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PARTIAL cDNA SEQUENCE OF THE GAMMA SUBUNIT OF TRANSDUCIN

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A 151 bp cDNA segment that encodes the amino-terminal portion of the gamma subunit of bovine transducin was isolated from a retinal cDNA library constructed in the expression vector λgt11. The base sequence of this cDNA confirms the sequence of the first 39 amino-terminal amino acids reported for the native protein (McConnell et al. (1984) Fed. Proc. 43, 1585). Northern blot analysis indicates that the complete mRNA is approximately 650 bases long and that its expression is limited to the retina.

The GTP-binding protein transducin couples photolysis of rhodopsin to activation of cyclic GMP-phosphodiesterase in retinal rod outer segments (1). Transducin is a heterotrimer consisting of alpha, beta and gamma subunits (1). The alpha subunit contains the binding site for GTP (1) and the sites for ADP-ribosylation by cholera toxin (2) and pertussis toxin (3). The beta and gamma subunits are essential for rebinding of the alpha subunit to the membrane and rhodopsin (4) and remain tightly complexed to one another under physiological conditions.

McConnell et al. (5) recently reported the amino acid sequence for the gamma subunit of bovine transducin. Using antibodies to the beta and gamma subunits of transducin, we screened a retinal cDNA library in the expression

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vector λgt11 (6) and have isolated a partial cDNA[1] segment that encodes the amino-terminal portion of the gamma subunit of transducin.

METHODS AND MATERIALS:

Preparation of antibodies. The beta-gamma subunit complex of transducin was isolated as previously described (1) and used to immunize rabbits. Serum titers to the beta-gamma subunit complex, measured by an enzyme-linked immunosorbent assay (7), were approximately 1:6000. The binding of rabbit antibody to antigen was detected with goat anti-rabbit immunoglobulin antibody coupled to horseradish peroxidase (Miles Laboratories). Prior to screening the λgt11 retinal library, the antisera were pre-purified on an *E. coli* lysate (strain BNN97 (8)) coupled to cyanogen bromide-activated Sepharose 6B, in order to remove antibodies that cross-reacted with *E. coli* or phage proteins, following the protocol described by Young and Davis (8).

Construction and screening of the λgt11 retinal cDNA expression library. A previously constructed retinal cDNA library inserted into the vector λgt10, containing 2.5 x 10^5 recombinants (9), was transferred into the expression vector λgt11, permitting screening of the library with antibody probes. The λgt10 library was amplified, fully digested with the restriction enzyme EcoRI, and then size-fractionated by sucrose gradient sedimentation (5-20% sucrose in 10 mM Tris-Cl pH 7.2 and 1 mM EDTA, centrifuged for 10 h at 38,000 rpm in a Beckman SW41 rotor) to separate λgt10 vector arms from the cDNA inserts. Gradient fractions containing cDNA inserts ranging in size from 0.1 to 8 kb were pooled, ethanol precipitated, and then ligated into the unique EcoRI restriction site of λgt11, which is located within the β-galactosidase gene. The ligated λgt11 was then packaged and the expression library subsequently amplified. The library was then screened with antisera according to the protocol developed by Young and Davis (8).

Characterization of clones. The cDNA inserts from plaque-purified clones were transferred into EcoRI insertion sites in the plasmid pUC8 (10) and the phage M13mp10 (11). Both strands of the cDNA insert were subcloned into M13 phage and their base sequences were determined using the dideoxynucleotide method of Sanger et al. (12), as modified (13).

Northern blot. Freshly excised bovine tissues were collected at a local abbatoir and immediately frozen in liquid nitrogen. Total RNA was prepared (14) and poly(A)$^+$RNA selected by chromatography on oligo(dT)cellulose (15). The poly(A)$^+$RNA was denatured and size-fractionated on 0.8% agarose-formaldehyde gels (16) and transferred to nitrocellulose (17). The pUC8 plasmid containing the 151 bp insert was nick-translated (18) using [α-^{32}P]dATP and used to probe the Northern blot (42°C, 3x SSC, 50% formamide, 1x Denhart's solution and 5% dextran sulfate).

Materials. The retinal λgt10 cDNA library and a full-length rhodopsin cDNA clone were donated by J. Nathans, Stanford University. The expression vector λgt11 and its host strains were donated by Dr. R. W. Davis, Stanford University. The plasmid pUC8 and its host strains were donated by Dr. J. Messing, University of Minnesota. [α-^{32}P]dATP and [α-^{35}S]dATP were purchased from Amersham Inc. The restriction enzyme EcoRI, T_4 DNA ligase, and the large fragment of DNA polymerase were purchased from Bethesda Research Laboratories or New England Biolabs. Packagene Lambda Packaging Extract was purchased from Promega Biotec (Madison, WI). Other reagents were obtained commercially.

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[1] Abbreviations: cDNA-complementary DNA, mRNA-messenger RNA, bp-base pairs.

RESULTS:

Using serum from a rabbit immunized with the beta-gamma subunit complex of transducin, we screened 7 x 10^5 recombinant plaques from the λgt11 retinal cDNA library, and identified five cDNA clones that reacted most strongly with anti-beta-gamma antibody. Serum from a second rabbit immunized with the beta-gamma subunits also reacted with these clones. The protein expressed by these cDNA clones did not react with sera from rabbits that had been immunized with the alpha subunit of transducin; (anti-alpha subunit titers of these sera were approximately 1:6000, as measured by enzyme-linked immunosorbent assay). The three clones that gave the strongest signals during screening were characterized further. Both strands of the insert were subcloned into the phage M13mp10 (11) and sequenced. The base sequences of these three cDNA inserts were identical, with a length of 151 bp (Fig. 1).

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                [-10]
        glu  phe  gly  lys  gln  phe  ala  tyr  arg  gln
  1     GAA  TTC  GGC  AAA  CAG  TTT  GCT  TAC  AGG  CAG

                [1]
        lys  met  pro  val  ile  asn  ile  glu  asp  leu
 31     AAG  ATG  CCA  GTG  ATC  AAT  ATT  GAG  GAC  CTG

           [10]
        thr  glu  lys  asp  lys  leu  lys  met  glu  val
 61     ACA  GAA  AAG  GAC  AAA  TTG  AAG  ATG  GAA  GTC

           [20]
        asp  gln  leu  lys  lys  glu  val  thr  leu  glu
 91     GAC  CAG  CTC  AAG  AAA  GAA  GTG  ACG  CTG  GAA

           [30]
        arg  met  leu  val  ser  lys  cys  cys  glu  glu
121     AGA  ATG  CTG  GTG  TCC  AAA  TGT  TGT  GAA  GAA

          [40]
        phe  arg  asp  tyr  val  glu  glu  arg  ser  gly
151     TTC

           [50]
        glu  asp  pro  leu  val  lys  gly  ile  pro  glu

           [60]
        asp  lys  his  pro  phe  lys  glu  leu  lys  gly


        gly
```

Figure 1. Nucleotide sequence of the cDNA for the gamma subunit of bovine retinal transducin and the corresponding amino acid sequence. Nucleotides are numbered in the 5' to 3' direction, beginning with the EcoRI restriction site. Numbers in brackets indicate the amino acid position relative to the amino-terminal proline determined by McConnell et al. (5). The overline indicates the 39-residue amino acid sequence encoded by bases 37-151 of the cDNA fragment.

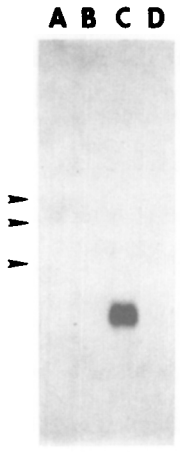

Figure 2. Autoradiogram of poly(A)$^+$mRNA from bovine tissues hybridized to ^{32}P-labelled γ-cDNA probe. Lanes contained 1.5 µg poly(A)$^+$mRNA from heart (A), brain (B), retina (C), and liver (D). Arrows indicate positions of markers, including 28S rRNA (4.7 kb), rhodopsin mRNA (2.65 kb), and 18S rRNA (1.7 kb).

The correct reading frame of the insert is indicated by the reading frame of the β-galactosidase gene at the EcoRI insertion site of λgt11 (6). Because the insert did not contain a poly(A)$^+$ segment at either end, the coding strand was determined by comparing the deduced amino acid sequence from both strands with the previously published amino acid sequence of the gamma subunit of transducin (5). The 5' and 3' ends of the cDNA fragment correspond to the occurrence of EcoRI restriction sites in the cDNA.

A Northern blot of poly(A)$^+$mRNA prepared from b vine retina, brain, heart and liver, probed with the 151 bp fragment, showed only one band, which was unique to the retina (Fig. 2). This RNA was approximately 650 bases in length.

DISCUSSION:

We have isolated a partial cDNA that encodes the gamma subunit of bovine transducin. This cDNA hybridizes with a mRNA that is unique to the retina (Fig. 2). Because 650 bases, the approximate length of the mRNA, is too short to encode both the beta (M_r-35,000) and gamma (M_r-8,000) subunits, we conclude that the beta and gamma subunits of transducin are synthesized separately, and not as a precursor protein that undergoes post-translational cleavage to yield the individual peptides.

The base sequence of the cDNA fragment confirms the amino-terminal amino acid sequence of the gamma subunit of transducin (5). An AUG codon occurs immediately 5' to the amino-terminal proline. Following analysis of 211 eukaryotic mRNA's, Kozak (19) concluded that: 1) Translation begins at the 5'-proximal AUG triplet in 95% of cases, and 2) There is a strong preference for a purine (usually adenosine) at the -3 position preceding the AUG triplets that serve as initiator codons. In addition, site-directed mutagenesis has confirmed the importance of the purine in position -3 (20). The AUG codon that immediately precedes the amino-terminal proline in the cDNA we sequenced could thus serve as the initiator codon for the gamma subunit. However, because we do not know the sequence of the bases that are 5' to the cDNA fragment, we cannot exclude the occurrence of an AUG codon further upstream. Furthermore, the pattern of codon utilization for amino acid residues predicted from bases 1-33 of our cDNA fragment is in close accord with the pattern determined for nucleic acid sequences that encode mammalian proteins (21); therefore, it is possible that this sequence encodes part of a precursor polypeptide. In conclusion, the base sequence indicates that post-translational modification of the amino-terminus does occur, and involves removal of either a methionine or a larger peptide.

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