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Plant Gene Register

A cDNA from *Medicago sativa* Encodes a Protein Homologous to Small GTP-Binding Proteins

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The superfamily of monomeric small GTP-binding proteins comprises members of 20 to 30 kD that can switch between an inactive (GDP bound) and an active (GTP bound) conformation. They are divided into different groups according to their structure, function, and subcellular location. The Ras family interacts with plasma membrane receptors mediating growth and differentiation processes. Rho and Rac are also activated by membrane receptors but appear to be involved in the control of actin polymerization. The Rab and ARF subfamilies control intracellular vesicle transport. In mammals and yeast, Rab proteins serve as important regulators of endocytic and secretory pathways. Distinct members of the Rab family are localized at specific subcellular compartments and they are necessary for different steps of membrane transport (for review, see Pfeffer, 1992; Hall, 1993).

Despite the crucial role of small GTP-binding proteins in diverse cellular processes, little is known about these proteins in plants (Terryn et al., 1993). Here we report the isolation of a cDNA potentially encoding a small GTP-binding protein from *Medicago sativa*, denoted as MsRab1 (Table I). A homology search in current protein sequence data banks revealed up to 90% identity to GTP-binding proteins of various plant species (e.g. Nagano et al., 1993; Yi and Guerinot, Guerinot, 1994). Comparison to animal proteins revealed high homology to the Rab family of GTP-binding proteins: 70% identity to ORA3 from electric ray (Ngsee et al., 1991), 60% to Rab11, and 50% to Rab2 from human (GenBank accession numbers P24410 and B34323, respectively).

The predicted MsRab1 protein sequence shows all important features of GTP-binding proteins. The four GTP-binding domains as well as the effector regions, known to interact with GTPase-activating proteins, are conserved. The MsRab1 sequence also contains two C-terminal Cys residues indispensible for membrane association. Southern analysis indicates that MsRab is a member of a gene family in *M. sativa*. Several related Rab proteins are also present in other plant species (e.g. Nagano et al., 1993). Recently, it has been demonstrated that two, small GTP-binding proteins from *Arabidopsis thaliana* and soybean are able to complement yeast YPT null mutants, indicating functional homology to yeast Rab proteins (Cheon et al., 1993; Bednarek et al., 1994). Studies on nodule formation in rhizobium-legume symbiosis revealed that plant Rab proteins are essential for development of the peribacteroid membrane (Cheon et al., 1993). These data suggest that plants contain a highly conserved family of Rab proteins, which appear to be involved in vesicle transport like their animal and yeast homologs.

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Table I. *Characteristics of the MsRab1 cDNA from M. sativa*

Organism:
Medicago sativa line RA3.
Clone Type; Designation:
cDNA; MsRab1.
Source:
cDNA library, prepared from suspension cultured cells that were induced by auxin treatment to form somatic embryos. The library was constructed using the λ Zap kit from Stratagene.
Method of Isolation:
DNA hybridization screening of 360,000 recombinant phages with the radiolabeled fragment of the MsK2 gene (Páy et al., 1993). Positive clones were sequenced on both strands using the Pharmacia double-strand sequencing kit.
Features of the cDNA:
The MsRab1 clone is 894 nucleotides in length containing a 193-nucleotide 3′ untranslated region and a poly(A) tail.
Structural Features of the Protein:
The 217-amino-acid-long polypeptide potentially encoded by the MsRab1 open reading frame shows the highest sequence similarity to different small GTP-binding proteins from plants. Highest homology to animal proteins on the amino acid level: ORA3 from electric ray and Rab11 and Rab2 from mammals. MsRab1 expressed in *Escherichia coli* gives rise to a protein of 24 kD.
Antibodies:
Antibodies against MsRab1 have not been generated.