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Rodent and Human β_3-Adrenergic Receptor Genes Contain an Intron within the Protein-Coding Block

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SUMMARY

DNA blot analysis of the cloned rat β_3-adrenergic receptor gene revealed unexpected restriction enzyme cleavage sites that suggested the presence of one or more introns near the end of the coding block. This region of the rat gene was mapped and sequenced and was found to contain two introns. The first intron occurs 12 amino acids from the end of the coding block, as deduced by comparison with the β_3 receptor cDNA. Sequence analysis of the first intron indicates that it might contain enhancer elements that could be important in the adipose tissue-specific expression of this gene. The mouse and human β_3 receptor genes have been assumed to be intronless; however, these genes contain potential splice sites that are homologous to those present in the rat gene. The relevant regions of the mouse and human β_3 receptor cDNAs were cloned and, by comparing them to the respective genomic sequences, it was concluded that these genes also contain one or more introns. Sequence analysis of the mouse and human β_3 receptor cDNAs indicates that they code for proteins that are, respectively, 12 and 6 amino acids larger than previously deduced from genomic clones.

β-Adrenergic receptors are members of the family of guanine nucleotide-binding protein-coupled receptors, which all contain the hallmark of seven transmembrane regions. The three β-adrenergic receptor subtypes that have been cloned are related structurally and functionally and appear to have evolved from a common ancestor gene. Comparison of the rat β_1- and the human β_2-adrenergic receptors with their respective cDNAs (1, 2)[1] has demonstrated that the genes encoding these receptors each contain a single protein-coding exon. The human and murine β_3 receptor genes each contain a continous open reading frame that encodes a protein with seven membrane-spanning regions and these genes, too, were assumed to consist of a single protein-coding exon (3, 4). However, the assumption that the β_3-adrenergic receptor gene is intronless has not been proven because the sequences of the gene and cDNA have not been compared for any single species.

We recently reported the cloning of the rat β_3-adrenergic receptor (5). As part of that study we isolated 1.8 kb of the rat β_3 receptor cDNA as well as the corresponding gene. Southern blot analysis of the rat β_3 receptor gene with the rat cDNA probe revealed unexpected restriction enzyme cleavage sites, which suggested that the rat β_3 receptor gene contained one or more introns near the 3′ end of the coding block. We, therefore, mapped and sequenced the rat β_3 gene in this region and found that it contains two introns. The first intron interrupts the protein-coding block 12 amino acids from the carboxyl terminus. This intron contains sequences that are homologous to those that bind transcription factors involved in tissue-specific expression, suggesting that elements in the first intron may act as adipose tissue-specific enhancers.

Like the rat, the sequences of the murine and human β_3 receptor genes contain potential donor splice signals at homologous sites. Because no data are available regarding the sequence of the corresponding murine or human β_3 receptor cDNAs, it is unclear whether the splice signals that are present in the primary transcripts are used and, therefore, whether these genes also contain introns. Using genomic and cDNA probes generated by the PCR, we have analyzed β_3 receptor mRNA from human and murine sources. Data from these experiments strongly indicated that the protein-coding block of the human and mouse genes was interrupted by one or more introns. We, therefore, cloned partial mouse and human β_3 receptor cDNAs for comparison with the published genomic sequences. Sequence analysis of the cDNAs demonstrated that mouse and human β_3 receptor genes also contain introns. Because these introns interrupt the protein-coding block, the putative cytoplasmic tails of these proteins are predicted to be larger than previously supposed (3, 4).

Materials and Methods

General recombinant DNA methods. Standard cloning techniques were used, as described by Maniatis *et al.* (6). The cloning of

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[1] J. G. Granneman and K. N. Lahners, unpublished observations.

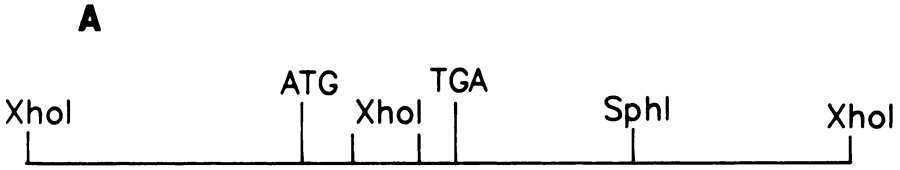
ABBREVIATIONS: Kb, kilobase(s); bp, base pair(s); PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends.

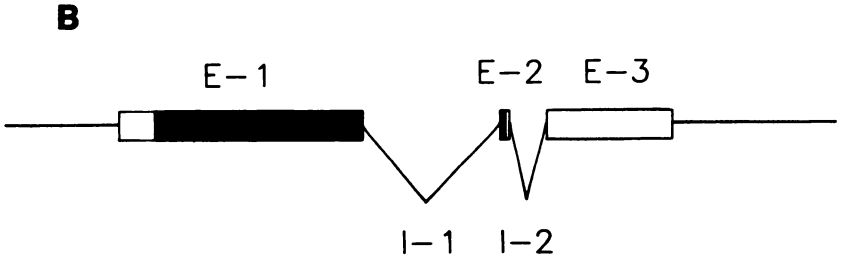
the rat β_3 receptor cDNA and gene was described previously (5). RNA extraction, reverse transcription of tissue RNA, and PCR amplification were performed as described previously (7).

Analysis of β_3 receptor mRNA by RNase protection assay. Rat and human β_3 receptor mRNAs were analyzed by RNase protection assay with species-specific probes. The rat probe used (p152) was the *Bss*HII to *Bgl*II fragment of the cloned rat β_3 cDNA cloned into pGEM-7z (5). This sequence spans the junction of the first and second exons.

Human mRNA was mapped with a β_1 receptor probe and two β_3 receptor probes that were amplified from human genomic DNA. A β_3 ceptor (p146) and the β_1 (p145) probes were amplified by "nested" PCR (7) from total nucleic acids with primers based upon the published sequences (4, 8). The resulting receptor DNAs were shortened and cloned into pGEM-7z for the generation of riboprobes. These probes are exact matches of the published sequences and encode amino acids 178–271 (β_1) and 151–223 (β_3). The second human β_3 receptor probe was amplified from genomic DNA (Promega) with a primer set that was designed to amplify a 256-bp DNA fragment that spanned the putative donor splice site. The coding strand primer was 5′-TGCGAATTCTGCCTTCAACCCGCTC-3′ and the noncoding strand primer was 5′-GCAGGATCCACGGACACATCGCATGCTTCC-3′. Both primers were based upon the published human sequence and contained engineered restriction sites on the 5′ ends for cloning into pGEM-7z (p174). The sequence of p174 was an exact match of the published human β_3 receptor gene sequence except for a discrepancy of adenosine for guanosine in the published sequence at bp 1193 (4) (GenBank accession number M29932). This potential discrepancy does not affect the nuclease protection assay because the T-1 ribonuclease used does not cleave at adenosine (9), and no fragments indicative of cleavage at this site were detected.

Cloning of a partial mouse β_3 receptor cDNA. The mouse β_3 receptor cDNA was obtained from mouse white adipose tissue RNA by reverse transcription/PCR (5). Reverse transcription of total RNA was performed with the oligonucleotide primer 5′-ATAAA-AGGTTTGCATC-3′, which was based upon the rat cDNA (5). The resulting cDNA was then amplified by PCR. The coding strand primer





C

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                 374
EXON 1 Pro Ala Ser Pro Val Ala Ser Arg Gln Asn Ser Pro Leu Asn Ar
       CCA GCT AGC CCT GTT GCG TCC AGG CAG AAC TCA CCG CTC AAC AG

GTAGGCGACGCAGGCAGAGGACTGGAGTCTGGGTGGGGACGCCTCTGTCTCTATTTTTGAGTTTG
AGGGTTGGGGGAGGAGAAGGTGTAGACAGGGCTTTTGTCTCGAGAGGACAGAAAAGGAGTAAGAA
CAGAATCGGGATCTAGGGCCCTTCCTTTTATTGGATCCAATCCCTGGGTCTGAGGCAAAGGAGGA
AAGGGAAATTTGTTCACCTTGGGACCAGGTGAGCCCCACAGGTTTCTGCCAGCAGGTTTCTGACC
TCTCTGGTTGCCTCTAGTTTGGATCTTTTTAGTTCTATTCTCCAGGCGCCCAGGTATCACTAACT
TGTCTGGGACATCCATAGACAGCAATGGACATGTCAAGTCCTCTGCCTCAGTTCCGCTTTCTTTC
AAAG

EXON 2   g Phe Asp Gly Tyr Glu Gly Glu Arg Pro Phe Pro Thr ---
         G TTT GAT GGC TAT GAA GGT GAG CGT CCA TTT CCC ACA TGA
 AGGACCATGGAGATCTAGCAAGGAGCCT


GTGAGTTGAATTTGAGCTGCTTTTCTCCCTCAGGGACTGGATTCGAGGTGTAGGGTGGGATGAGG
GAGGGTGCAGGATGATCCCTATATCTTTGAAAAGTAAATATGCTATTCAGGGTTCCTGAGTCACT
CCCCTCTTACCTCCAGTGCTTGATCCGCACCTCCTTGACTGGTTACCCCA
AGAAATATTGTTTCCGTTTTGCAG

EXON 3 GACTTCTGGAGAAATTTTTTTTTAAGACAGAAAGA...
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Fig. 1. Structure of the rat β_3 receptor gene. A, Map of the rat β_3 receptor gene clone, illustrating the locations of restriction enzyme cleavage sites and the translation initiation (ATG) and termination (TGA) codons. B, Schematic representation of the rat β_3 receptor gene, with mature mRNA as *blocks* and the coding sequence *filled*. *E*, exon; *I*, intron. C, Nucleic acid and amino acid sequences of exon/intron junctions of the rat β_3 receptor gene, beginning with Pro^{374} (5). *Underlined*, donor and acceptor splice sites; *bold*, inverted repeat that has homology with NF-1.

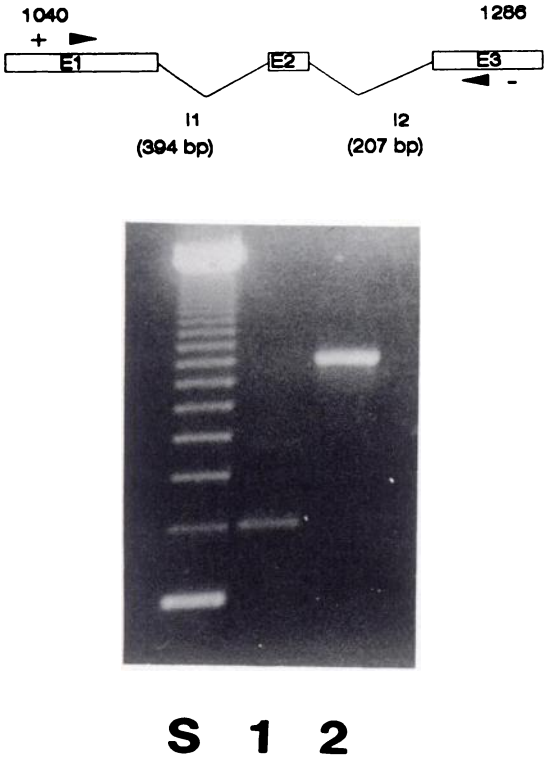

Fig. 2. PCR analysis of rat β_3 receptor cDNA and genomic DNA. PCR primers were complementary to sequences in the first and third exons (*top*). When cDNA was used as a template a 246-bp PCR product was amplified (*lane 1*), whereas a genomic template resulted in a 847-bp product (*lane 2*). S, 123 bp ladder.

was 5′-GGACTTTCGCGACGCCT-3′ and the noncoding strand primer was 5′-GCATCCATGGACGTTGCTTGTC-3′, which were also based upon the rat sequence. Samples were denatured at 94° for 2 min, annealed at 63° for 1.5 min, and extended at 72° for 2 min for 30 cycles. The resulting PCR product was shortened to 180 bp, cloned into pGEM-7z (p158), and sequenced.

PCR analysis of mouse and rat genomic DNA. To estimate the size of the mouse intron(s), PCR analysis was conducted on mouse and rat genomic DNA. The primer set used was the same that was used above to amplify the mouse cDNA. PCR was carried out for 30 cycles using 1 μg of mouse or rat genomic DNA (Promega), as described above. PCR products were resolved on 1% agarose gels containing ethidium bromide and were visualized with UV light.

Cloning of a partial human β_3 receptor cDNA. A partial human β_3 receptor cDNA was cloned by the RACE technique (10). Total RNA from SK-N-MC cells was reverse-transcribed as described above, with the oligonucleotide primer/adapter 5′-ACTATAGGGTCTAGAG-GATCCGTTTTTTTTTTTTTTTTT-3′, which contains engineered *Xba*I and *Bam*HI restriction sites. The resulting cDNA was amplified with human β_3 coding strand primer 5′-TGCGAATTCTGCCTT-CAACCCGCTC-3′ and noncoding strand primer 5′-ACTA-TAGGGTCTAGAGGATCCG-3′, which was the adapter sequence of the primer/adapter oligonucleotide described above. PCR was performed for 30 rounds as described above, and the resulting products were digested with *Eco*RI and *Xba*I and then cloned into pGEM-7z. Twelve recombinants were screened to determine insert size. Analysis of two clones by RNase protection assay with the p174 cRNA probe indicated that the 900-bp inserts they contained encoded a human β_3 receptor cDNA. These clones were then analyzed by restriction mapping and dideoxynucleotide sequencing and were found to be the same.

Cell culture. SK-N-MC cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Hyclone), penicillin (100,000 units/liter), and streptomycin (100 mg/liter). Cells were subcultured at a ratio of 1:10 and were harvested when about 80% confluent.

Mammalian tissues. Rat tissues were obtained from male Sprague-Dawley rats and mouse tissues were from male outbred mice (Hilltop Labs). Human adipose tissue was obtained from surgical specimens, with informed consent.

Results

We previously screened a rat genomic library with the rat β_3 receptor cDNA and isolated a clone containing a 7.7-kb insert that was flanked by *Xho*I sites (5). This clone was then subjected to Southern blot analysis by digesting it with *Xho*I and probing the resolved *Xho*I fragments with a radiolabeled rat β_3 cDNA probe. Results from this analysis revealed prominent bands of 3, 4, and 0.65 kb that hybridized to the rat β_3 receptor cDNA. Because the rat β_3 receptor cDNA contains only a single *Xho*I site, these data suggested the existence of one or more introns in the rat β_3 receptor. Further analysis utilizing selective cDNA probes suggested the existence of intron(s) near the 3′ end of the coding region. The *Xho*I fragments derived from the genomic clone were then isolated and sequenced.

Shown in Fig. 1A is a restriction map of the rat β_3 receptor gene and the exon/intron structure of the rat β_3 receptor gene that was deduced by comparing the genomic and cDNA sequences (Fig. 1B). The rat β_3 receptor gene contains three

Fig. 3. Analysis of rat adipose tissue β_3 receptor mRNA by RNase protection assay. *Top*, location of cRNA probe relative to first exon/exon junction. *Bottom*, autoradiogram of probe protected by white (*WAT*) and brown (*BAT*) adipose tissues and liver (*LIV*). The cRNA probe was fully protected, indicating lack of alternative splicing in these rat tissues.

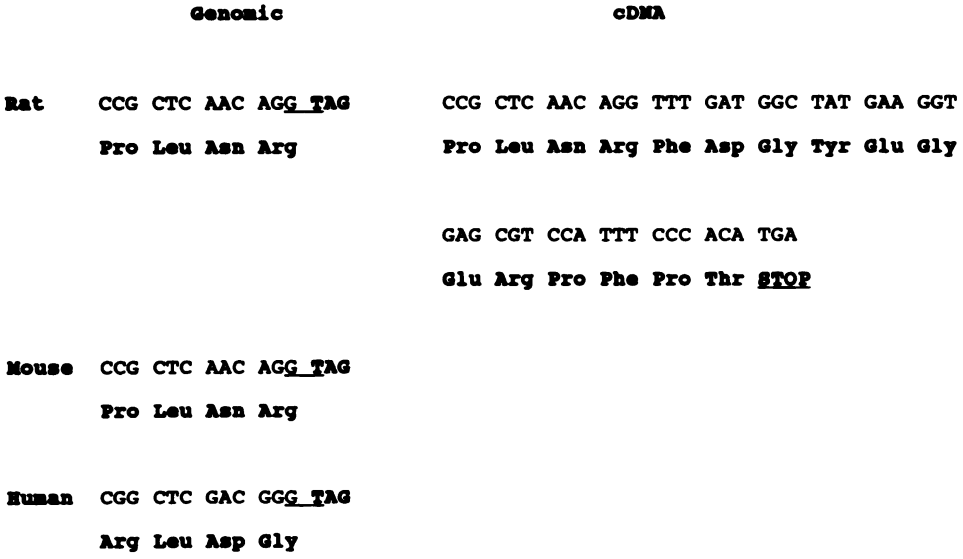

Fig. 4. Comparison of the mouse and human β_3 receptor gene sequences with the homologous sequence of the first exon/intron junction in the rat gene. *Underlined*, donor splice signals; *bold*, translation termination codons proposed by Emorine *et al.* (4) and Nahmias *et al.* (3).

exons and two introns. The first intron interrupts the open reading frame 12 amino acids from the carboxyl end (Fig. 1C). This intron is 394 bp and contains both 5′ donor and 3′ acceptor splicing signals. The second exon is 68 bp long and encodes the translation termination codon and 28 bp of nontranslated sequence. The second intron is 207 bp long and also contains donor and acceptor splice signals. The final exon contains sequence through the polyadenylation signal (11). In this regard, it should be noted that the 117-bp segment of the 3′ nontranslated sequence that extends beyond the polyadenylation site in the rat β_3 receptor cDNA recently reported by Muzzin *et al.* (11) is not authentic but is in fact the Stratagene cloning vector.

To verify further that the rat β_3 receptor gene contains introns, we performed PCR analysis of rat β_3 receptor cDNA (prepared by reverse transcription of total RNA from adipose tissue) and genomic DNA. The coding strand primer was placed upstream of the first splice junction, whereas the noncoding primer was placed in the third exon. Thus, the expected PCR product spanned the introns. As expected, this primer set amplified a 847-bp fragment from genomic DNA (Fig. 2). When tissue cDNA was used as a template, the product was 246 bp, as was expected if the primary transcript contained introns that had been removed. No other PCR products were observed, indicating that the β_3 pre-mRNA is not alternatively spliced. To verify this conclusion further, a nuclease protection assay was performed on rat β_3 receptor mRNA. The probe used (p152) in this instance was derived from the cloned rat β_3 receptor cDNA and spanned the junction of the first and second exons (see Fig. 4). If both introns of the rat β_3 receptor are removed by RNA splicing, then tissue mRNA should protect the full (281-nucleotide) complementary probe. However, if the first donor site is not used or if the first exon is spliced to any exon other than exon 2 (i.e., is alternatively spliced), then a fragment of 232 nucleotides would be protected by tissue β_3 receptor mRNA. As shown in Fig. 3, RNA from both white and brown adipose tissues protected the full probe, and no smaller fragments indicative of alternative splicing were observed. As expected, RNA from liver failed to protect the β_3 receptor probe (5).

The murine and human β_3 receptor genes have been cloned recently, and it was assumed that these receptors, like the β_1 and the β_2 receptor genes, lacked introns (3, 4). However, analysis of the genomic sequence alone is not sufficient to decide whether this is so. As shown in Fig. 4, the first exon/intron junction of the rat gene contains the sequence AGGTAG. In the absence of information derived from cDNA, it might be concluded erroneously that the final amino acid is arginine (encoded by AGG), followed by a translation termination codon (TAG). In this regard, we noticed that the sequence of the mouse β_3 receptor gene is identical to that of the rat gene in this region (3). In addition, the human gene also contains the sequence GGTAG in a homologous site, and this sequence could contain a donor splice site (GT), in which case the coding sequence could continue, or it could be a termination codon (TAG), as originally deduced (4).

In order to determine whether the mouse gene contains introns, we cloned the relevant region of the mouse β_3 receptor cDNA from mouse adipose tissue by reverse transcription of RNA followed by PCR. The nucleic acid sequence and deduced

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... GTT GAA GCC AGG CAG AGT CCA CCG CTC AAC AGG TTT GAT GGC TAT
    Val Glu Ala Arg Gln Ser Pro Pro Leu Asn Arg Phe Asp Gly Tyr

GAA GGT GCG CGT CCG TTT CCC ACG TGA AGGGCCGTGAAGATCCAGCAAG
Glu Gly Ala Arg Pro Phe Pro Thr ---

GAAGCTGACTTCTGGGGATTTTTTTTTTCCTCCAGAAAGACAAGCAACGTCCAT...
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Fig. 5. Nucleic acid and deduced amino acid sequences of a partial mouse β_3 receptor cDNA. The partial cDNA was cloned by reverse transcription of mouse adipose tissue mRNA followed by PCR. Shown is sequence beginning with the codon for Val^{378}. The cDNA exactly matches the genomic sequence reported by Nahmais *et al.* (3) until Arg^{388}. The open reading frame continues for 12 more amino acids (*bold*), which are similar to the rat sequence. The 45 bases of nontranslated sequence in this clone are 71% identical to the nontranslated sequence of the rat β_3 receptor cDNA.

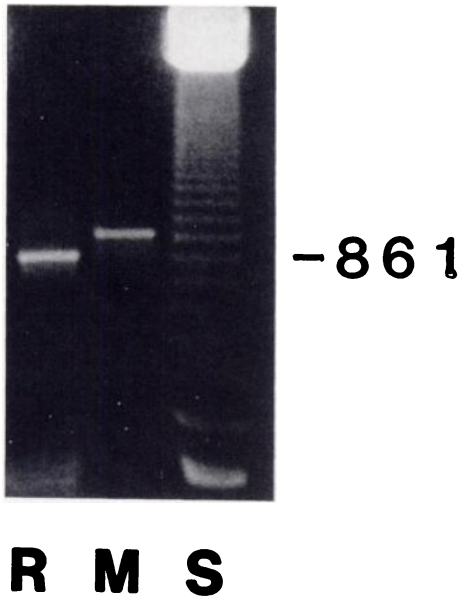

Fig. 6. PCR analysis of rat and mouse genomic DNA with cDNA-derived primers. *R*, rat; *M*, mouse; *S*, 123-bp ladder. See Fig. 2 for location of PCR primers.

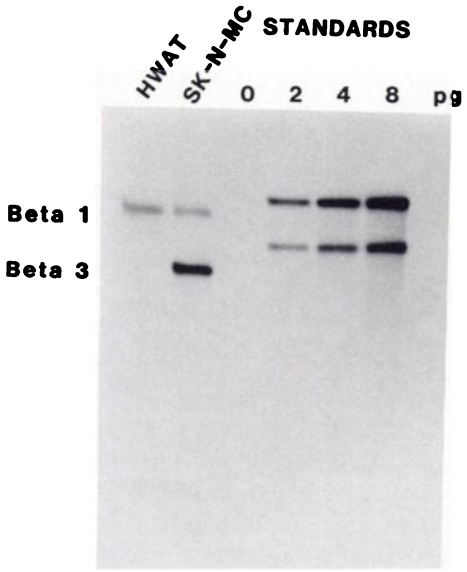

Fig. 7. Analysis of β_1 and β_3 receptor mRNA in human omental adipose tissue (*HWAT*) and in SK-N-MC cells by nuclease protection assay. Fifty micrograms of total RNA were hybridized to human β_1 (p145) and β_3 (p146) receptor probes simultaneously. SK-N-MC cells contain both β_1 and β_3 receptor mRNA, whereas human omental adipose tissue contains only β_1 receptor transcripts. *Right*, synthetic human β_1 and β_3 receptor RNA standards.

amino acid sequence of the mouse β_3 receptor cDNA are shown in Fig. 5. The open reading frame matches that of the gene (3) exactly until the genomic donor splice signal. In the cDNA, the open reading frame continues for 12 additional amino acids, exactly like the rat β_3 receptor cDNA. The remaining 45-bp nontranslated sequence in this clone (not including the primer sequence) is 71% identical to the rat β_3 receptor cDNA.

To verify further that the mouse β_3 receptor gene contains introns and to estimate their size, PCR analysis of genomic DNA was performed with oligonucleotide primers that were based upon the cDNA and were designed to span the intron(s).

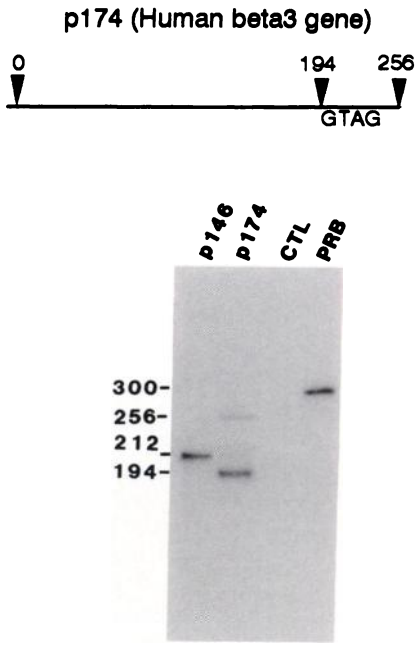

Fig. 8. RNase protection analysis of human β_3 receptor mRNA expressed in SK-N-MC cells. The cRNA probe derived from p146 is complementary to sequence within the first exon of the human β_3 receptor and is fully protected by SK-N-MC mRNA. The cRNA probed derived from p174 is complementary to genomic DNA sequence that spans the putative first exon/intron junction (*top*). *CTL*, p174 hybridized to tRNA; *PRB*, undigested p174 probe. Although some SK-N-MC β_3 mRNA protects the full p174 cRNA probe (256 nucleotides), most β_3 transcripts utilize the donor splice signal, as indicated by the protected fragment of 194 nucleotides. *Bottom*, autoradiogram of probe fragments that were protected from nuclease digestion.

In the mouse cDNA there are 208 bp between the primers in this set. Amplification of genomic DNA with this primer set resulted in a PCR product that was about 985 bp, confirming that the mouse gene contains introns and further indicating that the introns present in the mouse gene are about 120 bp larger than those in the rat gene (Fig. 6).

We next turned our attention to the human gene. In order to determine whether the human gene contains introns, one must first identify a source of human β_3 receptor mRNA for comparison. In rats, the β_3 adrenergic receptor is expressed abundantly only in adipose tissue, where β_3 receptor mRNA is about 5–7 times more abundant than β_1 receptor mRNA (7). We, therefore, examined mRNA from human omental adipose tissue in an RNase protection assay. Although β_1 receptor mRNA could be readily detected by nuclease protection assay, transcripts encoding the β_3 receptor were absent at the detection limit of the assay (about 4 copies/cell) (Fig. 7). Although the β_3 receptor does not appear to be abundantly expressed in human subcutaneous or omental adipose tissue, we have recently discovered that the β_3 receptor is abundantly expressed along with the β_1 receptor in the human neuroblastoma cell line SK-N-MC (12). Thus, these cells provide an excellent source for analysis of the human β_3 mRNA (Fig 7).

We then sought to map the 3′ end of the β_3 receptor mRNA from SK-N-MC cells. The probe used (p174) was derived from human genomic DNA and was designed to span the putative translation termination site/donor splice site (Fig. 8, *top*; see also Fig. 4). If the β_3 receptor gene is intronless, then SK-N-MC RNA should fully protect the complementary 256 nucleo-

```
(162 bp to beginning of clone)... GCG CAG CCC AGG CTT TGC CAA
                                  Ala Gln Pro Arg Leu Cys Gln

CGG CTC GAC GGG GCT TCT TGG GGA GTT TCT TAG GCCTGAAGGACAAGAAGC
Arg Leu Asp Gly Ala Ser Trp Gly Val Ser ---

AACAACTCTGTTGATCAGAACCTGTGGAAAACCTCTGGCCTCTGTTCAGAATGAGTCCCATG
GGATTCCCCGGCTGTGACACTCTACCCTCCAGAACCTG...(536 bp to poly A)
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Fig. 9. Nucleotide and amino acid sequences of a human β_3 receptor cDNA. Shown is the human β_3 receptor cDNA (p184) that was obtained from SK-N-MC cells using RACE, beginning with the codon for Ala^{392}. The 5′ cDNA sequence of the clone is identical to the published sequence of the human gene (4) for 194 bp and then diverges (*bold*) exactly at the predicted 5′ donor site. The open reading frame continues for 6 amino acids, followed by 657 bp of nontranslated sequence.

tide probe. However, if the 5′ donor splicing signal contained in the human β_3 receptor pre-mRNA is utilized in the SK-N-MC cells, then cellular RNA should protect exactly 194 nucleotides of the probe. We found that both 256 and 194 nucleotides of the probe were protected by SK-N-MC RNA (Fig. 8). The ability of SK-N-MC RNA to protect 194 nucleotides of the probe indicated that the splice signals in the human β_3 receptor primary transcript are used by SK-N-MC cells and that the gene contains at least one intron. However, unlike the expression of the rat β_3 receptor gene in adipocytes, the efficiency of splicing was not complete, as indicated by the 256-nucleotide fragment. Densitometric scanning of the protected fragments indicated that less than one fourth of the total β_3 receptor pre-mRNA failed to undergo splicing. Translation of the unspliced mRNA would be predicted to terminate at Gly^{402}, as originally deduced (4). Nevertheless, the great majority of the transcripts were spliced by these cells, and it seemed likely that the human β_3 receptor gene encoded additional amino acids.

To determine whether the spliced human β_3 mRNA encodes additional amino acids, the relevant region of the human β_3 receptor cDNA from SK-N-MC cells was cloned using RACE. Shown in Fig. 9 are the nucleic acid and deduced amino acid sequences of the human β_3 receptor cDNA obtained. The nucleic acid sequence of the 5′ end of the β_3 receptor cDNA is identical to that reported for the human β_3 receptor gene for 194 bp. As expected from the nuclease protection analysis of SK-N-MC RNA (Fig. 8), the genomic and cDNA sequences diverge exactly at the predicted donor splice site. The open reading frame of the cDNA continues for six additional amino acids, followed by 657 bp of 3′ nontranslated sequence.

Discussion

Comparison of the genomic sequences of the rat β_1 and the human β_2 receptors with the corresponding cDNAs demonstrated that these genes do not contain introns (1, 2).[1] The human (4) and murine (3) β_3 receptor genes were likewise thought to be intronless, despite the presence of a 5′ donor splice signal and the lack of confirmation with the corresponding cDNA. We have analyzed the rat β_3 receptor gene, cDNA, and mRNA and have demonstrated that the rat β_3 receptor gene is a split gene that contains three exons and two introns.

Because the mouse and human receptor genes contain homologous donor splicing signals, our results with the rat gene prompted us to examine the mouse and human genes. The cloning and sequencing of the relevant regions of the mouse and human β_3 receptor cDNAs clearly demonstrate that they encode proteins that are predicted to be larger than previously supposed. It is presently unclear whether the additional amino acids alter the pharmacological profile or regulatory properties of the human and mouse sequences. In this regard, the pharmacological properties of the β_3 receptor expressed in SK-N-MC cells (12) appear to be more similar to those reported for the cloned rat β_3 receptor (5) than to those reported for the receptor expressed in cells transfected with the first exon of the human β_3 receptor gene (4). Furthermore, because the 5′ donor sites present in the β_3 receptor pre-mRNA clearly can be used, there is the potential for the first exon to be spliced to an artificial 3′ acceptor site in the expression vector or site of integration, especially if the natural β_3 receptor acceptor sites have been eliminated. Indeed, we have found that such a situation occurs in Chinese hamster ovary cells that have been transfected with a construct bearing only the first exon of the human β_3 gene.[2] It is thus unclear whether the proteins expressed in the previous studies (3, 4) contained only amino acid sequences derived from the first exon or whether they also contained translated sequences that were derived from the expression vector or site of integration. In any event, pharmacological data from cells transfected with only the first exon of the mouse or human β_3 receptor genes should be viewed cautiously until these issues have been clarified.

The presence of introns in the β_3 gene was unexpected, given that neither β_1 nor β_2 receptor genes contain introns. This unique structure among the β receptor subtypes raises questions regarding the evolution and potential function of the additional exons and introns. For example, we have recently demonstrated that β_3, but not β_1, receptor mRNA is dramatically downregulated in adipose tissue by agents that elevate intracellular cAMP levels (7). The reduction in β_3 receptor mRNA may involve destabilization of the message, and it is possible that exons 2 and 3, which encode the 3′ nontranslated sequence, contain sequences that are involved in this modulation of mRNA stability (13).

Inspection of the sequences of the two introns has identified two inverted repeats in the first intron. Inverted repeats sometimes serve as binding sites for transcription factors, and it is conceivable that these sequences could operate as enhancers of β_3 receptor gene transcription. Tissue-specific enhancers have been identified in the first intron of several genes (14, 15), and, in this regard, the sequence within and surrounding one of the inverted repeats in the first intron of the rat β_3 receptor gene bears striking homology with NF-1 (16) and with ARF6 (17) (Fig. 1). Sequences related to NF-1 and ARF6 have been implicated recently in the control of adipose tissue-specific gene expression (17, 18). It is, therefore, tempting to speculate that these sequences play a role in the adipose tissue-specific

[2] J. G. Granneman, unpublished observations.

expression of the β_3 receptor (5). Experiments are currently in progress to evaluate this hypothesis.

Unlike rodents, it is presently unclear whether humans normally express the β_3 receptor. We have analyzed several human tissues (including brain, skeletal muscle, colon, and adipose tissue) for β_3 receptor mRNA, by RNase protection assay, and have yet to detect β_3 receptor mRNA at levels more than 1–2% of β_1 or β_2 receptor mRNA levels.[3] In contrast, rodent β_3 receptors are abundantly expressed only in adipose tissue, where levels of β_3 receptor mRNA are about 5–7 times greater than β_1 receptor mRNA levels (7). The lack of abundant expression in human adipose tissue suggests that the genetic elements controlling expression differ between species. It will be of interest, therefore, to compare the structure of the human and rat genes, especially with respect to the elements that control fat-specific expression in the rat. In this regard, the intron sequences may hold important clues regarding the tissue-specific expression of the β_3 receptor in humans and in rats.

Acknowledgments

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[3] Unpublished observations.

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