Plant Physiol. (1993) 102: 1051–1052

Plant Gene Register

The cDNA Sequence of Two MADS Box Proteins in *Petunia*[1]

Anil Kush[2], Alan Brunelle, Diane Shevell, and Nam-Hai Chua*

Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021

Several homeotic genes that are involved in floral differentiation have recently been isolated and characterized from *Antirrhinum majus* and *Arabidopsis thaliana* (Sommer et al., 1990; Yanofsky et al., 1990). Based on genetic and molecular studies, a model has been proposed to explain the roles of different homeotic genes in the specification of floral organ identity (Bowman et al., 1991). To see whether this model might also apply to *Petunia*, we initiated a project to isolate *Petunia* genes that show sequence homology to known floral homeotic genes in *Antirrhinum* and *Arabidopsis*.

All of the floral homeotic genes isolated to date contain a putative DNA-binding domain that shows homology with that of other so-called MADS box proteins (Schwarz-Sommer et al., 1990; Angenent et al., 1992). We used a polymerase chain reaction technique to synthesize a DNA fragment encoding the MADS box region (amino acids 2–93) of *Antirrhinum* DEF A (Sommer et al., 1990). This 280-bp polymerase chain reaction fragment was used as a probe to screen a *Petunia hybrida* W115 petal cDNA library. Twelve independent clones were purified and excised from λ Zap II according to the manufacturer's instructions (Stratagene). Nine clones were found to differ in their restriction patterns. The nucleotide sequences of two clones (*Petunia* MADS 1 and 2, Tables I and II) were determined using the United States Biochemical Sequenase kit.

The nucleotide sequence of the PMADS 1 cDNA is 881 bp in length and encodes a protein with 231 amino acid residues and a predicted molecular mass of 27,022 D. Sequence comparison shows that PMADS 1 is highly homologous to the DEF A gene of *Antirrhinum majus* (Sommer et al., 1990). The amino acid homology is 93% within the MADS box region (amino acids 3–60) and 77% in the remainder of the protein. Northern blot analysis showed that PMADS 1 is preferentially expressed in the second and third whorl of *Petunia* flowers (van der Krol et al., 1993). No expression of PMADS 1 was observed in leaves. Consistent with this expression pattern, we found that the PMADS 1 gene is deleted from a *green petal* mutant that shows a homeotic conversion of petal to sepal (van der Krol et al., 1993). Accordingly, the PMADS 1 gene is designated as the *green petal* (*gp*) gene (van der Krol et al., 1993).

The PMADS 2 cDNA clone is 972 bp in length and encodes a protein containing 213 amino acid residues, giving a molecular mass of 24,769 D. Comparison of the amino acid sequence of PMADS 2 with those of other MADS box proteins reveals strong homology, especially between PMADS 2 and FBP1 (Angenent et al., 1992) of *Petunia* and to the MADS box region of *globosa* (Schwarz-Sommer et al., 1990). Like MADS 1, PMADS 2 is also expressed predominantly in petals and stamens (van der Krol et al., 1993). The function of PMADS 2 in *Petunia* flower development is being investigated.

Table I. *Characteristics of the PMADS 1 cDNA from Petunia*

Organism:
Petunia hybrida, line W115 (Mitchell).
Mutant:
gp (*green petal*) deletion.
Location on Chromosome:
Chromosome number 4.
Function:
Encodes a transcription factor in the MADS box family of proteins. Necessary for the normal development of petals in *Petunia*.
Techniques:
λZap II library, polymerase chain reaction, dideoxy sequencing.
Method of Isolation:
A *Petunia* cDNA library in λZap II (Stratagene) was screened with a 280-bp polymerase chain reaction-generated fragment of the DEF A MADS domain from *Antirrhinum majus* (Sommer et al., 1990). Bluescript SK plasmid rescue was carried out as described (Stratagene). Sequencing of both strands was done with United States Biochemical Sequenase using gene-specific primers.
Characteristics of Deduced Amino Acid Sequence:
This protein has high homology to the *A. majus* DEF A (Sommer et al., 1990) protein throughout. Homology is especially high in the region from residue 3 to 60, as it is in most proteins of the MADS family.
Regulation Information:
PMADS 1 is expressed at the highest levels in petal and stamen and less so in carpel and sepal and is undetected in leaves, as seen in northern analysis (van der Krol et al., 1993).
Antibodies:
Antibodies against PMADS 1 have not been generated.

[1] This work was supported in part by a grant from Monsanto Co. A.B. and D.S. were supported by National Institutes of Health postdoctoral fellowships 5F32-GM13761-01 and 1F32-GM13761-01, respectively. A.K. was on leave from and supported by Institute of Molecular and Cell Biology, Singapore.

[2] Present address: Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511.

* Corresponding author; fax 1-212-327-8327.

Table II. *Characteristics of the PMADS 2 cDNA from Petunia*

Organism:
Petunia hybrida, line W115 (Mitchell).
Mutant:
Unknown.
Location on Chromosome:
Unknown.
Function:
This putative MADS box transcription factor is likely to be involved in some aspect of flower development.
Techniques:
λZap II library, polymerase chain reaction, dideoxy sequencing.
Method of Isolation:
Same as for PMADS 1.
Characteristics of Deduced Amino Acid Sequence:
This protein has high homology to the *A. majus* GLO (Schwarz-Sommer et al., 1990) and the *P. hybrida* FBP1 (Angenent et al., 1992).
Regulation Information:
PMADS 2 is expressed at the highest levels in petals and stamen and less so in carpel and sepal and is undetected in leaves, as seen in northern analysis (van der Krol et al., 1993).
Antibodies:
Antibodies against PMADS 2 have not been generated.

In summary, we have isolated and sequenced two *Petunia* cDNA clones encoding MADS box proteins. These clones should be useful in the characterization of *Petunia* mutants that are altered in floral development.

Received January 11, 1993; accepted January 13, 1993.

The EMBL accession numbers for the sequences described in this article are X69946 and X69947 for PMADS 1 and PMADS 2, respectively.

LITERATURE CITED

Angenent GC, Busscher M, Franken J, Mol JNM, van Tunen AJ (1992) Differential expression of two MADS box genes in wild type and mutant *Petunia* flowers. Plant Cell **4:** 983–993

Bowman JL, Smyth DR, Meyerowitz EM (1991) Genetic interactions among floral homeotic genes of *Arabidopsis*. Development **112:** 1–20

Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H (1990) Genetic control of flower development: homeotic genes in *Antirrhinum majus*. Science **250:** 931–936

Sommer H, Beltán JP, Huijser P, Pape H, Lönnig, Saedler H, 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

van der Krol S, Brunelle A, Tsuchimoto S, Chua NH (1993) Functional analysis of petunia floral homeotic MADS box gene pMADS1. Genes Dev **7:** (in press)

Yanofsky MH, Ma H, Bowman JL, Drews GN, Feldman KA, Meyerowitz E (1990) The protein encoded by the *Arabidopsis thaliana* homeotic gene *agamous* resembles transcription factors. Nature **346:** 35–39