Minireview

# The ins and outs of leptin receptor activation

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**Abstract The adipocyte-derived hormone leptin signals the status of body energy stores by activating its receptor in hypothalamic nuclei. In contrast to the initial expectations, leptin treatment of human obesity was largely unsuccessful. One explanation for this is the marked leptin resistance, which likely operates in part at the receptor level. The leptin receptor is a member of the class I cytokine receptor family, which uses the Janus kinase/signal transducer and activator of transcription pathway as a major signaling route. In this review, we focus on the molecular mechanisms underlying leptin receptor activation. Different modes of leptin-induced clustering of the ectodomains and the subsequent signaling events will be discussed.**


*Key words:* Leptin; Leptin receptor; Receptor clustering; Signal transduction

## 1. Introduction

Leptin and its receptor are essential components in the complex genetic wiring diagram underlying energy homeostasis and body weight. Spontaneous mutations in leptin or in its receptor result in marked obesity in both mice [1,2] and man [3,4]. Leptin is mainly produced in white adipose tissue [2] although expression has also been demonstrated in the fundus of the stomach [5] and in skeletal muscle [6]. The long, signaling-competent isoform of the leptin receptor (LR) shows high expression peaks in the feeding centers of the hypothalamus [7], consistent with leptin being the afferent signal informing the central nervous system of the body fat status. This concept is further supported by the observation that leptin-deficient ($ob^{-/-}$) mice and men can be successfully treated with leptin [8,9]. Leptin was therefore initially considered a miracle drug for treatment of obesity. However, obese people often have elevated leptin levels [10] and leptin administration showed only very limited effects [11]. Recent data have indicated that this is likely due to desensitization for the leptin signal, a phenomenon now often referred to as leptin resistance. This may be situated at least at two distinct levels: saturable transport of leptin across the blood–brain barrier, and abnormalities at the level of LR activation and/or signal transduction [12]. Besides its role via the central nervous system, leptin also has direct effects on a series of peripheral tissues, implying a much more complex leptin axis than was originally anticipated [13].

To date, six splice variants of the LR have been identified. The long isoform or Ob-Rb (further referred to as LRlo) consists of 1162 amino acids and is the only LR isoform with clearly demonstrated signaling capability. It is highly expressed in hypothalamic centers although expression at functional levels has also been demonstrated in a number of other tissues including liver, lung, testis, etc. Neuronal-specific ablation of Ob-Rb results in obesity, clearly indicating that the weight-reducing properties of leptin are exerted centrally [14]. Four short isoforms (Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Rf; Ob-Ra will be further referred to as LRsh) with shortened intracellular tails have been identified. High expression levels of Ob-Ra and Ob-Rc can be found in choroid plexus and brain microvessels [7], suggesting their role in blood–brain barrier transport. This idea is further supported by observations in mouse models for obesity [15] and by the use of an in vitro leptin transport assay [16]. A secreted isoform can be generated either by alternative splicing (Ob-Re) or by ectodomain shedding, and may be involved in modulating leptin activity [17].

## 2. Structure and evolutionary relationships of the LR extracellular domain

The LR was first cloned from a mouse choroid plexus cDNA library using an expression cloning strategy by Tartaglia and co-workers [7]. Based on sequence homology, this receptor belongs to the class I cytokine receptor family, which typically contains a so-called CRH (cytokine receptor homol-

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*Abbreviations:* CRH, cytokine receptor homology; FN III, fibronectin type III; G-CSF, granulocyte colony-stimulating factor; gp130, glycoprotein 130; Grb-2, growth receptor-bound-2; Ig, immunoglobulin; IL, interleukin; IRS, insulin receptor substrate; JAK, Janus kinase; LIF, leukemia inhibitory factor; LR, leptin receptor; MAPK, mitogen-activated protein kinase; OSM, oncostatin M; PI3K, phosphatidylinositol 3-kinase; PTP1B, phosphotyrosine phosphatase 1B; SHP-2, SH2-containing phosphatase-2; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription

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ogy) domain in its extracellular domain. This structure consists of two barrel-like domains, each approximately 100 amino acids in length, which resemble the fibronectin type III (FN III) fold. Two conserved disulfide bridges are found in the N-terminal domain, while a WSXWS motif is characteristic for the C-terminal part. The LR shares highest sequence similarity with the granulocyte colony-stimulating factor (G-CSF) receptor and the glycoprotein 130 (gp130) family receptors, including gp130, the leukemia inhibitory factor (LIF) and oncostatin M (OSM) receptors. Moreover, structural superposition shows that also leptin is structurally most similar to G-CSF and cytokines of the gp130 family, such as interleukin-6 (IL-6).

Fig. 1A shows a consensus phylogenetic tree, based upon multiple sequence alignment of the membrane-proximal CRHs. The tree shown in Fig. 1 was calculated using the neighbor-joining distance method [18] with the Seqboot, Protdist and Neighbor programs of the PHYLIP package [19]. In this tree, the LR is most closely related to the OSM and LIF receptors, although the bootstrap support value for this evolutionary relationship is very low (40%). A parsimony tree, calculated using PAUP [20], shows an identical image, and supports the close evolutionary relationship between the LR and the LIF and OSM receptors (bootstrap support value 39%). A parsimony tree, calculated using the Seqboot and Protpars programs of the PHYLIP package [19] however, suggests that the LR would be more closely related to the G-CSF receptor and gp130 (bootstrap support value = 25%).

This relationship is also clearly reflected in the overall architecture of the ectodomains of these receptors (Fig. 1B): besides a canonical CRH domain, the receptors for leptin, G-CSF, LIF, OSM and gp130 all contain an immunoglobulin-like (Ig-like) domain (Fig. 1B). The LR and LIF receptors have an additional N-terminal CRH module, the OSM receptor has a truncated N-terminal CRH module. While gp130 and the G-CSF, LIF and OSM receptors each contain three membrane-proximal FN III domains, the LR stands out, having only two.

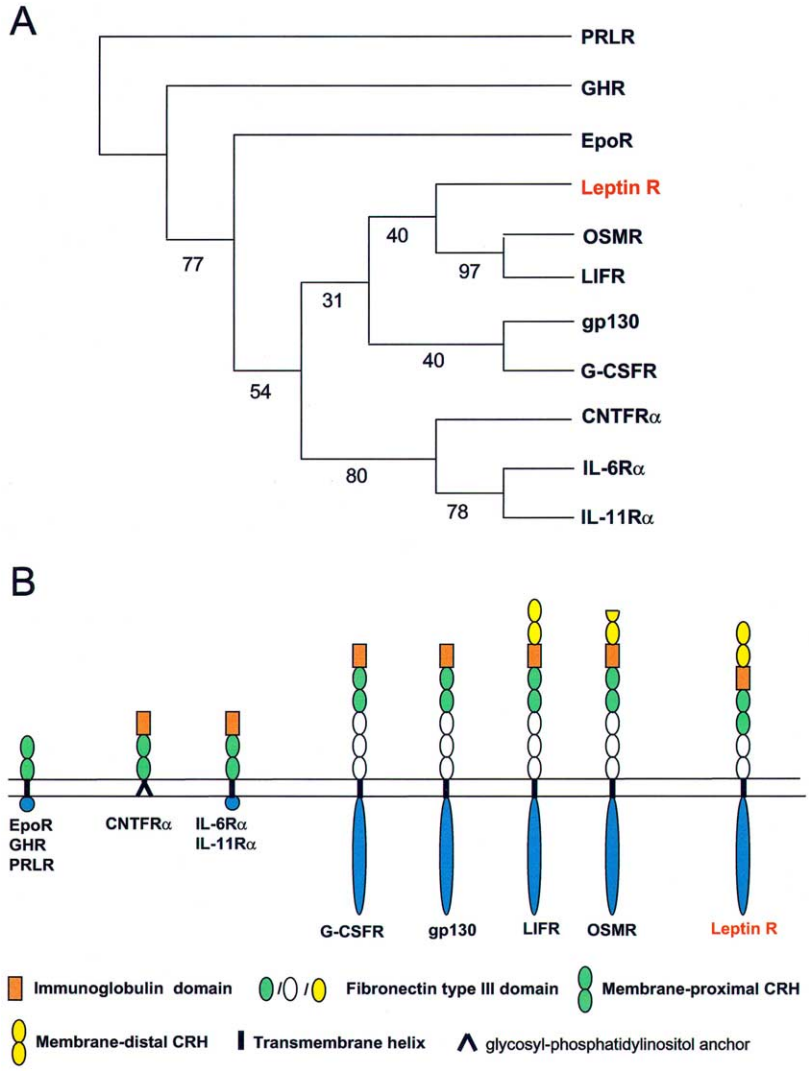


Fig. 1. A: Protdist phylogenetic tree of leptin and other long-chain cytokine receptors, based on an alignment of the membrane-proximal CRH. Numbers under the branches indicate the percent bootstrap support values out of 1000 bootstraps. PRLR: prolactin receptor; GHR: growth hormone receptor; EpoR: erythropoietin receptor; OSMR: oncostatin M receptor; LIFR: leukemia inhibitory factor receptor; G-CSFR: granulocyte colony-stimulating factor receptor; CNTFRα: ciliary neurotrophic factor receptor; IL-6Rα: interleukin 6α receptor; IL-11Rα: interleukin 11α receptor. B: Schematic presentation of the overall structure of long-chain cytokine receptors. The different domains are represented by colored squares or ovals, as explained below the figure.

### 3. Models for leptin/LR complex formation

Fong and co-workers generated a panel of LR deletion and substitution mutants, and showed that the membrane-proximal CRH domain is necessary and sufficient for leptin binding. These authors further provide evidence that the two FN III domains have no affinity for the ligand, but nevertheless are essential for receptor activation [21]. Recently, we could define a critical role for the Ig-like domain in receptor activation. Receptors lacking this domain are properly expressed on the cell surface, and bind leptin comparable to the wild type receptor, but are unable to activate the associated Janus kinases (JAKs) and fail to generate a signal transducer and activator of transcription (STAT)-3-dependent signal (see below; Zabeau et al., submitted).

Like all other class I cytokines, leptin adopts a four helical bundle structure. These cytokines usually interact with their receptor with two binding sites located on the helical faces of helices D (site I) and A and C (site II). A unique feature of G-CSF and of the gp130 family of cytokines is the presence of an additional binding site III, at the N-terminus of helix D, in one tip of the four helix bundle [22]. The function of this binding site III and of the Ig-like domain in the gp130 receptor systems was recently clarified by the crystal structure of the membrane-proximal CRH and Ig domain of gp130 in a 2:2 complex with Kaposi sarcoma herpesvirus IL-6 (vIL-6) [23]. This complex contains two copies of the gp130 fragment and of vIL-6. Each vIL-6 molecule interacts with two gp130 molecules by two interactions: binding site II in vIL-6 interacts with the CRH of one gp130 molecule, while binding site III in vIL-6 interacts with the Ig-like domain of a second gp130 molecule. This 2:2 tetrameric type of complex formation is very likely also a good model for the G-CSF:G-CSFR complex (Fig. 2A) [24]. Human IL-6 binds to its receptor in a 2:2:2 hexameric complex consisting of IL-6, IL-6Rα and gp130. In a generally accepted model, two human IL-6 molecules first bind to the CRH domains of dimeric IL-6Rα with their binding site I, and then bind to two gp130 receptor molecules with binding sites II and III [23,25] (Fig. 2B). It is tempting to speculate that similar clustering mechanisms also take place in the leptin/LR complex (Fig. 2C,D).

In gp130, the G-CSF receptor and the LIF receptor, the Ig-like domain interacts with binding site III of their cognate cytokine ligands. It is therefore very likely that a similar binding site III in leptin will interact with the Ig-like domain of the LR, which would help explain the deleterious effect of removal of this domain in the LR (Zabeau et al., submitted). Comparison with the IL-6 and G-CSF receptor systems suggests that leptin will bind its receptor either by its site I in the helical face of helix D, or by its site II in the helical faces of helices A and C. One of these sites probably interacts with the membrane-proximal CRH, as this constitutes the major leptin binding site [21]. Interactions between a binding site II in leptin and the membrane-proximal CRH were also suggested by molecular modeling of the leptin/LR complex [26,27].

Like the OSM and LIF receptors, LR contains an additional N-terminal CRH. In the LIF receptor, deletion of this CRH leads to a constitutively active receptor [28]. This is reminiscent of the constitutive activation seen in the fatty Zucker rat LR, which carries a mutation in this domain [29]. In the LIF receptor, this N-terminal CRH is able to interact with the ciliary neurotrophic factor receptor [30], suggesting a possible role for receptor/receptor interaction for the N-terminal CRH in the LR.

The membrane-proximal FN III domains of gp130 and of the LIF receptor interact with each other [31]. Although Devos et al. [32] showed that the membrane-proximal FN III domains are not necessary for dimerization of a soluble recombinant extracellular LR domain, similar interactions in the membrane-anchored LR cannot be excluded.

An important, unresolved question in LR biology is how LRlo can signal in the presence of excess LRsh. In many cell types, mRNA for the latter may account for up to 95% of all LR transcripts [33]. One possible explanation for this relative signaling insensitivity of LRlo to the effect of co-expression of dominant negative LR isoforms may be the formation of higher order clusters [29]. Based on the behavior of signal-

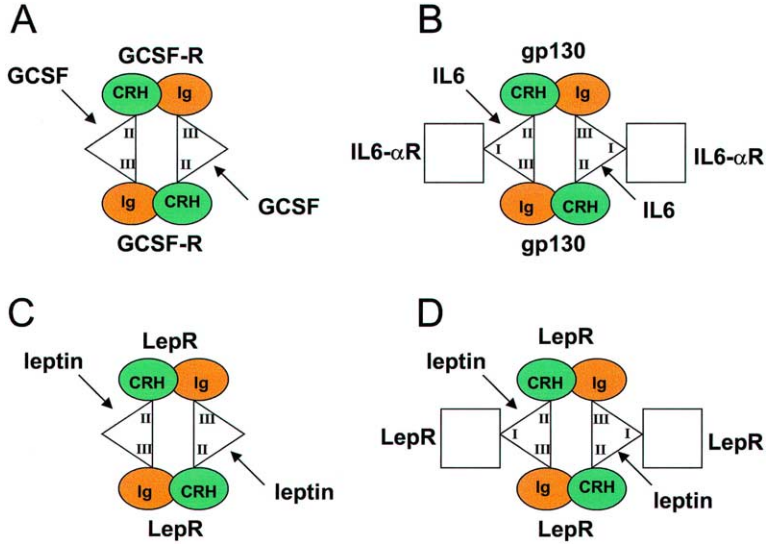


Fig. 2. Schematic representation of the complexes between cytokines and the extracellular domains of their receptors. A: G-CSF/G-CSF receptor 2:2 complex. B: IL-6/gp130/IL-6α receptor 2:2:2 complex. C: Model for a 2:2 leptin/LR complex. D: Model for a 2:4 leptin/LR complex.

ing-deficient LR mutants, we recently provided additional biochemical data to support this hypothesis (Zabeau et al., submitted). Like all other class I cytokine receptors, the LR lacks any intrinsic kinase activity, and uses cytoplasmic-associated kinases of the JAK family. Leptin binding results in formation of a receptor complex leading to cross-phosphorylation and activation of the JAKs. These activated JAKs then rapidly phosphorylate tyrosine residues in the cytosolic domain of the receptor. Such phosphorylated residues provide binding sites for signaling molecules including members of the STAT family. STATs themselves are also subject to JAK-mediated phosphorylation, inducing their homo- or heterodimerization, their release from the receptor complex, and subsequent translocation to the nucleus, where they can modulate transcription of specific target genes (for more details, see below). Two LR mutants, one deficient in the activation of JAK kinases (LR Δbox 1), the other unable to recruit STAT3 molecules (LR-F3), are only able to signal when they are co-expressed. Based on the requirements for JAK/STAT signaling, and on the lack of signaling complementation with similar receptor constructs, but containing the extracellular domain of the homodimeric erythropoietin receptor, this observation implies that more than two receptors must be clustered by leptin. When LRlo and LRsh forms are co-expressed, such higher order clustering can be expected to generate relatively more signaling-competent receptor complexes, when compared to simple homodimeric clustering. It is of note that the additional N-terminal CRH (or even the FN III domains) may contribute to even more complex patterns of receptor clustering.

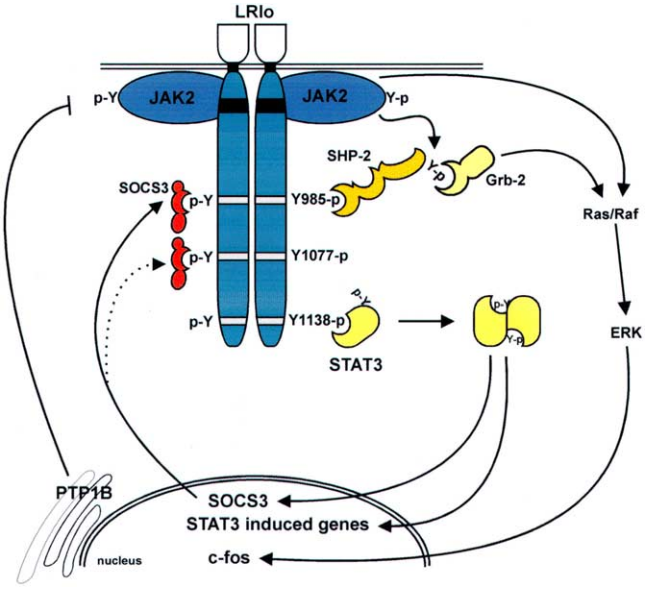


Fig. 3. LR signaling. The LR carries three conserved tyrosines in its cytoplasmic domain. JAK2, which associates membrane-proximally with the receptor, becomes activated upon ligand binding and phosphorylates these tyrosine residues. The membrane-distal Y1138 functions as a docking site for STAT3 which itself is a substrate of JAK2. Upon subsequent dimerization, it translocates to the nucleus and induces expression of SOCS3 and other genes. The membrane-proximal Y985 and Y1077 are involved in regulation and attenuation of the leptin signal. SOCS3 is taking part in a feedback loop inhibiting leptin signaling by binding to both tyrosines although binding affinity for Y1077 is much weaker. SHP2 is recruited to the Y985 position and activates the MAPK pathway through the adapter protein Grb-2, ultimately inducing c-*fos* expression. PTP1B is localized on the surface of the endoplasmic reticulum, and is also involved in negative regulation of LR signaling through dephosphorylation of JAK2 after internalization of the LRlo complex.

An alternative explanation was suggested by White and coworkers [34]. These authors showed ligand-independent homo-oligomerization of LRlo and LRsh. Hetero-oligomerization between the two isoforms was only observed in the presence of ligand. Therefore, differential sorting, perhaps based on the ability to bind JAKs, of LRsh and LRlo may permit efficient leptin signaling by the latter form. Differential sorting of the two isoforms is also suggested by the observation that LRsh could be involved in active transcytosis of leptin [16].

## 4. Activation of the JAK/STAT signaling pathway

A general overview of cellular events following LR activation is shown in Fig. 3. Leptin signaling occurs typically through the JAK/STAT pathway (see above). After ligand-induced clustering, the LRlo predominantly activates JAK2 [35], although JAK1 activation has also been demonstrated in some settings [36]. JAKs associate constitutively with a conserved box 1 motif, which is characterized by two essentially invariant prolines, in the class I cytokine receptors. Some receptors also contain an additional sequence, box 2, which is required for (maximal) JAK activation. In the murine LRlo, a box 1 motif (intracellular amino acids 6–17) critical for JAK2 activation and a putative box 2 motif (intracellular amino acids 49–60) have been identified. The latter may be required for maximal activation [35,37].

The LRlo has three conserved tyrosines in its cytoplasmic domain, which correspond in the murine receptor to positions Y985, Y1077 and Y1138. Y1138 is situated in a typical STAT3 recruitment or YxxQ motif, similar to motifs found in the gp130 family of receptors. After phosphorylation of this site, STAT3 is recruited via its SH2 (Src homology) domain. Activation, homo-dimerization and nuclear translocation of STAT3 will then lead to specific gene induction. The critical role of this site is underscored by the dramatic obese phenotype observed in knock-in mice containing a Y1138S mutation in LRlo [38]. It is unclear whether STAT3 is the only STAT factor that is activated upon stimulation. Vaisse and colleagues could demonstrate the activation of STAT3, but not of other STAT factors in the hypothalamus of leptin-treated *ob* mice [39]. In cell lines however, STAT1 and STAT5B activation was also shown [40]. The fact that LRlo Y1138S knock-in mice clearly have defects in body weight regulation but not in fertility, as opposed to mice lacking functional LRs, suggests alternative pathways, possibly via other STAT factors [38].

## 5. Role of phosphatases and of SOCS proteins

Mutation of the Y985 site in the receptor leads to enhanced signaling after leptin stimulation [36,41,42]. The Y985 site was identified as a recruitment site for the receptor-associated SH2-containing phosphatase-2 (SHP-2) [36,41]. It remains unclear whether negative effects are exerted via the phosphatase activity of SHP-2, or via the suppressor of cytokine signaling (SOCS)-3 protein, as it was later shown that this strong inhibitor of cytokine signaling also binds to the Y985 site [43,44]. SHP-2-dependent dephosphorylation of JAK2, how-

ever, also suggests an inhibiting function for SHP-2 in regulation of LRlo signaling [45]. SOCS proteins act in a typical negative feedback loop: they are rapidly induced after cytokine stimulation, and directly inhibit the receptor via various mechanisms, including receptor targeting to the proteasome. Both SOCS1 and SOCS3 can inhibit leptin signaling, and the observation that SOCS3 expression levels are elevated in the lethal yellow ($A^y$/a) obese mouse strain makes it a potential mediator of leptin resistance in vivo [46]. SOCS3 gene transcription is very rapidly induced in vitro and in vivo after leptin treatment [46,47], and even serves as a marker to map leptin-responsive neurons in the hypothalamus [48].

The function of the highly conserved Y1077 site is still unclear, mainly because phosphorylation of this site remains to be demonstrated. Remarkably, the Y985 and Y1077 sites are highly similar, suggesting binding of common signaling molecules. The Y1077 site shows weak interaction with SOCS3 and can therefore have an additive effect in signal termination [44].

SHP-2 is proposed as a positive regulator of leptin signaling through mitogen-activated protein kinase (MAPK) activation. Docking to Y985 is followed by recruitment of the adapter protein growth receptor bound 2 (Grb-2) and activation of the Ras/Raf pathway. A secondary pathway for leptin-induced MAPK signaling, directly via JAK2, probably requires the phosphatase activity of SHP-2. The MAPK pathway is responsible for leptin-induced c-*fos* activation [49]. It is of note that in LRlo signal transduction SOCS3 may also function as an adapter protein to other pathways [50].

Mice lacking PTP1B (phosphotyrosine phosphatase 1B) are hypersensitive to insulin and leptin and exhibit resistance to high fat diet obesity [51]. PTP1B recognizes a specific consensus substrate motif, (E/D)-$_p$Y-$_p$Y-(R/K), which was identified in JAK2 and the kinase activation loop of the insulin receptor [52,53]. Over-expression of PTP1B resulted in hypophosphorylation of endogenous JAK2 and blocked the leptin-induced transcription of endogenous SOCS3 and c-*fos* in a hypothalamic cell line. PTP1B appears to be a negative mediator of both the JAK/STAT and MAPK pathways in LR signaling and may be implicated in leptin resistance [54]. PTP1B is localized exclusively on the endoplasmic reticulum [55]. How PTP1B interacts with its substrates is not yet clear, although prior internalization of the receptor complex is suggested, especially since JAK2 has been detected at the endoplasmic reticulum [56].

## 6. Role of PI3 kinase

A strong correlation is assumed between the leptin and insulin signaling pathways since leptin and insulin resistance occur coincidentally in the majority of obese humans. Cross-talk between these pathways can be readily observed in various cell lines and in vivo. Phosphorylation of the insulin receptor substrates 1 and 2 (IRS1 and 2) as well as their interaction with Grb-2 and phosphatidylinositol 3-kinase (PI3K) show clear modulation by leptin in various hepatocytic cell lines [13,57]. Kim and colleagues performed a detailed in vivo study in rats showing STAT3 activation in insulin-responsive tissues after intraperitoneal injection of leptin. They also observed modulation of the PI3K and Grb-2 interaction with both IRS1 and IRS2 [58]. The apparent discrepancies described by various authors suggest that leptin and insulin signaling pathways interact in different ways depending on tissue type and cell line.

PI3K is also activated via IRS2 in the hypothalamus of rats, and appears to be crucial for the weight-reducing properties of leptin. Impaired PI3K signaling in peripheral tissues of obese individuals may also contribute to obesity-induced insulin resistance. A similar mechanism may explain the desensitization of leptin signaling in the hypothalamus, ultimately resulting in leptin resistance and obesity [59].

## 7. Concluding remarks

It is generally accepted that leptin plays a central role in regulating body weight. However, treatment of obesity using recombinant leptin seems to be only effective in individuals with a rare homozygous mutation in the gene for leptin or its receptor, or who exhibit subnormal secretion of the hormone, ruling out its current use as a generic drug. In most cases, obese humans have elevated leptin levels, indicative of leptin resistance. Unraveling the molecular mechanisms underlying this leptin resistance is therefore of great clinical interest. Given the complexity of the physiological processes controlling body weight, many defects may underlie leptin resistance. One possibility is a defect in the passage of leptin through the blood–brain barrier. It was suggested that the LR short isoform plays a role in this transport, but the precise mechanism is still a matter of debate and some evidence points to a hitherto uncharacterized leptin transporter in the brain capillary endothelium. Also, leptin resistance might result from a defect at the level of LR activation in the hypothalamic nuclei causing inappropriate sensing of the leptin levels. A better understanding of LR activation may help in understanding this, and may also provide a molecular explanation for the relative insensitivity of LR signaling in the presence of excess dominant negative LRsh receptors. Defects in LR signal transduction could be important as well, and in this light, negative regulators of signaling like SOCS3 and PTP1B are of special interest and may represent targets for the treatment of human obesity.

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## References

[1] 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. and Morgenstern, J.P. (1996) Cell 84, 491–495.
[2] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Nature 372, 425–432.
[3] Montague, C.T., Farooqi, I.S., Whitehead, J.P., Soos, M.A., Rau, H., Wareham, N.J., Sewter, C.P., Digby, J.E., Mohammed, S.N., Hurst, J.A., Cheetham, C.H., Earley, A.R., Barnett, A.H., Prins, J.B. and O'Rahilly, S. (1997) Nature 387, 903–908.
[4] Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M. and Dina, C. (1998) Nature 392, 398–401.
[5] Bado, A., Levasseur, S., Attoub, S., Kermorgant, S., Laigneau, J.P., Bortoluzzi, M.N., Moizo, L., Lehy, T., Guerre-Millo, M., Le Marchand-Brustel, Y. and Lewin, M.J. (1998) Nature 394, 790–793.
[6] Wang, J., Liu, R., Liu, L., Chowdhury, R., Barzilai, N., Tan, J. and Rossetti, L. (1999) Nat. Med. 5, 895–899.
[7] Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper,

J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T. and Deeds, J. (1995) Cell 83, 1263–1271.
[8] Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R. and Burn, P. (1995) Science 269, 546–549.
[9] Farooqi, I.S., Jebb, S.A., Langmack, G., Lawrence, E., Cheetham, C.H., Prentice, A.M., Hughes, I.A., McCamish, M.A. and O'Rahilly, S. (1999) New Engl. J. Med. 341, 879–884.
[10] Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R. and Ranganathan, S. (1995) Nat. Med. 1, 1155–1161.
[11] Heymsfield, S.B., Greenberg, A.S., Fujioka, K., Dixon, R.M., Kushner, R., Hunt, T., Lubina, J.A., Patane, J., Self, B., Hunt, P. and McCamish, M. (1999) J. Am. Med. Assoc. 282, 1568–1575.
[12] El-Haschimi, K., Pierroz, D.D., Hileman, S.M., Bjorbaek, C. and Flier, J.S. (2000) J. Clin. Invest. 105, 1827–1832.
[13] Margetic, S., Gazzola, C., Pegg, G.G. and Hill, R.A. (2002) Int. J. Obes. Relat. Metab. Disord. 26, 1407–1433.
[14] Cohen, B., Novick, D. and Rubinstein, M. (1996) Science 274, 1185–1188.
[15] Hileman, S.M., Pierroz, D.D., Masuzaki, H., Bjorbaek, C., El-Haschimi, K., Banks, W.A. and Flier, J.S. (2002) Endocrinology 143, 775–783.
[16] Hileman, S.M., Tornoe, J., Flier, J.S. and Bjorbaek, C. (2000) Endocrinology 141, 1955–1961.
[17] Ge, H.F., Huang, L., Pourbahrami, T. and Li, C. (2002) J. Biol. Chem. 277, 45898–45903.
[18] Saitou, N. and Nei, M. (1987) Mol. Biol. Evol. 4, 406–425.
[19] Felsenstein, J. (1993) PHYLIP Version 3.5.c Department of Genetics, University of Washington, Seatle, WA, distributed by the author.
[20] Swofford, D. (2001) PAUP 4.beta10, Sinaur Associates, Sunderland, MA.
[21] Fong, T.M. et al. (1998) Mol. Pharmacol. 53, 234–240.
[22] Bravo, J. and Heath, J.K. (2000) EMBO J. 19, 2399–2411.
[23] Chow, D., He, X., Snow, A.L., Rose-John, S. and Garcia, K.C. (2001) Science 291, 2150–2155.
[24] Layton, J.E., Hall, N.E., Connell, F., Venhorst, J. and Treutlein, H.R. (2001) J. Biol. Chem. 276, 36779–36787.
[25] Varghese, J.N. et al. (2002) Proc. Natl. Acad. Sci. USA 99, 15959–15964.
[26] Hiroike, T., Higo, J., Jingami, H. and Toh, H. (2000) Biochem. Biophys. Res. Commun. 275, 154–158.
[27] Sandowski, Y. et al. (2002) J. Biol. Chem. 277, 46304–46309.
[28] Voisin, M.B., Bitard, J., Daburon, S., Moreau, J.F. and Taupin, J.L. (2002) J. Biol. Chem. 277, 13682–13692.
[29] White, D.W., Kuropatwinski, K.K., Devos, R., Baumann, H. and Tartaglia, L.A. (1997) J. Biol. Chem. 272, 4065–4071.
[30] He, W., Gong, K., Zhu, G., Smith, D.K. and Ip, N.Y. (2002) FEBS Lett. 514, 214–218.
[31] Timmermann, A., Kuster, A., Kurth, I., Heinrich, P.C. and Muller-Newen, G. (2002) Eur. J. Biochem. 269, 2716–2726.
[32] Devos, R., Guisez, Y., Van der Heyden, J., White, D.W., Kalai, M., Fountoulakis, M. and Plaetinck, G. (1997) J. Biol. Chem. 272, 18304–18310.
[33] Lee, G.H., Proenca, R., Montez, J.M., Carroll, K.M., Darvishzadeh, J.G., Lee, J.I. and Friedman, J.M. (1996) Nature 379, 632–635.
[34] White, D.W. and Tartaglia, L.A. (1999) J. Cell Biochem. 73, 278–288.
[35] Kloek, C., Haq, A.K., Dunn, S.L., Lavery, H.J., Banks, A.S. and Myers Jr., M.G. (2002) J. Biol. Chem. 277, 41547–41555.
[36] Carpenter, L.R., Farruggella, T.J., Symes, A., Karow, M.L., Yancopoulos, G.D. and Stahl, N. (1998) Proc. Natl. Acad. Sci. USA 95, 6061–6066.
[37] Bahrenberg, G., Behrmann, I., Barthel, A., Hekerman, P., Heinrich, P.C., Joost, H.G. and Becker, W. (2002) Mol. Endocrinol. 16, 859–872.
[38] Bates, S.H. et al. (2003) Nature 421, 856–859.
[39] Vaisse, C., Halaas, J.L., Horvath, C.M., Darnell Jr., J.E., Stoffel, M. and Friedman, J.M. (1996) Nat. Genet. 14, 95–97.
[40] Baumann, H., Morella, K.K., White, D.W., Dembski, M., Bailon, P.S., Kim, H., Lai, C.F. and Tartaglia, L.A. (1996) Proc. Natl. Acad. Sci. USA 93, 8374–8378.
[41] Li, C. and Friedman, J.M. (1999) Proc. Natl. Acad. Sci. USA 96, 9677–9682.
[42] Eyckerman, S., Waelput, W., Verhee, A., Broekaert, D., Vandekerckhove, J. and Tavernier, J. (1999) Eur. Cytokine Netw. 10, 549–556.
[43] Bjorbak, C., Lavery, H.J., Bates, S.H., Olson, R.K., Davis, S.M., Flier, J.S. and Myers Jr., M.G. (2000) J. Biol. Chem. 275, 40649–40657.
[44] Eyckerman, S., Broekaert, D., Verhee, A., Vandekerckhove, J. and Tavernier, J. (2000) FEBS Lett. 486, 33–37.
[45] Banks, A.S., Davis, S.M., Bates, S.H. and Myers Jr., M.G. (2000) J. Biol. Chem. 275, 14563–14572.
[46] Bjorbaek, C., Elmquist, J.K., Frantz, J.D., Shoelson, S.E. and Flier, J.S. (1998) Mol. Cell 1, 619–625.
[47] Waelput, W., Verhee, A., Broekaert, D., Eyckerman, S., Vandekerckhove, J., Beattie, J.H. and Tavernier, J. (2000) Biochem. J. 348, 55–61.
[48] Elias, C.F. et al. (1999) Neuron 23, 775–786.
[49] Bjorbaek, C. et al. (2001) J. Biol. Chem. 276, 4747–4755.
[50] Cacalano, N.A., Sanden, D. and Johnston, J.A. (2001) Nat. Cell Biol. 3, 460–465.
[51] Elchebly, M. et al. (1999) Science 283, 1544–1548.
[52] Myers, M.P. et al. (2001) J. Biol. Chem. 276, 47771–47774.
[53] Salmeen, A., Andersen, J.N., Myers, M.P., Tonks, N.K. and Barford, D. (2000) Mol. Cell 6, 1401–1412.
[54] Kaszubska, W. et al. (2002) Mol. Cell. Endocrinol. 195, 109–118.
[55] Frangioni, J.V., Beahm, P.H., Shifrin, V., Jost, C.A. and Neel, B.G. (1992) Cell 68, 545–560.
[56] Lavoie, C., Chevet, E., Roy, L., Tonks, N.K., Fazel, A., Posner, B.I., Paiement, J. and Bergeron, J.J. (2000) Proc. Natl. Acad. Sci. USA 97, 13637–13642.
[57] Szanto, I. and Kahn, C.R. (2000) Proc. Natl. Acad. Sci. USA 97, 2355–2360.
[58] Kim, Y.B., Uotani, S., Pierroz, D.D., Flier, J.S. and Kahn, B.B. (2000) Endocrinology 141, 2328–2339.
[59] Niswender, K.D., Morton, G.J., Stearns, W.H., Rhodes, C.J., Myers Jr., M.G. and Schwartz, M.W. (2001) Nature 413, 794–795.