterodimers, and in whorl 1, sepals would be specified by AP1 homodimers (perhaps interacting with other, as yet unidentified, MADS domain dimers).

Ferrario et al. show that petunia FBP2 is functionally equivalent to the Arabidopsis SEP proteins, based on the high degree of similarity of the mutant phenotype in the two species and on the functional complementation of the Arabidopsis *sep* triple mutant by transformation with the petunia *FBP2* gene. Furthermore, petunia was found to contain a small family of *FBP2*-like genes, similar to the Arabidopsis *SEP* subfamily, and it was shown that the phenotype of *FBP2*-cosuppressed plants is caused by the downregulation of *FBP2* and at least one other gene in this family, *FBP5*. Next, the authors used yeast two-hybrid assays and more complex three- and four-hybrid assays to test the ability of FBP2 and related MADS domain proteins, including Arabidopsis SEP3, to interact

with each other as well as their ability to form higher order complexes. They found that petunia FBP2, FBP5, and SEP3 all interacted similarly with class-C (and class-D) proteins and that higher order complexes formed between C/E-class heterodimers and B-class heterodimers. These results provide strong support for the quartet model and the idea that the formation of MADS box multiprotein complexes is a highly evolutionarily conserved facet of flower development. Thus, homeotic monsters continue to "unveil the secrets" of the genetic control of organ identity.

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