

Plant Gene Register

A cDNA Encoding an HD-Zip Protein from Sunflower¹

Raquel L. Chan and Daniel H. Gonzalez*

Area Biología Molecular, Departamento de Ciencias Biológicas, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina

The homeodomain is a 61-amino acid protein motif present in a number of eukaryotic transcription factors (Gehring, 1987). This motif, first recognized to be encoded by some *Drosophila* genes involved in development (Gehring, 1987), folds into a characteristic helix-turn-helix structure that participates in DNA binding (Kissinger et al., 1990). In plants, different strategies have led to the isolation and characterization of homeoboxes (DNA sequences encoding homeodomains). In *Arabidopsis thaliana*, degenerate oligonucleotides deduced from conserved regions of animal homeodomains have been used to screen cDNA libraries, resulting in the isolation of several clones that represent four to five different genes coding unequivocally for homeodomains (Ruberti et al., 1991; Mattson et al., 1992; Schena and Davis, 1992; Carabelli et al., 1993). The encoded proteins are related to each other and contain a Leu zipper motif adjacent to the homeodomain. One of the members of this family of proteins, named HD-Zip proteins, is a developmental regulator (Schena et al., 1993).

We have undertaken the study of homeobox-containing genes in sunflower. For this purpose we have used a degenerate oligonucleotide [5'-CTTGGATCCGCNC(TG)-NC(GT)(GA)TT(TC)TG(AG)AACCA-3'] derived from the conserved sequence WFQNRRA from helix 3 of the homeodomain to screen a stem cDNA library. Portions of cDNA clones were amplified by PCR using this degenerate oligonucleotide together with λ sequencing primers (Gonzalez and Chan, 1993). One of the clones isolated was used to screen the original stem cDNA library in λ gt10 (Table I). A cDNA clone (named Hahb-1, after *Helianthus annuus* Homeobox) containing a 1.2-kb insert was subcloned into pUC119 and sequenced. Hahb-1 contains a typical HD-Zip domain. The spacing between the Leu zipper and the homeodomain is the same in all HD-Zip proteins analyzed to date, suggesting a role for the Leu zippers in the formation of dimers that position two homeodomains for DNA binding. This structure has been described only for plant proteins. The Leu zipper of Hahb-1 has five Leu and one Val (in position d₃). Another interesting feature in the deduced protein sequence is the presence of an acidic domain adjacent to the amino terminus of the homeodomain. Acidic domains are present in many transcription factors (Ptashne and Gann, 1990) and are lo-

Table I. Characteristics of an HD-Zip protein cDNA from sunflower

Organism:	<i>Helianthus annuus</i> L. (sunflower) line HA401B.
Clone Type:	1.2-kb cDNA cloned in pUC119.
Source:	cDNA library in λ gt10 prepared from sunflower stem mRNA; kindly provided by A. Steinmetz and colleagues (Herdenberger et al., 1990).
Sequencing Strategy:	Deletion subcloning using restriction enzymes. Double-stranded sequencing of both strands using the dideoxy chain-termination method.
Method of Identification:	Sequence comparison to homeodomain consensus sequences.
Features of cDNA Structure:	1216 bp including 5' and 3' untranslated regions.
Features of the Deduced Protein:	Open reading frame of 939 bp encoding a polypeptide of 313 amino acid residues. The HD-Zip domain comprises amino acids 88 to 187. There is an acidic domain adjacent to the amino-terminal end of the homeodomain.
Expression Characteristics:	RNA size is approximately 1.2 kb as determined by northern blots. Low-level expression as deduced from northern blot and frequency of positive clones in the cDNA library. Expression in stem, roots, leaves, and hypocotyls.

cated in similar positions in all HD-Zip proteins for which information on this region is available.

Sunflower genomic DNA digested with various restriction enzymes was probed with labeled Hahb-1 at moderate stringency. Several hybridizing bands of different intensity were observed with all enzymes, even though the conditions used for the hybridization are likely to identify only a subclass of homeodomain coding sequences. Our results extend previous observations on the existence in plants of a family of proteins that contain homeodomains linked to Leu zipper motifs.

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* Correspondence to either author; fax 54-41-300309.

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