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Isolation, Sequencing, and Analysis of a 14-3-3 Brain Protein Homolog from Pea (Pisum sativum L.)

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Plant Gene Register

Isolation, Sequencing, and Analysis of a 14-3-3 Brain Protein Homolog from Pea (*Pisum sativum* L.)[1]

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Initially identified as acidic, homodimeric proteins abundantly and preferentially present in mammalian brain neurotransmitter complexes, the eukaryotic 14-3-3 homologs appear to be ubiquitous and highly conserved among highly diverse organisms, including *Xenopus*, *Drosophila*, and *Saccharomyces* (Aitken et al., 1992). They have also been isolated, cloned, and sequenced from various plants, such as *Arabidopsis* (Lu et al., 1992), *Oenothera*, *Spinacea* (Hirsch et al., 1992), *Zea* (De Vetten et al., 1992), *Lycopersicon* (Laughner et al., 1994), *Hordeum* (Brandt et al., 1992), and *Oryza* (Kidou et al., 1993). Although there are no available sequence data in the GenBank (version 94–5), immunoprecipitation experiments suggest their existence in *Pisum* (Hirsch et al., 1992).

A cDNA library prepared from wounded pea epicotyls was screened with a ^{32}P-labeled probe prepared using *Arabidopsis* 14-3-3 homolog (Lu et al., 1992) as a template. The clone designated PSGF14a was isolated, sequenced, and identified. Its high identity at the nucleotide level and the amino acid level (Table I) suggests that it is a pea homolog to 14-3-3 mammalian brain proteins. Further examination of the screened positives suggests that this clone represents only one member of a larger gene family (data not shown).

There is increasing evidence for multiple functional properties and activities of this group of proteins, the majority being related to involvement in and regulation of signal transduction (Aitken et al., 1992). The understanding of their functional significance in plants is still at an early stage. Their potential signaling function in plants is corroborated by the demonstrated inhibition of protein kinase C (Hirsch et al., 1992), their phosphorylation properties, and calcium-binding ability (Lu et al., 1993), as well as involvement in transcriptional regulation (Lu et al., 1992; De Vetten et al., 1993). These proteins are modulated by high salt and low temperature (Kidou et al., 1993) and by pathogens (Brandt et al., 1992); thus, they may be important signaling molecules in plant responses to stress.

Table I. *Characteristics of the pea cDNA encoding brain protein 14–3-3 homolog*

Organism:
Pisum sativum L. var Alaska.

Source:
cDNA library prepared from wounded etiolated epicotyls.

Techniques:
A λgt11 cDNA library was constructed using poly(A)$^+$ RNA from wounded pea epicotyls. The library was screened with ^{32}P-labeled probe prepared using *Arabidopsis* brain protein 14-3-3 homolog, kindly provided by Dr. Robert J. Ferl. Positive plaques were isolated after three screenings and phage eluates were amplified by PCR (Stratagene, La Jolla, CA) using λgt11 primers. Following subcloning into pCR-Script SK(+) (Stratagene), both DNA strands were sequenced at the University of Nebraska, Lincoln, Center for Biotechnology DNA Sequencing Facility using a modified dideoxy method (Brumbaugh et al., 1988).

Method of Identification:
Similarity of the nucleotide and the deduced amino acid sequences to mammalian 14-3-3 brain proteins and to respective plant homologs. The degree of identity to mammalian 14-3-3 proteins ranges from 63 to 68% at the nucleotide level to 60 to 64% at the amino acid level. The degree of identity with other sequenced plant 14-3-3 homologs ranges between 74 and 81% at the nucleotide level and between 82 and 91% at the amino acid level.

Features of the cDNA Structure:
The full length of the clone PSGF14a is 1113 nucleotides. The predicted coding sequence has 783 nucleotides. There is an 85-nucleotide nontranslated region upstream of the first ATG. In addition there is a 245-nucleotide 3′ nontranslated region. The G+C content of the coding region is 48.9%.

Features of the Deduced Protein:
The open reading frame is 261 amino acids long, encoding a protein with a predicted molecular mass of 29,330 D and a pI of 4.54. The protein contains the N terminus 14-3-3 protein signature (RNLLSVAYKNV), as well as a C terminus 14–3-3 protein signature (SYKDSTLIMQLLRDNLTLWTS).

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