UC Davis
UC Davis Previously Published Works

Title
Protein-tyrosine phosphatase 1B substrates and metabolic regulation

Permalink
https://escholarship.org/uc/item/3n51f5bq

Authors
Bakke, Jesse
Haj, Fawaz G

Publication Date
2015

DOI
10.1016/j.semcdb.2014.09.020

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

NIH Public Access
Author Manuscript
Semin Cell Dev Biol. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:
Semin Cell Dev Biol. 2015 January ; 0: 58–65. doi:10.1016/j.semcdb.2014.09.020.

Protein-Tyrosine Phosphatase 1B Substrates and Metabolic Regulation

Jesse Bakke[1] and **Fawaz G. Haj**[1,2,3,*]

[1]Department of Nutrition, University of California Davis, One Shields Ave, Davis, CA 95616

[2]Division of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, University of California Davis, Sacramento, CA 95817

[3]Comprehensive Cancer Center, University of California Davis, Sacramento, CA 95817

Abstract

Metabolic homeostasis requires integration of complex signaling networks which, when deregulated, contribute to metabolic syndrome and related disorders. Protein-tyrosine phosphatase 1B (PTP1B) has emerged as a key regulator of signaling networks that are implicated in metabolic diseases such as obesity and type 2 diabetes. In this review, we examine mechanisms that regulate PTP1B-substrate interaction, enzymatic activity and experimental approaches to identify PTP1B substrates. We then highlight findings that implicate PTP1B in metabolic regulation. In particular, insulin and leptin signaling are discussed as well as recently identified PTP1B substrates that are involved in endoplasmic reticulum stress response, cell-cell communication, energy balance and vesicle trafficking. In summary, PTP1B exhibits exquisite substrate specificity and is an outstanding pharmaceutical target for obesity and type 2 diabetes.

Keywords

PTP1B; substrate; obesity; diabetes; ER stress; pyruvate kinase; Eph kinase

1. Introduction

Tyrosine phosphorylation is a key post-translational mechanism that regulates a plethora of cellular processes and is required for maintaining homeostasis. Aberrant changes in tyrosine phosphorylation are often associated with disease states such as metabolic disorders, cancer and cardiovascular disease. Tyrosine phosphorylation is tightly controlled by the dynamic and opposing actions of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs) [1, 2]. PTPs are a superfamily of receptor-like and non-transmembrane proteins, whose members are highly specific, tightly regulated and important modulators of cellular

© 2014 Elsevier Ltd. All rights reserved.

*Corresponding author: Fawaz Haj, D.Phil. University of California Davis, Department of Nutrition, 3135 Meyer Hall, Davis, CA 95616, Tel: (530) 752-3214, fghaj@ucdavis.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

signal initiation and termination [2-4]. Classical PTPs contain a 240-250 amino acid catalytic domain specific for phosphotyrosine (pTyr) residues, with a central "signature motif" containing an essential cysteine residue [5]. This review will focus on the prototypical non-receptor protein-tyrosine phosphatase 1B (PTP1B), which has become the subject of intense study and pharmaceutical interest initially owing to its role in metabolic diseases such as obesity and type 2 diabetes. We examine PTP1B cellular location, regulation of enzymatic activity and experimental approaches to identify its substrates. We then highlight the significant findings that implicate PTP1B in metabolic regulation. In particular, insulin and leptin signaling will be discussed as well as PTP1B substrates that are involved in endoplasmic reticulum (ER) stress response, cell-cell communication, energy balance and vesicle trafficking.

2. PTP1B: cellular location and post-translational regulation

PTP1B is an abundant, widely expressed non-receptor phosphatase that was originally purified from human placenta 25 years ago [6]. PTP1B is encoded by the *PTPN1* gene [7] that produces a 435 amino acid protein with a hydrophobic C-terminal sequence that targets PTP1B to the ER [8, 9]. Similar to other members of the PTP family, PTP1B contains the conserved sequence [I/V]H**C**XXGXXR[S/T] (X is any amino acid) that contains the essential catalytic cysteine [3]. Due to the unique chemical environment of the phosphatase active site, the catalytic cysteine that is located at the base of the active site cleft, has an unusually low acid dissociation constant (pKa) (~5.4). The low pKa enhances the catalytic function of the cysteine as a nucleophile but renders it susceptible to oxidation [5, 10-12], a modification that will be discussed later. Since PTP1B regulates many signaling pathways its function is tightly controlled to avoid aberrant cellular signaling. PTP1B sub-cellular location and post-translational modifications are key modulators of its function.

PTP1B is localized on the cytoplasmic face of the ER by means of a hydrophobic (35 residues) C-terminal sequence [8, 9] that imposes topological constraint on PTP1B ability to access substrates [13]. Despite its sub-cellular location, PTP1B can access substrates during (**a**) endocytosis, (**b**) biosynthesis and (**c**) by the ER network movement in close proximity to the plasma membrane (PM) at apparently specialized regions. (**a**) Genetic and biochemical studies establish that PTP1B dephosphorylates receptor PTKs (RTKs) including the activated epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and insulin receptor (IR) [14-21]. These findings raised a question: how can the ER-resident PTP1B dephosphorylate RTKs? Fluorescence resonance energy transfer (FRET) studies demonstrate that most of the activated EGFR and PDGFR interact with PTP1B after their endocytosis at specific sites on the surface of the ER [15]. Subsequent studies extended these findings to the IR [17, 21, 22]. In addition, PTP1B is a potential regulator of RTKs endocytosis. PTP1B-EGFR interaction occurs through membrane contact between multivesicular bodies (MVB) and the ER, with PTP1B promoting the sequestration of EGFR to MVB [23]. In line with these findings, the endosomal protein STAM2 which regulates sorting of activated RTKs is a PTP1B substrate [24]. Therefore, PTP1B regulates RTKs dephosphorylation and endocytosis that are two key modulators of RTKs signaling. (**b**) PTP1B is ideally placed to prevent tyrosine phosphorylation of RTKs during their biosynthesis in the ER. FRET and bioluminescence resonance energy transfer (BRET)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

studies demonstrate basal interaction between PTP1B and IR, which can be enhanced by tunicamycin (an inhibitor of RTKs cell surface maturation) [17]. (**c**) PTP1B also can access PM substrates at regions of cell-cell contact [25-29]. Why can PTP1B dephosphorylate some substrates (such as activated RTKs) after endocytosis, whereas others are targeted on the PM? Quantitative imaging and modeling of protein mobility demonstrate that the ER network comes in close proximity to the PM at apparently specialized sites of cell-cell contact, enabling PTP1B to engage substrates at these regions [30]. Collectively, these studies establish that PTP1B location limits its ability to access substrates but is still capable of interacting with numerous substrates at diverse sub-cellular locations.

Several post-translational modifications regulate PTP1B function including: (**a**) oxidation, (**b**) nitrosylation, (**c**) sulfyhydration, (**d**) sumoylation, (**e**) phosphorylation and (**f**) proteolytic cleavage. We will briefly highlight some key findings and recent advances (extensively reviewed in [31, 32]). (**a**) PTP1B, similar to other classical PTPs subfamily members, is susceptible to oxidation by reactive oxygen species (ROS) due to the chemical environment of its catalytic cleft [5, 12]. Oxidation of the active site cysteine abrogates its nucleophilic properties and inhibits PTP1B activity. Oxidation of PTP1B catalytic cysteine rapidly converts the sulphenic acid (S-OH) to a cyclic sulphenamide and is accompanied by significant changes in the architecture of the active site [33, 34]. These structural changes are reversible under physiological conditions and can help protect the enzyme from higher order ($S\text{-}O_2H$ and $S\text{-}O_3H$) irreversible oxidation and facilitate reduction back to its active form. Of note, these conformational changes were utilized to generate conformation-sensing intracellular antibodies that maintain PTP1B in an inactive state, and may have therapeutic applications [35]. ROS functions as an intracellular second messenger and activation of RTKs leads to transient production of H_2O_2 which is needed for receptor activation [36]. Stimulus-induced ROS production transiently inactivates PTP1B, which usually exerts inhibitory effects on the system, to initiate a response to the stimulus. Indeed, epidermal growth factor [37] and insulin [38] stimulation leads to reversible oxidation of PTP1B and attenuation of its enzymatic activity. (**b**) Similar to ROS, reactive nitrogen species inactivate PTP1B [39]. In particular, *S*-nitrosylation prevents PTP1B active site cysteine from subsequent oxidation when subjected to oxidative stress, and thus plays a protective role against irreversible oxidation [40]. (**c**) In addition, PTP1B is reversibly inactivated by endogenously generated hydrogen sulphide during the ER stress response through sulfhydration of the active site cysteine [41]. Sulfyhydration of PTP1B provides a potential mechanism for regulating ER stress response. (**d**) PTP1B is sumoylated on two lysine residues (Lys335 and 347) and its ER-targeting domain is required for maximum sumoylation [42]. Importantly, insulin-induced sumoylation of PTP1B transiently attenuates its enzymatic activity and inhibits the negative effect of PTP1B on insulin signaling [42]. Further, this modification also affects PTP1B-mediated dephosphorylation of emerin, an inner nuclear membrane protein that regulates nuclear architecture [43]. (**e**) PTP1B is phosphorylated on tyrosine and serine residues, which can either enhance or attenuate its enzymatic activity. Insulin stimulates PTP1B tyrosine phosphorylation (Tyr66, 152 and 153) [44], and results in increased phosphatase activity [45]. On the other hand, insulin induces PTP1B tyrosine phosphorylation and decreases its activity in skeletal muscle and adipose tissue in mice [46]. In addition, PTP1B is phosphorylated on multiple serine residues during

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

mitosis [47] and stress [48]. Moreover, phosphorylation of PTP1B (Ser50) by Akt attenuates PTP1B enzymatic activity and ability to dephosphorylate the IR [49], possibly as a positive feedback mechanism to potentiate insulin signaling. (**f**) In activated platelets, calpain-mediated cleavage frees PTP1B from the ER and generates an activated, soluble enzyme in the cytoplasmic space [50]. The reversibly oxidized PTP1B is more vulnerable to calpain proteolysis [51], illustrating how different post-translational modifications may work in tandem to regulate PTP1B activation.

PTP1B sub-cellular location and tight control of enzymatic activity by diverse post-translational modifications illustrate the dynamic regulation of this enzyme and its ability to modulate numerous signaling pathways, likely in a cell/tissue- and stimulus-dependent manner, with high specificity and precision.

3. Identification of PTP1B substrates

PTP1B-substrate interaction is regulated by its non-catalytic motifs and its catalytic domain. PTP1B contains proline-rich motifs that mediate its association with several Src-homology 3 (SH3) domain-containing substrates such as $p130^{Cas}$ and Src [52, 53]. In addition, PTP1B catalytic domain while closely related to other PTPs has intrinsic specificity and preference for select substrates. Crystallographic studies define the molecular basis of PTP1B interaction with the IR and establish that the sequence E/D-pY-pY-R/K is needed for optimal substrate recognition [54]. Indeed, PTP1B exhibits higher affinity (~70 fold) for tandem pTyr-containing peptides compared with mono-pTyr derivatives [54]. Remarkably, this motif can be utilized to predict PTP1B substrates as demonstrated with Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) [55]. Although the tandem pTyr-tyrosine sequence is important for optimal substrate recognition it is not a requirement, as several well-established PTP1B substrates do not harbor it.

Identification of PTP1B substrates is instrumental for understanding its physiological functions. Wild type PTP1B has a catalytic constant (k_{cat}) of ~2×10^3 molecules/min [56] rendering its association with substrates transient and hard to detect. However, structural studies helped identify PTP1B residues that are important for substrate recognition and catalysis. This enabled researchers to develop the substrate-trapping mutant that retains substrate binding but is catalytically impaired and forms stable complexes with substrates allowing their identification [57]. PTP1B substrate-trap entails mutating the invariant catalytic acid (Asp181) that is conserved in all members of the PTP family [57]. The initial trapping mutant was subsequently improved upon by combining it with another mutation (such as D181A-Q262A) [58-60]. This approach proved powerful in identifying numerous PTP1B substrates and revealed that PTP family members display remarkable substrate specificity in a cellular context [57, 61-66].

4. PTP1B substrates and metabolic regulation

Metabolic syndrome and type 2 diabetes are complex disorders that are associated with sedentary life style, obesity and genetic predisposition [67, 68]. The increased prevalence of these diseases highlights the urgent need to elucidate the underlying molecular mechanisms to aid in therapeutic intervention. PTP1B is an established metabolic regulator in mammals

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and a pharmacological target for obesity and type 2 diabetes. We will examine key findings that implicate PTP1B in insulin and leptin signaling, ER stress response, cell-cell communication as well as energy balance and vesicle trafficking (PTP1B substrates that are implicated in metabolism, cancer and immune function are extensively reviewed in [32, 69, 70]).

4.1. Insulin signaling

Insulin is secreted from pancreatic (β-cells and acts as a major regulator of glucose homeostasis through a complex and integrated signaling network [71]. Insulin binding induces IR autophosphorylation on tyrosines (Tyr1158, 1162 and 1163) leading to the recruitment of insulin receptor substrates (IRSs) (Figure 1). These are phosphorylated by the IR and can activate several downstream pathways including the phosphatidylinositol 3-kinase (PI3K)-Akt and MAPK pathways [72, 73]. Numerous studies illustrate the tissue-specific effects of insulin and establish that impaired insulin signaling is central to the development of the metabolic syndrome [74]. PTP1B is an established regulator of insulin signaling as evidenced by biochemical, genetic and inhibitor studies. Initial evidence in *Xenopus* suggests a role for PTP1B in regulating IR phosphorylation and antagonizing insulin signaling [75, 76]. Subsequent studies utilizing overexpression and neutralizing antibodies provide further support for the role of PTP1B in regulating insulin signaling [77, 78]. In addition, substrate-trapping studies identify direct association between PTP1B and IR [44, 79] and IRS1 [80, 81]. Together, these studies implicate PTP1B in IR signaling however, the most compelling evidence was provided by targeted disruption of *PTPN1* in mice [19]. Whole-body PTP1B knockout (KO) mice exhibit increased systemic insulin sensitivity and enhanced glucose tolerance compared with controls [19]. At the molecular level, PTP1B KO mice display enhanced insulin-induced IR phosphorylation in liver and muscle establishing PTP1B as a regulator of insulin signaling *in vivo*. When fed high fat diet, PTP1B KO mice exhibit resistance to weight gain whereas control mice gain weight and develop insulin resistance [19]. These findings were rapidly confirmed and extended in an independently generated PTP1B KO mouse line [20]. Subsequent studies using tissue-specific expression and deletion approaches provide additional insights into the role of PTP1B in insulin signaling. PTP1B over-expression in muscle impairs insulin-stimulated IR activation and decreases muscle glucose uptake [82]. In addition, adenovirus-mediated re-expression of PTP1B in the liver of knockout mice reduces insulin sensitivity, attenuates IR phosphorylation and reveals preferential dephosphorylation of hepatic IR at Tyr1162/1163 by PTP1B [83]. Moreover, mice with muscle-specific PTP1B deficiency have comparable body weight to control mice but exhibit improved systemic insulin sensitivity that is likely due to increased IR phosphorylation [84]. Similarly, mice with liver-specific PTP1B deficiency exhibit increased insulin sensitivity independent of changes in adiposity [85, 86]. Further, mice with myeloid cell-specific PTP1B deficiency exhibit improved glucose tolerance and protection against lipopolysaccharide-induced hyperinsulinemia [87]. These findings sparked interest in developing PTP1B inhibitors for the treatment of obesity and type 2 diabetes. While this led to the generation of potent inhibitors *in vitro*, they did not progress beyond the pre-clinical stage thus far due to delivery and specificity problems [88-90]. However, PTP1B antisense oligonucleotides (ASO; ISIS 113715) reduced PTP1B in liver and adipose, enhanced insulin signaling in the liver and improved systemic insulin

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

sensitivity in murine models of obesity [91]. Furthermore, ASO administration in monkeys reduces PTP1B expression in liver and adipose and improves insulin sensitivity [92]. Collectively, these studies establish PTP1B as a physiological regulator of insulin signaling and as an outstanding pharmacological target.

4.2. Leptin signaling

Leptin is an adipocyte-derived hormone that regulates feeding and energy homeostasis [93, 94]. Leptin signals through its receptor LepRb, which is associated with JAK2 [95]. Upon leptin binding to LepRb, JAK2 is activated, autophosphorylates and subsequently phosphorylates tyrosine residues along the intracellular tail of LepRb enabling the recruitment of downstream effectors such as signal transducer and activator of transcription 3 (STAT3) [96] (Figure 1). Phosphorylated STAT3 dimerizes and translocates to the nucleus to regulate transcription of target genes. Physiological evidence implicating PTP1B in attenuating leptin signaling through dephosphorylating JAK2 was provided by knockout studies [97, 98]. Mice with combined leptin and PTP1B deficiency display decreased weight gain, reduced adiposity and increased resting metabolic rates [97, 98]. In addition, specific deletion of PTP1B in neurons [99], LepRb-expressing cells [100] and pro-opiomelanocortin (POMC) neurons leads to reduced body mass and adiposity and increased energy expenditure [99]. Furthermore, hypothalamus-specific deletion suggests that the metabolic effects of central PTP1B-deficiency are due to action within the hypothalamus through a leptin receptor-dependent pathway [101]. Together, these studies highlight PTP1B as a target for improving leptin sensitivity.

4.3. Endoplasmic reticulum stress response

Emerging evidence establishes that ER dysfunction is a significant contributor to metabolic diseases, particularly obesity and type 2 diabetes [102, 103]. The ER plays an essential role in protein folding and accumulation of unfolded proteins within the ER lumen causes ER stress [104]. Cells deploy adaptive mechanisms to counter the deleterious effects of ER stress known as the unfolded protein response (UPR) [104]. UPR utilizes three ER transmembrane sensors: protein kinase-like ER kinase (PERK), inositol requiring enzyme 1α (IRE1α) and activating transcription factor 6 (ATF6) [103, 105]. These sensors synergize to attenuate stress and maintain homeostasis through increasing the folding capacity of the ER, translational attenuation, ER biogenesis, and ER-associated protein degradation. For example, PERK phosphorylates eukaryotic translation initiation factor 2 (eIF2α), a modification that blocks initiation of mRNA translation (Figure 1) [106]. Of note, PERK is a serine/threonine kinase but is also tyrosine phosphorylated [107] and its activity is regulated by phosphorylation at Tyr615 [108]. A link between PTP1B and ER stress was established using PTP1B-null fibroblasts which exhibit resistance to IRE1α-mediated signaling and attenuation of ER stress-induced apoptosis [109]. Subsequent studies showed that mice with liver-specific PTP1B deficiency are protected against high fat diet-induced ER stress and exhibit attenuation of PERK and eIF2α phosphorylation [85, 110]. In contrast to these findings, PTP1B deficiency in pancreatic β-cell lines leads to increased chemical-induced PERK/eIF2α signaling [111]. Similarly, PTP1B deficiency in adipose tissue and adipocytes leads to upregulation of PERK-eIF2α phosphorylation and sensitizes adipocytes to chemical-induced ER stress [112]. In addition, Krishnan *etal*, demonstrated a direct link

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

between PTP1B and PERK and modulation of ER stress response [41]. ER stress-induced production of hydrogen sulphide in fibroblasts reversibly inactivates PTP1B by sulfhydration of the active site cysteine (see earlier). Importantly, PTP1B inhibition directly promotes PERK phosphorylation and activity during the response to ER stress. Furthermore, this study identifies PERK as a direct substrate of PTP1B [41]. This finding was rapidly confirmed in adipocytes where direct interaction between PTP1B and PERK was established and PERK Tyr615 identified as a mediator of the association [112]. More recently, studies examining the role of PTP1B in ER stress using cultured myotubes point to a requirement for PTP1B for activating PERK [113]. The reason(s) for the seemingly contradictory findings regarding the role of PTP1B in ER stress remain unclear. The effects of PTP1B on ER stress signaling can be general (indirect regulation of one or more pathway) and/or specific (direct regulation of a key target such as PERK). In addition, PTP1B can conceivably regulate the same pathway in a tissue-specific manner to yield different outcomes. This highlights the need to further elucidate the tissue- and stimulus-dependent regulation of ER stress response by PTP1B and its implications in obesity and type 2 diabetes.

4.4. Cell-cell communication

Cell-cell communication is important for metabolic regulation and maintaining homeostasis. For example, pancreatic β cell-cell communication ensures secretion of low amounts of insulin during starvation and high levels during feeding [114]. Eph RTKs and their PM-bound ephrin ligands exhibit a unique feature of bidirectional signaling and are thus well suited for regulating cell-cell communication (Figure 1) [115]. Eph and ephrins are divided into A and B subclasses, with most EphAs binding to ephrin As and most EphBs binding to ephrin Bs [115]. EphA5 signaling is critical for β cell-cell communication and insulin secretion [116]. Under basal conditions, EphA5 signaling inhibits insulin secretion, but after glucose stimulation, EphA5 tyrosine dephosphorylation inhibits its signaling and favors ephrin A5 signaling and insulin secretion [116]. Indeed, Eph RTK pharmacological inhibition enhances glucose-stimulated insulin secretion (GSIS) from mouse and human pancreatic islets [117]. As indicated earlier, ER-resident PTP1B engages PM substrates at regions of cell-cell contact and can regulate cell-cell communication. PTP1B regulates EphA3 function and trafficking [118] and EphA2 tyrosine phosphorylation specifically at regions of cell-cell contact [119]. In addition, pancreatic (endocrine and exocrine) PTP1B deficiency enhances basal and glucose-stimulated EphA5 tyrosine phosphorylation in islets [66]. Further, substrate trapping identifies EphA5 as a PTP1B substrate in pancreatic β-cell line. Mice with pancreatic PTP1B deficiency display comparable glucose tolerance to controls [66]. However, when subjected to a robust challenge (such as prolonged high fat feeding or aging) pancreatic PTP1B knockout mice exhibit mild glucose intolerance and attenuated GSIS, in contrast to the salutary effects of whole-body PTP1B deletion [19, 20] and its deletion in IRS2 knockout mice [120]. Nevertheless, these findings establish association between PTP1B and EphA5; however its contribution to GSIS remains to be determined. It is worth noting that PTP1B likely regulates cell-cell communication by engaging numerous substrates at regions of cell-cell contact. Indeed, Zonula Occludens (ZO1) and p120 catenin are hyper-phosphorylated in PTP1B-null fibroblasts and are thus

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

putative PTP1B substrates [62]. Regulation of cell-cell communication by PTP1B and its potential contribution to metabolic regulation warrants additional investigation.

4.5. Energy balance

Energy balance is an important component in metabolic homeostasis and emerging evidence suggests regulation of key modulators by PTP1B. AMP-activated protein kinase (AMPK) is a fuel sensing enzyme complex and a regulator of adipose function [121]. PTP1B-deficient brown adipose tissue [122] and brown adipocytes [81] exhibit increased AMPK activity. In addition, recent studies identify pyruvate kinase M2 (PKM2) as a PTP1B substrate [64]. Pyruvate kinase (PK) is a rate-limiting glycolytic enzyme that catalyzes the generation of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP [123] (Figure 1). Four isoforms of pyruvate kinase exist in mammals with the M2 isoform expressed in proliferative adult cells and some differentiated tissues such as lung, adipose and pancreatic islets [124]. In addition, most cancer cells have increased expression of PKM2 that facilitates aerobic glycolysis and provides a growth advantage [125, 126]. Indeed, PKM2 deficiency or pharmacological inhibition elicits anticancer effects [126-128]. Moreover, PKM2 pyruvate kinase activity is regulated by binding to phosphotyrosine or becoming phosphorylated under growth stimulation [125, 129]. Of note, PKM2 Tyr105 phosphorylation suppresses its pyruvate kinase activity [129]. Recently, we identified PKM2 as a novel PTP1B substrate in adipocytes and demonstrated that PTP1B deficiency and pharmacological inhibition lead to increased PKM2 total tyrosine and Tyr105 phosphorylation in adipocytes and *in vivo* [64]. Moreover, PKM2 Tyr105 is a key residue that mediates PKM2 interaction with PTP1B. Further, decreased PKM2 Tyr105 phosphorylation in adipose tissue correlates with glucose intolerance and insulin resistance in rodents, non-human primates and humans [64]. These findings establish PKM2 as an exciting PTP1B substrate and highlight the need to investigate the interplay between PTP1B and PKM2 and its potential contribution to the normal metabolic state and to cancer metabolism.

4.6. Vesicle trafficking

Insulin-stimulated glucose uptake in peripheral tissues is mediated by translocation of glucose transporter GLUT4-containing vesicles to the cell periphery and fusion with the PM leading to externalization of GLUT4 [130]. Fusion of vesicles with the PM is mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which is tightly regulated to ensure precise trafficking [131]. Munc18 proteins are essential regulators of SNARE-mediated vesicle budding and fusion, and mammalian cells express three isoforms (a, b and c) [132]. Munc18c is solely utilized by adipocytes to regulate insulin-stimulated GLUT4 vesicle exocytosis [133]. In addition, Munc18c tyrosine phosphorylation regulates its interactions and function, and the IR phosphorylates Munc18c (Tyr521) thereby linking insulin signaling to SNARE exocytosis [134] (Figure 1). Munc18c was recently identified as a PTP1B substrate and PTP1B deficiency in adipocytes and adipose tissue leads to increased Munc18c tyrosine phosphorylation [65]. In addition, Munc18c Tyr218/219 and Tyr521 mediate its interaction with PTP1B. Importantly, biochemical and functional studies establish that insulin-induced Munc18c tyrosine phosphorylation in general, and Tyr218/219 and Tyr521 in particular, regulate its

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

associations and facilitate SNARE-mediated delivery of GLUT4 to the PM [65]. Collectively, these studies identify PTP1B as a regulator of Munc18c phosphorylation and function in adipocytes. Additional studies that decipher PTP1B regulation of Munc18 isoforms in insulin-responsive tissues will likely yield new insights into GLUT4 trafficking and may provide modalities to increase glucose transport.

5. Concluding remarks

The alarming increase in metabolic syndrome highlights the urgent need for effective therapeutic interventions. As this review illustrates, PTP1B is an outstanding pharmacological target for obesity and type 2 diabetes. Numerous PTP1B substrates have been identified with some serving as key components in metabolic signaling including insulin, leptin, ER stress, cell-cell communication, energy balance and vesicle trafficking, and it is likely that additional metabolic substrates will be identified. Deciphering PTP1B-substrates interactions and their biological significance will yield valuable insights into metabolic regulation. While PTP1B inhibition is actively pursued for therapeutic intervention and novel approaches will likely aid in developing small molecule inhibitors for clinical evaluation, targeting PTP1B substrates may be easier and should be considered as a complementary approach to combat obesity and type 2 diabetes.

Acknowledgments

Thanks to Ahmed Bettaieb for comments on the manuscript and preparing the figure. Research in the Haj laboratory is supported by grants from Juvenile Diabetes Research Foundation, American Diabetes Association and National Institutes of Health (R56 DK084317, R01 DK090492 and R01 DK095359 to F.G.H and K99 DK100736 to A.B).

References

1. Sun H, Tonks NK. The coordinated action of protein tyrosine phosphatases and kinases in cell signaling. Trends in biochemical sciences. 1994; 19:480–5. [PubMed: 7855891]
2. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. Nat Rev Mol Cell Biol. 2006; 7:833–46. [PubMed: 17057753]
3. Tonks NK, Neel BG. Combinatorial control of the specificity of protein tyrosine phosphatases. Curr Opin Cell Biol. 2001; 13:182–95. [PubMed: 11248552]
4. Mustelin T, Feng GS, Bottini N, Alonso A, Kholod N, Birle D, et al. Protein tyrosine phosphatases. Front Biosci. 2002; 7:d85–142. [PubMed: 11779706]
5. Denu JM, Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. Current opinion in chemical biology. 1998; 2:633–41. [PubMed: 9818190]
6. Charbonneau H, Tonks NK, Kumar S, Diltz CD, Harrylock M, Cool DE, et al. Human placenta protein-tyrosine-phosphatase: amino acid sequence and relationship to a family of receptor-like proteins. Proceedings of the National Academy of Sciences of the United States of America. 1989; 86:5252–6. [PubMed: 2546149]
7. Brown-Shimer S, Johnson KA, Lawrence JB, Johnson C, Bruskin A, Green NR, et al. Molecular cloning and chromosome mapping of the human gene encoding protein phosphotyrosyl phosphatase 1B. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87:5148–52. [PubMed: 2164224]
8. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell. 1992; 68:545–60. [PubMed: 1739967]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

9. Woodford-Thomas TA, Rhodes JD, Dixon JE. Expression of a protein tyrosine phosphatase in normal and v-src-transformed mouse 3T3 fibroblasts. The Journal of cell biology. 1992; 117:401–14. [PubMed: 1373143]
10. Lohse DL, Denu JM, Santoro N, Dixon JE. Roles of aspartic acid-181 and serine-222 in intermediate formation and hydrolysis of the mammalian protein-tyrosine-phosphatase PTP1. Biochemistry. 1997; 36:4568–75. [PubMed: 9109666]
11. Barford D, Das AK, Egloff MP. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu Rev Biophys Biomol Struct. 1998; 27:133–64. [PubMed: 9646865]
12. Salmeen A, Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. Antioxidants & redox signaling. 2005; 7:560–77. [PubMed: 15890001]
13. Mauro LJ, Dixon JE. 'Zip codes' direct intracellular protein tyrosine phosphatases to the correct cellular 'address'. Trends in biochemical sciences. 1994; 19:151–5. [PubMed: 8016862]
14. Liu F, Chernoff J. Protein tyrosine phosphatase 1B interacts with and is tyrosine phosphorylated by the epidermal growth factor receptor. The Biochemical journal. 1997; 327(Pt 1):139–45. [PubMed: 9355745]
15. Haj FG, Verveer PJ, Squire A, Neel BG, Bastiaens PI. Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. Science. 2002; 295:1708–11. [PubMed: 11872838]
16. Haj FG, Markova B, Klaman LD, Bohmer FD, Neel BG. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatase-1B. The Journal of biological chemistry. 2003; 278:739–44. [PubMed: 12424235]
17. Boute N, Boubekeur S, Lacasa D, Issad T. Dynamics of the interaction between the insulin receptor and protein tyrosine-phosphatase 1B in living cells. EMBO Rep. 2003; 4:313–9. [PubMed: 12634852]
18. Dadke S, Kusari J, Chernoff J. Down-regulation of insulin signaling by protein-tyrosine phosphatase 1B is mediated by an N-terminal binding region. The Journal of biological chemistry. 2000; 275:23642–7. [PubMed: 10807907]
19. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science. 1999; 283:1544–8. [PubMed: 10066179]
20. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Molecular and cellular biology. 2000; 20:5479–89. [PubMed: 10891488]
21. Romsicki Y, Reece M, Gauthier JY, Asante-Appiah E, Kennedy BP. Protein tyrosine phosphatase-1B dephosphorylation of the insulin receptor occurs in a perinuclear endosome compartment in human embryonic kidney 293 cells. The Journal of biological chemistry. 2004; 279:12868–75. [PubMed: 14722096]
22. Cromlish WA, Tang M, Kyskan R, Tran L, Kennedy BP. PTP1B-dependent insulin receptor phosphorylation/residency in the endocytic recycling compartment of CHO-IR cells. Biochemical pharmacology. 2006; 72:1279–92. [PubMed: 16956584]
23. Eden ER, White IJ, Tsapara A, Futter CE. Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. Nature cell biology. 2010; 12:267–72.
24. Stuible M, Abella JV, Feldhammer M, Nossov M, Sangwan V, Blagoev B, et al. PTP1B targets the endosomal sorting machinery: dephosphorylation of regulatory sites on the ESCRT component STAM2. The Journal of biological chemistry. 2010; 31:23899–907. [PubMed: 20504764]
25. Balsamo J, Leung T, Ernst H, Zanin MK, Hoffman S, Lilien J. Regulated binding of PTP1B-like phosphatase to N-cadherin: control of cadherin-mediated adhesion by dephosphorylation of beta-catenin. The Journal of cell biology. 1996; 134:801–13. [PubMed: 8707857]
26. Pathre P, Arregui C, Wampler T, Kue I, Leung TC, Lilien J, et al. PTP1B regulates neurite extension mediated by cell-cell and cell-matrix adhesion molecules. Journal of neuroscience research. 2001; 63:143–50. [PubMed: 11169624]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

27. Xu G, Craig AW, Greer P, Miller M, Anastasiadis PZ, Lilien J, et al. Continuous association of cadherin with beta-catenin requires the non-receptor tyrosine-kinase Fer. Journal of cell science. 2004; 117:3207–19. [PubMed: 15226396]
28. Anderie I, Schulz I, Schmid A. Direct interaction between ER membrane-bound PTP1B and its plasma membrane-anchored targets. Cellular signalling. 2007; 19:582–92. [PubMed: 17092689]
29. Nakamura Y, Patrushev N, Inomata H, Mehta D, Urao N, Kim HW, et al. Role of protein tyrosine phosphatase 1B in vascular endothelial growth factor signaling and cell-cell adhesions in endothelial cells. Circulation research. 2008; 102:1182–91. [PubMed: 18451337]
30. Haj FG, Sabet O, Kinkhabwala A, Wimmer-Kleikamp S, Roukos V, Han HM, et al. Regulation of signaling at regions of cell-cell contact by endoplasmic reticulum-bound protein-tyrosine phosphatase 1B. PloS one. 2012; 7:e36633. [PubMed: 22655028]
31. Tonks NK. PTP1B: From the sidelines to the front lines! FEBS letters. 2003; 546:140–8. [PubMed: 12829250]
32. Yip SC, Saha S, Chernoff J. PTP1B: a double agent in metabolism and oncogenesis. Trends in biochemical sciences. 2010; 35:442–9. [PubMed: 20381358]
33. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, et al. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature. 2003; 423:769–73. [PubMed: 12802338]
34. Van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature. 2003; 423:773–7. [PubMed: 12802339]
35. Haque A, Andersen JN, Salmeen A, Barford D, Tonks NK. Conformation-sensing antibodies stabilize the oxidized form of PTP1B and inhibit its phosphatase activity. Cell. 2011; 147:185–98. [PubMed: 21962515]
36. Rhee SG, Bae YS, Lee SR, Kwon J. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. Sci STKE. 2000; 2000:pe1. [PubMed: 11752613]
37. Lee SR, Kwon KS, Kim SR, Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. The Journal of biological chemistry. 1998; 273:15366–72. [PubMed: 9624118]
38. Meng TC, Buckley DA, Galic S, Tiganis T, Tonks NK. Regulation of insulin signaling through reversible oxidation of the protein-tyrosine phosphatases TC45 and PTP1B. The Journal of biological chemistry. 2004; 279:37716–25. [PubMed: 15192089]
39. Chen YY, Huang YF, Khoo KH, Meng TC. Mass spectrometry-based analyses for identifying and characterizing S-nitrosylation of protein tyrosine phosphatases. Methods. 2007; 42:243–9. [PubMed: 17532511]
40. Chen YY, Chu HM, Pan KT, Teng CH, Wang DL, Wang AH, et al. Cysteine S-nitrosylation protects protein-tyrosine phosphatase 1B against oxidation-induced permanent inactivation. The Journal of biological chemistry. 2008; 283:35265–72. [PubMed: 18840608]
41. Krishnan N, Fu C, Pappin DJ, Tonks NK. H2S-Induced Sulfhydration of the Phosphatase PTP1B and Its Role in the Endoplasmic Reticulum Stress Response. Science signaling. 4:ra86. [PubMed: 22169477]
42. Dadke S, Cotteret S, Yip SC, Jaffer ZM, Haj F, Ivanov A, et al. Regulation of protein tyrosine phosphatase 1B by sumoylation. Nature cell biology. 2007; 9:80–5.
43. Yip SC, Cotteret S, Chernoff J. Sumoylated protein tyrosine phosphatase 1B localizes to the inner nuclear membrane and regulates the tyrosine phosphorylation of emerin. Journal of cell science. 125:310–6. [PubMed: 22266903]
44. Bandyopadhyay D, Kusari A, Kenner KA, Liu F, Chernoff J, Gustafson TA, et al. Proteintyrosine phosphatase 1B complexes with the insulin receptor in vivo and is tyrosine-phosphorylated in the presence of insulin. The Journal of biological chemistry. 1997; 272:1639–45. [PubMed: 8999839]
45. Dadke S, Kusari A, Kusari J. Phosphorylation and activation of protein tyrosine phosphatase (PTP) 1B by insulin receptor. Mol Cell Biochem. 2001; 221:147–54. [PubMed: 11506178]
46. Tao J, Malbon CC, Wang HY. Insulin stimulates tyrosine phosphorylation and inactivation of protein-tyrosine phosphatase 1B in vivo. The Journal of biological chemistry. 2001; 276:29520–5. [PubMed: 11395511]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

47. Flint AJ, Gebbink MF, Franza BR Jr, Hill DE, Tonks NK. Multi-site phosphorylation of the protein tyrosine phosphatase, PTP1B: identification of cell cycle regulated and phorbol ester stimulated sites of phosphorylation. The EMBO journal. 1993; 12:1937–46. [PubMed: 8491187]

48. Shifrin VI, Davis RJ, Neel BG. Phosphorylation of protein-tyrosine phosphatase PTP-1B on identical sites suggests activation of a common signaling pathway during mitosis and stress response in mammalian cells. The Journal of biological chemistry. 1997; 272:2957–62. [PubMed: 9006942]

49. Ravichandran LV, Chen H, Li Y, Quon MJ. Phosphorylation of PTP1B at Ser(50) by Akt impairs its ability to dephosphorylate the insulin receptor. Molecular endocrinology. 2001; 15:1768–80. [PubMed: 11579209]

50. Frangioni JV, Oda A, Smith M, Salzman EW, Neel BG. Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP-1B) in human platelets. The EMBO journal. 1993; 12:4843–56. [PubMed: 8223493]

51. Trumpler A, Schlott B, Herrlich P, Greer PA, Bohmer FD. Calpain-mediated degradation of reversibly oxidized protein-tyrosine phosphatase 1B. The FEBS journal. 2009; 276:5622–33. [PubMed: 19712109]

52. Liu F, Hill DE, Chernoff J. Direct binding of the proline-rich region of protein tyrosine phosphatase 1B to the Src homology 3 domain of p130(Cas). The Journal of biological chemistry. 1996; 271:31290–5. [PubMed: 8940134]

53. Liang F, Lee SY, Liang J, Lawrence DS, Zhang ZY. The role of protein-tyrosine phosphatase 1B in integrin signaling. The Journal of biological chemistry. 2005; 280:24857–63. [PubMed: 15866871]

54. Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Molecular cell. 2000; 6:1401–12. [PubMed: 11163213]

55. Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien JP, et al. TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. The Journal of biological chemistry. 2001; 276:47771–4. [PubMed: 11694501]

56. Zhang M, Van Etten RL, Stauffacher CV. Crystal structure of bovine heart phosphotyrosyl phosphatase at 2.2-A resolution. Biochemistry. 1994; 33:11097–105. [PubMed: 7537084]

57. Flint AJ, Tiganis T, Barford D, Tonks NK. Development of “substrate-trapping” mutants to identify physiological substrates of protein tyrosine phosphatases. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94:1680–5. [PubMed: 9050838]

58. Garton AJ, Flint AJ, Tonks NK. Identification of p130(cas) as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. Molecular and cellular biology. 1996; 16:6408–18. [PubMed: 8887669]

59. Xie L, Zhang YL, Zhang ZY. Design and characterization of an improved protein tyrosine phosphatase substrate-trapping mutant. Biochemistry. 2002; 41:4032–9. [PubMed: 11900546]

60. Boubekeur S, Boute N, Pagesy P, Zilberfarb V, Christeff N, Issad T. A new highly efficient substrate-trapping mutant of protein tyrosine phosphatase 1B (PTP1B) reveals full autoactivation of the insulin receptor precursor. The Journal of biological chemistry. 2011; 286:19373–80. [PubMed: 21487008]

61. Stuible M, Dube N, Tremblay ML. PTP1B regulates cortactin tyrosine phosphorylation by targeting Tyr446. The Journal of biological chemistry. 2008; 283:15740–6. [PubMed: 18387954]

62. Mertins P, Eberl HC, Renkawitz J, Olsen JV, Tremblay ML, Mann M, et al. Investigation of protein tyrosine phosphatase 1B function by quantitative proteomics. Mol Cell Proteomics. 2008; 9:1763–77. [PubMed: 18515860]

63. Bettaieb A, Matsuo K, Matsuo I, Wang S, Melhem R, Koromilas AE, et al. Protein tyrosine phosphatase 1B deficiency potentiates PERK/eIF2alpha signaling in brown adipocytes. PloS one. 2012; 7:e34412. [PubMed: 22509299]

64. Bettaieb A, Bakke J, Nagata N, Matsuo K, Xi Y, Liu S, et al. Protein tyrosine phosphatase 1B regulates pyruvate kinase M2 tyrosine phosphorylation. The Journal of biological chemistry. 2013; 288:17360–71. [PubMed: 23640882]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

65. Bakke J, Bettaieb A, Nagata N, Matsuo K, Haj FG. Regulation of the SNARE-interacting protein Munc18c tyrosine phosphorylation in adipocytes by protein-tyrosine phosphatase 1B. Cell communication and signaling. 2013; 11:57. [PubMed: 23937695]

66. Liu S, Xi Y, Bettaieb A, Matsuo K, Matsuo I, Kulkarni RN, et al. Disruption of protein-tyrosine phosphatase 1B expression in the pancreas affects beta-cell function. Endocrinology. 2014 en20132004.

67. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell. 2001; 104:531–43. [PubMed: 11239410]

68. Biddinger SB, Kahn CR. From mice to men: insights into the insulin resistance syndromes. Annual review of physiology. 2006; 68:123–58.

69. Tiganis T. PTP1B and TCPTP--nonredundant phosphatases in insulin signaling and glucose homeostasis. The FEBS journal. 2013; 280:445–58. [PubMed: 22404968]

70. Feldhammer M, Uetani N, Miranda-Saavedra D, Tremblay ML. PTP1B: a simple enzyme for a complex world. Critical reviews in biochemistry and molecular biology. 2013; 48:430–45. [PubMed: 23879520]

71. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol. 2006; 7:85–96. [PubMed: 16493415]

72. Avruch J. Insulin signal transduction through protein kinase cascades. Mol Cell Biochem. 1998; 182:31–48. [PubMed: 9609112]

73. Luo J, Cantley LC. The negative regulation of phosphoinositide 3-kinase signaling by p85 and it's implication in cancer. Cell cycle. 2005; 4:1309–12. [PubMed: 16131837]

74. Rask-Madsen C, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. Arteriosclerosis, thrombosis, and vascular biology. 2012; 32:2052–9.

75. Cicirelli MF, Tonks NK, Diltz CD, Weiel JE, Fischer EH, Krebs EG. Microinjection of a proteintyrosine-phosphatase inhibits insulin action in Xenopus oocytes. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87:5514–8. [PubMed: 2164686]

76. Tonks NK, Cicirelli MF, Diltz CD, Krebs EG, Fischer EH. Effect of microinjection of a low-Mr human placenta protein tyrosine phosphatase on induction of meiotic cell division in Xenopus oocytes. Molecular and cellular biology. 1990; 10:458–63. [PubMed: 2153916]

77. Ahmad F, Li PM, Meyerovitch J, Goldstein BJ. Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. The Journal of biological chemistry. 1995; 270:20503–8. [PubMed: 7544790]

78. Kenner KA, Anyanwu E, Olefsky JM, Kusari J. Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signaling. The Journal of biological chemistry. 1996; 271:19810–6. [PubMed: 8702689]

79. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, et al. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes. 1996; 45:1379–85. [PubMed: 8826975]

80. Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. The Journal of biological chemistry. 2000; 275:4283–9. [PubMed: 10660596]

81. Matsuo K, Bettaieb A, Nagata N, Matsuo I, Keilhack H, Haj FG. Regulation of brown fat adipogenesis by protein tyrosine phosphatase 1B. PloS one. 2011; 6:e16446. [PubMed: 21305007]

82. Zabolotny JM, Haj FG, Kim YB, Kim HJ, Shulman GI, Kim JK, et al. Transgenic overexpression of protein-tyrosine phosphatase 1B in muscle causes insulin resistance, but overexpression with leukocyte antigen-related phosphatase does not additively impair insulin action. The Journal of biological chemistry. 2004; 279:24844–51. [PubMed: 15031294]

83. Haj FG, Zabolotny JM, Kim YB, Kahn BB, Neel BG. Liver-specific protein-tyrosine phosphatase 1B (PTP1B) re-expression alters glucose homeostasis of PTP1B-/-mice. The Journal of biological chemistry. 2005; 280:15038–46. [PubMed: 15699041]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

84. Delibegovic M, Bence KK, Mody N, Hong EG, Ko HJ, Kim JK, et al. Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B. Molecular and cellular biology. 2007; 27:7727–34. [PubMed: 17724080]

85. Delibegovic M, Zimmer D, Kauffman C, Rak K, Hong EG, Cho YR, et al. Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress. Diabetes. 2009; 58:590–9. [PubMed: 19074988]

86. Owen C, Lees EK, Grant L, Zimmer DJ, Mody N, Bence KK, et al. Inducible liver-specific knockdown of protein tyrosine phosphatase 1B improves glucose and lipid homeostasis in adult mice. Diabetologia. 2013; 56:2286–96. [PubMed: 23832083]

87. Grant L, Shearer KD, Czopek A, Lees EK, Owen C, Agouni A, et al. Myeloid-cell protein tyrosine phosphatase-1B deficiency in mice protects against high-fat diet and lipopolysaccharide-induced inflammation, hyperinsulinemia, and endotoxemia through an IL-10 STAT3-dependent mechanism. Diabetes. 2014; 63:456–70. [PubMed: 24186864]

88. Zhang S, Zhang ZY. PTP1B as a drug target: recent developments in PTP1B inhibitor discovery. Drug discovery today. 2007; 12:373–81. [PubMed: 17467573]

89. Barr AJ. Protein tyrosine phosphatases as drug targets: strategies and challenges of inhibitor development. Future medicinal chemistry. 2010; 2:1563–76. [PubMed: 21426149]

90. He R, Zeng LF, He Y, Zhang S, Zhang ZY. Small molecule tools for functional interrogation of protein tyrosine phosphatases. The FEBS journal. 2013; 280:731–50. [PubMed: 22816879]

91. Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, et al. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:11357–62. [PubMed: 12169659]

92. Swarbrick MM, Havel PJ, Levin AA, Bremer AA, Stanhope KL, Butler M, et al. Inhibition of protein tyrosine phosphatase-1B with antisense oligonucleotides improves insulin sensitivity and increases adiponectin concentrations in monkeys. Endocrinology. 2009; 150:1670–9. [PubMed: 19164474]

93. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372:425–32. [PubMed: 7984236]

94. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995; 269:540–3. [PubMed: 7624776]

95. Tartaglia LA. The leptin receptor. The Journal of biological chemistry. 1997; 272:6093–6. [PubMed: 9102398]

96. Ahima RS, Osei SY. Leptin signaling. Physiology & behavior. 2004; 81:223–41. [PubMed: 15159169]

97. Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, et al. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Developmental cell. 2002; 2:497–503. [PubMed: 11970899]

98. Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, et al. PTP1B regulates leptin signal transduction in vivo. Developmental cell. 2002; 2:489–95. [PubMed: 11970898]

99. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, et al. Neuronal PTP1B regulates body weight, adiposity and leptin action. Nature medicine. 2006; 12:917–24.

100. Tsou RC, Zimmer DJ, De Jonghe BC, Bence KK. Deficiency of PTP1B in leptin receptor-expressing neurons leads to decreased body weight and adiposity in mice. Endocrinology. 153:4227–37. [PubMed: 22802463]

101. Tsou RC, Rak KS, Zimmer DJ, Bence KK. Improved metabolic phenotype of hypothalamic PTP1B-deficiency is dependent upon the leptin receptor. Molecular metabolism. 2014; 3:301–12. [PubMed: 24749060]

102. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004; 306:457–61. [PubMed: 15486293]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

103. Hummasti S, Hotamisligil GS. Endoplasmic reticulum stress and inflammation in obesity and diabetes. Circulation research. 107:579–91. [PubMed: 20814028]

104. Kaufman RJ, Scheuner D, Schroder M, Shen X, Lee K, Liu CY, et al. The unfolded protein response in nutrient sensing and differentiation. Nat Rev Mol Cell Biol. 2002; 3:411–21. [PubMed: 12042763]

105. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007; 8:519–29. [PubMed: 17565364]

106. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmicreticulum-resident kinase. Nature. 1999; 397:271–4. [PubMed: 9930704]

107. Ma Y, Lu Y, Zeng H, Ron D, Mo W, Neubert TA. Characterization of phosphopeptides from protein digests using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and nanoelectrospray quadrupole time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2001; 15:1693–700. [PubMed: 11555868]

108. Su Q, Wang S, Gao HQ, Kazemi S, Harding HP, Ron D, et al. Modulation of the eukaryotic initiation factor 2 alpha-subunit kinase PERK by tyrosine phosphorylation. The Journal of biological chemistry. 2008; 283:469–75. [PubMed: 17998206]

109. Gu F, Nguyen DT, Stuible M, Dube N, Tremblay ML, Chevet E. Protein-tyrosine phosphatase 1B potentiates IRE1 signaling during endoplasmic reticulum stress. The Journal of biological chemistry. 2004; 279:49689–93. [PubMed: 15465829]

110. Agouni A, Mody N, Owen C, Czopek AJ, Zimmer D, Bentires-Alj M, et al. Liver-specific deletion of protein tyrosine phosphatase (PTP) 1B improves obesity- and pharmacologically-induced endoplasmic reticulum stress. The Biochemical journal. 2011; 2:369–78. [PubMed: 21605081]

111. Bettaieb A, Liu S, Xi Y, Nagata N, Matsuo K, Matsuo I, et al. Differential regulation of endoplasmic reticulum stress by protein tyrosine phosphatase 1B and T cell protein tyrosine phosphatase. The Journal of biological chemistry. 2011; 11:9225–35. [PubMed: 21216966]

112. Bettaieb A, Matsuo K, Matsuo I, Wang S, Melhem R, Koromilas AE, et al. Protein tyrosine phosphatase 1B deficiency potentiates PERK/eIF2alpha signaling in brown adipocytes. PloS one. 7:e34412. [PubMed: 22509299]

113. Panzhinskiy E, Hua Y, Culver B, Ren J, Nair S. Endoplasmic reticulum stress upregulates protein tyrosine phosphatase 1B and impairs glucose uptake in cultured myotubes. Diabetologia. 2013; 56:598–607. [PubMed: 23178931]

114. Ravier MA, Guldenagel M, Charollais A, Gjinovci A, Caille D, Sohl G, et al. Loss of connexin36 channels alters beta-cell coupling, islet synchronization of glucose-induced Ca2+ and insulin oscillations, and basal insulin release. Diabetes. 2005; 54:1798–807. [PubMed: 15919802]

115. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. Cell. 2008; 133:38–52. [PubMed: 18394988]

116. Konstantinova I, Nikolova G, Ohara-Imaizumi M, Meda P, Kucera T, Zarbalis K, et al. EphA-Ephrin-A-mediated beta cell communication regulates insulin secretion from pancreatic islets. Cell. 2007; 129:359–70. [PubMed: 17448994]

117. Jain R, Jain D, Liu Q, Bartosinska B, Wang J, Schumann D, et al. Pharmacological inhibition of Eph receptors enhances glucose-stimulated insulin secretion from mouse and human pancreatic islets. Diabetologia. 2013; 56:1350–5. [PubMed: 23475368]

118. Nievergall E, Janes PW, Stegmayer C, Vail ME, Haj FG, Teng SW, et al. PTP1B regulates Eph receptor function and trafficking. The Journal of cell biology. 2010; 191:1189–203. [PubMed: 21135139]

119. Haj FG, Sabet O, Kinkhabwala A, Wimmer-Kleikamp S, Roukos V, Han HM, et al. Regulation of signaling at regions of cell-cell contact by endoplasmic reticulum-bound protein-tyrosine phosphatase 1B. PloS one. 2012; 7:e36633. [PubMed: 22655028]

120. Kushner JA, Haj FG, Klaman LD, Dow MA, Kahn BB, Neel BG, et al. Islet-sparing effects of protein tyrosine phosphatase- 1b deficiency delays onset of diabetes in IRS2 knockout mice. Diabetes. 2004; 53:61–6. [PubMed: 14693698]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

121. Vila-Bedmar R, Lorenzo M, Fernandez-Veledo S. Adenosine 5'-monophosphate-activated protein kinase-mammalian target of rapamycin cross talk regulates brown adipocyte differentiation. Endocrinology. 2010; 151:980–92. [PubMed: 20133456]

122. Xue B, Pulinilkunnil T, Murano I, Bence KK, He H, Minokoshi Y, et al. Neuronal protein tyrosine phosphatase 1B deficiency results in inhibition of hypothalamic AMPK and isoform-specific activation of AMPK in peripheral tissues. Molecular and cellular biology. 2009; 29:4563–73. [PubMed: 19528236]

123. Kayne FJ, Price NC. Amino acid effector binding to rabbit muscle pyruvate kinase. Archives of biochemistry and biophysics. 1973; 159:292–6. [PubMed: 4784462]

124. Yamada K, Noguchi T. Regulation of pyruvate kinase M gene expression. Biochemical and biophysical research communications. 1999; 256:257–62. [PubMed: 10079172]

125. Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature. 2008; 452:181–6. [PubMed: 18337815]

126. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature. 2008; 452:230–3. [PubMed: 18337823]

127. Goldberg MS, Sharp PA. Pyruvate kinase M2-specific siRNA induces apoptosis and tumor regression. The Journal of experimental medicine. 2012; 209:217–24. [PubMed: 22271574]

128. Macintyre AN, Rathmell JC. PKM2 and the tricky balance of growth and energy in cancer. Molecular cell. 2011; 42:713–4. [PubMed: 21700216]

129. Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Science signaling. 2009; 2:ra73. [PubMed: 19920251]

130. Bryant NJ, Govers R, James DE. Regulated transport of the glucose transporter GLUT4. Nat Rev Mol Cell Biol. 2002; 3:267–77. [PubMed: 11994746]

131. Cheatham B, Volchuk A, Kahn CR, Wang L, Rhodes CJ, Klip A. Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:15169–73. [PubMed: 8986782]

132. Tellam JT, McIntosh S, James DE. Molecular identification of two novel Munc-18 isoforms expressed in non-neuronal tissues. The Journal of biological chemistry. 1995; 270:5857–63. [PubMed: 7890715]

133. Thurmond DC, Ceresa BP, Okada S, Elmendorf JS, Coker K, Pessin JE. Regulation of insulin-stimulated GLUT4 translocation by Munc18c in 3T3L1 adipocytes. The Journal of biological chemistry. 1998; 273:33876–83. [PubMed: 9837979]

134. Jewell JL, Oh E, Ramalingam L, Kalwat MA, Tagliabracci VS, Tackett L, et al. Munc18c phosphorylation by the insulin receptor links cell signaling directly to SNARE exocytosis. The Journal of cell biology. 2011; 193:185–99. [PubMed: 21444687]

Abbreviations

PTKs	protein-tyrosine kinases
PTPs	protein-tyrosine phosphatases
pTyr	phosphotyrosine
PTP1B	protein-tyrosine phosphatase 1B
ER	endoplasmic reticulum
EGFR	epidermal growth factor receptor
PDGFR	platelet-derived growth factor receptor
IR	insulin receptor

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

FRET	fluorescence resonance energy transfer
MVB	multivesicular bodies
BRET	bioluminescence resonance energy transfer
ROS	reactive oxygen species
SH3	Src-homology 3
JAK2	Janus kinase 2
TYK2	tyrosine kinase 2
IRS	insulin receptor substrate
PI3K	phosphatidylinositol 3-kinase
KO	knockout
LepRb	leptin receptor
STAT3	signal transducer and activator of transcription 3
POMC	pro-opiomelanocortin
UPR	unfolded protein response
PERK	protein kinase-like ER kinase
IRE1α	inositol requiring enzyme 1α
ATF6	activating transcription factor 6
eIF2α	eukaryotic translation initiation factor 2
GSIS	glucose-stimulated insulin secretion
ZO1	Zonula Occludens
AMPK	AMP-activated protein kinase
PKM2	pyruvate kinase M2
PEP	phosphoenolpyruvate
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

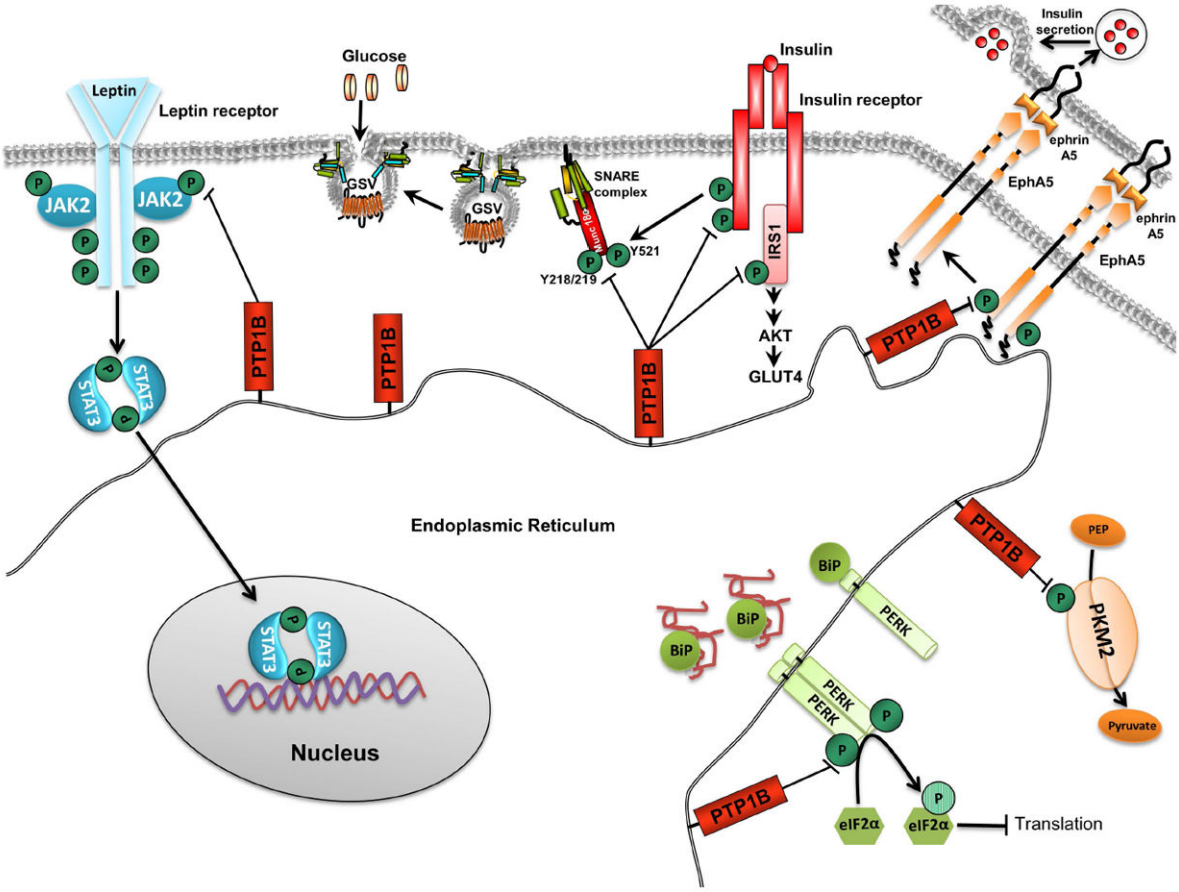

Figure 1. Schematic representation of PTP1B-substrates interaction and metabolic regulation

Insulin signaling and vesicle trafficking: Insulin activates the IR leading to IRS1 phosphorylation and subsequent activation of downstream signaling pathways. PTP1B attenuates insulin signaling by dephosphorylating the endocytosed IR and IRS1. GLUT4-containing vesicles (GSV) interact with several molecules as they are delivered towards the cell periphery then are tethered and fused to the plasma membrane. In adipocytes, the IR phosphorylates Munc18c while PTP1B dephosphorylates it thereby linking insulin signaling directly to SNARE exocytosis. Leptin signaling: Leptin binds to the LepRb and leads to JAK2 activation and subsequent phosphorylation of LepRb tyrosine residues enabling STAT3 recruitment. Phosphorylated STAT3 translocates to the nucleus to regulate transcription. PTP1B attenuates leptin signaling by dephosphorylating JAK2. Cell-cell communication: The ER network comes in close proximity to the plasma membrane at specialized regions of cell-cell contact (right corner), enabling ER-resident PTP1B to engage substrates (such as EphA5) at these sites. Energy balance: PKM2 is a rate-limiting glycolytic enzyme that catalyzes the generation of pyruvate and ATP from PEP and ADP. PKM2 is a PTP1B substrate in adipocytes, and PTP1B deficiency increases PKM2 overall and site-specific tyrosine phosphorylation. ER stress response: Under basal conditions PERK is maintained inactive by its association with the molecular chaperone BiP. When improperly folded proteins accumulate in the ER, BiP dissociates from PERK which is activated. PERK phosphorylates eIF2α, a modification that blocks initiation of translation. PTP1B directly

interacts with and dephosphorylates PERK. Tyrosine phosphorylated residues are indicated by green ovals and serine phosphorylated residue is indicated by green oval with white stripes.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript