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# A novel leptin receptor isoform in rat

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**Abstract Five mouse and human leptin receptors (Ob-R) have recently been identified, a long isoform (Ob-Rb), preferentially expressed in hypothalamus, and 4 short isoforms, Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Re. We have identified a new short isoform in the rat, r-OB-Rf, with 6 C-terminal amino acids and a 3′ untranslated region without homology to other Ob-R isoforms. Its higher expression in rat liver and spleen compared to brain, stomach, kidney, thymus, heart, lung and hypothalamus, contrasts with Ob-Ra and Ob-Rb homologues and raises possibilities of as yet unidentified roles for members of the growing Ob-R gene family.**

*Key words:* Leptin receptor isoform; Ob-R gene

## 1 Introduction

The recent cloning of the mouse leptin receptor (Ob-R) [1] has led to the identification of a mutation in the Ob-R of the db/db mouse [2,3], thereby fulfilling a prediction made 30 years ago by Hummel et al. [4] of a mutation in the fourth chromosome. The phenotype of obesity and diabetes in db/db mice has been ascribed to a mutant Ob-R mRNA that contains a 106 bp insertion. This insertion occurs because of a point mutation that creates a consensus splice donor site, and results in the apparent failure to synthesize the long form of Ob-R. The 106 bp insertion is identical to the short form Ob-R sequence, including a stop codon prior to the C-terminal cytoplasmic tail of the long form of the receptor. The implication of these findings is that the long form of Ob-R is responsible for transducing the leptin-induced signals that regulate food intake, thermogenesis, and body weight [5–7], leaving the physiological significance of forms of Ob-R with short cytoplasmic tails undefined. Since rats may well be the species of choice for the study of leptin receptor functions and regulation, we have cloned cDNAs encoding several isoforms of Ob-R, including a novel one, from normal rat brain and have characterized the tissue expression of their corresponding transcripts.

## 2 Materials and methods

### 2.1. Screening of a rat brain cDNA library

A cDNA library prepared from Sprague-Dawley rat brain in lambda ZAP II phage (Stratagene, Inc) was plated at a density of $10^4$ plaques per 150 mm petri dish, lifted onto Hybond-N plus membranes (Amersham), and then screened with a mixture of six synthetic oligonucleotides derived from human [1] and mouse [1,2] leptin receptor cDNA sequences. The sequences of the oligonucleotides used are 5′TGTGGTTTTGTTACACTGGGAATTTCTTTA-3′, 5′ACAGATGATGGTAATTTAAAGATTTCTTGG-3′, 5′CCCAAAAACTGCGTCTTACAGAGAGACGG-3′, 5′GGATATTGGAGTAATTGGAGCAATCCAGCC-3′, 5′GGGGATAAGCACTGAGTGACTCC-3′, and 5′GATGTTCCAAACCCCAAGAATTGTTCCTGG-3′. These oligonucleotides were end-labeled using [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase. Hybridization of the probes with filters was performed overnight at 42°C in Rapid-hyb buffer (Amersham). The membranes were then successively washed once in 2×SSC and 0.1% SDS at room temperature for 15 min and twice in 0.1×SSC and 0.1% SDS at 42°C for 30 min. Following washing, they were exposed overnight to XAR-5 film (Eastman Kodak) with an intensifying screen at −70°C. Several positive clones were identified and plaque-purified. The cDNA inserts in the lambda vector of these clones were either directly sequenced using the fmol DNA cycle sequencing kit (Promega) or automated sequencing (Applied Biosystems Model 377) using oligonucleotides annealing to the T3 and T7 promoter sequences of the lambda zap vector, or excised and subcloned into pBluescript SK(−) (Stratagene). In the latter case, the desired cDNAs were sequenced on both strands using oligonucleotide primers with sequences derived from internal regions of known leptin receptor cDNAs.

### 2.2. Reverse transcription-polymerase chain reaction (RT-PCR) amplification of leptin receptor transcripts

Total RNA was extracted from freshly dissected tissues from Wistar rats (150–250 g animals), using TRIzol Reagent (Life Technologies). Reverse transcription was carried out using 1 μg of total RNA isolated from various tissues. First-strand cDNA, reverse-transcribed with MMLV reverse transcriptase (Clontech) using oligo $d(T)_{18}$ as primer, was PCR-amplified using a primer from the transmembrane region of the mouse leptin receptor sequence [1] opposed with various primers corresponding to C-terminal regions of mouse or rat leptin receptor isoforms. The specific oligonucleotide pairs used were as follows: (a) for rat leptin receptor isoform a, 5′TATGTCATTGTACCGATAATTATT-3′ (termed primer TM), and 5′AGTGATCTTTAATTAAAATAGGTT-3′ (termed primer rRa); (b) for rat isoform b, primer TM and 5′CAGAGAAGTTAGCACTGTT-3′ (primer rRb); (c) for rat isoform f, primer TM and 5′GGGTACCTGCACACATATGTG-3′ (primer rRf). As a control, 'mock' amplifications were carried out in the presence of RNA template and Pfu polymerase, but in the absence of reverse transcriptase. As a control for RNA quality and quantity, β-actin mRNA was amplified from all RNA samples using oligonucleotides 5′CGTAAAGACCTCTATTGCCAA-3′ and 5′AGCCATGCCAAATGTGTCAT-3′, based upon the sequence of rat β-actin [8]. The 50 μl of PCR reactions contained, in addition to first strand cDNA as template, 0.5 μM of each primer, 0.2 mM each of dNTP, and 1.25 units of Pfu DNA polymerase in 1×Pfu polymerase buffer (Stratagene). All PCR reactions were performed with a RoboCycler Gradient temperature Cycler (Stratagene). The scheme for Ob-R PCR reactions was 94°C for 2 min, followed by 50 cycles, each consisting of 30 s at 92°C, 30 s at 50°C, and 1 min at 72°C, followed by single-cycle extension for 10 min at 72°C. RT-PCR of β-actin was performed by similar methods, except that the reaction was limited to 30 cycles.

### 2.3. Sequence analysis of RT-PCR products

RT-PCR reaction products encompassing the intracellular segment of the rat Ob-R long isoform were first electrophoresed on a 2% low melting-point agarose gel (FMS). Gel bands of interest were excised, and DNAs were isolated by using a QIAEX II Gel extraction kit (QIAGEN). The gel-purified PCR products were directly sequenced with primers originally used in the PCR reactions.

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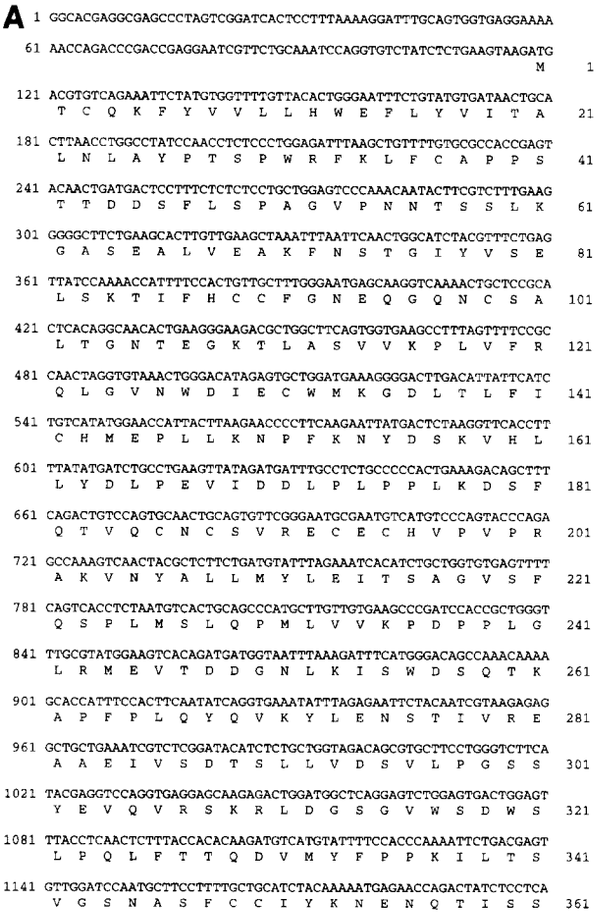
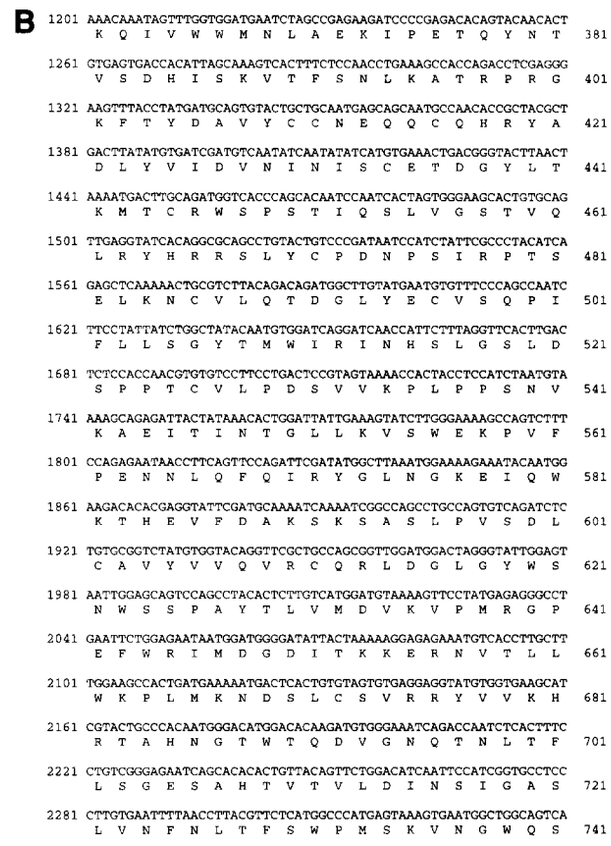
## 3. Results and discussion

A Sprague-Dawley rat brain cDNA library was screened with a mixture of six oligonucleotides from the mouse and human leptin receptor cDNA sequences. Among a total of eight cDNA clones that were isolated and characterized, one



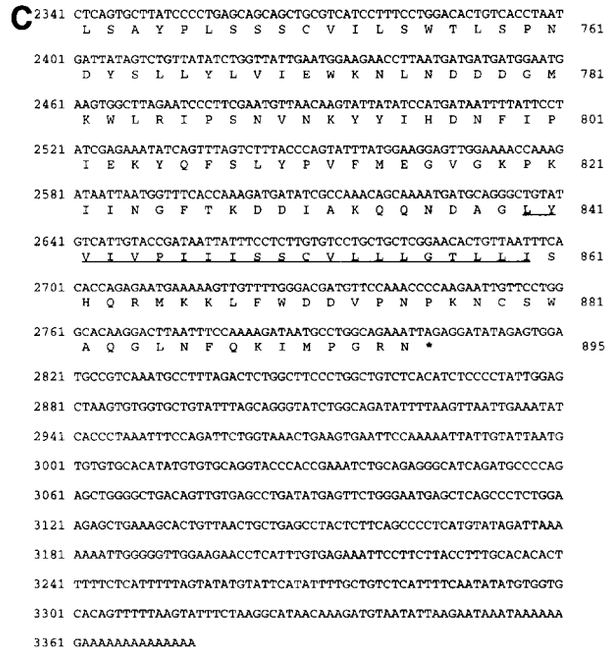




Fig. 1. Nucleotide sequence and deduced amino acid sequence of a novel rat leptin receptor isoform cDNA (r-Ob-Rf). The complete coding region along with portions of 5′- and 3′-noncoding sequences (Genband accession no. U53144) is shown with the predicted amino acid sequence in standard single-letter code. The numbers of the nucleotide and amino acid sequences are shown at the far left and right sides, respectively. The stop codon is marked by an asterisk. The putative membrane-spanning region is underlined.

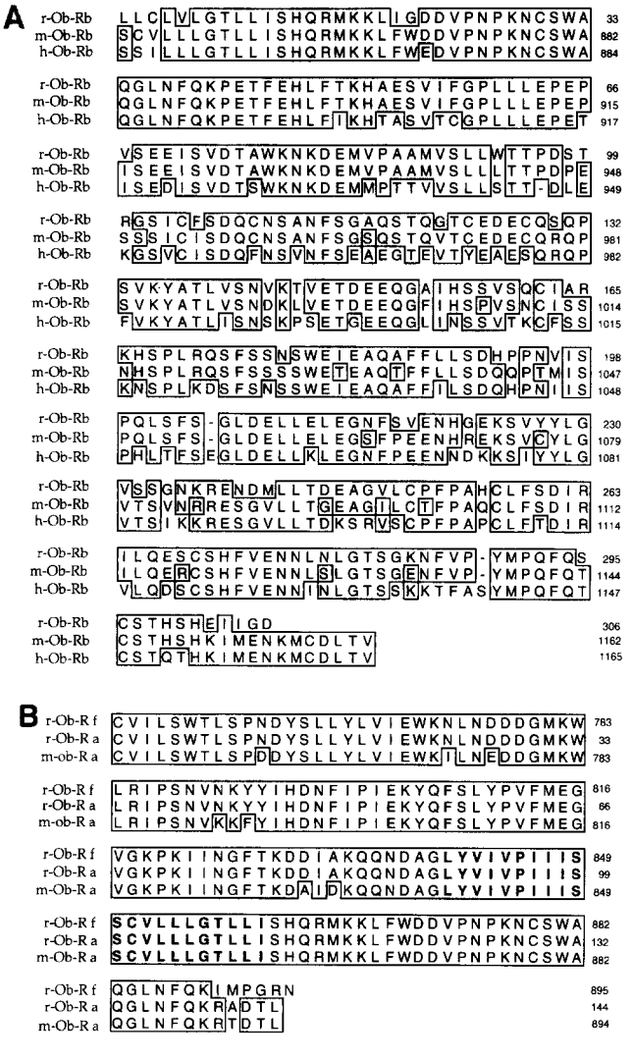


Fig. 2. Rat leptin receptor isoforms are highly homologous to human and mouse receptors. (A) Alignment of amino acid sequence of the rat leptin receptor short isoform (r-Ob-Ra), deduced from a partial cDNA sequence, the corresponding segment of the novel rat leptin receptor short isoform (r-Ob-Rf) shown in its entirety in Fig. 1, and the mouse leptin receptor short isoform (Ob-Ra) (1,2) sequence. The numbering of the mouse Ob-Ra amino acid sequence is from [1]. (B) Alignment of amino acid sequence of the rat leptin receptor long isoform (r-Ob-Rb), predicted from sequence of a RT-PCR fragment, with the corresponding segment of the mouse (Ob-Rb) and human (h-Ob-Rb) leptin receptor long isoforms. In A and B identical amino acids among any two sequences shown are boxed. Gaps in the sequences are introduced as dashes for optimal alignment.

was found to encode an intact open reading frame with strong homology to the mouse and human leptin receptors, but including a short intracellular domain unlike any previously cloned isoform [1–3]. The nucleotide and deduced amino acid sequence of this novel Ob-R cDNA (designated r-Ob-Rf, in keeping with the nomenclature of Friedman and colleagues [2]), is shown in Fig. 1. The clone predicts a protein of 895 amino acids, with a predicted molecular mass of 101.3 kDa. The first 889 amino acids of r-Ob-Rf clearly align with the corresponding region of the mouse and human Ob-R proteins (this region is identical in the long and short isoforms), with 92 and 77% identity, respectively. Several alternative splice forms of human and mouse Ob-R with unique C-terminal segments and 3′ untranslated regions have been described, all of which are produced by splicing of discrete 3′ exons downstream of the codon for amino acid 889 [2,3]. Our rat clone appears to represent a novel splice variant of this class, in that it contains, distal to the codon for amino acid 889, a sequence that is different from any form thus far described in the mouse or human [1–3]. Specifically, the new sequence predicts a novel six amino acid C-terminal peptide (IMPGRN), followed by a stop codon and a 3′ untranslated region without homology to any of the published mouse or human sequences.

In addition to the novel r-Ob-Rf form, we obtained other Ob-R clones from the rat brain cDNA library or by RT-PCR amplification with primers from the transmembrane and intracellular domains of the mouse Ob-R. One of these cDNA clones was 1.8 kB in length, with a partial open reading frame of 144 amino acids. This fragment was 94.4% identical to the Ob-Ra (short) isoform of the mouse [1,2], and has been designated r-Ob-Ra (Fig. 2A). A second rat isoform was 918 nucleotides in length, encoding 306 amino acids, and was 88 percent identical to the Ob-Rb (long) isoform of the mouse and 69% identical to the human isoform [2,3]. This clone has been designated r-Ob-Rb (Fig. 2B). The three other splice variants identified in the mouse, all encoding short C-terminal segments and termed Ob-Rc, Ob-Rd and Ob-Re [2] were not detected in our screen of the rat brain cDNA library. Since no attempt was made to identify these isoforms by RT-PCR amplification from rat tissues, it remains possible that these splice variants are also expressed in the rat.

Tissue expression of the previously known (r-Ob-Ra, r-Ob-Rb) and novel (r-Ob-Rf) leptin receptor isoforms was evaluated by RT-PCR analysis of RNA isolated from Wistar rat brain, liver, stomach, kidney, spleen, lung, thymus, heart, testis, and hypothalamus. An oligonucleotide corresponding to a segment of the transmembrane domain (oligo TM) was used as the 5′ primer in all three sets of reactions, while oligonucleotides derived from the isoform-specific alternatively spliced exons were used as the 3′ primers. Fig. 3 shows that each of the three primer pairs amplified products of predicted size (487 bp for r-Ob-Ra, 370 bp for r-Ob-Rb, and 390 bp for r-Ob-Rf) in a variety of tissues. The amount of RNA used for RT-PCR was the same for each tissue based on spectrophotometric determination. Furthermore, a similar level of amplified β-actin transcript was obtained in all 11 of the lanes containing rat RNA, providing assurance that RNA of similar quality and quantity was used for each of the amplification reactions. These methods allow assessment of the relative levels of a specific isoform of Ob-R in different tissues, but do not permit comparison of levels of the three different isoforms within a single tissue, since the efficiency of the isoform-specific primer pairs may be different.

Interestingly, the three types of Ob-R clearly have a different pattern of tissue expression. r-Ob-Ra is relatively abundant in rat brain, liver, stomach, kidney, lung, heart, and hypothalamus, is present at significantly lower levels in testis, and is undetectable or at very low levels in thymus and spleen, respectively. r-Ob-Rb is relatively abundant in brain and hypothalamus, is expressed at lower levels in stomach, spleen, lung, thymus, and heart, and is undetectable in liver, kidney, and testis.

Amplification with primers specific for the novel r-Ob-Rf demonstrates that this leptin receptor isoform is in fact ex-

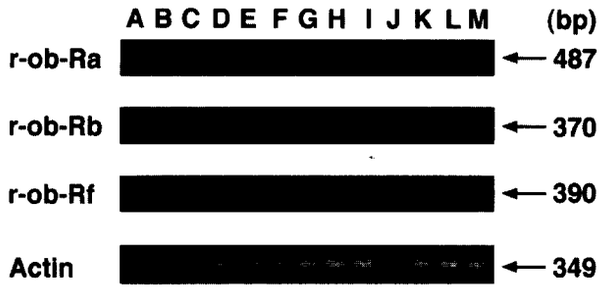



Fig. 3. Tissue distribution of rat leptin receptor isoforms. Amplified products corresponding to r-Ob-Ra, r-Ob-Rb and r-Ob-Rc isoforms were detected by RT-PCR using the primer pairs described in Section 2. The sizes of the PCR products are 487, 370, and 390 bp for the r-Ob-Ra, r-Ob-Rb, and r-Ob-Rf isoforms, respectively. RT-PCR analysis of actin mRNA is included as a control for RNA quality and amount. Lanes contain amplified RNA from the following tissue sources: A, 'mock' RT-PCR control (RT-PCR performed in the absence of reverse transcriptase); B, mouse brain; C, Zucker diabetic fatty lean rat hypothalamus; D, Wistar rat hypothalamus; E, Wistar rat testis; F, Wistar rat heart; G, Wistar rat thymus; H, Wistar rat lung; I, Wistar rat spleen; J, Wistar rat kidney; K, Wistar rat stomach; L, Wistar rat liver; M, Wistar rat brain. The size of the amplified bands was estimated by alignment with 100-bp DNA ladder markers (LIFE Tech; not shown).

pressed in a wide variety of tissues, and is therefore not an artifact of cDNA library construction (Fig. 3). Our data also show that the oligonucleotide pair chosen for amplifying r-Ob-Rf in the rat amplifies a band of similar size from mouse brain, indicating that this new isoform is expressed in species other than the rat. r-Ob-Rf is expressed at detectable levels in all of the tissues examined, with a relatively low level of expression in testis, and moderate to higher levels in brain, liver, stomach, kidney, lung, heart, thymus, spleen, and hypothalamus.

Interesting differences in tissue expression pattern are observed when comparing r-Ob-Rf with r-Ob-Ra and r-Ob-Rb. Firstly, the two short isoforms, r-Ob-Ra and r-Ob-Rf, are relatively abundant in liver and kidney, while the long isoform Ob-Rb is undetectable in these two tissues. For unexplained reasons, our findings in the rat differ from previous RT-PCR analysis in mouse tissues, where expression of Ob-Ra and three other short Ob-R isoforms (c, d, and e) was not detected in kidney, and with the exception of Ob-Rc, in liver either [2]. Secondly, r-Ob-Rf is expressed at high levels in spleen relative to other tissues in which it is expressed, while r-Ob-Ra and r-Ob-Rb are expressed at relatively low or undetectable levels, respectively, in this tissue. Finally, r-Ob-Rf is expressed at a moderate level in thymus, while the other short form analyzed in this study, r-Ob-Ra is undetectable in this tissue.

The differences in the patterns of tissue expression of the r-Ob isoforms may reflect differences in their functions. The fact that the intact C-terminal tail of the Ob-Rb isoform appears to be required for transducing the leptin-induced signals that regulate food intake, thermogenesis and body weight [2,3] does not necessarily signify that Ob-R isoforms with short C-terminal segments have no signalling function. The leptin receptor sequence [1] has been shown to be closely related to the gp130 signal-transducing component of the IL-6, G-CSF, and LIF receptors [9–12]. Some members of this general class of cytokine receptor/signal transducing molecules have short or even absent cytoplasmic tails, including the IL-3, IL-5, and IL-6, and GM-CSF receptors, and mutagenesis or deletion of this short cytoplasmic tail abrogates signalling in at least some cases [13]. It has been proposed that variations in structure of cytoplasmic domains may be a critical determinant for formation of heterodimeric receptor/signal transducer complexes or for interaction with other types of cellular effectors such as tyrosine kinases. In summary, while functional roles for the novel leptin receptor isoform reported here and the previously described short variants [2,3] remain to be elucidated, the expression of the r-Ob-Rf isoform in tissues involved in immune regulation such as spleen and thymus and the prevalence of short forms such as r-Ob-Ra and r-Ob-Rf in liver and kidney may provide important clues for future studies.

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