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The leptin receptor promoter controls expression of a second distinct protein

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ABSTRACT

The leptin receptor (OB-R) is a single membrane-spanning protein that mediates the weight-regulatory effects of leptin (OB protein). Several mRNA splice variants have been described which either encode OB-R proteins with cytoplasmic domains of different length or the *OB-R* and *B219/OBR* variants, which have different 5′-untranslated regions. Here we report evidence for the synthesis of a human mRNA splice variant of the *OB-R* gene that potentially encodes a novel protein, leptin receptor gene-related protein (OB-RGRP), which displays no sequence similarity to the leptin receptor itself. This *OB-RGRP* transcript contains the first two *OB-R* gene 5′-untranslated exons, but then is alternatively spliced to two novel exons which were mapped to a yeast artificial chromosome containing the leptin receptor gene. First identified by analysis of a large human expressed sequence tag database, the *OB-RGRP* transcript has now also been found in human and mouse tissues by the use of PCR. Preliminary experiments suggest that *OB-RGRP* and the *OB-R* variants share similar patterns of expression that are distinct from that of the *B219/OBR* variant. OB-RGRP is highly homologous to putative open reading frames in both yeast and *Caenorhabditis elegans*, suggesting a phylogenetically conserved role for this novel protein.

INTRODUCTION

Leptin and the leptin receptor have recently been reported to play key roles in the regulation of body weight of rodents. The *obese* phenotype of *ob/ob* mice was thus shown to result from a single mutation in the *ob* gene (1), which codes for leptin. The leptin receptor (OB-R) is encoded by a gene found to be defective in obese *db*/*db* mice and in *fa*/*fa* Zucker and fa^k/fa^k Koletsky rats (2–5). The OB-R is a single membrane-spanning receptor homologous to members of the class I cytokine receptor family (6,7). Two 5′-untranslated regions (5′-UTRs) and several 3′-alternative splice variants encoding OB-R with cytoplasmic domains of different length have been described in mouse, rat and human (2,3,6,8–10). Two major isoforms, *B219/OBR* and *OB-R*, differ in their 5′-UTR and in their expression patterns (10).

A single transcription unit may serve to generate more than one protein. For instance, several isoforms can be derived from a single gene locus by alternative pre-mRNA splicing (11). The use of alternative promoters or polyadenylation sites may also generate proteins with different N- or C-terminal regions.

Leaky reading at the first AUG during initiation of translation has been described as another potential mechanism to generate different gene products. Initiation at the first or second AUG generates either long and short isoforms or unrelated proteins when the AUGs are in different, overlapping reading frames (12).

We report here that alternative splicing in the *OB-R* gene may generate either the *OB-R* transcripts or another transcript containing the 5′-UTR of *OB-R* in which an alternative AUG initiation codon starts a distinct open reading frame (ORF). This newly identified human and murine *OB-R* mRNA encodes a putative 14 kDa protein, named OB receptor gene-related protein (OB-RGRP), which is homologous to yeast and *Caenorhabditis elegans* putative ORFs. Genomic organization and cDNA comparison show that the *OB-RGRP* gene shares its promoter and two exons with the *OB-R* gene. The OB-RGRP amino acid sequence is, however, entirely different from that of OB-R.

The double alternative utilization of exons and promoter in this manner has not, until now, been reported for the mammalian genome. The fact that we have cloned similar cDNAs from a mouse library shows that this feature is conserved in humans and rodents. This may suggest that there is a requirement for a coordinate expression of *OB-R* and *OB-RGRP* to elicit the full physiological response to leptin *in vivo*.

MATERIALS AND METHODS

Expression studies

Premade Northern blots were obtained from Clontech Laboratories Inc. and prehybridized at 42°C for 6 h in a hybridization cocktail containing 50% formamide, 5× SSPE, 10× Denhardt's solution, 2.0% (w/v) SDS and 100 µg/ml sheared salmon sperm. The blots were hybridized with a [^{32}P]dCTP-labelled DNA generated by PCR and corresponding to nucleotides 29–979 of huOB-RGRP (see Fig. 1) for 16 h. Northern blots were rinsed twice at room

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temperature with 2× SSC, 0.05% SDS and twice at 50°C for 20 min in 0.1× SSC, 0.1% SDS. Overnight autoradiography was performed using Biomax X-ray film (Kodak).

Reverse transcription–PCR (RT–PCR)

Total cellular RNA from HeLa cells, from a panel of hematopoietic cell lines and from immortalized brown adipocytes (13) were assayed by RT–PCR. For adipose cell line differentiation, cells were cultivated for 3 days in medium containing 6% fetal calf serum to obtain confluence, then cells were refed with ITT medium supplemented with 0.1 µM dexamethasone, 850 nM insulin, 1 nM triiodothyronine, 1 µM pioglitazone and IBMX (0.25 mM for 4 days) for 15–21 days. Reverse transcription was performed on 1 µg mRNA with Superscript™ II reverse transcriptase (Gibco BRL) using random hexamers in a 50 µl reaction. The primer sequences for P1–P4 are 5′-AAGGCCGCAGGCTCCCCCATT-3′, 5′-AGCAGCCGCGGCCCCAGTTC-3′, 5′-TGACAAGTTAAACGCAGTTATCACAT-3′ and 5′-TCTCTGCCTTCGGTCGAGTTG-3′ respectively. The concentrations of the four primers were as follows: P1, 500 nM; P2, 250 nM; P3, 500 nM; P4, 100 nM. The 50 µl PCR reaction contained 10 µl first-strand cDNA, 200 µM each dNTP and 0.3 U Taq polymerase (Promega). The PCR profile was 94°C 3 min, 94°C 20 s; 62°C 30 s, 72°C 30 s for 34 cycles; 72°C 4 min for one cycle. To measure the ratio of the PCR products, quantification on ethidium bromide stained agarose gels was performed for several independent experiments using the Adobe Photoshop program.

Long template PCR

Long template PCRs were performed using the Expand™ Long Template 2 PCR system (Boehringer Mannheim) with human placental DNA. Several PCRs were performed using sense or antisense primers for the four *OB-RGRP* exons.

Sequencing analysis of PCR product

PCR products were precipitated with 0.3 M NaCl and 2.5 vol ethanol, resuspended in water and directly sequenced with both primers. DNA sequencing was performed on an ABI 377 DNA sequencer using the Taq cycle sequencing kit (Applied Biosystems) and dye-terminator sequencing reactions.

RESULTS

The 5′-end of *OB-R* is found in the *OB-RGRP* cDNA, which encodes a putative protein of 14 kDa

The accelerating pace of high-throughput sequencing has led to the production of large expressed sequence tag (EST) databases, both public and proprietary, that are powerful resources for gene discovery. Direct sequence homology searching of EST databases has a significant advantage relative to primer-directed PCR cloning, as the results are inclusive rather than selective. We have used the human leptin receptor cDNA sequence to search both a private (Incyte Pharmaceuticals) and a public (Washington University/Merck) database for matching EST sequences.

A number of EST sequences were identified that exactly matched the published leptin receptor sequence from +12 to +173 (6) and then abruptly diverged in sequence. For human mRNA different RT–PCRs were performed to confirm the assembly of the EST sequence (data not shown). Assembly of the EST matching sequences and RT–PCR product sequences revealed a consensus alternative transcript (accession no. Y12670) which contains an AUG at nucleotides 71–73 flanked by Kozak consensus sequences. Translation from this initiation site would yield a polypeptide of 131 amino acid residues with a molecular mass of 14.255 Da (p14 $^{OB–RGRP}$, leptin receptor gene-related protein or OB-RGRP) (Fig. 1A). Surprisingly, the AUG of *OB-RGRP* is present in the 5′-UTR of the *OB-R* transcript. The putative translation from this AUG ends 4 nt upstream of the AUG of *OB-R* and yields a truncated OB-RGRP polypeptide of 36 amino acid residues, molecular mass 3.652 Da (Fig. 1B). The predicted strength of these two AUG signals, calculated according to the score described in Lida *et al.* (14), showed that both scores are compatible with a strong initiation of translation (4.6 and 5.2 for the AUG of *OB-RGRP* and *OB-R* respectively).

The divergence in sequence between *OB-RGRP* and *OB-R* occurs at position 163 of the *OB-RGRP* nucleotide sequence, which marks the alternative 5′ regions found in the hematopoeitic-specific form, *B219/OB-R* (10). Downstream of position 163, *OB-RGRP* mRNA shares no sequence homology with the *OB-R* transcripts. This suggests that the 5′-UTR of *OB-R* may be alternatively spliced to novel exons to generate the *OB-RGRP* mRNA (Fig. 1B). Similarly, at the same position the *B219/OB-R* and *OB-R* transcripts correspond to alternative splicing of different 5′-UTRs to the downstream coding exons (Fig. 1B). Differences in promoter usage and splicing have been proposed to explain the different 5′-ends of these two leptin receptor mRNAs (10). In contrast, the fact that the *OB-R* and *OB-RGRP* cDNA 5′-ends are nearly identical (Fig. 1A) suggests that they are transcribed from the same promoter. The new type of leptin receptor gene splice variant described here has eluded investigators working with traditional cloning methods because it actually contains no sequence overlapping with the coding region of the receptor.

To determine whether *OB-RGRP* mRNA was conserved in evolution, cDNA prepared from mouse lung and kidney cDNA was PCR amplified using degenerate primers; the 5′-ends of the sense and antisense primers are located at the initial and the stop codons of hu*OB-RGRP* respectively (Fig. 1A). In both cases PCR products of predicted size (396 bp) were produced (data not shown). Sequencing of the mu*OB-RGRP* cDNA revealed a strong conservation of the ORF with the human cDNA (Fig. 1A). The sequence of the C-terminus of the muOB-RGRP protein was confirmed using a genomic clone. The N-terminus of the muOB-RGRP protein, although not determined unambiguously, is likely to be similar to that of huOB-RGRP, since a degenerate primer used for PCR was complementary to this region in hu*OB-RGRP*. To identify a murine *OB-R* 5′ splice mRNA variant containing identical sequence to the mu*OB-RGRP* 5′ sequence, cDNA was PCR amplified using the above 5′ degenerate primer and an antisense primer in the mouse *OB-R* ORF. The partial sequence of the amplified product is represented in Figure 1 and shows the expected homology. This product corresponds to a third 5′-alternative variant of murine *OB-R*. Indeed, two different 5′-UTRs of the *OB-R* have been described in the mouse (6,10), but they display no homology with the *OB-RGRP* cDNA. In rat two groups of cDNAs which encode OB-R differ in the 5′-UTR (8,9) and, as demonstrated here in mouse and human, one 5′-UTR

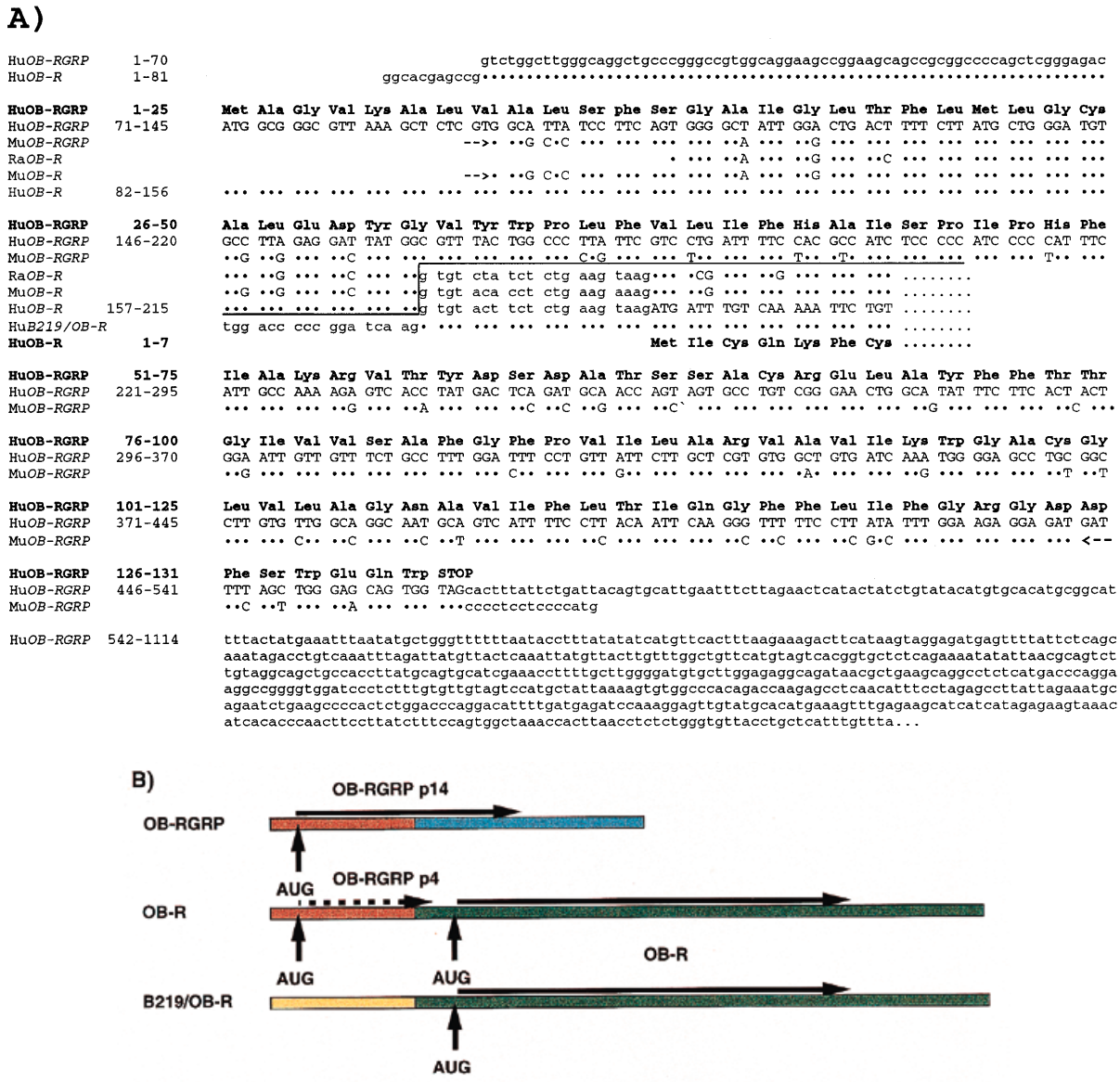

Figure 1. Comparison of cDNA of the human and mouse *OB-RGRP* gene (hu*OB-RGRP* and mu*OB-RGRP*) and the human, rat and mouse leptin receptor (hu*OB-R*, hu*B219/OB-R*, ra*OB-R* and mu*OB-R*). (**A**) The sequence identity of cDNA of the human and murine *OB-RGRP* (hu*OB-RGRP* and mu*OB-RGRP*) and the rat, murine and human *OB-R* (ra*OB-R*, mu*OB-R* and hu*OB-R*) are indicated by dots (•). For each species the *OB-R* and *OB-RGRP* sequences are identical upstream of position 163. The potential translation of this region in the three species is identical. A vertical line indicates the end of this identity. Upstream of this position, hu*B219/OB-R* shares no homology, as indicated by the partial sequence. However, downstream of position 163, hu*B219/OB-R* is identical to hu*OB-R* and shares no homology with *OB-RGRP*. The unrelated sequences between the *OB-RGRP* cDNAs and the *OB-R* cDNAs are separated by a horizontal line. Translation of the human OB-RGRP protein (huOB-RGRP) is indicated. The sequences of hu*OB-R*, hu*B219/OB-R* and ra*OB-R* correspond to published data (6,9,10). The mu*OB-RGRP* cDNA has been obtained by RT–PCR using degenerate primers; the 3′-ends of these primers are indicated by -->. Mu*OB-R* was amplified using the above sense degenerate primer and antisense primers in the *OB-R* coding sequence. (**B**) Schematic representation of the hu*OB-RGRP*, hu*OB-R* and hu*B219/OBR* transcripts. The AUG initiation codons and the ORFs of *OB-RGRP* p14 and *OB-R* are indicated by arrows. For the ORF the arrows end at the stop codon. The putative ORF of *OB-RGRP* p4 is indicated by a dashed arrow. The colours represent identical sequence in the cDNA.

which is likely to be partial (9) has a sequence that encodes the N-terminal residues of the OB-RGRP protein (Fig. 1A). We retain the name OB-R for the OB-R transcripts with a 5′-UTR identical to the *OB-RGRP* mRNA 5′-end. Thus in human, mouse and probably in rat *OB-RGRP* and one *OB-R* share 5′ exons, while *B219/OB-R* has different 5′ exons.

Human genomic organization of OB-RGRP

Sense and antisense primers from the *OB-RGRP* cDNA were used to amplify genomic DNA from human placental DNA by long template PCR. The restriction map and partial sequence of the amplified product were determined to elucidate the genomic

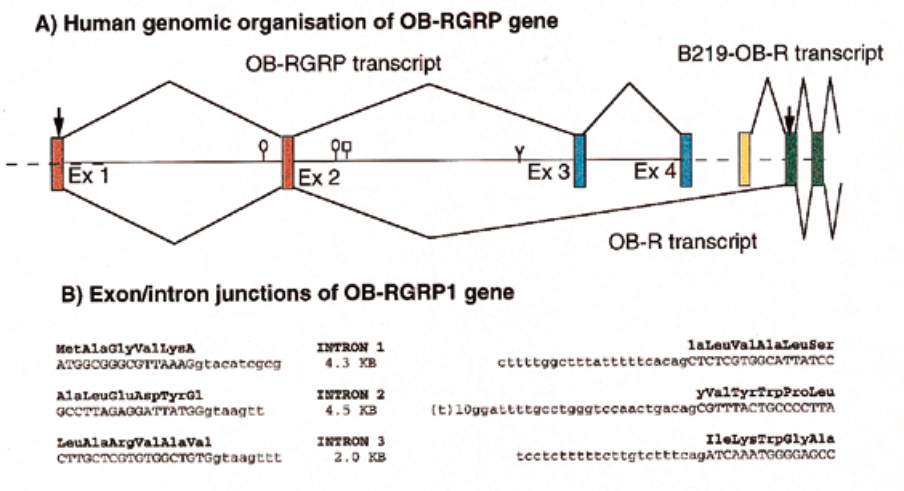

Figure 2. Genomic organization of *OB-RGRP* and exon/intron junction sequences. (**A**) The genomic organization (exon position) and the restriction sites (the *Eco*RI, *Bam*HI and *Hin*dIII restriction sites are represented by circle, square and Y symbols respectively) of the human *OB-RGRP* gene are indicated. Schematic representation of *OB-R* exons is indicated downstream to the *OB-RGRP* gene. *OB-R* contains 18 coding exons (32). The yellow exon represents the *B219/0B-R* 5′ exon; its localization is unknown. The exon colours are identical to Figure 1B. (**B**) The genomic sequence at the 3′- and 5′-ends of the *OB-RGRP* exons are represented by partial intronic sequences. The size of the introns is indicated.

organization and the exon/intron boundaries of the *OB-RGRP* gene (Fig. 2). The sequence which is common to *OB-RGRP* and *OB-R* mRNAs corresponds to the first two exons of *OB-R* (Thompson, D.B., Ossowski,V., Sutherland,J., Apel,W. and Biesterdfeld,J., accession numbers U59246–U59248). The second exon of the *OB-R* gene may be spliced alternatively to generate either *OB-RGRP* or the *OB-R* transcripts. Long template PCR, effective for a 10 kb amplification, fails to amplify the DNA between the *OB-RGRP* exons and either *OB-R* exon 3 or *B219/OBR* exon 1, suggesting that the distance between the *OB-RGRP* and downstream *OB-R* exons is probably >10 kb. This is not unexpected, since the *OB-R* gene has been shown to span >100 kb (4).

We also confirmed that *OB-RGRP* is located in the vicinity of *OB-R* by PCR amplification of *OB-RGRP* exon 4 from CEPH-B YAC 897 G11 DNA, which is known to contain human *OB-R* (15; data not shown).

The initial AUG codon of *OB-RGRP* is located in the first exon and the terminal UAX stop codon is located in exon 4. About 750 bases of the 3′-end of the *OB-RGRP* cDNA are located in exon 4, suggesting that this exon is probably last (Figs 1 and 2). The splice site sequences of *OB-RGRP* reveal that the acceptor splice site of exon 3 is unusual. Indeed, the pyrimidine tract upstream of the invariant PyAG trinucleotide (16) of this acceptor splice site is not present. There are only 50% pyrimidines in the 12 nt upstream of the nCAG. We observed, however, a pyrimidine tract further upstream (Fig. 2B). The alternative use of this splice site is likely to determine the initial ratio between *OB-RGRP* and *OB-R* transcripts.

OB-RGRP mRNA expression

The tissue distribution of *OB-RGRP* was analysed on poly(A)$^+$ mRNA Northern blots of several human tissues. The *OB-RGRP* mRNA appears as a band between the 1.3 and 2.4 kb markers (Fig. 3) and is detected in heart, placenta, lung, liver, skeletal muscle, kidney and pancreas. Heart and placenta express

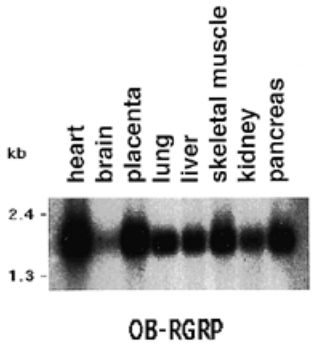

Figure 3. Expression of *OB-RGRP* visualized in a human multiple tissue Northern blot. The Northern blot was probed with a 950 bp *OB-RGRP*-specific probe.

OB-RGRP at the highest levels, whereas brain and kidney express it at the lowest levels. These data suggest that expression of *OB-RGRP* is relatively widespread.

The relative abundances of *OB-R*, *B219/OB-R* and *OB-RGRP* mRNAs were examined by PCR analysis of cDNA prepared from a panel of hematopoietic and other cell lines. The three transcripts were PCR amplified in the same PCR reaction containing the four oligonucleotides, as shown in Figure 4A. This method allows assessment of variation in the relative levels of these transcripts from different cell types, but does not permit comparison of the absolute expression levels of these transcripts within a single cell line, since the efficiency of the PCR reactions with the isoform-specific primer pairs may be different. Figure 4B shows that the *B219/OB-R* transcript was only observed in human granulocytic erythroleukaemia K562 cells and in human brown adipose PAZ-6 cells (13). Expression of *B219/OB-R* increases during differentiation of human adipose cells. However, both *OB-RGRP* and *OB-R* transcripts were expressed in all cell lines

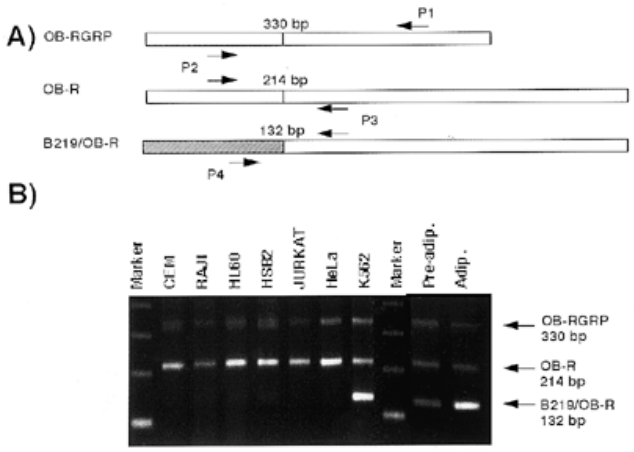

Figure 4. Differential expression of *OB-RGRP*, *OB-R* and *B219/OB-R*. *OB-RGRP*, *OB-R* and *B219/OB-R* expression was determined by RT–PCR in a panel of hematopoietic cells (CEM, Raji, HL60, HSB2, Jurkat and K562), in HeLa cells and in human brown pre-adipocyte and adipocyte cells using four primers to amplify the three transcripts in the same PCR reaction. (**A**) Schematic representation of the PCR. The P2 and P3 primers are common for two transcripts. (**B**) The PCR products were compared on agarose gels with a 100 bp ladder. The size of the amplified products is indicated by arrows.

tested, as shown by the presence of both 330 and 214 bp PCR products (Fig. 4B) and the relative abundance of the two PCR products is relatively invariant (330/214 bp of 0.4–0.7). The apparent similarity in the relative abundance of the *OB-RGRP* and *OB-R* transcripts suggests that post-transcriptional splicing of the pre-mRNA that generates the two mature mRNAs is subject to little if any cell-specific regulation.

Homology of OB-RGRP with putative ORFs

The sequence alignment of mouse and human OB-RGRP (Fig. 5) reveals differences at six positions, 42, 43, 49, 87, 92 and 119. Two of these are conserved substitutions.

Searches for amino acid sequence similarity (BLASTP and TFASTA programs) between translated *OB-RGRP* and various databases yielded no matches in the primate, rodent or vertebrate protein databases except for human and rat *OB-R* 5′-UTRs (6,9). However, significant homologies were observed with putative ORFs identified in *C.elegans* (18) and *Saccharomyces cerevisiae* (Fig. 5). The best match was found with *C.elegans* C30B5.2 and extends over nearly the entire length of both predicted proteins. Two domains are highly homologous. Domain 1 starts from amino acid +9 of OB-RGRP to +27 (corresponding to +24 to +42 of C30B5.2), with 14/19 identical residues (74% identity) and three non-polar conserved residues, yielding an overall homology of 90% for this domain, which is highly hydrophobic and contains no charged amino acids. Domain 2, from residues +65 to + 88 of OB-RGRP, contains 17/25 identical and five non-polar conserved amino acids yielding a combined homology of 92%. OB-RGRP also has a significant but weaker match with yeast ORF YJR 044c. Interestingly, all three proteins begin and end at approximately the same positions.

DISCUSSION

We describe here the translation of two putative unrelated proteins from alternatively spliced mRNAs transcribed under the control of the same promoter. One of these proteins is the leptin receptor. The other is a new 131 residue protein, OB-RGRP, found in both man and mouse. Only six residues distinguish OB-RGRP in the two species. This protein is quite homologous to putative ORFs in yeast and *C.elegans*. Two domains of ~20 residues are each 90% homologous between huOB-RGRP and *C.elegans* C30B5.2. The predicted ORF of the *C30B5.2* gene is

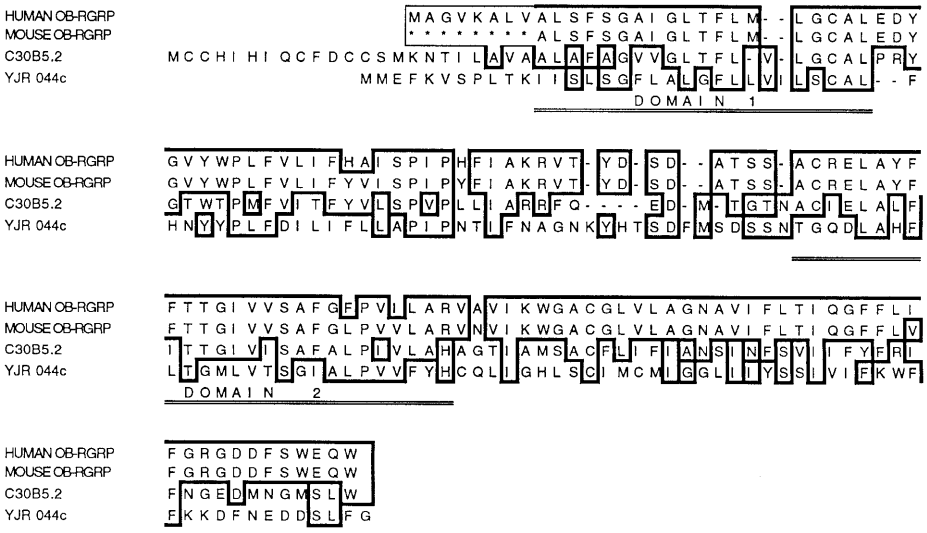

Figure 5. Sequence alignment of the human and mouse OB-RGRP, C30b5.2 (*C.elegans*, accession no. U23450) and YJR O44c (yeast) putative ORFs. Those amino acid residues of C30B5.2 and YJR 044C identical to either the human or mouse OB-RGRP are boxed. The N-terminal ends of human and mouse OB-RGRP are also boxed, since they are likely to be homologous based on efficient amplification using the degenerate primer. Although the actual sequence of the mouse was not obtained, the homologous residues are indicated by stars. Two domains of high homology between mammalian OB-RGRP and C30B5.2 are overlined.

split by two small introns (17) and the relative position of these introns is identical in the hu*OB-RGRP* gene. These data strongly suggest that the *OB-RGRP* cDNA encodes a protein well conserved in mammals and related to an ancestral gene retained in an invertebrate and in a lower eukaryote.

The genomic organization of the 5′-part of the *OB-R* gene and the exon/intron sequences are in agreement with transcription in the same direction of the first two exons to yield *OB-RGRP* and *OB-R* mRNA (Figs 1 and 2). As a common 3′-alternative splicing mechanism (11), the polyadenylation signal recognition in *OB-RGRP* exon 4 may leak and introns 2 and 3 may remain unspliced to trigger transcription through the *OB-R* gene and maturation of the *OB-R* transcript. Separation of the strong pyrimidine tract associated with the branch site from the acceptor splice site of *OB-RGRP* exon 3 (Fig. 2) fits with a difficult to excise intron 2, as already described in the sequence upstream of exon 3 of α-tropomyosin and exon 7 of β-tropomyosin, in which a negative regulatory element lies just upstream of the acceptor splice site (18,19).

Formation of the *B219/OB-R* transcript is likely to be due to the use of an alternative promoter, as proposed earlier (10). *OB-RGRP*, *OB-R* and *B219/OB-R* expression, examined by RT–PCR, supports such a genomic organization. Indeed, in the cells we examined the percentage of immature transcripts initiated from a single promoter from either *OB-RGRP* or *OB-R* is nearly constant, as shown by only slight variation in the ratio of specific PCR products (Fig. 3). This suggests that there is no strong post-transcriptional regulation. In contrast, the *B219/OB-R* transcript shows a distinct pattern of expression. Unlike *OB-RGRP* and *OB-R*, *B219/OB-R* is expressed in haematopoietic K562 cells (10) and is induced in PAZ-6 adipocytes as they differentiate, suggesting different transcriptional regulation of the *B219/OB-R* and *OB-R* promoters.

There are few examples of dual utilization of a single promoter generating unrelated proteins in eukaryotic genes. Overlapping genes have been shown, however, to be controlled by a common promoter with transcription in opposite directions, as occurs for example at the complex mouse *surfeit* locus, in which the *surf1* and *surf2* genes are transcribed in opposite directions from a common 73 bp promoter (20). Transcription of genes may diverge by specific use of initiation sites of transcription in opposite directions (21). Another example is provided by the *calcitonin/calcitonin gene-related peptide* (*CGRP*) locus, which, by alternative splicing and polyadenylation (22,23) followed by N-terminal proteolytic cleavage, yields two unrelated products with different functions. In this case the promoter, the first three exons and the initiation codon are identical and tissue-specific post-transcriptional regulation occurs to yield expression of these proteins in different cell types (23). Recently, alternative utilization of two promoters and two reading frames within the second exon has been described for the *INK4a* gene (24). This process yields two polypeptides that are entirely different in their amino acid sequences. The resulting proteins nevertheless have similar biological functions in cell growth arrest in mammalian fibroblasts. Concerning *OB-RGRP* and *OB-R* transcripts, classic alternative splicing and polyadenylation generate two transcripts with different ORFs. The initiation codon for the second protein is present in the 5′-UTR of the alternative transcript.

Such a genomic organization, allowing transcription of both *OB-RGRP* and *OB-R* mRNA from the same promoter with little or no cell-specific post-transcriptional regulation may yield coordinated OB-R and OB-RGRP synthesis. Indeed, OB-R may be synthesized from the transcript containing two AUGs by leaky scanning of the first AUG, as observed in several virus genes and in cDNAs with leader sequences with several AUGs (12). The selective pressure to conserve this unusual overlapping gene organization in mouse, human and probably rat suggests a functional importance for interdependent regulation of expression of these genes. Coordinated expression of *OB-RGRP* and *OB-R* may be necessary to maintain a constant basal expression of the OB-RGRP and OB-R proteins. Indeed, Northern blot analysis of various human tissues (Fig. 3) reveals that expression of *OB-RGRP* is as widespread as that of *OB-R* (10). In addition, the *B219/OB-R* transcript may allow cell- or differentiation-specific variation of the OB-RGRP and OB-R protein expression ratios.

In the OB-RGRP protein sequence several stretches of hydrophobic residues suggest the possible presence of transmembrane domains. Proximal to the transmembrane domain of several members of the cytokine receptor family, including the leptin receptor (6), one finds a Pro–X–Pro sequence preceded by a cluster of hydrophobic residues, called box 1 (25,26). Substitution of the two Pro by Ser residues results in loss of tyrosine phosphorylation of JAK2 induced by the activated receptor (25). In the OB-RGRP protein a similar box 1 (Pro^{46}–Ile–Pro^{48}) is observed which is conserved in the various species studied so far (Fig. 5). It is noteworthy that the full-length leptin receptor has been shown to modulate the JAK/STAT pathway, as do the interleukin 6-type cytokine receptors (27), whereas the short form expressed in *db/db* mice is unable to activate this signalling pathway (28). Box 1 is present in both OB-R forms, suggesting that this motif may not be sufficient for JAK/STAT activation. However, under leptin stimulation both OB-R forms are able to induce mRNA expression of immediate early genes (29).

It has been shown recently that leptin can homodimerize the OB-R receptor extracellular domains (30,31). It is tempting to suggest that *OB-RGRP* could encode an accessory protein, involved in leptin signalling.

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