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Plant Gene Register

Sequences of Three *Arabidopsis* General Regulatory Factor Genes Encoding GF14 (14–3–3) Proteins[1]

Michael F. Rooney and Robert J. Ferl*

Program in Plant Molecular and Cellular Biology, Horticultural Sciences Department, Institute for Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611

Plants must be able to adapt quickly to unfavorable changes in their environment to ensure survival. This adaptation is initiated by events at the molecular level that produce a rapid increase in the expression pattern of genes necessary to protect the plant from life-threatening environmental stresses. One such gene is *Arabidopsis* alcohol dehydrogenase, which increases expression with the onset of hypoxic conditions (Dolferus and Jacobs, 1991). The alcohol dehydrogenase gene contains a *cis*-acting promoter element, the G-box (5'-CCACGTGG-3') (Ferl and Laughner, 1989). When the G-box is mutated, alcohol dehydrogenase expression is reduced and in vitro protein binding is disrupted (McKendree and Ferl, 1992). The G-box also appears in the promoters of other stress-induced plant genes (Williams et al., 1992), including the light-regulated small subunit of Rubisco, Chl *a/b*-binding protein and chalcone synthase genes and the hormone-regulated early Met-labeled protein and the *rab-16* family of genes.

A nuclear protein complex binds to the *Arabidopsis* G-box (McKendree et al., 1990), and genes encoding G-box binding factors have been cloned from *Arabidopsis* (Schindler et al., 1992). A monoclonal antibody (anti-GF14) generated against the partially purified G-box-binding protein complex was used to identify and isolate five cDNA clones of proteins associated with the G-box-binding complex (Lu et al., 1992). These five proteins, which do not directly bind to the G-box element, were designated GF14χ, GF14ω, GF14ψ, GF14ϕ, and GF14ν (Lu et al., 1994a). These plant proteins were named GF14 because they exhibited greater than 60% identity with a highly conserved class of proteins known as 14–3–3 proteins that were found in yeast, plants, and animals. Mammalian brains also contain 14–3–3 homologs that function to activate Tyr and Trp hydroxylases

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* Corresponding author; e-mail robferl@nervm.nerdc.ufl.edu; fax 1–904–392–4072.

Abbreviation: GRF, general regulatory factor.

Table I. *Characteristics of three GRF genes from Arabidopsis*

Organism:
Arabidopsis thaliana cv Columbia.

Location of Gene:
Nuclear genome.

Gene Products:
GRF1 encodes the GF14χ protein, GRF2 encodes the GF14ω protein, and GRF3 encodes the GF14ψ protein. The GF14 proteins display 74% identity at the amino acid level.

Cloning and Sequencing Techniques:
The three genomic clones were isolated from an *A. thaliana* genomic library (Clontech) in λEMBL-3 Sp6/T7 by probing with the near full-length GF14χ, GF14ω, and GF14ψ cDNAs. Restriction fragments were subcloned into plasmid vectors and both strands were subjected to automated dideoxy sequencing.

Expression:
RNA transcripts of 1.2 kb were detected for all three genes in suspension cells.

Gene Copy Number:
Southern blot analysis of *Arabidopsis* genomic DNA indicated that GF14 was a small family of several genes. This was verified by western blot analysis, which detected five polypeptides using anti-GF14 antibody.

Features of Gene Structure:
GRF1-GF14χ contains an open reading frame of 801 bp interrupted by three introns ranging from 76 to 799 bp. GRF2-GF14ω contains an open reading frame of 777 bp interrupted by three introns ranging from 101 to 176 bp. GRF3-GF14ψ has an open reading frame of 765 bp and contains four introns ranging in size from 75 to 438 bp. The first intron of GRF3-GF14ψ occurs upstream of the start codon.

Features of Gene Sequence:
A total of 3250 bp composing the GRF1-GF14χ gene were sequenced, including 985 bp upstream and 495 bp downstream of the coding region. A total of 2250 bp composing the GRF2-GF14ω gene were sequenced, including 583 bp upstream and 497 bp downstream of the coding region. A total of 2700 bp composing the GRF3-GF14ψ gene were sequenced, including 1247 bp upstream and 445 bp downstream of the coding region. No recognizable TATA box was observed in any of the three GRF genes.

Antibody:
An anti-GF14 monoclonal antibody is available.

(Ichimura et al., 1988) and to inhibit protein kinase C (Toker et al., 1990).

The cDNAs of three *Arabidopsis* GF14 proteins, GF14χ, GF14ω, and GF14ψ, were used as probes to isolate genomic clones from an *Arabidopsis* genomic library. Three genes, GRF1-GF14χ, GRF2-GF14ω, and GRF3-GF14ψ, were cloned and sequenced (Table I). To avoid confusion, the gene name was followed by the protein that it encodes. The GF14ω protein encoded by the GRF2 gene was demonstrated to bind calcium (Lu et al., 1994b) and have potential protein kinase A and C phosphorylation sites (Lu et al., 1992). This would suggest that GF14 may be involved in a much more diverse role than being just a transcription factor, so the three genes were designated GRF.

Both the GRF1-GF14χ and GRF2-GF14ω genes contained four exons separated by three introns. The GRF3-GF14ψ gene contained four exons and three introns in the same location as the two other GRF genes, but it also contained an additional intron positioned between the transcriptional and the translational start site. Upstream of the coding region of the GRF1-GF14χ, GRF2-GF14ω, and GRF3-GF14ψ genes, 985, 583, and 1247 bp have been sequenced, respectively. There was no recognizable TATA box for any of the three GRF genes. There widespread distribution of the GRF-encoded GF14 homologs throughout the plant and animal kingdoms suggests an important evolutionarily conserved cellular function for these proteins.

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The GenBank accession numbers for the three GRF clones are U09377, U09376, and U09375.

LITERATURE CITED

Dolferus R, Jacobs M (1991) Another ADH system from . . . *Arabidopsis thaliana:* an overview. Maydica **36:** 169–187

Ferl RJ, Laughner BH (1989) *In vivo* detection of regulatory factor binding sites of *Arabidopsis thaliana Adh.* Plant Mol Biol **12:** 357–366

Ichimura T, Isobe T, Okuyama T, Takahashi N, Araki K, Kuwano R, Takahashi Y (1988) Molecular cloning of cDNA coding for brain-specific 14–3-3 protein, a protein kinase-dependent activator of tyrosine and tryptophan hydroxylases. Proc Natl Acad Sci USA **85:** 7084–7088

Lu G, DeLisle AJ, De Vetten NC, Ferl RJ (1992) Brain proteins in plants: an *Arabidopsis* homolog to neurotransmitter pathway activators is part of a DNA binding complex. Proc Natl Acad Sci USA **89:** 11490–11494

Lu G, Rooney MF, Wu K, Ferl RJ (1994a) Five cDNAs encoding *Arabidopsis* GF14 proteins. Plant Physiol **105:** 1459–1460

Lu G, Sehnke P, Ferl RJ (1994b) *Arabidopsis* GF14ω: a calcium binding protein and substrate of endogenous protein kinase. Plant Cell **6:** 501–510

McKendree WL, Ferl RJ (1992) Functional elements of the *Arabidopsis* Adh promoter include the G-box. Plant Mol Biol **19:** 859–862

McKendree WL, Paul A-L, Delisle AJ, Ferl RJ (1990) In vivo and in vitro characterization of protein interactions with the dyad G-box of the *Arabidopsis Adh* gene. Plant Cell **2:** 207–214

Schindler U, Menkens AE, Beckmann H, Ecker JR, Cashmore AR (1992) Heterodimerization between light-regulated and ubiquitously expressed *Arabidopsis* GBF bZIP proteins. EMBO J **11:** 1261–1273

Toker A, Ellis CA, Sellers LA, Aitken A (1990) Protein kinase C inhibitor protein: purification from sheep brain and sequence similarity to lipocortins and 14–3-3 protein. Eur J Biochem **191:** 421–429

Williams ME, Foster R, Chua N-H (1992) Sequence flanking the hexameric G-box core CACGTG affect the specificity of protein binding. Plant Cell **4:** 485–496