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Identification of a GTP-binding protein α subunit that lacks an apparent ADP-ribosylation site for pertussis toxin

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ABSTRACT Recent molecular cloning of cDNA for the α subunit of bovine transducin (a guanine nucleotide-binding regulatory protein, or G protein) has revealed the presence of two retinal-specific transducins, called Tα and Tα, which are expressed in rod or cone photoreceptor cells. In a further study of G-protein diversity and signal transduction in the retina, we have identified a G-protein α subunit, which we refer to as Gα, by isolating a human retinal cDNA clone that cross-hybrids at reduced stringency with bovine Tα subunit cDNA. The deduced amino acid sequence of Gα is 41-67% identical with those of other known G-protein α subunits. However, the 355-residue Gα lacks a consensus site for ADP-ribosylation by pertussis toxin, and its amino acid sequence varies within a number of regions that are strongly conserved among all of the other G-protein α subunits. We suggest that Gα, which appears to be highly expressed in neural tissues, represents a member of a subfamily of G proteins that mediate signal transduction in pertussis toxin-insensitive systems.

Guanine nucleotide-binding regulatory proteins (G proteins) are a family of receptor-coupled signal-transducing proteins that regulate a variety of second-messenger systems and ion channels. Members of this family of structurally and functionally homologous proteins are involved in the regulation of retinal cyclic GMP phosphodiesterase, adenylate cyclase, phospholipase C, phospholipase A2, and subsets of K+ and Ca2+ channels (1-3). G proteins are generally found as heterotrimers composed of diverse α, β, and γ subunits. Diversity in the structure of G proteins has been shown by the identification of at least seven α-subunit cDNA clones that are encoded by separate genes (reviewed in ref. 1). Distinct β subunits, called β1 and β2, also exist (4, 5), as well as at least two forms of γ subunits (6-8).

G proteins are often referred to by the type of α subunit found in the holoprotein. The α subunit can bind GDP or GTP depending upon the state of its associated receptor and undergoes reversible association with βγ complexes. Different subfamilies of α subunits have been found and classified based upon their similarities in amino acid sequence. Some of the Gα proteins, such as the α subunits of the retinal rod and cone transducins, Tα and Tα, are present only in a single cell type (9), while others, such as those of Gα (which stimulates adenylate cyclase), the Gα group (Gα1, Gα2, and Gα3, which are structurally related G proteins), and Gα (whose function is unknown), appear to be widely expressed. The amino acid sequence of each Gα protein is extremely well conserved through evolution. For example, the bovine, rodent, and human Gα proteins have at least 98% amino acid sequence identity. All of the Gα proteins have characteristically conserved amino acid sequences, and it has been argued that these mediate Gα-specific function. Furthermore, all of the Gα proteins found thus far have sites that act as substrate for ADP-ribosylation by either chola toxin, pertussis toxin, or both. However, there are reports of signal-transduction processes that appear to require GTP but are not affected by toxins (10-14). We sought to further explore the diversity of Gα proteins, and in this paper we report the characterization of a cDNA clone that represents a G-protein α subunit distinct from Gα, Gα1-3, Gα, Tα, and Tα.

METHODS

Screening of the Human Retinal cDNA Library. Clone λc161 was isolated from a human retinal λgt10 cDNA library that was constructed by Jeremy Nathans (Johns Hopkins University School of Medicine, Baltimore). The library was screened with a bovine Tα cDNA plasmid probe (pML7) that contained a 2.19-kilobase (kb) Nco I cDNA fragment including the entire protein-coding region of bovine Tα. Hybridization was performed at 42°C in 20% (vol/vol) formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM NaH2PO4, pH 7.0/2× concentrated Denhardt’s solution/0.1% NaDodSO4 containing 50 μg of salmon sperm DNA per ml. (Denhardt’s solution is 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone.) The filters were washed at 37°C in 0.3 M NaCl/0.03 M sodium citrate/0.1% NaDodSO4 for low stringency and at 50°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% NaDodSO4 for high stringency. Selected clones that hybridized to Tα cDNA under conditions of low stringency only were rescanned by hybridization to a 32P-labeled synthetic oligodeoxynucleotide probe (TαZ) and cDNA probes for rat Gα, Gα1, Gα2, Gα3, and Gα (kindly provided by David Jones and Randall Reed, Johns Hopkins University School of Medicine, Baltimore) and bovine Tα. The nucleotide sequence of TαZ, which was based on the amino acid sequence of Gα (residues 42-53; Fig. 2), is 5’-d(CCCTTAGCICCTTCTGTTGTTTTTAC) 3’ [with deoxynosine (I) substituted at some of the third-base codon positions].

Hybridization Analysis of mRNA and Genomic DNA. Mouse spleen DNA was obtained from the Mouse DNA Resource (The Jackson Laboratory). DNA from human placenta was prepared as described (15). Hybridization to genomic DNA blots was carried out at 65°C in 0.9 M NaCl/0.09 M sodium citrate/5× Denhardt’s solution/1% NaDodSO4/10% dextran.

Abbreviations: G protein, guanine nucleotide-binding regulatory protein; Tα, rod transducin; Tα, cone transducin; Gα, G proteins that stimulate and inhibit adenylate cyclase; Gα1, Gα2, and Gα3, structurally related G proteins; Gα, a G protein of undefined function; Gα, G-protein α subunit.

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†This sequence is being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03260).
sulfate. Final washing conditions were 65°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% NaDodSO₄.

Total poly(A)⁺ RNA samples from quick-frozen bovine tissues were prepared, size-fractionated by 1.0% agarose/2.2 M formaldehyde gel electrophoresis, and transferred directly to a nylon membrane as previously described (4). The filter was hybridized to nick-translated probe at 42°C in 50% formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM NaH₂PO₄/2× Denhardt’s solution/0.1% NaDodSO₄ containing 50 μg of salmon sperm DNA per ml. Washing was done at 50°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% NaDodSO₄.

**RESULTS**

Bovine Tα cDNA was hybridized at reduced stringency to about 42,000 plaques from a human retinal A410 cDNA library, and 309 preliminary positive clones were detected. About 230 of the positive clones remained hybridized to the Tα cDNA probe after washing under conditions of high stringency. The other candidate positive clones, which hybridized only under conditions of lower stringency, were analyzed further. DNA was prepared from 16 of these cDNA clones, blotted onto nylon membranes, and hybridized to a labeled synthetic oligonucleotide, TaZ. The sequence of TaZ (see Methods) was derived from a strongly conserved amino acid sequence within all Ga proteins. It hybridized strongly to four of the cDNA clones, three of which were classified as Gα by hybridization to a rat Gα cDNA probe and by partial nucleotide sequencing. However, the fourth, TaZ-hybridizing clone, α161, failed to show strong hybridization to any of the cDNA probes for known α subunits (Tcα, Gα₁, Gα₂1, Gα₃, or Gα₂). α161 was analyzed further by complete nucleotide sequencing of the 2.7-kb cDNA insert (Fig. 1).

Translation of the cDNA sequence in one open reading frame gave a 355-residue protein sequence (Mₚ = 40,920) that showed 66–67% amino acid sequence identity with Gα₁α, Gα₂α, and Gα₃α; 60% identity with Gα₃α; 55–57% identity with Tα and Tα₂; and about 41% sequence identity with Gαβ. By analogy with the homologous Ga proteins the initiation codon was assumed to be the ATG at nucleotide position 1 (Fig. 1). Two consensus polyadenylation signals (AATAAA) are present in the cDNA sequence at nucleotide positions 2156 and 2649. The second polyadenylation site is closely followed by a 17-nucleotide-long poly(A) tract at the 3' terminus. The protein encoded by the cDNA is closely homologous to members of the Ga gene family, and we refer to it as Gα₁α.

A comparison of the amino acid sequence of Gα₁α with those of other Ga proteins, Gα₁, Gα₂, Gα₃, and Tα₂, each representative of a particular class of Ga proteins, is shown.
These results encode pear with other known Ga changes these common (Fig. 2). Amino acid sequence differences between Ga and the other G-protein α subunits suggests that Ga is not derived by differential splicing from a known Ga gene. To test this notion further we hybridized radiolabeled Ga cDNA to blots of human placental and mouse spleen DNA cut with various restriction enzymes (Fig. 3). The pattern of restriction fragments hybridizing with the Ga probe was compared with that detected with cDNA probes for all the other Ga proteins derived from rat or human tissues (unpublished data). The Ga pattern was clearly distinct, with few if any restriction fragments in common with the patterns obtained with the other probes. These results suggest that there is a unique gene that encodes Ga both in humans and in mice.

Since Ga protein sequences and mRNA distribution appear to be well-conserved between humans and a number of other mammals, we used bovine RNA from different tissues to study the expression of Ga. Hybridization of a Ga cDNA probe to bovine poly(A)⁺ RNA revealed Ga expression in a number of tissues (Fig. 4). A major mRNA transcript about 3.0 kb long and minor transcripts of 2.5 and 2.2 kb were detected. The abundance of Ga mRNA was highest in neural tissues (i.e., retina and brain) and relatively low or absent in other tissues. The 3.0-kb transcript predominated in retina, brain, adrenal gland, kidney, and liver; however, it was not detected in testis, lung, and spleen. A faint 2.5-kb band was also present in retina RNA. The different mRNA species may represent different gene products that result from tissue-specific alternative splicing or the selection of alternative polyadenylation or transcriptional initiation sites. The results also do not exclude the possibility that the individual transcripts derive from separate but highly conserved genes.

**DISCUSSION**

On the basis of its unique primary structure, the size and pattern of its expressed mRNA, and its distinct array of hybridizing genomic fragments, the Ga cDNA represents a novel Ga protein. Ga is most homologous to the Ga-like α subunits, having 66–67% identity in amino acid sequence to Ga₁, Ga₂, and Ga₃ subunits. However, with respect to one another, the Ga-like α proteins are 85–94% identical in amino acid sequence and appear to form a separate subfamily or
class of $\alpha$ subunits. $T_\alpha$ and $T_\alpha$, which are about 80% identical in amino acid sequence, form another group of $\alpha$ chains. $G_\alpha$ is as divergent from the $G_\alpha$ group as are $G_\alpha$, $T_\alpha$, and $T_\alpha$, and is therefore best categorized within a separate class of $\alpha$ proteins.

Fig. 3. Hybridization analysis of human and mouse genomic DNA. Samples (8 $\mu$g) of DNA from human placenta or mouse spleen were cut with the indicated restriction enzymes and analyzed by blot hybridization to the 2.7-kb $G_\alpha$ cDNA probe. These same filters and other similarly prepared blots were hybridized to other $\alpha$ subunit cDNA probes for $G_3$, $G_1$, $G_2$, $G_3$, $G_4$, $T_\alpha$, and $T_\alpha$. No overlapping patterns of restriction fragments were found (unpublished data). HindIII fragments of phage $\lambda$ DNA were used as size markers (lengths in kb at left).

In all known $G$ proteins, several regions or domains have been identified as ones that may be involved in guanine nucleotide binding or hydrolysis (1, 22). These regions are homologous in amino acid sequence to the putative GDP-binding regions, designated the A, C, E, and G regions in the sequence of translation elongation factor EF-Tu and p21$^{ras}$ proteins (23). Like other $G_\alpha$ proteins, $G_\alpha$ shows highly conserved amino acid sequences in these regions (Fig. 2). However, there are a few notable variations in the $G_\alpha$ sequence. In particular, in the A region $G_\alpha$ is different in sequence from other $G_\alpha$ proteins, all of which contain a perfectly conserved 18-amino acid stretch. This conserved region includes an amino acid sequence motif, Gly-(Xaa)-Gly-Lys (specifically Gly-Thr-Ser-Asn-Ser-Gly-Lys in $G_\alpha$ and Gly-Ala-Gly-Glu-Ser-Gly-Lys in all other $G_\alpha$ proteins), that is found in many mononucleotide-binding proteins, including p21$^{ras}$ and elongation factors EF-Tu and EF-1 $\alpha$ (26, 27). Mutations in the corresponding sequence motif in p21$^{ras}$ have been correlated with decreased GTPase activity, enhanced oncogenic capacity, and tumor progression (28). The mononucleotide-binding and -hydrolysis properties of $G_\alpha$ remain to be determined.

In some cases sequence differences in $G_\alpha$ may be interpreted in terms of potential effects on its properties. For example, $G_\alpha$ does not contain a typical amino acid sequence for ADP-ribosylation by pertussis toxin near the carboxyl terminus. $G_\alpha$ proteins that are pertussis toxin substrates, including $T_\alpha$, $G_\alpha$, and $G_\alpha$-like $\alpha$ subunits, terminate with the common sequence Cys-Gly-Leu-(Phe or Tyr) in which the conserved cysteine is the ADP-ribose acceptor site (24). This common sequence and the conserved cysteine are absent in $G_\alpha$ as well as in $G_\alpha$, which is known not to be modified by pertussis toxin. The ability of $G_\alpha$ to be ADP-ribosylated by cholera toxin might also be compromised due to an amino acid difference in $G_\alpha$ adjacent to Arg-179, which corresponds to the site of ADP-ribosylation by cholera toxin in $T_\alpha$ (25).

Since $G_\alpha$ does not contain a typical pertussis toxin modification site, it may mediate transduction in signaling systems that are not blocked by the toxin. There are several examples of receptor-mediated stimulation of phospholipase C that appear to involve $G$ proteins but are insensitive to the effects of pertussis toxin, including, for example, the action of vasopressin, muscarinic agonists, and thyrotropin-releasing hormone in various cell types (10-14). Thus, one possible function of $G_\alpha$ may be to control phospholipase C activity in specific cells. The identification and characterization of $G_\alpha$ by molecular cloning provides an important means to purify the $G_\alpha$ gene product and study its distribution, biochemistry, and function.

Note Added in Proof. Kaziro and his colleagues (29) have isolated a human genomic clone and characterized a rat brain cDNA clone that correspond to the human $G_\alpha$ cDNA reported in this paper.

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