Plant Physiol. (1994) 105: 1459–1460

Plant Gene Register

Five cDNAs Encoding *Arabidopsis* GF14 Proteins[1]

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The transcriptional regulation of gene expression depends to a large degree on the interaction of *cis*-acting elements and *trans*-acting factors. The G-box (5′-CCACGTGG-3′) is an important *cis*-acting element present in the *Arabidopsis Adh* promoter (McKendree et al., 1990; McKendree and Ferl, 1992). In addition, the G-box motif is also found in the promoter of other environmentally inducible plant genes (Williams et al., 1992), such as genes encoding the *Arabidopsis* Chl *a*/*b*-binding protein, the early Met-labeled polypeptide in wheat, chalcone synthase in parsley, and the small subunit of Rubisco in *Arabidopsis* and tomato. The G-box binding factor has been demonstrated in a vast array of plants, such as *Arabidopsis* (McKendree et al., 1990) and maize (De Vetten et al., 1992), and has been cloned from *Arabidopsis* (Schindler et al., 1992) and other plants (Brunelle and Chua, 1993) by means of G-box oligonucleotide screening.

To detect all of the components of the G-box-binding complex, monoclonal antibodies against the partially purified G-box-binding protein complex were prepared (Lu et al., 1992). Using one of the monoclonal antibodies (anti-GF14), we have isolated cDNA clones of proteins (termed GF14) that are involved in the G-box-binding complex from maize (De Vetten et al., 1992) and *Arabidopsis* (Lu et al., 1992). Based on the western assay, anti-GF14 detects at least five polypeptides in extracts of *Arabidopsis* plant or suspension cells (Lu et al., 1992), indicating that GF14 is probably a family of proteins. We found another four distinct clones by using the anti-GF14 monoclonal antibody to re-screen the λgt11 cDNA expression library (Clontech, Palo Alto, CA) constructed from *Arabidopsis thaliana* suspension cell culture mRNA. Positively reacting plaques were purified, and the *Eco*RI inserts were amplified, cloned, and sequenced on an Applied Biosystems (Foster City, CA) 373A DNA sequencer (Lu et al., 1992).

The four additional full-length cDNAs exhibit approximately 60% identity with pLU14 in the coding region at the nucleotide level (Lu et al., 1992) (Table I). To uniquely identify the original GF14 clone and subsequent homologs, a Greek letter designation has been added in keeping with the precedent set in the literature of mammalian homologs of this protein family (Aitken et al., 1992). The original GF14 clone is now GF14ω. The other four clones are GF14ψ, GF14χ, GF14φ, and GF14υ. Each member of the GF14 cDNAs contains a poly(A) tail (from 8–22 nucleotide) at the 3′ end. Like the GF14ω cDNA clone, the four new GF14 clones contain an open reading frame encoding a protein of about 30 kD, with various lengths of untranslated regions at 5′ and 3′ ends of the cDNAs. Genomic clones for GF14ω,

Table I. *Characteristics of five GF14 cDNA clones from Arabidopsis*

Organism:
Arabidopsis thaliana Columbia.
Location of Gene:
Nuclear genome.
Clone Type:
Five cDNA clones all contain entire coding sequences, designated as GF14ω, GF14ψ, GF14χ, GF14φ, and GF14υ, respectively.
Techniques:
A λgt11 cDNA expression library (Clontech), prepared from poly(A)$^+$ RNA of an *Arabidopsis* suspension cell culture, was screened with anti-GF14 monoclonal antibody. Positive clones were plaque purified and the *Eco*RI inserts were amplified by PCR using λgt11 primers and subcloned into pUC18. Clones were sequenced on both strands by automated dideoxy methods.
Method of Identification:
Similarity of deduced amino acid sequences to mammaliam brain 14-3-3 proteins (approximately 60%), KCIP (approximately 60%), and maize GF14 (approximately 65%).
Features of cDNA Structure:
Full-length cDNA. GF14ω: 1133 nucleotides, deduced translation start site as nucleotide 67 and stop site at nucleotide 844; GF14ψ: 1108 nucleotides, deduced translation start site at nucleotide 133 and stop site at nucleotide 898; GF14χ: 1126 nucleotides, deduced translation start site at nucleotide 1 and stop site at nucleotide 802; GF14φ: 1126 nucleotides, deduced translation start site at nucleotide 52 and stop site at nucleotide 853; GF14υ: 991 nucleotides, deduced translation start site at nucleotide 7 and stop site at nucleotide 781.
Features of Deduced Proteins:
GF14ω: 259 amino acids; M_r 29,165. GF14ψ: 255 amino acids; M_r 28,611. GF14χ: 267 amino acids; M_r 29,921. GF14φ: 267 amino acids; M_r 30,146. GF14υ: 258 amino acids; M_r 28,893. Each isoform contains a sequence resembling a Leu zipper preceded by protein kinase C and A recognition sites, as well as a potential EF-hand calcium-binding domain.
Antibody:
Anti-GF14 monoclonal antibody.

[1] This work was supported by National Institutes of Health grant R01 GM40061 and U.S. Department of Agriculture/National Research Initiative grant 93-3704-9608 to R.J.F. This is manuscript number R-03702 of the Florida Agricultural Experiment Station.

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GF14ψ, and GF14χ support the accuracy of the corresponding cDNA sequences with approximately 99.8% identity. The five GF14 proteins exhibit 74% identity at the amino acid level.

These GF14 proteins are homologs of a small class of mammalian brain proteins that were originally described as kinase-dependent activators of Tyr and Trp hydroxylases and inhibitors of protein kinase C (Aitken et al., 1992). Recently, GF14/14-3-3 homologs were isolated from many eukaryotes including yeast, insects, animals, and plants, and additional potential functions have been ascribed to this evolutionarily conserved protein family (Aitken et al., 1992; Ferl et al., 1994; Lu et al., 1994).

ACKNOWLEDGMENT

We thank Dr. Ernie Almira in the Interdisciplinary Center for Biotechnology Research at the University of Florida DNA Sequencing Core Laboratory for DNA sequencing.

Received March 4, 1994; accepted April 4, 1994.

The GenBank accession numbers for the cDNA clones described in this article are M96855, L09109, L09110, L09111, and L09112.

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