

β -Subunits of the human liver G_s/G_i signal-transducing proteins and those of bovine retinal rod cell transducin are identical

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The complete cDNA encoding the β -subunit of the human liver signal transducing proteins G_s/G_i (β_G) has been cloned from a λ gt11 library using an oligonucleotide as a screening agent. The cDNA has 3088 nucleotides and an 11 nucleotide poly(A) tail, of which 280 nucleotides constitute the 5'-untranslated region, 1023 form the open reading frame (ORF) and its stop codon, and 1785 are the 3'-untranslated region with two AATAAA cleavage and polyadenylation signals separated by 1467 nucleotides. The ORF codes for a 340 amino acid polypeptide that is identical to that encoded by bovine retinal rod cell cDNA of the β -subunit of transducin. Yet, it does so by using 87 different codons. Curiously, the 280 nucleotide 5' leader sequence obtained starts with an ATG that is part of another ORF encoding a putative peptide X of 75 amino acids (nucleotide 280 to 55). This work proves for the first time that the β -subunits of all signal-transducing G-proteins, including transducin, are the same.

G-protein β -Subunit Signal transduction Adenylyl cyclase Photoreceptor Hormone receptor

1. INTRODUCTION

G-proteins (also N-proteins) are a family of membrane GTPases responsible for the transduction of hormone or neurotransmitter receptor occupancy, which occurs on the outer surface of cells, into altered activity of an effector system, the active site of which is located on the inner surface of the plasma membrane of cells (reviews [1-3]). Of the seven or eight such G-proteins that currently can be defined, four have been purified to better than 90% purity: G_s , the stimulatory regulatory component of adenylyl cyclase [4-6]; G_i , the inhibitory regulatory component of adenylyl cyclase [7,8]; G_t (also T or transducin), the mediator between photoactivation of rhodopsin and stimulation of cGMP-specific phosphodiesterase in outer segments of retinal rod cells [9-11]; and the so-called G_o , a pertussis toxin substrate of neural origin able to interact with brain muscarinic recep-

tors, but having an as yet undefined effector [12,13]. All these proteins share a common subunit organization, being formed of α -, β - and γ -subunits [4,6,14] and react to the presence of Mg^{2+} and a non-hydrolyzable GTP analog (such as GTP γ S or GMP-P(NH)P) by binding the nucleotide to their α -subunits and undergoing a subunit dissociation reaction, with products α^G and the complex of $\beta\gamma$ [15-17]. Although functional, as well as structural, characterizations of the different G-proteins mentioned above reveal that each protein differs from the other by the type of α -subunit they have (e.g. α_{s1} , α_{s2} , α_i , α_t , α_o) the situation is not as clear when their $\beta\gamma$ complexes are considered. Thus, functional assays involving stimulation of GTPase activity of α_t by rhodopsin [18] and inhibition of reconstituted G_sC complexes in phospholipid vesicles [19,20] show apparent complete interchangeability between $\beta\gamma$ of G_t vs G_s vs G_i or G_o , and suggest these complexes to be very

similar if not identical. Yet, $\beta\gamma$ complexes from retinal rod outer segments, where they comprise close to 1% of the protein mass, are water-soluble [9,21,22], while those derived from any other tissue, in which they exist in 10–100-fold lower abundance, are not [17,23] and require detergents to remain in solution. This difference in behavior may be due to differences in either one or both of the two subunits that constitute the $\beta\gamma$ complex.

Recently, Sugimoto et al. [24] and Hurley et al. [25] determined by molecular cloning the complete amino acid sequence of the β -subunit of bovine transducin (β_T). This subunit, which migrates on SDS-PAGE as a polypeptide with apparent M_r 35000–36000, has 340 amino acids and a calculated M_r of 37375. Northern analyses of poly(A)⁺ RNA from retina, brain and liver using β_T cDNA fragments as hybridization probes revealed the existence of both retina- and liver/brain-specific RNA species [24,25]. Thus two bands of hybridization-positive RNA were detected in each tissue, but they differed between retina and brain/liver in size as well as composition in their 5' leader sequences.

The present work was initiated to determine the primary amino acid composition of liver β_G -subunit, and what the difference, if any, might be between it and β_T . Molecular cloning and sequencing of a full length cDNA clone of 3099 nucleotides revealed it to be quite different from the cDNA encoding β_T in both its 5' leader sequence and in its 3'-untranslated region. Yet the deduced amino acid sequence encoded by the open reading frame of the human liver cDNA is identical to that encoded by the bovine β_T cDNA. This proves that β -subunits of all G-proteins are the same and functional differences of $\beta\gamma$ complexes must reside in the structure of differing α -subunits.

2. MATERIALS AND METHODS

A cDNA library, constructed as in [26,27] in the cloning vector λ gt11, was made using poly(A)RNA [28,29] from a human liver [30]. 100000 recombinant phages of this library were screened at a density of 20000–25000 phages per 150 mm petri dish, using a replicate plaque amplification technique [31], for the presence of nucleotide sequences complementary to the synthetic 27-meric

oligodeoxynucleotide (kindly prepared for us by Vega, Tucson, AZ):

5'-AGAGCTGGTAACAATCTGATTGTCATC-3'

(oligonucleotide V)

which is part of the antisense strand of the coding sequence of bovine β_T (amino acids 153–161) [24]. To this end filters were prehybridized in $6 \times$ SSC, $5 \times$ Denhardt's solution, 300 mM sodium phosphate, pH 6.8, 0.1% SDS and 0.1 mg/ml sheared herring sperm DNA [32], for 3 h at 32°C, and then hybridized overnight at 32°C with the same solution plus 0.1×10^6 cpm/ml of oligonucleotide V phosphorylated with T₄ polynucleotide kinase using [α -³²P]ATP of 4500 Ci/mmol. The filters were then washed extensively at 32°C with $6 \times$ SSC, dried and subjected to autoradiography for 17 h at –70°C in the presence of two Dupont Cronex Lighting Plus enhancing screens using Kodak X-Omat AR X-ray films.

Phages giving duplicate signals in the replicate screening procedure were plaque purified [32]. Their inserts were excised with *Eco*RI, isolated by electrophoresis in 1% low melting point agarose and subcloned into M13 mp18 for sequencing as in [33] using the buffer system of [34]. Southern blots [35], using oligonucleotide V, as well as two other oligonucleotides, as probes, were done onto nitrocellulose sheets prehybridizing for 4 h at 37°C and hybridizing overnight at 37°C as in [36]. Prior to autoradiography, the sheets were extensively washed with $6 \times$ SSC [36] at 4°C.

3. RESULTS AND DISCUSSION

Primary screening of the λ gt11 human liver cDNA library with oligonucleotide V led to the identification of 63 possible candidates. Of these, one gave a significantly stronger signal on secondary screening and was plaque purified. It contained an insert of approx. 3000 nucleotides (λ b1) which could be excised with *Eco*RI from the recombinant phage as a single fragment. Insert λ b1 was transferred onto nitrocellulose and tested for hybridization to three probes. One was the screening probe, i.e. ³²P-labeled oligonucleotide V (probe A). The second, probe B, tested for the

presence of sequences coding for a portion closer to the carboxy-terminus of a putative β_G polypeptide and the third, probe C, tested for the presence of sequences coding for the amino end of a putative β_G polypeptide. Specifically, probe B was the 17-mer 5'-ATG GGA GTA GGT CAT GA-3' which is complementary to nucleotides 792-808 (spanning amino acids 261-266) of the sense strand of the same cDNA. Probe C was the 17-mer 5'-TA ACT GGT CAA GTT CAC T-3' which is complementary to nucleotides 3-19 (spanning amino acids 1-7) of the sense strand of bovine β_T cDNA. As shown in fig.1, the single insert $\lambda b1$ was hybridization-positive with all three nucleotides, strongly suggesting that a full-length cDNA had been isolated. $\lambda b1$ was subcloned into M13 mp18

and sequenced. Fig.2 presents the complete nucleotide sequence of $\lambda b1$ and the deduced amino acid sequence of the long open reading frame (ORF) spanning from what is numbered as nucleotide 1 to 1020. The amino acid composition of the polypeptide encoded by this open reading frame corresponds exactly to that of the cDNA published for β_T [24,25].

The first nucleotides (-280 to -278) of the 5'-untranslated leader sequence present in this cDNA are ATG and a potential translation initiation codon. As shown in fig.3, this ATG is part of another ORF (ORF of peptide X) extending from -280 to -54, followed by codon TGA. This is then followed by two additional TGAs in the same reading frame. The ORF of this putative peptide X

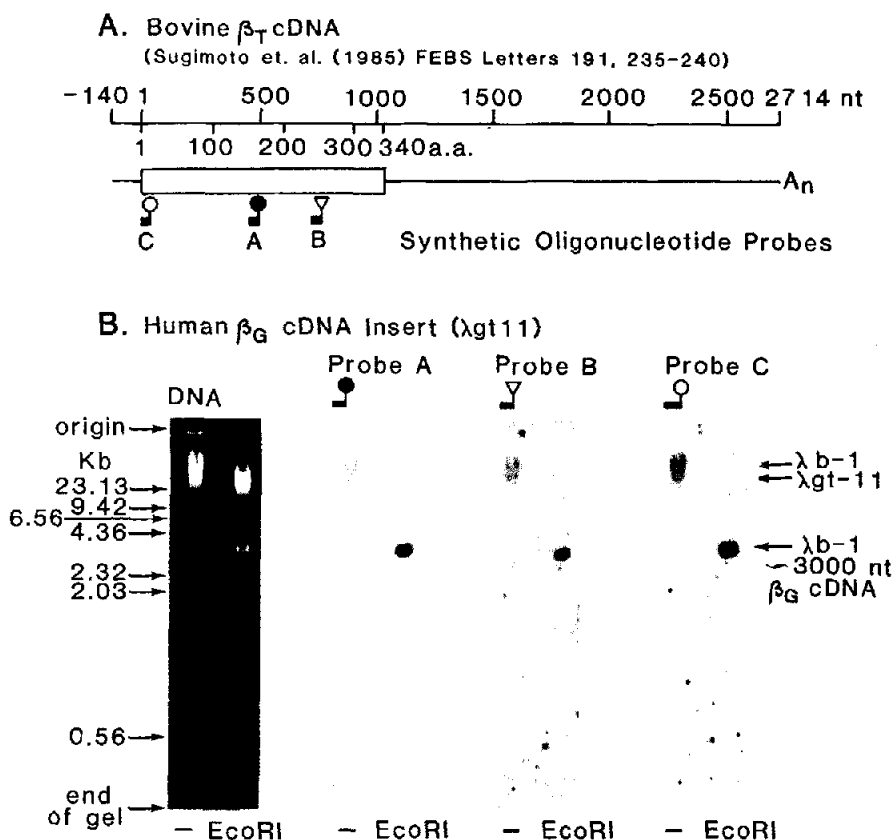


Fig.1. (A) Scheme of β_T cDNA cloned by Sugimoto et al. [25] and location of nucleotide sequences on the basis of which synthetic oligonucleotide probes were made for screening (probe A) and confirmation (probes B,C) of identity of a putative cDNA for human liver β_G . (B) Ethidium bromide stain of DNA of plaque purified recombinant lambda with putative insert ($\lambda b1$) coding for β_G without and after digestion with *EcoRI*, and Southern blots [35] of the digests after transfer onto nitrocellulose hybridized sequentially with ^{32}P -labeled probe A, B and C.

-280
5'-----ATGGGCGGCGAGTGGGAGCGGGGCCGGGAGTGGAGCAGCAGCGCGGCGGACTGGACCGAGCCTCGCGCGGCGGCGACCT

-200 GCCCGCAGCGCCCGCGGACGCGAGCGCGGCCGAGCGGACGACCTTCGAGCGGCGCGCGAGCGCGGCTGTGGGCGCTCAGGCGCGGACGAGGCGG

-100 CTGAGACAAATTACATGTATTGGAGACCAAGCCAGAGCCCTTCTGAATTAAGATCTCAATTCTTGAAGGTGGCATTGAAGAGCAGTAAGATCGGAAG -1

1	10	20	30	
ATG AGT GAG CTT GAC CAG TTA CGG CAG GAG GCC GAG CAA CTT AAG AAC CAG ATT CGA GAC GCC AGG AAA GCA TGT GCA GAT GCA ACT CTC				90
Met Ser Glu Leu Asp Arg Glu Arg Gln Glu Ala Glu Gln Leu Lys Asn Gln Ile Arg Asp Ala Arg Lys Ala Cys Ala Asp Ala Thr Leu				
40	50	60	70	
TCT CAG ATC ACA AAC AAC ATC GAC CCA GTG GGA AGA ATC CAA ATG CGC ACQ AGG AGG ACA CTG CGG GGG CAC CTG GCG AAG ATC IAC GCC				180
Ser Gln Ile Thr Asn Asn Ile Asp Pro Val Gly Arg Ile Gln Met Arg Thr Arg Arg Thr Leu Arg Gly His Leu Ala Lys Ile Tyr Ala				
80	90	100	110	
ATG CAC TGG GGC ACA GAC TCC AGG CTT CTG GTC AGT GCC TCG CAG GAT GGT AAA CTT ATC ATC TGG GAC AGC TAC ACC ACC AAC AAG GTC				270
Met His Trp Gly Thr Asp Ser Arg Leu Leu Val Ser Ala Ser Gln Ser Arg Lys Leu Ile Ile Trp Asp Ser Tyr Thr Thr Asn Lys Val				
120	130	140	150	
CAC GCC ATC CCT CTG CCG TCC TCC TGG GTC ATG ACC TGI GCA TAT GCC CCT TCT GGG AAC TAI GTG GCC TGC GGI GGC CTG GAT AAC ATI				360
His Ala Ile Pro Leu Arg Ser Ser Trp Val Met Thr Cys Ala Tyr Met Thr Cys Gly Lys Leu Ala Cys Gly His Thr Thr Asn Leu Asp Asn				
160	170	180	190	
TGC TCC ATT TAC AAT CTG AAA ACT CGT GAG GGG AAC GTG CGC GTG AGT CGT GAG CTG GCA GGA CAC ACA GGT TAC CTG TCC TGC TGC CGA				450
Cys Ser Ile Tyr Asn Leu Lys Thr Arg Glu Gly Asn Val Arg Val Ser Arg Gln Leu Ala Gly His Thr Gly Tyr Leu Ser Cys Cys Arg				
200	210	220	230	
TTC CTG GAT GAC AAT CAG ATC GTC ACC ATC TCT GGA GAC ACC AGC TGT GCC CTG TGG GAC ATC GAG ACC GGC CAG CAG ACG ACC ACG TTI				540
Phe Leu Asp Asp Asn Gln Ile Val Thr Ser Ser Gly Asp Thr Thr Cys Ala Leu Trp Asp Ile Gln Thr Gly Gln Gln Thr Thr Thr Phe				
240	250	260	270	
ACC GGA CAC ACT GGA GAT GTC ATG AGC CTI TCT CTT GCT CCT GAC ACC AGA CTG TTC GTC TCT GGT GCT TGT GAI GCT TCA GCC AAA CTC				630
Thr Gly His Thr Gly Asp Val Met Ser Leu Ser Leu Ala Pro Asp Thr Arg Leu Phe Val Ser Gly Ala Cys Asp Ala Ser Ala Lys Leu				
280	290	300	310	
TGG GAT GTG CGA GAA GGC ATG TGC CGG CAG ACC TTC ACT GGC CAC GAG TCI GAC ATC AAT GCC ATA TGC TTC TTI CCA AAT GGC AAT GCA				720
Trp Asp Val Arg Glu Gly Met Cys Arg Gln Thr Phe Thr Gly His Glu Ser Asp Ile Asn Ala Ile Cys Phe Phe Pro Asn Gly Asn Ala				
320	330	340	350	
TTT GCC ACT GGC TCA GAC GAC GCC ACC TGC AGG CTG TTI GAC CFI CGT GCI GAC CAG GAG CTC ATG ACT TAC TCC CAT GAC AAC ATC ATC				810
Phe Ala Thr Gly Ser Asp Asp Ala Thr Cys Arg Leu Phe Asp Leu Arg Ala Asp Gln Glu Leu Met Thr Tyr Ser His Asp Asn Ile Ile				
360	370	380	390	
TGC GGG ATC ACC TCT GTC TCC TTC TCC TCC AAG AGC GGG CGC CTC CTC CTI GCI GGG TAC GAC TTC AAC TGC AAC GTC TGG GAT GCA CTC				900
Cys Gly Ile Thr Ser Val Ser Phe Ser Lys Ser Gly Arg Leu Leu Leu Ala Gly Tyr Asp Asp Phe Asn Cys Asn Val Trp Asp Ala Leu				
400	410	420	430	
AAA GCC GAC CGG GCA GGT GTC TTG GCI GGG CAT GAC AAC CGC GTC AGC TGC CTG GGC GTG ACT GAC GAT GGC ATG GCI GTG GCG ACA GGG				990
Lys Ala Asp Arg Ala Gly Val Leu Ala Gly His Asp Asn Arg Val Ser Cys Leu Gly Val Thr Asp Asp Gly Met Ala Val Ala Thr Gly				
440	450	460	470	
TCC TGG GAT AGC TTC CTC AAG ATC TGG AAC TAA CGCCAGTAGCATGTGGATGCCATGGAGACTGGAAGACATTCCAACCTGGACGCGTTACCATGAGAG				1090
Ser Trp Asp Ser Phe Leu Lys Ile Trp Asn Stop				

Fig.2. Nucleotide sequence and deduced amino acid composition of human liver β -subunit of G_s/G_i (β_G). The CG content of the 5' leader sequence, the long open reading frame and the 3'-untranslated region is 58, 56 and 42%, respectively. Two AATAA cleavage and polyadenylation signals [37] are highlighted. The 87 bases in β_G that differ from those of the retinal rod cell β_T cDNA are underscored.

and that of β_G are not in frame, and the β_G ORF is preceded in frame by two TAA stop codons 9 and 48 nucleotides upstream of the ATG β_G initiation codon. In a recent survey of 211 5' leader sequences of eukaryotic messages [37], only 10 were found to have one or more upstream ATGs, only 7 were longer than 200 nucleotides and only 1 was both longer than 200 nucleotides and had one or more upstream ATGs. 75% of the leader sequences were between 20 and 80 nucleotides long. Upstream ORFs ranged from 3 (ATG followed by a stop codon) to a maximum of 75 nucleotides, encoding for a possible polypeptide of 25 amino acids. In this context, the leader sequence of the human liver β_G mRNA is rather out of the common both in nucleotide length (280 nucleotides) and in the length of the upstream open reading frame it presents (encoding for 75 amino acids). To our knowledge, there are no reports of polycistronic messages in higher eukaryotes. Yet,

the relatively long ORF present in the ~280 long leader sequence of β_G opens the possibility that it may be translated. We are now raising antibodies to fragments of the putative peptide X to test this hypothesis. We are also attempting to determine the total length of the mRNA species that gave rise to the cDNA reported here.

Structural aspects of β -subunits of different G-proteins have been compared previously by various indirect means, including total amino acid composition, mono- and two-dimensional peptide mapping and immunoreactivity. All these studies suggested them to be very similar, if not identical, and that functional interchangeability among β -complexes of different G-proteins is a reflection of the similarity between their β -subunits. The present work supports this concept by proving that G_s and G_i have β -subunits that are identical to that of transducin.

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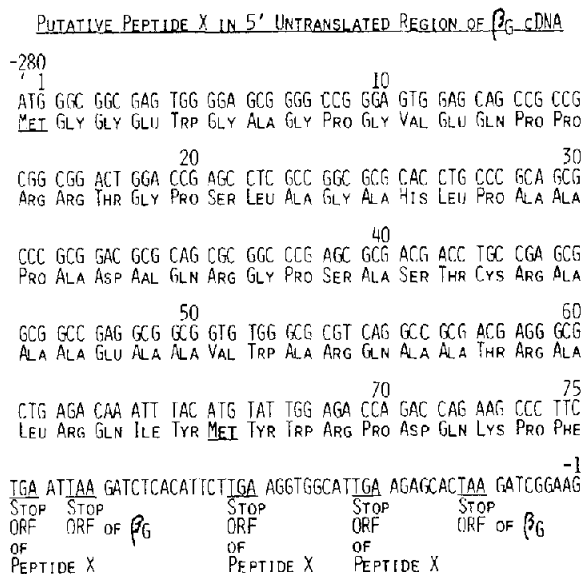


Fig.3. Deduced amino acid sequence of the putative peptide X that is coded for by sequences -280 to -55 of fig.2, and location of all stop codons present in the -280 to -1 leader sequence of β_G . ORF, open reading frame.

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