Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3792–3796, June 1987
Genetics

Distinct forms of the β subunit of GTP-binding regulatory proteins identified by molecular cloning

(signal transduction/molecular evolution/human myeloid HL-60 cells)

HENRY K. W. FONG, THOMAS T. AMATRUDA, III, BRUCE W. BIRREN, AND MELVIN I. SIMON

Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125

Contributed by Melvin I. Simon, February 27, 1987

ABSTRACT **Two distinct β subunits of guanine nucleotide-binding regulatory proteins have been identified by cDNA cloning and are referred to as β_1 and β_2 subunits. The bovine transducin β subunit (β_1) has been cloned previously. We have now isolated and analyzed cDNA clones that encode the β_2 subunit from bovine adrenal, bovine brain, and a human myeloid leukemia cell line, HL-60. The 340-residue M_r 37,329 β_2 protein is 90% identical with β_1 in predicted amino acid sequence, and it is also organized as a series of repetitive homologous segments. The major mRNA that encodes the bovine β_2 subunit is 1.7 kilobases in length. It is expressed at lower levels than β_1 subunit mRNA in all tissues examined. The β_1 and β_2 messages are expressed in cloned human cell lines. Hybridization of cDNA probes to bovine DNA showed that β_1 and β_2 are encoded by separate genes. The amino acid sequences for the bovine and human β_2 subunit are identical, as are the amino acid sequences for the bovine and human β_1 subunit. This evolutionary conservation suggests that the two β subunits have different roles in the signal transduction process.**

Guanine nucleotide-binding regulatory proteins (G proteins) are involved in the transduction mechanisms of a variety of signaling systems. Members of this family of structurally and functionally homologous proteins serve to transfer stimulatory or inhibitory signals to intracellular targets in response to activation of specific cell-surface receptors by light, hormones, neurotransmitters, or other chemical signals (1–3). G proteins are involved in the regulation of retinal cyclic GMP phosphodiesterase, adenylate cyclase, phospholipase C, and ion channels and are generally found as heterotrimers composed of α, β, and γ subunits. Diversity in the structure of the α subunit has been shown by the isolation of at least six distinct α-subunit cDNA clones. These include cDNA clones for the retinal specific transducins, $T_{r\alpha}$ and $T_{c\alpha}$ (where T_r and T_c are rod transducin and cone transducin), at least two distinct cDNA clones homologous to $G_{i\alpha}$, and distinct clones for $G_{s\alpha}$ and $G_{o\alpha}$ (where G_i and G_s are G proteins that mediate inhibition and stimulation of adenylate cyclase and G_o is a G protein of unknown function) (reviewed in ref. 1).

Biochemical and immunological studies have demonstrated that the β subunits of specific G proteins are highly similar or identical and their structures are strongly conserved among different species (4–8). Two forms of the β subunit with M_rs of 35,000 and 36,000 have been described (9–12). In each known case the β subunit forms a tightly associated complex with a smaller γ subunit. Among different G proteins the γ subunits appear to be diverse (12–14). Several biochemical activities of the βγ complex have been found by using *in vitro* reconstitution systems. In the visual system $T_{\beta\gamma}$ is required for the binding of T_α to photolyzed rhodopsin and is necessary for GTP–GDP exchange (15). The βγ subunit also deactivates the α subunit of G_s upon reassociation to form heterotrimers (16–19). In addition, the βγ subunit may directly inhibit adenylate cyclase (20), and recently, it has been reported that βγ subunits activate the muscarinic K^+ channel in heart (21).

The transducin β subunit has been studied by molecular cloning and found to be a highly acidic 340-amino acid protein with a M_r of 37,375 (22, 23). It is composed of repeated homologous segments arranged in tandem and has significant homology in primary structure and segmental repetitive sequence to the COOH-terminal region of a yeast cell division cycle gene (*CDC4*) product. The pattern of hybridization of transducin β probes with bovine mRNA and with bovine genomic DNA suggested the existence of multiple β-subunit genes (23). In this paper we report the structure of a distinct β subunit that is homologous with bovine transducin β and examine the expression of the β-subunit genes in bovine and human tissues.

MATERIALS AND METHODS

Isolation and Analysis of mRNA and Genomic DNA. Human HL-60 myeloid leukemia cells (generously provided by H. P. Koeffler) were cultured as described (24). RNA from HL-60 cells or bovine tissues was prepared (25), size-fractionated by formaldehyde/agarose gel electrophoresis (26), and transferred directly to nylon or nitrocellulose filters (27). High molecular weight DNA was prepared from bovine liver and kidney (28). DNA samples were cut with restriction enzymes, electrophoresed in 0.8% agarose gels, and blotted onto nylon or nitrocellulose filters (29). Hybridization of nick-translated probes to filters was carried out as described (23). Filters were exposed to Kodak XAR film at −70°C with an intensifying screen (DuPont). Autoradiograms in a linear range of exposure were analyzed by densitometry using an LKB2202 Ultroscan.

Construction of λgt10 cDNA Libraries. Ten micrograms of poly(A)$^+$ RNA from HL-60 cells was primed with oligo-$(dT)_{12-18}$ (Pharmacia), and double-stranded cDNA was constructed using avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) and S1 nuclease (30). After ligation with *Eco*RI linkers cDNA was introduced into the *Eco*RI site of bacteriophage λgt10. Bacteriophage DNA was then packaged *in vitro* (Amersham) and plated on *Escherichia coli* strain C600. Recombinant phage (4×10^5) with an average insert size of 0.9 kilobase (kb) were obtained. After isolation of partial-length β-subunit cDNA clones, a second cDNA library was constructed. Ten micrograms of poly(A)$^+$ RNA was incubated with methylmercuric hydrox-

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, guanine nucleotide-binding regulatory protein; T_r, rod transducin; T_c, cone transducin; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylate cyclase; G_o, a G protein of unknown function.

ide (10 mM) for 5 min; this was followed by incubation with 2-mercaptoethanol (100 mM). cDNA was then synthesized by the method of Gubler and Hoffman (31), and a cDNA library was constructed as described above. Recombinant clones (1.5×10^6) with an average insert size of 1.7 kb were obtained.

Isolation of cDNA Clones and DNA Sequence Analysis. cDNA libraries were screened with radiolabeled cDNA probes and synthetic oligodeoxynucleotides as described (32, 33). DNA sequencing was performed by the dideoxynucleotide chain-termination method using synthetic oligodeoxynucleotides, 21 bases in length, as sequencing primers (34–36).

RESULTS

Identification and Sequence of Bovine cDNA Clones Homologous to the Transducin β Subunit. A bovine brain λgt10 cDNA library was screened under low-stringency hybridization conditions with a transducin β subunit cDNA probe, pTB112, which consisted of a 1.4-kb $T_{r\beta}$ cDNA (ref. 23, Fig. 1) subcloned in the pSP64 plasmid vector. Eighteen positive clones were isolated and characterized by varying the stringency of hybridization conditions. DNA from two clones, λBH2 and λBH14, was found to hybridize relatively weakly to $T_{r\beta}$ cDNA. Clone λBH14 was analyzed by nucleotide sequencing and found to contain a 389-base-pair (bp) cDNA insert with the sequence from nucleotide 402 to 790 as shown in Fig. 1. To obtain longer cDNA clones the λBH14 insert was used as a probe to screen a bovine adrenal λgt10 cDNA library and isolate nine additional clones. The adrenal cDNA clones were then characterized by restriction enzyme mapping and nucleotide sequencing and found to encode a protein highly homologous to, but distinct from, the β subunit of transducin. Clone λBH309 contained the longest open reading frame. The open reading frame of the cDNA in λBH309 extends for 980 bp and is followed by 359 bp of 3′-untranslated sequence (Fig. 1). However, the cDNA lacks the NH_2 terminus of the encoded protein since an appropriate ATG initiation codon was not found. We refer to the transducin β subunit as β_1 and the novel protein encoded by cDNA clone λBH309 as the β_2 subunit.

Homology between the β_1 and β_2 subunits is shown in Fig. 1; the proteins are about 90% identical in amino acid sequence. A comparison of the available protein sequence indicates 32 substitutions that include divergent as well as conserved amino acid replacements. Nucleotide sequence differences between β_1 and β_2 cDNAs extend throughout the clones.

Expression of β_1- and β_2-Subunit mRNAs. Hybridization of β_1- and β_2-specific cDNA probes to bovine poly(A)$^+$ RNA defined the mRNA species for each subunit (Fig. 2). The specific probes consisted of corresponding 3′-untranslated regions [nucleotides 971–1339 of β_2 (Fig. 1) and nucleotides 1083–1386 of β_1 (ref. 23, Fig. 1)] that are <40% homologous throughout their entire sequence. Hybridization at high stringency with the 3′-β_1-specific probe revealed two major mRNAs in each tissue that are 3.0 kb and 1.5 kb long in retina and 3.3 kb and 1.7 kb long in other tissues (Fig. 2*A*). The larger mRNA in each tissue is more abundant. The smaller mRNA transcript in each tissue may result from utilization of an alternative poly(A) site that can be correlated with an AATAAA signal sequence in the 3′-untranslated region of β_1 cDNA (22).

In contrast, the 3′-β_2-specific probe hybridized predominantly to a mRNA transcript about 1.7 kb in length (Fig. 2*B*). In some tissues this probe showed faint hybridization to a 3.0-kb mRNA. The specificity of this probe was demonstrated by the lack of hybridization to β_1 mRNA in retina (Fig. 2*B*, lane 5) and by the absence of hybridization to the 3.3-kb β_1

```
                                                                TC CGG AAC CAG ATC CGG GAT GCC CGA AAA GCA TGT GGG GAT TCA ACA CTG ACC CAG ATC ACA GCT    65
                                                                   Arg Asn Gln Ile Arg Asp Ala Arg Lys Ala Cys Gly Asp Ser Thr Leu Thr Gln Ile Thr Ala
  1 Met Ser Glu Leu Asp Gln Leu Arg Gln Glu Ala Glu Gln Leu Lys  -   -   -   -   -   -   -   -   -   -  Ala  -  Ala  -   -  Ser  -   -   -  Asn

    GGG CTG GAC CCA GTG GGG AGA ATC CAG ATG AGG ACA CGG AGG ACC CTC CGT GGG CAC CTG GCC AAA ATC TAT GCC ATG CAC TGG GGG ACA GAC TCA AGG CTG CTG   170
    Gly Leu Asp Pro Val Gly Arg Ile Gln Met Arg Thr Arg Arg Thr Leu Arg Gly His Leu Ala Lys Ile Tyr Ala Met His Trp Gly Thr Asp Ser Arg Leu Leu
 36 Asn Ile  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

    GTC AGC GCC TCC CAG GAC GGC AAG CTC ATC ATT TGG GAC AGC TAC ACC ACC AAC AAG GTC CAC GCC ATC CCG CTG CGC TCC TCC TGG GTC ATG ACC TGT GCC TAC   275
    Val Ser Ala Ser Gln Asp Gly Lys Leu Ile Ile Trp Asp Ser Tyr Thr Thr Asn Lys Val His Ala Ile Pro Leu Arg Ser Ser Trp Val Met Thr Cys Ala Tyr
 71  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

    GCG CCC TCA GGG AAC TTC GTG GCC TGC GGG GGA CTG GAC AAC ATA TGC TCC ATC TAC AGC CTC AAG ACC CGT GAG GGC AAT GTC AGG GTC AGC CGG GAG CTG CCT   380
    Ala Pro Ser Gly Asn Phe Val Ala Cys Gly Gly Leu Asp Asn Ile Cys Ser Ile Tyr Ser Leu Lys Thr Arg Glu Gly Asn Val Arg Val Ser Arg Glu Leu Pro
106  -   -   -   -   -  Tyr  -   -   -   -   -   -   -   -   -   -   -   -   -  Asn  -   -   -   -   -   -   -   -   -   -   -   -   -  Ala

    GGC CAC ACC GGG TAC CTG TCA TGC TGC CGC TTT CTG GAT GAC AAC CAA ATC ATC ACC AGC TCT GGG GAC ACC ACC TGT GCC CTG TGG GAC ATT GAG ACC GGC CAG   485
    Gly His Thr Gly Tyr Leu Ser Cys Cys Arg Phe Leu Asp Asp Asn Gln Ile Ile Thr Ser Ser Gly Asp Thr Thr Cys Ala Leu Trp Asp Ile Glu Thr Gly Gln
141  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -  Val  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

    CAG ACT GTG GGC TTT GCT GGA CAT AGT GGT GAT GTG ATG TCC CTG TCC CTG GCC CCC GAC GGC CGC ACC TTT GTG TCA GGC GCC TGC GAC GCC TCC ATC AAG CTG   590
    Gln Thr Val Gly Phe Ala Gly His Ser Gly Asp Val Met Ser Leu Ser Leu Ala Pro Asp Gly Arg Thr Phe Val Ser Gly Ala Cys Asp Ala Ser Ile Lys Leu
176  -   -  Thr Thr  -  Thr  -   -  Thr  -   -   -   -   -   -   -   -   -   -   -  Thr  -  Leu  -   -   -   -   -   -   -   -  Ala  -   -

    TGG GAC GTG CGG GAC TCC ATG TGC CGA CAG ACC TTC ATC GGC CAC GAA TCC GAC ATC AAC GCC GTG GCT TTC TTC CCC AAC GGC TAC GCC TTC ACC ACG GGC TCT   695
    Trp Asp Val Arg Asp Ser Met Cys Arg Gln Thr Phe Ile Gly His Glu Ser Asp Ile Asn Ala Val Ala Phe Phe Pro Asn Gly Tyr Ala Phe Thr Thr Gly Ser
211  -   -   -   -  Glu Gly  -   -   -   -   -   -  Thr  -   -   -   -   -   -   -   -  Ile Cys  -   -   -   -   -  Asn  -   -  Ala  -   -   -

    GAT GAC GCC ACA TGC CGC CTC TTT GAC TTG CGG GCC GAC CAG GAG CTC CTC ATG TAT TCG CAC GAC AAC ATC ATC TGC GGC ATC ACC TCT GTT GCC TTC TCG CGC   800
    Asp Asp Ala Thr Cys Arg Leu Phe Asp Leu Arg Ala Asp Gln Glu Leu Leu Met Tyr Ser His Asp Asn Ile Ile Cys Gly Ile Thr Ser Val Ala Phe Ser Arg
246  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -  Met Thr  -   -   -   -   -   -   -   -   -   -   -   -  Ser  -   -  Lys

    AGC GGC AGG CTG CTG CTC GCG GGC TAC GAC GAC TTC AAC TGC AAC ATC TGG GAT GCC ATG AAG GGC GAC CGT GCA GGT GTC CTC GCC GGC CAT GAC AAC CGT GTG   905
    Ser Gly Arg Leu Leu Leu Ala Gly Tyr Asp Asp Phe Asn Cys Asn Ile Trp Asp Ala Met Lys Gly Asp Arg Ala Gly Val Leu Ala Gly His Asp Asn Arg Val
281  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -  Val  -   -   -  Leu  -  Ala  -   -   -   -   -   -   -   -   -   -   -

    AGC TGC CTC GGG GTC ACT GAC GAT GGC ATG GCT GTG GCC ACA GGT TCC TGG GAC TCC TTC CTC AAG ATC TGG AAC TAA CAGCC CCGAC CCCAA CTGGG CCCAG GCTAG  1013
    Ser Cys Leu Gly Val Thr Asp Asp Gly Met Ala Val Ala Thr Gly Ser Trp Asp Ser Phe Leu Lys Ile Trp Asn END
316  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

    GAGGG  GCCCT GCCCG TGCCC ACACT ACAGG CCGGG AGCTC  TGGGG CTGGG TGCAC ACCGA GCCTC CCTCC CCGGG CCACG GGGCC TTGGG TCCCT GCTCC CCTAC CCAGG TTTGG  1128

    TTCCT  CCCGG GGCCC CCACT GTGGA GATTA AGATG GGGAT  AGAAT GGGGA AAGAG GGGCA GAAGG CCCTC ATCCT TTTGC TGCCC TGGGG TTGAG GCCTC ATCCC GCTGG AGGGC  1243

    CAGAG  GCAGG AGGTG GAAAC TCCAG GGGCT GGCTT TTTTA  AAACT GGTTT TATTT TAATT TTTAT TATAT TTTCA GTTTT TCCAT AAAGG AACCA A                        1339
```

FIG. 1. Nucleotide and partial protein sequence of the bovine β_2 subunit determined from cDNA clone λBH309. For comparison, the amino acid sequence of the bovine transducin β subunit (β_1) is given below the β_2 sequence. Amino acid substitutions are given, and conserved residues are indicated by dashes. The DNA sequence is numbered on the right, and the protein sequence is numbered on the left. The NH_2 terminus is missing from the β_2 protein sequence, which is arbitrarily numbered relative to the β_1 subunit.

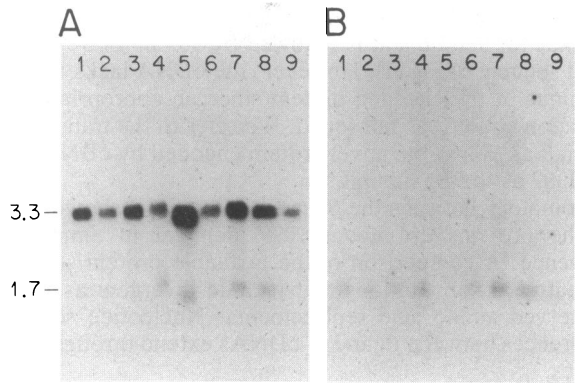

FIG. 2. Hybridization analysis of β_1 and β_2 mRNA in bovine tissues. Bovine poly(A)$^+$ RNAs were probed first with a 3′-β_2-specific cDNA probe. After exposure to x-ray film the filter was stripwashed of bound probe (confirmed by exposure to film) and reprobed with a 3′-β_1-specific cDNA probe. The mRNAs used were selected either twice (lanes 1–3) or once (lanes 4–9) on oligo(dT)-cellulose. The tissues represented and the amounts loaded are as follows: lane 1, adrenal, 1 μg; lane 2, heart, 2 μg; lane 3, brain, 1 μg; lane 4, testis, 4 μg; lane 5, retina, 0.75 μg; lane 6, liver, 4 μg; lane 7, spleen, 3 μg; lane 8, lung, 2 μg; lane 9, kidney, 2 μg. (*A*) Bovine mRNA homologous to the 3′-β_1-specific probe. (*B*) mRNA homologous to the 3′-β_2-specific probe. The probes were of comparable specific activity, and the autoradiographic exposure times were identical. RNA lengths were determined relative to bovine 28S and 18S rRNA size markers (kilobases).

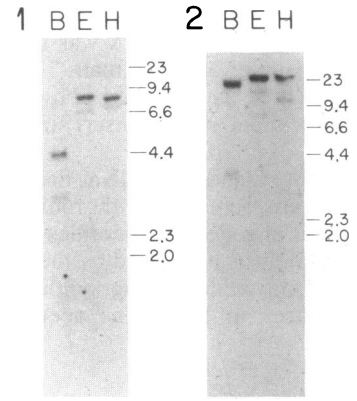

FIG. 3. Hybridization analysis of bovine genomic DNA. Ten micrograms of bovine DNA was cut to completion with *Bam*HI (B), *Eco*RI (E), and *Hin*dIII (H) and analyzed by blot hybridization to bovine β_1 cDNA probe, 1.4-kb β_1 cDNA (ref. 23, Fig. 1) subcloned into plasmid pSP64 (*1*) and bovine β_2 cDNA probe, 1.3-kb β_2 cDNA (Fig. 1) subcloned into plasmid pUC18 (*2*). λ*Hin*dIII-digested DNA size markers (kilobases) are shown for each panel.

mRNA in other tissues. The β_2 mRNA was estimated to be less abundant (by a factor of ≈10) than the β_1 mRNA on the basis of hybridization with the 3′-specific probes. In particular, little β_2 mRNA is expressed in retina where the level of β_1 mRNA is highest. The fraction of poly(A)$^+$ RNA that hybridized with the β_1 and β_2 3′-specific probes was estimated by densitometric scanning of the autoradiograms. The level of both β mRNAs varied by as much as 5-fold between the different tissues analyzed in Fig. 2.

Hybridization Analysis of Genomic DNA. Although the β_1 and β_2 proteins have long regions of identical amino acid sequence, the extensive divergence in nucleotide sequence exhibited by the cDNAs suggests that the β_1 and β_2 subunits are the products of separate genes. Hybridization of bovine genomic DNA with cDNA probes indicated that β_1 and β_2 are encoded by separate genomic regions. Each probe hybridized to a complex but distinct array of restriction fragments (Fig. 3).

Isolation of Human β_1 and β_2 cDNAs. Though it is clear that two highly homologous β genes are expressed in bovine tissues, it was of interest to determine if β_1 and β_2 genes are expressed in the same cell and to examine the relationships between β_1 and β_2 in different mammals. We therefore isolated human β_1 and β_2 subunit cDNA clones from a cDNA library constructed from HL-60 myeloid leukemia cells. Screening of 1.5×10^5 recombinant clones from the HL-60 cDNA library with the bovine transducin β cDNA probe, pTB112, yielded 10 clones. The cDNA insert from 1 of these clones (λ115.1) hybridized to a single 1.9-kb mRNA from HL-60 cells (see Fig. 6). Sequence analysis of the cDNA insert revealed that it encoded a protein that was about 90% homologous to the transducin β subunit. The λ115.1 cDNA sequence, nucleotides 240–1397 (Fig. 4), encoded the portion of the human β_2 subunit homologous with residues 80–340 of the transducin β subunit. In an attempt to isolate full-length cDNA clones, a second cDNA library was constructed by the method of Gubler and Hoffman (31). Clones (7×10^5) from this library were screened in duplicate with a human β_2-subunit cDNA probe corresponding to nucleotides 240–440 (Fig. 4) and with synthetic oligodeoxynucleotides derived from the 5′ nucleotide sequence of the bovine β_2 cDNA. Nine clones were isolated, and the longest cDNA clone (λ4C4) was shown to encode a β_2-protein sequence that aligns with residues 6–340 of the transducin β subunit. Further screening of the HL-60 library with the synthetic oligodeoxynucleotide probes yielded clone λ123. The cDNA insert in λ123 was identical in nucleotide sequence with clone λ4C4 in the region corresponding to nucleotides 18–114 (Fig. 4). In addition, clone λ123 provided the 5′ cDNA sequences from nucleotides −57 to 18, including the putative ATG initiation codon at position 1. The three overlapping cDNA clones (λ115.1, λ4C4, λ123) that were isolated from the HL-60 cDNA library encode a continuous open reading frame of 340 codons (Fig. 4). This corresponds to a predicted protein of M_r 37,329.

The human and bovine β_2 cDNA clones are identical in predicted amino acid sequence and 93% homologous in nucleotide sequence. There are 33 amino acid changes between the human β_2 and human liver β_1 (37) proteins, which are indicated in Fig. 4. The repeated sequence motif described in the bovine transducin β subunit (23) is also found in the human and bovine β_2 proteins (Fig. 5).

A partial cDNA clone, λ284, that encoded the human β_1 subunit was also isolated. The β_1 cDNA contained a 744-bp open reading frame followed by 180 bp of 3′-untranslated sequence (results not shown). The predicted amino acid sequence of the human β_1 subunit was identical with the corresponding sequence of the bovine transducin β subunit (β_1) from amino acid residues 94 to 340. The human HL-60 β_1 cDNA was also compared with the sequence of a β_1 cDNA isolated from a human liver cDNA library (37). The two human β_1 cDNA sequences diverged in only four regions. There was a conservative change in codon 232 (ATT for ATA) and three changes in the 3′-untranslated region: insertion of a guanine at position 1107, insertion of ATATC-CTATC after position 1091, and deletion of CATATCCTAT after position 1170. Nucleotide positions refer to the sequence of the β_1 cDNA from human liver (37).

Expression of β_1 and β_2 mRNA in HL-60 Cells. The β_1 and β_2 genes are expressed in HL-60 cells since cDNA clones for β_1 and β_2 were isolated from the HL-60 cDNA library. Furthermore, specific cDNA probes for β_1 and β_2 hybridized with mRNA from these cells (Fig. 6). Human β_1 cDNA probes hybridized to mRNA transcripts 3.4 kb and 1.9 kb in length and to a minor species of 2.5 kb. These mRNA species also hybridized at high stringency with a 150-bp human β_1 cDNA probe that consisted primarily of 3′-untranslated sequences with <50% homology to the corresponding 3′

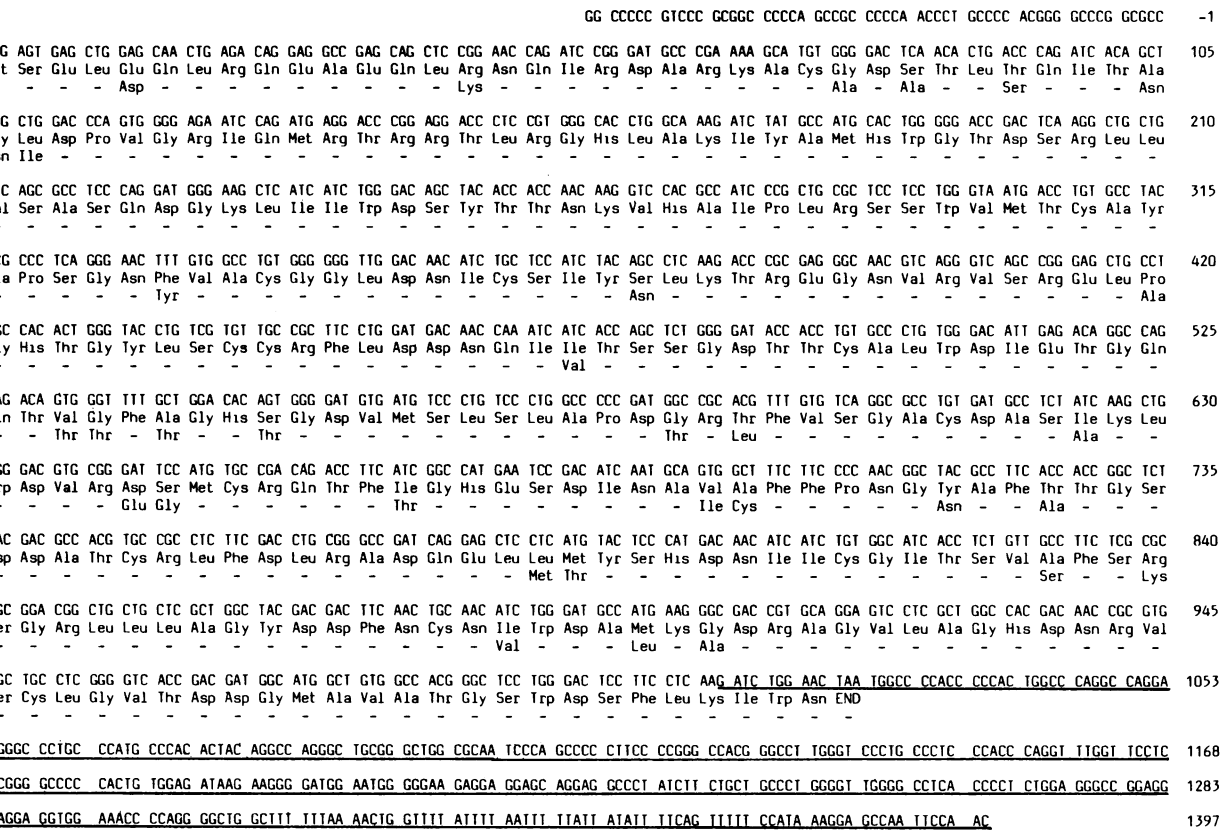

FIG. 4. Nucleotide and protein sequence of the human β_2 subunit derived from cDNA clones λ115.1, λ4C4, and λ123. For comparison, the amino acid sequence of the human β_1 subunit (37), which is identical to that of the bovine transducin β subunit (β_1), is indicated below the β_2 sequence. Amino acid substitutions are given, and conserved residues are indicated by dashes. The DNA sequence is numbered at the right, and the protein sequence is numbered at the left. The region used as a 3′-β_2-specific probe (nucleotides 1011–1397) is underlined.

region of human β_2 cDNA. In contrast, the human β_2 cDNA hybridized to a single 1.9-kb transcript. A 3′-β_2-specific cDNA probe, corresponding to nucleotides 1011–1397 (Fig. 4), hybridized only with the 1.9-kb message under high stringency (Fig. 6) and low stringency hybridization conditions (data not shown). The relative level of β_2 mRNA expression was less than that of β_1 mRNA. Additional human cell lines were screened, including HeLa, melanoma (M14), and hepatoma (HepG2), as well as normal cultured human fibroblasts. All of these cell types express β_1 and β_2 mRNA (data not shown).

DISCUSSION

The β subunits of guanine nucleotide regulatory proteins are encoded by at least two highly homologous but distinct genes. The β_1 and β_2 subunits have been unambiguously identified on the basis of molecular cloning, nucleotide sequencing, and hybridization analyses of mRNA and genomic DNA. The β_1 and β_2 genes have also been shown to map to different human chromosomes (unpublished data).

Like the β_1 subunit, the β_2 protein sequence contains four homologous repeats ≈86 residues in length, and there is significant homology in primary structure and repeated consensus sequence with the COOH-terminal portion of the yeast *CDC4* gene product (23). The β_1 and β_2 proteins are about 90% identical, but there are divergent as well as conserved amino acid substitutions between them. The divergent substitutions do not occur in the residues of the identifiable repeat motif and are clustered only in two regions, between amino acid residues 26–37 and residues 178–184. It also appears that divergent substitutions are found more often in the NH_2-terminal half of the homologous repeats. The importance of the repeated consensus sequence in the β subunits is unknown.

Reports on the purification of G proteins from various tissues have described the presence of two β subunits with M_rs of 36,000 (β_{36}) and 35,000 (β_{35}) (9–12). The β subunit

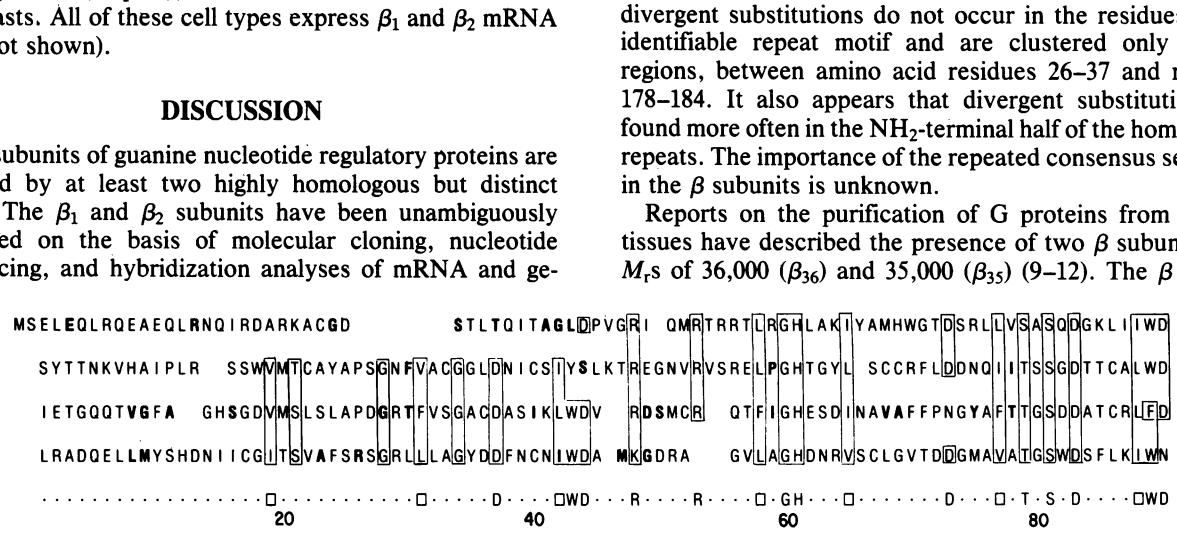

FIG. 5. Homologous repeat segments of the β_2 subunit. The amino acid sequence of the human β_2 subunit was aligned as ≈86-residue repeat units based on computer programs applied to the bovine transducin β subunit (23). Residues that differed between β_2 and β_1 are indicated as boldface letters. Identical or highly conserved residues that align three or more times in the homologous segments are boxed. A consensus sequence including some of the most highly conserved residues is presented below the aligned sequence; large hydrophobic residues are symbolized with a square.

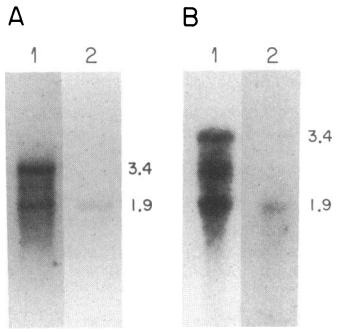

FIG. 6. Expression of β_1 and β_2 mRNA in human HL-60 cells. Total RNA (10 μg) from HL-60 cells was hybridized with β cDNA probes. (*A*) Filter that was probed with a human β_1 cDNA, the insert from λ284 (lane 1), and then stripwashed and probed with a human β_2 cDNA, the insert from λ115.1 (lane 2). (*B*) Filter that was probed with a 3′-β_1-specific probe (lane 1) and then stripwashed and probed with a 3′-β_2-specific probe (lane 2). RNA lengths were determined relative to human and bacterial rRNA size markers (kilobases).

associated with retinal transducin is a single M_r 36,000 polypeptide, whereas many other purified preparations of G proteins were found to include β_{36} and β_{35}. With regard to mRNA expression, the most abundant β-subunit transcripts in retina correspond to β_1 and little β_2 mRNA is expressed. β_2 mRNA is expressed in all other tissues that have been examined. However, the relative abundance of β_2 mRNA is significantly lower than β_1 mRNA. Direct comparison of the amino acid sequence of β_{35} with the predicted amino acid sequence of β_2 (M_r 37,329) is necessary to establish the relationship of these two molecules.

As previously reported, the amino acid sequences of the bovine and human β_1 subunits are identical (22, 23, 37). In addition, the available predicted protein sequences of the bovine and human β_2 subunits are also identical, reflecting evolutionary conservation of even the most divergent substitutions between the two subunits. The interspecies sequence conservation strongly supports the hypothesis that the β_1 and β_2 subunits have distinct but related functions in transmembrane signaling systems.

What are the roles of β_1 and β_2 in regulating the cellular response to receptor-mediated signals? The fact that both subunits are expressed in all of the cloned human cell lines that we tested suggests that both function in cells with diverse signaling systems. Recently, it has been reported that $\beta\gamma$ subunits alone can regulate an ion channel, the muscarinic K^+ channel in heart cells (21), and an effector enzyme, adenylate cyclase (20). Thus, the $\beta\gamma$ complex may have an active role in regulating different effectors in addition to modulating the activity of α subunits. β structural diversity may allow $\beta\gamma$ subunits of different G proteins to interact with distinct subsets of effectors and receptors.

We express sincere thanks to Dr. Barbara Wold and Dr. Ryn Miake-Lye for helpful discussions and for providing us with the bovine adrenal and brain cDNA libraries, to Cathy H. W. Chang and Kenneth K. Yoshimoto for technical assistance, and to Cathy Elkins for help in preparation of the manuscript. This work was supported by a Physician's Research Training fellowship from the American Cancer Society (to T.T.A.), National Institutes of Health Postdoctoral Fellowship Award GM10974 (to B.W.B.), and Grant GM34236 from the National Institutes of Health.

1. Stryer, L. & Bourne, H. R. (1986) *Annu. Rev. Cell Biol.* **2**, 391–419.
2. Bourne, H. (1986) *Nature (London)* **321**, 814–816.
3. Gilman, A. G. (1984) *Cell* **36**, 577–579.
4. Manning, D. R. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 7059–7063.
5. Audiger, Y., Pantaloni, C., Bigay, J., Deterre, P., Bockaert, J. & Homburger, V. (1985) *FEBS Lett.* **189**, 1–7.
6. Gierschik, P., Codina, J., Simons, C., Birnbaumer, L. & Spiegel, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 727–731.
7. Huff, R. M., Axton, J. M. & Neer, E. J. (1985) *J. Biol. Chem.* **260**, 10864–10871.
8. Tsuda, M., Tsuda, T., Terayama, Y., Fukada, Y., Akino, T., Yamanaka, G., Stryer, L., Katada, T., Ui, M. & Ebrey, T. (1986) *FEBS Lett.* **198**, 5–10.
9. Sternweis, P. C., Northup, J. K., Smigel, M. D. & Gilman, A. G. (1981) *J. Biol. Chem.* **256**, 11517–11526.
10. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813.
11. Neer, E. J., Lok, J. M. & Wolf, L. (1984) *J. Biol. Chem.* **259**, 14222–14229.
12. Evans, T., Fawzi, A., Fraser, E. D., Brown, M. L. & Northup, J. K. (1987) *J. Biol. Chem.* **262**, 176–181.
13. Hildebrandt, J. D., Codina, J., Rosenthal, W., Birnbaumer, L., Neer, E. J., Yamazaki, A. & Bitensky, M. W. (1985) *J. Biol. Chem.* **260**, 14867–14872.
14. Roof, D. J., Applebury, M. L. & Sternweis, P. C. (1985) *J. Biol. Chem.* **260**, 16242–16249.
15. Fung, B. K.-K. (1983) *J. Biol. Chem.* **258**, 10495–10502.
16. Northup, J. K., Smigel, M. D. & Gilman, A. G. (1982) *J. Biol. Chem.* **257**, 11416–11423.
17. Northup, J. K., Sternweis, P. C. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 11361–11368.
18. Katada, T., Bokoch, G. M., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3568–3577.
19. Katada, T., Northup, J. K., Bokoch, G. M., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3578–3585.
20. Katada, T., Oinuma, M. & Ui, M. (1986) *J. Biol. Chem.* **261**, 5215–5221.
21. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J. & Clapham, D. E. (1987) *Nature (London)* **325**, 321–326.
22. Sugimoto, K., Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Minamino, N., Kangawa, K., Matsuo, H., Hirose, T., Inayama, S. & Numa, S. (1985) *FEBS Lett.* **191**, 235–240.
23. Fong, H. K. W., Hurley, J. B., Hopkins, R. S., Miake-Lye, R., Johnson, M. S., Doolittle, R. F. & Simon, M. I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2162–2166.
24. Koeffler, H. P., Ranyard, J., Yelton, L., Billing, R. & Bohman, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4080–4084.
25. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
26. Lehrach, H., Diamond, D., Wozney, J. M. & Broedtker, H. (1977) *Biochemistry* **16**, 4743–4751.
27. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
28. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3**, 2303–2308.
29. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
30. Huynh, T. V., Young, R. A. & Davis, R. W. (1985) in *DNA Cloning Techniques: A Practical Approach*, ed. Glover, D. M. (IRL, Oxford, U.K.), Vol. 1, pp. 49–78.
31. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
32. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
33. Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H. & Itakura, K. (1981) *Nucleic Acids Res.* **9**, 879–894.
34. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
35. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3963–3965.
36. Williams, S. A., Slatko, B. E., Moran, L. S. & De Simone, S. M. (1986) *Biotechniques* **4**, 138–148.
37. Codina, J., Stengel, D., Wood, S. L. C. & Birnbaumer, L. (1986) *FEBS Lett.* **207**, 187–192.