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CURRENT PROBLEMS OF DEVELOPMENTAL BIOLOGY

MADS-Box Genes Controlling Inflorescence Morphogenesis in Sunflower

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Abstract—*MADS*-box genes play an important role in plant ontogeny, particularly, in the regulation of floral organ induction and development. Eight full-length cDNAs of *HAM* genes (*Helianthus annuus MADS*) have been isolated from sunflower. They encode MADS-box transcription factors expressed in inflorescence tissues. In the frames of the ABCDE model, the HAM proteins were classified according to their structural homology to known MADS-box transcription factors. The *HAM45* and *HAM59* genes encode the homeotic C function and are involved in the control of the identity of pistil and stamens, while the *HAM75* and *HAM92* genes determine the A function and identity of floral and inflorescence meristems and petal identity. The *HAM31*, HAM2, *HAM63*, and *HAM91* genes encode the B function and are involved in the formation of petals and stamens; and the *HAM137* gene encodes the E function. Analysis of the expression of *HAM* genes in sunflower has demonstrated that the structural and functional differences between the ray and tubular flowers in the inflorescence could be a consequence of the lack of *HAM59* expression during ray flower initiation

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The proper ontogeny of any organism is maintained by the strict and multilevel control of gene expression. Eukaryotic *MADS*-box genes encode a family of transcription factors involved in different key biological functions (Messenguy and Dubois, 2003). These proteins control plant transition from vegetative growth to reproductive development and determine the identity of floral meristem and floral organs as well as the root and ovule development (Angenent and Colombo, 1996; Burgeff et al., 2002; Kim et al., 2002; de Folter et al., 2006; Ferrario et al., 2006). Several hundreds of putative *MADS*-box genes are known in plants; the proteins encoded by them have conserved DNA-binding domain (*MADS*-box) and K-domain, which determine their functional specificity (Krizek and Meyerowitz, 1996). Phylogenetic analysis has demonstrated that the family of plant *MADS*-box genes includes several branches (Becker and Theissen, 2003). Overall, 14 subfamilies of different paralogs have been identified in angiosperms (Alvarez-Buylla et al., 2000; Becker and Theissen, 2003), and members of the same subfamily have a similar expression pattern and interrelated functions (Ng and Yanofsky, 2001).

Studies on floral homeotic mutants of model plants *Arabidopsis thaliana*, *Antirrhinum majus*, and *Petunia hybrida* have defined the main principles of the control of the floral organs identity—the ABCDE model (Coen and Meyerowitz, 1991; Angenent et al., 1995; Pelaz et al., 2001). According to this model, the floral organs in each whorl are specified by a combination of different activities: A + E determine the identity of sepals; A + B + E, petals; C + E + D, ovules; B + E + C, stamens; and E + C, carpels. These activities correspond to genes largely encoding MADS-box transcription factors. Studies of these proteins in different plants have demonstrated a strict correspondence between their structure and functional activity. The subfamilies of plant MADS-box proteins were named after proteins that represent these groups in model plants *A. thaliana* and *A. majus* For instance, the A activity proteins belong to the SQUAMOSA/APETALA1 subfamily; B activity, GLOBOSA/PISTILLATA and DEFICIENS/APETALA3; C and D activities, AGAMOUS/PLENA; and E activity, SEPALLATA. The identity of floral organs in *A. thaliana* proved to be determined by the following quartets of MADS-box proteins: sepals, AP1/AP1/SEP4/SEP4; petals, AP1/PI/AP3/SEP3; stamens, PI/AP3/SEP3/AG; and carpels, SEP3/SEP3/AG/AG (Honma and Goto, 2001; Pelaz et al., 2001).

We studied the genetic control of inflorescence morphogenesis in sunflower and the role of *MADS*-box genes in this process. Sunflower is a typical member of the Asteraceae family. The sunflower inflorescence capitulum includes hundreds of small flowers of two types. The peripheral outer whorl includes ray zygo-

morphic (lacking stamens and pistil) sterile flowers, while the inner part of the head is occupied by fertile tubular flowers. Despite numerous studies on the flower morphology and development in Asteraceae (Palmer and Steer, 1985; Hernandez and Green, 1993; Harris, 1995), the development of the inflorescence and flower in this huge angiosperm family remains unclear in terms of molecular genetics (Yu et al., 1999; Kotilainen et al., 2000; Dezar et al., 2003; Shchennikova et al., 2003, 2004). The identification and study of the genes controlling sunflower morphogenesis can shed light on the simultaneous presence of morphologically and functionally different flowers in the same inflorescence as well as on the origin of floral organs and evolutionary changes in the flower structure.

MATERIALS AND METHODS

Sunflower cultivar Peredovik used in this research was grown in a greenhouse at 20–25°C and 16 h photoperiod. RNA was isolated from the whole inflorescence no more than 1 cm in diameter without the involucre, and from individual flowers picked from the inflorescence no more than 4 cm in diameter.

The cDNA library construction, molecular cloning, and gene expression assay were carried out as described previously (Maniatis et al., 1982). The cDNA library and MADS-box proteins of sunflower were analyzed using the GAL4 system according to the manufacturer's instructions (Stratagene, United States) and protocol by Honma and Goto (2001).

Plasmid DNA was sequenced using an ABI PRISM sequencer and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, United States).

(Stratagene, United States). Sequence data were analyzed with the BLAST search program (Altschul et al., 1997) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). All obtained *MADS*-box gene sequences were aligned with those of known *MADS*-box genes from other plants and phylogenetically analyzed using the ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX) and Tree-View programs (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

RESULTS AND DISCUSSION

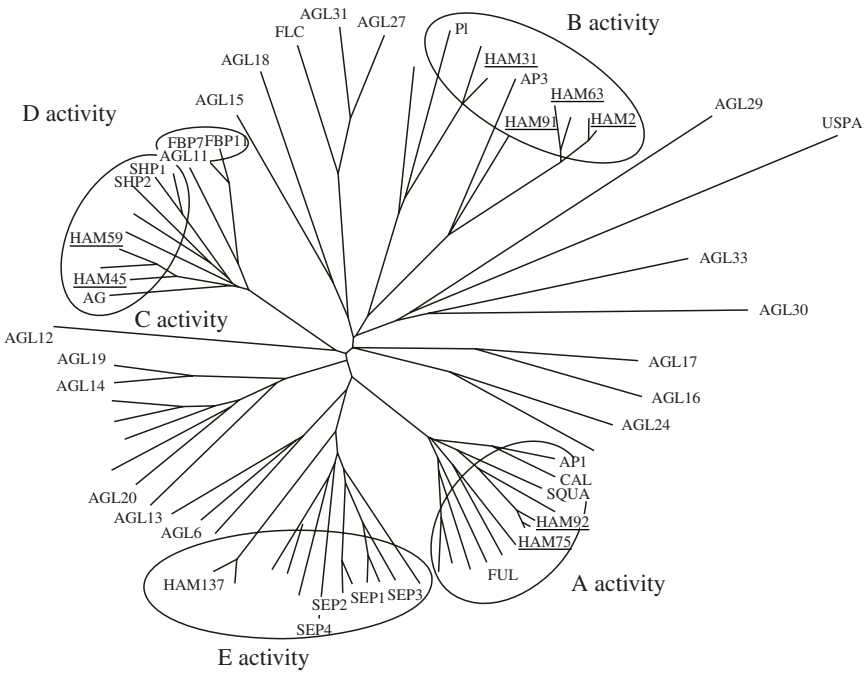
In order to isolate the full-length cDNAs of *MADS*-box genes expressed in the sunflower inflorescence, a cDNA library was constructed from mRNA isolated from the inflorescence at the stage of flower initiation and from closed (preanthesis) tubular flowers (Maniatis et al., 1982). The library screening with the petunia *pMADS3* (X72912) and *A. thaliana CAULIFLOWER* (NM_102395) genes allowed us to isolate seven full-length cDNAs of sunflower MADS-box genes named *HAM* (***H****elianthus* ***a****nnuus* ***M****ADS*) genes. Further analysis of the cDNA library using the two-hybrid yeast GAL4 system allowed us to identify full-length cDNAs of HAM2 and HAM63 proteins interacting with the HAM31 protein (table). BLAST comparison of the amino acid sequences of HAM proteins has demonstrated the identity of 84–99 and 52–80% with known MADS-box proteins in the composite family and other angiosperm families, respectively. Blot-hybridization of total RNA isolated from different flower parts of sunflower has shown that the *HAM75* and *HAM92* genes are expressed in the petals and seed coat; *HAM45* in the ovule; and *HAM59* in the ovule, stamens, and pistil style and stigma (unpublished). Since the structural homologs of *MADS*-box genes have similar functions, we proposed that the *HAM75* and *HAM92* genes encode the A activity; *HAM45* and *HAM59*, C activity; *HAM137* E activity; and *HAM31*, *HAM2*, *HAM63* and *HAM91* B activity.

Description of cDNAs of sunflower *HAM* genes

cDNA	GenBank accession number	Protein length, amino acids	Homologous genes in *A. thaliana* and *A. majus*
HAM2	EF612597	239	*AP3, DEF*
HAM31	AAO18230	196	*PI, GLO*
HAM45	AAO18228	267	*AG, PLENA*
HAM59	AAO18229	247	*AG, PLENA*
HAM63	EF612598	239	*AP3, DEF*
HAM75	AAL83209	248	*AP1, SQUA*
HAM91	AAO18231	210	*AP3, DEF*
HAM92	AAO18232	251	*AP1, SQUA*
HAM137	AAO18233	253	*AGL9 (SEP3)*

The evolutionary relationships between these and other MADS-box proteins were evaluated by phylogenetic analysis of the MIK fragments of HAM and MADS-box proteins from *Arabidopsis thaliana*, *Petunia hybrida*, and *Chrysanthemum morifolium* as well as the key proteins from *Antirrhinum majus* using the ClustalX and Tree-View programs. The obtained data indicate that most of isolated *HAM* genes code for proteins that belong to different subfamilies of the MADS-box transcription factors (figure). These data suggests sunflower genome duplication in evolution (Sossey-Alaoui et al., 1998; Alvarez-Buylla et al., 2000).

We analyzed the expression of the identified genes in different inflorescence parts at different developmental stages (unpublished). The pattern of *HAM59* expression is the most curious for us. In situ hybridization of a section of the sunflower inflorescence at the stage of flower initiation and early differentiation of floral organs has demonstrated the absence of *HAM59* transcripts in the central part of the ray flower primordia. At the same time, the strong signal was observed in the central part of the tubular flowers, where the sta-



Phylogenetic tree based on the comparative analysis of amino acid sequences of the MIK fragments of HAM and MADS-box proteins from *Arabidopsis thaliana, Petunia hybrida*, and *Chrysanthemum morifolium.* The USP A protein represents a different phylogenetic group. (Only HAM proteins and group-specific MADS-box proteins of *A. thaliana, P. hybrida*, and *A. majus* are indicated on the picture.)

mens and pistil style developed later. It is possible that the absence of the HAM59 protein is responsible for the ray flower sterility. We plan to test this hypothesis by producing transgenic plants with constitutive *HAM59* expression. The data obtained in this work concerning the MADS-box homologs controlling flower morphogenesis in sunflower indicate that the development of individual flowers in the Asteraceae family proceeds according to the ABCDE model.

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