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Identification of a Putative γ-Aminobutyric Acid (GABA) Receptor Subunit rho_2 cDNA and Colocalization of the Genes Encoding rho_2 (GABRR2) and rho_1 (GABRR1) to Human Chromosome 6q14–q21 and Mouse Chromosome 4

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Screening of a genomic DNA library with a portion of the cDNA encoding the γ-aminobutyric acid (GABA) receptor subunit rho_1 identified two distinct clones. DNA sequencing revealed that one clone contained a single exon from the rho_1 gene (GABBR1) while the second clone encompassed an exon with 96% identity to the rho_1 gene. Screening of a human retina cDNA library with oligonucleotides specific for the exon in the second clone identified a 3-kb cDNA with an open reading frame of 1395 bp. The predicted amino acid sequence of this cDNA demonstrates 30 to 38% similarity to α, β, γ, and δ GABA receptor subunits and 74% similarity to the GABA rho_1 subunit suggesting that the newly isolated cDNA encodes a new member of the rho subunit family, tentatively named GABA rho_2. Polymerase chain reaction (PCR) amplification of rho_1 and rho_2 gene sequences from DNA of three somatic cell hybrid panels maps both genes to human chromosome 6, bands q14 to q21. Tight linkage was also demonstrated between restriction fragment length variants (RFLVs) from each rho gene and the Tsha locus on mouse chromosome 4, which is homologous to the CGA locus on human chromosome 6q12–q21. These two lines of evidence confirm that GABRR1 and newly identified GABRR2 map to the same region on human chromosome 6. This close physical association and high degree of sequence similarity raises the possibility that one rho gene arose from the other by duplication. © 1992 Academic Press, Inc.

INTRODUCTION

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the central nervous system. In the brain, pharmacological binding and electrophysiological studies indicate that GABA interacts with two major receptor classes, termed A and B. Binding of GABA to type A receptors activates a chloride conductance that is antagonized by bicuculline and modulated by barbiturates and benzodiazepines. Type B receptors are agonized by baclofen and are insensitive to barbiturates, benzodiazepines, and bicuculline. See Sivilotti and Nistri (1991) for review.

Cloning of the cDNAs encoding type A GABA ($GABA_A$) receptor subunits reveals diverse subunit types that can associate into functional heterooligomeric receptors with subtly varying properties depending on subunit composition. Four major subunit classes of $GABA_A$ receptors have been identified and cDNAs encoding six α, four β, three γ, and one δ subunit have been cloned (Olsen and Tobin, 1990; Bateson *et al.*, 1991; Wilson-Shaw *et al.*, 1991). The predicted amino acid sequences of each $GABA_A$ subunit display regions of significant sequence similarity suggesting that these areas encode functionally important domains (Olsen and Tobin, 1990). Expression of a number of these subunits singly in *Xenopus* oocytes produces GABA-activated chloride conduction. Examination of the genomic organization of four of these subunit genes, β_1, β_3, β_4, and δ, reveals conservation of intron–exon boundaries raising the possibility that these $GABA_A$ genes arose from a common ancestral gene (Kirkness *et al.*, 1991; Lasham *et al.*, 1991).

To determine whether molecular defects in a particular subunit may be associated with human disease, several subunit genes have been mapped to human chromosomes. Genes encoding α_1, α_3, β_3, and δ subunits family have been found dispersed throughout the genome although the α_2 and β_1 genes are both located on chromosome 4 in bands p12–p13 (Bell *et al.*, 1989; Buckle *et al.*,

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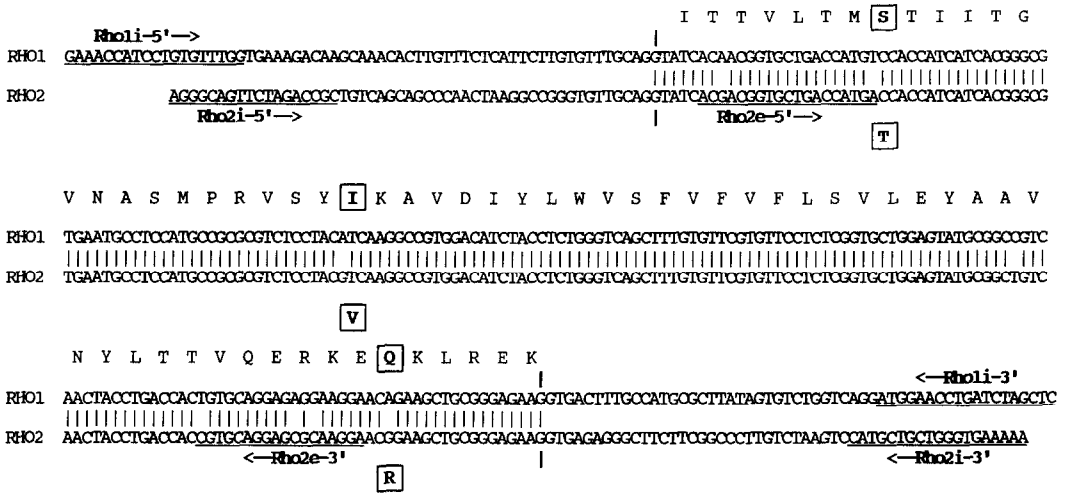

FIG. 1. Alignment of the nucleotide sequences of an exon and flanking introns from the rho_1 and rho_2 genes. Identical nucleotides are shown by a vertical line (|) between the two sequences while vertical lines above and below the nucleotide sequences indicate the 5′ and 3′ extent of each exon. Predicted amino acid sequence of the rho_1 exon is shown above the nucleotides using the single-letter code. Residues that differ between rho_1 and rho_2 are boxed. The location of oligonucleotides used for PCR amplification and library screening (see text) are underlined.

1989; Sommer *et al.*, 1990; Dean *et al.*, 1991; Kirkness *et al.*, 1991; Wagstaff *et al.*, 1991).

A related member of the GABA receptor subunit family rho_1 displaying high levels of expression in the retina has recently been identified (Cutting *et al.*, 1991). Expression of this subunit in *Xenopus* oocytes demonstrates a pharmacologic profile distinct from that exhibited by type A and type B GABA receptors (Shimada *et al.*, 1992). Northern blotting studies suggested that additional members of the rho receptor subtype may be expressed in the retina (Cutting *et al.*, 1991). To further investigate this unique subclass, we have screened libraries to identify additional rho receptor subunits and determine their location in the human genome. Screening of a human genomic library confirmed that a second member exists and provided sequence information to allow the isolation of a cDNA closely related to rho_1, which has tentatively been termed rho_2. Mapping studies using somatic cell hybrids and linkage analysis indicate that the genes encoding rho_1 and rho_2 may exist as a gene cluster on human chromosome 6q14–q21.

MATERIALS AND METHODS

Isolation and sequencing of genomic fragments. A human genomic library (Clontech HL1006d) was screened using standard methods for genomic sequences hybridizing to a PCR-amplified fragment (clone D) previously used to isolate the GABA rho_1 cDNA (Cutting *et al.*, 1991). DNA isolated from positive recombinant phage was digested with *Sal*I, *Hin*dIII, *Bam*HI, *Kpn*I, *Sst*I, and *Eco*RI, electrophoresed in 1% agarose gels, then transferred to nitrocellulose filters by the Southern method (Maniatis *et al.*, 1982). Restriction fragments of two different phage hybridizing to radioactively labeled clone D were subcloned into pGem3Z+ (Promega) and designated pKpn4.2 and pSst4.4. Plasmid DNA was prepared from these two subclones and sequenced by the dideoxynucleotide method with primers 5′D (5′-ATCATCACGGGOGTGAATGCC-3′) and 3′D (5′-GCTGACCCA-GAGGTAGATGTC-3′) derived from clone D sequence (Cutting *et al.*, 1991).

Identification and sequencing of the rho_2 cDNA. A human retina cDNA library was plated and transferred to nitrocellulose filters using standard methods (Maniatis *et al.*, 1982). Oligonucleotides (New England Nuclear) rho2e-5′ (5′-ACGACGGTGCTGACCATGA-3′) and rho2e-3′ (5′-TCCTTGCGCTCCTGCACG-3′) (see Fig. 1) were end-labeled with 150 μCi [γ-^{32}P]ATP and 5 units T4 kinase (Bethesda Research Labs) in a reaction mixture recommended by the manufacturer. Phage DNA bound to the nitrocellulose filters was hybridized at 42° for 16 h with 1×10^6 cpm of end-labeled oligonucleotide per milliliter of hybridization buffer (5× SSPE [0.9 *M* NaCl, 5 m*M* NaH_2PO_4, 5 m*M* NaEDTA, pH 7.4]; 0.5% sodium dodecyl sulfate (SDS), 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, and 0.1% Ficoll). The filters were washed for 30 min at room temperature and 30 min at 54°C in 2× SSPE, 0.1% SDS, dried, and autoradiographed for 16 h at −70°C with intensifying screens.

The cDNA inserts were isolated from positively hybridizing phage subcloned into the pBluescript KS(−) (Stratagene) vector. The entire 1.4-kb coding region of the longest insert (3 kb), designated pR24, was sequenced in both directions using automated fluorescently labeled dideoxynucleotide sequencing (Applied Biosystems 373A).

Chromosomal assignment. DNA from two human/rodent somatic cell hybrid panels (BIOS Corporation, New Haven, CT and the National Institute of General Medical Sciences, Camden, NJ) was amplified by the polymerase chain reaction (PCR) using primers selected from intron sequences of the rho_1 and rho_2 genes (rholi-5′ and rholi-3′ and rho2i-5′ and rho2i-3′ shown in Fig. 1, respectively). Primers 834D (5′-TTCTTCAchATGGGACGGAGCG-3′) and 835D (5′-GCCGCT-GCACTGTGAAGCTCTC-3′) chosen from intron sequence of the HLA DR β_1 gene on human chromosome 6p were used to verify the chromosomal assignment of the rho_1 and rho_2 genes. Fifty nanograms of gene sequences were amplified from genomic DNA from each hybrid cell line using 20 pmol of each primer pair in a 100-μl reaction as previously described (Cutting *et al.*, 1991). Thirty cycles of denaturation were performed at 94°C for 45 s, annealing of primers at 56°C (61°C for primers 834D and 835D) for 45 s, extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. Successful amplifications were documented by electrophoresis of 15 μl in 1% agarose (Bethesda Research Laboratories) and 3% NuSieve (FMC BioProducts, ME) gels, staining with ethidium bromide and visualizing fragments under UV transillumination.

Hybrid cell lines and regional mapping. The following human–hamster hybrid cell lines were used for regional mapping of the GABA subunit rho_1 and rho_2 genes: HHW484 containing human chromosome 6 and a piece of chromosome 12 (Nakamichi *et al.*, 1986), I-7

containing the short arm of chromosome 6 as its only human chromosome (Zoghbi *et al.*, 1990), and CF34-10-2/11, with a derivative chromosome 15 containing the long arm of chromosome 6 from a 6/15 translocation, 46, XX, t(6:15)(cen:p13) (Mohandas *et al.*, 1980). The somatic cell hybrid mapping panel for 6q consisted of cell line RAGSU 3-1-2-3, which contains 6pter–q14, cell line ITA 9-1-2, which contains 6q12–qter, cell line GM610 RAG-4-5-1, which contains 6pter–q21, and cell line GM610 RAG-5-23, which contains 6q21–qter (Naylor *et al.*, 1983a).

Linkage mapping in mice. C3H/HeJ-*gld* and *Mus spretus* (Spain) mice and [C3H/HeJ-*gld* × *M. spretus*)F_1 × C3H/Hej-*gld*] interspecific backcross mice were bred and maintained as previously described (Seldin *et al.*, 1988). *M. spretus* was chosen as the second parent in this cross because of the relative ease of detection of informative restriction fragment length variants (RFLV) in comparison with crosses using conventional inbred laboratory strains.

Mouse DNA was digested with restriction endonucleases and 10-μg samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schull, Inc., Keene, NH), hybridized at 65°C and washed under stringent conditions (Maniatis *et al.*, 1982). A 400-bp *Eco*RI–*Hin*dIII cDNA fragment from the rho_1 cDNA (corresponding to transmembrane regions 1–3), which detects sequences from rho_1 and rho_2 in human and mouse genomic DNA (G. R. Cutting and B. F. O'Hara, unpublished observation) was used as a probe for the GABA rho subunits. Other probes used include pMS-1 of the most protooncogene (Mos) (Canaani *et al.*, 1983), a cDNA probe of the alpha subunit of the thyroid-stimulating hormone (Tsha) (Kourides *et al.*, 1984), and Jac.1 of c-jun protooncogene (Ryder and Nathans, 1988).

Linkage was determined by segregation analysis (Green, 1981). Gene order was determined by analyzing all haplotypes and minimizing crossover frequency between all genes. This method resulted in determination of the most likely gene order (Bishop, 1985).

RESULTS

Identification of Genomic Sequences from rho_1 and a Related Gene

Screening of 5×10^5 phage from the human genomic library identified seven positively hybridizing clones. Restriction mapping of five of these clones revealed that four had the same insert while the fifth clone had a different insert. Partial sequence of genomic subclone pKpn4.2 demonstrated a single exon containing a sequence identical to that found in the GABA rho_1 cDNA. Subclone pSst4.4 encompassed a single exon with 96% nucleotide homology to the corresponding region of the rho_1 cDNA and intron/exon splice sites that were conserved with the rho_1 exon in pKpn4.2 (Fig. 1). The differences in flanking intron sequence and restriction digestion pattern of the insert indicated that pSst4.4 contained sequences from a gene related to, but distinct from, GABA rho_1.

Cloning and Sequencing of the rho_2 cDNA

Screening of 5×10^5 recombinants from the retina cDNA library identified three clones hybridizing with oligonucleotides rho2e-5′ and rho2e-3′. Partial sequencing of all three clones revealed one incompletely spliced cDNA, one aberrantly spliced cDNA, while the third clone contained a 3-kb cDNA insert with an open reading frame of 1395 bp encoding a predicted protein of 465 amino acids (Fig. 2). The first in-frame AUG codon has a purine at position −3 suggesting that it could serve as the start of translation (Kozak, 1989). The first 20 amino acids conform to predictions for a secretory signal sequence, with cleavage producing a mature protein of 445 amino acids (von Heijne, 1986). Alignment of the proposed amino acid sequence of this cDNA with members of the four $GABA_A$ subunit classes (α, β, δ, and γ) and with GABA rho_1 demonstrates 30 to 38% similarity to the $GABA_A$ sequences (data not shown) and 74% identity with rho_1 (Fig. 3). The lowest degree of amino acid similarity between rho_1 and the sequence predicted by this cDNA occurs in the region bounded by transmembrane regions M3 and M4. The very high degree of similarity between this cDNA and GABA rho_1 at the nucleotide and amino acid level suggests that this cDNA represents the second member of the GABA rho subunit family. Preliminary expression studies using Northern blotting reveal that rho_2 is expressed at much lower levels than rho_1 in human retina RNA (data not shown).

Chromosomal Assignment and Regional Localization of the rho_1 and rho_2 Genes to Chromosome 6, Bands q14 to q21

Amplification of the BIOS panel with primers specific for rho_1, rho_2, and HLA DR β1 produced the correct size DNA fragments in the same four cell lines, all of which contain human chromosome 6. Amplification did not occur in any cell line lacking chromosome 6. A similar result was obtained with the NIGMS panel.

Cell lines retaining chromosome 6 were used initially to confirm the localization of the GABA subunit genes rho_1 (GABRR1) and rho_2 (GABRR2) to chromosome 6. Sublocalization to chromosome 6q was confirmed by the presence of the expected amplified fragments in the cell lines retaining 6q and the absence of these fragments from the chromosome 6p-specific cell line (Fig. 4). The results of the regional localization on 6q are also shown in Fig. 4. Both GABRR1 and GABRR2 map to 6q14–q21 as evident by the presence of the expected PCR product in cell line ITA9-1-2 (6q12–qter) and in cell line GM610 RAG-4-5-1 (6pter–q21). No amplified product was detected in cell line RAGSU 3-1-2-3 (6pter–q14) nor in cell line GM610 RAG-5-23 (6q21–qter) (Fig. 4).

Linkage of Both GABA rho Subunit Genes to Tsha on Mouse Chromosome 4

A panel of DNA samples from an interspecific cross that has been characterized for over 400 genetic markers throughout the genome was used for genetic mapping. The genetic markers included in this map span between 50 and 80 cM on each mouse autosome and the X chromosome (for examples see Watson *et al.*, 1991 and Saunders and Seldin, 1990). Initially, DNA from the two parental mice [C3H/HeJ-*gld* and (C3H/HeJ-*gld* × *M. spretus*) F_1] were digested with various restriction endo-

nucleases and hybridized with the 400-bp cDNA probe to determine RFLVs that would allow haplotype analyses. Informative *Taq*I RFLVs from each of 114 [C3H-*gld* × (C3H-*gld* × *M. spretus*)F_1] interspecific backcrosses displayed either the homozygous (C3H-*gld*) or heterozygous (C3H-*gld* × *M. spretus*) F_1 pattern indicating cosegregation of the RFLVs. Since this probe detects both GABA rho subunits, these results demonstrate that the two genes are tightly linked.

Comparison of the haplotype distribution of the RFLVs detected with the 400-bp cDNA probe indicated that in all 114 meiotic events examined, the *Gabrr* locus cosegregated with *Tsha*, a locus previously mapped to mouse chromosome 4 (Kourides *et al.*, 1984; Ceci *et al.*, 1990). These data indicated that these two loci were linked (probability of linkage >0.99; upper 95% confidence limit by binomial distribution was 3.2 cM). The distribution of haplotypes determined by typing for several mouse chromosome 4 markers (Fig. 5) indicated the following gene order (± standard error): (centromere) *Mos*–16.6 ± 3.5 cM–*Gabrr*/*Tsha*–22.8 cM ± 3.9 cM–*Jun* placing the *Gabrr* locus on distal mouse chromosome 4. The human homolog of *Tsha* is the α chain of chorionic gonadotropin (CGA), which has been previously mapped to human chromosome 6, bands q12–q21 (Naylor *et al.*, 1983b).

DISCUSSION

The identification of a second potential member of the GABA rho receptor subunit further increases the diversity of the GABA-gated ion channel family. The degree of nucleotide and predicted amino acid sequence similarity between the rho_1 cDNA and the newly isolated cDNA is comparable to the degree of conservation exhibited among $GABA_A$ subunits from the same class (i.e., α, β, or γ). The region of lowest similarity occurs between transmembrane domains 3 and 4. This area is also least conserved among the $GABA_A$ subunits. It therefore appears appropriate that this cDNA be designated rho_2. The rho_2 cDNA appears to be full-length, since the first methionine residue is preceded by a sequence fitting the consensus for initiation of translation and is followed by a 20-amino-acid signal peptide sequence. Preliminary expression studies reveal that *Xenopus* oocytes injected with *in vitro* transcribed RNA from this cDNA produce GABA-gated chloride currents which are significantly lower than those observed in oocytes injected with similar amounts of rho_1 RNA (T.-L. Wang, G. R. Cutting, and W. B. Guggino, unpublished observations). Expression of single $GABA_A$ receptor subunit cDNAs in *Xenopus* oocytes reveals considerable variation in their efficiency at forming GABA-gated ion channels (Blair *et al.*, 1988). Coexpression of subunits often produces substantial potentiation of the GABA responsiveness, suggesting that the wild-type receptors formed from these sub

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                                                                    tgtgagg
-71  gcaagscaggggggatttgctctgccacaggctactggaaagcagctttttgcctcacagatgtccacaaa

1   ATG CCT TAT TTT ACA AGA CTC ATT TTG TTC TTG TTT TGC TTG ATG GTT CTC GTG
1   MET Pro Tyr Phe Thr Arg Leu Ile Leu Phe Leu Phe Cys Leu Met Val Leu Val

55  GAG AGC AGA AAA CCC AAG AGG AAG CGA TGG ACA GGG CAG GTG GAA ATG CCC AAG
19  Glu Ser Arg Lys Pro Lys Arg Lys Arg Trp Thr Gly Gln Val Glu Met Pro Lys
    ↑                                  ▲
109 CCA AGT CAC TTA TAT AAG AAG AAC CTT GAT GTG ACC AAG ATC CGG AAG GGA AAG
37  Pro Ser His Leu Tyr Lys Lys Asn Leu Asp Val Thr Lys Ile Arg Lys Gly Lys

163 CCT CAG CAG CTT CTC AGA GTG GAC GAG CAC GAC TTC AGC ATG AGA CCC GCC TTC
55  Pro Gln Gln Leu Leu Arg Val Asp Glu His Asp Phe Ser Met Arg Pro Ala Phe
                                                  *
217 GGA GGC CCT GCC ATC CCG GTG GGC GTG GAC GTA CAG GTG GAG AGC CTG GAC AGC
73  Gly Gly Pro Ala Ile Pro Val Gly Val Asp Val Gln Val Glu Ser Leu Asp Ser

271 ATC TCC GAG GTG GAC ATG GAC TTC ACT ATG ACC CTG TAC CTG CGG CAT TAC TGG
91  Ile Ser Glu Val Asp Met Asp Phe Thr Met Thr Leu Tyr Leu Arg His Tyr Trp

325 AAG GAT GAG AGG CTA GCT TTC TCC AGC GCC AGC AAC AAG AGC ATG ACC TTC GAT
109 Lys Asp Glu Arg Leu Ala Phe Ser Ser Ala Ser Asn Lys Ser Met Thr Phe Asp
                                    *      ^^^^^^^^^
379 GGC CGG CTG GTG AAG AAG ATC TGG GTC CCT GAT GTC TTC TTT GTT CAC TCC AAA
127 Gly Arg Leu Val Lys Lys Ile Trp Val Pro Asp Val Phe Phe Val His Ser Lys
                                                                    *
433 AGA TCG TTC ACT CAT GAC ACC ACC ACT GAC AAC ATC ATG CTG AGG GTG TTC CCA
145 Arg Ser Phe Thr His Asp Thr Thr Thr Asp Asn Ile Met Leu Arg Val Phe Pro

487 GAT GGA CAC GTG CTG TAC AGC ATG AGG ATT ACG GTC ACT GCC ATG TGC AAC ATG
163 Asp Gly His Val Leu Tyr Ser Met Arg Ile Thr Val Thr Ala Met Cys Asn Met
                          *                                         |--------
541 GAC TTC AGC CAC TTT CCC CTG GAC TCC CAG ACC TGT TCT TTG GAG CTG GAG AGC
181 Asp Phe Ser His Phe Pro Leu Asp Ser Gln Thr Cys Ser Leu Glu Leu Glu Ser
    ----------------------------------------|
595 TAT GCC TAT ACA GAT GAA GAT CTA ATG CTG CAC TGG AAG AAT GGG GAT GAA TCC
199 Tyr Ala Tyr Thr Asp Glu Asp Leu Met Leu His Trp Lys Asn Gly Asp Glu Ser
                                                                        *
649 CTA AAA ACA GAT GAG AAG ATC TCC TTG TCT CAG TTT CTG ATT CAG AAA TTT CAC
217 Leu Lys Thr Asp Glu Lys Ile Ser Leu Ser Gln Phe Leu Ile Gln Lys Phe His

703 ACA ACT TCC AGG CTG GCC TTC TAC AGC AGC ACT GGC TGG TAC AAC CGT CTG TAC
235 Thr Thr Ser Arg Leu Ala Phe Tyr Ser Ser Thr Gly Trp Tyr Asn Arg Leu Tyr
      *
757 ATT AAC TTC ACG TTG CGT CGC CAC ATC TTC TTC TTC TTG CTC CAA ACA TAT TTC
253 Ile Asn Phe Thr Leu Arg Arg His Ile Phe Phe Phe Leu Leu Gln Thr Tyr Phe
        ^^^^^^^^*
811 CCT GCC ACT CTG ATG GTC ATG CTG TCC TGG GTG TCC TTC TGG ATC GAC CGC AGA
271 Pro Ala Thr Leu Met Val Met Leu Ser Trp Val Ser Phe Trp Ile Asp Arg Arg

865 GCT GTG CCT GCC AGA GTT TCA CTG GGT ATC ACG ACG GTG CTG ACC ATG ACC ACC
289 Ala Val Pro Ala Arg Val Ser Leu Gly Ile Thr Thr Val Leu Thr Met Thr Thr

919 ATC ATC ACG GGC GTG AAT GCC TCC ATG CCG CGC GTC TCC TAC GTC AAG GCC GTC
307 Ile Ile Thr Gly Val Asn Ala Ser Met Pro Arg Val Ser Tyr Val Lys Ala Val
                    ^^^^^^^^^
973 GAC ATC TAC CTC TGG GTC AGC TTT GTG TTC GTG TTC CTC TCG GTG CTG GAG TAT
325 Asp Ile Tyr Leu Trp Val Ser Phe Val Phe Val Phe Leu Ser Val Leu Glu Tyr

1027 GCG GCT GTC AAC TAC CTG ACC ACC GTG CAG GAG CGC AAG GAA CGG AAG CTG CGG
343  Ala Ala Val Asn Tyr Leu Thr Thr Val Gln Glu Arg Lys Glu Arg Lys Leu Arg

1081 GAG AAG TTC CCG TGC ATG TGT GGA ATG CTT CAC TCA AAA ACC ATG ATG CTG GAT
361  Glu Lys Phe Pro Cys Met Cys Gly Met Leu His Ser Lys Thr Met Met Leu Asp

1135 GGA AGC TAC AGT GAG TCT GAG GCC AAC AGC CTG GCT GGG TAC CCC AGA AGC CAT
379  Gly Ser Tyr Ser Glu Ser Glu Ala Asn Ser Leu Ala Gly Tyr Pro Arg Ser His

1189 ATC CTG ACA GAA GAA GAA AGG CAA GAC AAA ATA GTG GTC CAC CTG GGC CTG AGT
397  Ile Leu Thr Glu Glu Glu Arg Gln Asp Lys Ile Val Val His Leu Gly Leu Ser

1243 GGT GAA GCC AAC GCT GCC AGA AAG AAG GGG CTT CTG AAG GGC CAG ACG GGT TTT
415  Gly Glu Ala Asn Ala Ala Arg Lys Lys Gly Leu Leu Lys Gly Gln Thr Gly Phe

1297 CGT ATC TTC CAG AAT ACC CAT GCC ATT GAC AAA TAC TCT AGG TTG ATA TTC CCT
433  Arg Ile Phe Gln Asn Thr His Ala Ile Asp Lys Tyr Ser Arg Leu Ile Phe Pro

1351 GCC TCC TAC ATA TTT TTC AAC TTA ATT TAT TGG TCA GTG TTT TCC TAG ggg ctc
451  Ala Ser Tyr Ile Phe Phe Asn Leu Ile Tyr Trp Ser Val Phe Ser ---

1405 caaggctgttcctagaagagggcatagacatcgaggggggctggccagtcattg
1459 acagacggacttgttgaccacacgcccctcaccaaacaatgcagcagctactgg
1513 accacoctgagcagcactcatctctcagagaagccc
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FIG. 2. GABA rho_2 cDNA and predicted amino acid sequences. Sequence corresponding to oligonucleotides Rho2e-5′ and Rho2e-3′ used to detect this cDNA is found at nucleotides 895 to 913 and 1050 to 1067, respectively. Symbols below the amino acid sequence represent the following: predicted site of cleavage of signal peptide (↑); potential phosphorylation sites by cAMP-dependent protein kinase (▲) and protein kinase C (*); cysteine–cysteine bridge (|--|); potential N-linked glycosylation sites (^^^); hydrophobic domains (___). One nucleotide in the 5′-untranslated region could not be determined and is designated S indicating that either G or C exists at that position.

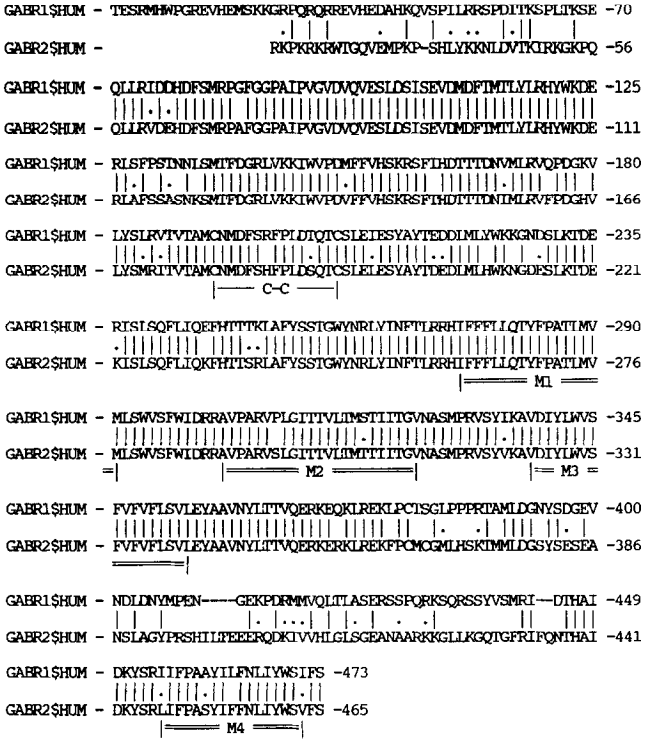

FIG. 3. Alignment of the predicted mature amino acid sequences (in single-letter code) of the rho_1 and rho_2 cDNAs. Identical residues are indicated by (|) and conservative residues by (.). The relative locations of the Cys–Cys loop and predicted transmembrane regions M1 to M4 are shown below the aligned sequences.

units are heterooligomeric (Blair *et al.*, 1988; Levitan *et al.*, 1988). These findings might thus suggest that rho_2 may require coexpression with other subunits to form fully functional receptors.

Bicuculline and benzodiazapine insensitivity of homooligomeric GABA rho_1 receptors expressed in *Xenopus* oocytes suggests that the rho class may represent a novel type of GABA receptor (Shimada *et al.*, 1992). This hypothesis is supported by the apparent proclivity of the rho_1 subunit to self-associate (Shimada *et al.*, 1992). Analysis of the genomic organization of genes

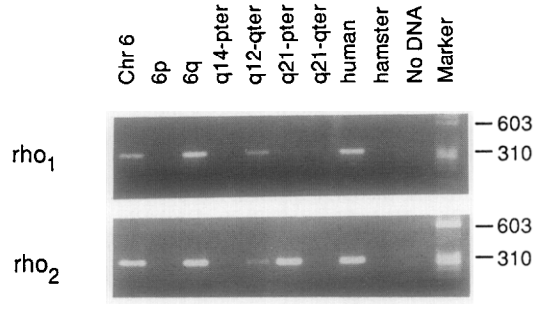

FIG. 4. DNA fragments stained with ethidium bromide PCR amplified from somatic cell hybrid DNA using primers specific for the rho_1 and rho_2 subunit genes. The human component present in each hybrid cell line is shown above the amplified fragments. Amplification of genomic DNA with rho_1 primers produces a fragment of 313 bp and with rho_2 primers a fragment of 299 bp. The sizes of two *Hae*III-digested fragments (310 and 600 bp) are shown to the right of the marker lanes.

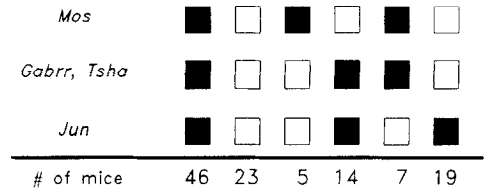

FIG. 5. Segregation of *Gabrr* among distal mouse chromosome 4 loci in [C3H/HeJ-*gld* × *Mus spretus*)F1 × C3H-HeJ-*gld*] interspecific backcross mice. Solid boxes represent the homozygous C3H pattern and open boxes represent the F1 pattern. The number of backcross mice with each haplotype pattern is shown below the line. For *Mos*, *Eco*R1-restricted DNA indicated the following RFLVs (C3H-*gld*: 18.0 kb; *Mus spretus*: 21.0 kb). For *Tsha*, *Msp*I-restricted DNA indicated the following RFLVs (C3H-*gld*; 6.0 kb; *Mus spretus*: 3.4 kb). For Jun, *Bam*H1-restricted DNA indicated the following RFLVs (C3H-*gld*: 5.8 kb; *Mus spretus*: 7.0 kb). The reference genetic markers (Mos, Tsha, and Jun) have been mapped previously (Ceci *et al.*, 1990).

from two $GABA_A$ subunit classes β and δ demonstrates conservation of intron/exon structure suggesting that these genes have evolved from a common ancestor. The location of the 5′ intron/exon splice site of the rho_1 and rho_2 exons shown in Fig. 1 corresponds precisely to the 5′ splice site of exon 8 of the β_1, β_4, and δ subunits (Sommer *et al.*, 1990; Kirkness *et al.*, 1991). This raises the possibility that the rho and $GABA_A$ subunit genes arose from a common ancestor but evolved with different functional properties.

Colocalization of a cloned gene and a disease locus can provide the first clue as to the identity of the defective protein in a disorder. To this end, several of the $GABA_A$ subunit genes have been mapped in the human genome, including the α_3 subunit gene to a region of the X chromosome believed to be involved in affective disorders and the β_3 gene to the Angelman/Prader-Willi locus on chromosome 15 (Bell *et al.*, 1989; Buckle *et al.*, 1989; Wagstaff *et al.*, 1991). The regional localization of the rho genes does not coincide with a known disease locus.

Four other $GABA_A$ subunit genes have been mapped: α_1 to chromosome 5 and α_2 to chromosome 4 bands p12–p13, coincident with the location of the β_1 subunit gene (Buckle *et al.*, 1989; Kirkness *et al.*, 1991; Dean *et al.*, 1991) and the δ subunit gene to human chromosome 1 (Sommer *et al.*, 1990). To our knowledge, the colocalization of the rho subunits is the first example of members of the same subunit class mapping to the same chromosomal location. The chromosomal assignment and sublocalization of the rho subunit genes to chromosome 6 bands q14 to q21 by somatic cell hybrids is consistent with the linkage analysis in mice and suggests that the rho genes may be physically close in both species. Close physical association and sequence similarity suggests that one gene arose from the other by duplication. This hypothesis is supported by the conservation of intron/exon boundaries analyzed thus far. Further analysis and comparison of the genomic organization and relative orientation of these two genes in various species should help determine the evolutionary origins of this intriguing branch of GABA receptors.

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