Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8732–8736, September 1993
Plant Biology

Molecular cloning and characterization of rho, a ras-related small GTP-binding protein from the garden pea

(*Pisum sativum* L./polymerase chain reaction/cDNA cloning/*Rho1Ps*)

ZHENBIAO YANG* AND JOHN C. WATSON†

Department of Botany and Maryland Agricultural Experiment Station, University of Maryland, College Park, MD 20742-5815

Communicated by Nina Fedoroff, June 24, 1993 (received for review May 14, 1993)

ABSTRACT **The rho proteins, members of the ras superfamily of small GTP-binding proteins, play a central role in the modulation of cellular functions involving the actin cytoskeleton such as in the establishment of cell polarity and morphology. As a first step in elucidating signal transduction pathways leading to processes mediated by the actin cytoskeleton in plants, we initiated cloning and characterization of rho proteins from pea. One rho-related, partial cDNA clone of 167 bp was isolated utilizing a polymerase chain reaction-based cloning strategy, using degenerate primers that correspond to conserved domains within the rho proteins. A full-length cDNA was isolated by screening a pea cDNA library using the 167-bp cDNA as a probe. The *Rho1Ps* cDNA contains an open reading frame encoding a polypeptide (Rho1Ps) of 197 amino acids that shows 45–64% sequence identity to members of the rho family and about 30% identity to other members of the ras superfamily. In addition to the nucleotide-binding and GTPase domains, Rho1Ps shares conserved residues and motifs unique to the rho proteins. Purified Rho1Ps protein expressed in *Escherichia coli* retains specific GTP-binding activity. These data indicate that *Rho1Ps* encodes a small GTP-binding protein of the rho family. The *Rho1Ps* transcript is expressed in all organs of pea seedlings, being more abundant in root tips and apical buds. DNA gel blot analyses show that the rho proteins in pea are encoded by a multigene family.**

The ras superfamily of small GTP-binding proteins is composed of low molecular mass proteins (20–29 kDa) that are structurally and biochemically similar to the α subunit of the trimeric G proteins. Their biological activity is regulated by cycling between GTP- and GDP-bound forms, being activated upon binding of GTP and inactivated when the bound GTP is hydrolyzed to GDP by the intrinsic GTPase activity. The small GTP-binding proteins modulate diverse processes, including cell growth, division, differentiation and morphogenesis, cytoskeletal organization, and vesicle trafficking (1, 2). Based on amino acid sequence similarity, the ras superfamily is divided into at least three major families: ras, rho, and rab/YPT, each family generally having distinct functions in the control of cell growth and differentiation, microfilament organization, and vesicle trafficking, respectively (2–4). Although all three classes of small GTP-binding proteins are found in a number of eukaryotes (1, 3), to our knowledge, only the rab/YPT proteins have been detected in plants (5, 6).

Members of the rho family of small GTP-binding proteins share several unique structural and functional features. The amino acid sequences of these proteins are >45% identical to each other and 30% or less identical to ras or rab/YPT proteins (2, 3, 7). Moreover, the rho proteins possess many motifs and residues absent in other members of the ras superfamily (7–10). In fungi and animals, the rho proteins play a central role in the control of microfilament organization and are key components of signal transduction pathways regulating functions mediated by the actin cytoskeleton (4, 11–16). In plants, there is increasing evidence that the actin cytoskeleton plays a key role in the spatial control of cell growth and division and cell morphogenesis (17–25). As a first step in understanding the regulation of plant processes linked to the actin cytoskeleton, we initiated cloning and characterization of the rho proteins from plants. In this paper, we describe the molecular cloning of one member of the rho family from pea, *Rho1Ps*, using polymerase chain reaction (PCR)-based cloning that involves degenerate primers corresponding to conserved domains within the yeast and mammalian rho proteins.‡ Our results indicate that *Rho1Ps* encodes an active GTP-binding protein. We also show that pea contains a family of rho genes and that the *Rho1Ps* gene is preferentially expressed in the meristematic tissues of pea seedlings.

MATERIALS AND METHODS

Plant Material. Growth of pea seedlings (*Pisum sativum* cv. Alaska or Extra Early Alaska) and harvest of the apical buds were as described (26, 27). In experiments with different organs, 3-day-old dark-grown seedlings were dissected into apical buds, stems, root tips (about 1.5–2 mm), and roots (excluding root tips).

PCR Amplification, cDNA Cloning, and Sequencing. Two degenerate oligonucleotides, R1 (5′-GTI GGN GAC/T GGI GCI GTI GGN AAA/G AC-3′; in which I is inosine and N represents all four nucleotides) and R2 (5′-TA A/GTC C/TTC C/TTG ICC IGC NGT A/GTC-3′), encoding VGDGAVGKT and DATGWEDY, respectively, were used as PCR primers. These two amino acid sequences are located within the nucleotide-binding and GTPase domains conserved in all known rho proteins (1, 7). Using primer R2 and murine leukemia virus reverse transcriptase (GIBCO/BRL), first strand cDNA was synthesized from total RNA isolated from the apical buds of dark-grown pea seedlings (28). The single-stranded cDNA was used as template for PCR amplification as described (27). PCR amplification was performed under the following conditions: 1 min at 94°C for denaturation, 1 min at 58°C for annealing, and 30 sec at 72°C for synthesis. PCR products were separated on a 3% NuSeive (FMC) agarose gel. DNA fragments of expected size (≈165 bp) were cloned into the *Sma* I site of the Bluescript II SK$^-$ vector as described (27) and sequenced by the dideoxynucleotide chain-termination method (29).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.
†To whom reprint requests should be addressed.
‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. L19093).

Two of 11 clones sequenced contained an identical 167-bp DNA sequence whose predicted amino acid sequence exhibited >60% identity to the rho proteins and about 40% identity to other members of the ras superfamily. This rho-related PCR fragment was used as a probe to screen a pea cDNA library purchased from Clontech as described (27). Of $\approx 1 \times 10^6$ plaques screened, two positive clones (designated as Psrho1.7R and Psrho1.8R) were obtained. The inserts of these clones were subcloned into the *Eco*RI site of pUC18, mapped with restriction endonucleases, and sequenced as described above.

Filter Hybridizations. Procedures for the purification of pea nuclear DNA (30) and for DNA and RNA blot hybridizations have been described (27). DNA probes used for these hybridizations were the 167-bp PCR clone, the 400-bp *Hin*dIII/*Eco*RI fragment of the cDNA clone Psrho1.7R (see Fig. 1), and the insert of pB0.7 containing 700 bp of pea nuclear 25S rRNA gene (31).

Expression of Rho1Ps Protein in *Escherichia coli* and GTP-Binding Assay. To express recombinant Rho1Ps protein in *E. coli*, we used the expression vector pDS-MCS (a gift from A. R. Cashmore, University of Pennsylvania), which has six histidine codons inserted downstream of the initiation codon and a multiple cloning site (32). The cDNA insert from Psrho1.7R, encoding a truncated Rho1Ps polypeptide that lacks the N-terminal three amino acids, was first subcloned into the *Eco*RI site of the Bluescript II SK$^-$ vector. The insert was then excised and ligated in frame into the *Eco*RV–*Bam*HI sites of pDS-MCS. The resulting plasmid, pDS-RHO1, encodes a Rho1Ps fusion protein containing the N-terminal sequence MHHHHHHRGSIFRA. pDS-RHO1 was transformed into *E. coli* strain M15 containing the repressor plasmid pREP4 carrying the neomycin phosphotransferase and *lacI* genes (Qiagen, Chatsworth, CA). Transformants were screened for isopropyl β-D-thiogalactoside-inducible expression of the recombinant protein. One such transformant expressing high levels of the Rho1Ps fusion protein was used for protein purification. Soluble protein was extracted from *E. coli* cell lysates and used for the purification of Rho1Ps by affinity chromatography on His-Bind resin according to the manufacturer's procedures (Novagen).

Purified protein (5 μg) was separated by 12% SDS polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was used for a GTP-binding assay as described (33). Briefly, the membrane was washed twice (10 min each) in the GTP-binding buffer (0.3% Tween 20/5 mM dithiothreitol/1 mM $MgCl_2$/20 mM Tris·HCl, pH 7.9) and then incubated at room temperature for 30 min in the same buffer containing 0.3% bovine serum albumin (BSA) and 1.0 μCi of [α-^{32}P]GTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) per ml. For binding competition, 10 mM GTP[γ-S] or ATP (Boehringer Mannheim) was included in the binding buffer. Following two washes with the binding buffer lacking BSA and nucleotides, the membrane was autoradiographed at −80°C with an intensifying screen.

RESULTS

cDNA Cloning and Sequencing of *Rho1Ps*. Two degenerate oligonucleotides (R1 and R2), corresponding to the conserved nucleotide-binding and GTPase domains within the rho proteins, were used as primers for PCR amplification. Primer R2 was used to initiate the synthesis of cDNA from total RNA isolated from the apical buds of 7-day-old dark-grown pea seedlings. PCR amplification of the cDNA, using primers R1 and R2, resulted in several DNA products. One PCR product is ≈165 bp, the expected length of an amplified rho cDNA sequence (data not shown). Sequencing of this PCR product shows that it is a 167-bp fragment encoding 55 amino acids. A search of the SwissProt data base using the FASTA program (34) reveals that the predicted amino acid sequence is 60–80% identical to the rho proteins from various organisms but only about 40% identical to ras or rab/YPT proteins. This indicates that the 167-bp PCR product indeed represents a rho-related sequence.

To obtain a full-length rho cDNA from pea, the 167-bp PCR fragment was used as a hybridization probe to screen a λgt10 pea cDNA library (27). Two positive clones, designated Psrho1.7R and Psrho1.8R, containing inserts of 0.7 kb and 1.2 kb, respectively, were isolated (Fig. 1). Sequence analyses reveal that these two clones correspond to the same *Rho1Ps* mRNA. Clone Psrho1.8R appears to represent a full-length *Rho1Ps* cDNA and contains an open reading frame encoding a polypeptide of 197 amino acids (Rho1Ps) with a calculated molecular mass of 21.3 kDa and an isoelectric point of 9.8 (Fig. 2*A*). Clone Psrho1.7R begins at nucleotide position 269 and encodes a truncated Rho1Ps polypeptide lacking the N-terminal three amino acids (Fig. 2*A*). A search of the SwissProt data base using FASTA (34) shows that the predicted amino acid sequence of Rho1Ps is more similar to the rho proteins (45–64% identity) than other members of the ras superfamily (30% identity or less).

Sequence Comparison and Phylogenetic Analysis. To analyze Rho1Ps structural features and their relationship to members of the ras superfamily, a multiple sequence alignment using parsimony analysis (35) was performed (Fig. 2*B*). Rho1Ps contains all four conserved domains (I, II, III, and IV) and residues that are critical for nucleotide binding or GTPase activity (1, 36, 37). Residues Lys-19, Thr-20, Thr-118, Lys-119, Asp-201, Cys-158, and Ser-160 correspond to residues involved in nucleotide binding by the ras proteins. Residues Gly-15, Ala-16, Ala-62, Gln-64, and Asp-66 correspond to those required for GTPase activity. Rho1Ps also has a conserved motif (positions 35–43) equivalent to the ras effector domain that appears to interact with a GTPase-activating protein (1, 37).

Importantly, Rho1Ps contains all conserved residues or motifs unique to members of the rho family (Fig. 2*B*). These include Asn-42, the site for specific ADP-ribosylation by the C3 exoenzyme toxin from *Clostridium botulinum* (9, 10), and the C-terminal CAAL (A is an aliphatic amino acid), the signature sequence for posttranslational modification by protein geranylgeranyltransferase I (7, 8). Other rho-specific residues are clustered between domains II and III and near the C terminus of the polypeptide (Fig. 2*B*). Deletion of the corresponding regions in ras proteins does not affect their activity (37), suggesting these residues are critical for regulatory functions unique to the rho proteins. Compared to the ras and rab/YPT proteins, Rho1Ps has a 10 amino acid insertion at residues 128–137 that is present only in the rho proteins. Rho1Ps also contains a typical poly(lysine) domain next to the CAAL motif, thought to be involved in targeting of the ras and rho proteins to the plasma membrane (38).

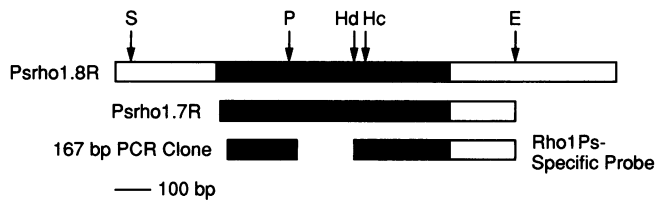
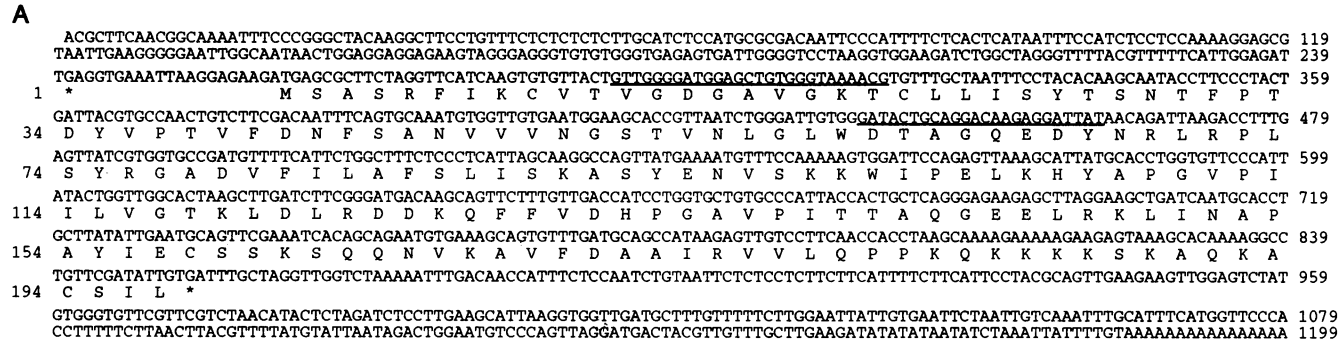


FIG. 1. Structure of *Rho1Ps* cDNA clones. Structure of the two *Rho1Ps* cDNAs (Psrho1.7R and Psrho1.8R) is shown schematically. The protein coding region is shown in black; the 5′ and 3′ untranslated regions are shown in white. The cDNAs were cloned into the *Eco*RI site of pUC18 and mapped with restriction endonucleases (S, *Sma* I; P, *Pst* I; Hd, *Hin*dIII; Hc, *Hin*cII; E, *Eco*RI). Locations of the 167-bp PCR clone and the *Hin*dIII–*Eco*RI fragment used as a *Rho1Ps* gene-specific probe are also shown.

A



B

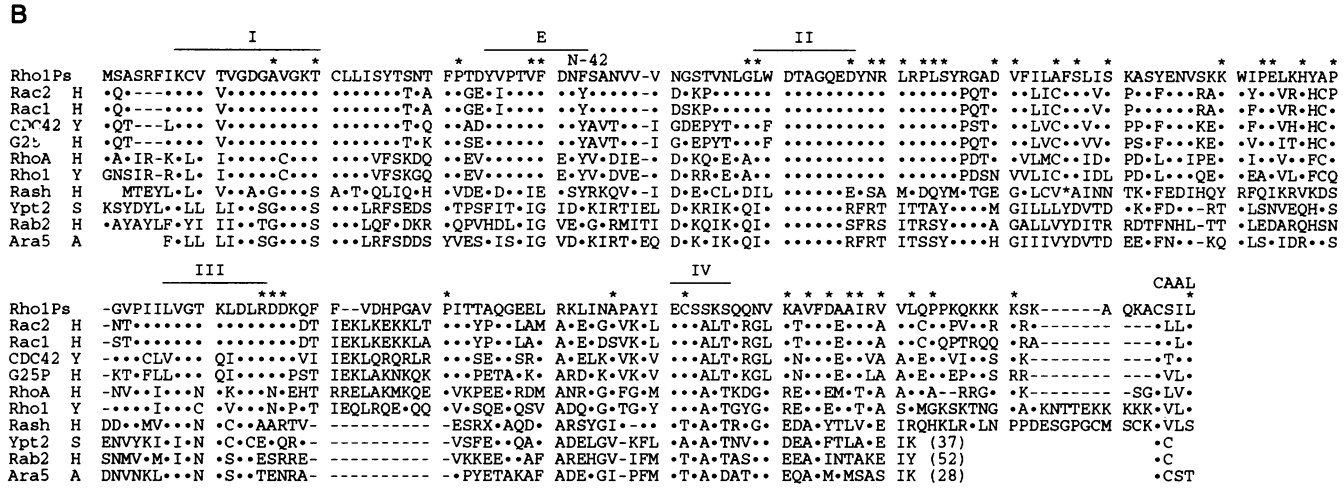


FIG. 2. Sequence analysis of Rho1Ps. (*A*) Nucleotide and predicted amino acid sequences of the cDNA clone Psrho1.8R. DNA sequencing reveals that the two clones (Psrho1.8R and Psrho1.7R) are overlapping and the first nucleotide of Psrho1.7R corresponds to nucleotide 269 of Psrho1.8R. The deduced amino acid sequence is shown using the single-letter code. Numbers on the right refer to the nucleotide sequence; numbers on the left refer to the amino acid sequence. The DNA sequences corresponding to PCR primers R1 and R2 are underlined. The TGA stop codon upstream of the putative initiation codon is marked with an asterisk, as is the stop codon at the end of the long open reading frame. (*B*) Comparison of the Rho1Ps amino acid sequence with members of the rho family. Sequences were aligned using parsimony analysis (35). Conserved domains referred to as I, II, III, IV, and E (effector domain) are shown above the sequence alignment. Conserved amino acids unique to the rho proteins are marked with asterisks. CAAL is a motif recognized by protein geranylgeranyltransferase I. N-42 is the site for ADP-ribosylation by C3 ribosyltransferase. Dashes represent gaps introduced into the alignment; dots represent exact matches with the Rho1Ps sequence. Numbers in the last three lines indicate the number of amino acids not shown so that the C-terminal sequences can be aligned with Rho1Ps. Species abbreviations: H, human; Y, *Saccharomyces cerevisiae*; S, *Schizosaccharomyces pombe*; A, *Arabidopsis thaliana*. All sequences used for these analyses are from the SwissProt and GenBank data bases (34).

These sequence comparisons clearly indicate that Rho1Ps belongs to the rho family of small GTP-binding proteins.

To trace the phylogenetic relationship of Rho1Ps to other members of the rho family, an unrooted gene tree (Fig. 3) was constructed using parsimony analysis (35). Based on this analysis, Rho1Ps clearly diverges from the ras and rab/YPT families early in evolution even before the origin of plants. Previously identified members of the rho family fall into two major subfamilies (rac and rho), with >70% amino acid sequence identity within each subfamily (Fig. 3) (7). Rho1Ps is most closely related to the rac subfamily, with 64% identity to human rac2 and about 55% identity to yeast or human CDC42, but is more distantly related to the rho subfamily (about 50% identity).

GTP-Binding Activity of Rho1Ps Protein Expressed in *E. coli*. The presence of conserved GTPase and nucleotide-binding domains in Rho1Ps predicts that it is an active GTP-binding protein. To verify this, Rho1Ps was expressed in *E. coli* as a fusion protein for use in GTP-binding assays. Psrho1.7R cDNA was cloned into the *E. coli* expression vector pDS-MCS (32). An *E. coli* strain carrying the recombinant plasmid pDS-RHO1 expressed a Rho1Ps fusion protein of ≈22 kDa that was tagged with six histidine residues to facilitate purification. The fusion protein was affinity-purified to near homogeneity using nickel-bound resin from soluble extracts of *E. coli* strain carrying pDS-RHO1.

To determine GTP-binding activity of Rho1Ps, the purified protein was subjected to SDS/PAGE and transferred from the gel to a nitrocellulose membrane. The membrane was incubated in GTP-binding buffer containing [α-^{32}P]GTP with or without nonradioactive competitors. As shown in Fig. 4, the Rho1Ps fusion protein binds [α-^{32}P]GTP efficiently and specifically; this binding is essentially eliminated when GTP[γ-S] is included in the binding buffer as a competitor. In contrast, ATP at a concentration of 10 mM had no effect on GTP binding. GTP binding to Rho1Ps is Mg^{2+}-dependent (data not shown). These results demonstrate that Rho1Ps expressed in *E. coli* is an active GTP-binding protein.

Multiplicity of rho Genes in Pea. To determine if the pea nuclear genome contains a family of rho genes as found in yeast and animals, pea genomic DNA blots were hybridized with the ^{32}P-labeled 167-bp PCR clone. Under high stringency, three *Hin*dIII fragments hybridized (9.3, 5.0, and 3.6 kb) and under low stringency hybridization an additional three *Hin*dIII fragments (6.3, 2.1, and 1.5 kb) were detected (Fig. 5*A*). An equivalent number of DNA fragments hybridized when pea genomic DNA was digested with *Bam*HI or *Eco*RI, suggesting that the pea genome contains a family of rho genes. Since the 167-bp PCR clone does not contain any of these restriction sites, it seems likely that each hybridizing fragment represents an isoform of *Rho1Ps*. The multiplicity of the rho genes in pea was confirmed by the cloning and

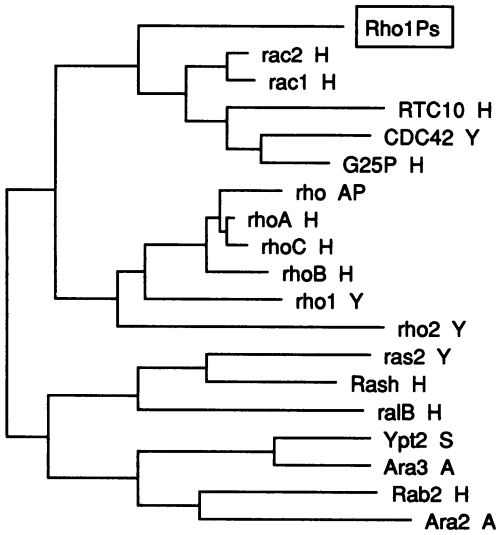


FIG. 3. Phylogenetic relationships within the ras superfamily. An unrooted phylogenetic gene tree was constructed using parsimony analysis (35). Species abbreviations: AP, *Aplasia californica*; otherwise as listed in the legend to Fig. 2*B*.

sequencing of two additional rho-related 167-bp PCR products (unpublished data). The template cDNA for PCR amplification in this case was derived from poly(A)$^+$ RNA isolated from the apical buds of light-grown pea seedlings. The predicted amino acid sequences of these partial cDNAs are 85–90% identical to each other and to Rho1Ps (unpublished data), indicating that these pea rho proteins are closely related and perhaps represent a subfamily of the rho proteins unique to plants.

Single restriction fragments were observed when the DNA gel blot was hybridized (Fig. 5*B*) with the 3′ fragment of Psrho1.7R (400-bp *Hin*dIII–*Eco*RI fragment, see Fig. 1). This result demonstrates that the 400-bp *Hin*dIII–*Eco*RI fragment is a gene-specific probe.

Expression of the *Rho1Ps* Gene in Pea Seedlings. RNA gel blot hybridization of poly(A)$^+$ RNA isolated from the apical buds of 8-day-old dark-grown pea seedlings and the *Rho1Ps* gene-specific DNA probe detected a transcript of ≈1.3 kb (Fig. 6*A*), showing that the Psrho1.8R cDNA clone is full-length, or nearly so. *Rho1Ps* transcript levels did not differ significantly in the apical buds of seedlings grown in complete darkness for 7 days or grown in darkness and then transferred to continuous white light for 2, 4, 6, 16, and 24 hr prior to harvest (data not shown), suggesting that the accumulation of the *Rho1Ps* transcript is not regulated by light.

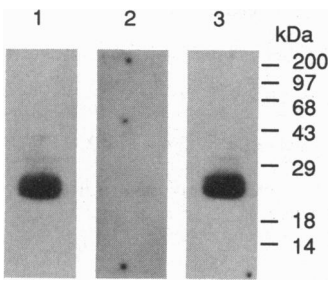


FIG. 4. GTP-binding assay for the Rho1Ps fusion protein expressed in *E. coli*. The expression, purification, polyacrylamide gel electrophoresis, and transfer to nitrocellulose membrane of the Rho1Ps fusion protein is described in the text. The membranes were incubated with [α-^{32}P]GTP in the absence (lane 1) or presence (lane 2) of an excess of unlabeled GTP[γ-S] or ATP (lane 3). Following washing, the membranes were autoradiographed for 3 hr (lanes 1 and 3) or 15 hr (lane 2).

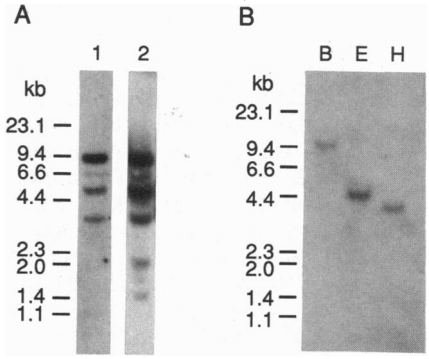


FIG. 5. DNA gel blot analyses of the pea rho genes. (*A*) Five micrograms of pea nuclear DNA was digested with *Hin*dIII, separated on 1.0% agarose gel, and transferred to Nytran membranes. The membranes were hybridized with the ^{32}P-labeled 167-bp PCR clone under high (lane 1) or low (lane 2) stringency conditions. Hybridization conditions were as described (27). (*B*) Pea nuclear DNA was digested with *Bam*HI (lane B), *Eco*RI (lane E), and *Hin*dIII (lane H), separated on an agarose gel, transferred to membranes, and hybridized with the 400-bp *Hin*dIII–*Eco*RI fragment of the Psrho1.7R cDNA clone (see Fig. 1) under high stringency conditions.

To examine the spatial distribution of *Rho1Ps* transcripts in pea seedlings, total RNA was isolated from the apical buds, stems, roots (excluding root tips), and root tips (1.5–2.0 mm) of 3-day-old dark-grown seedlings. *Rho1Ps* transcript levels were determined by slot blot hybridization with the *Rho1Ps*-gene specific DNA probe. Transcript levels are higher in the apical buds and root tips than in the stems and roots (Fig. 6*B*). The same blot was stripped and rehybridized with probe for 25S rRNA to verify that consistent amounts of RNA were transferred to each well. These results indicate that the *Rho1Ps* gene is preferentially expressed in regions of the pea seedling that are active in cell division (39).

DISCUSSION

An ever-increasing number of the ras superfamily of small GTP-binding proteins are being identified in eukaryotes (1, 3). In the past several years, members of the rab/YPT family of small GTP-binding proteins have also been described in diverse species within the plant kingdom (3, 5, 6). Until this report the presence of other members of the ras superfamily in plants remained elusive. We have cloned and characterized

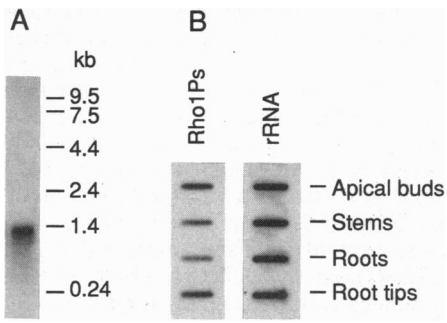


FIG. 6. Analysis of *Rho1Ps* transcripts. (*A*) RNA gel blot analysis. Nine micrograms of poly(A)$^+$ RNA isolated from the apical buds of dark-grown pea seedlings was separated on a 1.0% formaldehyde/agarose gel, transferred to Nytran membranes, and hybridized with ^{32}P-labeled *Rho1Ps*-specific probe (400-bp *Hin*dIII–*Eco*RI fragment, see Fig. 1). (*B*) Spatial distribution of *Rho1Ps* transcripts in pea seedlings. Twenty micrograms of total RNA isolated from apical buds, stems, roots, and root tips of 3-day-old dark-grown seedlings was transferred to Nytran membrane by slot-blotting and hybridized with the *Rho1Ps*-specific probe (left lane) or with a DNA probe specific for 25S rRNA (right lane).

a cDNA encoding Rho1Ps, a member of the rho family of small GTP-binding proteins from the garden pea. Rho1Ps possesses all of the key structural features unique to members of the rho family, including the C-terminal sequence CSIL (Fig. 2), typical of the CAAL signature motif for protein geranylgeranyltransferase I (7, 8). Protein geranylgeranyltransferase I activity has recently been identified in cultured tobacco cells (40), and *E. coli*-expressed Rho1Ps is geranylgeranylated *in vitro* using an *Atriplex nummularia* cell extract (J.-K. Zhu, Z.Y., P. M. Hasegawa, and J.C.W., unpublished results). Moreover, the Rho1Ps fusion protein expressed in *E. coli* exhibits specific GTP-binding activity (Fig. 4), indicating that it is a functional protein. We conclude that Rho1Ps encodes a member of the rho family of small GTP-binding proteins.

Members of the rho family characterized to date are divided into two subfamilies: the rho subfamily, including human rhoA, -B, and -C, *Aplasia* rho, and yeast rho1; and the rac subfamily, including human rac1, rac2, CDC42 (G25P), TC10, and yeast CDC42 (3, 7). Our phylogenetic analysis suggests that Rho1Ps diverges significantly from both of these subfamilies, although it is more closely related to the rac subfamily (Figs. 4 and 5). We hypothesize that plants have evolved a unique subfamily of rho proteins. This hypothesis is supported by our cloning of two additional partial rho cDNA sequences from pea that show greater sequence similarity to Rho1Ps than any other rho protein described to date (data not shown). This may reflect roles in the modulation of the structure and function of the actin cytoskeleton in plants that are different from those in fungi and animals. Nonetheless, verification of this hypothesis awaits characterization of other rho family members from pea and other plant species. *Rho1Ps*-related sequences are present in diverse plant species such as *Arabidopsis*, tobacco, and rice, as shown by DNA gel blot analyses (unpublished data).

The rho proteins may be an attractive system for elucidating signal transduction pathways regulating cell division and differentiation in plants. First of all, the multiplicity of rho proteins in pea raises the possibility that each member is involved in a different or a distinct set of cellular or developmental processes. In human cells, rac1 mediates actin filament accumulation on the plasma membrane leading to membrane ruffling, rhoA is involved in the formation of actin stress fibers and focal adhesions induced by growth factors, and rhoC modulates cell morphology (4, 11, 13). In the budding yeast, CDC42Sc is essential for the asymmetrical distribution of actin filaments and thus controls the establishment of cell polarity, whereas rho3 and rho4 regulate directional bud growth involving the actin cytoskeleton (14–16). Because the functions of the rho proteins in regulating the spatial organization of actin filaments are conserved in such diverse organisms as yeast and human, it is likely that the plant rho proteins also play a similar role. In plants, the actin cytoskeleton has been implicated in various cellular and developmental processes, such as the formation of cell division planes, the establishment of cell polarity in *Fucus*, cell morphogenesis, cytoplasmic streaming, and geotropism (17, 18, 22, 23, 25, 41). An intriguing possibility is that each rho protein modulates a different actin-mediated function in plants. Our results showing that the *Rho1Ps* gene is preferentially expressed in meristematic tissues of pea seedlings (Fig. 6) suggest that Rho1Ps may play a role in the regulation of cell division. More detailed analyses of expression patterns and subcellular localization of the rho proteins will provide clues as to their specific functions.

We are grateful to Timothy P. Frielander for his assistance with the parsimony analysis of Rho1Ps sequence. We also thank Steve Wolniak for critical reading of this manuscript, Thomas La Rosa for providing the pea nuclear DNA, and Paul Bottino for use of facilities.

This work was supported by grants from the U.S. Department of Agriculture (89-37280-4818) and from the National Science Foundation (IBN-9118226) to J.C.W. and by the Maryland Agricultural Experiment Station (Scientific Article A6472 and contribution no. 8675).

1. Takai, Y., Kaibuchi, K., Kikuchi, A. & Kawata, M. (1992) *Int. Rev. Cytol.* **133**, 187–230.
2. Hall, A. (1990) *Science* **249**, 635–640.
3. Kahn, R. A., Der, C. J. & Bokoch, G. M. (1992) *FASEB J.* **6**, 2512–2513.
4. Ridley, A. J. & Hall, A. (1992) *Cell* **70**, 389–399.
5. Matsui, M., Sasamoto, S., Kunieda, T., Nomura, N. & Ishizaki, R. (1989) *Gene* **76**, 313–319.
6. Palme, K., Diefenthal, T., Vingron, M., Sander, C. & Schell, J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 787–791.
7. Vincent, S., Jeanteur, P. & Fort, P. (1992) *Mol. Cell. Biol.* **12**, 3138–3148.
8. Reiss, Y., Stradley, S. J., Gierasch, L. M., Brown, M. S. & Golstein, J. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 732–736.
9. Aktories, K., Braun, U., Rosener, S., Just, I. & Hall, A. (1989) *Biochem. Biophys. Res. Commun.* **158**, 209–213.
10. Sekine, A., Fujiwara, M. & Narumiya, S. (1989) *J. Biol. Chem.* **264**, 8602–8605.
11. Chardin, P., Boquet, P., Maduale, P., Popoff, M. R., Rubin, E. J. & Gill, D. M. (1989) *EMBO J.* **8**, 1087–1092.
12. Paterson, H. F., Self, A. J., Garrett, M. D., Just, I., Aktories, K. & Hall, A. (1990) *J. Cell Biol.* **111**, 1001–1007.
13. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. & Hall, A. (1992) *Cell* **70**, 401–410.
14. Johnson, D. I. & Pringle, J. R. (1990) *J. Cell Biol.* **111**, 143–152.
15. Matsui, Y. & Toh-e, A. (1992) *Mol. Cell. Biol.* **12**, 5690–5699.
16. Ziman, M., O'Brien, J. M., Ouellette, L. A., Church, W. R. & Johnson, D. I. (1991) *Mol. Cell. Biol.* **11**, 3537–3544.
17. Quatrano, R. S. (1989) in *Genetics of Pattern Formation and Growth Control*, ed. Mahowald, A. P. (Wiley/Liss, New York), pp. 31–46.
18. Seagull, R. W. (1989) *CRC Crit. Rev. Plant Sci.* **1**, 131–167.
19. Cho, S.-O. & Wick, S. M. (1991) *Cell Motil. Cytoskel.* **19**, 25–36.
20. Wick, S. M. (1991) in *The Cytoskeletal Basis of Plant Growth and Form*, ed. Lloyd, C. W. (Academic, New York), pp. 231–244.
21. Lloyd, C. W. (1991) in *The Cytoskeletal Basis of Plant Growth and Form*, ed. Lloyd, C. W. (Academic, New York), pp. 245–257.
22. Eleftheriou, E. P. & Palevitz, B. A. (1992) *J. Cell. Sci.* **103**, 989–998.
23. Seagull, R. W. & Falconer, M. M. (1991) in *The Cytoskeletal Basis of Plant Growth and Form*, ed. Lloyd, C. W. (Academic, New York), pp. 183–194.
24. Seagull, R. W. (1991) *Protoplasma* **159**, 44–59.
25. Kobayashi, H., Fukuda, H. & Shibaoka, H. (1988) *Protoplasma* **143**, 29–37.
26. Lin, X., Feng, X.-H. & Watson, J. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6951–6955.
27. Yang, Z., Cramer, C. L. & Watson, J. C. (1993) *Plant Physiol.* **101**, 667–674.
28. Thompson, W. F., Everett, M., Polans, N. O., Jorgensen, R. A. & Palmer, J. D. (1983) *Planta* **158**, 487–500.
29. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
30. Watson, J. C. & Thompson, W. F. (1986) *Methods Enzymol.* **118**, 57–75.
31. Watson, J. C., Kaufman, L. S. & Thompson, W. F. (1987) *J. Mol. Biol.* **193**, 15–26.
32. Klimczak, L. J., Schindler, U. & Cashmore, A. R. (1992) *Plant Cell* **4**, 87–98.
33. Doucet, J.-P. & Tuana, B. S. (1991) *J. Biol. Chem.* **266**, 17613–17620.
34. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 397–395.
35. Swofford, B. L. (1991) PAUP Version 3.0r (Illinois Natural History Survey, Champaign, IL).
36. Downward, J. (1992) *BioEssays* **14**, 177–184.
37. Bollag, G. & McCormick, F. (1991) *Annu. Rev. Cell Biol.* **7**, 601–632.
38. Hancock, J. F., Cadwallader, K., Paterson, H. & Marshall, C. J. (1991) *EMBO J.* **10**, 4033–4039.
39. Thomson, B. F. & Miller, P. M. (1962) *Am. J. Bot.* **49**, 383–387.
40. Randall, S. K., Marshall, M. S. & Crowell, D. N. (1993) *Plant Cell* **5**, 433–442.
41. Sievers, A., Buchen, B., Volkmann, D. & Hejnowicz, Z. (1991) in *The Cytoskeletal Basis of Plant Growth and Form*, ed. Lloyd, C. W. (Academic, New York), pp. 169–182.