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Plant Gene Register

A cDNA Encoding a New GTP-Binding Protein of the ADP-Ribosylation Factor Family from *Arabidopsis*[1]

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The low mol wt GTP-binding protein superfamily *ras* includes ARFs. Their function depends on their ability to alternate between active and inactive states by association to GTP and GDP, respectively. The first ARF protein was identified in mammalian cells as a cofactor required for ADP-ribosylation of the adenylate cyclase $G_{s\alpha}$ subunit catalyzed by cholera toxin in vitro (Kahn and Gilman, 1984). Widespread expression of the highly conserved ARF proteins in eukaryotic cells raised the question of their physiological role in cell regulation. Recent studies have shown that these proteins were involved in various vesicular traffic steps of the exocytic and endocytic pathways (Serafini et al., 1991; Balch et al., 1992; Kahn et al., 1992; Lenhard et al., 1992) and in nuclear envelope assembly (Boman et al., 1992). In higher plants, ARFs were first described when we reported the isolation of a cDNA coding for an ARF homolog (ARF1) from *Arabidopsis* (Regad et al., 1993; accession No. M95166). Recently, an ARF-type protein has been detected in *Pisum sativum* (Memon et al., 1993). On the other hand, genes of an ARF subfamily coding for structurally related proteins were characterized in *Drosophila* and human genomic libraries (Tamkun et al., 1991; Clark et al., 1993). These ARLs are functionally distinct from ARFs by virtue of their complete lack of in vitro ADP-ribosylation activity.

By sequencing of randomly selected cDNA clones from an *Arabidopsis* cultured cell library, we identified a clone (*Atadprf3*) encoding a new ARF protein homolog (ARF3) that is different from the previously reported *Arabidopsis* ARF1 protein (Table I). Both nucleotide- and DNA-deduced amino acid sequences of ARF3 exhibit a somewhat low homology with those of ARF1, corresponding to 62 and 61% identity, respectively. The *Arabidopsis* ARF3 protein has been compared to the 27 DNA-deduced amino acid sequences of the ARF family available to date in data bases. Surprisingly, ARF3 is closest to the *Drosophila* ARL called *arl* characterized by Tamkun et al. (1991). ARF3 and *arl* are 64% identical, their homology reaching 74% for the 100 amino acids of the amino-terminal region. This suggests that *Arabidopsis* ARF3 may belong to the ARL protein class. However, ARF3 is rather divergent from the other two ARL species identified by Clark et al. (1993) in humans (ARL2) and in *Drosophila* (ARL84F).

Table I. *Characteristics of the Atadprf3 clone from Arabidopsis*

Organism:
Arabidopsis thaliana L. (Heynh.), ecotype Columbia.
Gene Product and Function:
ADP-ribosylation factor-like protein. Member of the family of ARF proteins involved in intracellular transport regulation. Structurally related to an ARL of *Drosophila*. Function unknown in plants.
Techniques:
Sequencing of 210 randomly selected cDNA clones from a cDNA library in λ-ZAPII (Stratagene) prepared from *Arabidopsis* cell-suspension cultures (Regad et al., 1993). Nucleotide sequencing on double-stranded templates using the dideoxy chain-termination method.
Sequence Identification:
Sequence comparison in data bases using the search programs FASTA and BLASTN. Two different cDNA clones showing similarity to DNA sequences of the ARF/ARL family from other species. One cDNA clone (*Atadprf3*) fully sequenced on both strands. Analysis of *Atadprf3* deduced amino acid sequence using program BLASTX and programs of the University of Wisconsin Genetics Computer Group package.
Features of cDNA Structure:
Insert of 830 bp including an open reading frame of 546 nucleotides. Polyadenylation site at position 811.
Features of Predicted Amino Acid Sequence:
Open reading frame of 182 amino acids with a calculated M_r of 20,242 and a predicted isoelectric point of 5.11. Gly at position 2 (site of *N*-myristoylation for ARF proteins). GTP-binding motifs: GLDNAGKT from position 24 to position 31 for the P site (identical only to the human ARL2 and the *Drosophila* ARL84F sequences), DLGGQ from position 67 to position 71 for the G′ site (part of the sequence QVWDLGGQT, distinct from the conserved sequence TVWDVGGQD of all ARFs, although identical to the *Drosophila arl* sequence), and NKQD from position 126 to position 129 for the G site.

Animal ARF proteins have been shown to regulate individual steps of vesicular transport in vitro. In vivo, each ARF

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Abbreviations: ARF, ADP-ribosylation factor; ARL, ARF-like protein.

protein might play a specific role in vesicle membrane formation. The identification in *Arabidopsis* of at least two different gene products of the ARF and ARL types indicates that some of the mechanisms regulating membrane traffic are conserved in plants. Their function remains to be discovered.

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