Primary structure of the β-subunit of bovine transducin deduced from the cDNA sequence

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Received 7 September 1985

DNA complementary to the bovine retinal mRNA coding for the β-subunit of transducin has been cloned by screening a cDNA library with oligodeoxyribonucleotide probes. Nucleotide sequence analysis of the cloned cDNA has revealed that this polypeptide consists of 340 amino acid residues (including the initiating methionine). Furthermore, cDNA hybridizable with a transducin β-subunit cDNA probe has been cloned from a library derived from bovine brain poly(A)$^+$ RNA. Comparison of the cloned cDNAs, in conjunction with blot hybridization analysis and S_1 nuclease mapping of poly(A)$^+$ RNA from bovine retina, brain and liver, suggests that the mRNAs coding for the β-subunits of transducin and other guanine nucleotide binding proteins have the same protein-coding sequence but partly different 5′-noncoding sequences.

Transducin *Guanine nucleotide binding protein* *cDNA cloning* *Nucleotide sequence* *RNA blot hybridization analysis* *S_1 nuclease mapping*

1. INTRODUCTION

A family of membrane-associated guanine nucleotide binding proteins (G-proteins) play an essential role in transducing signals generated at cell surface receptors into changes in cellular function and metabolism [1]. These proteins are composed of 3 subunits termed α, β and γ. The α-subunit is unique to each G-protein, whereas the β-subunit is thought to be common to all the G-proteins that have been examined [2–4]. Transducin, a member of this protein family, mediates visual transduction by coupling the signal of photolysed rhodopsin with activation of a cyclic GMP phosphodiesterase [5]. The primary structures of the α- [6–9] and γ-subunit [10–12] of bovine transducin have been elucidated by cDNA or protein sequencing. Here we have deduced the complete amino acid sequence of the β-subunit of bovine transducin by cloning and sequencing cDNA encoding it. cDNA from bovine brain poly(A)$^+$ RNA that hybridizes with a transducin β-subunit cDNA probe has also been cloned. Using the cloned cDNAs, we have investigated the relationship between the β-subunits of transducin and other G-proteins.

2. MATERIALS AND METHODS

The β-subunit of bovine transducin was purified as in [6]. Total RNA was extracted from bovine tissues as in [13], and poly(A)$^+$ RNA isolated as in [14]. The Okayama-Berg cDNA library [15] screened for transducin β-subunit cDNA was the same as that used for cloning transducin α-subunit cDNA [6]. The transformation and screening procedures were as in [16,17]. The 2 oligodeoxyribonucleotide probes used were as follows: an equimolar mixture of 5′-GT$^{A}_{G}$TANGA$^{A}_{G}$TCCCA-3′ and 5′-GT$^{A}_{G}$TA$^{A}_{G}$CT$^{A}_{G}$TCCCA-3′ (probe a) and

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00145793/85/$3.30

5′-GTNCCCCA$^{A}_{G}$TGCAT-3′ (probe b), which correspond to the sequences Trp-Asp-Ser-Tyr-Thr (residues 82–86) and Met-His-Trp-Gly-Thr (residues 61–65), respectively. Each probe was synthesized by the triester method [18] as 2 pools containing A/T or G/C at the position indicated by N, which denotes a mixture of A, T, G and C. The probes were labelled with ^{32}P at the 5′-end and used for hybridization at 37°C. A cDNA library derived from bovine cerebral cortex poly(A)$^+$ RNA was constructed with 7.8 μg poly(A)$^+$ RNA and 4.2 μg vector-primer DNA according to [15]. Ampicillin-resistant transformants were screened by hybridization at 60°C with the *Aat*II(−51)-*Ava*II(268) fragment from clone pTβ6, labelled with [α-^{32}P]dCTP by nick-translation [19]; the restriction sites are identified by numbers indicating the 5′-terminal nucleotide generated by cleavage. DNA sequencing was carried out as in [20]. RNA blot hybridization analysis was performed by the procedure in [21], and S_1 nuclease mapping according to [22].

3. RESULTS AND DISCUSSION

Tryptic peptides from the purified bovine retinal transducin β-subunit were isolated by reverse-phase high-performance liquid chromatography (HPLC) and analysed for amino acid sequence with a gas-phase sequencer (fig.1). Ten peptide sequences were thus determined. Two oligodeoxyribonucleotide probes (probes a and b) corresponding to part of the amino acid sequences determined for fractions IX and VII were synthesized (see section 2). Screening of a cDNA library derived from bovine retinal poly(A)$^+$ RNA by hybridization with probe a yielded 10 positive clones from about 2.4×10^5 transformants. Six of them also hybridized with probe b. One of these 6 clones, pTβ6, was subjected to nucleotide sequence analysis.

Fig.2 shows the 2822-nucleotide sequence (excluding the poly(dA) tract) of the cDNA insert of clone pTβ6. All the 10 partial amino acid sequences determined by peptide analysis were found to be encoded by the cDNA sequence in the same reading frame (amino acid residues 24–37, 53–57, 58–68, 79–89, 130–134, 135–137, 210–214, 252–256, 305–314 and 338–340). This reading frame was used to deduce the primary structure of

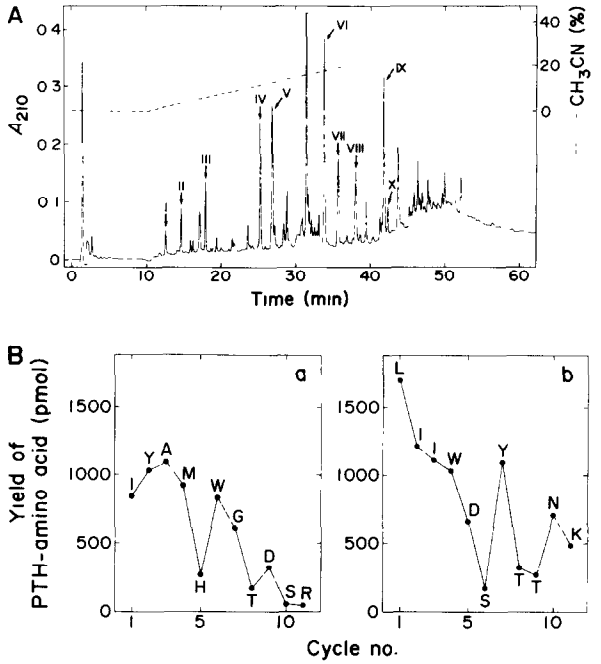

Fig.1. Partial amino acid sequence analysis of the β-subunit of bovine transducin. (A) Reverse-phase HPLC of tryptic peptides. Approx. 200 μg purified bovine transducin β-subunit was digested by trypsin and then subjected to reverse-phase HPLC. Ten fractions (I–X) corresponding to absorbance peaks were collected. (B) Sequence analysis of peptides. The yield of phenylthiohydantoin (PTH)-amino acids at each cycle of Edman degradation is shown for fractions VII (a) and IX (b); the one-letter amino acid notation [23] is used. The sequences determined for the other fractions (not shown) were as follows: I, VSR; II, EGNVR; III, GHLAK; IV, AGVLAGHDNR; V, IWN; VI, LWDVR; VIII, LFDLR; X, AXADATLSQITNNI. For experimental details, see [6].

the β-subunit of bovine transducin. The translational initiation site was assigned to the methionine codon composed of nucleotide residues 1–3 because this is the first ATG triplet that appears downstream of a nonsense codon (TAG at positions −6 to −4) found in frame and because the second ATG triplet encodes amino acid residue 45, which is preceded by one of the peptide sequences determined. A translational termination codon (TAA) occurs in frame after the 340th codon specifying asparagine. Thus, it is concluded that the β-subunit of bovine transducin consists of 340 amino

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                                                                                                    5'-----AGAGAAGGAGCCTAGGCC  -121
ACGTAACTGATGGTGGGCTTCCTGGAGGCTCTGGGGGACTGAGGACAGTGAGGAGAGCAACAGAGACGTCACATATCTCAGATTCTTTGAGGTGGCTTTGAAGAGCACTAAGATTAGAAG    -1
  1                                  10                                  20                                  30
Met Ser Glu Leu Asp Gln Leu Arg Gln Glu Ala Glu Gln Leu Lys Asn Gln Ile Arg Asp Ala Arg Lys Ala Cys Ala Asp Ala Thr Leu
ATG AGT GAA CTT GAC CAG TTA CGG CAG GAG GCC GAA CAG CTT AAA AAC CAA ATT AGA GAT GCT CGG AAG GCG TGT GCA GAT GCG ACT CTC     90
                                  40                                  50                                  60
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
TCT CAG ATC ACA AAC AAC ATC GAC CCT GTG GGA AGA ATC CAG ATG CGC ACC AGG AGG ACG CTG AGG GGA CAC TTG GCC AAG ATC TAT GCC    180
                                  70                                  80                                  90
Met His Trp Gly Thr Asp Ser Arg Leu Leu Val Ser Ala Ser Gln Asp Gly Lys Leu Ile Ile Trp Asp Ser Tyr Thr Thr Asn Lys Val
ATG CAC TGG GGC ACC GAC TCC AGG CTT CTA CTC AGT GCC TCG CAG GAT GGT AAA CTT ATT ATC TGG GAC AGC TAC ACC ACA AAC AAG GTC    270
                                 100                                 110                                 120
His Ala Ile Pro Leu Arg Ser Ser Trp Val Met Thr Cys Ala Tyr Ala Pro Ser Gly Asn Tyr Val Ala Cys Gly Gly Leu Asp Asn Ile
CAT GCC ATC CCT CTG CGC TCC TCG TGG GTC ATG ACG TGC GCT TAT GCC CCT TCT GGG AAC TAC GTG GCC TGT GGA GGC CTG GAT AAC ATC    360
                                 130                                 140                                 150
Cys Ser Ile Tyr Asn Leu Lys Thr Arg Glu Gly Asn Val Arg Val Ser Arg Glu Leu Ala Gly His Thr Gly Tyr Leu Ser Cys Cys Arg
TGC TCC ATT TAC AAT CTG AAA ACT CGT GAA GGG AAT GTA CGT GTG AGT CGT GAG CTG GCT GGA CAC ACA GGT TAC CTG TCC TGC TGC CGT    450
                                 160                                 170                                 180
Phe Leu Asp Asp Asn Gln Ile Val Thr Ser Ser Gly Asp Thr Thr Cys Ala Leu Trp Asp Ile Glu Thr Gly Gln Gln Thr Thr Thr Phe
TTT CTG GAT GAC AAT CAG ATT GTT ACC AGC TCT GGA GAC ACC ACC TGT GCC CTG TGG GAC ATC GAG ACT GGC CAG CAG ACG ACC ACA TTC    540
                                 190                                 200                                 210
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
ACT GGA CAC ACC GGA GAC GTC ATG AGC CTT TCA CTC GCT CCG GAC ACC AGA CTG TTT GTC TCT GGT GCT TGT GAC GCT TCA GCC AAA CTC    630
                                 220                                 230                                 240
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
TGG GAC GTG CGA GAA GGG ATG TGC CGG CAA ACC TTC ACC GGC CAT GAG TCC GAT ATC AAT GCC ATC TGT TTC TTC CCG AAC GGC AAT GCA    720
                                 250                                 260                                 270
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
TTT GCC ACT GGC TCA GAC GAT GCC ACC TGC CGG CTG TTC GAC CTC CGT GCG GAC CAG GAG CTC ATG ACC TAC TCC CAT GAC AAC ATC ATC    810
                                 280                                 290                                 300
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
TGC GGG ATC ACC TCC GTC TCC TTC TCC AAG AGC GGG CGC CTC CTT CTC GCA GGG TAC GAC GAC TTC AAC TGC AAC GTC TGG GAC GCC CTC    900
                                 310                                 320                                 330
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
AAG GCC GAC AGG GCA GGG GTC TTG GCC GGG CAT GAC AAC CGC GTG AGC TGC CTG GGG GTA ACT GAT GAC GGG ATG GCC GTG GCG ACG GGA    990
                                 340
Ser Trp Asp Ser Phe Leu Lys Ile Trp Asn
TCC TGG GAC AGC TTC CTC AAA ATC TGG AAC TAA CCCTGACAGCAGGTGGACGCCACAGTGACTGGAAGACCATTCCAGCCTTGGAGCGTTGCAGATAGCATTTCCCATC   1099
TACCCCTACTAACGCGGACGCCCTGCACCCCTCAGAACTTCAAAAGGGCAAGACCTTCCCCTCACTTATTGCTGAAACCAAGAGCACAATTCCCACTGAGAGAAGAATCTCTGTGCTGTA   1219
ACTAAAACGAATCGTGCATTCCTTCCGGGACCATTGTCTTTGTTTTCTTTTTTTGTTTGTTTTGAATGAATTTAAAAGGAAATACTATAATAAAAATGTTAACAAGAAGGTAAACTTGAG   1339
TGTAATTGTAAGACAGACACACCTTTTTACTAGTTTATTTGAAATCTTGATCAATAATCCTGTTTGGTGATGTTCATGCAAAACAATTTGAGGACTTCATCTTGTAATTGACTCTGAATT   1459
CTAGATTGTGTTTGTAACAGGATTTTGCAGACATGATTCATATTTCCTTCTTAAAATATATTTCTTTCTGCTCGTAGTTAAACAGAGAAGGAGGGTCTGGTGTGGCCTCTCTGAGATTGT   1579
TAATGATGTAAATTTAGTCCCTGGTTTTTTTTACTTCCGTCTCGTGTCACATGACCGTCGTGCGCATGGTGTAGAAGGATCATGCTGAGAAGTCAAGGAACTGAGCACGCGGACGAACAG   1699
TGGCCGCGTCAGGCGCCTTTCCCCCAACCCACCCTCTCCCCTCAGTTTGGTTTTTAGTCTCACCCAGTCTCCTTGTGAAGTTGGAGGGAGGCTGACACAACAGCGCGACACTACCCTGTG   1819
CCCCGCCCGGCCATCACGAGCCTCCGTCCTCAAGAGCGGTGCCGTCCTGTGCGTGGAGAGTCAGTCAGTTATTTGTGTATGAAACAATGTACAAATCAATGTTTTGAAAATAATGATCTC   1939
AAACTTTCTAAGTTAAATTTTAAAAAACCTGATCGTTTGCCATATGGGGTGGGTTTGCTCTTAGAACCGCATGCTGTAGAAATGCTCACAGTGCATATAGGACTCAGTCCTTAGATGTTC   2059
CTTTTCTTTTAAGAAATAACCTCTTTGAAGTTGTGACGTCGCGCTCTGTCCACTTCGCGTCGCCCCTCTGTGCACACGCATCGCATTGGAGGCCGTCCAGTTAGCGGCTCTACACGCTCG   2179
CCTGGCGAGATGTCACTGTTTTCTTTCTTTAAAAAAACAAGTCCTGTTCCAAAATCGTTGTTGGATGGGGTGGGGGGTGTACAGGGAACGGAGTCCCTGGCCTCCCTTATGGATTCCTCA   2299
CGGCCCACCTCTCCCGGGAACCAGCCTTTGTGCTGAGGCAGACCACTTTTCTAACACGCAGATGTCCCTGGTTCCGTGCGCGTCCAGTGTTGGGCAGCGGTGCTGGGACAATGCGGGCCT   2419
CCTCCCCGTACCAGCGCCCCCGTAGCGGCAGCAGTGGCTTCTCCACCTGTGTTTGCACCATCCTGTGCGCCCTGTGCCCTGGACCCGCAGAGAGTGGGATGAGCCCCCGCCCCCGTCGGC   2539
ACCTCTGTAGAGCTCTCTGCACCCTGTCCCTCACCTTTTGTATTTAATTTTAAAGTCAATGTACTGCAAGGAAGCTGGATGCAAGATAGATCCTATATTCAACTGTACTGTTATTTAAGA   2659
TGTAATAAAGCAGTTTGACATGAGG-----3'
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Fig.2. Nucleotide sequence of the cDNA encoding the β-subunit of bovine transducin. Nucleotide residues are numbered in the 5'- to 3'-direction, beginning with the first residue of the ATG triplet encoding the initiating methionine, and the nucleotides on the 5'-side of residue 1 are indicated by negative numbers; the number of the nucleotide residue at the right-hand end of each lane is given. The deduced amino acid sequence of the transducin β-subunit is shown above the 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. The 3'-terminal sequence shown is followed by a poly(dA) tract connected with the vector DNA sequence [15].

acid residues (including the initiating methionine) and has a calculated M_r of 37375, which agrees with the reported value [5,24]. The 3′-noncoding region contains 2 copies of the polyadenylation signal AATAAA [25] (residues 1308–1313 and 2663–2668). Nucleotide sequence analysis showed that one of the 6 clones isolated carried a DNA insert complementary to the mRNA polyadenylated at residue 1326.

G-proteins other than transducin have been purified from mammalian tissues including brain and liver [26–29]. Poly(A)$^+$ RNA from bovine retina, brain and liver was subjected to blot hybridization analysis with a transducin β-subunit cDNA probe comprising most of the protein-coding sequence and a downstream portion of the 5′-noncoding sequence (fig.3A). Two hybridization-positive bands, corresponding to approximate sizes of 3200 and 1700 nucleotides (retinal RNA) or 3300 and 1800 nucleotides (brain and liver RNA),

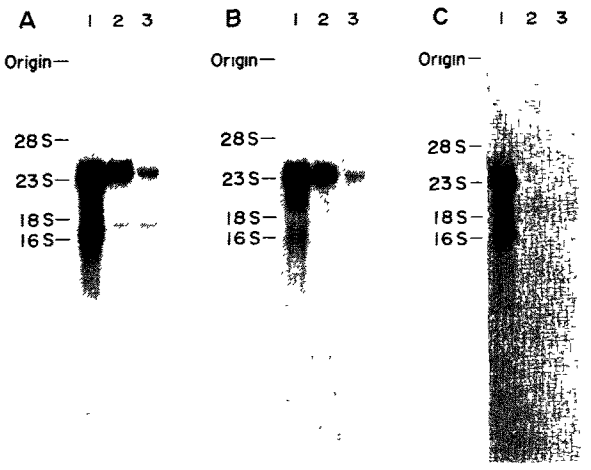

Fig.3. Blot hybridization analysis of poly(A)$^+$ RNA from bovine retina (lane 1), cerebral cortex (lane 2) and liver (lane 3). The amount of poly(A)$^+$ RNA used was 5 μg (lane 1), 10 μg (lane 2) or 20 μg (lane 3). The hybridization probes used, derived from clone pTβ6, were an equimolar mixture of the *Aat*II(−51)-*Aat*II(561) and *Bgl*II(171)-*Bam*HI(989) fragments (A), the *Hpa*II(1827)-*Hpa*II(2314) fragment (B) and the *Hae*III(−122)-*Hin*fI(−39) fragment (C); the probes were labelled by nick-translation [19] with [α-^{32}P]dCTP (A and B) or by 5′-end labelling (C). The size markers were bovine and *Escherichia coli* rRNA.

were observed. When a 3′-noncoding sequence downstream of the polyadenylation site at residue 1326 was used as probe, only the larger RNA bands were hybridizable (fig.3B). Thus, the 2 hybridization-positive bands seem to represent the mRNA species polyadenylated at the different sites (see above and below). When a further upstream portion of the 5′-noncoding sequence was used as probe, however, brain and liver poly(A)$^+$ RNA exhibited no hybridization signal at all (fig.3C). Furthermore, S_1 nuclease mapping with a probe containing the 5′-end of the cDNA insert of clone pTβ6 (fig.4) showed that the major fragment protected by retinal poly(A)$^+$ RNA had a size of ~408 nucleotides as expected, whereas a major fragment with a size close to 316 nucleotides (see below) was protected by brain and liver poly(A)$^+$ RNA; retinal RNA also protected the 316-nucleotide fragment as a minor band. The results of RNA blot hybridization analysis and S_1 nuclease mapping indicate that the brain as well as the liver contains an mRNA species homologous with the retinal transducin β-subunit mRNA but different from it in 5′-noncoding sequence. The absence of transducin α-subunit mRNA in bovine brain and liver [8] was confirmed by blot hybridization analysis of poly(A)$^+$ RNA from these tissues with a cDNA probe obtained from clone pTα108 [6].

To characterize the G-protein β-subunit mRNA in the brain, we screened a cDNA library derived from bovine cerebral cortex poly(A)$^+$ RNA, using a transducin β-subunit cDNA probe. Two hybridizable clones (pGβ2 and pGβ4) were isolated from about 1.2×10^5 transformants. Restriction mapping with 7 endonucleases (*Bst*NI, *Hin*fI, *Nci*I, *Rsa*I, *Sau*3AI, *Sau*96I and *Taq*I) indicated that the 2 clones were indistinguishable from clone pTβ6, except in the 5′-noncoding region. DNA sequencing revealed that at least the sequence of nucleotide residues −46 to 264 of clone pTβ6 was shared by clone pGβ4, but that the sequence upstream of residue −46 was completely different between clones pTβ6 and pGβ4. This agrees with the size of the DNA fragment (316 nucleotides) protected by brain poly(A)$^+$ RNA upon S_1 nuclease mapping (see above). It is to be noted that at least 4 of the 5 remaining clones isolated from the retinal cDNA library apparently carried a transducin-type 5′-noncoding sequence because they were hybridizable with the probe comprising

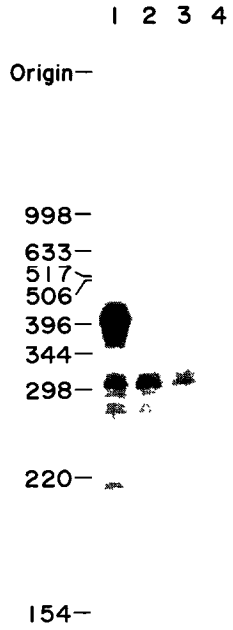

Fig.4. S_1 nuclease mapping of the 5′-terminal region of the mRNAs coding for the β-subunits of transducin and brain and liver G-proteins. For preparing the probe, the 1367-base-pair *Ava*II fragment excised from clone pTβ6 was labelled with ^{32}P at the 5′-ends and cleaved with *Hin*dIII. The resulting 703-base-pair fragment, extending from the *Hin*dIII site on the vector [15] to the *Ava*II(268) site, was used as probe. Poly(A)$^+$ RNA from bovine retina (5 μg), cerebral cortex (10 μg) or liver (20 μg) was hybridized at 50°C to the probe (20 fmol, 50000 cpm). The products of S_1 nuclease digestion were analysed by electrophoresis on an 8 M urea/6% polyacrylamide gel: lanes: 1, retinal RNA; 2, cerebral cortex RNA; 3, liver RNA; 4, control (20 μg of yeast tRNA as carrier). The size markers were the 5′-end-labelled *Eco*RI and *Hin*fI double cleavage products of pBR322 DNA, the sizes of which are given in nucleotides.

the upstream portion of the 5′-noncoding sequence of clone pTβ6 (see fig.3C).

Thus, our results suggest that the mRNAs coding for the β-subunits of transducin and other G-proteins have the same protein-coding sequence but partly different 5′-noncoding sequences. This is consistent with the previous finding that the β-subunits of all the G-proteins examined are virtually identical in amino acid composition and proteolytic pattern [2,3]. The difference in 5′-noncoding sequence may result from transcriptional initiation at different promoters and/or alternative RNA splicing [30,31].

ACKNOWLEDGEMENTS

We thank Dr Paul Berg and Dr Hiroto Okayama for providing their high-efficiency cloning system. This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation and the Japanese Foundation of Metabolism and Diseases.

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