



Characterization of DRGs, developmentally regulated GTP-binding proteins, from pea and *Arabidopsis*

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Abstract

Developmentally regulated GTP-binding proteins (DRGs) from animals and fungi are highly conserved but have no known function. Here we characterize DRGs from pea (PsDRG) and *Arabidopsis* (AtDRG). Amino acid sequences of AtDRG and PsDRG were 90% identical to each other and about 65% identical to human DRG. Genomic Southern blotting indicated that *AtDRG* and *PsDRG* probably are single-copy genes. PsDRG mRNA accumulated preferentially in growing organs (root apices, growing axillary buds and elongating stems) compared with their non-growing counterparts. At DRG mRNA was relatively abundant in *Arabidopsis* leaves, stems and siliques, less abundant in flowers and flower buds, and barely detectable in roots. Histone mRNAs are known to accumulate predominantly during S phase of the cell cycle and are markers for proliferating cells. The patterns of histone H2A mRNA accumulation in pea and *Arabidopsis* organs were very similar to those of DRG mRNAs. An antiserum raised against a PsDRG N-terminal fusion protein recognized 43 and 45 kDa proteins. PsDRG proteins were more abundant in growing pea roots and stems than in non-growing organs, but they were equally abundant in growing and dormant axillary buds. After differential centrifugation, PsDRG proteins were found primarily in the microsomal ($150\,000 \times g$ pellet) and soluble ($150\,000 \times g$ supernatant) cell fractions.

Introduction

GTP-binding protein (G proteins) regulate many important cellular processes. For example, Ras and heterotrimeric G α proteins are involved in signal transduction [1, 9], prokaryotic EF-Tu and eukaryotic EF1 assure fidelity of protein translation [14], Rabs are involved in vesicle transport [24], Ran regulates transport through nuclear pores [21] and Rho is involved with organization of the cytoskeleton [12]. Homologues of several classes of G proteins have been identified in plants by gene cloning of their presence inferred from physiological studies [35]. Other characterized plant G protein include: dynamin-like proteins from *Arabidopsis* and soybean [8, 11]; an auxin re-

sponsive gene, *arcA*, which encodes the β -subunit of a heterotrimeric G protein [15]; and 29 different Rab-related genes from *Lotus* [2].

G proteins contain five 'G domains' that form the guanosine nucleotide binding pocket [4]. All known G proteins are active in the GTP-bound state and inactive in the GDP-bound state [3]. Many cellular factors influence the conversion of one form into the other, which in turn regulates G protein activity. Certain classes of G protein have lipidic groups attached at specific residues. Ras protein is farnesylated at a CAAX sequence near the C-terminus and Rab proteins are geranylgeranylated at a XCCX or a related sequence, which also occurs near the C-terminus [10]. These prenylations are essential for targeting Ras to the plasma membrane and Rab proteins to various classes of microsomal membranes. ADP-ribosylation factors (ARFs), another subclass of G proteins, participate in coating and uncoating of membrane vesicles.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers AF014821 (PsDRG) and AF014822 (AtDRG).

ARFs need to be myristoylated on a Gly residue near the N-terminus in order to function properly [22].

Developmentally regulated GTP-binding proteins (DRGs) are a subclass of G proteins that has been discovered rather recently. Genomic or cDNA clones have been isolated from mouse [27], man [28], *Xenopus* [18], *Caenorhabditis elegans* [36], *Drosophila* [30], *Schizosaccharomyces pombe* [13], *Saccharomyces cerevisiae* [5], and *Halobacterium* [29]. Amino acid sequence identity among DRGs is very high [28]. For example, deduced sequences of human and *S. pombe* DRGs are 66% identical to each other. Very little is known about the cellular functions of DRGs.

In this report we describe DRG cDNA clones from pea (PsDRG) and *Arabidopsis* (AtDRG). PsDRG mRNA accumulated preferentially in growing organs (elongating stems, growing axillary buds and root apices). AtDRG mRNA accumulated to rather high levels in all organs assayed except roots. An antiserum raised against a PsDRG fusion protein was used to assess PsDRG protein accumulation in pea organs by western blotting. These antibodies also were used to analyze DRG localization in subcellular fractions upon differential centrifugation.

Materials and methods

Plant material and growth conditions

Pea seeds (*Pisum sativum* L. cv. Alaska) were imbibed overnight in running tap water and grown in trays in a greenhouse. Ambient temperature ranged from about 20 to 30 °C, depending on the season. Natural light was supplemented with artificial light to maintain a 16 h light/8 h dark photoperiod. Node 2 of intact 7-day-old plants contains four dormant axillary buds, all of which begin to grow within a few hours of decapitating the terminal bud [33]. Buds were isolated from intact plants (0 h) and a several time points during the 24 h dormancy-to-growth transition upon decapitation. Growing stem tissue was isolated from internode 5 of 10-day-old plants, which measured ≤ 7 mm at the time of collection. Fully elongated ('mature') stem tissue was from internode 3 of 10-day-old plants. Expanded leaflets were collected from leaves at node 3 or node 4 of 10-day-old plants. Root apices (terminal 2 mm, including root cap) were isolated from 3-day-old seedlings sown on moist paper towels. Fully elongated roots (10 to 20 mm from the apex) were collected from the same seedlings. Floral organs (sepals,

petals, stamens and carpels) were from flowers one day prior to pollen anthesis, which occurred about 21 days after sowing under these growth conditions. These organs were frozen in liquid nitrogen and stored at -80°C .

Arabidopsis thaliana plants (ecotype Columbia) were grown in a growth chamber under a 12 h light/12 h dark photoperiod. Roots, rosette leaves from 1-week-old and 6-week-old plants, flower buds, open flowers, inflorescence stems and siliques were collected.

cDNA clones

PsDRG was isolated during a differential screen of a dormant axillary bud cDNA library, which was constructed in pSPORT1 [32]. Although PsDRG mRNA accumulates preferentially in growing axillary buds, reduced amounts of this message are present in dormant buds as well. A full-length *Arabidopsis* DRG cDNA clone, which we call AtDRG, was obtained from the *Arabidopsis* Biological Resource Center (EST clone 45B8T7 [23]; GenBank T14006). Each clone contained an insert of ca. 1.5 kb. Nested deletion clones were generated using the Erase-A-Base kit (Promega). Both strands of these clones were sequenced manually (Sequenase II kit, US Biochemical) or using an Applied Biosystems PRISM 373 automated sequencer (Biotechnology Core Facility, Northern Illinois University).

RNA gel blotting

Total RNA was isolated using guanidinium isothiocyanate and phenol extraction followed by LiCl precipitation [33]. RNA was separated by denaturing formaldehyde gel electrophoresis and blotted onto nylon membranes. Random-primed ^{32}P -labeled probes were prepared from gel-purified cDNA inserts (DecaPrime II kit, Ambion). Single-stranded RNA riboprobes were prepared by *in vitro* transcription in the presence of ^{32}P -UTP (MaxiScript kit, Ambion). Blots were prehybridized in 50% formamide, $5\times$ Denhardt's reagent, 0.5% SDS, $5\times$ SSPE and 100 $\mu\text{g/ml}$ salmon sperm DNA for 2 h at 42 °C, and hybridized overnight under the same conditions. Blots were washed in $0.2\times$ SSC and 0.1% SDS at 65 °C and exposed to X-ray film. Some blots were stripped and reprobed up to two times. RNA loadings were assessed by ethidium bromide staining of rRNAs or by northern blot analysis using clone BB695, which corresponds to 28S rRNA from pea.

Antibody generation and western blotting

The PsDRG clone contained a *Sall* site 45 bases upstream of the ATG start codon (within pSPORT1), which was used to subclone PsDRG into bacterial expression vector pQE31 (Qiagen). Fusion proteins were generated that correspond to the entire 399 amino acid coding domain (PsDRG-399) or to the N-terminal 202 amino acids of PsDRG (PsDRG-202). An internal *HindIII* site was used to make the latter construct. Bacterial extracts from PsDRG-399 transformants contained a large number of His-tagged proteins, which are presumed to be breakdown products of PsDRG (these proteins were not present in extracts from vector controls). PsDRG-202, which could be purified to apparent homogeneity, was used to immunize rabbits. PsDRG-202 contained a 35 residue N-terminal tag with the sequence: MRGSHHHHHHTDPHAS-SVPR [from pQE31] -VDPRVRCGDCIFRGK [from pSPORT1 and the 5'-noncoding region of PsDRG]. His-tagged fusion proteins were purified on Ni-NTA resin. Initially, the manufacturer's (Qiagen) suggested protocol was used to purify fusion proteins: fusion proteins were extracted from bacteria using guanidine-HCl, bound to Ni-NTA resin, washed with guanidine-HCl and urea buffers, eluted with imidazole and dialyzed against phosphate-buffered saline (PBS). Under these conditions, the purified fusion proteins precipitated during dialysis. Precipitation could be prevented by gradually replacing the chaotropes with PBS before the protein was eluted from the Ni-NTA column. A rabbit was immunized by injecting purified PsDRG-202 into a sterile whiffle ball chamber that had been implanted beneath the epidermis of its back [6]. About 400 μ g of PsDRG-202 was injected directly into the ball chamber for the primary immunization and for each booster injection. Ca. 15 ml of cell-free serum could be isolated from the chamber every two weeks. PsDRG-202 was conjugated to Affigel AG-10 resin (BioRad) to affinity purify PsDRG-specific antibodies. A 1:1000 dilution of the antiserum could detect 1.0 ng or less of purified fusion protein.

Frozen plant tissues were ground under liquid nitrogen and soluble proteins were extracted with homogenization buffer (HB; 50 mM Tris-HCl pH 7.5, 2 mM EDTA, 28 mM 2-mercaptoethanol) containing protease inhibitors (5 mM 6-aminocaproic acid, 1 mM benzamidine, 2 mM PMSF, 1 mg/ml w/v antipain). Protein content was determined with the BioRad dye binding assay. Proteins were separated on 12% SDS-PAGE gels. BioRad low-molecular-weight pre-stained

markers were used on all gels. For western blotting, proteins (50 μ g/lane) were electrophoretically transferred to nitrocellulose membranes using a semi-dry transfer apparatus. Equal protein loadings were confirmed by staining equivalent gels with Coomassie or silver. Affinity-purified primary antibodies were used at a 1:500 dilution. A donkey anti-rabbit IgG conjugated to horse radish peroxidase (DAR-HRP) secondary antibody was used at a 1:3000 dilution. HRP activity was detected on X-ray film using a chemiluminescent substrate (ECL kit, Amersham).

Root apices were homogenized and subjected to differential centrifugation in order to obtain fractions enriched in specific cell organelles. HB plus protease inhibitors was supplemented with 400 mM sucrose. The initial homogenate was passed through a 25 gauge needle five times and then filtered through three layers of Miracloth (CalBiochem). The homogenate was centrifuged at $3000 \times g$ for 10 min (pellet P3). The resulting supernatant was centrifuged at $20\,000 \times g$ for 10 min (pellet P20). The resulting supernatant was centrifuged at $150\,000 \times g$ for 18 h (pellet P150 and supernatant S150). At each step, a small volume of supernatant directly above the pellet was not collected and was discarded before the pellet was resuspended. Pellets were resuspended in HB plus 150 mM NaCl, 1% v/v Triton X-100 and 1% v/v Sarcosyl. Samples were kept at 4 °C during all steps. Cell fractions were analyzed by SDS-PAGE and western blotting as described above.

Results

During a screen for dormancy-associated clones from pea axillary buds [32], several growth-associated cDNA clones also were isolated. The deduced amino acid sequence of one such clone contained domains that are present in all members of the G protein superfamily (G1 to G5 domains [4]; Figure 1). This clone was most similar to the DRG subfamily of G proteins, which was characterized first in mouse [17, 26, 27]. We called the pea clone PsDRG. We obtained an *Arabidopsis* homologue (AtDRG) and determined the complete sequence of both cDNAs (Figure 1). Deduced amino acid sequences of the plant clones were 90% identical and 97% similar to each other. The plant sequences were compared to human and mouse DRGs, which represent two subfamilies of DRGs [28]. The deduced amino acid sequences of plant and human DRGs were identical at about 65% of the overlapping

	#		
Ps		MGIIEKIKEIEFEMARTQKNKATEYHLGQLKAKIAKLRTQLLEP-PKGSSGGGDGFVETKFGHGRVALIGFPPSVGKSTLLTLLTGTHSEAASYEFTTLTTCI	100
At	R.....A.....-.....A.....E.....Y.....M.....	100
Hs		..L..S..K..I.....L..L..Y..A.....-S..SA..SK..E..D..M..S..DA.....F..S..M..S..A.....	100
Mm		MS.TLA..A.....A.....AH.....L.....RL.....RE.IT.KGG.GG.P.E..D.A.T.DA.IGFV.....SN.A.VY..V.A.....TV	102
		G1	G2
Ps		PGIIHYNKTKIQLLDLPGLIEGASEGKGRGRQVIIVAKSSDIVLMVLDASKSEGRQILTKLEAVGLRLNKRPFQIYFKKKKTGGISFNSTMPLTHVDE	200
At		..V...D.....T.....T.A...I..	200
Hs		..V.E.KGAN.....AQ.....RTA.VII.M...T.G.VQ.SL.E...S..I...HK.N...P..G.....VT..QCS.	200
Mm		..V.R.KGA.....KD.....RTCNLI..I...VL..PL..KK.IEN...GF.I...SK..N.G...DK...NLTA.C.QSEL.A	202
		G3	
		**	
Ps		KLICYQILHEYKIHNAEILFREDATVDDFIDVIEGRKMYKCVVYVKNKIDVVGIDDVRLPRQPNSVVISCNLKNLDRLLSRMWDDEMLVRVYTKPQQGQ	300
At	V.....N.....I.....I.....S.....I.....A.....S.....	300
Hs		..VQL.....F..V.....CSP.E..V..V..P.L.....QISMEE...A.K.....GM.....Y..EML.EYLA.TCI...KR..R	300
Mm		ETVKS..A.....DVTLS.S...A..L..VV...V.IP.I..L.....QIS.EEL..IYKV.HC.P..AHRW.FDD..EKI..YLK...I...K..L	302
		G4	
Ps		PDFSDPVLVSADRGCSVEDFCNHIHRSLVKDKYVLVWVGIMARHYPQHCGLSHVLRDEDEVVQIVKKKETDEGGRGRFKSHSDAPARISDRQKAPLKQ	399
At		...DE.F...S...T...M.A...TST..N..N...QH.E.....R.....N...A..E.....	399
Hs		..T.A.II.--K.A...HV.HR...ASQF..A...TSTKYS..RV..T.TMEH...I.....	364
Mm		..YTSP...--PYSRTT...MK..KN..I..EF..A...L.SVK.N..KV.KD.T.E...I.....	367
		G5	

Figure 1. Comparison of deduced amino acid sequences of DRGs. The PsDRG and AtDRG cDNAs each contained about 1500 bp and encoded deduced proteins containing 399 amino acid residues. Amino acid sequence of PsDRG (Ps) and DRGs from *Arabidopsis* (At), human (Hs, [28]) and mouse (Mm, [27]) are compared. The guanosine nucleotide binding domains (G1 to G5) were identified according to Schenker *et al.* [28]. Gly-2, a potential N-myristoylation site, is marked (#). Dots (.) indicate identity; dashes (–) indicate gaps. The Lys-Leu residues at positions 201–202 (*) are encoded by AAGCTT, which was digested with *Hind*III to generate the N-terminal fusion protein, PsDRG-202.

positions, whereas plant and mouse DRGs were 58% identical. PsDRG and AtDRG encoded 32 residues at their C-termini that are not found in other eukaryotic DRGs (Figure 1). The plant DRG sequences did not contain C-terminal prenylation sites characteristic of Ras and Rab proteins [10]. DRGs also lacked a conserved Arg residue between domains G2 and G3 that is a target in heterotrimeric $G\alpha$ for ADP ribosylation by cholera toxin [19]. PsDRG and AtDRG did contain a Gly residue at amino acid position 2, which is a potential N-myristoylation site [10, 16].

Pea and *Arabidopsis* genomic DNA was digested with each of four different restriction enzymes. Southern blots were probed with the appropriate DRG clone and washed at moderately low stringency (42 °C, 0.2 × SSC). Each lane contained a single hybridizing band in all lanes of both blots (data not shown).

Patterns of PsDRG mRNA accumulation were analyzed by Northern blotting. A PsDRG probe recognized a 1.6 kb transcript (Figure 2). Dormant axillary buds on intact plant (0 h) and buds from plants 1 and 3 h after decapitation contained low levels of PsDRG mRNA; buds contained about 3-fold more mRNA at 6 h and longer times after decapitation (Figure 2A). PsDRG mRNA accumulation was assayed in other pea organs (Figure 2B). Growing root apices and elongating stems contained more PsDRG mRNA than fully elongated ('mature') roots and stems. This transcript

was present at relatively high levels in petals, stamens and carpels, but it was barely detectable in sepals and leaves. Histone mRNAs accumulate predominantly during S phase in proliferating cells [34] and are useful markers for growing organs [7]. The pattern of histone H2A mRNA accumulation was qualitatively similar to that of PsDRG mRNA.

Accumulation of AtDRG mRNA in *Arabidopsis* organs was assayed (Figure 3). Rosette leaves from 1- or 6-week-old plants, inflorescence stems and siliques contained somewhat higher levels of this message than flower buds or open flowers. AtDRG message was barely detectable in roots. A pea His2A probe hybridized to a corresponding *Arabidopsis* message. Similar relative levels of AtDRG and His2A mRNAs were present in all of the organs tested (Figure 3).

A rabbit antiserum was raised against the N-terminal 202 amino acid residues of PsDRG, about one half of the total 399 residues (see Figure 1). This antiserum recognized protein band of 43 and 45 kDa (Figure 4). The 45 kDa protein was more abundant in all of the samples shown here, but occasionally the 43 kDa band was more abundant. In axillary buds, the abundance of both bands was very similar at all stages of bud development (Figure 4A). DRG proteins were more abundant in growing root apices and elongating stems than in leaves and mature stems. DRG proteins were not detected in roots (Figure 4B).

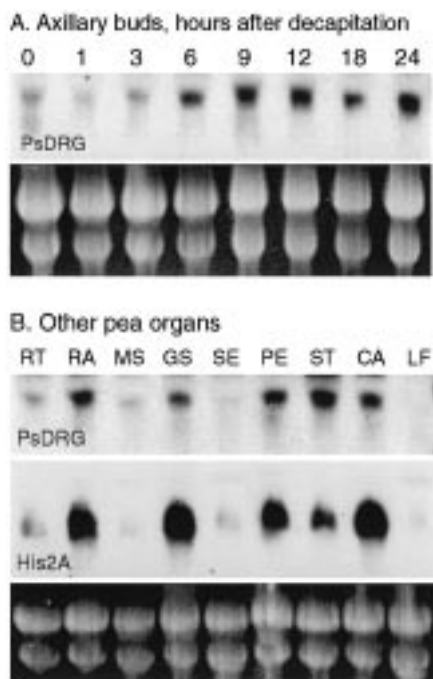


Figure 2. Northern blot analysis of pea organs. **A.** Axillary buds. Low levels of PsDRG mRNA (ca. 1.6 kb) were present in dormant buds on intact plants (0 h) and at 1 and 3 h after decapitation. Higher levels were present at 6 h (about the time that visible growth could be detected) and at later times. **B.** Other organs. PsDRG mRNA accumulated preferentially in growing root apices (RA) and growing stems (GS) compared with mature roots and stems (RT, MS). Relatively large amounts of this mRNA were present in petals (PE), stamens (ST) and carpels (CA); reduced amounts were present in sepals (SE) and leaflets (LF). The qualitative pattern of histone H2A mRNA accumulation in these organs was similar to that of PsDRG. Ethidium bromide staining of rRNAs demonstrated similar loadings of total RNA in each lane. Total RNA (25 μg per lane) was separated by denaturing formaldehyde gel electrophoresis and transferred onto a nylon membrane. PsDRG mRNA was detected using a riboprobe and histone mRNA was detected using a DNA probe.

Root apices were homogenized and then fractionated by differential centrifugation (Figure 5). DRG proteins were found predominantly in the soluble and microsomal fractions (S150 and P150, respectively). Centrifugation at $150\,000 \times g$ was performed for 18 h to assure that essentially all microsomal membranes would be pelleted. DRG proteins were not detected in the P3 fraction (enriched in nuclei) and were barely detectable in the P20 fraction (enriched in mitochondria and plastids).

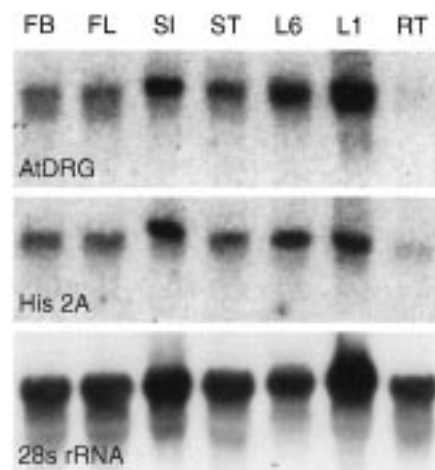


Figure 3. Northern blot analysis of *Arabidopsis* organs. Relatively high levels of AtDRG mRNA (ca. 1.6 kb) were present in siliques (SI), inflorescence stems (ST), and rosette leaves from 1-week-old plants (L1) and 6-week-old plants (L6); reduced amounts were present in flower buds (FB) and open flowers (FL); and very little message was present in roots (RT). The blot was stripped and hybridized with a pea His2A probe. The qualitative pattern of histone H2A mRNA accumulation in these organs was similar to that of AtDRG. The 28S rRNA probe indicated similar RNA loadings in each lane. Each lane contained 10 μg of total RNA. The blots were hybridized with DNA probes.

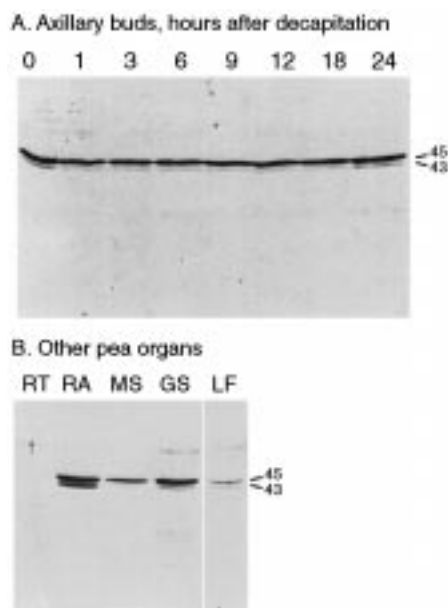


Figure 4. Western blot analysis of pea organs using PsDRG antibodies. PsDRG antibodies recognized protein bands of 45 and 43 kDa. **A.** Axillary buds contained similar amounts of DRG proteins at each stage of development. **B.** Root apices (RA) and growing stems (GS) contained relatively large amounts of PsDRG proteins; mature stems (MS) and leaflets (LF) contained reduced amounts of these proteins; and these proteins were not detected in fully elongated roots (RT).

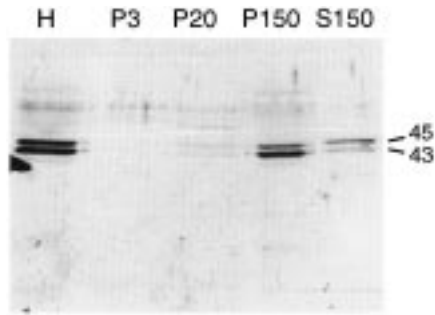


Figure 5. Accumulation of PsDRG in subcellular cell fractions of root apices. Root apices were homogenized and fractionated by differential centrifugation (H, homogenate; P3, 3000 \times g pellet; P20, 20 000 \times g pellet; P150, 150 000 \times g pellet; and S150, 150 000 \times g supernatant). PsDRG proteins were found predominantly in the microsome-enriched fraction (P150) and the post-microsomal supernatant fraction (S150). PsDRG proteins were detected by western blotting as described in Figure 4.

Discussion

Full-length DRG cDNA clones from pea (PsDRG) and *Arabidopsis* (AtDRG) are described in this report (Figure 1). PsDRG was isolated from a pea axillary bud library and AtDRG was obtained from the ABRC. Another *Arabidopsis* DRG clone, called AtDRG1, has been sequenced (J.R. Botella, GenBank U66408). The nucleotide sequences of the two AtDRG clones are identical except for three bases in the 3' non-coding region and are presumed to be derived from the same gene. Five additional *Arabidopsis* EST clones also appear to be derived from the same gene. Genomic Southern blotting (data not shown) indicated that the pea and *Arabidopsis* genomes probably contain only one DRG-like gene. To date, only one type of DRG has been isolated from any species. Nonetheless, the fact that human DRG is more similar to plant DRGs than it is to mouse DRG leaves open the possibility that mammals, at least, may contain more than one DRG-like gene (Figure 1; [28]). Plant DRGs contain an additional 32 amino acids at their C-termini that are not found in other DRGs. This domain may be responsible for unique functions of plant DRGs.

Mouse and *Drosophila* DRG fusion proteins can bind GTP [27, 30]. Presumably, all DRGs bind and hydrolyze GTP *in vivo* and thereby regulate some cellular activity. DRG genes show a wide variety of expression patterns in animals, making it difficult to identify their role(s) in development or cell physiology. *Drosophila* DRG accumulates at similar levels in adult tissues, embryos and cultured cells [30]. In *Xenopus*, DRG transcripts generally increase in abun-

dance during embryonic development and are more abundant in the developing brain than in other organs [18]. Mouse DRG was cloned based on its relatively high level of expression in early stages of brain development. Mouse DRG mRNA is expressed at moderate levels in several mature organs (brain, spleen, liver, lung, muscle and kidney) and at very high levels in testis; it is not expressed in heart [27]. Human DRG was cloned based on its reduced level of expression in fibroblasts that had been transformed with SV40, which might suggest a role for DRG in inhibiting cell proliferation in untransformed cells [28]. Human DRG also is expressed in several organs; unlike mouse, however, expression is relatively low in brain and high in heart. Human DRG protein interacts with TAL1, an oncoprotein that is over-expressed in leukemic cells [20]. Human DRG also is capable of stimulating the co-transforming activities of *c-myc* and *ras*. Together these results suggest a role for human DRG in promoting cell proliferation.

PsDRG mRNA accumulated preferentially in growing regions of pea stems and roots (Figure 2). The root apex includes zones of rapid cell division and cell elongation [25]; and the region of internode-5 designated as 'growing' was about 7 mm long when it was isolated and would have elongated to about 100 mm. Axillary buds released from apical dominance are an excellent system for analyzing temporal patterns of gene expression during the transition from the non-growing (dormant) to the growing developmental state [7]. Dormant buds contained a small amount of PsDRG mRNA compared to buds from plants 6 h or more after decapitation (Figure 2A). Transcripts corresponding to MAP kinase, *cdc2* kinase, histones, and ribosomal proteins genes increase within one hour of decapitation [7, 33], so increased PsDRG expression is not among the earliest events in the dormancy-to-growth transition. Histone mRNA accumulation is closely correlated with S-phase and is an excellent marker for proliferating cells [34]. Root apices and growing stems accumulated high levels of histone H2A mRNA (Figure 2B), as did growing axillary buds [7]. The 'growth state' of the floral organs has not been well characterized. Nevertheless, organs that accumulated relatively high levels of PsDRG mRNA also accumulated high levels of histone H2A mRNA (Figure 2B). It was not practical to collect growing versus non-growing tissues from *Arabidopsis*. However, the patterns of AtDRG and His2A mRNA accumulation were very similar (Figure 3),

which extends the correlation between DRG mRNA accumulation and growth.

The predicted translation product of PsDRG would have a molecular mass of 44 998 Da. PsDRG antibodies recognized proteins with apparent molecular weights of 43 and 45 kDa (Figure 4). As indicated above, we believe that plants probably contain a single DRG-like gene. It is possible that rather distantly related proteins share common epitopes or that the 43 kDa band is a breakdown product of 45 kDa. We are testing these possibilities.

Root apices and growing stems contained relatively high levels of DRG proteins, whereas mature stems contained less and none was detected in mature roots (Figure 4B). These patterns of protein accumulation are generally similar to patterns of mRNA accumulation in these organs (Figure 2B). In contrast, the relative abundance of PsDRG proteins was similar at each stage of bud development, a result that was unexpected based on the pattern of mRNA expression (Figures 2A and 4A). It may be significant that dormant buds are able to resume growing whereas mature stems and roots are not. The molecular basis for this difference might be resolved by determining relative rate of synthesis and degradation of PsDRG mRNA and protein.

Cell fractionation experiments showed that PsDRG proteins occur predominantly in microsomal and cytoplasmic fractions and that they were barely detectable in fractions enriched in nuclei, mitochondria and plastids (Figure 5). Other classes of G proteins also are membrane-associated. Ras proteins are associated with the plasma membrane via farnesyl groups and Rab proteins are associated with the ER, Golgi apparatus and other membranes by geranylgeranyl groups [10, 31]. Deduced sequences of DRG proteins lack conserved sites near the C-terminus that are necessary for these lipid modifications. ADP-ribosylation factors are myristoylated on a Gly residue near the N-terminus [10]. There is a Gly residue at position 2 in plant and human DRGs and a position 3 in mouse DRG (Figure 1) which might be a target for myristoylation. Some myristoylated proteins reversibly associate with and dissociate from their target membranes. We are determining whether DRG proteins are myristoylated *in vivo* and whether the association of PsDRG with cellular membranes is correlated with the GTP/GDP binding state of the protein.

Summary

G proteins utilize the GTP cycle as a molecular switch to regulate many cellular processes [3]. DRG genes from plants, animals and fungi encode remarkably similar proteins. While this might indicate a single conserved function, the disparate patterns of gene expression observed in various organisms challenge this simple hypothesis. Accumulation of DRG mRNA and protein in plants cells is likely to contribute to the overall control of DRG function. In addition, protein movement between cellular compartments, perhaps mediated by binding to specific guanine nucleotides in a manner similar to that of ARF proteins [10], and the presence and activity of upstream regulators and downstream effector proteins also are expected to affect DRG function. Both pea and *Arabidopsis* will continue to be useful for deciphering the cellular function of plant DRGs. Pea is highly tractable for biochemical and physiological studies, for cell fractionation experiments, and for determining the effects of G protein inhibitors on PsDRG activity and subcellular localization. Transgenic *Arabidopsis* plants that over-express sense, antisense or dominant-negative DRG constructs also might provide clues to understanding DRG function and expression, as would plants transformed with the AtDRG promoter fused to a marker gene.

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