Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6101–6105, August 1989
Biochemistry

Molecular cloning, characterization, and expression of human ADP-ribosylation factors: Two guanine nucleotide-dependent activators of cholera toxin

(guanine nucleotide-binding proteins/adenylyl cyclase/phospholipase C)

DAVID A. BOBAK*, MARIA S. NIGHTINGALE, JAMES J. MURTAGH, S. RUSS PRICE, JOEL MOSS, AND MARTHA VAUGHAN

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Martha Vaughan, May 23, 1989

ABSTRACT **ADP-ribosylation factors (ARFs) are small guanine nucleotide-binding proteins that enhance the enzymatic activities of cholera toxin. Two ARF cDNAs, ARF1 and ARF3, were cloned from a human cerebellum library. Based on deduced amino acid sequences and patterns of hybridization of cDNA and oligonucleotide probes with mammalian brain poly(A)$^+$ RNA, human ARF1 is the homologue of bovine ARF1. Human ARF3, which differs from bovine ARF1 and bovine ARF2, appears to represent a newly identified third type of ARF. Hybridization patterns of human ARF cDNA and clone-specific oligonucleotides with poly(A)$^+$ RNA are consistent with the presence of at least two, and perhaps four, separate ARF messages in human brain. *In vitro* translation of ARF1, ARF2, and ARF3 produced proteins that behaved, by SDS/PAGE, similar to a purified soluble brain ARF. Deduced amino acid sequences of human ARF1 and ARF3 contain regions, similar to those in other G proteins, that are believed to be involved in GTP binding and hydrolysis. ARFs also exhibit a modest degree of homology with a bovine phospholipase C. The observations reported here support the conclusion that the ARFs are members of a multigene family of small guanine nucleotide-binding proteins. Definition of the regulation of ARF mRNAs and of function(s) of recombinant ARF proteins will aid in the elucidation of the physiologic role(s) of ARFs.**

The family of guanine nucleotide-binding proteins includes the following: the protein translation initiation and elongation factors, such as elongation factor Tu (1); the heterotrimeric signal-transducing G proteins—e.g., G_s, the stimulatory G protein of adenylyl cyclase (2); yeast Ypt1p and Sec4p (3); the *ras* oncogene products (4); the *ras*-related proteins—e.g., *rap* and *rho* (5–7); and a group of similar proteins known as ADP-ribosylation factors, or ARFs (8–11). The ARFs stimulate the enzymatic activities of cholera toxin in a GTP-dependent manner (8–11). They enhance toxin-catalyzed ADP-ribosylation of the α subunit of G_s, resulting in persistent activation of the catalytic subunit of adenylyl cyclase (2, 12).

Kahn and Gilman (8, 9) originally purified ARF from rabbit liver and bovine brain membranes. Tsai *et al.* (10, 11) purified one membrane-associated (mARF) and two soluble forms (sARF I and sARF II) of ADP-ribosylation factors from bovine brain. These proteins have molecular weights of ≈20,000 and appear to be very similar in structure and function. Based on activity and immunoreactivity, ARFs appear to be present in many, if not most, types of eukaryotic cells and are especially abundant in brain, where they represent nearly 1.0% of total protein (11, 13). Deduced amino acid sequences of ARFs include structural elements similar to those found in other guanine nucleotide-binding proteins (1, 14, 15).

Sewell and Kahn (14) isolated one ARF clone from a bovine adrenal chromaffin cell cDNA library and another from a yeast genomic library, referred to here as bovine ARF1 and yeast ARF, respectively. Price *et al.* (15) characterized a bovine retinal cDNA clone, referred to here as bovine ARF2; the nucleotide and deduced amino acid sequences are closely related to, but distinct from, bovine ARF1.

We describe here the molecular cloning of two forms of ARF cDNA from human cerebellum. One is the human homologue of the bovine ARF1, whereas the other appears to be a newly identified form of ARF very similar to but clearly different from the two reported bovine cDNAs.†

MATERIALS AND METHODS

Materials. A human brain cDNA library in λgt11 was kindly provided by Edward Ginns (National Institutes of Health, Bethesda, MD). The λZAP human cerebellum cDNA library, mRNA capping kit, and rabbit reticulocyte lysate *in vitro* translation kit were purchased from Stratagene; random-primed DNA labeling kits and *Eco*RI restriction enzyme were from Boehringer Mannheim; nylon colony/plaque-hybridization filters, [α-^{32}P]dATP (6000 Ci/mmol; 1 Ci = 37 GBq), [α-^{32}P]dCTP (3000 Ci/mmol), and deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate (500 Ci/mmol) were from New England Nuclear; *Thermus aquaticus* DNA polymerase for polymerase chain reaction amplifications and Gene Amp kit were from Perkin–Elmer/Cetus; oligonucleotide primers corresponding to the λgt11 nucleotide sequence flanking the *Eco*RI cloning site (no. 1218-"forward" and no. 1222-"reverse") were from New England Biolabs; Sequenase 2.0 T7 DNA polymerase was from United States Biochemicals; *Thermus aquaticus* DNA polymerase for sequencing reactions was from Promega; nylon membranes (Nytran) were from Schleicher & Schuell; terminal deoxynucleotidyltransferase, RNA standards, and prestained protein standards were from Bethesda Research Laboratories; protein standards were from Bio-Rad; L-[^{35}S]methionine (1159 Ci/mmol) was from Amersham; and polyvinylidene difluoride membrane (Immobilon) was from Millipore. Human, rabbit, and

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.

Abbreviations: G protein, heterotrimeric guanine nucleotide-binding protein; G_s, the stimulatory G protein of adenylyl cyclase; G_o, the G protein in brain that may regulate ion flux; ARF, ADP-ribosylation factor; mARF and sARF, membrane-associated and soluble forms of ARF, respectively; PLC, phospholipase C.

*To whom reprint requests should be addressed.

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M25203, human ARF1; M25204, human ARF3).

mouse brain poly(A)$^+$ RNAs were purchased from Clontech and custom preparations of rat and bovine brain poly(A)$^+$ RNAs were from Lofstrand Laboratories (Gaithersburg, MD). Oligonucleotides were synthesized by Pharmacia or within our laboratory using an Applied Biosystems 380B DNA synthesizer.

Cloning and Sequencing of Human ARF cDNA. Phage from the λgt11 human brain cDNA library were mixed with *Escherichia coli* Y1090, plated, and lifted in duplicate onto nylon colony/plaque-hybridization filters. Filters were denatured, neutralized, baked at 80°C for 2 hr, and prehybridized in solution A (40% formamide/1 M NaCl/50 mM Tris·HCl, pH 7.5/10% dextran sulfate/1% SDS with denatured salmon sperm DNA at 100 μg/ml) for ≈6 hr at 42°C. Hybridization was carried out for ≈16 hr at 42°C using fresh solution A containing 2–5 × 10^5 cpm of the bovine ARF2 cDNA per ml (15) labeled with [α-^{32}P]dCTP (3000 Ci/mmol) by the random-primer method (16). Filters were washed in 2× SSC/0.5% SDS at room temperature, and 1× SSC/0.5% SDS at 42°C and 60°C before exposure to Kodak XAR-2 film at −70°C (1× SSC = 0.15 M NaCl/0.015 M sodium citrate).

Inserts from plaque-purified clones were amplified by the polymerase chain reaction (17) with oligonucleotide primers complementary to the λgt11 nucleotide sequences flanking the *Eco*RI cloning site (18). Inserts were ≈900 base pairs (bp) long as assessed by electrophoresis in 1% agarose. Direct sequencing (19, 20) of one insert, clone A, was performed in both directions by the dideoxynucleotide chain-termination method (21) with deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate and T7 DNA polymerase (Sequenase). The nucleotide sequence of this clone exhibited a high percentage of identity with both bovine ARF cDNAs (14, 15) but was truncated at the extreme 5′ end of the predicted coding region (data not shown). Limited sequencing of several other positive clones indicated inserts identical to that of clone A.

To obtain a human ARF cDNA containing the complete coding region, a λZAP human cerebellum cDNA library in *E. coli* strain BB4 was screened (≈4 × 10^5 plaques) using clone A; hybridization and washing conditions were as outlined for the λgt11 library and protocols supplied by the manufacturer. Six positive clones were isolated and subcloned into Bluescript phagemid (22). Two clones, B and E, were sequenced by using the Sequenase reagents and protocols described above. Portions of the clones were also sequenced by the dideoxynucleotide chain-termination method (21) with deoxyadenosine 5′-[^{35}S]thio]triphosphate and *Thermus aquaticus* DNA polymerase according to protocols supplied by the manufacturer (Promega). Sequencing primers were synthesized based on determined sequences. Sequencing reactions overlapped, resulting in the generation of continuous sequence in sense and antisense directions for each clone.

Northern Blot Analysis. Mammalian brain poly(A)$^+$ RNA was fractionated by electrophoresis in 1% agarose/formaldehyde gels and transferred to nylon filters, which were baked at 80°C for 2 hr and prehybridized in solution A for ≈6 hr at 42°C. Hybridization was carried out for ≈16 hr at 42°C with fresh solution A containing 2–5 × 10^5 cpm of cDNA labeled with [α-^{32}P]dCTP (3000 Ci/mmol) per ml by the random-primer method (16). Filters were washed in 2× SSC/0.5% SDS at room temperature, and 1× SSC/0.5% SDS at 42°C and 60°C. Filters were stripped with two washes in 0.1× SSC/0.5% SDS at 100°C and checked for residual radioactivity by autoradiography before reuse. The 48-base 5′-flanking sequences immediately upstream of the start codons of the two human cDNAs (71% mismatch; see Fig. 1) were used to construct antisense direction clone-specific oligonucleotide probes. Hybridizations with these oligonucleotides, labeled with [α-^{32}P]dATP (6000 Ci/mmol) using terminal deoxynucleotidyltransferase (23), were performed as described for the cDNAs except that filters were washed in 2× SSC/0.5% SDS

```
-424  GTGCAGATCTTGGTGGTAGGTTGAGGCCATCTCCTGGGCCGCCTGGCGGCCATCGTGGCTAAACAGGTACTGCTGGGCC -346

-345 GGAAGGTGGTGGTCGTACGCTGTGAAGGCATCAACATTTCTGGCAATTTCTACAGAAACAAGTTGAAGTACCTGGCTTTC -266

-265 CTCCGCAAGCCGGTGCAGATCTGGGTGCTAGGTGGAGGGAAGGAAGGAGGGAGCCGGGGAGTGGGCCAGGGCAGCAGCCG -186

-185 GGCTGAAGTCGGCTTGGGGAGCCAGAGGGAGCGGAGCGGAACCTGCGGGGCAGAGGCGGCGGCCGCAGCGGCGCAGCTGA -106

-105 TAGCAGGAACAGGATCAAGTCTTCCATACCAGGGACCAGGCCAAAACCAGACTGCCACTGCCCCTACTTGGGCCCCACTG  -26
                                                          -49 G A   T  GAGGT T   TGGCC

 -25 TCCCCAGAAAGCAGCTGCTGTGATCATGGGCAATATCTTTGGAAACCTTCTCAAGAGCCTGATTGGGAAGAAGGAGATG   54
     AGTGTCCTTCCACCTGT CACA G     G C    C CC    CT    G   TT   C A A A

  55 CGCATCCTGATGGTGGGCCTGGATGCCGCAGGAAAGACCACCATCCTATACAAGCTGAAACTGGGGGAGATCGTCACC  132
           C              T   G      G    C       T G    T       G

 133 ACCATCCCTACCATTGGGTTCAATGTGGAGACAGTGGAGTATAAGAACATCAGCTTTACAGTGTGGGATGTGGGTGGC  210
       T C    A C    C    A C       C            C T       C

 211 CAGGACAAGATTCGACCCCTCTGGAGACACTACTTCCAGAACACCCAAGGGTTGATATTTGTGGTCGACAGCAATGAT  288
           C G    G  C C                 A    CC   C C    G          C

 289 CGGGAGCGAGTAAATGAGGCCCGGGAAGAGCTGATGAGAATGCTGGCGGAGGACGAGCTCCGGGATGCTGTACTCCTT  366
     A A    T G C       T G    C    G       C                    C    G

 367 GTCTTTGCAAACAAACAGGATCTGCCTAATGCTATGAACGCTGCTGAGATCACAGACAAGCTGGGCCTGCATTCCCTT  444
       G C C    G    C C C C C    T G C                   G    C A A

 445 CGTCACCGTAACTGGTACATTCAGGCCACCTGTGCCACCAGCGGGGACGGGCTGTACGAAGGCCTGGACTGGCTGGCC  522
       C  A G                    C          C       C T    A          T

 523 AATCAGCTCAAAAACAAGAAGTGAAAGCCAGACAGCCCTAACAAAGCACCCCACCCACCCCTGACATACCTACTGTCAC  601
           CGG   C       C  G CC CC T CCT TC CTC T TTG  CT TGCTTTACT TC TGTGGCA

602 CCTGCCCCAGTCCTACCCCTTCCTCTCCATGCA 634 End Clone B, Human ARF3
    AAC TG GGC  G GGTGTGAGTG CAG A  TGCCTCCGTGGTTTGGTCACCGTGTGCATCGCACCGTGCTGTAAATGT  681

682 GGCAGACGCAGCCTGCGGCCAGGCTTTTTATTTAATGTAAATAGTTTTTGTTTCCAATGAGGCAGTTTCTGGTACTCCTA  761

762 TGCAATATTACTCAGCTTTTTTTATTGTAAAAAAAAAAAAAAAAAAAA 809 End Clone E, Human ARF1
```

FIG. 1. Nucleotide sequences of human ARF cDNAs. Human ARF3 (clone B) begins on the first line. Immediately beneath is the corresponding region of human ARF1 (clone E); only those nucleotides that differ from clone B are displayed. Initiation and termination codons are boxed. Sequences are numbered starting with the first nucleotide of the start codon (+1).

Table 1. Percentage identity of coding region nucleotide sequences and deduced amino acid sequences of ARF clones

	hARF3	hARF1	bARF1	bARF2	yARF
hARF3		84	84	80	63
hARF1	96		91	79	64
bARF1	96	100		80	66
bARF2	94	96	96		67
yARF	76	77	77	77	

Percentage identity of nucleotide sequences of the coding regions of the indicated ARF clones is above the diagonal and percentage identity of deduced amino acid sequences is below. hARF3, human clone B; hARF1, human clone E; bARF1, bovine ARF1 (14); bARF2, bovine ARF2 (15); yARF, yeast ARF (14).

at room temperature, 1× SSC/0.5% SDS at 42°C, and 0.2× SSC/0.5% SDS at 60°C before exposure to Kodak XAR-2 film at −70°C.

Expression of ARF cDNAs. With an mRNA capping kit and instructions provided by the manufacturer (Stratagene), ARF cDNAs cloned in the Bluescript phagemid were used to synthesize mRNA in the sense and antisense directions for each clone. mRNAs were incubated for 60 min at 30°C in 20 μl of rabbit reticulocyte lysate containing L-[^{35}S]methionine (≈18 × 10^6 cpm) according to the manufacturer's protocol (Stratagene). Reaction products (5 μl) were analyzed by SDS/PAGE (16% polyacrylamide; ref. 24), with purified bovine brain sARF II (11) as a reference marker, transferred to polyvinylidene difluoride membrane (25), and exposed to Kodak XAR-2 film for 8 hr at room temperature.

RESULTS AND DISCUSSION

Two independent clones were isolated from the λZAP human cerebellum cDNA library using human brain ARF clone A. Human ARF clone B (1058 bp) contains an open reading frame that encodes a protein of 181 amino acids with a predicted molecular weight of 20,587 (Fig. 1). The nucleotide sequence of this clone is identical to overlapping regions of the truncated clone A from the human brain λgt11 library (data not shown). Human ARF clone E (858 bp) also encodes a protein of 181 amino acids with a predicted molecular weight of 20,683 (Fig. 1). The two clones are 84% identical over their putative coding regions, indicating that although quite similar, they are different gene products. There is little, if any, similarity of nucleotide sequences in the 5′- and 3′-untranslated regions; no potential polyadenylylation signals were identified in either clone.

Because clone E encodes a protein with an amino acid sequence identical to that predicted for bovine ARF1 (14), it appears to be the homologue of bovine ARF1 and is referred to as human ARF1. Clone B encodes an ARF protein different from both ARF1 and ARF2. This newly identified form of ARF is referred to as human ARF3. Nucleotide sequences in the coding regions of the human and bovine ARF1 are 91% identical (Table 1). Among the other human and bovine ARFs there is 94–96% identity of amino acid and 79–84% identity of nucleotide (coding region) sequences. Yeast ARF sequences are less similar when compared with any of the others. The LFASTA computer program (26), with a *ktup* of 2, was used to assess similarities in untranslated regions of the ARF clones. The 5′- and 3′-untranslated regions of human and bovine ARF1 are 72% and 84% identical, respectively. Among other ARF clones, there is little, or no, similarity in sequences of corresponding untranslated regions (data not shown).

Deduced amino acid sequences of human, bovine, and yeast ARF clones are aligned in Fig. 2. Most of the differences among the mammalian ARFs are at the amino and carboxyl termini. Human ARF3 has differences at three positions (positions 9, 13, and 174) that are identical in the other ARFs, including yeast. ARF3 also has lysines at positions 178, 180, and 181, creating a relatively lysine-rich carboxyl terminus. Sequences of CNBr peptides from bovine brain sARF II (15) are identical with the deduced amino acid sequences of bovine and human ARF1, and human ARF3, but differ in 2 (of 60) positions from that of bovine ARF2. Bovine sARF II, then, is likely to be the bovine ARF1 or, perhaps, bovine ARF3 gene product. Deduced amino acid sequences of ARF1, ARF2, and ARF3, each differ in several positions from sequences of CNBr peptides of mARF purified from bovine brain (13). Thus, mARF may be the product of a gene not yet cloned.

Proposed consensus amino acid sequences for GTP binding and hydrolysis (1, 27) are present in the two human ARF forms. These are GXXXGK (positions 24–30), DXXG (positions 67–70), and NKXD (positions 126–129) as shown in Fig. 2. Except for Gly-27 in yeast ARF, all ARFs are identical in these putative GTP-binding regions. Because of these regions, all of the ARF forms are significantly related to both

```
                    10        20        30        40        50
Human ARF3  MGNIFGNLLKSLIGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Human ARF1  -----A--F-G-F--------------------------------------
Bovine ARF1 -----A--F-G-F--------------------------------------
Bovine ARF2 ---V-EK-F---F--------------------------------------
Yeast  ARF  --LFASK-FSN-F-N----------G-----V--------VI-------

                    60        70        80        90       100
Human ARF3  FNVETVEYKNISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Human ARF1  ----------------------------------------------------
Bovine ARF1 ----------------------------------------------------
Bovine ARF2 ----------------------------------------------------
Yeast  ARF  ------Q---------------R--S-----YR--E-V---------S-I

                   110       120       130       140       150
Human ARF3  NEAREELMRMLAEDELRDAVLLVFANKQDLPNAMNAAEITDKLGLHSLRH
Human ARF1  ----------------------------------------------------
Bovine ARF1 ----------------------------------------------------
Bovine ARF2 -------T----------------V-----------------------Q
Yeast  ARF  G----VMQ---N-----N-AW----------E--S-----E------I-N

                   160       170       180
Human ARF3  RNWYIQATCATSGDGLYEGLDWLANQLKNKK
Human ARF1  ----------------------S---R-Q-
Bovine ARF1 ----------------------S---R-Q-
Bovine ARF2 ----------------------S-----Q-
Yeast  ARF  -P-F---------E------E--S-S---ST
```

FIG. 2. Comparison of deduced amino acid sequences of human, bovine, and yeast ARF clones. The deduced amino acid sequences of the human ARF cDNAs were aligned. Amino acid identities, compared with the sequence of human ARF3 in the top line, are indicated by a hyphen and amino acid differences are shown with the corresponding single-letter amino acid code. References for sequences are as follows: bovine ARF1, ref. 14; bovine ARF2, ref. 15; yeast ARF, ref. 14.

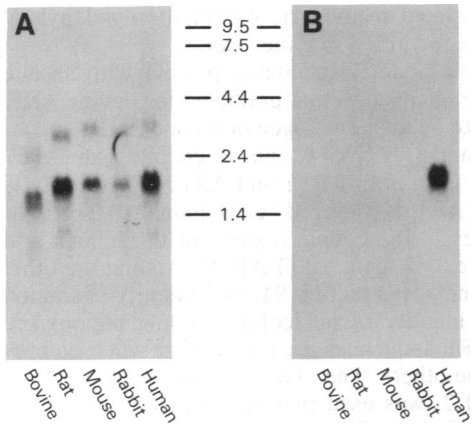

FIG. 3. Hybridization of poly(A)$^+$ RNA from mammalian brain with cDNA and oligonucleotide probes specific for human ARF1. (*A*) Human ARF1 cDNA was hybridized with poly(A)$^+$ RNA from bovine, rat, mouse, rabbit, and human brain. (*B*) The filter was stripped and hybridized with a human ARF1-specific oligonucleotide. Positions of RNA standards (kb) are shown.

the heterotrimeric and small G proteins (14, 15), although they appear not to share effector domains.

Human brain ARF1 cDNA was hybridized with poly(A)$^+$ RNA from bovine, rat, mouse, rabbit, and human brain (Fig. 3*A*). It hybridized strongly with an ≈1.8-kilobase (kb) mRNA from human brain, and weakly with an mRNA of ≈3.7 kb. mRNAs of similar sizes were detected in brain poly(A)$^+$ RNA from other species. To identify the specific mRNA from human brain corresponding to the ARF1 cDNA, the filter was stripped and reprobed with a human ARF1-specific oligonucleotide using higher stringency washes (Fig. 3*B*). This probe hybridized only with the 1.8-kb mRNA. Bovine ARF1-specific probes also identified the 1.8-kb mRNA from human brain (data not shown). These hybridization studies support the conclusion that the human ARF1 cDNA clone is the homologue of bovine ARF1.

Human ARF3 cDNA was also used to probe poly(A)$^+$ RNA from mammalian brain (Fig. 4*A*). It hybridized strongly with a human brain mRNA of ≈3.7 kb and moderately with mRNAs of ≈1.2 and ≈1.0 kb. Patterns of hybridization with mRNA from brain tissue of other mammalian species were similar. The filter was stripped and reprobed with a human ARF3-specific oligonucleotide, which hybridized, in human brain, strongly with the 3.7-kb mRNA and moderately with

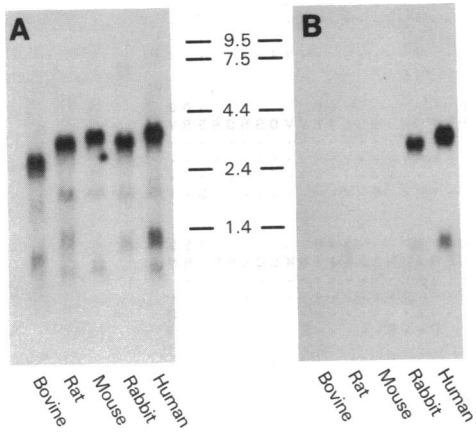

FIG. 4. Hybridization of poly(A)$^+$ RNA from mammalian brain with cDNA and oligonucleotide probes specific for human ARF3. (*A*) Human ARF3 cDNA was hybridized with poly(A)$^+$ RNA from bovine, rat, mouse, rabbit, and human brain. (*B*) The filter was stripped and hybridized with the human ARF3-specific oligonucleotide. Positions of RNA standards (kb) are shown.

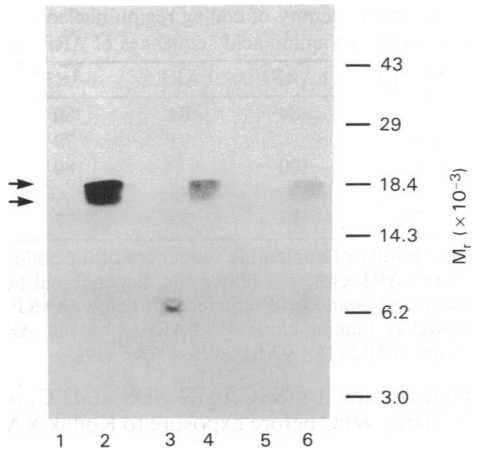

FIG. 5. Translation of mRNAs synthesized from ARF cDNAs. Sense and antisense capped mRNAs produced from human ARF1, human ARF3, and bovine ARF2 cDNAs were translated in rabbit reticulocyte lysate in the presence of [^{35}S]methionine, and products were analyzed as described. Lanes: 1, human ARF1, antisense; 2, human ARF1, sense; 3, human ARF3, antisense; 4, human ARF3, sense; 5, bovine ARF2, antisense; 6, bovine ARF2, sense. Mobility of purified bovine brain sARF II is indicated by the upper arrow. In the presence of lysate alone, sARF II migrated as a broader band (indicated by the area between the upper and lower arrows). Positions of prestained protein standards are shown. When unstained standards were used as reference, purified and recombinant ARFs behaved as proteins of M_r ≈20,000 (data not shown).

the 1.2-kb mRNA (Fig. 4*B*). The 1.0-kb mRNA identified with the ARF3 cDNA did not hybridize with the ARF3 clone-specific oligonucleotide. Bovine ARF1- and ARF2-specific probes did not recognize either the ≈3.7- or ≈1.2-kb mRNA species identified with the human ARF3 probes (data not shown), consistent with the view that human ARF3 represents a newly identified form of ARF. The 3.7- and 1.2-kb mRNAs in human brain likely represent either very closely related, but distinct, ARF species or, perhaps, alternatively spliced forms of ARF3 mRNAs. The 1.0-kb mRNA, which hybridized with the ARF3, but not the ARF1, cDNA may represent an ARF3-related form in human brain.

Sense and antisense mRNAs, synthesized from human ARF1, human ARF3, and bovine ARF2 (15) cDNAs, were translated in rabbit reticulocyte lysate containing L-[^{35}S]-methionine. Sense-oriented mRNA generated from each ARF cDNA resulted in the production of labeled proteins with apparent mobilities on SDS/PAGE identical to that of purified bovine brain sARF II (Fig. 5). Functional activity of these putative recombinant ARF proteins is being investigated.

Using the TFASTA program (*ktup* of 1), the deduced amino acid sequence of human ARF3 was compared with the entries in the Protein Identification Resource (National Biomedical

Table 2. Similarities of sequences of ARF proteins to those of G proteins and PLC II

Clone	% amino acid sequence identity		
	$G_{o\alpha}$	*smg21/rap1*	PLC II
hARF3	31 (74)	20 (69)	23 (60)
h/bARF1	32 (74)	20 (69)	22 (60)
bARF2	31 (72)	20 (69)	23 (60)

Deduced amino acid sequences of the ARF clones were compared with those of bovine $G_{o\alpha}$ (138-amino acid overlap), *smg21/rap1* (121-amino acid overlap), and PLC II (148-amino acid overlap) by the LFASTA computer program (26) with *ktup* of 1. The percentage identity is shown. References for sequences are as follows: bovine ARFs, Table 1 legend; $G_{o\alpha}$, ref. 29; *smg21/rap1*, ref. 30; PLC II, ref. 28. Numbers in parentheses represent percentage identity plus conservative substitutions.

Research Foundation) and Swiss-Prot (European Molecular Biology Laboratory) protein sequence data bases (26). The highest scores, indicating potentially significant homology with ARF3, were produced by other guanine nucleotide-binding proteins, as reported by Sewell and Kahn (14) for bovine ARF1 and Price *et al.* (15) for bovine ARF2. The ARFs also exhibited a modest, but significant, percentage of identity with the deduced amino acid sequence of bovine phospholipase C II (PLC II) reported by Stahl *et al.* (28). Percentage identity of the deduced amino acid sequences of the ARFs with that of bovine PLC II (Table 2) is less than that with the α subunit of bovine G_o ($G_{o\alpha}$), a heterotrimeric G protein (29), but similar to that with bovine *smg21/rap1*, a *ras*-related protein (30). The basis for the homology between the ARFs and other guanine nucleotide-binding proteins is primarily the presence of sequences believed to be involved in GTP binding, whereas the PLC II contains no such sequences. Evaluation of the significance of the possible homology between the ARF and PLC families of proteins awaits characterization of the functional protein domains of the ARFs and PLCs.

The ARFs, then, are members of a family of closely related guanine nucleotide-binding proteins whose participation in the pathophysiological ADP-ribosylation of $G_{s\alpha}$ by cholera toxin is now well described but whose role(s) in normal cells remains to be elucidated. The availability of clones representing multiple forms of ARF and definition of the properties of corresponding recombinant proteins should facilitate identification of the physiologic function(s) of ARFs.

We thank Dr. Lloyd Mitchell for advice regarding direct sequencing of polymerase chain reaction-amplified DNA.

1. Allende, J. E. (1988) *FASEB J.* **2,** 2356–2367.
2. Casey, P. J. & Gilman, A. G. (1988) *J. Biol. Chem.* **263,** 2577–2580.
3. Bourne, H. R. (1988) *Cell* **53,** 669–671.
4. Barbacid, M. (1987) *Annu. Rev. Biochem.* **56,** 779–827.
5. Pizon, V., Chardin, P., Lerosey, I., Olofsson, B. & Tavitian, A. (1988) *Oncogene* **3,** 201–204.
6. Narumiya, S., Sekine, A. & Fujiwara, M. (1988) *J. Biol. Chem.* **263,** 17255–17257.
7. Yamamoto, K., Kondo, J., Hishida, T., Teranishi, Y. & Takai, Y. (1988) *J. Biol. Chem.* **263,** 9926–9932.
8. Kahn, R. A. & Gilman, A. G. (1984) *J. Biol. Chem.* **259,** 6228–6234.
9. Kahn, R. A. & Gilman, A. G. (1986) *J. Biol. Chem.* **261,** 7906–7911.
10. Tsai, S.-C., Noda, M., Adamik, R., Moss, J. & Vaughan, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5139–5142.
11. Tsai, S.-C., Noda, M., Adamik, R., Chang, P., Chen, H.-C., Moss, J. & Vaughan, M. (1988) *J. Biol. Chem.* **263,** 1768–1772.
12. Moss, J. & Vaughan, M. (1988) *Adv. Enzymol.* **61,** 303–379.
13. Kahn, R. A., Goddard, C. & Newkirk, M. (1988) *J. Biol. Chem.* **263,** 8282–8287.
14. Sewell, J. L. & Kahn, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4620–4624.
15. Price, S. R., Nightingale, M., Tsai, S.-C., Williamson, K. C., Adamik, R., Chen, H.-C., Moss, J. & Vaughan, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5488–5491.
16. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
17. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
18. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1194–1198.
19. Gyllensten, U. B. & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7652–7656.
20. Mitchell, L. G. & Merril, C. R. (1989) *Anal. Biochem.* **178,** 239–242.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
22. Short, J. M., Fernandez, J. M., Sorge, J. A. & Huse, W. D. (1988) *Nucleic Acids Res.* **16,** 7583–7600.
23. Eschenfeldt, W. H., Puskas, R. S. & Berger, S. L. (1987) *Methods Enzymol.* **152,** 337–342.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
26. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
27. Halliday, K. R. (1983) *J. Cyclic Nucleotide Protein Phosphorylation Res.* **9,** 435–448.
28. Stahl, M. L., Ferenz, C. R., Kelleher, K. L., Kriz, R. W. & Knopf, J. L. (1988) *Nature (London)* **332,** 269–272.
29. Van Meurs, K. P., Angus, C. W., Lavu, S., Kung, H.-F., Moss, J. & Vaughan, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3107–3111.
30. Kawata, M., Matsui, Y., Kondo, J., Hishida, T., Teranishi, Y. & Takai, Y. (1988) *J. Biol. Chem.* **263,** 18965–18971.