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Short sequence-paper

Molecular cloning, organization and localization of the gene for the mouse neuropeptide Y-Y5 receptor [1]

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Abstract

A cDNA clone homologous with the human neuropeptide Y (NPY)-Y5 receptor has been isolated from a mouse brain cDNA library. Analysis of the predicted amino acid sequence indicates that the polypeptide encoded by this cDNA is 89% and 97% identical to the human and rat NPY-Y5 receptors, respectively, but has a repeat sequence of 21-amino acid residues, unlike the human and rat NPY-Y5 receptors, in its N-terminal region. Northern blot analysis indicated that the mouse NPY-Y5 receptor mRNA was highly expressed in the brain, but not in the heart, kidney, spleen, lung, liver, skeletal muscle, and testis. A genomic DNA clone encoding the mouse NPY-Y5 receptor has also been cloned and used as a probe for fluorescence in situ hybridization analysis. The mouse NPY-Y5 receptor gene consists of at least two exons with a 7.0 kbp-long intronic sequence and is localized in the chromosome 8B3-C2 region. Restriction mapping, partial sequencing, and hybridization analysis of the mouse NPY-Y5 and NPY-Y1 receptor genes were used to determine the relationship between the two genes in the chromosome 8B3-C2 region. The mouse NPY-Y5 receptor gene can map about 10 kbp upstream of the transcriptional initiation sites of the NPY-Y1 receptor gene with an opposite orientation.

Keywords: Chromosomal localization; Neuropeptide Y; Y5 receptor; cDNA sequencing; (Mouse)

Neuropeptide Y (NPY) is an important regulator in the central and peripheral nervous system [1,2]. It belong to a family of homologous peptides including the gut peptide YY (PYY) and pancreatic polypeptide (PP), all of which are 36-amino acid peptides characterized by a hairpin loop [3,4]. Studies of various organs and cell types with peptide fragments of NPY have indicated that multiple NPY receptor subtypes exist [1,2]: (1) the Y1 type binds NPY, PYY, and an analog of NPY modified at residues 31 and 34 ([Leu^{31}, Pro^{34}]NPY) > PP and the NPY peptide C-terminal fragment (NPY-(13–36)); (2) the Y2 type binds NPY, PYY, and NPY-(13–36) > PP and [Leu^{31}, Pro^{34}]NPY; (3) the Y3 type binds NPY > PYY; and (4) the Y4 type binds PP > [Leu^{31}, Pro^{34}]NPY > NPY. Recently, Y1 [5–11], Y2 [12–16] and Y4-type [17–19] receptor cDNAs were isolated and found to have sequences similar to members of a G-protein

Abbreviations: NPY, neuropeptide Y; kbp, kilobase pair(s); bp, base pair(s); FISH, fluorescence in situ hybridization; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; ORF, open reading frame

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[1] The nucleotide sequence(s) reported in this paper have been submitted to the GenBank/EMBL Data Bank under the accession number(s) AB001346.

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coupled receptor superfamily. Moreover, the cloning of the human and rat NPY-Y5 [20,21] and the mouse NPY-Y6 [22] receptors was recently reported. The mouse NPY-Y6 receptor has significant homology (56% identity) with the NPY-Y1 receptor, and has universally lost its receptor function in primate species due to a frame shift mutation occurring early in primate evolution [23,24]. The pharmacological characteristics of the NPY-Y6 receptor are distinct from those described for the NPY-Y2, -Y3 and -Y4 receptors and are similar to those described for the NPY-Y1 and 'atypical Y1' receptors. On the other hand, the NPY-Y5 receptor has little identity with other previously cloned NPY receptors. This NPY-Y5 receptor seemed to be of the NPY feeding receptor subtype based on the following criteria: (1) the NPY-Y5 receptor mRNA was detected primarily in the central nervous system, including the paraventricular hypothalamic nucleus and the lateral hypothalamus, which have been implicated in the control of feeding behavior [1], (2) after intracerebroventricular injection of some peptides (e.g. human (3–36)PYY, porcine (2–36)NPY, porcine NPY and human PP) which activated the NPY-Y5 receptor (in vitro profile based on negative coupling to cAMP concentration), these peptides produced a statistically significant stimulation of food intake [20]. Furthermore, the human NPY-Y5 receptor gene is in fact just a few kbp apart from the human NPY-Y1 receptor gene on chromosome 4q(31.3-32) [20]. The *Pst*I polymorphism near the human NPY-Y1 receptor gene was also reported [25]. However, the close relationship between the NPY-Y1 and NPY-Y5 receptor genes, for example short distance between the mouse NPY-Y1 and NPY-Y5 receptor genes, has not been investigated. We therefore isolated the cognate mouse NPY-Y5 receptor cDNA from a brain cDNA library using the rat NPY-Y5 receptor cDNA as a probe, and then obtained the mouse NPY-Y5 receptor gene from a mouse genomic library. We describe here the chromosomal localization of the mouse NPY-Y5 receptor gene, and provide novel information on the relationship between the mouse NPY-Y1 and NPY-Y5 receptor genes.

To obtain a mouse homologue of the NPY-Y5 receptor cDNA, a mouse brain cDNA library (Uni-ZAP XR, 1.0×10^6 plaques) was screened with a 946-base pair (bp) PCR-derived probe spanning amino

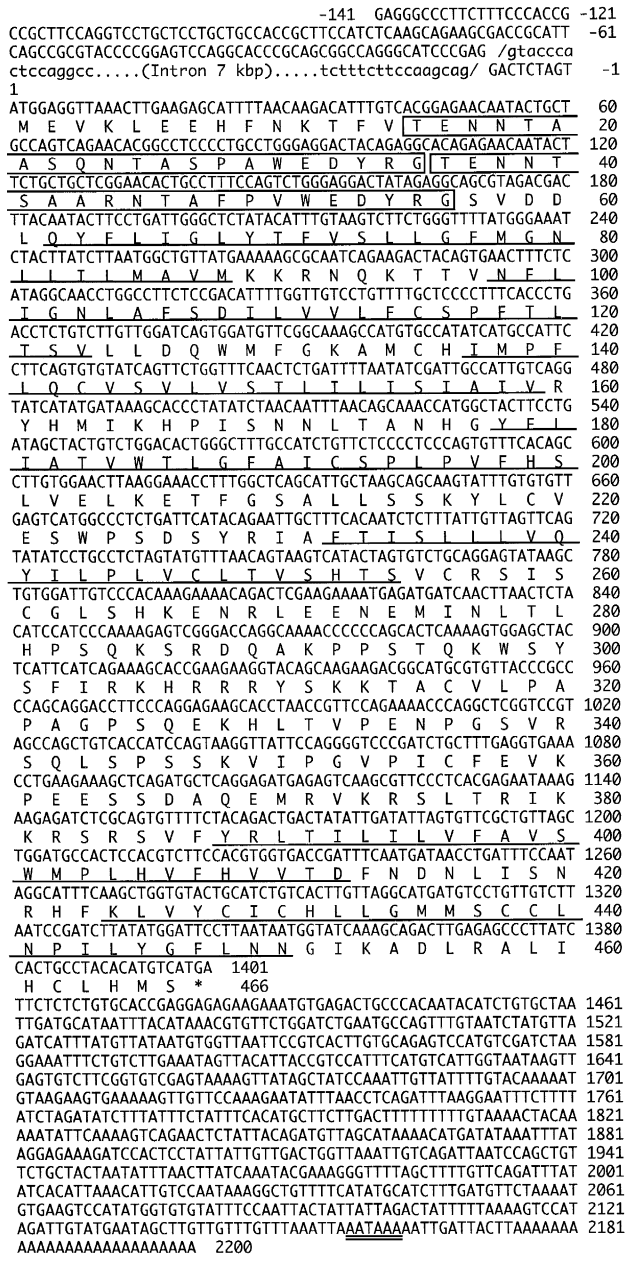

Fig. 1. Nucleotide sequence of the mouse NPY-Y5 receptor (mY5) cDNA (containing the boundary nucleotide sequences of the exon/intron of the NPY-Y5 receptor gene). The mY5 cDNA was sequenced using the dideoxynucleotide chain termination method on a double-stranded template [27]. The deduced amino acids of the mouse NPY-Y5 receptor are shown in single-letter codes under its nucleotide sequence. The sequences of the exon and intron (position $-9/-10$) are represented by capital and lower case letters, respectively. The seven putative transmembrane domains are underlined. The repeat sequences in the N-terminal region are boxed. The potential polyadenylation signal is double-underlined.

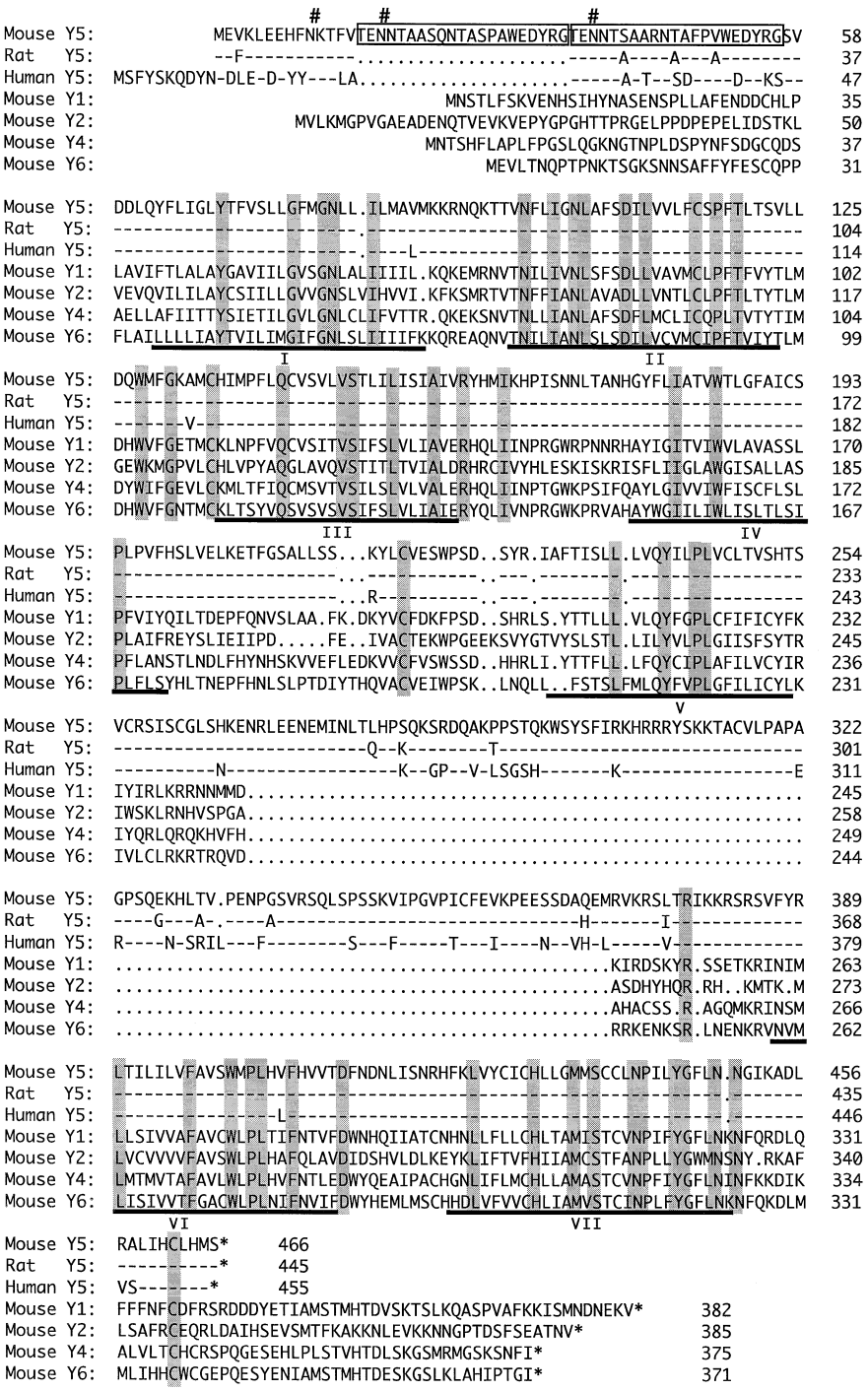

Fig. 2. Alignment of the predicted amino acid sequence of the mouse NPY-Y5 receptor with the rat NPY-Y5, human NPY-Y5, mouse NPY-Y1, the mouse NPY-Y2, the mouse NPY-Y4, and the mouse NPY-Y6 receptor sequences. Periods represent spaces added for proper alignment. Numbers correspond to the amino acid positions of each receptor. The seven putative transmembrane domains are underlined and numbered I–VII. Identical amino acids of the human and rat NPY-Y5 receptors to mouse protein are shown by dashes. The '#' above an amino acid sequence indicates a potential N-linked glycosylation site in the N-terminal extracellular region of the mouse NPY-Y5 receptor. The repeat sequence in the N-terminal region of the mouse NPY-Y5 receptor are boxed. Residues identical among the seven receptors are shaded in *gray*.

acids 26–341 of the rat NPY-Y5 receptor [20,21]. One positive clone, designated mY5, has a 2341-bp insert DNA containing a 1398-bp open reading frame (ORF) (Fig. 1). Since analysis of the predicted amino acid sequence indicates that the polypeptide encoded by this cDNA has seven transmembrane regions typical of G-protein coupled receptors and is 89% and 97% homologous to the human and rat NPY-Y5 receptors, respectively, it is the mouse homologue of the NPY-Y5 receptor (Fig. 2). The amino acid sequences of the rat NPY-Y5 receptor reported by Gerald et al. [20] is shown as containing eleven extra N-terminal residues as compared with that published by Hu et al. [21]. The reading frame of the N-terminal of the mouse NPY-Y5 receptor is in agreement with the latter. Interestingly, the 21-amino acid repeat sequences that were not observed in the human and rat proteins [20,21] were contained in the N-terminal extracellular region (Figs. 1 and 2). Furthermore, there seem to be three *N*-linked glycosylation sites (NxS/T) in its N-terminal domain (two of these sites are in the 21-amino acid repeat sequences). A long polyadenylation sequence was found at the 3′-end of the clone, suggesting that the cDNA clone encompasses most of the mRNA species observed in the Northern blot analysis (Fig. 3B). Comparison of the amino acid sequence of the mouse NPY-Y5 receptor to those of the mouse NPY-Y1 [7,10], mouse NPY-Y2 [16], mouse NPY-Y4 [19] and mouse NPY-Y6 [22] receptors reveals extensive differences. As shown in Fig. 2, at the amino acid level the mouse NPY-Y5 receptor exhibits low overall identities of 26%, 22%, 25% and 25% with the NPY-Y1, NPY-Y2, NPY-Y4 and NPY-Y6 receptors, respectively. The alignment scores for the transmembrane (TM) domains are 45%, 45%, 44% and 40% identity with the NPY-Y1, NPY-Y2, NPY-Y4 and NPY-Y6 receptors, respectively. Interestingly, a high identity was observed in the TM7 domain of these receptors. Southern blot analysis of the mouse genomic DNA indicated the pres-

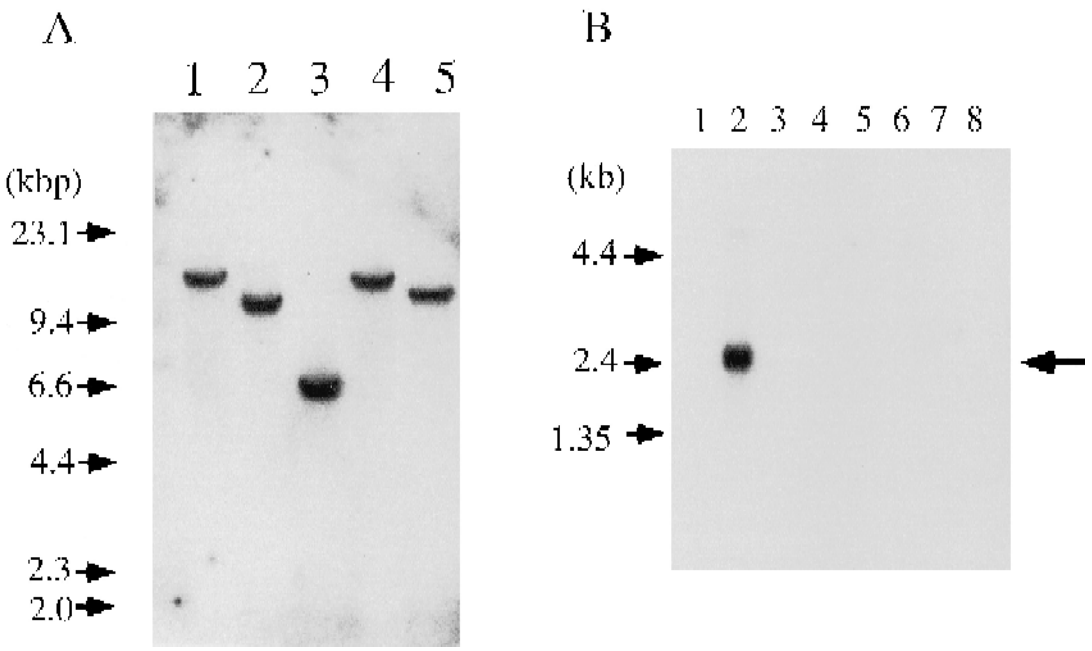

Fig. 3. Southern and Northern blot analyses. (A) Southern blot analysis of a mouse genomic DNA. Mouse genomic DNA was digested with *Hin*dIII (lane 1), *Kpn*I (lane 2), *Sac*I (lane 3), *Bam*HI (lane 4), or *Eco*RI (lane 5). By using the ^{32}P-labeled mY5 cDNA as a probe, hybridization was carried out at 65°C for 2 h in Rapid Hybridization Buffer (Amersham) and the filter was washed at 65°C for 30 min in 0.2 × SSC containing 0.1% SDS. (B) Northern blot analysis of the mouse NPY-Y5 receptor mRNA. Mouse Multiple Tissue Northern Blot that contained 2 μg of mRNA from various sources was purchased from Clontech. By using the mY5 cDNA as a probe, hybridization was carried out at 65°C for 2 h in Rapid Hybridization Buffer, and the filter was successively washed at 65°C for 20 min in 2 × SSC containing 0.1% SDS and then at 65°C for 30 min in 0.2 × SSC containing 0.1% SDS. Each lane contains 2 μg of mRNA. Lane 1, heart; lane 2, brain; lane 3, spleen; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, testis.

ence of single *Hin*dIII, *Kpn*I, *Sac*I, *Bam*HI or *Eco*RI restriction fragments, when probed with the full-length cDNA of the mY5 clone (Fig. 3A). This result means that there is a single NPY-Y5 receptor gene. The expression of the receptor mRNA in various mouse tissues was investigated by Northern blot analysis (Fig. 3B). Among them, only brain showed a band in the range of about 2.5 kilobases. No signal was obtained from any of the other peripheral tissues, such as heart, spleen, lung, liver, skeletal muscle, kidney, or testis. This expression pattern is almost identical to that of the rat NPY-Y5 receptor [20,21].

To further investigate the genomic structure of the mouse NPY-Y5 receptor gene, we isolated its genomic clones. Upon screening approximately 1.0×10^6 plaques of a mouse genomic DNA library (λFIX II) with the mY5 cDNA (full-lenght) as a probe, we obtained three positive clones. Two of them, λgY5-1 (approximately 12 kbp long) and λgY5-2 (approximately 20 kbp long), were subjected to extensive characterization. Restriction mapping of these clones are shown in Fig. 4A. Partial sequence and Southern blot analyses revealed that λgY5-2 contained the entire NPY-Y5 receptor ORF, including the 5′- and 3′-untranslated regions (Figs. 1 and 4). As shown in Figs. 1 and 4, the mouse NPY-Y5 receptor gene has at least one intron. This intron, approximately 7.0 kbp long, was located at position $-9/-10$ in the 5′-untranslated region of the NPY-Y5 receptor cDNA. Sequences around the putative junction are shown in Fig. 1. The nucleotide sequence of the intron adjoining the splice junction is the recognized consensus sequence GT/AG. Hu et al. reported that one of the isolated rat cDNA clones contained a 123-bp insertion at position $-9/-10$ in the 5′-untranslated region and suggested the existence of intron(s) in this portion of the rat NPY-Y5 receptor gene [21]. The position of this insertion is consistent with the location of the intron of the mouse NPY-Y5 receptor gene.

Recently, Gerald et al. [20] reported that the human NPY-Y5 and NPY-Y1 receptor genes could map to the same locus but with an opposite orientation, because the reverse complement sequences of the human NPY-Y5 coding region (transmembrane domain VI-VII and carboxy tail regions) showed a 99.6% match with the exon 1C region (including its flanking sequences) located in the 5′-noncoding region of the Y1C alternate splice variant mRNA of the human NPY-Y1 receptor [25,26]. To confirm the close relationship between the mouse NPY-Y5 and NPY-Y1 receptor genes, we compared the restriction mapping and sequences of the isolated NPY-Y5 receptor genomic clones with those of the previously

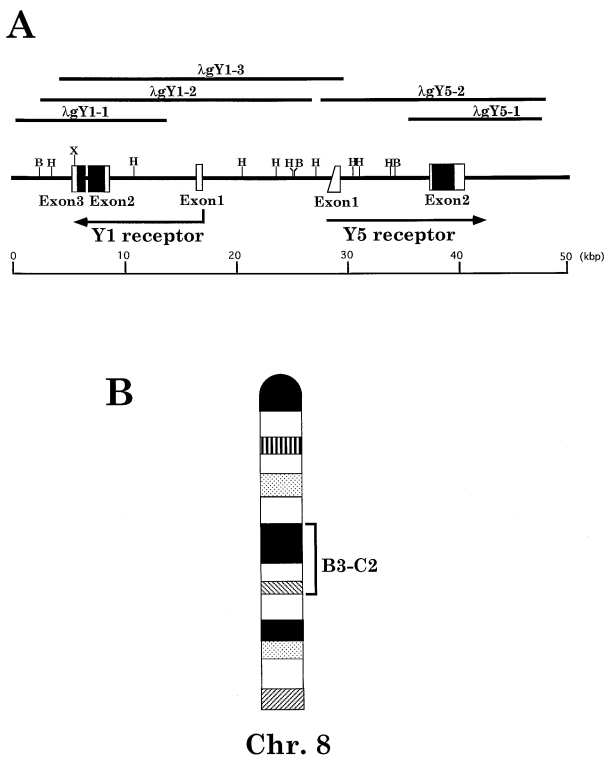

Fig. 4. (A) Structure of the mouse NPY-Y5 and NPY-Y1 receptor genes. The isolated mouse genomic clones (λgY5-1 and λgY5-2 for the NPY-Y5 receptor gene, and λgY1-1, λgY1-2 and λgY1-3 for the NPY-Y1 receptor gene) are indicated by horizontal bars above the gene. The closed and the open boxes indicate coding and noncoding regions in the exons, respectively. Pertinent restriction enzyme sites are noted (B, *Bam*HI; H, *Hin*dIII; X, *Xho*I). The transcriptional start points of the NPY-Y1 receptor gene were determined by Eva et al. [7], whereas those of the NPY-Y5 receptor gene are still unknown. (B) Chromosomal mapping of the mouse NPY-Y5 and NPY-Y1 receptor genes by fluorescence in situ hybridization (FISH) analysis. Mouse chromosome 8 is shown schematically. The R-banding for direct mapping with FISH was carried out according to the method of Takahashi et al. [28,29]. Plasmid containing the mouse NPY-Y5 or NPY-Y1 receptor genomic clones, λgY5-2 or λgY1-2, was labeled with biotin-16-dUTP using a nick translation kit (Boehringer Mannheim) according to the supplier's instruction. The FISH procedure was carried out according to the method of Trask et al. [30,31].

isolated mouse NPY-Y1 receptor genomic clones (λgY1-1, λgY1-2 and λgY1-3) [10]. We previously showed that the mouse NPY-Y1 receptor gene comprised three exons with a 6.5 kbp 5′-noncoding and a 108-bp internal intronic sequences [10]; the multiple transcriptional initiation sites of the mouse NPY-Y1 receptor gene were determined by Eva et al. [7]. Partial sequence, restriction mapping, and Southern blot analysis of these genomic clones revealed that the 5′-end region (approximately 2 kbp) of one of the isolated NPY-Y1 receptor genomic clone (λgY1-3) was an exact match for the reverse sequence of the 5′-end region of the λgY5-2 clone (Fig. 4A). These results taken together indicate that the 5′-noncoding exon (referred to as exon1 in Fig. 4A) of the mouse NPY-Y5 receptor gene can map about 10 kbp upstream of the transcriptional initiation sites of the NPY-Y1 receptor gene with an opposite orientation. Next, to further determine the chromosomal localization of the mouse NPY-Y5 gene, metaphase chromosome from mouse lymphocytes showing typical R-bands were examined using the DNA fragment of λgY5-2 as a probe. The fluorescence signals observed with the probe for the NPY-Y5 gene were specific to chromosome 8B3-C2 (99 signals out of a total of 137 signals, $P < 0.01$), as identified from R-banding pattern (Fig. 4B). There was no other significant signal cluster on any other chromosomes. This result demonstrates that there is a single gene for the mouse NPY-Y5 receptor localized in the region of chromosome 8B3-C2. The same localization was observed by using the mouse NPY-Y1 receptor genomic clone, λgY1-2, as a probe (95 signals out of a total of 134 signals, $P < 0.01$). Interestingly, the regions where the mouse NPY-Y5 and NPY-Y1 receptor genes are localized are not in a region homologous with human chromosome 4q(31.3-32) where the gene for the human NPY-Y1 receptor maps [25].

In summary, we have presented the structure and genomic localization of the mouse NPY-Y5 receptor. These observation might have important implications and it will be interesting to determine whether this locus is associated with eating disorders or obesity in mouse.

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