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Defective STAT signaling by the leptin receptor in *diabetic* mice

(*janus* kinase/signal transducers and activators of transcription signaling pathway/*obese* mutant/obesity/alternative splicing)

NICO GHILARDI*, SANDRA ZIEGLER*, ADRIAN WIESTNER*, RUEDI STOFFEL*, MARKUS H. HEIM†, AND RADEK C. SKODA*‡

*Department of Pharmacology, Biozentrum, University of Basel, 4056 Basel, Switzerland; and †Department of Research and Internal Medicine, University Hospital of Basel, 4056 Basel, Switzerland

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ABSTRACT **Leptin and its receptor, *obese* receptor (OB-R), comprise an important signaling system for the regulation of body weight. Splice variants of OB-R mRNA encode proteins that differ in the length of their cytoplasmic domains. We cloned a long isoform of the wild-type leptin receptor that is preferentially expressed in the hypothalamus and show that it can activate signal transducers and activators of transcription (STAT)-3, STAT-5, and STAT-6. A point mutation within the OB-R gene of *diabetic* (*db*) mice generates a new splice donor site that dramatically reduces expression of this long isoform in homozygous *db/db* mice. In contrast, an OB-R protein with a shorter cytoplasmic domain is present in both *db/db* and wild-type mice. We show that this short isoform is unable to activate the STAT pathway. These data provide further evidence that the mutation in OB-R causes the *db/db* phenotype and identify three STAT proteins as potential mediators of the anti-obesity effects of leptin.**

Leptin, the product of the *obese* (*ob*) gene, is a 16-kDa secreted protein primarily produced by adipocytes (1). There is a good correlation between the percentage of body fat and serum leptin levels suggesting that leptin production is regulated by the mass of adipocytes (2, 3). Leptin levels were normal or elevated in obese individuals (2, 4) arguing against a simple leptin deficiency as the cause of obesity in the majority of humans (5). Serum leptin concentrations increased under a fatty diet but failed to prevent weight gain (3). Therefore, insensitivity to the action of leptin appears to be a common mechanism in obese individuals and in several rodent models. This suggests that dysregulation at the level of the leptin receptor, the downstream signaling pathway, or an unknown modifying mechanism may constitute the basis for weight disorders. The crucial role of leptin and its receptor in obesity is well illustrated by two phenotypically very similar mutants *obese* (*ob*) and *diabetes* (*db*) (6). Mice homozygous for a loss of function mutation of *ob* display obesity, hyperglycemia, and insulin resistance resembling type II diabetes. Administration of recombinant leptin to *ob* mice corrected these abnormalities (7–9). Based on early parabiosis experiments it was expected that *db* would be caused by a mutation in the *ob* receptor (OB-R) (6).

OB-R was cloned by virtue of its high affinity to leptin through an expression cloning strategy (10). The OB-R gene was mapped to the same 5-centimorgan interval on mouse chromosome 4 to which *db* had been localized (10). Surprisingly, no mutation in the coding region of OB-R cDNA of *db/db* mice was found and leptin binding sites were unaltered in *db/db* mice (10). However, the cloned mouse OB-R cDNA encoded a protein with a much shorter cytoplasmic domain than the human homologue, suggesting that a longer mouse isoform exists. We cloned this longer form of OB-R from wild-type mice and found that the mRNA for this isoform is dramatically reduced in *db/db* mice. A G to T mutation in *db* mice generates a new splice donor and suppresses the expression of this long isoform through competition for a splice acceptor. Very recently, two other groups also described the longer OB-R isoform and the splicing defect in *db* (11, 12). Because the short form of OB-R protein is expressed at high levels in *db/db* mice, it was not clear whether the splicing defect would be sufficient to interrupt the OB-R signaling pathway by solely decreasing the long isoform.

The leptin receptor is homologous to members of the cytokine receptor superfamily (10, 13). These receptors lack enzymatic motifs in their cytoplasmic domains and instead associate with members of the *janus* kinase (Jak) family (14), a class of cytoplasmic tyrosine kinases. Binding of ligand to the receptor activates the Jak kinase and leads to phosphorylation of cytoplasmic target proteins. Among these are the intracellular domains of the receptors and a class of cytoplasmic transcription factors called signal transducers and activators of transcription (STAT) (15). Six members of the STAT family have been identified to date (16–22). Phosphorylation of STAT proteins induces dimerization and translocation into the nucleus and results in specific activation of gene transcription (15, 23).

Here we demonstrate that the short form of OB-R is unable to activate STAT proteins and thereby provide further evidence that reduced expression of the long form is sufficient to cause the *db/db* phenotype. In addition, we identified three STAT proteins that can be activated by the long isoform of OB-R and therefore represent candidates for mediating the anti-obesity effects of leptin.

MATERIALS AND METHODS

Cloning of the OB-R cDNA and Gene. The human expressed sequence tag clone 84708, accession number T73849, was obtained from the IMAGE Consortium (Lawrence Livermore National Laboratory). The mouse homologue was cloned by reverse transcription (RT)-PCR from mouse spleen 1st strand cDNA using a primer pair derived from the human expressed sequence tag sequence. This mouse OB-R cDNA fragment was used to screen a mouse spleen cDNA library. Six independent positive clones were obtained and sequenced on a Applied Biosystems 373 DNA sequencer (Perkin–Elmer). The 5′ end and 3′ end of the OB-R cDNA was cloned by the rapid amplification of cDNA ends (RACE) protocol using the

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Abbreviations: OB-R, *obese* receptor; *ob*, *obese* mutant; *db*, *diabetes* mutant; STAT, signal transducers and activators of transcription; Jak, *janus* kinase; RT, reverse transcription.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. U58861, U58862, and U58863).

‡To whom reprint requests should be addressed at: Biozentrum, Department of Pharmacology, University of Basel, Klingelbergstrasse 70, Switzerland. e-mail: skoda@ubaclu.unibas.ch.

Marathon cDNA amplification Kit (Clontech). The full-length cDNA for the short form of OB-R was constructed by ligation of a 681- bp 5′ RACE fragment to the longest cDNA clone using a unique *Kpn*I site. The full-length cDNA for the long form of OB-R was generated by substitution of a 852-bp 3′ RACE fragment. The *db* OB-R cDNA was cloned from lung mRNA prepared from C57BLKS/J +/+ m *db/db* mice by RT-PCR using the primer pair 5′-TTCCCATC-GAGAAATATCAG-3′ (forward) and 5′-GAATGGAAC-CTTGAGGCTTC-3′ (reverse) derived from the OB-R cDNA and an OB-R genomic clone, respectively. Genomic clones containing the OB-R gene were isolated from a mouse λ Fix II library (Stratagene) and from a bacterial artificial chromosome library (Genome Systems, St. Louis). The clone GS 9301 (clone address: 168:O23) was chosen for further analysis because it hybridized to probes from both the 5′ and 3′ end of the OB-R cDNA. Fragments of the OB-R gene were subcloned into pBluescript KS II (Stratagene) and sequenced. Genomic DNA from C57BL/6J-db +/+ m *db/db* and control mice were purchased from the The Jackson Laboratory. Genomic fragments containing the intron/exon boundaries of the 3′ end of OB-R from *db/db* and C57BL/6J control were amplified by PCR using the primers 5′-CCTTGCTTTGAACCTTACAC-3′ (forward) and 5′-GAATGGAACCTTGAGGCTTC-3′ (reverse) or 5′-TGACCACTACAGATGAACCC-3′ (forward) and 5′-ATCTCACCAGCCCCCTAATG-3′ (reverse). The fragments were sequenced directly or after subcloning into pBluescript II.

RNA Analysis. Ribonuclease (RNase) protection analysis was performed as described (24). Total RNA from FVB/N mice was prepared by the acid phenol method (25) and 20 μg from each tissue were used for analysis. For the detection of OB-R isoforms we constructed a riboprobe by PCR using the primers 5′-TTCCCATCGAGAAATATCAG-3′ (forward) and 5′-GGGACCATCTCATCTTTATT-3′ (reverse) derived from the long form of OB-R cDNA. This riboprobe protects a 403-nt fragment for the long form and a 270-nt fragment for the short form of OB-R mRNA. RNA loading was normalized with a riboprobe for mouse hypoxanthine-guanine phosphoribosyltransferase, a house keeping gene (26). The hypoxanthine-guanine phosphoribosyltransferase riboprobe was mixed with the OB-R probe and added as an internal standard to each sample. Radioactive bands were quantitated on a PhosphorImager 425 using the IMAGEQUANT software (Molecular Dynamics) and are indicated below each lane. Total RNA from hypothalamus or lung of *db/db* or heterozygous *db/+* mice (C57BLKS/J +/+ m genetic background) was reverse transcribed and amplified by PCR using the primers 5′-GAATTGTTCCTGGGCACAAG-3′ (forward) and 5′-GGGACCATCTCATCTTTATT-3′ (reverse) derived from the long form of OB-R cDNA. These primers amplify a 133-bp fragment for the wild-type long isoform and a 239-bp fragment for the *db/db* OB-R transcript.

Cell Culture and Signal Transduction Analysis. COS cells were transfected by the DEAE-Dextran method (27) with cDNAs for OB-R alone or cotransfected with cDNA for individual STATs. COS cells (5×10^5) were transfected with 8 μg of either human STAT-1 and human STAT-2 in pcDNA3 (Invitrogen), mouse STAT-3 and mouse STAT-4 in pRc/CMV (Invitrogen), sheep STAT-5 and human STAT-6 in pXM, cDNA for the long form and short form of mouse OB-R in pcDNAI/Amp (Invitrogen). Transfected COS cells were starved for 4 hr in medium without serum and then stimulated for 15 min with 0.2 μM of leptin. Electrophoretic mobility-shift assay was performed as described (28) with the oligonucleotide probes m67 5′-CATTTCCCGTAAATCAT-3′ (29) and βCAS 5′-GATTTCTAGGAATTCAATCC-3′ (30). Cell surface expression of transfected OB-R protein was determined by binding of ^{125}I-leptin. Transfected COS cells and controls were seeded in 24-well plates in triplicates, washed once with binding buffer (DMEM containing 1% BSA and 0.05% sodium azide to prevent receptor internalization) and incubated at room temperature in binding buffer containing 2×10^5 cpm of ^{125}I-leptin (final concentration of leptin = 1 nM) labeled with the Bolton–Hunter reagent (a generous gift from M. Chiesi, Ciba–Geigy, Basel). For competition, 0.1 μM of unlabeled leptin was added in some samples. Cells were washed 3 times with binding buffer and bound ^{125}I-leptin was measured in a gamma counter. To verify expression of transfected STATs, cytoplasmic extracts of COS cells were separated by SDS/PAGE, transferred to nitrocellulose by Western blotting and incubated with the following specific antibodies against individual STAT proteins: anti STAT-1 (16), anti STAT-2 (31), anti STAT-3 and STAT-4 (18), rabbit polyclonal antibodies against amino acids 687–794 of sheep STAT-5 (M.H.H., unpublished results), and rabbit polyclonal antibodies against amino acids 633–837 of mouse STAT-6 (M.H.H., unpublished results).

RESULTS AND DISCUSSION

We cloned the mouse homologue of a human expressed sequence tag, which showed sequence similarity to gp130 (32) and LIF-Rβ (33), two members of the cytokine receptor superfamily (13). Screening of a spleen cDNA library yielded clones representing two mRNA isoforms. Six independent

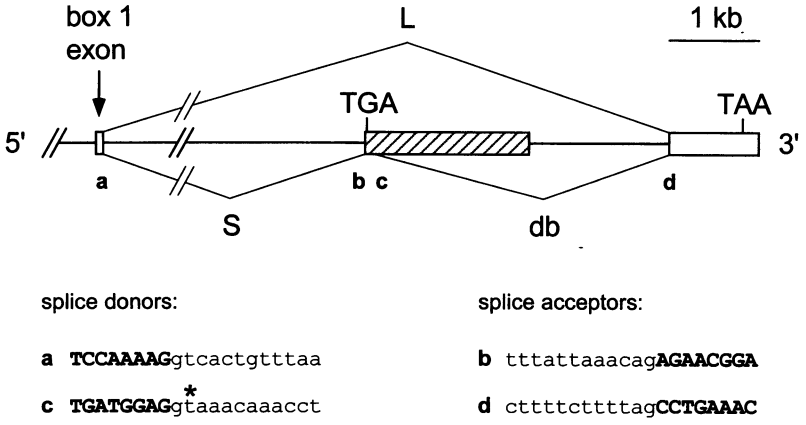

FIG. 1. Genomic structure of the OB-R gene and alternative splicing. Boxes represent exons. A large intron of ≈15 kb is spliced-out to generate the long form (L) of the OB-R mRNA. An alternative splice acceptor joins an untranslated exon (hatched box) to generate the short form (S) of the OB-R mRNA. A point mutation within this exon generates a new splice donor in *db* mice. Sequences of the intron/exon boundaries of the OB-R gene from wild-type and *db* mice are shown. The G to T mutation in *db* is marked by an asterisk.

clones were identical to the mouse OB-R reported by Tartaglia *et al.* (10). This cDNA encodes a transmembrane receptor with a short cytoplasmic domain of only 34 amino acids. A second isoform encodes an OB-R protein that terminates prematurely in the second fibronectin III domain (amino acid 796) of the extracellular domain and generates a potentially soluble and secreted protein (not shown). We cloned a third isoform with a longer cytoplasmic domain of 302 amino acids that shows 72% identity at the amino acid level to the C terminus of the human OB-R (10). Sequencing the intron/exon boundaries of a genomic clone for OB-R revealed that the short and long isoforms are generated by differential splicing within the last intron of the OB-R gene (Fig. 1). The size of this intron, as determined by long range PCR, is ≈15 kb. An alternative splice acceptor site within this intron produces mRNA encoding the short form of OB-R. In the OB-R gene of *db/db* mice, a G to T mutation generates a new splice donor within the exon for the short OB-R isoform. This additional splice donor competes with the upstream splice donor that is used to generate the long form for the same downstream splice acceptor. The resulting *db* transcript contains the first 106 bp of the exon encoding the short isoform (Fig. 2*b*). However, because a stop codon is present within this 106-bp insertion, the same protein with a short cytoplasmic domain is produced in *db/db* and wild-type mice.

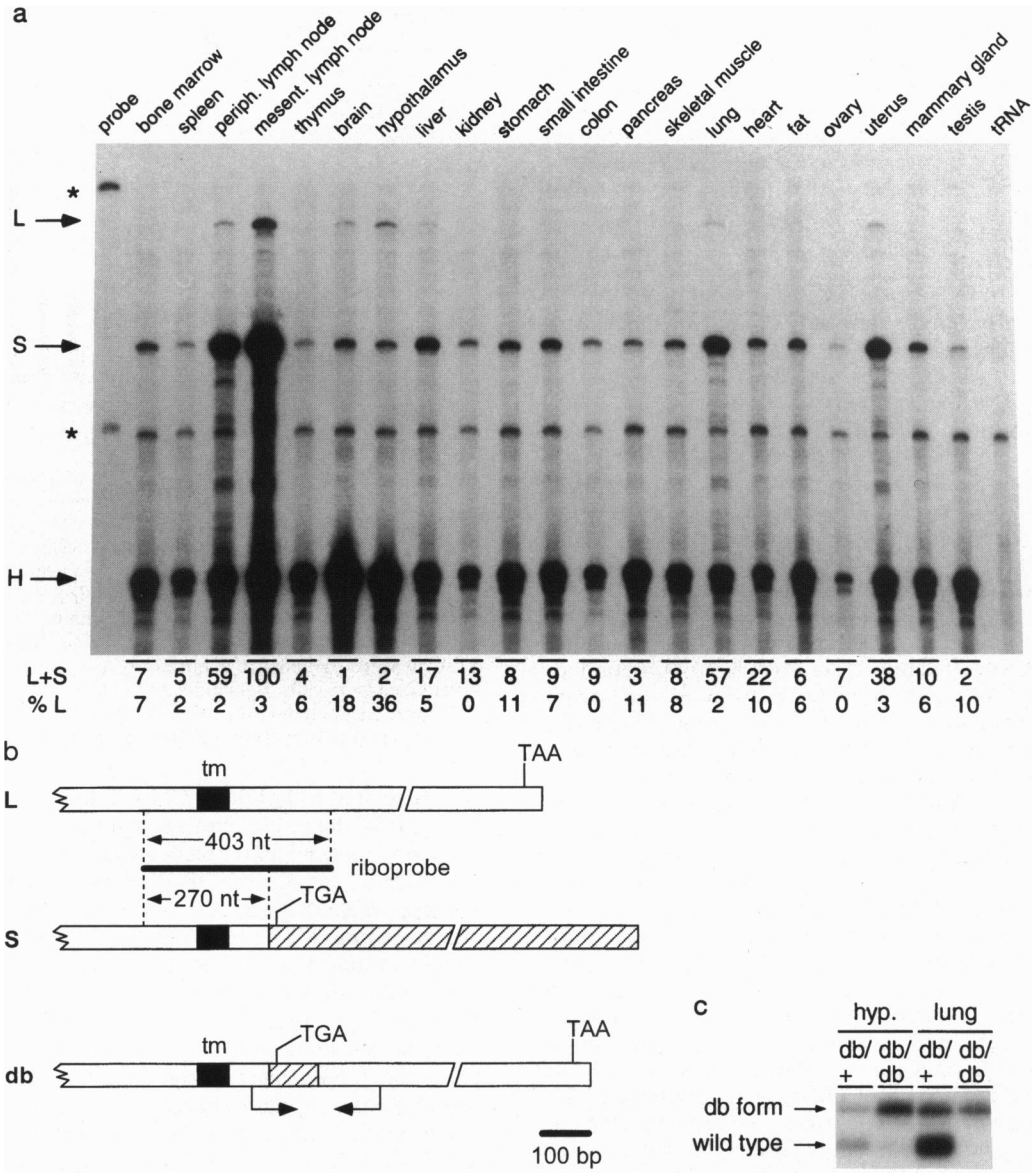

FIG. 2. Analysis of differential expression of OB-R mRNA isoforms in tissues from wild-type and *db/db* mice. (*a*) RNase protection assay of 20 μg of total RNA from wild-type mouse tissues with a riboprobe that can distinguish between the long form (L) and short form (S) of the OB-R mRNA. A riboprobe for hypoxanthine-guanine phosphoribosyltransferase (H) was used to normalize RNA loading. Positions of bands resulting from undigested riboprobes are marked with an asterisk. Numbers in the upper row (L+S) represent the abundance of the total OB-R mRNA in arbitrary units after normalization for RNA loading using the hypoxanthine-guanine phosphoribosyltransferase band. The value for the highest expressing tissue was set to 100. Numbers in the lower row represent the relative amount of the mRNA for the long form expressed as percentage of the total OB-R mRNA (%L). (*b*) Position of the OB-R riboprobe and location of the PCR primers in respect to the OB-R mRNA isoforms. The riboprobe is drawn as a thick line and the length in nt of the protected fragments is given. The black box represents the position of the transmembrane domain (tm) of OB-R, the hatched box represents the alternative untranslated exon for the short form (S). The location of the stop codons (TGA and TAA) is shown. Arrows indicate the position of PCR primers that distinguish the long form (L) from the *db* mutant. (*c*) Determination of the relative abundance of mutant OB-R isoforms in *db/db* mice by RT-PCR. Hypothalamus (hyp) and lung total RNA was reverse transcribed and amplified by PCR with primers shown in *b*. The autoradiogram of a Southern blot hybridized to a ^{32}P-labeled internal OB-R probe is shown.

To examine the distribution of the two transmembrane isoforms of OB-R in mouse tissues we used a sensitive RNase protection assay that enabled us to simultaneously determine the abundance of the two isoforms (Fig. 2*A*). We found that the mRNA for the short form of OB-R was expressed ubiquitously with highest levels in lymph nodes, lung, and uterus. In contrast, the longer form accounts for only 3–5% of the OB-R mRNA in these tissues. Interestingly, in the hypothalamus the relative abundance of the long form was clearly higher. The same result was also found by RT-PCR (12). Quantitation of the radioactive bands revealed that the long form accounts for 30–40% of the OB-R mRNA in the hypothalamus (Fig. 2*a*). This further suggested that this isoform might be physiologically more important than the ubiquitously expressed short isoform. To test if any correctly spliced transcripts for the long form existed in *db/db* mice we analyzed expression of the leptin receptor in hypothalamus and lung by RT-PCR. We used primers that amplify a 133-bp fragment for the wild-type long form and 239-bp for the *db* transcript (Fig. 2*c*). We observed a dramatic reduction of the 133-bp fragment in *db/db* mice indicating that expression of the long isoform of OB-R is efficiently suppressed in *db/db* mice. In two other reports (11, 12), the new splice donor apparently completely suppressed expression of the long isoform as assessed by RT-PCR and visualization of PCR fragments by ethidium bromide staining. We found the same result on ethidium bromide stained gels. However, when the specific OB-R bands were visualized by hybridization to an internal ^{32}P-labeled probe, we detected very low levels of expression of the long isoform in *db/db* mice (Fig. 2*c*). At these reduced levels of expression, the long form of OB-R is apparently unable to prevent the *db* phenotype.

The Jak/STAT pathway is a prime candidate to transmit the anti-obesity signal generated by leptin. Therefore, we examined the capacity of the leptin receptor isoforms to activate individual STAT proteins. We transfected COS cells with expression constructs for OB-R along with each of the known STAT isoforms (Fig. 3 *a* and *b*). Nuclear extracts were analyzed for the presence of activated STATs by electrophoretic mobility-shift assay. We used a mutated form of the serum inducible element of the fos promoter (m67-probe) (29) to test for STAT-1, STAT-3, and STAT-4 activation, the mammary gland factor response element of the β-casein gene (βCAS probe) (30) to test for STAT-5 and STAT-6 activation, and the interferon stimulated response element of the ISG15 gene (34) to test for the presence of the interferon stimulated gene factor 3 (ISGF3). We found that the long isoform of OB-R upon ligand binding activated STAT-3, STAT-5, and STAT-6 but not STAT-1 and STAT-4 (Fig. 3 *a* and *b*). These results were obtained in two independent transfections. Identity of the proteins producing gel shifts was confirmed by supershifts with specific antibodies to individual STAT proteins (not shown). Furthermore, we detected no activation of interferon stimulated gene factor 3 when cells cotransfected with STAT-1, STAT-2, and the long form of OB-R were stimulated with leptin and probed with interferon stimulated response element (not shown). In contrast, the short form of OB-R protein, common to *db/db* and wild-type mice, was unable to activate any of the six STAT proteins. Cell surface expression of OB-R protein was verified for each transfection by binding of ^{125}I-labeled leptin (Fig. 3*c*) and expression of transfected STAT proteins was confirmed by immunoblot analysis of cytoplasmic lysates with specific antibodies against STAT-1 through STAT-6 (not shown).

The failure of leptin to activate STAT proteins through the short OB-R isoform may explain why *db/db* mice display a phenotype despite abundant expression of this isoform. The capacity of the long OB-R isoform to activate three different STAT proteins (Fig. 3) may constitute the basis for the pleiotropic effects of leptin. As the leptin receptor is most

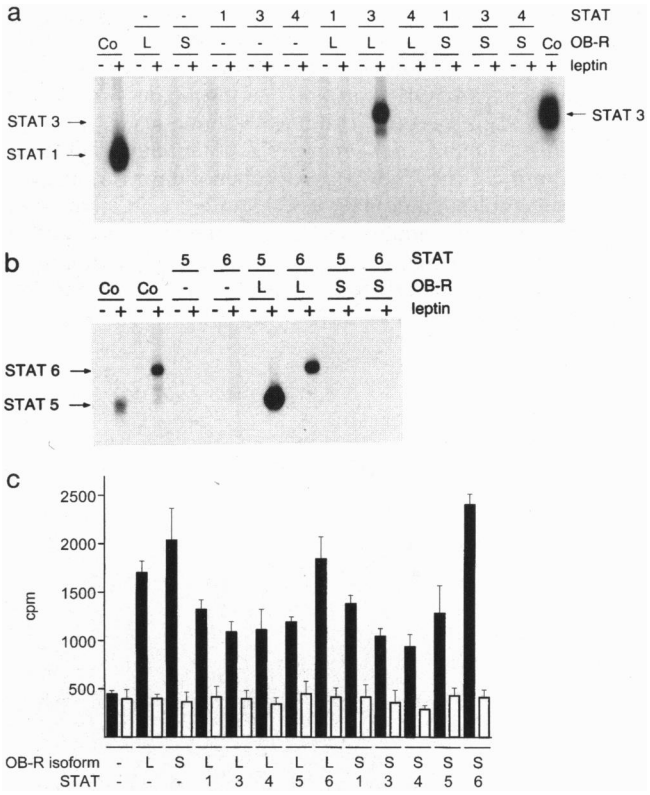

FIG. 3. Differential activation of STAT proteins by OB-R isoforms in response to leptin binding. Electrophoretic mobility-shift assay analysis with the m67 (*a*) or βCAS probe (*b*). COS cells were transfected with expression constructs encoding STAT and/or OB-R isoforms and stimulated with leptin as indicated above the lanes. Numbers stand for STAT-1 through STAT-6. L, long form; S, short form of OB-R. Presence or absence of leptin in the media is indicated (+ or −). Positive controls (Co) for STAT-1, STAT-3, STAT-5, and STAT-6 were loaded for comparison. (*c*) Binding of ^{125}I-leptin to transfected COS cells. Bars indicate the median ± SEM of triplicates. Cells were incubated with 1 nM ^{125}I-leptin in the absence (solid bars) or presence (open bars) of 100 nM of unlabeled leptin.

closely related to gp130, G-CSF-R, and LIF-Rβ (10), and all members of the gp130 family signal through STAT-3, it is not surprising that the leptin receptor also activates STAT-3. The cytoplasmic domain of OB-R contains a putative binding sequence YXXQ for STAT-3 (35). Activation of STAT-5 has not been described for gp130 family members. Also unexpected was the ability of OB-R to stimulate STAT-6, which was previously only known to be activated by interleukin 4 (22). High levels of expression of OB-R in lymph nodes (Fig. 2*a*) suggests that leptin might play a role in the lymphoid compartment. Interestingly, an immunodeficiency syndrome was described as a primary phenotype of *db/db* mice (36).

The STAT pathway may play an important role in mediating the lipostat/fat controller function of leptin. Although cytokine receptors can also activate the ras and the PI3 kinase pathways, there is increasing evidence that the activation of the STAT proteins might be essential for most if not all of the specific biological effects. This is best illustrated in the interferon system where a targeted disruption of the *Stat1* gene in mice resulted in a complete deficiency in interferon mediated biologic responses (37, 38). Furthermore, loss of function mutants of *Drosophila* STAT showed the same phenotype as loss of function mutants of hopscotch (*hop*), the *Drosophila* Jak homologue, and suppressed the effect of *hop*$^{\text{Tum-l}}$, a dominant gain of function allele (39, 40), indicating that STAT is an essential component downstream of *hop*.

Control of leptin signaling may be achieved in part by regulation of the differential tissue distribution of OB-R isoforms. The potentially secreted soluble isoform, truncated at amino acid 796, could act as a leptin-binding protein to reduce the free serum leptin concentration. Because the short form of the leptin receptor is unable to activate STATs or prevent obesity in *db/db* mice, leptin sensitivity, and as a consequence body weight, might be influenced by differential expression of the long and short isoform of the leptin receptor.

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1. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. & Friedman, J. M. (1994) *Nature (London)* **372,** 425–432.
2. Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., Kern, P. A. & Friedman, J. M. (1995) *Nat. Med.* **1,** 1155–1161.
3. Frederich, R. C., Hamann, A., Anderson, S., Loellmann, B., Lowell, B. B. & Flier, J. S. (1995) *Nat. Med.* **1,** 1311–1314.
4. Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciumas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., Bauer, T. L. & Caro, J. F. (1996) *N. Engl. J. Med.* **334,** 292–295.
5. Considine, R. V., Considine, E. L., Williams, C. J., Nyce, M. R., Magosin, S. A., Bauer, T. L., Rosato, E. L., Colberg, J. & Caro, J. F. (1995) *J. Clin. Invest.* **95,** 2986–2988.
6. Coleman, D. L. (1978) *Diabetologia* **14,** 141–148.
7. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T. & Collins, F. (1995) *Science* **269,** 540–543.
8. Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. & Friedman, J. M. (1995) *Science* **269,** 543–546.
9. Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R. & Burn, P. (1995) *Science* **269,** 546–549.
10. Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Woolf, E. A., Monroe, C. A. & Tepper, R. I. (1995) *Cell* **83,** 1263–1271.
11. Chen, H., Charlat, O., Tartaglia, L. A., Woolf, E. A., Weng, X., Ellis, S. J., Lakey, N. D., Culpepper, J., Moore, K. J., Breitbart, R. E., Duyk, G. M., Tepper, R. I. & Morgenstern, J. P. (1996) *Cell* **84,** 491–495.
12. Lee, G.-H., Proenca, R., Montez, J. M., Caroll, K. M., Darvishzadeh, J. G., Lee, J. I. & Friedman, J. M. (1996) *Nature (London)* **379,** 632–635.
13. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6934–6938.
14. Ihle, J. N. (1995) *Nature (London)* **377,** 591–594.
15. Darnell, J. E., Jr., Kerr, I. M. & Stark, G. R. (1994) *Science* **264,** 1415–1421.
16. Schindler, C., Fu, X. Y., Improta, T., Aebersold, R. & Darnell, J. E., Jr. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7836–7839.
17. Fu, X. Y., Schindler, C., Improta, T., Aebersold, R. & Darnell, J. E., Jr. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7840–7843.
18. Zhong, Z., Wen, Z. & Darnell, J. E., Jr. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4806–4810.
19. Akira, S., Nishio, Y., Inoue, M., Wang, X. J., Wei, S., Matsusaka, T., Yoshida, K., Sudo, T., Naruto, M. & Kishimoto, T. (1994) *Cell* **77,** 63–71.
20. Yamamoto, K., Quelle, F. W., Thierfelder, W. E., Kreider, B. L., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., Silvennoinen, O. & Ihle, J. N. (1994) *Mol. Cell. Biol.* **14,** 4342–4349.
21. Wakao, H., Gouilleux, F. & Groner, B. (1994) *EMBO J.* **13,** 2182–2191.
22. Hou, J., Schindler, U., Henzel, W. J., Ho, T. C., Brasseur, M. & McKnight, S. L. (1994) *Science* **265,** 1701–1706.
23. Ihle, J. N. (1996) *Cell* **84,** 331–334.
24. Krieg, P. A. & Melton, D. A. (1987) *Methods Enzymol.* **155,** 397–415.
25. Chomczynski, P. & Sachi, N. (1987) *Anal. Biochem.* **162,** 156–159.
26. Chinault, A. C., Brennand, J., Konecki, D. S., Nussbaum, R. L. & Caskey, C. T. (1984) *Adv. Exp. Med. Biol.* **165,** 411–415.
27. Seed, B. & Aruffo, A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3365–3369.
28. Levy, D. E., Kessler, D. S., Pine, R. & Darnell, J. E., Jr. (1989) *Genes Dev.* **3,** 1362–1371.
29. Wagner, B. J., Hayes, T. E., Hoban, C. J. & Cochran, B. H. (1990) *EMBO J.* **9,** 4477–4484.
30. Schmitt, N. M., Doppler, W., Ball, R. K. & Groner, B. (1991) *Mol. Cell. Biol.* **11,** 3745–3755.
31. Qureshi, S. A., Salditt-Georgieff, M. & Darnell, J. E., Jr. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3829–3833.
32. Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. & Kishimoto, T. (1990) *Cell* **63,** 1149–1157.
33. Gearing, D. P., Thut, C. J., VandeBos, T., Gimpel, S. D., Delaney, P. B., King, J., Price, V., Cosman, D. & Beckmann, M. P. (1991) *EMBO J.* **10,** 2839–2848.
34. Reich, N., Evans, B., Levy, D., Fahey, D., Knight, E. J. & Darnell, J. E., Jr. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6394–6398.
35. Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., Jr., & Yancopoulos, G. D. (1995) *Science* **267,** 1349–1353.
36. Montgomery, L. B., Loria, R. M. & Chinchilli, V. M. (1990) *Diabetes* **39,** 675–682.
37. Meraz, M. A., White, J. M., Sheehan, K. C. F., Bach, E. A., Rodig, S. J., Dighe, A. S., Kaplan, D. H., Riley, J. K., Greenlund, A. C., Campbell, D., Carver-Moore, K., DuBois, R. N., Clark, R., Aguet, M. & Schreiber, R. D. (1996) *Cell* **84,** 431–442.
38. Durbin, J. E., Hackenmiller, R., Simon, M. C. & Levy, D. E. (1996) *Cell* **84,** 443–450.
39. Hou, X. S., Melnick, M. B. & Perrimon, N. (1996) *Cell* **84,** 411–419.
40. Yan, R., Small, S., Desplan, C., Dearolf, C. R. & Darnell, J. E. (1996) *Cell* **84,** 421–430.