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Genetics

Molecular cloning of β3 subunit, a third form of the G protein β-subunit polypeptide

(signal transduction/molecular cloning)

MICHAEL A. LEVINE*†, PHILIP M. SMALLWOOD*, PHILLIP T. MOEN, JR.‡, LEE J. HELMAN§, AND THOMAS G. AHN*

*Division of Endocrinology and Metabolism, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; ‡E. I. Du Pont de Nemours and Co., Boston, MA 02118; and §Pediatric Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **The signal-transducing guanine nucleotide-binding regulatory (G) proteins are heterotrimers composed of three subunits—α, β, and γ. Although multiple distinctive forms of the α subunit have been described, only two forms of the β subunits of the G proteins have been identified. To investigate further the structural diversity of the β subunits, we screened bovine and human retina cDNA libraries and isolated clones encoding three distinct types of G protein β subunit. One form was identical to previously isolated β1-subunit cDNA clones that encode the 36-kDa form of the β subunit, whereas a second form was identical to previously described β2 cDNAs that encode the 35-kDa β isoform. In addition, we identified another species, designated β3 subunit, which encodes a third distinct form of the β subunit. The β3-subunit cDNA corresponds to a 2.0-kilobase mRNA expressed in all tissues and clonal cell lines examined. Nucleotide sequence analysis indicates that the encoded peptide consists of 340-amino acid residues with a M_r of 37,221. The amino acid sequences of the three β subunits are closely related: 83% identity between β1 and β3 subunits and 81% identity between β2 and β3 subunits. By contrast, the 3′-untranslated regions of the three cDNAs show no significant homology. Our data support the hypothesis that a family of β-subunit polypeptides exists and extend understanding of β-subunit structure.**

The guanine nucleotide-binding regulatory proteins, referred to as G proteins, are a family of signal-coupling proteins that mediate numerous transmembrane hormonal and sensory transduction processes. G proteins are essential for carrying extracellular signals generated by activated membrane receptors to intracellular effector enzymes and ion channels (for review, see ref. 1). The G proteins share a heterotrimeric structure composed of α, β, and γ subunits. The α subunit contains the guanine nucleotide-binding site, has intrinsic GTPase activity, and is unique to each G protein, conferring specificity for receptor–effector interactions. Through the combined approaches of molecular cloning and biochemical characterization it has become clear that there are multiple genes that encode a complex superfamily of homologous α-subunit proteins. There are at least two genes for the retinal G protein transducin, encoding $G_t\alpha$-1 in rods and $G_t\alpha$-2 in cones (2), which regulate cGMP phosphodiesterase activity in response to light activation of the photoreceptor rhodopsin (3). At least two G proteins control activity of the hormone-sensitive adenylyl cyclase system: the α subunit of G_s is responsible for stimulation of catalytic activity ($G_s\alpha$), whereas another group of G α subunits (Gα) represented by at least three forms of G_i (4) mediates inhibition of the enzyme ($G_i\alpha$) (1, 5). In olfactory tissue the G protein $G_{olf}\alpha$ mediates sensory signal transduction (6). The target functions of other Gα subunits, including $G_o\alpha$, a G protein originally found in bovine brain (7), and $G_z\alpha$ (or $G_x\alpha$) (8, 9), remain largely unknown.

In contrast to the diversity present among the Gα proteins, structural (and functional) differences among the other components of the G protein heterotrimer are less well understood. The β and γ subunits of the G proteins are tightly associated with each other as a βγ complex. Studies using several different assays to measure βγ activity suggest that the βγ complexes of the various G proteins are functionally interchangeable (10–12). Nevertheless, several important structural differences have been found. Thus, among the different G proteins, the γ subunits appear to be structurally distinct (13–15). By contrast, only two forms of the β subunits of the G proteins have been identified. These two isoforms are highly conserved but distinct polypeptides with molecular masses of 36 kDa (β1) and 35 kDa (β2). The β1 and β2 polypeptides are immunologically (16, 17) and structurally (18–22) distinct and are encoded by separate genes (23).

Under conditions of high stringency, the β1- and β2-subunit cDNAs hybridize with mRNAs that have molecular lengths of 2.9 kilobases (kb) and 1.8 kb, respectively, in all tissues examined (18, 19). However, under reduced stringency conditions these cDNAs detect multiple hybridizing bands, corresponding to mRNAs that range in size from 1.4 to nearly 5.0 kb (21). These observations have led us and others to hypothesize that additional forms of the β subunit exist. We now report the primary structure of a third human β-subunit polypeptide that we have termed β3 subunit.¶ Based on our data, the human genome has at least three nonallelic genes encoding distinct forms of the β subunit of the G proteins.

MATERIALS AND METHODS

Isolation of cDNA Clones and DNA Sequence Analysis. The 45-base oligonucleotide 5′-CTGGGAGGCGCTGACCAG-CAGCCTTGAGTCTGTCCCCCAGTGCAT-3′ (probe A), which is complementary to the mRNA region encoding the

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Abbreviations: G protein, guanine nucleotide-binding regulatory proteins; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylyl cyclase; G_t, transducin, the major G proteins of retinal rods and cones; G_{olf}, the major G protein of olfactory neuroepithelium; G_o, a G protein of unknown function purified from the brain; Gα, α chain of G; $G_s\alpha$, $G_i\alpha$, $G_t\alpha$, $G_{olf}\alpha$, and $G_o\alpha$, α subunits of G_s, G_i, transducin, olfactory G, and G protein originally found in brain, respectively.

†To whom reprint requests should be addressed: Division of Endocrinology and Metabolism, The Johns Hopkins University School of Medicine, 816 Hunterian Building, 725 North Wolfe Street, Baltimore, MD 21205.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M31328).

amino acid sequence 61–75 of bovine β1 subunit (20), was used for the initial screening of a bovine retinal cDNA library. This oligonucleotide and others were synthesized with a DuPont Coder 300 DNA synthesizer by using modified phosphoramide chemistry (24). The oligonucleotide probes were labeled at the 5′-OH end with T4 polynucleotide kinase (P-L Biochemicals) and [γ-^{32}P]ATP (7000 Ci/mmol) (1 Ci = 37 GBq).

Bovine and human retinal cDNA libraries in the λgt10 phage vector (provided by J. Nathans, The Johns Hopkins University) were screened by plaque hybridization (25) with use of a radiolabeled probe (see text). After hybridization, the filters were washed three times in a solution containing 6× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate)/1% SDS at 23°C for 10 min per wash and then washed either twice in 0.2× SSC/1% SDS at 60°C for 30 min per wash or twice at 50°C in 0.2× SSC/0.5% SDS for 15 min per wash (reduced stringency). Plaques exhibiting positive hybridization signals were purified through several rounds of plating. The purified phage clones were amplified in liquid culture, and the DNA was isolated and analyzed by gel electrophoresis, as described (26). The cloned inserts were subcloned into plasmid pUC13 for subsequent DNA sequence analysis. The DNA sequence was determined by sequencing both strands by the dideoxynucleotide chain-termination method of Sanger *et al.* (27) with double-stranded templates (28). Universal and sequence-specific synthetic oligonucleotides, 15–21 bases in length, were used as sequencing primers.

Preparation and Analysis of DNA and RNA. High-molecular-weight genomic DNA was extracted from peripheral blood leukocytes (29). Total cellular RNA was isolated from tissues by the guanidinium isothiocyanate–cesium chloride technique (30). Poly(A)$^+$ RNA was obtained by oligo(dT)-cellulose chromatography (31). Total RNA (10 μg) or poly(A)$^+$ RNA (10 μg) was denatured with formaldehyde, size-fractionated by electrophoresis in agarose gels containing 2.2 M formaldehyde, and transferred to nylon membranes (GeneScreen*Plus*) (32). Mobilities of bovine 28S and 18S RNA standards (Bethesda Research Laboratories) were determined by ethidium bromide staining before transfer. The filters were hybridized, as described above, with radiolabeled hybridization probes (1 × 10^6 cpm/ml) prepared by random primer-extension of excised cDNA inserts (33). Filters were washed four times at 25°C in 2× SSC/0.5% SDS for 5 min and either up to 50°C in 2× SSC/0.5% SDS two times for 15 min (reduced stringency), or to 60°C in 1× SSC/0.5% SDS two times for 15 min (moderate stringency), or to 65°C in 0.1× SSC/0.5% SDS two times for 30 min (high stringency).

For analysis of genomic DNA, restriction endonucleases were purchased from Bethesda Research Laboratories or New England Biolabs and used according to the manufacturer's directions. Each DNA sample (10 μg) was digested with an excess of the various restriction enzymes, size-fractionated by electrophoresis on 1% agarose gels, and transferred to nitrocellulose membranes (34). DNA blots were hybridized with radiolabeled DNA probes as described (26).

RESULTS AND DISCUSSION

Identification and Characterization of β-Subunit Polypeptide cDNA Clones. Twelve positive clones were identified using probe A to screen 200,000 recombinant clones from the bovine retina λgt10 cDNA library. Digestion of the DNA from these clones with *Eco*RI showed that all twelve clones contained a similar-sized cDNA fragment of 1.8 kb. One clone, BTd1, was analyzed further. Restriction mapping and nucleotide sequence analysis confirmed that this cDNA was identical to previously isolated β1-subunit cDNA clones encoding the 36-kDa form of the β-subunit polypeptide (20–22). This partial-length cDNA contained the complete coding sequence of β1 subunit but lacked nucleotide sequences corresponding to the complete 5′ and 3′ untranslated regions.

To examine expression of the β1-subunit mRNA and related β-subunit transcripts, the bovine β1-subunit cDNA was hybridized to bovine retinal RNA. Under conditions of reduced stringency multiple hybridizing bands were detected, which ranged in size from 1.4 kb to nearly 5.0 kb (Fig. 1). With increasing stringency of hybridization the bovine β1-subunit cDNA probe identified fewer bands; under conditions of high stringency the β1-subunit cDNA probe hybridized specifically to only one major mRNA transcript with an approximate molecular size of 2.9 kb (Fig. 1).

To isolate cDNA clones corresponding to the other mRNAs that hybridized to the bovine β1-subunit cDNA probe, we used the bovine β1-subunit cDNA to screen bovine and human retinal λgt10 cDNA libraries under hybridization conditions of reduced stringency. Seventeen positive clones were isolated from 200,000 plaques of the bovine retina cDNA library. These clones contained cDNA inserts identical to that of clone BTd1. Screening of 200,000 recombinant plaques from the human retina cDNA library yielded 24 hybridizing clones. DNA was prepared from 10 selected clones and was characterized by restriction endonuclease analysis. Each clone contained a single *Eco*RI cDNA insert that ranged in size from 800 base pairs (bp) to 1.4 kb and under conditions of reduced stringency showed specific hybridization with the bovine β1 subunit cDNA probe. Radiolabeled probes prepared from purified *Eco*RI inserts of several of the clones were individually used to screen the entire collection at high stringency. This analysis revealed that the 24 clones comprised three independent classes. To characterize further these cDNA inserts, the largest cDNA insert of each class was subcloned into the unique *Eco*RI site of the plasmid pUC13, and part of the nucleotide sequence of each cDNA was determined. This analysis revealed that the 24 clones included 12 clones encoding β1 subunit, 8 clones encoding β2 subunit, and 4 clones encoding a third distinct form of β subunit; we have termed this form of the β subunit β3. The β-subunit cDNA inserts were radiolabeled and hybridized under conditions of high stringency to human retinal RNA to

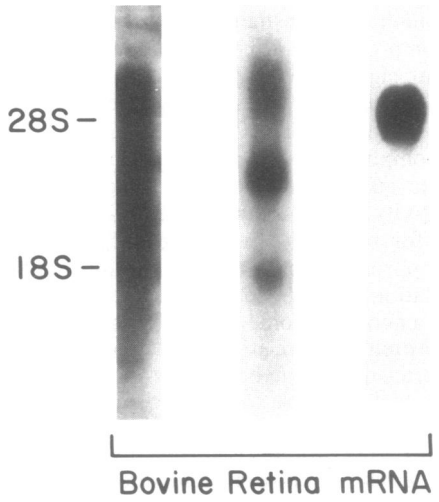

FIG. 1. Blot hybridization analysis of β-subunit-like mRNAs. Each lane contained 10 μg of total RNA from bovine retina. The hybridization probe was the radiolabeled bovine β1-subunit cDNA derived from clone BTd1. The filter was hybridized at 42°C and washed either to 50°C in 2× SSC/0.5% SDS twice for 15 min (left lane), or to 60°C in 1× SSC/0.5% SDS twice for 15 min (middle lane), or to 65°C in 0.1× SSC/0.5% SDS twice for 30 min (right lane). RNA size markers are indicated at left.

define the mRNA species for each β-subunit cDNA form (Fig. 2). The β1-subunit cDNA hybridized with a major mRNA of ≈2.9 kb, whereas the β2-subunit cDNA hybridized specifically to a single mRNA transcript of 1.8 kb. In contrast, the β3-subunit cDNA hybridized predominately to an unusual mRNA species of ≈2.0 kb in length and faintly hybridized to a 3.0-kb mRNA (Fig. 2).

The human retinal β3-subunit cDNA isolated from clone β22 was analyzed by nucleotide sequencing and found to contain 1293 bp (Fig. 3). Translation of the partial cDNA yielded amino acid sequence similar but not identical to amino acid residues 85–340 of the β1 and β2 subunit proteins (18–22). To obtain longer cDNA clones encoding the β3 protein we synthesized 45-base-long oligonucleotide probes, corresponding to the 5′ end (probe B, Fig. 3) and the 3′-untranslated region (probe C, Fig. 3) of the β3 cDNA, and used these probes to rescreen the human retinal cDNA library. The nucleotide sequence and the predicted amino acid sequence of the longest cDNA clone (β46) that we isolated from this library are shown in Fig. 3. The cDNA insert in clone β46 was identical in nucleotide sequence to clone β22 in the region corresponding to nucleotides +252 to +1545 (Fig. 3). In addition, clone β46 provided the 5′ cDNA sequences from −3 to +251, including the putative ATG initiation codon at position +1. The composite sequence typified by clone β46 contains 3 bp of 5′ untranslated region, a 340-codon open reading frame, and 525 bp of 3′ untranslated region, including a single copy of the cleavage and polyadenylylation signal AATAAA and a 28-nucleotide-long poly(A) tract (Fig. 3). The size of the β3 transcript is 2.0 kb, whereas the size of the β3 clone is only 1545 bp. Thus the β3 clone is not full-length and is missing nearly 500 bp of 5′-untranslated sequences. By comparison, unusually long 5′-untranslated regions have been reported in studies of other genes (35).

Two-hundred and thirty-eight (23%) of the nucleotide residues scattered throughout the coding portion of human retinal β3-subunit cDNA differ from those of human β2-subunit cDNAs isolated from human adrenal (18) and human HL-60 cell (19) cDNA libraries. These nucleotide substitutions include silent as well as functional mutations and result in the replacement of 64 of the 340 amino acids compared

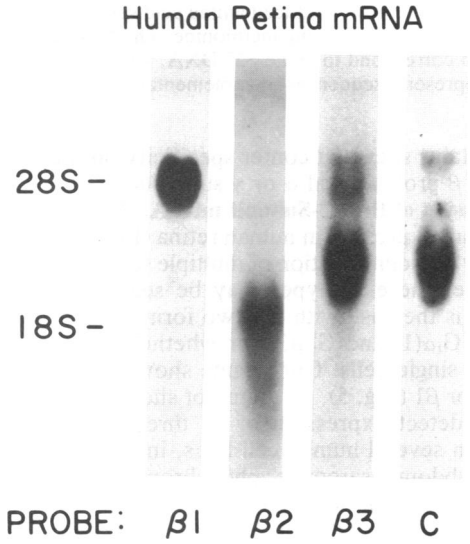

FIG. 2. Blot hybridization analysis of β-subunit mRNAs. Each lane contained 10 μg of total RNA from human retina. Hybridization probes were the radiolabeled cDNAs derived from human retina clones encoding β1, β2, or β3 subunit or the 45-base-long oligonucleotide (probe C, shown in Fig. 3) derived from the 3′-untranslated region of β3 subunit. Hybridization was at high stringency. RNA size markers are indicated at left.

(19%). Qualitatively similar results are observed when the β1 (20–22) and β3-subunit cDNAs are compared; nucleotide variations account for differences in 58 of the 340 amino acids compared (17%). Although the nucleotide sequences corresponding to the coding regions of the three cDNAs show considerable identity, there is little or no homology in the nucleotide sequences of the 3′-untranslated regions of the three human β-subunit cDNAs. The extensive nucleotide divergence among the three cDNAs is consistent with the notion that the three mRNAs are transcribed from different genes. Evidence in favor of this prediction has been presented by Blatt and coworkers (36), who have recently mapped the β1- and β2-subunit genes to discrete loci present on human chromosomes 1 and 7, respectively. To extend these observations, we hybridized human genomic DNA with cDNA probes corresponding to the three forms of β subunit. The β3-subunit probe hybridized to a complex but distinct array of restriction fragments that differed markedly from those identified by the β1- and β2-subunit probes, indicating that β3 subunit is encoded by a separate genomic region. Preliminary studies indicating that the human β3 subunit-encoding gene is found on chromosome 12 (M.A.L., W. S. Modi, and S. J. O'Brien, unpublished data) provide additional evidence that the three forms of β subunit are encoded by distinct genetic loci.

Analysis of β-Subunit Amino Acid Sequences. Translation of the nucleotide sequence of the human retinal β3-subunit cDNA reveals an open reading frame of 340 amino acid residues that encode a protein with M_r 37,221. The size of the deduced protein product of this clone agrees closely with the apparent molecular weight of G protein β subunits purified from various tissues. Purified preparations of retinal G_t contain only one form of the β subunit with an apparent molecular mass of 36 kDa. In contrast, the β subunits of the other described G proteins can be resolved electrophoretically into a doublet of proteins with apparent molecular masses of 36 kDa and 35 kDa. The 36-kDa β-subunit protein is encoded by β1-subunit cDNA, whereas the 35-kDa form of β subunit is encoded by the β2-subunit cDNA (23, 37). The human retinal β3-subunit cDNA clone has not yet been expressed. In the absence of information regarding the size of the protein expressed by this clone, it is tempting to speculate that this retinal clone may encode an additional form of the 36-kDa β subunit.

The predicted amino acid sequence of human retinal β3 subunit is compared in Fig. 4 with amino acid sequences translated from the nucleotide sequence of cDNAs that encode the β1 and β2 forms of the β subunit. The deduced amino acid sequences of the human adrenal β2 (18) and human liver β1 subunits (22) are closely related and differ in only 34 of 340 residues (10%). Generally, these differences represent conservative amino acid substitutions, and appear to be found more often in the N-terminal half of the proteins. In contrast, the human β3 protein differs from β1 in 17% and from β2 in 19% of amino acid residues. These differences represent divergent as well as conservative substitutions and occur not only in the N-terminal region of the protein but also randomly throughout the sequence (Fig. 4).

The N-terminal region contains the greatest diversity in amino acid sequence in the β family of proteins. Indeed, the predicted secondary structures of β1, β2, and β3 subunits based on the parameters of Chou and Fasman (38) indicate that these three proteins differ most markedly in the N-terminal region. Amino acid residues 30–40 of the β1 and β2 subunits are predicted to form β-turns not present in the β3 protein. Conversely, amino acid residues 45–50 of human β3 subunit are predicted to form an α-helix not present in human bovine β1 and β2 subunits. These differences in secondary structure within the N-terminal region may indicate that this

```
                                                                                                       GGGTCG   -1

1                                   10                                      20                                      30
MET Gly Glu MET Glu Gln Leu Arg Gln Glu Ala Glu Gln Leu Lys Lys Gln Ile Ala Asp Ala Arg Lys Ala Cys Ala Asp Val Thr Leu
ATG GGG GAG ATG GAG CAA CTG CGT CAG GAA GCG GAG CAG CTC AAG AAG CAG ATT GCA GAT GCC AGG AAA GCC TGT GCT GAC GTT ACT CTG   90

                                    40                                      50                                      60
Ala Glu Leu Val Ser Gly Leu Glu Val Val Gly Arg Val Gln MET Arg Thr Arg Arg Thr Leu Arg Gly His Leu Ala Lys Ile Tyr Ala
GCA GAG CTG GTG TCT GGC CTA GAG GTG GTG GGA CGA GTC CAG ATG CGG ACG CGG CGG ACG TTA AGG GGA CAC CTG GCC AAG ATT TAC GCC  180

                                    70                                      80                                      90
MET His Trp Ala Thr Asp Ser Lys Leu Leu Val Ser Ala Ser Gln Asp Gly Lys Leu Ile Val Trp Asp Ser Tyr Thr Thr Asn Lys Val
ATG CAC TGG GCC ACT GAT TCT AAG CTG CTG GTA AGT GCC TCG CAA GAT GGG AAG CTG ATC GTG TGG GAC AGC TAC ACC ACC AAC AAG GTG  270

                                    100                                     110                                     120
His Ala Ile Pro Leu Arg Ser Ser Trp Val MET Thr Cys Ala Tyr Ala Pro Ser Gly Asn Phe Val Ala Cys Gly Gly Leu Asp Asn MET
CAC GCC ATC CCA CTG CGC TCC TCC TGG GTC ATG ACC TGT GCC TAT GCC CCA TCA GGG AAC TTT GTG GCA TGT GGG GGG CTG GAC AAC ATG  360

                                    130                                     140                                     150
Cys Ser Ile Tyr Asn Leu Lys Ser Arg Glu Gly Asn Val Lys Val Ser Arg Glu Leu Ser Ala His Thr Gly Tyr Leu Ser Cys Cys Arg
TGT TCC ATC TAC AAC CTC AAA TCC CGT GAG GGC AAT GTC AAG GTC AGC CGG GAG CTT TCT GCT CAC ACA GGT TAT CTC TCC TGC TGC CGC  450

                                    160                                     170                                     180
Phe Leu Asp Asp Asn Asn Ile Val Thr Ser Ser Gly Asp Thr Thr Cys Ala Leu Trp Asp Ile Glu Thr Gly Gln Gln Lys Thr Val Phe
TTC CTG GAT GAC AAC AAT ATT GTG ACC AGC TCG GGG GAC ACC ACG TGT GCC TTG TGG GAC ATT GAG ACT GGG CAG CAG AAG ACT GTA TTT  540

                                    190                                     200                                     210
Val Gly His Thr Gly Asp Cys MET Ser Leu Ala Val Ser Pro Asp Phe Asn Leu Phe Ile Ser Gly Ala Cys Asp Ala Ser Ala Lys Leu
GTG GGA CAC ACG GGT GAC TGC ATG AGC CTG GCT GTG TCT CCT GAC TTC AAT CTC TTC ATT TCG GGG GCC TGT GAT GCC AGT GCC AAG CTC  630

                                    220                                     230                                     240
Trp Asp Val Arg Glu Gly Thr Cys Arg Gln Thr Phe Thr Gly His Glu Ser Asp Ile Asn Ala Ile Cys Phe Phe Pro Asn Gly Glu Ala
TGG GAT GTG CGA GAG GGG ACC TGC CGT CAG ACT TTC ACT GGC CAC GAG TCG GAC ATC AAC GCC ATC TGT TTC TTC CCC AAT GGA GAG GCC  720

                                    250                                     260                                     270
Ile Cys Thr Gly Ser Asp Asp Ala Ser Cys Arg Leu Phe Asp Leu Arg Ala Asp Gln Glu Leu Ile Cys Phe Ser His Glu Ser Ile Ile
ATC TGC ACG GGC TCG GAT GAC GCT TCC TGC CGC TTG TTT GAC CTG CGG GCA GAC CAG GAG CTG ATC TGC TTC TCC CAC GAG AGC ATC ATC  810

                                    280                                     290                                     300
Cys Gly Ile Thr Ser Val Ala Phe Ser Leu Ser Gly Arg Leu Leu Phe Ala Gly Tyr Asp Asp Phe Asn Cys Asn Val Trp Asp Ser MET
TGC GGC ATC ACG TCC GTG GCC TTC TCC CTC AGT GGC CGC CTA CTA TTC GCT GGC TAC GAC GAC TTC AAC TGC AAT GTC TGG GAC TCC ATG  900

                                    310                                     320                                     330
Lys Ser Glu Arg Val Gly Ile Leu Ser Gly His Asp Asn Arg Val Ser Cys Leu Gly Val Thr Ala Asp Gly MET Ala Val Ala Thr Gly
AAG TCT GAG CGT GTG GGC ATC CTC TCT GGC CAC GAT AAC AGG GTG AGC TGC CTG GGA GTC ACA GCT GAC GGG ATG GCT GTG GCC ACA GGT  990

                                    340
Ser Trp Asp Ser Phe Leu Lys Ile Trp Asn END
TCC TGG GAC AGC TTC CTC AAA ATC TGG AAC TGA GGAGGCTGGAGAAAGGGAAGTGGAAGGCAGTGAACACACTCAGCAGCCCCCTGCCCGACCCCATCTCATTCAGGT 1095

GTTCTCTTCTATATTCCGGGTGCCATTCCCACTAAGCTTTCTCCTTTGAGGGCAGTGGGGAGCATGGGACTGTGCCTTTGGGAGGCAGCATCAGGGACACAGGGGCAAA         1205
GAACTGCCCCATCTCCTCCCATGGCCTTCCCTCCCCACAGTCCTCACAGCCTCTCCCTTAATGAGCAAGGACAACCTGCCCCTCCCCAGCCCTTTGCAGGCCCAGCAGA         1315
CTTGAGTCTGAGGCCCCAGGCCCTAGGATTCCTCCCCCAGAGCCACTACCTTTGTCCAGGCCTGGGTGGTATAGGGCGTTTGGCCCTGTGACTATGGCTCTGGCACCAC         1425
TAGGGTCCTGGCCCTCTTCTTATTCATGCTTTCTCCTTTTTCTACCTTTTTTTCTCTCCTAAGACACCTGCAATAAAGTGTAGCACCCTGGT(A)28  1545
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FIG. 3. Nucleotide sequence of human β3-subunit cDNA and predicted amino acid sequence. Nucleotide residues are numbered in 5′ → 3′ direction, beginning with the first residue of the ATG triplet encoding the initiating methionine. Nucleotides on the 5′ side of residue 1 are indicated by negative numbers. Number of the nucleotide residue is indicated at right of each row. Deduced amino acid sequence of β3 subunit appears above nucleotide sequence, and amino acid residues are numbered beginning with the initiating methionine. The 5′-terminal sequence presented does not extend to the 5′ end of the mRNA. Nucleotide residues 252–1545 correspond to clone β22 DNA, whereas clone β46 DNA contained the entire nucleotide sequence presented. Underlined nucleotides represent sequences complementary to the 45-base-long oligonucleotides termed probe B (5′ region) and probe C (3′ region) in text.

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β1   MSELDQLRQE AEQLKNQIRD ARKACADATL SQITNNIDPV GRIQMRTRRT LRGHLAKIYA   60
β2   ····E····· ····R····· ·····G·S·· T···AGL··· ·········· ··········   60
β3   ·G·ME····· ·····K··A· ·······V·· AELVSGLEV· ··V······· ··········   60

β1   MHWGTDSRLL VSASQDGKLI IWDSYTTNKV HAIPLRSSWV MTCAYAPSGN YVACGGLDNI  120
β2   ·········· ·········· ·········· ·········· ·········· F·········  120
β3   ···A···K·· ·········· V········· ·········· ·········· F········M  120

β1   CSIYNLKTRE GNVRVSRELA GHTGYLSCCR FLDDNQIVTS SGDTTCALWD IETGQQTTTF  180
β2   ····S····· ·········P ·········· ·······I·· ·········· ·······VG·  180
β3   ·······S·· ···K·····S A········· ·····N···· ·········· ······K·V·  180

β1   TGHTGDVMSL SLAPDTRLFV SGACDASAKL WDVRQGMCRQ TFTGHESDIN AICFFPNGNA  240
β2   A··S······ ·····G·T·· ·······I·· ····DS···· ··I······· ·VA·····Y·  240
β3   V·····C··· AVS··FN··I ·········· ····E·T··· ·········· ········E·  240

β1   FATGSDDATC RLFDLRADQE LMTYSHDNII CGITSVSFSK SGRLLLAGYD DFNCNVWDAL  300
β2   ·T········ ·········· ·LM······· ······A··R ·········· ·····I···M  300
β3   IC······S· ·········· ·ICF··ES·· ······A··L ·····F···· ········SM  300

β1   KADRAGVLAG HDNRVSCLGV TDDGMAVATG SWDSFLKIWN   340
β2   ·G········ ·········· ·········· ··········   340
β3   ·SE·V·I·S· ·········· ·A········ ··········   340
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FIG. 4. Amino acid sequence of β3 subunit from human retina compared with sequences for β1 (20–22) and β2 (18, 19) subunits. Letters represent single-letter abbreviations for amino acids. ·, Amino acid residue identical to residue in β1 subunit.

area contains sites that confer specificity on the interactions between β proteins and α or γ subunits.

Expression of the β3-Subunit mRNA. All three forms of β subunit are expressed in human retina (Fig. 2). One question raised by the identification of multiple forms of the β subunit is whether these subtypes may be segregated in different cells, as is the case with the two forms of the transducin α subunit, $G_t\alpha(1)$ and $G_t\alpha(2)$, or whether these subtypes are found in single cells. Our results show that specific cDNA probes for β1 (Fig. 5), β2 (data not shown), and β3 subunits (Fig. 5) detect expression of all three forms of β-subunit mRNA in several human cell lines, including those derived from rhabdomyosarcoma, pheochromocytoma, neuroblastoma, and dermal skin fibroblasts. For β1 subunit we detected the 2.9-kb (18, 20, 21) and the 2.0-kb (20) mRNA species reported to arise from use of alternative polyadenylation sites (20). The β3-subunit probe detected a predominant mRNA of ≈2.0 kb with a minor band at ≈2.9 kb. Both of these mRNA species also hybridized at high stringency with a 45-base oligonucleotide (probe C, Fig. 3) used to isolate the β3-subunit-containing clone (β46) (Fig. 2). Because this probe is complementary to 3′-untranslated se-

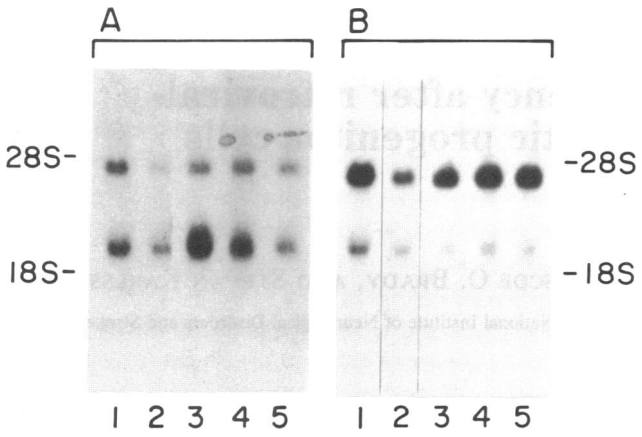

FIG. 5. Blot hybridization analysis of β1- and β3-subunit mRNA in human cell lines. Poly(A)$^+$ RNA was isolated from human cell lines and hybridized first with the radiolabeled cDNA from the human β3-subunit clone β22 (*A*). After exposure to x-ray film the filter was stripwashed of the bound probe (confirmed by reexposure of the blot to film) and rehybridized with a radiolabeled cDNA from the human β1-subunit clone (*B*). The probes were of similar specific activities, and the autoradiographic exposure times were 48 hr. Ten micrograms of poly(A)$^+$) RNA was used in each lane. Lanes: 1 and 2, rhabdomyosarcoma; 3, pheochromocytoma; 4, neuroblastoma; and 5, dermal fibroblast. RNA size markers are indicated at left and right.

quences unique to β3-subunit cDNA, the 2.9-kb hybridization-positive band is unlikely to represent cross-hybridization with β1 subunit mRNA. By analogy to β1 subunit, we speculate that the two forms of β3-subunit mRNA may arise from alternative processing of a single-gene product.

Relative abundance of the mRNA for the three subtypes of β subunit is variable. Moreover, the ratio of the two mRNAs detected by the β3 probe also differs in a tissue-specific manner. In each tissue and cell type the β2 mRNA is the least abundant β-subunit mRNA detected (ref. 19 and data not shown). Fig. 5 shows the results of probing the same nylon filter with cDNA probes specific for β1 and β3 subunits; under the hybridization conditions used, no crossreactivity of the probes occurs. Clearly, the relative abundance of mRNA for these two subtypes varies among the cell types studied. Nevertheless, the greatest abundance of each mRNA is expressed in the retina.

The existence of multiple β-subunit cDNAs raises several significant questions. Most importantly, what is the role of the β subunit in regulating cellular responses to signal transduction? Moreover, do different subtypes of the β subunit perform specific functions? What part of the β-subunit structure is likely to determine that specificity? The comparative analysis of amino acid sequences among β subunits reveals structural differences that may provide the basis for specificity in their interactions with α and/or γ subunits. The functional consequences of these differences are not yet known, but differential expression of β subunit genes may permit characterization of distinct physiological roles for the β subunits.

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