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Characterization of the cDNA and Genomic Sequence of a G Protein γ Subunit (γ_5)

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A cDNA from human placenta and liver tissues that contained both sequence for the lysosomal glycosidase di-*N*-acetylchitobiase and sequence homologous to the γ subunit of GTP-binding proteins was previously isolated. Here we have shown that the γ-subunit-homologous portion of this unusual cDNA is derived from a member of the γ-subunit multigene family. The partial human γ-subunit sequence was used to isolate the corresponding full-length cDNA clones from bovine and rat livers. The two cDNAs encode identical 68-amino-acid proteins (7.3 kDa) homologous to previously cloned G protein γ subunits. The bovine gene sequence encoding this new γ-subunit isoform (γ_5) was determined and found to have an intron-exon structure consistent with the original human chitobiase–γ_5-subunit hybrid mRNA being a product of alternative splicing. Genomic cloning also resulted in the isolation of a human γ_5 pseudogene.

Heterotrimeric G proteins are important regulators of receptor-mediated signal transduction, linking extracellular signals to cellular responses (5, 7, 16). These proteins are composed of tightly associated β and γ subunits with a GDP-bound α subunit. Formation of a ligand-receptor complex stimulates the catalytic exchange of GTP for GDP, thus activating the α subunit and causing its dissociation from the G protein proper. In response to the freed α subunit and $\beta\gamma$ heterodimer, a cascade of events that ultimately results in a cellular function occurs. Molecular cloning, biochemical, and immunological studies indicate that the α, β, and γ subunits of heterotrimeric G proteins are derived from large multigene families (21). It has been proposed that additional diversity in G protein structure and function may be obtained by bringing together different combinations of the subunit isoforms expressed by each family (6). Within the γ-subunit family, three complete cDNA clones representing three different isoforms have been isolated: one from the bovine retina (γ_1) (8), the second from the bovine brain and adrenal gland (γ_2) (17), and the third from the bovine brain (γ_3) (6). A partial cDNA sequence of a fourth isoform (γ_4) from mouse kidney and retina tissues has also been reported (6).

During our studies on the molecular genetics of a species-specific lysosomal glycosidase involved in Asn-linked glycoprotein degradation, di-*N*-acetylchitobiase (chitobiase), a chitobiase-related cDNA was cloned from both human placenta and liver λgt11 libraries (4). This cDNA specifies the first 319 of 385 amino acids for human chitobiase and a 41-amino-acid carboxyl terminus with 50% identity to the γ subunit of a heterotrimeric G protein. Using the γ-subunit-homologous sequence as a hybridization probe, we have isolated full-length γ-subunit cDNAs from bovine and rat livers and a bovine γ-subunit genomic clone that encode a previously undescribed member of the γ-subunit multigene family, γ_5. Preliminary evidence based on the bovine γ_5 gene intron-exon structure suggests that the chitobiase-related cDNA results from alternative splicing of a pre-mRNA that contains exons from both the chitobiase and the γ_5 genes. The novel occurrence of sequence for two different cellular proteins in a single mRNA further suggests that the structural genes for chitobiase and the G protein γ_5 subunit form a complex genetic locus.

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MATERIALS AND METHODS

Library screening. The protocol for cloning rat and human chitobiase and human chitobiase-related cDNAs has been described elsewhere (4). A 200-bp *Pvu*II-*Hin*dIII restriction fragment specific for the γ-subunit-homologous region of a human placenta chitobiase-related cDNA, HPCB10a (4), was gel purified and random primed labeled with biotin-11-dUTP (Sigma). λgt11 cDNA libraries from bovine liver (Clontech) and rat liver (C.-P. David Tu, Pennsylvania State University) were screened with the γ-subunit-homologous probe as described previously (4). One of the cDNAs that was isolated from the bovine library (BLGTPγ.6) was random primed and labeled with [5′-^{32}P]dCTP and used to screen a λgt11 library from human placenta tissue (Clontech). The bovine cDNA was also used to screen a bovine liver EMBL-4 genomic library (Katherine L. Knight, Loyola University of Chicago) and a human placenta FIX II genomic library (Stratagene). Nylon membrane plaque lifts were prehybridized in a solution containing 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, 1.0% sodium dodecyl sulfate (SDS), and 0.05 mg of sheared herring sperm DNA per ml at 65°C for 1 h and hybridized in a solution containing 5× SSC, 1× Denhardt's, 1% SDS, 0.05 mg of sheared herring sperm DNA per ml, and 2 ng of labeled probe (10^6 cpm) per ml at 65°C for 12 to 16 h. Posthybridization washes were performed in the following order: 2× SSC–0.1% SDS twice for 15 min at 23°C, 0.2× SSC–0.1% SDS twice for 15 min at 23°C, and 0.2× SSC–0.1% SDS for 15 min at 65 or 50°C, depending on the desired stringency.

Northern (RNA) blot analysis. The same 200-bp γ-subunit-homologous sequence from HPCB10a that was used for library screening was random primed labeled with [5′-^{32}P]dCTP (Du Pont-New England Nuclear) to a specific activity of approximately 10^9 cpm/μg. Total RNA was isolated from tissue by the acid guanidinium thiocyanate-

phenol-chloroform extraction method (3). Poly(A)$^+$ selected RNA was prepared by oligo(dT) chromatography (12). RNA samples (1 to 2 μg) were heated to 65°C for 5 min, quick cooled in an ice bath, resolved on a 1.2% agarose–2.2% formaldehyde gel, and electroblotted from the gel onto a nylon membrane. Blots were hybridized in a solution containing 5× SSC, 1× Denhardt's solution, 1.0% SDS, 10% polyethylene glycol, 50 μg of sheared herring sperm DNA per ml, and 2 ng (10^6 cpm) of labeled probe per ml at 60°C for 16 h. Posthybridization washes were conducted in the following order: 2× SSC–0.1% SDS twice for 15 min at 23°C, 0.2× SSC–0.1% SDS twice for 15 min at 23°C, and 0.2× SSC–0.1% SDS for 15 min at 60°C.

Subcloning, nucleotide sequence analysis, and Southern blot mapping. The bacteriophage DNA from positive clones was purified from plate lysates, endonuclease digested, and resolved on a 1.0% agarose gel. Cloned sequences were purified by electroelution into dialysis tubing and ligated into pUC18, M13mp18, or M13mp19. Nucleotide sequence analysis was done by the dideoxynucleotide chain termination method with the Sequenase Version 2.0 DNA sequencing kit (U.S. Biochemicals). Sequencing reaction mixtures were labeled with ([5′-α-^{35}S]thio)-dATP (Du Pont-New England Nuclear) and separated on a wedge 6% polyacrylamide–7.5 M urea gel. Southern blot mapping of genomic clones was performed as previously described (12).

RESULTS

Isolation of a chitobiase-related cDNA with sequence homologous to a G protein γ subunit. Twelve cDNA clones were isolated from a human placenta λgt11 library when it was screened with a rat cDNA encoding the lysosomal glycosidase di-*N*-acetylchitobiase (chitobiase) (4). The sequence from four of the cDNAs was found to encode human chitobiase on the basis of its homology to rat chitobiase and on the basis of expression studies in COS-1 cells (4). DNA sequencing and restriction analysis of the remaining eight clones, however, revealed a sequence that was identical to that of human chitobiase through bp 958 of the open reading frame but that terminated with a 3′ end different from that of chitobiase. The sequence of a representative of the eight cDNAs, HPCB10a (1.3 kb), is shown in Fig. 1, partially aligned to the sequence from a full-length human chitobiase cDNA, HPCB5b (4). The open reading frame of the divergent 3′ end from HPCB10a continues for 41 amino acids, thereby replacing the carboxyl-terminal 67 amino acids of normal chitobiase. Expression of HPCB10a in COS-1 cells did not produce an increase in chitobiase activity (data not shown), making it appropriate to refer to this sequence as chitobiase related. A protein data base search with the 41-amino-acid carboxyl terminus of the chitobiase-related sequence revealed 56% sequence identity to the γ subunit of a heterotrimeric GTP-binding protein from bovine brain and adrenal gland tissues (Fig. 1) (17).

To determine the occurrence of the chitobiase-related cDNA in a tissue other than placenta, a human liver λgt11 library was screened with HPCB10a. A total of six cDNAs were isolated from the liver library, five of which encoded normal chitobiase. The sixth liver cDNA, HLCB2a, encoded the same chitobiase-related sequence originally discovered in placenta tissue. While this established the presence of the chitobiase-related sequence in two different tissues, the ratio of chitobiase-related cDNA to chitobiase cDNA in the liver library was much lower than that for the placenta library (1:5 for liver versus 8:4 for placenta).

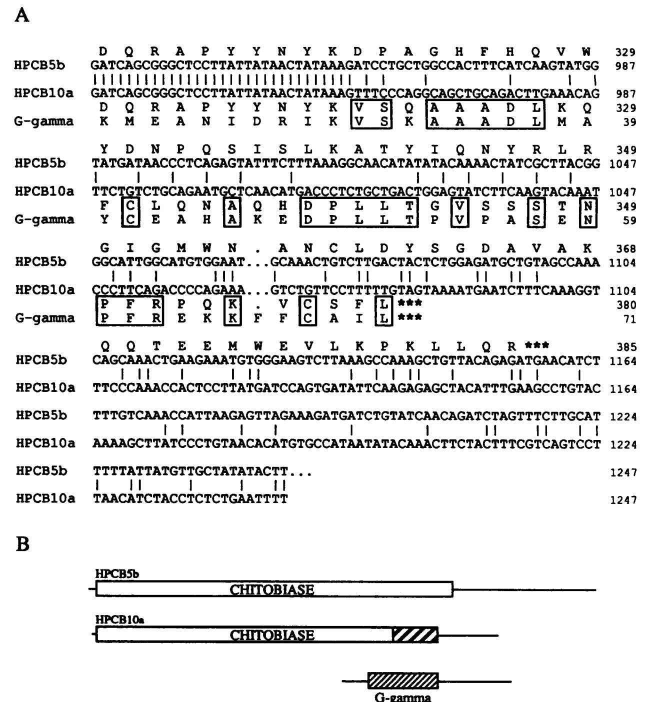

FIG. 1. Divergent 3′ end from a human placenta chitobiase-related cDNA and its homology to a G protein γ subunit. (A) The nucleotide and deduced amino acid sequences from the 3′ end of a human placenta chitobiase cDNA, HPCB5b (4), and a chitobiase-related cDNA, HPCB10a (see text) are aligned, with nucleotide matches indicated by vertical lines. The two sequences are identical through bp +958 of the chitobiase open reading frame (where +1 is A of the ATG initiation codon for chitobiase). A portion of the deduced amino acid sequence from a bovine retina and adrenal gland G protein γ subunit (17) cDNA is aligned with that from HPCB10a, with matches boxed. A single codon gap has been introduced in HPCB5b and HPCB10a to maintain alignment with the γ-subunit sequence. Translational stop codons are indicated by asterisks. (B) Graphical alignment of the human and bovine cDNA sequences of panel A. Boxed regions represent the protein coding sequences, while untranslated sequences are indicated by solid lines. Open boxes indicate chitobiase sequence; the box hatched with thin lines represents the bovine G protein γ-subunit sequence (17); and the box hatched with thick lines indicates the divergent 3′ end of HPCB10a that shows homology to the bovine G protein γ-subunit sequence.

cDNA cloning of bovine and rat liver γ_5 subunits. A Northern blot containing mRNA from human placenta, rat liver, and bovine liver tissues was probed with the γ-subunit-homologous sequence from the human placenta chitobiase-related cDNA HPCB10a. The probe was expected to recognize at least one of three chitobiase mRNAs (1.5, 1.8, and 3.7 kb) that have been detected in human placenta tissue (4). Surprisingly, a 550-bp message was observed in all three species, with the quantities greatest in bovine liver, intermediate in rat liver, and low in human placenta (Fig. 2). Although the hybridization conditions for the blot in Fig. 2 were not sufficient to detect the parent mRNA responsible for the chitobiase-related cDNA in human placenta, we have since shown that the γ-subunit-homologous probe will hybridize to the 1.5-kb chitobiase message (data not shown).

The γ-subunit-homologous probe was next used to screen bovine and rat liver λgt11 cDNA libraries. Six positive clones were isolated from the bovine library, and a single

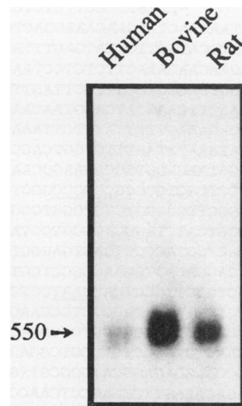

FIG. 2. Northern blot analysis with the γ-subunit-homologous 3′ end from a human chitobiase-related cDNA. The probe was a ^{32}P-labeled 200-bp *Pvu*II-*Hin*dIII fragment specific for the unique 3′ end of HPCB10a (bp 968 to 1167, Fig. 1A). Poly(A) selected mRNAs (0.5 μg) from human placenta, bovine liver, and rat liver tissues were resolved on a 1.2% agarose–2.2% formaldehyde gel, blotted onto a nylon membrane, and hybridized. The 550-bp message that was detected is indicated.

clone was isolated from the rat library. Restriction analysis of the purified phage DNA revealed that all six bovine cDNAs were approximately 550 bp in length, while the rat cDNA was approximately 1.3 kb. Figure 3 shows a complete alignment between one of the bovine cDNAs (BLGTPγ.6), the rat cDNA (RLGTPγ.1), and the open reading frame from the γ-subunit-homologous portion of the human chitobiase-related cDNA. The bovine and rat liver cDNAs were found to encode identical 68-amino-acid proteins. The corresponding 41-amino-acid sequence deduced from the human chitobiase-related cDNA sequence also matched precisely. A sequence conforming to the CC(A/G)CC consensus sequence for translational initiation (10) is present immediately 5′ to the proposed start codon ATG in both the bovine and the rat cDNAs. Polyadenylation signals appear 15 bp upstream of the poly(A) tail in the bovine cDNA and at bp 432, 1260, and 1342 in the rat cDNA. The predominance of the 550-bp message in rat liver determined by Northern blotting (Fig. 2) suggests that the polyadenylation site at bp 432 is the most frequently utilized. The homology between the bovine and rat cDNAs extends into the 3′ untranslated region, where 70% of the nucleotides are identical. This same degree of homology also applies to the 3′ untranslated sequence from the human chitobiase-related cDNA when it is compared with either bovine or rat cDNA (data not shown).

Alignment of the deduced protein sequence from BLGTPγ.6 and RLGTPγ.1 with those of G protein γ subunits that have been cloned strongly suggests that the cDNA from bovine and rat tissues is a new isoform of the γ subunit, which we have tentatively designated γ_5 (Fig. 4). The degree of sequence identity between γ_5 and other γ subunits ranges from 25% (γ_1 from bovine retina tissue) to 49% (γ_2 from bovine brain and adrenal gland tissues). As with the other members of the γ-subunit family, γ_5 maintains a cysteine residue four positions from the carboxyl terminus. This important amino acid has been shown to be a target for the posttranslational addition of polyisoprenoid groups, which are thought to be needed for membrane anchoring (11, 14, 18). Since the γ-subunit-homologous sequence from the human chitobiase-related cDNA identically encodes 41 of 68

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Bovine                                                ccgccgactggcc    -61

Bovine   cagcggacccatcgcagacctgccgacggcccgcggatctgccatcctttcgagtgcgcc     -1
                                              |  |||   || ||  | |||||
Rat                                         agttggcccggctgtcccgagcgcc     -1

          M  S  G  S  S  S  V  A  A  M  K  K  V  V  Q  Q  L  R  L  E      20
Bovine   ATGTCTGGTTCATCCAGCGTCGCGGCTATGAAAAAAGTGGTTCAACAGCTCCGGCTGGAG     60
         ||||| ||||| || |||||||| || ||||| || |||||||| || ||||||||||||
Rat      ATGTCGGGTTCTTCTAGCGTCGCCGCCATGAAGAAGGTGGTTCAGCAACTCCGGCTGGAG     60

          A  G  L  N  R  V  K  V  S  Q  A  A  A  D  L  K  Q  F  C  L      40
Bovine   GCCGGGCTCAATCGGGTGAAGGTTTCCCAGGCAGCTGCAGATTTGAAACAGTTCTGTCTG    120
         ||||||||||| || |||||||||||||||||||||||||| ||||||||||||||||||
Rat      GCCGGGCTCAACCGCGTGAAGGTTTCCCAGGCAGCTGCAGACTTGAAACAGTTCTGTCTG    120
                              |||||||||||||||||||| ||||||||||||||||||
Human                         GTTTCCCAGGCAGCTGCAGACTTGAAACAGTTCTGTCTG     39

          Q  N  A  Q  H  D  P  L  L  T  G  V  S  S  S  T  N  P  F  R      60
Bovine   CAGAATGCTCAACATGACCCTCTGCTGACTGGAGTATCTTCAAGTACAAATCCCTTCAGG    180
         ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||| 
Rat      CAGAATGCTCAACATGACCCTCTGCTGACTGGAGTGTCTTCAAGTACAAATCCCTTCAGA    180
         ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||| 
Human    CAGAATGCTCAACATGACCCTCTGCTGACTGGAGTGTCTTCAAGTACAAATCCCTTCAGA     99

          P  Q  K  V  C  S  F  L ***                                      68
Bovine   CCCCAGAAAGTCTGTTCCTTTTTGTAGtaagacgaatcttgacaaggttttccaaaccac    240
         |||||||||||||| ||||||||||||| | |       |  | ||||||  ||||| ||
Rat      CCCCAGAAAGTCTGCTCCTTTTTGTAGtcata.....tatctcgaggtttctcaaactac    235
         |||||||||||||| ||||||||||||
Human    CCCCAGAAAGTCTGTTCCTTTTTGTAG...

Bovine   gtttcataaaccagtgaatattcaaaggaa.............agctaaatttgaagcct    287
          |||||| ||||||||||||||||   |||               |||| ||||||| ||
Rat      ttttcatgaaccagtgaatattcacgagaactaagttcgaagtcactaagtttgaagtct    295

Bovine   gtacaaaagcctctctgtaacgtgccatactatacaaacttctacttttgtcagtcctta    347
         ||||| |||| ||||| ||||  |    | || ||| | |  |    | |||||||||||
Rat      gtacagaagcttctctttaacacgtgcca.tacacataatcttctactcgtcagtcctta    354

Bovine   atgtctacctctctgaatattcataattttctgtttcacaagg.gtaattattttatata    406
         |  |||||||||||| || |||||   | ||||||||||||||  ||| | |||||||||
Rat      acatctacctctctggattttcatggatctctgtttcacaaggtttaactgttttatata    414

Bovine   cactgatggcaacatacaataaaatacttagtaaaaaaaaaaaaaaa                 438
         |||||   | | |||||||||||  |      |||
Rat      cactggctgtagcatacaataaagcagcatacaaactttggccttgttattgatatgaa    474

Rat      atgtgctgtatactaatttttcaacatcaggactcactgccttattggcaaggcttcta    534
         ggaatttacagaacaactgcaaatctttgttcaaaggccggaagacttaagagtttctaa    594
         tccttcagtcagttatgggaattatcttaaatatcccaaatataggtagggagatggctc    654
         ggtggctaagagcacttgctctgcagttagttatgctgagttcagatcctgccacccatg    714
         taaaaagctgggcgtggctgtacatgcccgtggccacagcttcggggagatggtttgttg    774
         gctgccagcgagggtaaggttgtaattagctccgtgagaacgaggcagaaagggatacag    834
         gtgcctgacactgccatgtgggctcacacaggcaacaaacaactctagtggcgtcagcag    894
         ttagtgctaccaagaaggtggctgcttccatctggaaaaagagttaaagattcacagaat    954
         caagaccttgaggacttacgacaatgcctcaagtaggcaagtggaggtaattaggtagaa   1014
         aggaacaagaaaacaggttaacctctgtgacctgtaactttgctccaagtcccaataacc   1074
         tgtcctttagaactggtatattaaatcagggtcatacactatctaccaacaagcctttt    1134
         tctagcctacaagttctttgggaatgaaaattataaagtttgaatcgtcattcctaagaa   1194
         attattacaactaatccaaaatgacaacagcttttatgactttcatacataatttttcag   1254
         acaaaaataaaattatatttatttatatttactatatgcagtggaaactcatagcacttg   1314
         gtcatttcttcaaacacaggatttataaaataaaatcccatttgaaaagtaa           1367
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FIG. 3. Nucleotide and deduced amino acid sequences of bovine and rat liver cDNA clones isolated with the γ-subunit-homologous sequence from a human chitobiase-related cDNA. The bovine sequence is from clone BLGTPγ.6, and the rat sequence is from RLGTPγ.1. The sequences have been aligned, with nucleotide matches indicated by vertical lines. The open reading frame from the γ-subunit-homologous 3′ end of a human chitobiase-related cDNA (4) is also included in the alignment. In the region where all three species have been aligned, conserved nucleotides are indicated by vertical lines. Gaps have been introduced in the 3′ untranslated region of the bovine and rat sequences to optimize the alignment. Potential polyadenylation signals (aataaa) have been underlined. The translational stop codon is indicated by asterisks.

amino acids of the bovine and rat γ_5 subunit, we propose that the human chitobiase-related cDNA is a chitobiase–γ_5-subunit hybrid.

Characterization of the bovine γ_5 gene and a human γ_5 retropseudogene. Having established the existence of a γ_5 cDNA sequence in bovine and rat liver tissues, we were interested in the structure of the γ_5 gene and whether it would provide any clues as to how the chitobiase-γ_5 cDNA

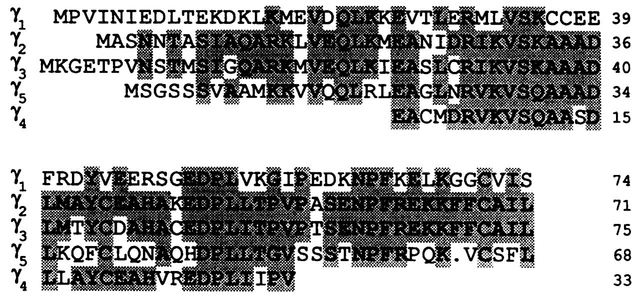

FIG. 4. Alignment of G protein γ-subunit isoforms. The amino acid sequences deduced from previously cloned γ-subunit cDNAs have been aligned with the sequences from the bovine and rat liver cDNAs BLGTPγ.6 and RLGTPγ.1. Designations: γ_1, isoform from bovine retina tissue (8); γ_2, isoform from bovine brain and adrenal gland tissues (17); γ_3, a second isoform from bovine brain tissue (6); γ_4, partial sequence from an isoform that was amplified by polymerase chain reaction from mouse kidney and retina tissues (6); and γ_5, bovine and rat liver sequences from BLGTPγ.1 and RLGTPγ.1. Exact matches that occur in two or more isoforms are shaded. Gaps have been introduced to obtain optimal alignment.

sequence from human placenta and liver tissues may have arisen. Bovine and human genomic libraries were screened with the bovine γ_5 cDNA BLGTPγ.6, and several positive clones were isolated. Southern mapping of the bacteriophage DNA from two bovine genomic clones revealed that each contained three *Eco*RI fragments (4.5, 4.0, and 2.2 kb) that hybridized to the cDNA probe. The three *Eco*RI fragments from clone BGGTPγ.2a were sequenced and found to encode the entire bovine γ_5 gene. The gene spans approximately 7 kb and is divided into three exons, with the open reading frame distributed between the first two while the third comprises only the 3′ untranslated sequence (Fig. 5). Interestingly, the 5′ end of exon 2 was found to correspond exactly to the 5′ end of the γ_5-subunit sequence from the human chitobiase-γ_5 hybrid cDNA. All intron-exon boundaries of the bovine γ_5 gene conform to the GT-AG rule for intron splice sites.

The 5′ transcriptional regulatory domain of the bovine γ_5 gene contains elements characteristic of housekeeping genes (13, 19), including three GC boxes located at bp −166, −528, and −616 (where +1 is A of the initiation codon ATG). Two CAAT boxes are present at positions −304 and −859, but there is no TATA box. Although the transcriptional start site was not experimentally determined, we predict that it corresponds approximately to bp −73 (i.e., the 5′ end of BLGTPγ.6), since all six bovine cDNAs that were isolated initiate near this site. Within the immediate vicinity of this putative start site are several blocks of sequence that are nearly exact matches to the weak consensus PyPyCAPyPyPyPy (where Py is C or T and A is where transcription starts) that has been proposed to be a recognition determinant for transcriptional initiation (22).

Southern blot analysis of two human genomic clones that were isolated revealed that both contained a single 2.3-kb *Eco*RI fragment that hybridized to the bovine γ_5 cDNA probe. This fragment was sequenced and was discovered to contain a processed retropseudogene that appears to be derived from human γ_5 mRNA. In Fig. 6, the human pseudogene H$\psi\gamma_5$ has been aligned with the bovine cDNA BLGTPγ.6 instead of with the partial human placenta γ_5 sequence in order to obtain a complete comparison. Through the open reading frame, the bovine γ_5 and human H$\psi\gamma_5$

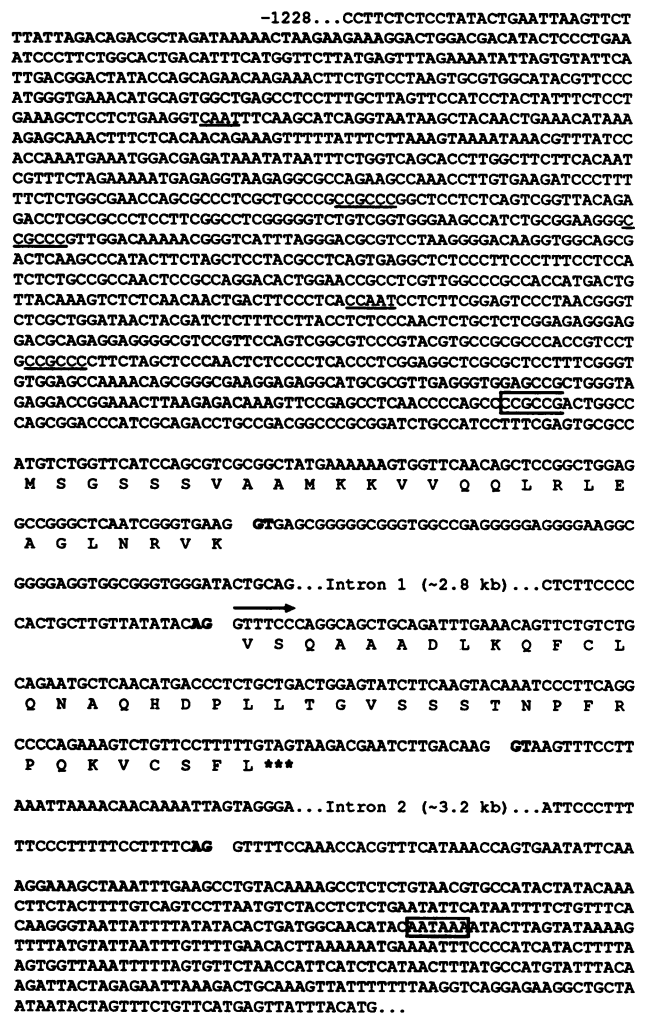

FIG. 5. Nucleotide and deduced amino acid sequences of the bovine γ_5 structural gene. The translated amino acid sequence (in single-letter designations) is given below the nucleotide sequence. Potential regulatory elements in the 5′-flanking region are underlined. The sequence enclosed in an open-ended box indicates the 5′ end of the bovine cDNA BLGTPγ.6 and corresponds to the vicinity where RNA transcription is proposed to initiate. The GT-AG intron splice sites are in bold print and separated from the exon sequence by a space. The translational stop codon is indicated by asterisks. The boxed sequence in the 3′-flanking region is a putative polyadenylation site (AATAAA). The 5′ end of exon 2 overscored with a horizontal arrow denotes the beginning of the γ_5 sequence in the human chitobiase-γ_5 hybrid cDNA (Fig. 1).

nucleotide sequences are 86% identical, compared with 78% for the complete alignment. The identity between the partial human γ_5 cDNA sequence and H$\psi\gamma_5$ is 90% (data not shown). In addition to several nucleotide substitutions that result in amino acid changes, deletions of 1 and 2 bp have occurred in the open reading frame of H$\psi\gamma_5$, which further alters the predicted protein sequence as well as introducing a premature translational stop codon (Fig. 6). Among the amino acid changes that have occurred because of nucleotide substitutions is the loss of the carboxyl-terminal cysteine residue believed to be involved in polyisoprenylation. Although a few processed retropseudogenes have been shown to remain functional (23), the mutations that have

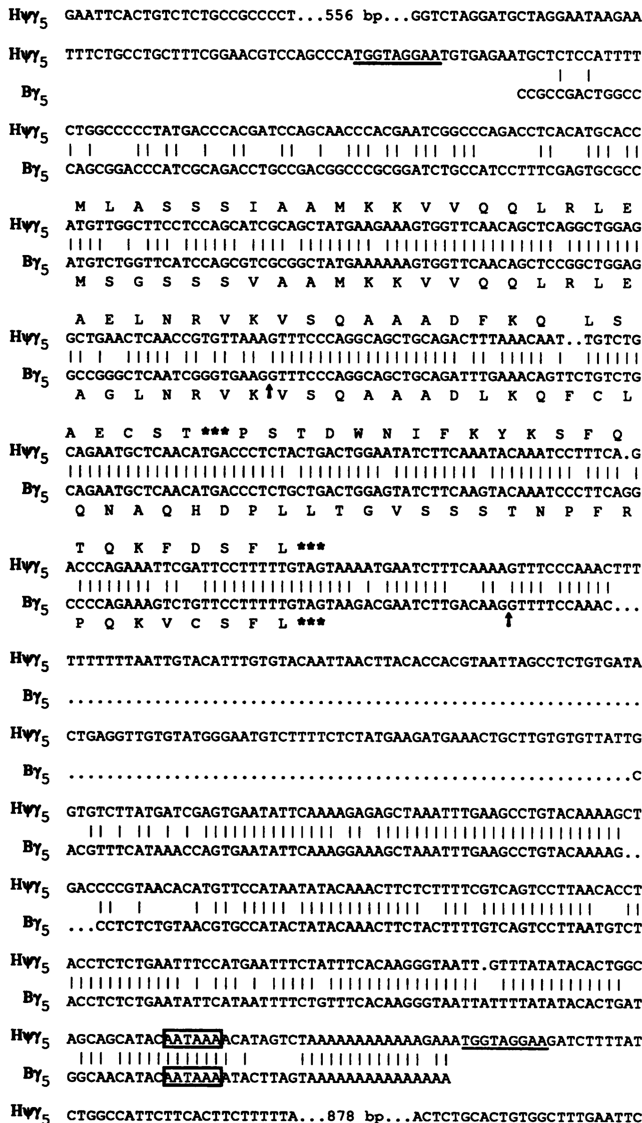

FIG. 6. Nucleotide and deduced amino acid sequences of a human γ_5 retropseudogene. The sequence of a 2.3-kb *EcoRI* fragment from a human genomic clone is shown aligned with the bovine γ_5 cDNA sequence from BLGTPγ.6. Nucleotide matches are indicated by vertical lines. The translated amino acid sequence is given in single-letter designations. Gaps have been introduced in the human sequence to maintain alignment with the bovine sequence. Vertical arrows indicate introns in the bovine γ_5 gene (Fig. 5). Stop codons are labeled by asterisks. The underlined sequence is a direct repeat proposed to be a consequence of retrotransposition.

occurred in H$\psi\gamma_5$ would argue against it remaining functional.

Features of the H$\psi\gamma_5$ sequence that are characteristic of processed retropseudogenes include a poly(A) tail with its associated polyadenylation signal and the absence of introns (Fig. 6) (for a review, see reference 25). Immediately following the poly(A) tail, there is a 9-bp sequence, TGGTAG GAA, that is also present as a direct repeat at a site that nearly corresponds to the 5′ end of the bovine cDNA BLGTPγ.6. Short direct repeats similar to this are thought to be a consequence of the retrotransposition mechanism. Because these repeats often flank the 5′ cap site and poly(A) tail of the processed mRNA, the location of the 5′ repeat in H$\psi\gamma_5$ is consistent with our prediction that the transcriptional start site in the bovine γ_5 gene (and likely in the human gene as well) approximates the 5′ end of BLGTPγ.6.

DISCUSSION

The cDNA clones from bovine (BLGTPγ.6) and rat (RLGTPγ.1) liver tissues that were characterized in this study display significant homology to the different G protein γ subunits that have been previously described (Fig. 4) (6, 8, 17). This suggests that the bovine and rat cDNAs encode a new isoform, which we have designated γ_5. An attempt to clone from human placenta the remainder of the human γ_5-subunit sequence originally cloned as a hybrid chitobiase–γ_5-subunit cDNA was also made. However, no full-length γ_5 cDNAs, only chitobiase–γ_5-subunit hybrids, were detected. Although the Northern blot in Fig. 2 shows a 550-bp message in human placenta cDNA homologous to the human γ_5-subunit probe, the signal is reduced relative to that in bovine and rat liver tissues. This may indicate a low copy number of the γ_5-subunit mRNA in placenta or a cross-hybridization with homologous γ-subunit isoforms. In light of the tissue-specific pattern of expression displayed by most G protein γ subunits (6), γ_5 may be liver specific and not expressed in the placenta.

A striking feature of the γ_5 protein coding sequence is its precise conservation among bovine, rat, and human (Fig. 3). This cross-species identity has also been demonstrated for the γ_2 and γ_3 subunits (6) and may reflect the different structure-function relationships of the various isoforms. The γ_5 subunit is both the smallest of the isoforms that have been cloned and the only one which possesses a carboxyl-terminal sequence that deviates from the Cys-A-A-X (where A is an aliphatic residue and X is unspecified) motif believed to be the recognition determinant for posttranslational addition of polyisoprene groups. Instead of aliphatic residues occupying the −2 and −3 positions relative to the carboxyl terminus, γ_5 contains an aromatic amino acid (Phe) and a hydroxy amino acid (Ser), respectively (Fig. 4). Another class of GTP-binding protein that has nonaliphatic amino acids at the −2 and −3 positions is the low-molecular-mass G proteins of the *rac* and *ral* families (9). These proteins have been shown, however, to successfully undergo polyisoprenylation. The Cys-Ser-Phe-Leu motif in the γ_5 subunit therefore fits the proposal of Kinsella et al. (9) that proteins containing the carboxyl-terminal sequence Cys-X-X-X (where X is unspecified) may be targeted for polyisoprenoid modification as well.

The structure of the bovine γ_5 gene provides strong evidence that the chitobiase-γ_5 hybrid cDNA from human placenta and liver tissues is a product of alternative splicing. Comparing the two sequences reveals that exon 2 matches precisely to the site that defines the start of the human γ_5 sequence within the chitobiase-γ_5 hybrid cDNA (Fig. 1 and 4). In addition to exon 2, sequence homologous to exon 3 is present in the γ_5 portion of the hybrid cDNA. Assuming γ_5 gene structure conservation between bovine and human genes, exons 2 and 3 from the human γ_5 gene therefore appear to have been spliced together with exons 1 through N (where the exact linear position of exon N is unknown because the structure of the chitobiase gene has not been determined) from the chitobiase gene. Excluding complex mechanisms such as *trans* splicing (15, 24), the most likely explanation to account for the formation of a chitobiase-γ_5 mRNA is that the two sequences originate from a common

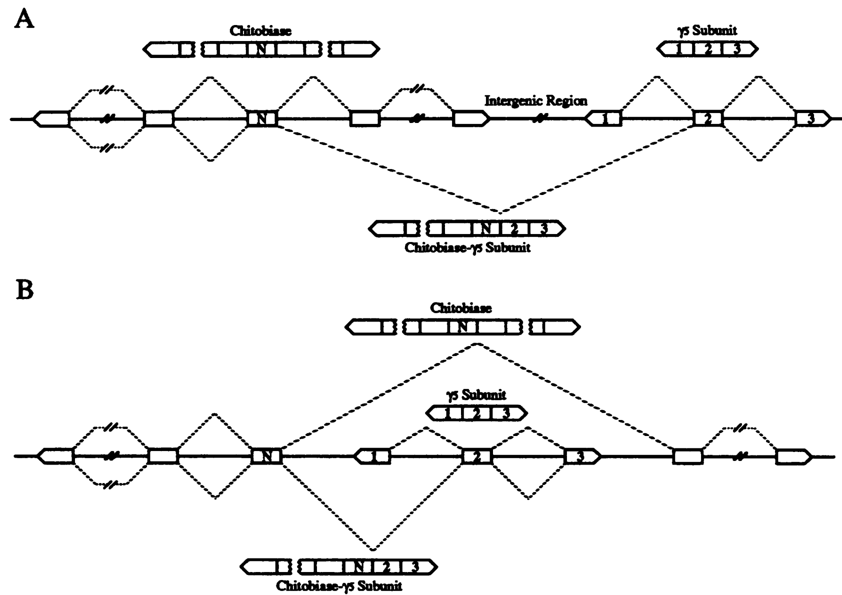

FIG. 7. Two mechanisms for generation of a chitobiase-γ_5 hybrid mRNA. Chitobiase exons are represented by open boxes, while the γ_5-subunit exons (Fig. 5) are labeled 1 through 3. Chitobiase exon N encodes the 3′ end of the chitobiase sequence in the chitobiase-γ_5 hybrid cDNA (Fig. 1). The actual number of chitobiase exons has not been determined, as indicated by the broken exons. Dashed lines represent splicing patterns. (A) Chitobiase and γ_5 genes separated by an intergenic region sufficient to allow RNA polymerase II read-through are shown. (B) The γ_5 gene is placed within an intron of the chitobiase gene. In both scenarios, the splicing products include chitobiase, the γ_5 subunit, and a chitobiase–γ_5-subunit hybrid.

primary RNA transcript and are joined during RNA processing. Consistent with an alternative splicing mechanism is the existence of at least three different chitobiase mRNAs (1.5, 1.8, and 3.7 kb) in human placenta tissue (4). The sequence of the bovine γ_5 gene presented here (Fig. 5) is the first report of a genomic sequence from the γ-subunit multigene family.

For the chitobiase and γ_5 sequences to have been combined into a single RNA transcript suggests that the two genes may form a complex genetic locus. Such an association has not yet been determined; however, there are at least two possible genomic organizations. The first model envisions the two genes juxtaposed such that the distance between them is small enough to allow RNA polymerase read-through from the chitobiase gene into the γ_5 gene (Fig. 7A). Eukaryotic RNA polymerase II is known to extend from approximately 0.5 to 2.0 kb beyond the polyadenylation site (2). If the polymerase were to establish contact with the γ_5 gene during this extension, it might continue through until the transcriptional termination signal in the γ_5 gene. In this regard, it is interesting that the 3′ untranslated sequences from human and rat chitobiase display a high level of sequence identity (4). While this may reflect the presence of a sequence(s) that is important for aspects of the mRNA, such as stability (20), it may also indicate a functional importance of the putative intergenic region that links the chitobiase and γ_5 genes. Our second model places the entire γ_5 gene within the intron that corresponds to the chitobiase splice site in the human hybrid cDNA (Fig. 7B). Considering the small size of the γ_5 gene (7 kb), such an overlap is feasible. This scenario has an advantage over the first mechanism in that it would not require RNA polymerase read-through from the chitobiase to the γ_5 gene, since γ_5 would lie within the chitobiase transcription unit. Southern blot analysis of the bovine γ_5 gene has revealed no evidence of chitobiase-homologous sequence positioned within either of the two γ_5 introns, indicating that exons from the two genes are unlikely to be interspersed. Currently we are trying to characterize these and any other possible structural organizations for the bovine, rat, and human chitobiase and γ_5 genes.

An intriguing hypothesis that has emerged from this study addresses the possibility that the unique organization of the chitobiase and γ_5 genes may be related to the deficient levels of chitobiase expression in ungulates and carnivores (1). In other studies, we have shown that species that represent these two groups, bovines and dogs, maintain the chitobiase gene as part of their genetic load, yet bovines express very reduced levels of chitobiase mRNA in their livers (4). Because the loss of chitobiase expression is traceable along the lines of mammalian radiation (1), an evolutionarily acquired genetic alteration in the ancestor of ungulates and carnivores may have changed the context of the chitobiase-γ_5 locus sufficiently to result in positional interference between the two genes. Although in this report we have presented no evidence that directly supports this view, the occurrence of two unusual events at a single genetic locus (i.e., suppressed chitobiase expression and a complex organization of the chitobiase and γ_5 genes) suggests a possible cause-and-effect relationship that will be important to elucidate.

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