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Pulling Strings Below the Surface

Hormone Receptor Signaling Through Inhibition of Protein Tyrosine Phosphatases

Xavier Espanel, Sébastien Wälchli, Rosanna Pescini Gobert, Mohamed El Alama, Marie-Laure Curchod, Nicole Gullu-Isler, and Rob Hooft van Huijsduijnen

Serono Pharmaceutical Research Institute, Geneva, Switzerland

Hormones, cytokines, and related proteins (such as soluble hormone receptors) play an important role as therapeutic agents. Most hormone receptors signal through a mechanism that involves phosphorylation of the receptor's tyrosine residues. At any given moment, the receptor's phosphorylation state depends on the balance of kinase and phosphatase activities. Recent findings point to the exciting possibility that receptor signaling can be regulated by inhibition of protein tyrosine phosphatases (PTPs) that specifically hydrolyze receptor tyrosine-phosphates, or their immediate downstream effectors. This strategy has now been firmly validated for the insulin receptor and PTP1B; inhibiting PTP1B activity results in stimulation of the insulin receptor and signaling, even in the absence of insulin. This and similar findings suggest that PTP inhibitors have potential as hormone mimetics. In the present review, we outline this new paradigm for therapeutic regulation of the insulin receptor and discuss evidence that hints at other specific receptor-PTP pairs.

Key Words: Cytokines; insulin; protein tyrosine kinase; protein tyrosine phosphatase; PTP1B.

Introduction

Hormones form an integral part of today's medicine chest. A nonexhaustive list includes follicle-stimulating hormone, thyroxin, corticosteroids, insulin-like growth factor, interferons, luteinizing hormone, insulin, growth hormone (GH), oxytocin, estrogens, glucocorticoids, progesterone, and other steroids. Since the endocrine system involves fewer players than are involved in intracellular signaling events, therapeutic and side effects of hormone therapy are generally better understood than consequences of low molecular weight drug therapies. Some hormones, such as insulin, have been around as therapeutic drugs since the beginning of the twentieth century. Others, such as leptin and cytokines, are still under evaluation. With the exception of steroids, hormones as drugs suffer from the fact that exceeding the typical 500 Dalton oral drug size limit, they need to be administered intravenously. Furthermore, hormones have short in vivo half-lives. For example, the cytokines GH and β-interferon require about three injections per week when used in the treatment of dwarfism and multiple sclerosis, respectively. Insulin needs to be administered even more frequently to treat diabetes. Another issue is that proteins are costly to produce, especially if they require proper glycosylation. They may also evoke undesired immune responses or contain pathogenic contaminants. Attempts have been made by pharmaceutical companies to produce small-molecule hormone agonists that mimic hormonal action. With the exception of steroids, these efforts have mostly failed, presumably because the molecular surface of the protein-protein interactions involved is too large to be mimicked by a small molecule. In spite of recent efforts toward intradermal and pulmonary delivery technologies, there is clearly a medical need for alternative hormone therapies.

The elucidation of hormone signaling pathways has provided alternative targets for therapeutic intervention. With the exception of steroid hormones, which directly activate intracellular transcription factors, most hormones signal via cascades of tyrosine, serine, threonine, and lipid kinases. Since kinases are enzymes, they are, in principle, good drug targets. This approach has met with considerable success and has resulted in the development of protein tyrosine kinase inhibitors for the treatment of cancer (angiogenesis and growth factor signaling inhibitors, apoptosis-inducing agents), antiinflammatory treatments, viral infections, and malaria (reviewed in ref. *1*). Kinases as drug targets have as advantages the fact that they have been studied for a long time (starting with the characterization of retroviral oncogenes) and include many family members, suggesting low functional redundancy *(2,3)*. However, their inhibition is mostly associated with the *extinction* of hormone signaling. To develop drugs that mimic or stimulate hormonal activity, we need to inhibit the phosphatases that counteract the action of kinases. Inhibiting dephosphorylation of a hormone receptor or its immediate target should result in activation of the corresponding hormone signaling pathway

Author to whom all correspondence and reprint requests should be addressed: Rob Hooft van Huijsduijnen, Serono Pharmaceutical Research Institute, 14, chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland. E-mail: rob.hooft@serono.com

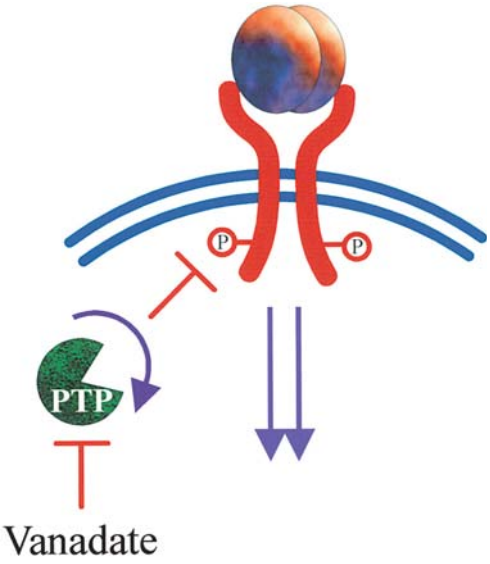

Fig. 1. Schematic representation of receptor stimulation through PTP inhibition. Inhibition of a specific PTP for a receptor by vanadate (or another inhibitor) results in receptor hyperphosphorylation and enhanced signaling.

(Fig. 1). Interesting results have been obtained with vanadate-based compounds, which are potent, generic inhibitors of protein tyrosine phosphatases (PTPs) *(4–10)*. These results suggest that tyrosine phosphorylation and dephosphorylation are highly dynamic processes and that inhibiting dephosphorylation can result in tyrosine phosphorylation even in the absence of a hormone-induced signaling cascade. PTPs were discovered much later than kinases (Fig. 2), and very little is known about their physiologic targets and regulation. Emerging evidence indicates that PTPs are valid drug targets for the industrial development of hormone analogs.

The purpose of this review is to summarize data that support this evidence and to evaluate the prospects. We limit our scope to receptors whose stimulation may have a therapeutic effect and ignore receptors for growth factors (fibroblast growth factor receptor, epidermal growth factor receptor [EGFR], platelet-derived growth factor receptor) because their constitutive activity is associated with cancers.

PTPs Family

The mammalian PTP family presently includes 50–60 members *(11)*. The proteins are characterized by a 250–300 amino acid catalytic domain that is conserved about 30% between family members. Many PTPs are type I membrane proteins; others are cytoplasmic or nuclear. Their ancestry has been traced to *Drosophila* and *Candida elegans (12)*, but only very few PTPs have been described in bacteria, yeast, and plants. The family has been the subject of several recent reviews *(11,13–17)*.

Insulin Signaling and PTP1B: The New Paradigm

The insulinR is a prototypical tyrosine kinase receptor whose ligand binding and dimerization results in autophosphorylation on multiple tyrosines. This is followed by the recruitment and phosphorylation of insulin receptor substrate 1–4 (IRS1–4) (depending on the tissue) and phosphoinositide-3-kinase (PI3K), as recently reviewed *(18)*. Although vanadium-containing compounds have been known since the nineteenth century to alleviate diabetes (cited in ref. *10*), it was understood only recently that these inhibitors stimulate the insulin signaling pathway by blocking PTP action *(7,19)*. Considerable efforts have been made to identify the PTPs that play a negative role in insulin signaling. PTP-SHP2 *(20–23)*, PTP-LAR *(24–27)*, PTP-ε *(28)*, PTP1B *(29–35)*, LMW-PTP *(36)*, PTEN *(37)*, TC-PTP *(35)*, and PTP-α *(28,38)* have all been associated with insulin signaling (see Table 1 for a listing of synonyms for PTPs). In addition, there are several observations of enhanced PTP activity in diabetic tissues *(39,40)*. Among these PTPs, PTP1B emerged as a key role player when two independent groups reported that mice mutated for this PTP show reduced serum glucose levels and are obesity resistant *(41, 42)*. Evidence for the involvement of the insulin receptor and insulin receptor substrate 1 (IRS1) in this phenotype was that both proteins show increased tyrosine phosphorylation in the PTP1B-mutated mice. From the knockout mice alone it is not certain which of these two phosphoproteins is PTP1B's substrate. The apparently dominant role for PTP1B came as a surprise, since PTP1B is associated with endoplasmatic reticulum—not cytoplasmic membranes. The mutant mice also display increased energy expenditure in muscle, which remains unexplained *(42)*. Another area of question is whether the observed phenotype results from enhanced glucose uptake in all major tissues that involve insulin signaling (liver, muscle, and adipose tissue) or from muscle alone. Nevertheless, these data strongly suggest that PTP1B is a suitable target for the development of drugs to treat diabetes and obesity. A second validated PTP target in insulin signaling is PTEN/MMac1. This phosphatase blocks the PI3K/PKB(Akt) signaling pathway through the dephosphorylation of phosphatidyinositol 3,4,5,-triphosphate *(43, 44)* (Fig. 3). Blocking either PTEN or PTP1B gene expression using antisense oligonucleotides restores glucose levels in diabetic mice *([45]*; Brett Monia, Isis Pharmaceuticals, personal communication). However, PTEN as a drug target is suspect because loss of this tumor suppressor gene is associated with cancers *(46–50)*.

What can be learned from the PTP1B/PTEN–diabetes connection is the difficulty of predicting biochemically which PTP is involved with what receptor. Although PTP1B is the first PTP that was discovered, many aspects of the PTP1B knockout mice had not been predicted and are still poorly understood. In the following sections, we examine a number of other hormone receptors that signal via tyrosine sig-

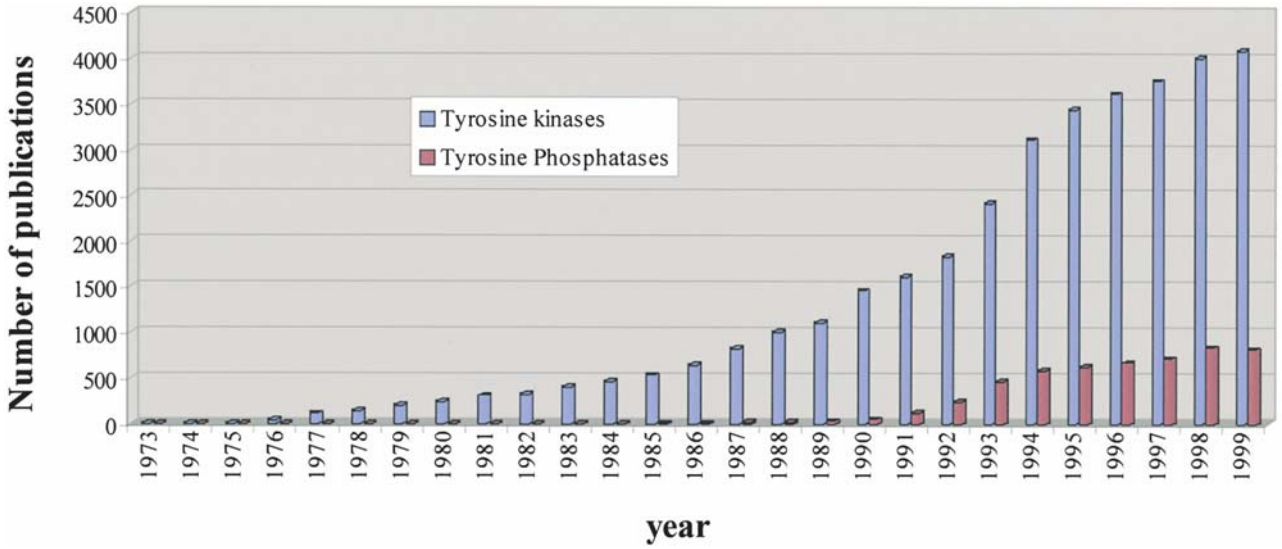

Fig. 2. The literature on PTPs lags 10 yr behind tyrosine kinases. Publications per year with key words *protein tyrosine phosphatase* or *tyrosine kinase* were obtained from the MedLine (Life Sciences) database.

naling and review what is known of PTPs associated with their signaling pathways; we limit our scope to receptors whose stimulation may be clinically useful.

The Erythropoietin Receptor and PTP SHP1

Erythropoietin (EPO) is a primary hormonal inducer of erythrocyte production. Its receptor is a classic tyrosine kinase that undergoes dimerization and autophosphorylation on ligand binding and initiates of Jak-STAT signaling. SHP1 binds the phosphorylated receptor, and mouse strains mutated in SHP1 (*motheaten*) show increased EpoR activity *(51)*. Although *motheaten* mice also suffer from immunologic disorders, and SHP1 plays diverse roles *(52)*, an SHP1 inhibitor could have clinical benefits as an EPO mimetic.

The Leptin Receptor

Leptin is a member of the cytokine family, whose receptor shows sequence similarity with gp130. Among the different receptor splice variants only the long form, called Ob-Rl, with two tyrosines (985 and 1138) was shown to be involved in intracellular signaling *(53,54)*. Short forms (Ob-Rs) have cytoplasmic tails of only 30–40 amino acids that lack tyrosines; the function of these truncated receptors is not yet known (*[55]*; reviewed in refs. *56* and *57*).

Leptin is produced in adipose tissue, placenta *(58)*, and the gastrointestinal tract *(59)*. The long form of the leptin receptor (Ob-Rl) is mostly found in the hypothalamus *(60)*, but it is also expressed at lower levels elsewhere *(61,62)*. The key target organ is the hypothalamus, and leptin injected into cerebrospinal fluid is much more potent than when injected intravenously. The physiology of leptin distribution has shown that the plasma level of leptin is correlated with the fat tissue mass *(63)*; that unlike insulin, there is no significant increase in its production after a meal; and that serum leptin levels follow a circadian rhythm *(64)*. Although leptin has been associated with activities such as T-cell activation *(65)*, growth (cited in ref. *56*), and reproduction *(64)*, its primary role is to provide a negative feedback mechanism between stored fat vs food uptake and energy conservation *(66)*. This effect is most clearly illustrated by the extreme obesity seen in mice mutated in leptin (*ob*/*ob*), or leptin receptor (*db*/*db*) genes. In humans, too, these mutations, although rare, result in obesity. However, most clinical obesity that is seen today is associated with excess serum leptin levels, in patients carrying normal leptin and leptinR genes. In analogy with diabetes, obesity is therefore associated with leptin resistance. It is not surprising that early results from clinical trials with recombinant leptin for the treatment of obesity have been disappointing. We speculate that, as in insulin resistance, leptin resistance may be overcome by inhibiting the PTPs that control the receptor's tyrosine phosphorylation state.

Ob-Rl signaling is related to the interleukin-6 (IL-6) signal transduction pathway (see below): upon interaction with ligand, the receptor is tyrosine phosphorylated by Jak2. This process leads to the exposure of a docking site for the SH2 domain containing protein STAT3 that recognizes phosphorylated Tyr^{1138} *(55)*. An important PTP associated with this signaling pathway is SHP2. SHP2 has (like SHP1) two SH2 domains *(67)* and has been shown to dock to Tyr^{985} phosphorylated leptinR. SHP2 itself is activated by tyrosine phosphorylation, probably by JAK2. The activated PTP then may dephosphorylate JAK2 but not STAT3 or ObRl *(68)*. It has been shown that inactivation of the SHP2 docking site (Tyr^{985} mutated to Phe; *[69]*) augments STAT3-mediated leptin signaling. In the absence of SHP2, Jak2

Table 1
List of Mammalian PTPs, Accession Number of Full-Length Sequences, and Their Synonyms

PTP[a]	Accession no.	Synonyms
A37109	A37109	—
BDP-1	X79568	—
CD45/LCA	Y00062	CD45, LCA, T200, B220; Ly-5 (mouse)
cdc25A	M81933	—
cdc25B	M81934	—
cdc25C	M34065	—
Cdi-1	L25876	—
DEP-1	D37781	hPTPη, PTP-U1, CD148
Esp	MMU36488	—
FAP-1	D21209	hPTP1E, PTPL1, PTP-BL (rat), PTP-BAS, RIP (mouse)
GLEPP-1	U20489	PTP-U2, PTP-ϕ
HPC-PTP	D64053	Ch-1PTPα, -δ, -γ, EC-PTP, NC-PTPCOM1, Cr1PTPase; PTP-SL, PTPBR7, CBPTP, TPPBS mouse); CBPTP, PCPTP1 (rat)
Hs14603	U14603	—
I32039	I32039	—
IA-2ß	AB002385	ICAAR, IA-2β, PTP-π, PTP NE6 or phogrin (in rat), PTP-IAR, PTP-NP, R-PTP-X
LAR	Y00815	—
LC-PTP	D11327	LPTPase, PTN7
Lyp-1	AF001846	PEP (mouse)
Meg-1	M68941	PTN4
Meg-2	M83738	PTN9
PEZ	X82676	PEZ, PTPN14 PTP
Prl-1	NM_003463	PTP4A1, PTP type IVA
PTEN	U92436	(MMAC1
PTP-1b	g190741	—
PTP-α	M34668	H(R)PTP-α, (H)LRP
PTP-β	X54131	HPTPβ, m/hRPTPμ, VE-PTP (mouse)
PTP-d1	X79510	PTP2E(1) in rat, PTP-RL10 (mouse)
PTP-δ	L38929	(R)PTPδ
PTP-ε	X54134	(R)PTPe
PTP-γ	L09247	Receptor-type PTPγ, RKPTP (rat), MPTP-PEST (mouse)
PTP-H1	M64572	—
PTP-IA2	L18983	ICA 512, PTP35
PTP-κ	L77886	—
PTP-μ	X58288	—
PTP-o	U71075	PTPRO, PTPRo, PCP-2, R-PTPΨ, hPTP-J, 4 FNIII
PTP-PEST	M93425	PTPG1, P19-PTP, PTPty43
PTP-ρ	AF043644	PTPλ (mouse); rPTPμ/κ-like
PTP-σ	U35234	(R)PTPσ, PTP-OB, LARPTP2, PTPN3, PTPP-1/P-S, PTPν-3, CPTP-1/3 BPTP-1 (?)
PTP-TD14	AF077000	(rat)
PTP-ζ	M93426	RPTPβ
SAP-1	D15049	PTPRH
SHP-1	X62055	PTP-1c, SHPTP1, HCP or SHP
SHP-2	D13540	SHPTP2, syp, PTP1D, PTP2C, SH-PTP3, PTP-SHβ, PTP-L1
STEP	U27831	PTN5, PC12-PTP1
TC-PTP	M25393	PTP-S, MPTP
TPTE	AF007118	—
B23	U16996	nucleophosmin, hVH-3
cdc14b	AF023158	—
cdc14b homolog		AF000367
EPMA2	AJ130763	Laforin
Hs6694	U01669	—
hVH-5	U27193	—
MKP-1	X68277	hVH1, CL100
MKP-2	HSU21108	hVH-2, Typ-1
MKP4	Y08302	—
MKP-5	AF179212	—
MTM1	U46024	Myotubularin
MTMR1	U58032	—
MTMR6	AF072928	—
MTMR7	AF073482	—
Pac-1	L11329	—
Pir1	AF023917	—
Pyst-1	X93920	MKP3, hVH6, rVH6 (rat)
Pyst-2	X93921	MKP-X, B59
VH-1	MVA091L	(*Vaccinia*)
VHR	L05147	VH1-related
YOPH	M30457	(*Yersinia*)
YVH1	AF119226	

[a]The dual-specificity PTPs are grouped at the bottom.

phosphorylation is increased. Although the precise role of SHP2 is not yet entirely understood, most evidence suggests that it plays a negative role in leptinR signaling.

Whether this means that SHP2 is a good target for drugs that reverse leptin resistance remains to be seen. Mice mutated in SHP2 die very early in development *(70)*. However, a gene-modulating approach in adult animals (e.g., using antisense oligonucleotides) may still validate SHP2 as a valid drug target. The fact that SHP2 is closely associated with Jak2 phosphorylation products is problematic, since Jak2 is involved in signaling through many cytokine receptors, such as IL-3, IL-5, granulocyte macrophage colony-stimulating factor, and GH *(71)*. The PTP responsible for dephosphorylating the leptinR itself remains unknown.

Angiopoietin Receptor Tie-2 and PTP-β

Angiopoietin-1 is a recently discovered tissue hormone *(72)* involved in the regulation of blood vessel differentiation and remodeling. It is thought to be involved in important pathologic processes such as ischemia *(73)* and vascu-

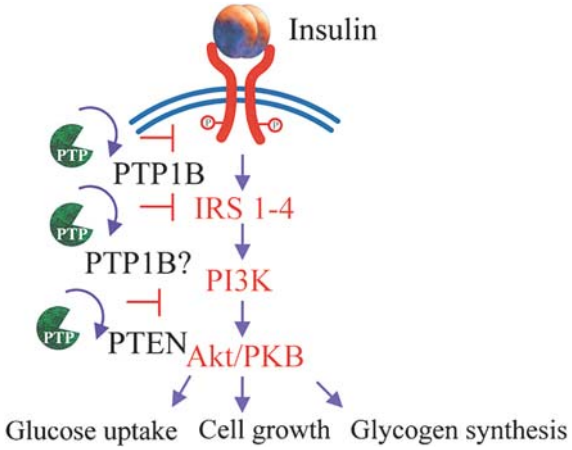

Fig. 3. Signaling through the insulin receptor and role of the PTPs PTP1B and PTEN.

lar permeability *(74,75)*. Although the signaling pathway of its receptor, tyrosine kinase Tie-2, has not yet been extensively studied, recent evidence shows that the autophosphorylated receptor is specifically dephosphorylated by PTP-β (VE-PTP in mouse), a blood vessel–specific PTP *(76)*. This would predict that a PTP-β-specific inhibitor induces Tie-2 signaling, leading to a reduction in vessel leakiness. By contrast, another study indicated that phenylarsine oxide, another generic PTP inhibitor, increased vessel permeability through proteolysis of tight junctional proteins *(77)*. Again, a more specific inhibitor such as an antisense reagent may validate PTP-β as a drug target.

GH Receptor

GH binds as a monomer to two receptors units *(78)*. Their dimerization triggers Jak2 recruitment followed by tyrosine phosphorylation on multiple residues of the GH receptor (GHR), and of Jak2 itself *(79)*. The phosphorylated GHR/Jak2 complex recruits and phosphorylates STAT1 *(80)*, STAT3 *(81)*, and STAT5 *(82)*; Shc *(83)*; and IRS1 and IRS2 *(84,85)*. Tyrosine-phosphorylated Shc further recruits Grb-2 and initiates the SOS, Ras, Raf, MEK mitogen-activated protein kinase (MAPK) cascade *(86)*. Activation of IRS1 and IRS2, similar to IGF-1 and insulin-initiated pathways, results in association with the regulatory subunit of PI3K, whose activation results in, among other things, glucose uptake. The GHR signaling pathway was recently reviewed *(87,88)*.

The human intracellular GHR domain has seven tyrosines. Which of these are involved in Jak2 recruitment and phosphorylation is only beginning to be elucidated. Early work pointed to Tyr^{333}, Tyr^{338}, Tyr^{391} and Tyr^{437} as phosphorylation targets (see ref. *89* for numbering). More recently, it was found that Tyr^{333} and/or Tyr^{338} are phosphorylated by Jak2 but are not required for Jak2 binding and activation *(90)*. Mutation of these tyrosines interfered with some GH responses (lipogenesis and protein synthesis), but not with others (SPI 2.1 transcription; *[91]*). Given that different phosphotyrosines mediate different responses, it seems reasonable to predict that dephosphorylation is mediated by different PTPs. Although it is, again, not known which PTPs dephosphorylate the activated GHR, much attention has focused on SHP2 *(92,93)*. As for the leptinR, SHP2 is activated in GH signaling. Another PTP associated with GH signaling is SHP1 (PTP-1c, SHPTP1, HCP, or SHP; not to be confused with SHIP). This PTP is activated three- to fourfold by GH *(94)*. It has been suggested that SHP1 is involved in male-pattern GH signaling: in male rat livers, STAT5b is activated through intermittent GH pulses. In females, GH expression is continuous and STAT5b is much less active. This downmodulation is inhibited by vanadate, consistent with a role for a PTP in this pathway *(95)*.

Finally, PTPs have been associated with kinases in the MAPK cascade triggered by GH. These are mostly dual-specificity PTPs (i.e., they also dephosphorylate phosphoserine and -threonine) such as MKP1-5 *(96–100)*. However, it is unlikely that these PTPs are good drug targets in a specific GH-signaling context.

Recent work has indicated that a single tyrosine mutation in the GHR results in prolonged signaling through Jak2 and STAT5b, which suggests that specific PTPs may act as GH mimetics *(101)*.

CD45/T- and B-Cell Antigen Receptor Signaling

PTP-CD45 is a receptor PTP that is abundantly expressed in lymphocytes. Early experiments with knockout mice indicated that it is essential for T-cell receptor (TCR) and B-cell antigen receptor signaling *(102,103)*. T-cells from mice lacking CD45 behave as if they have unsuccessfully rearranged their TCR genes and undergo massive apoptosis in the thymus; B-cells fail to mature and no immunoglobulin class switching is seen beyond the mIgM stage (see Table 2). This phenotype has been associated with an inhibition of the src-like kinases lck and fyn. Src-family kinases carry an autoinhibitory, C-terminal phosphotyrosine in a domain that folds back onto the kinase's SH2 domain. This mechanism of activation of lck by CD45 has been challenged *(102)*, and the CD3ζ chain was proposed instead as CD45's effective target *(104)*. Either way, it would seem from the knockout animals that a CD45 inhibitor would have strong antiinflammatory properties.

Pleiotropin and Its Receptor, PTP-ζ

PTP-ζ (RPTP-β) is only the first receptor PTP for which soluble ligands have been identified, namely the cytokines pleiotropin *(132)* and midkine *(133)*. Pleiotropin plays a role in tumor angiogenesis and proliferation *(134)*. In contrast to receptor kinases, binding of pleiotropin to its receptor (PTP-ζ) leads to an inactivation of receptor phosphatase activity *(135)*. The reduced PTP activity results in increased

Table 2
Biologic Effects of PTP Inhibition[a]

			References
LAR	Gene knockout	Insulin and glucose low; weak rate of hepatic glucose production; females unable to produce milk owing to abnormal terminal differentiation of mammary glands	*105,106*
	Stable cell line PC12 expressing antisense mRNA	Decreased cell death on serum-deprivation; increase in neurite outgrowth after stimulation by nerve growth factor	*107*
MTMR2	Human mutations	Charcot-Marie-Tooth type 4B disease (CMT4B)	*108*
PEP	Stable WEHI-231 cells expressing antisense mRNA	No G1 arrest and apoptosis on BCR activation in immature B-cell line (WEHI-231)	*109*
PEST	Gene knockout in murine fibroblasts	Hyperphosphorylation of $p130^{cas}$, FAK, PSTPIP, and paxillin; Faster spreading of cells on fibronectin but also show a migration defect on fibronectin	*110,111*
PTEN	Human mutations	Susceptibility to cancers: glioblastomas, endometrium, prostate	*112*
PTP-1B	Gene knockout	Increased insulin sensitivity, protection against obesity, low adiposity, elevated basal metabolism	*41,42*
	Antisense oligonucleotide in rat aortic smooth cells	Motility increased; hyperphosphorylation of focal adhesion proteins and FAK	*113*
PTP-γ	Stable embryonic stem cells expressing antisense mRNA	No hematopoietic precursors in embryoid bodies	*114*
PTP-δ	Gene knockout	Semilethal caused by insufficient food intake; impaired learning	*115*
PTP-ε	Gene knockout	Voltage-gated potassium channels hyperphosphorylated and more active; hypomyelination of sciatic nerve at early age	*116*
PTP-κ	Gene knockout by insertion	No detectable effect	*117*
PTP-μ	Viral antisense vector on rat retinal explants	Decrease in neurite outgrowth on N-cadherin substrate.	*118*
PTP-σ	Gene knockout	Elevated neomortality; retarded growth, hyposmia, hypofecundity; overall brain size is reduced; small anterior and posterior pituitary lobes	*119*
	Stable cell line A431 expressing antisense mRNA	EGFR phosphorylation increased as well as soft agar colonies.	*120*
HPC-PTP	Antisense oligonucleotide on embryonic explant culture	(PTPPBS γ-) Increase size of chondrogenic region and elevated proliferation of chondroblasts; Meckels cartilage patterned abnormally	*121*
PTP-RO	Antisense oligonucleotide	Inhibition of megakaryocyte progenitor development	*122*
PTP-ROt (truncated)	Stable B-cells expressing antisense mRNA	G_0/G_1 arrest	*123*
SHP1	Motheaten mice (me)	Lethal; hemopoietic dysregulation; splenomegaly, runting; autoimmune disease (viable motheaten mice, mev, inactive PTP domain); low bone density owing to elevated amount of activated osteoclasts	*124–126*
	Antisense oligonucleotide in erythroleukemic SK6 cells	Increased globin expression on EPO induction; augmentation of terminal differentiation	*127*
SHP2	Gene knockout	Lethal at E8.5–10; defect in mesoderm patterning; cells impaired in their motility on fibronectin; hyperphosphorylation of FAK	*70,128,129*
PTP-ζ	Gene knockout	No gross abnormalities	*130*
TC-PTP	Gene knockout	Death of mice 3–5 wk after birth; pathologies: runting, splenomegaly, lymphadenopathy, defect in erythropoiesis and lymphopoiesis	*131*

[a]Non-dual-specificity PTP knockout, mutants, and key antisense experiments are listed.

tyrosine phosphorylation of β-catenin. This type of regulation parallels PTP-α, for which a series of elegant experiments has shown that it is inactivated through dimerization *(136,137)*. An inhibitor of PTP-ζ would mimic pleiotropin and might have therapeutic benefits where angiogenesis needs to be induced.

Fap/Apoptosis

The membrane proteins Fas (APO-1/CD95) and Fas-ligand are key players in apoptosis. Apoptosis plays an important role in many disease processes; tumor cells and viruses often adopt apoptosis-evading mechanisms *(138)*, whereas apoptosis is an undesired neurodegenerative effect

of axon damage, immune insult or hypoxia *(139)*, as well as graft rejection *(140)*. Drugs that regulate apoptosis are therefore of considerable pharmaceutical interest. FAP (fas-associated PTP) has been shown to associate with the fas receptor *(141)*. A number of reports suggest that FAP inhibits apoptosis mediated through Fas-FasL signaling in humans *(142–145)*, but this could not be reproduced in mice *(146)*. Although the Fas-FasL signaling pathway and FAP's mode of action are still poorly understood, this PTP would seem a good drug target for proapoptotic drugs.

Gp130, a Regulated Signal Transducer of IL-6R Complex

Gp130 is a transmembrane receptor associated with different cytokine receptors *(147)* including IL-6 *(148)*, LIF, oncostatin M, and IL-11 and IL-12 receptors. Since gp130 is tyrosine-phosphorylated by Jak1, it is not surprising that this receptor is also associated with SHP2 *(149)*.

After stimulation with IL-6, Jak1 phosphorylates both itself and docking sites of STAT3 and STAT1 on gp130 (Tyr^{769}, Tyr^{814}, Tyr^{905} and Tyr^{915}), STAT3, SHP2, and its docking site on gp130 (Tyr^{759}). SHP2 then binds grb2 and initiates the MAPK pathway. SHP2 modulates the STAT3 pathway by an unknown mechanism. The docking sites for STAT3 have been postulated as target tyrosines for SHP2 *(150)*. SHP2 also moderates Jak1 activity *(151)*.

More recently, it was shown that a cytoplasmic isoform of PTPε, PTPεC *(152)*, negatively regulates IL-6 signaling when overexpressed *(153)*. Furthermore, Jak1, gp130, and STAT3 were identified as this PTP's substrates. Finally, a dominant negative mutant of PTPεC, PTPεC-DA, enhanced signaling of IL-6. IL-6 has therapeutic potential in immune system restoration after chemotherapy and bone marrow transplantations *(154)*. From these data, PTPε seems the best potential drug target for the development of IL-6 mimetics.

Prospects for Probing PTP Specificity

Several promising approaches have been initiated that allow the identification of PTPs that dephosphorylate a given receptor. One such approach involves the systematic testing of a full panel of PTPs for their reactivity in vitro with a phosphorylated test receptor *(35)*. This approach makes use of PTP "trapping mutants" *(155,156)*. The phosphorylated receptor is immobilized on a membrane and incubated in a 96-well format with trapping glutathione-*S*-transferase (GST)-PTP fusion proteins. PTPs that strongly interact are subsequently identified by an anti-GST antibody *(35)*. Other approaches are based on PTP knockout mice, the study of natural mutants, or the use of antisense technologies (listed in Table 2). The knockout mutants have yielded important insights, but the models do not predict accurately the effects of gene repression in adults (i.e., a drug effect). Conditional knockout models have been developed (e.g., the Cre-lox; *[157]*), but these are difficult to set up. Another technology, RNAi, was used to inhibit LAR in leech embryos *(158)*. Unfortunately RNAi seems not to work efficiently in adult mammals *(159)*. Studies that rely on overexpression of PTPs, in wild-type form, or as "trapping mutants" to probe their effect on a given signaling pathway suffer from the fact that overexpression may reduce a PTP's natural catalytic selectivity. It would seem, therefore, that antisense technologies, such as oligonucleotides or "DNA enzymes" *(160)*, would present the best approach to validate PTPs (and other genes) as drug targets.

Conclusion

As we have seen, one PTP (PTP1B) is now a well-validated drug target for a therapeutically highly relevant hormone receptor (insulinR), and several other PTPs have been implicated for other receptors. It is as yet too early to say if and how many further examples will be found. What is mostly known today is about PTPs that are involved in deactivating intracellular (nonreceptor) kinases in downstream signaling cascades (SHP2/Jak2, MKPs/MAPKs). Unfortunately, these pathways are shared by many receptors, and the associated PTPs may therefore not represent useful drug targets. By contrast, PTPs that directly dephosphorylate the receptors themselves may yield drug targets that are both specific and potent. Although there are hints, like for the GHR, that different receptor tyrosines signal different pathways and may be associated with different PTPs, the identification of these PTPs is still lagging.

Another area of question is the specificity of PTPs in receptor downmodulation. A pessimistic scenario is that most activated receptors simply "decay" to their resting state through the collective activity of cellular PTPs (no specificity). The issue of specificity is linked to the diversity of PTPs, which has been the subject of speculation (see ref. *11*). Earlier estimates ran more than 1500 PTPs, but with the recent downward revision of the number of mammalian (human) genes, their number is likelier to be at least an order of magnitude smaller. PTPs are highly regulated through differential splicing, protein-protein interactions, and posttranslational modifications. Both considerations lead to a reduction in the projected number of different catalytic domains and, thus, of potential drug targets.

Another scenario for receptor downregulation is that the activated receptor is not dephosphorylated, but, instead is recycled from the cell surface through endosomal or ubiquitination-dependent pathways, as has been proposed for the GHR *(161,162)*. If such a pathway dominates the fate of an activated receptor, it is unlikely to respond well to PTP modulation.

A final area of question concerns the technical suitability of PTPs as drug targets. Again, since this enzyme family only has a relatively short history, few specific inhibitors are available. Nevertheless, the development of specific PTP1B inhibitors has been reported recently *(163–166)*. It

is hoped that inhibitors such as these will help answer the question of the value of PTPs as drug targets.

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