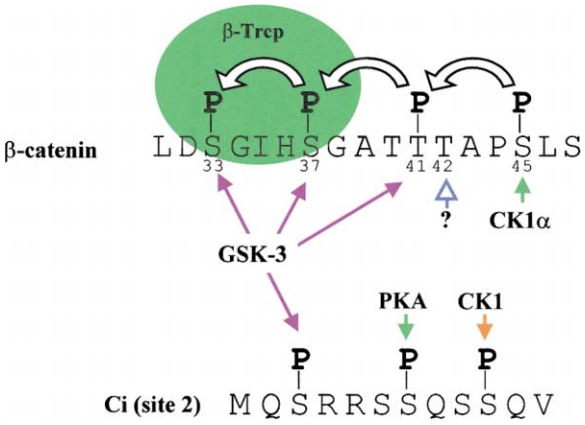

Phosphorylation Sites on β-Catenin and Ci (Site 2)

Priming phosphorylation is indicated by green arrows, and possible second priming site on β-catenin (T42) is marked with a blue open arrowhead.

after overexpression of Wg, a *Drosophila* Wnt homolog. This argues that the role of Axin is to bring together CK1α and GSK-3β to first prime and then fully phosphorylate β-catenin. In this way, only Axin-bound β-catenin is efficiently phosphorylated by GSK-3 and degraded. Interestingly, the CK1 family is the other kinase group that requires primed substrates, although they will also use a site rich in acidic residues at a 100-fold lower efficiency (Flotow et al., 1990). CK1 phosphorylates at a site 3 residues to the C terminus of priming phosphate. This position in β-catenin, residue 42, is a threonine (see Figure), raising the possibility of a second priming kinase that has yet to be identified.

Such a "triple key" mechanism occurs in the Hh signaling pathway. Here, binding of the secreted protein Hedgehog (Hh) to its receptor Patched removes inhibition from a second membrane protein Smoothened (Ingham and McMahon, 2001). This regulates the function of the transcription factor Cubitus interruptus (Ci), known as Gli in vertebrates. Ci is a 155 kDa protein, but in the absence of Hh is cleaved to form a 75 kDa transcriptional inhibitor. This proteolysis requires cAMP-dependent protein kinase (PKA) activity, but Hh signaling does not appear to regulate kinase activity. Price and Kalderon (2002) now show in *Drosophila* that PKA in fact is the priming kinase for both GSK-3 and CK1 phosphorylation of Ci (see Figure). All three kinases are then required for Ci proteolysis.

It is important to establish the identity of the CK1 subtype involved in Ci regulation, as overexpression only hints at Double-time (Dbt), a CK1δ/ϵ subtype. Interestingly, CK1ϵ can phosphorylate β-catenin, but not prime GSK-3 phosphorylation (Vielhaber and Virshup, 2001; Gao et al., 2002). Instead, CK1ϵ mediates Wnt signaling by binding to the protein Dishevelled and may cause dissociation of the Axin complex. It therefore acts antagonistically to CK1α and inhibits β-catenin degradation. This raises the possibility that global changes of CK1ϵ activity could control the balance between Wnt and Hh signaling; for example, increased activity could make cells more sensitive to Wnt, but less sensitive to Hh.

A bank has partial control over many deposit boxes, and likewise, could there be other regulatory elements in common between Wnt and Hh signaling? Smoothened and the Frizzled proteins share some sequence similarity; perhaps they interact with common effector proteins? Both pathways require β-Trcp, although it is not clear whether β-Trcp can bind to fully phosphorylated Ci as it contains no consensus binding site. Could Wnt and Hh pathways share other components to regulate GSK-3 or CK1 phosphorylation and the interaction with β-Trcp? What is clear is that these new observations open further questions in Wnt and Hh signaling.

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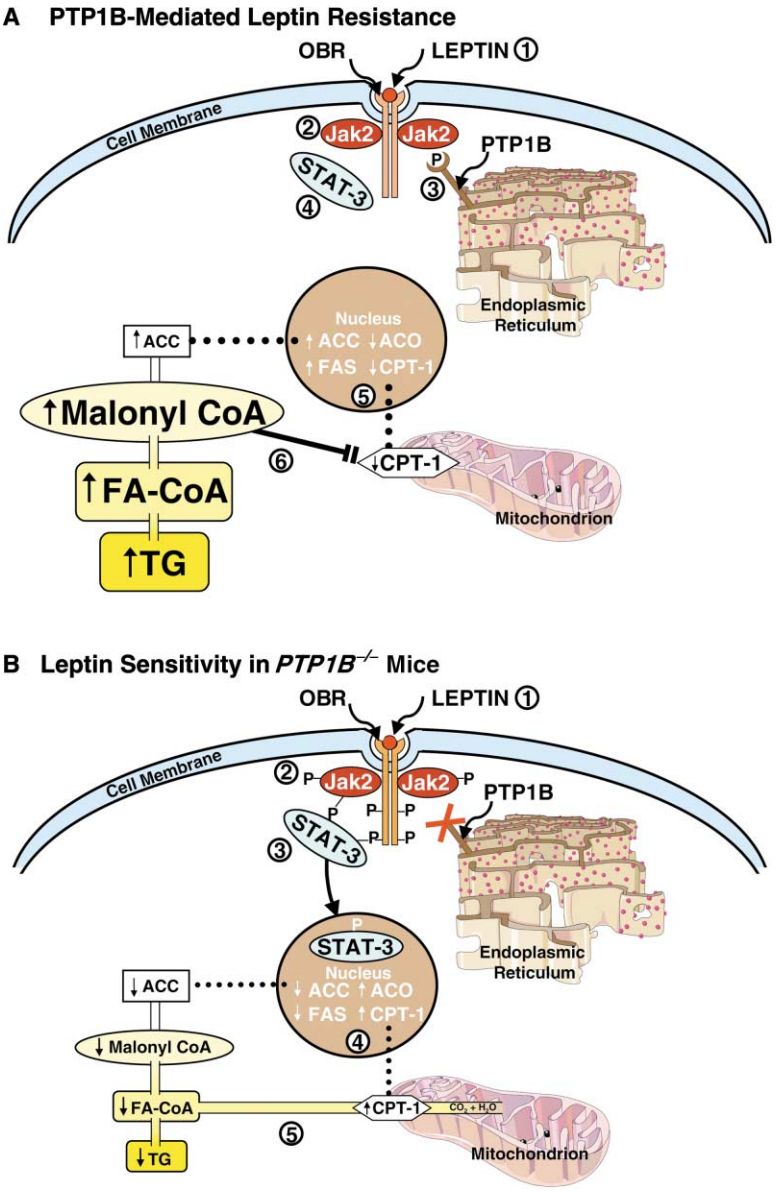
Protein Tyrosine Phosphatase 1B: A Potential Leptin Resistance Factor of Obesity

Indirect evidence implicates leptin resistance in the pathogenesis of the lipotoxicity that complicates obesity and results in the metabolic syndrome. In this issue of *Developmental Cell*, two groups identify protein tyrosine phosphatase 1B (PTP1B) as a cause of leptin resistance through dephosphorylation of Jak2.

The discovery of leptin in 1994 revolutionized concepts of energy metabolism and feeding behavior. Epidemiological and experimental evidence suggests that the role of leptin is to prevent ectopic lipid overaccumulation



How PTP1B Affects Leptin Signaling

(A) Depiction of the putative mechanism by which PTP1B causes resistance to leptin signaling believed to occur in diet-induced obesity. Although leptin binds normally to its cell surface receptor (1) and phosphorylates Jak2 (2), the receptor complex comes into proximity with PTP1B on the ER (3). There, PTP1B dephosphorylates Jak2, blocking the phosphorylation of the receptor and Stat3 (4). Unphosphorylated Stat3 is therefore unable to exert transcriptional control over its target genes that encode enzymes of lipid homeostasis. Consequently, ACC expression is abnormally high and CPT-1 is low (5); malonyl CoA levels are elevated and they inhibit CPT-1, thereby reducing oxidation of fatty acids (6). This is believed to lead to steatosis, lipotoxicity, and lipoapoptosis of nonadipocytes.

(B) Depiction of mechanism of leptin sensitivity in PTP1B knockout mice. Steps (1) and (2) are presumably the same as in (A). However, PTP1B is absent and Stat3, phosphorylated by Jak2 (3), enters the nucleus to alter the transcription of its target genes (4). ACC and FAS are downregulated and CPT-1 and ACO are upregulated. The reduction of ACC expression, coupled with inactivation of the enzyme by AMP-activated kinase, a crucial control system not depicted here, reduces malonyl CoA activity and thereby disinhibits CPT-1 (5). Fatty acid synthesis is reduced, FA-CoA oxidation increases, and normal intracellular liporegulation is restored. ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; CPT-1, carnitine palmitoyl transferase 1; ACO, acyl CoA oxidase; TG, triacylglycerol. (Figure by K. McCorkle.)

during the development of obesity via direct peripheral action on nonadipose tissues, thus sparing them the metabolic trauma of lipid overload (Unger, 2002). When leptin action is lacking, the lipid content of liver, muscle, and other nonadipose tissues is increased; derivatives of fatty acids, such as ceramide, impair their function (lipotoxicity) and ultimately cause apoptosis (lipoapoptosis; Shimabukuro et al., 1998). The clinical phenotype of leptin deficiency in congenital generalized lipodystrophy includes hyperlipidemia, hepatic steatosis, insulin resistance, diabetes, and cardiomyopathy, all of which improve during treatment with recombinant leptin (Shimomura et al., 1999; Oral et al., 2002). A very similar phenotype is observed in rodents with a loss-of-function mutation of the leptin receptor (OBR or LepR); gene transfer of wild-type OBR to the lipid-laden tissue of leptin-unresponsive rats reverses the abnormalities (Wang et al., 1998). These studies suggest that a role of leptin is to enhance tolerance to dietary fat and to maintain fatty acid homeostasis.

Diet-induced obesity (DIO), the most common of American health problems, is also associated with hyperlipidemia, insulin resistance, hepatic steatosis, fatty heart, coronary artery disease, and hypertension, collectively termed "metabolic syndrome" or "syndrome x." In contrast to the congenital syndromes mentioned above, the nonadipose tissues of individuals with DIO are assumed to be protected initially by the progressively increasing hyperleptinemia. The late appearance of lipotoxic manifestations such as diabetes would then reflect an acquired leptin resistance, presumably at a postreceptor level. Previously identified potential leptin resistance factors include Socs-3, Shp2, and others (Bjorbaek et al., 2000).

It was previously observed that increased expression of PTP1B in adipose tissue of obese patients with type 2 diabetes accounted for reduced tyrosyl phosphorylation of the insulin receptor and reduced insulin signaling (Ahmad et al., 1995). PTP1B knockout mice are hypersensitive to insulin, presumably as a result of increased tyrosyl phosphorylation of insulin receptors in skeletal muscle. They are resistant to obesity when fed a high-fat diet, suggesting an increase in leptin sensitivity, a suspicion that is now convincingly verified by two studies published in this issue (Zabolotny et al., 2002; Cheng et al., 2002).

Zabolotny et al. (2002) demonstrate that *PTP1B*$^{-/-}$ mice are hypersensitive to leptin, exhibiting reduced serum leptin levels even on a high-fat diet. Destruction of OBR-expressing cells in the hypothalamus of *PTP1B*$^{-/-}$ mice using gold thioglucose caused only half as much weight gain as in wild-type controls, despite an equal increase in food intake in both groups. These data suggest that PTP1B may directly regulate leptin signaling in peripheral tissues, such as in muscle, fat, and/or liver.

The mechanism of leptin signaling is depicted in the Figure. Extracellular leptin binds the OBR dimer, leading to intracellular Jak2 transphosphorylation and activation. Activated Jak2 phosphorylates Tyr1138 of OBR, providing a binding site for Stat3. When Stat3 binds to OBR, it is phosphorylated by Jak2 and then travels into the nucleus to activate transcription of target genes. A "substrate-trapping" assay was used by both groups to identify which member of the leptin signaling pathway is targeted by PTP1B. Cheng et al. used 293 cells, while Zabolotny et al. used fibroblasts derived from *PTP1B*$^{-/-}$ mice and immortalized with SV40 large T antigen, to identify Jak2 as the substrate of PTP1B in the leptin signaling pathway.

PTP1B is a cytoplasmic enzyme anchored to the membrane of the endoplasmic reticulum (ER) by a hydrophobic tail at the carboxyl terminus. This raises the question of how an ER-anchored phosphatase interacts with a cell surface receptor tyrosine kinase. Haj et al. (2002) observed that PTP1B binds to labeled EGF and PDGF receptors that had been internalized and had migrated in endocytotic vesicles to the ER where they were dephosphorylated. PTP1B may dephosphorylate the insulin receptor by the same mechanism, but this remains to be demonstrated. Since PTP1B antagonizes leptin signaling via dephosphorylation of Jak2 bound to OBR during signaling (Bjorbaek et al., 2000), the intensity and/or duration of leptin signaling may be influenced by the spatial and temporal relationships between the endocytosed receptor complex and the PTP1B on the ER (see Figure).

The leptin signaling pathway is a potential target for drug therapy in the treatment of obesity, type 2 diabetes, and other components of the metabolic syndrome. If the rising plasma leptin levels of obesity initially protect nonadipocytes from steatosis and lipotoxicity, the metabolic syndrome must represent resistance to leptin. Based on these studies, PTP1B is a highly plausible candidate for a leptin resistance factor and inhibiting its activity may provide a therapeutic strategy to restore leptin sensitivity and prevent disease in the nonadipose tissues. Additionally, if PTP1B activity is expressed in obese adipocytes, which are resistant to extremely high leptin concentrations present in their interstitial fluid, its suppression might lower their fat content and reduce obesity ("pharmacologic liposuction").

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