Regulation of *Arabidopsis thaliana* Flower Development

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An *Arabidopsis thaliana* flower is composed of four organs, sepals, petals, stamens, and carpels. Development of these organs is determined by three classes of homeotic genes. One member gene *PISTILLATA* (*PI*) contributes to the formation of both petals and stamens. We have now cloned the *PI* gene with its promoter region and clarified the time and locations of *PI* expression during floral organogenesis of the wild type plant and the flower mutant plants (*pistillata*, *apetala3*, and *superman*).

Keywords: ABC model / Floral development / Homeotic gene / MADS box / Transcription factor

Occurrence of cell divisions in higher plants such as *Arabidopsis thaliana* is generally restricted to the meristems after embriogenesis has been finished. The apical meristem is organized tissues of pluripotent "stem" cells. Reproductive development in *Arabidopsis* is controlled by the activities of the inflorescence and the floral meristems derived from the apical meristem. The inflorescence meristem displays a pattern of indeterminate growth, whereas the floral meristem shows a determinate pattern of cell division and organogenesis, always resulting in the production of four whorls of floral organs (i.e., sepals, petals, stamens, and carpels). Genetic studies have shown that the establishment of the floral meristem requires several genes including *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *APETALA2* (*AP2*), and that the floral organogenesis is directed by three classes of organ identity genes (A, B, and C). The class A genes of *Arabidopsis* that are presently known are *AP1* and *AP2*, the class B genes are *PISTILLATA* (*PI*) and *APETALA3* (*AP3*), and the only known class C gene is *AGAMOUS* (*AG*). Representative mutant flowers defective in each class of the genes are shown in Figure (right half). How these homeotic genes specify the four whorls is interpreted by the ABC model in which the organs of each whorl are determined by combinations of the three class genes: sepals and carpels are directed by the class A and the class C genes, respectively; petals and stamens are given by a combination between the class A and B genes and between the class B and C genes, respectively; and the class A and C genes are mutually exclusive (see Figure) [1].

To date, the molecular cloning of *PI*, *AG*, *AP1*, *AP2*, and *AP3* from *Arabidopsis* has been reported.

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Scope of research

Attempts have been made to elucidate structure-function relationships of genetic materials and various gene products. The major subjects are mechanisms involved in signal transduction and regulation of gene expression responsive to environmental stimuli, development of plant leaves and flowers, and plant-microbe interaction. As of December 1995, study is being concentrated on (1) roles of homeo domain proteins and MADS box proteins in developmental processes and transcriptional control in higher plants and (2) contribution of protein phosphorylation and dephosphorylation toward cell cycle control and signal transduction in plants and plant pathogens.

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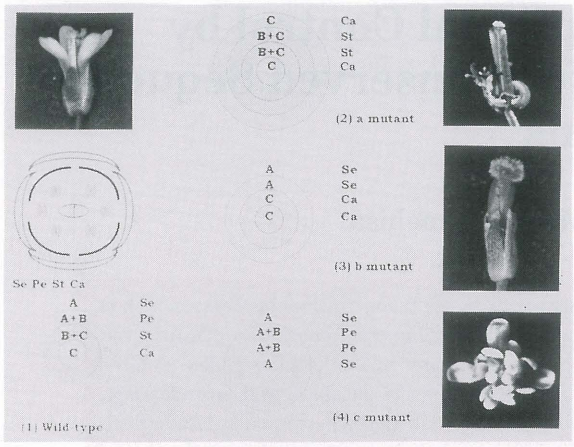

Figure. Arrangement of floral organs in the wild-type *Arabidopsis* is shown in the left (1). Flowers in the right indicate those of a (2), b (3), and c (4) homeotic mutants, respectively. Four rings correspond to the whorls (Se, sepals; Pe, petals; St, stamens; and Ca, carpels), and A, B, and C within them indicate an expressing gene(s).

All of these gene products except *AP2*, and also their snapdragon homologs, *GLOBOSA* (*GLO*), *PLENA* (*PLE*), *SQUAMOSA* (*SQUA*), and *DEFICIENS* (*DEF*), respectively, have been characterized by a highly conserved 58-amino-acid DNA binding domain called MADS box. This name is come from yeast MCM1 (minichromosome 1 maintenance), *Arabidopsis* AG, snapdragon DEF, and human SRF (serum response factor). MCM1 and SRF are known to function as homodimers to regulate gene expression in response to extracellular signals. Since *PI* has been expected to be a member of the MADS box genes on the analogy of other organ identity genes, we have cloned it by using PCR-amplified *GLO* cDNA as a probe. A flower cDNA library was screened under the low stringency conditions. Eight positive clones obtained were all derived from the same RNA species [2]. Also a genomic clone corresponding to these cDNAs was isolated. The cloned DNA actually corresponds to the *PI* gene was confirmed by RFLP mapping and by sequencing of three *PI* mutant alleles (*pi-1*, *pi-2*, and *pi-3*). The *PI* cDNA has a single protein coding frame of 208-amino-acid residues. This protein includes a MADS box domain and a K box domain at the amino-terminal and the central portions, respectively. The latter domain seems to be liable to coiled-coil formation because of the resemblance to the amphipathic α-helical region of keratin proteins [2].

To see the precise spatiotemporal expression pattern of *PI*, *in situ* hybridization was done with cross sections prepared from various developmental stages of flowers. Hybridization signals of *PI* mRNA appear at stage 3 flowers, the same stage when *AG* and *AP3* mRNA's are first detected. The signals are all located centripetally in a region of floral meristem, cells at which will later contribute the formation of not only the petal and stamen but also ovary. However, signals gradually become limited to the second and third whorl cells, and remain at high levels until stages 10 and 11 where differentiation of the cells in each organ type is finished. This *PI* expression pattern is different from that of another class B gene *AP3*: signals of *AP3* mRNA are always restricted in the cells forming the second and third whorls and never detected in the center (fourth whorl) of developing flowers [2].

PI expression was analyzed in the *ap3-3* and *pi-1* mutant backgrounds. *PI* expression in *ap3-3* flowers is normal in early stages but not in later stages, suggesting a role for AP3 in maintenance, but not establishment, of *PI* expression. *PI* mRNA in *pi-1* flowers seems to accumulate normally at stages 3-6, before the primordia of the second and third whorl organs begin to differentiate obviously, but then to decrease. The weaker mutant allele *pi-2* shows a less severe effect on the reduction of *PI* mRNA level. Thus, PI is likely to be necessary for maintaining its own expression [2]. This was confirmed by transient expression experiments in which the GUS reporter gene placed downstream of the *PI* promoter was introduced with a particle delivery procedure into the leaves of the wild-type plant and of a transgenic plant constitutively expressing *PI* [3]. The *superman* mutant displays the phenotype of reduction or elimination of the ovary, concomitant with appearance of many extra stamens in the forth whorl [1]. This phenotype is attributed to *PI* (and presumably *AP3*, too) expression at the forth whorl. Effects of overexpression of *PI* and *AP3* were tested with transgenic plants carrying the cauliflower mosaic virus 35S promoter-driven *PI* and *AP3* [3]. Plants overexpressing either gene are expected to show the normal phenotype, while those overexpressing both genes simultaneously are thought to convert homeotically sepal-to-petal and carpel-to-stamen, provided no other gene is required for the class B function. The latter gives the expected results, indicating PI and AP3 being sufficient for the class B function. However, the former phenotype does not become normal: sepal-to-petal conversion occurs in the 35S::*PI* plants, whereas carpel-to-stamen conversion does in the 35S::*AP3* plants. These results suggest that PI induces *AP3* expression at the first whorl but not the forth whorl and that AP3 induces *PI* expression at the forth whorl but not the first whorl. Although there is no direct biochemical evidence of *Arabidopsis* homeotic gene products being transcription factors at present, these experimental results suggest the existence of complicated regulatory network in control of flower morphogenesis. Assignment of DNA elements responsive to each factor on the *PI* promoter region is now in progress.

References

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