MINIREVIEW

Regulation of Growth Factor–Induced Signaling by Protein-Tyrosine-Phosphatases (44153)

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Abstract. **The binding of a growth factor to its specific receptor catalyzes a complex cascade of intracellular signaling events, characterized by changes in the phosphorylation state of many key proteins. Among these phosphorylation events, tyrosine phosphorylation plays a prominent role in the transmission of postreceptor signals. The state of tyrosine phosphorylation is regulated by the actions of protein-tyrosine kinases (PTKs) and protein-tyrosine-phosphatases (PTPs). Dysregulation of either event can lead to abnormal cellular responses. PTPs generally act to regulate negatively—that is, to turn off—any signals generated by PTKs. However, this is not always the case, as seen by the phosphatase SHP-2, which can either be a positive or negative regulator of signal transduction depending on the particular cellular context. In addition, a novel family of dual specificity phosphatases has been recently discovered. These enzymes are capable of dephosphorylating phosphotyrosine and phosphothreonine/phosphoserine residues, and seem to play a significant role in attenuating the action of MAP kinases. Several themes appear throughout PTP regulation of growth factor signaling, including positive or negative regulation, importance of cell/tissue type, identity of the receptor activated, and subcellular localization. Although only a handful of PTPs have been identified, the present work done in elucidating their function has revealed their significance in the maintenance of normal physiological responses to growth factors.** [P.S.E.B.M. 1997 Vol 216]

The study of protein-tyrosine kinases (PTKs) and protein-tyrosine-phosphatases (PTPases) has been intricately interwoven into the study of signal transduction processes. Hormones, encountering their receptors on the exterior surface of cells, trigger an internal series of molecular events, a signal transduction cascade, in which PTKs and PTPases play prominent and recurring roles. Activation of signal transduction cascades alters the phosphorylation state of key enzymatic proteins, increasing or decreasing their enzymatic potential, and thus potentiating the hormonal signal into biological responses.

Nearly two decades of research into the roles of tyrosine kinases have resulted in a growing understanding of their importance in growth factor signaling. Some hormone receptors, such as those for insulin, insulin-like growth factor–I (IGF-I), epidermal-derived growth factor (EGF) and platelet-derived growth factor (PDGF), contain ligand-stimulated tyrosine kinase activity. In other instances, ligand stimulation results in the receptor-mediated activation of intracellular kinases. Cytokine-, and B- and T-cell receptors utilize secondary kinases for the transduction of their intracellular signals. These growth factor stimulated kinases

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phosphorylate specific substrates. The set of intracellular substrates phosphorylated in response to a given hormone is believed to determine its biological response.

Growth factor signal transduction leads to alterations in cell growth and differentiation, and must be maintained within tightly regulated limits. Unregulated cellular kinase activity can lead to uncontrolled cell proliferation and malignancy. Strict control of cellular tyrosine phosphorylation is handled in one of two ways: by regulating the activity of tyrosine kinases and by regulating the phosphorylation states of substrate proteins. Tyrosine phosphatases are central players in both regulatory processes, playing the crucial role of opposing cellular tyrosine kinase activities. Although much is known about the activities of various tyrosine kinases, research into PTPases has only recently begun to gain momentum. Previous efforts have focused largely on the identification of members of the PTPase family. Clarifying the roles of each of the known PTPases is a much larger task.

PTPases can be categorized using one of several criteria. Under one set of criteria, PTPases have been categorized according to whether they contain extracellular and transmembrane regions (receptor-like) or are comprised entirely of intracellular domains. Another differentiating structural characteristic is the absence or presence of SH2 domains. SHP-2, a well-characterized SH2-containing PTPase, contains yet another defining characteristic, that of function. Of the PTPases whose functions are known, most have negative effects, while a few notable exceptions, such as SHP-2, exert positive effects within growth factor–signaling processes. Enzymatic activity is another defining characteristic. While most PTPases specifically dephosphorylate phospho-tyrosine residues, several PTPases, known as dual specificity PTPases, have a broader range of activity that includes phosphothreonine/phosphoserine. PTPases can even be grouped according to tissue specificity, determined by expression within a limited range of tissues, such as neuronal or hematopoietic tissues versus ubiquitous expression. As the number of known PTPases continues to grow, organization into the various subgroups has aided in the identification of structural and functional subfamilies, common origins, and the roles of individual PTPases within the biological signaling matrix.

This review will begin with a brief discussion of general PTPase structure, and then will discuss in turn the roles of some of the major PTPases and their putative functions in growth factor signaling. A common recurring theme will be the ability of various PTPases to act as either positive or negative regulators of signaling, depending on their specific context. Particular attention will be placed on the intracellular PTPase, PTP1B, and the work done in our laboratory in elucidating its role in insulin-mediated signaling.

PTPase Structure

The existence of PTPases was first hypothesized in 1979 to explain the loss of phosphorylation from *in vitro* phosphorylated membrane proteins (1). Several key findings within the last decade launched the recent deluge of work that has flooded the PTPase field. PTP1B, the major PTPase from human placenta, was purified to homogeneity (2), and its primary sequence was determined (3). The subsequent discovery that leukocyte common antigen (LCA, the CD45 gene product) shares homology with the catalytic domain of PTP1B demonstrated that these PTPases belong to a family of structurally related molecules. All PTPases contain at least one PTPase domain. PTPase domains span approximately 300 amino acid residues, including the highly conserved core sequence (I/V)HCXAGXXR(S/T)G, in which X can be any amino acid (4). The conserved PTPase domains have formed the basis for the hybridization and PCR techniques used to isolate cDNAs encoding members of an ever-expanding family of PTPases. Currently, more than 75 PTPases have been identified (5), and others are believed to exist. Interestingly, the functional interplay between PTPases and PTKs is complemented by structural similarities, suggesting common evolutionary paths for these two enzyme families: both PTKs and PTPases are comprised of either transmembrane, receptor-like proteins, or of proteins that are entirely intracellular. These loose distinctions form the basis of the classification of PTPases into either of two broad categories: the receptor-like transmembrane PTPases and the intracellular cytosolic PTPases. (Fig. 1).

Transmembrane PTPases are comprised of three general regions. The extracellular region of transmembrane PTPases contains great variability, suggesting the existence of as yet unidentified cognate ligands. Structural motifs found within the extracellular region include immunoglobulin like and fibronectin III repeats, as well as glycosylation sites and cysteine rich regions. These divergent motifs are the basis for several subcategories within the transmembrane PTPase group. A transmembrane section traverses the plasma membrane, connecting the extracellular and intracellular domains. The intracellular region, contains one or, more commonly, two PTPase domains. Of the two domains commonly present in transmembrane PTPases, the N-terminal domain is usually more active.

Other PTPases, such as PTP1B, are comprised entirely of intracellular residues. The cytosolic PTPases contain a single PTPase domain. Other regions of the PTPase may be involved in regulation, protein localization, or determining substrate specificity. For instance, PTP1B contains a C-terminal sequence that localizes it to the endoplasmic reticulum, possibly limiting its range of function (6).

A functional motif found in some of the intracellular PTPase family members is the *src* homology 2 (SH2) domain, which allows these PTPases to bind to proteins containing phosphorylated tyrosine residues. One or two SH2 domains may be present. SH2 domains have been shown to target the association of signal transducing molecules with activated growth factor receptors containing intrinsic pro-

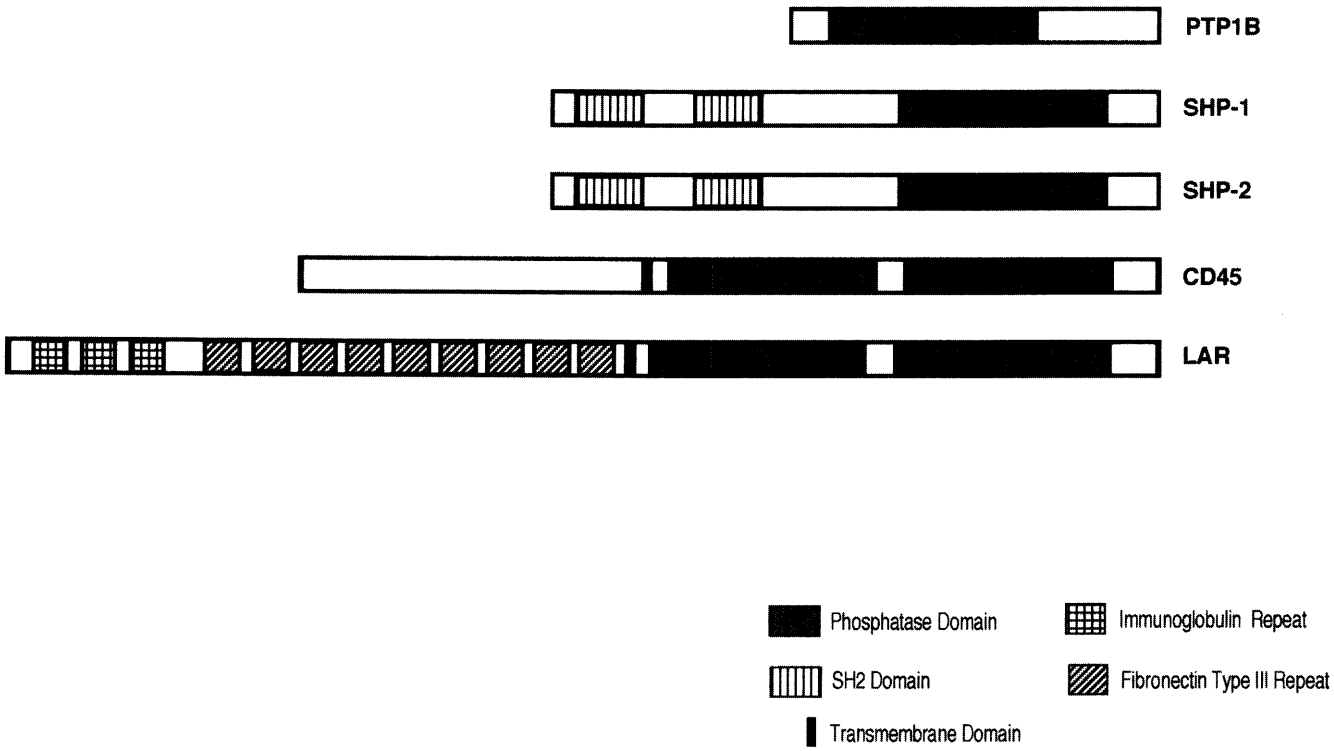

Figure 1. Primary structures of a representative number of protein-tyrosine-phosphatases. PTP1B, SHP-1, SHP-2, CD45, LAR.

tein kinase activity. SHP-1 and SHP-2 are two examples of SH2-containing PTPases.

CD45 (Leukocyte Common Antigen): The Prototypical Receptor-Like PTPase

Engagement of the T-cell receptor by peptide antigen bound to major histocompatibility complex (MHC) molecules initiates a tyrosine kinase cascade, probably involving the kinases Lck, Fyn, and ZAP-70. In T lymphocytes, CD45 dephosphorylates and activates these members of the Src-tyrosine kinase family. Without CD45 functions, T cells cannot respond to antigen. Therefore, CD45 functions as a positive mediator of lymphocyte activation.

Like most transmembrane receptor PTPases, CD45 is comprised of an extracellular domain, transmembrane domain, and two phosphatase domains (proximal and distal). Studies using chimeric receptors expressed in CD45-deficient cells have elucidated the roles of these various domains in regulating CD45's, phosphatase activity. Desai *et al.* have created chimeric proteins wherein the extracellular and transmembrate domains of CD45 have been replaced with those of the EGF receptor (EGFR/CD45) (7). In CD45-deficient cells, expression of this chimeric protein restored TCR signaling. Thus, the two phosphatase domains are necessary and sufficient to activate TCR signal transduction. However, what is most intriguing is that addition of EGF to these chimera expressing cells leads to inactivation of CD45 activity and loss of TCR-mediated signaling (7). This negative regulation is dependent on dimerization of two EGFR/CD45 molecules (7). These results indicate that receptor-PTPases may be regulated by ligand in a manner similar to that of the receptor-tyrosine kinases.

Multiple CD45 isoforms that differ in the length and glycosylation of their extracellular domains are generated through differential mRNA splicing. The differential distribution of CD45 isoforms defines functional subsets of T cells. Jurkat cell clones transfected with antisense CD45 cDNA or with cDNA encoding specific isoforms of CD45 exhibit isoform-dependent differences in interleukin-2 (IL-2) production and the tyrosine phosphorylation of cellular proteins, including Vav, after anti-CD3 stimulation. This result demonstrates that the different isoforms differentially regulate T-cell receptor–mediated signaling pathways (8).

The presence of different CD45 isoforms expressed on various T-cell populations, due to alternative mRNA splicing, may result in altered T-cell responsiveness. Overexpression of the isoform CD45RABC significantly increased $CD4^+$ T-cell proliferation both in a mixed lymphocyte reaction and after stimulation by anti-TCR monoclonal antibody (mAb). CD45RABC enhanced Ca^{2+} mobilization and phosphotyrosine accumulation, while suppressing the inhibitory effect of anti-CD4 antibodies. By contrast, overexpression of the CD45RO isoform did not enhance TCR signaling or phosphotyrosine levels in $CD4^+$ T cells, and required co-stimulation *via* the TCR to augment cellular proliferation. Alternative isoforms of CD45 appear to be functionally distinct and reveal a unique mechanism by which T-cell immunologic responsiveness can be modulated (9).

CD45 has a functional role in other signaling systems

as well. It has been known that the antigen responsiveness of T cells to signaling from antigen receptors is modulated by non–antigen-specific accessory membrane proteins for a full-range of cellular responses, such as differentiation, activation, and tolerance. Recently, this paradigm has been found to also apply to B cells. IgM signaling in these cells requires CD45 and is amplified by a complex containing CD19, complement receptor 2, and TAPA-1, which recruits phosphatidylinositol-3 kinase (PI-3 kinase) (10). IL-2 can restore the lytic potential of cytotoxic T lymphocytes and natural killer (NK) cells after interaction with sensitive target cells. The restorative effect of IL-2 is in part due to an increase in CD45 phosphatase activity, suggesting that CD45 plays an important role in modulating the lytic response of effector cells (11).

The generation of CD45 null (–/–) mice to conduct whole-animal or cell culture studies has greatly facilitated efforts to identify the physiological functions of CD45. Important information on CD45's effect in T-cell receptor–mediated signaling, lymphocyte development, and apoptosis has been uncovered. Site-directed mutagenesis of the individual catalytic residues of the two CD45 phosphatase domains indicates that the catalytic activity of the membrane-proximal domain is both necessary and sufficient for restoration of TCR signal transduction in a CD45-deficient cell. Thus, the catalytic activity of only the CD45 membrane-proximal phosphatase domain is required for TCR signaling and regulation (12).

Natural killer cells isolated from CD45 defective transgenic mice ($CD45^{-/-}$) possessed normal cytotoxic activity compared with NK cells of normal mice ($CD45^{+/+}$). These cells could still differentiate functionally into lymphokine-activated killer cells after incubation in high doses of IL-2, even though they lacked CD45 expression. In NK cells, it seems that CD45 is essential for development but not for cytotoxic activities and Fc-γ R-mediated signaling (13).

Stimulation of T cells through the Fas/APO-1 receptor results in apoptosis. Since CD45 and the Src-family kinase Lck are involved in T-cell receptor (TCR) signaling, it has been thought that these molecules may also be involved in the apoptotic response. However, it has now been shown that Fas induces apoptosis even in CD45- or Lck-deficient T cells, suggesting that they are not required for Fas signaling (14).

T-cell development of CD45-null mice is significantly inhibited at two very distinct stages. Development of $CD4^-CD8^-$ thymocytes into $CD4^+CD8^+$ thymocytes is reduced by 2-fold. Furthermore, the subsequent development of double-positive thymocytes into mature single-positive T cells is reduced by an additional 4- to 5-fold. These CD45-null thymocytes are also resistant to apoptotic signals mediated through the TCR, even though they remain responsive to apoptotic signals that are TCR-independent. CD45 is absolutely required for mitogenic signaling by IgM and IgD but not for CD40-mediated signals (15).

Although a cognate ligand for CD45 has yet to be discovered, employment of cross-linking studies and binding to the extracellular domain by anti-CD45 monoclonal antibodies have revealed a role for the extracellular portion of this receptor-type PTPase in mediating signaling events and regulating lymphocyte effector function. Cross-linking of CD45 to CD40 by biotynylated monoclonal antibodies and avidin strongly inhibited CD40-mediated IgE synthesis in interleukin-4 (IL-4)–treated human B cells. CD45/CD40 cross-linking also inhibited CD40-mediated phosphorylation and activation of the protein tyrosine kinase Lyn (16). Monoclonal antibodies to CD45 inhibited the IL-4–induced release of the soluble form of the CD23 (Fc-ε RIII) molecule by B lymphocytes (17). Stimulation of primary human monocytes caused cross-linking of Fc-γ RI or Fc-γ RII by CD45, and the induction of interleukin-6 (IL-6) production (18). Finally, when Jurkat T cells were exposed to an mAb to CD45 (CD45.2) prior to stimulation with OKT3, TCR were partially uncoupled from calcium signals and calcium-regulated events, without promoting a general inhibition of all TCR-mediated signaling (19).

Despite the fact that CD45 acts a positive regulator in T-cell transduction, it appears to act as a negative regulator of proliferation and mitogenesis in nonlymphocyte systems. When expressed in the murine cell line C127, hormone-dependent autophosphorylation of the PDGF and IGF-I receptors was markedly reduced. Tyrosine phosphorylation of other PDGF-dependent phosphoproteins (160, 140, and 55 kDa) and IGF-I–dependent phosphoproteins (145 kDa) was similarly decreased. Interestingly, the pattern of growth factor–independent tyrosine phosphorylation was comparable in cells expressing the PTPase and control cells. This suggests selectivity or limited access of the PTPase to cellular phosphotyrosyl proteins. The maximum mitogenic response to PDGF and IGF-I in cells expressing the PTPase was decreased by 67% and 71%, respectively. These results demonstrate that a transmembrane PTPase can both affect the tyrosine phosphorylation state of growth factor receptors and modulate proximal and distal cellular responses to the growth factors (20).

The ability of CD45 to affect downstream effector molecules of growth factor receptor tyrosine kinases has also been assessed. In one study, the impact of PTPase expression of IGF-I– and PDGF-dependent activation of PI-3 kinase was investigated. In PTPase+ cells, IGF-I– and PDGF-dependent PI-3 kinase activity in antiphosphotyrosine immunoprecipitates was decreased by 62% ± 13% and 50% ± 17%, respectively, compared with control cells. Similar decreases in the PI-3 kinase activities associated with anti-PDGF receptor and anti-insulin receptor substrate–1 (IRS-1) immunoprecipitates were also observed. The association of PI-3 kinase with the hormone-activated PDGF receptor decreased by approximately 55%, paralleling its loss of activation in PTPase+ cells. Tyrosine phosphorylation of the 85-kDa subunit of PI-3 kinase was also inhibited. Similarly, the IGF-I dependent tyrosine phosphorylation of IRS-1 was decreased by 45%, and its association with PI-3 kinase was

decreased by 65% in PTPase+ cells. Finally, the PDGF-dependent tyrosine phosphorylation of phospholipase C-γ 1 and GTPase-activating protein (GAP) was reduced by 60–70% in the PTPase+ cells as was tyrosine phosphorylation of the PDGF receptor associated with these proteins (21). Expression of CD45 decreased the PDGF-dependent activation of pp60-*src* without increasing basal pp60-*src* activity. There was no change in the phosphotyrosine content of pp60-*src* in either PDGF-treated control or PTPase+ cells. While PDGF caused an increase in pp60-*src* activity, the effect was smaller in PTPase+ cells. *In vitro,* hormone-stimulated pp60-*src* autophosphorylation was decreased in PTPase+ cells, and substrate phosphorylation by pp60-*src* was also reduced. Interestingly, pp60-*src* was an *in vitro* substrate of CD45, and subsequent dephosphorylation by CD45 increased pp60-*src* tyrosine kinase activity. These data suggest that the PDGF receptor is an *in vivo* substrate of CD45 while pp60-*src* is not, demonstrating the importance of intracellular localization and/or accessory proteins in PTPase-substrate interactions (22).

Insulin receptor signaling is increased when the CD45-related PTPase LAR is reduced by antisense suppression in a rat hepatoma cell line. To test whether the hematopoietic cell–specific PTPase CD45 is similarly capable of negatively modulating insulin receptor signaling in hematopoietic cells, the insulin-responsive human multiple myeloma cell line U266 was isolated into two subpopulations that differed in CD45 expression. In CD45 nonexpressing (CD45–) cells, insulin receptor autophosphorylation was increased by 3-fold after insulin treatment when compared with CD45 expressing (CD45+) cells. This increase in receptor autophosphorylation was associated with similar increases in insulin-dependent tyrosine kinase activation. Receptor level effects were paralleled by postreceptor responses. The insulin-dependent tyrosine phosphorylation of IRS-1 and Shc was 3-fold greater in CD45– cells. In addition, insulin-dependent IRS-1/phosphatidylinositol 3-kinase association and mitogen-activated protein (MAP) kinase activation were also 3-fold larger in CD45– cells. While expression of CD45 was associated with a decrease in the responsiveness of early insulin receptor signaling, interleukin-6–dependent activation of mitogen-activated protein kinase kinase (MEK) and MAP kinase was equivalent in CD45– and CD45+ cells. These observations indicate that CD45 can function as a negative modulator of growth factor receptor tyrosine kinases in addition to its well-established role as an activator of the Lck and Fyn tyrosine kinases during TCR-mediated signaling (23).

LAR: A Widely Expressed Receptor-Like PTPase

The PTPase LAR, or leukocyte common antigen–related molecule, was isolated from a human placental library based upon its similarity to CD45. Like CD45, LAR is a receptor-type PTPase, with an extracellular domain similar to the neural adhesion molecule, N-CAM (24), a transmembrane region, and two intracellular PTPase catalytic domains. While most of the catalytic activity seems to reside in the N-terminal phosphatase domain, the C-terminal domain may regulate the activity of the N-terminal domain as in addition to retaining some catalytic activity (25). Unlike CD45, LAR has been found to be expressed in a variety of tissues, with high levels found in various neural tissue types, rat liver, skeletal muscle, and adipocytes (26–30). Changes in LAR expression were found upon nerve growth factor–induced PC12 pheochromacytoma cell differentiation and with contact-mediated inhibition of fibroblast growth. LAR may represent one mechanism regulating neural development after exposure to neurotrophins (27).

The cellular responses to insulin are now known to result from the net effects of the insulin receptor tyrosine kinase and various phosphatases. This has led to a search for one or more physiological insulin receptor (IR) phosphatase(s). The prominent expression of LAR within the rat liver, skeletal muscle, and adipose tissue has made it a candidate IR PTPase (28–30). An *Escherichia coli* bacterial expression system was used to create recombinant LAR, PTP1B, and LRP. These recombinant proteins were incubated with purified insulin receptors activated by insulin, and resulting kinase activity was measured. LAR dephosphorylated the insulin receptor more rapidly than either PTP1B or LRP (31). Also, tryptic mapping revealed that LAR preferentially dephosphorylated Tyr^{1150} within the receptor's regulatory domain more rapidly than either PTP1B or LRP. The accelerated deactivation of the insulin receptor kinase by LAR and its preferential activity towards a key regulatory phosphotyrosine residue identify LAR as a potential regulator of insulin receptors in intact cells (31). Treatment of cells with LAR antisense oligonucleotides causes not only an increase in insulin-dependent PI-3 kinase activation, but also increases in insulin-dependent insulin receptor tyrosine kinase activity, as measured by the increased phosphorylation of the insulin receptor substrate–I (IRS-1) and Shc. The increases in IRS-1 and Shc phosphorylation are paralleled by an increase in the insulin-dependent association of PI-3 kinase with IRS-1 and with increased MAP kinase activity (32). LAR suppression also results in increased receptor autophosphorylation of the EGF and hepatocyte growth factor (HGF) receptors, as well as the increased tyrosine phosphorylation of their respective substrates. Thus, the transmembrane PTPase LAR is able to modulate the hormone-dependent activation of various growth factor receptor tyrosine kinases (32).

SHP-2: A Ubiquitous Regulator of Intracellular Signaling

SHP-2 (SYP, PTP1D, PTP2C, SH-PTP3, SAP-2, SH-PTP2) is a cytoplasmic PTPase, containing two SH2 domains (33–37). It is expressed widely throughout mammalian tissues (34). SHP-2 associates with activated EGF, PDGF, and erythropoietin receptors (38–40), becoming rapidly tyrosine phosphorylated upon ligand stimulation (33). Whether it interacts directly with receptors, or with down-

stream signaling intermediates, SHP-2 is involved in signaling from multiple other hormone receptors as well. *In vitro* evidence suggests that its SH2 domains interact with the activated IGF-I receptor at receptor site pY1316, a site favored by the p85 subunit of PI-3 kinase (41), as well as within the signaling pathways for insulin, granulocyte/macrophage colony–stimulating factor (GM-CSF) and several interleukins (42–44). Further evidence places SHP-2 under the influence of v-*src* transforming activity, since it is constitutively phosphorylated in v-*src*–transformed cells (33).

Although, the exact significance of SHP-2 tyrosine phosphorylation remains unknown, two prominent possibilities are currently under investigation: the phosphorylation of SHP-2 could regulate its phosphatase activity, or, alternatively, tyrosine phosphorylation might create binding sites for the SH2 domains of other signaling proteins. Evidence for the former stems from the observation that SHP-2 activity is stimulated by the addition of a phosphoryl peptide, comprised of the region surrounding Tyr 1009 of the PDGF receptor β subunit (45). Other studies have shown that PDGF receptor activation results in its association with SHP-2 at Tyr 1009 (39) and in the subsequent phosphorylation of SHP-2 (46). Thus, phosphorylation of both the growth factor receptor and SHP-2 may be necessary for maximal PTPase activity.

The latter possibility results from the observation that tyrosine phosphorylated SHP-2 associates with several SH2 domain containing proteins following ligand stimulation (42–45, 47). In response to interleukin-3 and granulocyte/macrophage colony–stimulating factor, but not IL-4, phosphorylated SHP-2 binds to the SH2 domain of Grb2, and the p85 subunit of PI-3 kinase (43). Through these interactions, SHP-2 may serve as an adaptor for signaling from growth factor receptors to the Ras and MAP kinase pathways, or as an integrator between Ras and PI-3 kinase (35). IL-11 stimulation results in SHP-2 tyrosine phosphorylation and its association with JAK2 and an unknown protein of 130 kDa (44). By using SHP-2 as bait and supplying an exogenous tyrosine kinase gene to yeast cells, it has been found that SHP-2 also interacts with the signaling protein Grb7. In this case, the region of interaction has been localized to tyrosine 580 in the carboxyl end of SHP-2 and to the SH2 domain in the carboxyl terminus of Grb7, thus demonstrating that Grb7 binds to SHP-2 *in vitro* under conditions in which the latter is tyrosine-phosphorylated (48).

In addition to establishing interactions through tyrosine phosphorylation, SHP-2 also associates with multiple tyrosine phosphorylated proteins through its own SH2 domains. As stated above, SHP-2 associates with multiple tyrosine phosphorylated hormone receptors (36–39). SHP-2 interacts with phosphorylated nonreceptor proteins as well. In HepG2 and NIH 3T3 cells, while only trace amounts of SHP-2 associate with the activated EGF receptor, it co-immunoprecipitates with a tyrosine-phosphorylated protein of 115 kDa that is not associated with Grb2. In comparison, activation of Jurkat T cells results in the co-immunoprecipitation of SHP-2 and two tyrosine-phosphorylated proteins of molecular weight 120 kDa and 105 kDa, respectively. While the 120-kDa protein has been found to associate with Grb2, the 105-kDa protein does not. Instead, the latter co-immunoprecipitated with SHP-2. Although the identities of the 120-kDa, 115-kDa, and 105-kDa proteins are currently unknown, the 115-kDa and 105-kDa proteins appear to be the major SHP-2 associated phospho-proteins in EGF- and T-cell receptor–activated cells (49). The ability to interact with tyrosine phosphorylated proteins *via* SH2 domains, and with SH2-containing proteins through tyrosine phosphorylation, makes SHP-2 an ideal component of tyrosine phosphorylation cascades, and may underlie its prevalence within diverse hormone signaling pathways.

In contrast to other growth factor receptors, insulin receptor activation leads to the association of SHP-2 with the intermediary phosphorylated protein, IRS-1, rather than with the receptor itself (47). The association of IRS-1 with SHP-2 has been used to probe the specificity of its SH2 domain binding interactions. Screening of several phosphotyrosyl (pY) IRS-1 peptides revealed that both the N- and C-terminal SH2 domains of SHP-2 can bind to IRS-1, but with different binding preferences: the N-terminal SH2 domain binds with greatest affinity to peptide sequences surrounding pY1172, while the C-terminal SH2 domain binds preferentially to pY1222. Interestingly, proteolysis and truncation studies have revealed an autoregulatory role for the C-terminal SH2 domain. Limited tryptic cleavage of this region results in a 27-fold activation of SHP-2 activity. Further activation of this tryptic fragment cannot be achieved by pY peptide binding to the SH2 domains, indicating the autoregulatory function of the C-terminal domain (45). SHP-2 uses its SH2 domains to bind to tyrosine phosphorylated IRS-1. *In vitro,* this phosphatase showed preferential activity towards a recombinant tyrosine-phosphorylated IRS-1 versus an activated-recombinant cytoplasmic insulin receptor kinase (CIRK). To assess the significance of the SH2 domains in this interaction, a truncation mutant of SHP-2 was constructed, which lacked the SH2 domains. Although the truncation mutant did dephosphorylate activated IRS-1, albeit at a slower rate than the wild type, it showed little preferential activity for IRS-1 over other phosphorylated substrates. These data seem to indicate that binding of the phosphorylated tyrosine residues of IRS-1 to the SH2 domains of SHP-2 enhances its activity towards IRS-1. Thus, SHP-2 is probably the *in vivo* phosphatase responsible for IRS-1 dephosphorylation (50).

The specificity of SHP-2 binding to various phosphorylated receptors or phosphopeptides is defined by amino acid residues immediately adjacent to the substrate phospho-tyrosine (pY) residue. The binding affinities between SHP-2 and various peptides, consisting of alternative residues surrounding the critical pY residues of the PDGF-receptor, EGF-receptor, and IRS-1, were examined. The results revealed that the N-terminal SH2 domain of SHP-2

bound with highest affinity to peptide sequences containing phosphotyrosine (pY), followed by a β-branched residue (Val, Ile, Thr) at pY + 1, and a hydrophobic residue (Val, Ile, Leu) at pY + 3. Phosphopeptides of 11 and 9 amino acids containing the correct SH2 recognition sequence bound tightly to SHP-2, whereas 6–amino acid peptides showed weak binding, revealing that residues outside of the pY-1 to pY + 4 motif are also important for high affinity interactions (51).

Like CD45, SHP-2 can act as either a negative or positive regulator in response to numerous growth factors. For example, wild-type SHP-2 negatively regulates EGF-mediated signaling in the human glioma cell line SNB19, which cannot proliferate in response to EGF stimulation, even though its receptors retain the ability to bind EGF, become activated, and associate with the PTPase. In these cells, overexpression of a dominant negative SHP-2 restores the proliferative effects of EGF, suggesting that the wild-type PTPase blocks signaling beyond the receptor (52). SHP-2 also downregulates T-cell activation, through its interaction with CTLA-4. CTLA-4 is homologous to the T-cell co-stimulatory molecule CD28, and its cell surface expression is increased upon T-cell receptor activation. In CTLA-4–deficient mice, there is uncontrolled T-cell proliferation due to constitutive activation of the T-cell receptor specific kinases Lck, Fyn, and ZAP-70, as well as the Ras pathway. SHP-2 has been shown to associate with the cytoplasmic tail of CTLA-4 and dephosphorylate the Ras regulator p52-Shc, thereby regulating T-cell receptor–mediated T-cell activation (53). The inhibitory effects of SHP-2 can be visualized at the microscopic level. When 293 cells are stimulated by PDGF, SHP-2 has been shown to translocate from the cytoplasm to membrane ruffles, where it co-localizes with actin and inhibits membrane ruffling activity (54).

By inhibiting cell proliferation, SHP-2 might be a potent inhibitor of malignant growth. Through its NH_2-terminal SH2 domain, SHP-2 interacts with the p210*bcr-abl* fusion protein, which has been implicated in the pathogenesis of chronic myelogenous leukemia. The p210*bcr-abl* complex stably associates with Grb2. SHP-2 is able to dephosphorylate p210*bcr-abl,* thereby possibly limiting the effects of p210*bcr-abl* tyrosine kinase activity (55).

SHP-2 is also able to function as a positive mediator of both metabolic and mitogenic events. In many instances, it appears to act in association with the Grb2-Sos complex, and upstream of the Ras/MAP kinase pathway. For example, the PDGF-stimulated phosphorylation of SHP-2 (Tyr-542) leads to its association with the Grb2-Sos complex, thus providing a mechanism for PDGF-stimulated Ras activation (56, 57). IRS-1–mediated cell transformation requires interactions with both Grb2 and SHP-2, leading to the activation of the MAP kinase signaling cascade (58). The c-*kit* oncogene encodes a receptor tyrosine kinase that is important in normal hematopoietic development. When bound by its ligand, steel factor, the autophosphorylated receptor associates with SHP-2, which also associates with the adaptor molecule Grb2. Since Ras is activated by steel-factor binding to the c-*kit* gene product, SHP-2 may be a downstream signaling component involved in Ras activation in hematopoietic cells (59). TEK is a newly cloned receptor tyrosine kinase, expressed predominantly in the endothelium of actively growing blood vessels. Transgenic mice defective in TEK function have profound defects in vascular development, resulting in embryonic lethality. Screening of a mouse embryo expression library with the radiolabeled soluble kinase domain of TEK resulted in the isolation of two associated signaling molecules, Grb2 and SHP-2. These final data suggest that SHP-2, in association with Grb2, critically affects embryonic development, mediating signaling from the TEK kinase (60).

Additional evidence places SHP-2 activity within the Ras/MAP kinase signaling pathway. Usually, the target of this PTPase is a molecule upstream of Ras, supporting the evidence that it interacts, at least in part, *via* the Grb2-Sos complex. Overexpression of catalytically inactive SHP-2 in 293 cells inhibits MAP kinase activation and subsequent Elk-1 transactivation following EGF stimulation. However, the inactive SHP-2 failed to inhibit transactivation by activated Ras, suggesting that at least in this case SHP-2 seems to act upstream of, or parallel to, Ras (61). Expression of dominant-negative mutants of SHP-2 inhibit insulin stimulated c-*fos* reporter gene expression and the activation of p42 and p44 MAP kinases. Co-transfection of dominant-negative SHP-2 mutants with v-*ras* resulted in attenuation of insulin stimulated SRE-reporter gene expression. However, co-expression of Grb2 with the mutant phosphatase was able to circumvent the inhibition of downstream insulin signaling. In this case, SHP-2 again seems to regulate insulin signaling either upstream of or parallel to Ras (42).

Interestingly, in several cases SHP-2 actually appears to target molecules downstream of Ras, suggesting that more complex signaling motifs determine the role of this PTPase. In NIH 3T3 cells stably overexpressing the insulin receptor, expression of dominant negative SHP-2 blocks the activation of MEK and Raf-1, with no detectable effect on insulin-stimulated Ras activity (62).

Corkscrew (Csw), the Drosophila homolog of SHP-2, appears to act in either a positive or negative manner, and upstream or downstream of Ras, depending upon the signaling system involved (63). The receptor tyrosine kinase Torso (Tor) is involved in Drosophila embryo development. Tor associates with, and phosphorylates Csw *via,* Tor residue Tyr 630. Mutation of this site to Phe decreases the efficiency of Tor signaling, suggesting a positive role for Csw in Tor signaling, upstream from Ras (64). Csw is also an essential signaling component of the sevenless (Sev) receptor tyrosine kinase signaling pathway, which is involved in eye development. In this system Csw is required for signaling by activated Ras and Raf-1, suggesting a substrate downstream of Ras (65). Recently, using catalytically inactive Csw as a trap, a new 115-kDa substrate was found, and

named daughter of sevenless (Dos). In the presence of constitutively active Sev, Dos is tyrosine phosphorylated. A screen of *dos* mutations revealed three striking phenotypes that could affect Csw function. One enhanced the phenotype of impaired Csw function, a second blocked the phenotype of constitutively active Csw, and the last mimicked the effect of nonfunctional Csw. All of these mutations impaired normal eye development. Thus, Dos seems to be a positive component of the Sev signaling pathway and, in this case, appears to be negatively regulated by Csw (66).

The signaling pathways in which SHP-2 acts as a positive mediator often result in mitogenic stimulation. In expression studies, while the overexpression of wild-type SHP-2, resulting from the stimulation of CCL39 cells by a variety of growth factors, did not appear to affect DNA synthesis, overexpression of a catalytically inactive form of SHP-2 inhibited the stimulatory effects of PDGF and α-thrombin on early gene transcription and DNA synthesis (67). Microinjection of interfering SHP-2 antibodies inhibited EGF (but not PDGF- or serum-)-induced entry into S phase. SHP-2 PTPase activity was also required for the immediate-early response to EGF, although, interestingly, the tyrosine phosphorylation of SHP-2 did not appear to play a role (61). Microinjection of anti–SHP-2 antibodies also significantly decreased insulin-IGF-I–, and EGF-stimulated DNA synthesis. Furthermore, disruption of SHP-2 inhibited cell-cycle progression in response to growth factor stimulation (68).

SHP-2 is not, however, exclusively a mediator of mitogenic signaling. The insulin-signaling system presents an example in which SHP-2 is involved in metabolic signaling. SHP-2 acts as a positive regulator of insulin action and insulin signaling, resulting in the dephosphorylation of tyrosine-phosphorylated pp125FAK, which correlates with a decrease in the number of actin stress-fibers formed (42). In addition, microinjection of a glutathione S-transferase (GST) fusion protein encoding the N- and C-terminal SH2 domains of SHP-2 or anti–SHP-2 antibodies into 3T3-L1 adipocytes inhibited the insulin-stimulated increase in GLUT1 expression but did not affect the insulin-mediated GLUT4 translocation. These data suggest that SHP-2 is necessary for insulin-stimulated GLUT1 expression but not for insulin-activated metabolic signaling involving GLUT4 translocation (69).

SHP-1: A Major Hematopoietic PTPase

Like SHP-2, SHP-1 (HCP, SHP, PTP1C) is an intracellular PTPase, containing two SH2 domains in the N-terminal region (70–72). However, its expression is primarily confined to cells of hematopoietic lineage; thus, its role is largely limited to cytokine-mediated signaling events (70–72).

The motheaten (*me/me*) mouse is a naturally occurring mutant, devoid of SHP-1 expression (73, 74). Phenotypically, it displays hyperproliferation and inappropriate activation of granulocytes and macrophages. It has been used as a model for dissecting the function of SHP-1. In primary bone marrow–derived macrophages from *me/me* mice (SHP-1 null), colony-stimulating factor 1 (CSF-1) stimulation results in hyperphosphorylation of the CSF-1 receptor (CSF-1R), suggesting that SHP-1 normally acts to dephosphorylate the CSF-1R. Upon CSF-1 stimulation, SHP-1 is tyrosine phosphorylated (75) and binds to the small adaptor molecule Grb2. In *me/me* macrophages, Grb2 is associated *via* its SH2 domains with several tyrosine-phosphorylated proteins. It is therefore possible that Grb2 recruits additional substrates for SHP-1. Whether it acts upon the CSF-1R or at downstream substrates, SHP-1 appears to be a critical negative regulator of CSF-1 signaling (76).

There is evidence that SHP-1 regulates B-cell signaling at the receptor level (77, 78). B cells isolated from motheaten (*me*) and viable-motheaten (*mev*) mice proliferate normally in response to lipopolysaccharide (LPS) but are hyperresponsive to sub-mitogenic concentrations of $F(ab')_2$ anti-Ig antibody, and they are less susceptible to the inhibitory effects of Fc-γ IIRB cross-linking on B-cell receptor (BCR)–induced proliferation. Further analysis by co-immunoprecipitation has revealed that SHP-1 is constitutively associated with the resting BCR complex. In the resting state, SHP-1 can dephosphorylate a 35-kDa BCR-associated protein, thought to be Ig-α. Upon receptor activation, SHP-1 dissociates from the BCR complex (77). Cross-linking of Fc-γ RIIB1 with the BCR leads to abortive B-cell signaling. This abortive signaling seems to be mediated by recruitment of SHP-1 to the Fc-γ RIIB1-BCR complex by Fc-γ RIIB1. The SH2 domains of SHP-1 bind to a 13–amino acid tyrosine-phosphorylated sequence of Fc-γ RIIB1. Binding to this sequence leads to SHP-1 phosphorylation and activation. Thus, SHP-1 is an effector of BCR–Fc-γ RIIB1 negative signal cooperativity (78). These results suggest that SHP-1 plays an important role in modulating BCR signaling in both resting and activated cells.

Interferons (IFNs) induce early-response genes *via* stimulation of the Janus family (JAK) of tyrosine kinases, which lead to tyrosine phosphorylation of the STAT transcription factors (79–81). Binding of IFN-α/β results in the activation of the Janus kinases JAK1 and Tyk-2, and the subsequent phosphorylation of STAT1-α and STAT2. PTPases have been implicated in controlling this pathway (82). SHP-1 reversibly associates with the IFN-α receptor complex, upon ligand-induced receptor activation, making SHP-1 a good candidate for involvement in at least some IFN-signaling events (83). In addition, SHP-1 seems to be able to selectively regulate distinct components of the JAK/STAT signaling pathways *in vivo*. Compared with macrophages from normal mice, macrophages from *me* mice showed dramatically increased JAK1 and STAT1-α tyrosine phosphorylation, whereas Tyk-2 and STAT2 activation was unchanged (83). However, the substrate specificity of SHP-1 may be tissue specific: when SHP-1 activity was studied in various human leukemia cell lines, it was found to associate with the tyrosine-phosphorylated form of Tyk-2

in vivo, suggesting a role for SHP-1 in regulating Tyk-2 activity (82).

IL-3 is a potent growth factor for many hematopoietic cells. Although the IL-3 receptor does not possess intrinsic tyrosine kinase activity, binding of its ligand induces rapid tyrosine phosphorylation of intracellular substrates as well as one or more of the receptor chains, and there is a direct correlation between this tyrosine-phosphorylation and ligand-stimulated cell growth (84–86). SHP-1 was found to interact with the β-chain of the IL-3 receptor (87). The role of SHP-1 in regulating IL-3–induced mitogenesis has been explored using dexamethasone-inducible SHP-1 cDNA constructs. SHP-1 overexpression decreased IL-3–induced receptor tyrosine phosphorylation and dramatically reduced cell growth. Decreasing SHP-1 levels had the opposite effect: increased IL-3 receptor tyrosine phosphorylation and a slight increase in cell growth rate. These data strongly suggest an important role for SHP-1 in the regulation of hematopoietic cell growth (87).

The Kit receptor tyrosine kinase is encoded by the dominant white spotting (*W*) locus, while SHP-1 is the gene product of the *me* gene. In mice homozygous for mutations in both *W* and *me* the pathological aspects of both the *me* and *W* phenotypes are ameliorated, most significantly the lethal lung disease associated with *me,* and the embryonic lethality and mast cell deficiency found with the *W* phenotype. These data indicate that the kit receptor plays a role in the pathology of the *me* phenotype and conversely, that SHP-1 negatively regulates Kit signaling *in vivo* (88).

Among the hematopoietic growth factor receptors with intrinsic tyrosine kinase activity are c-*fms,* which encodes the colony-stimulating factor 1 (CSF-1) receptor, and c-*kit,* which encodes the stem cell factor receptor. c-*fms* is normally expressed in committed macrophage progenitors and mature macrophages, while c-*kit* is expressed in mast cells and early hematopoietic progenitors (89). In normal cells, SHP-1 does not bind to the c-fms receptor but does transiently associate with the ligand-activated c-Kit receptor *via* its SH2 domains. Binding of SHP-1 to c-*kit* causes an increase in the phosphorylation state of SHP-1. *In vitro,* SHP-1 is capable of dephosphorylating autophosphorylated c-*kit* and c-*fms,* however, the *in vivo* kinetics of SHP-1 are not entirely consistent with this function, as c-*kit* phosphorylation can still be detected beyond the time of association with the phosphatase. An alternative function could be that phosphorylated c-*kit* acts to recruit SHP-1 to the appropriate cellular location (89).

The threshold at which a ligand will trigger T-cell activation depends on a dynamic balance between positive and negative regulatory events. Src family kinases and ZAP-70 are responsible for the initial tyrosine phosphorylation events upon T-cell activation *via* the TCR. Upon T-cell activation, SHP-1 associates with ZAP-70, resulting in increased SHP-1 phosphatase activity and subsequent ZAP-70 dephosphorylation (90). Expression of a dominant negative form of SHP-1 increases the sensitivity of T cells to antigen stimulation. Thus, SHP-1 acts as a negative regulator of the T-cell antigen receptor and may play a role in setting the T-cell receptor threshold response after antigen stimulation (90).

More recently, SHP-1 has been found to associate with the activated erythropoietin receptor (EPO-R) *via* its SH2-domains. EPO-R has no intrinsic tyrosine kinase activity; however, activation by ligand induces phosphorylation of several intracellular substrates, including the receptor itself and the tyrosine kinase JAK2 (91, 92). The mechanism of JAK2 inactivation has long been unclear, but it is now evident that it involves SHP-1. Tyrosine 429 in the EPO-R appears to be the potential SHP-1 binding site, and this interaction recruits the phosphatase to the general vicinity of JAK2 where it then mediates the dephosphorylation and inactivation of JAK2 (93). Cells expressing a mutant EPO-R unable to bind SHP-1 are hypersensitive to erythropoietin (EPO) and exhibit an increased proliferative response. Thus, SHP-1 may serve an important function as a negative regulator of JAK2 signaling by mitigating the ligand induced mitogenic response (93).

In IM-9 lymphoblastic cells, rat H35 hepatoma cells, and in Chinese hamster ovary cells overexpressing SHP-1 and the insulin receptor, SHP-1 has been found to interact with the insulin receptor upon insulin stimulation. The IR phosphorylated Tyr 538 in the C-terminal region of SHP-1 in response to insulin. Interestingly, SHP-1 binds to autophosphorylated receptors only *via* its C-terminal SH2 domain (94). This leads to a subsequent increase in SHP-1 phosphatase activity. Thus, like that of SHP-2, phosphorylation of SHP-1 seems to increase its phosphatase activity.

SHP-1 may also play a role in cell-specific differentiation. Rat PC12 cells treated with nerve growth factor (NGF) exit the cell cycle and differentiate into a neuronal phenotype, whereas treatment with EGF leads to proliferation. SHP-1 becomes specifically phosphorylated after NGF treatment: NGF stimulation does not promote the phosphorylation of SHP-2, nor does EGF stimulation result in the phosphorylation of SHP-1. In addition, SHP-1 binds to the NGF receptor TrkA *in vitro.* The specific activation and phosphorylation of SHP-1 by NGF suggests a role for SHP-1 in NGF-induced differentiation (95). In another example, the human monoblastic cell line U937 undergoes differentiation into monocytes/macrophages upon treatment with the phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). This chemically induced differentiation event is associated with the genetic upregulation and enzymatic activation of PTPases. The TPA-resistant U937 clone UT16 produced very little induction of SHP-1, PTP-MEG2, P19-PTP, HPTP-ε, or PTP-U1, and subsequently failed to differentiate into monocytes after TPA treatment (96). Similar findings have been reported in HL-60 cells (97). Upon phorbol 12-myristate 13-acetate (PMA)–induced differentiation of HL-60 cells to macrophages, SHP-1 activity and expression was increased. In addition, SHP-1 was found to translocate from the cytoplasm to the plasma membrane in

a manner that temporally correlated with the differentiation process (97). These data also leave open the possibility that SHP-1 is involved in cell differentiation.

Dual-Specificity Phosphatases: A Novel PTPase Family

Recently, a novel family of protein phosphatases has been discovered, possessing dual-specificity for phosphorylated threonine/serine and tyrosine residues, and can be divided into two subfamilies, the VH1-like and the cdc25 phosphatase family. VH1 (*vaccinia virus* late H1 gene) was the first dual-specificity phosphatase identified by its ability to hydrolyze phosphoserine and phosphotyrosine residues of a viral histone-like protein required for encapsidation (98). However, most of the attention has focused on members of this family that can hydrolyze phosphothreonine and phosphotyrosine residues of activated MAP kinases *in vitro* and *in vivo,* thereby inactivating them. These members include MAP-kinase phosphatase-1 (MKP-1), PAC1, HVH2, Pyst1 MKP-2, and MKP-3. Cdc25 (cell-division control) was first identified in the fission yeast *Schizosacharomyces pombe,* where it functions to dephosphorylate Tyr15 of the cyclin-dependent kinase cdc2 (99). There are three mammalian homologues of cdc25 (A, B, and C), which regulate distinct cyclin-dependent kinase (CDK)-cyclin complexes in order to regulate different cell-cycle checkpoints. The mammalian cdc25s dephosphorylate Thr14 and Tyr15 of the CDKs (100). Since Cdc25 is mainly a regulator of the cell cycle, emphasis will be placed on the VH1-like family of dual-specificity phosphatases, in keeping with the theme of this review.

Perhaps the most thoroughly studied phosphatase within this family is MKP-1, also known as CL100, 3CH134, Erp, and hVH-1 (101–104). MKP-1 was originally described as an immediate-early gene that is serum-inducible in normal fibroblasts. Several lines of evidence link it to MAP kinase inactivation: the kinetics of p42 MAP-kinase inactivation coincide with the expression of newly synthesized MKP-1 protein (105); treatment with cycloheximide, an inhibitor of new protein synthesis, which inhibits MKP-1 protein expression, also leads to sustained MAP-kinase activity (105). Through the overexpression of a catalytically inactive mutant of MKP-1 (Cys 258 to Ser), MKP-1 was found to associate physically with phosphorylated p42 MAP kinase (105). These data all indicate that MKP-1 most likely attenuates MAP kinase signaling by interacting with activated MAP kinases.

In fibroblasts overexpressing the human insulin receptor (Hirc B), we have seen that insulin induces a time- and dose-dependent increase in MKP-1 mRNA expression. Insulin receptor tyrosine kinase activity is necessary for the insulin-mediated induction of MKP-1. The overexpression of MKP-1 causes a decrease in hormone-stimulated MAP kinase activity compared with control cells. Furthermore, the overexpression of MKP-1 causes a dramatic decrease in MAP kinase-regulated reporter gene expression (105a). In another set of studies, the regulatory effects of MKP-1 on the insulin-dependent gene transcription of AP-1 were studied by coupling microinjection with luciferase imaging. Overexpression of MKP-1 inhibited insulin-stimulated gene transcription, as measured by luciferase expression, demonstrating the importance of MAP-kinase in insulin-stimulated gene transcription, and defining a physiological role for MKP-1 (106).

MKP-1 has also been implicated in angiotensin II–signaling events. In vascular smooth muscle cells (VSMCs), MKP-1 expression is induced by angiotensin II. In these cells the attentuation of MKP-1 expression by either actinomycin D or specific antisense oligonucleotides prolongs the activation of p42 and p44 MAP kinases (107). The newly discovered angiotensin II type 2 (AT_2) receptor is widely expressed in fetal tissue but is found only in the brain, adrenal medulla, and atretic ovary in adults (108–111). The AT_2 receptor mediates apoptosis in rat PC12W and mouse R3T3 cells, which express high levels of AT_2 but not AT_1 receptors (112, 113). The cellular mechanism whereby it mediates this effect seems to involve the dephosphorylation of MAP kinase: MKP-1 antisense oligonucleotide treatment inhibited the apoptosis of these cells in response to angiotensin II, whereas the use of sense oligonucleotides to MKP-1 restored its apoptotic effects (114). Thus, MKP-1 plays a role in angiotensin II–mediated events in multiple tissues.

MKP-1 also seems to be inducible by stimuli other than growth factors. Treatment of mammalian cells with short-wavelength ultraviolet radiation (UV-C) or chemical genotoxic agents, such as methyl-methanesulfonate (MMS), results in induced MKP-1 expression. In response to UV-C but not to MMS treatment, elevated MKP-1 expression coincides with a decline in c-Jun–N-terminal kinase (JNK) activity, suggesting that MKP-1 can also dephosphorylate activated JNK (115). In Drosophila, heat shock and chemical stress activate a MAP kinase-specific phosphatase, which also inhibits MAP kinase activity, which may or may not be MKP-1 (116).

In general, MKP-1 is a negative regulator of MAP kinase's cellular effects. In a Xenopus oocyte cell-free system, MKP-1 is capable of suppressing the activation of MAP kinase induced by oncogenic Ras (V12-Ras) (117). In quiescent rat embryonic fibroblasts (REF-52), co-microinjection of a MKP-1 plasmid along with a plasmid containing a constitutively active mutant of Ras (V12-Ras) results in the inhibition of DNA synthesis (118). The constitutive expression of wild-type MKP-1 has also been shown to inhibit G1-specific gene transcription and entry into S-phase (119). In this case, MKP-1 inactivation of MAP kinase was found to occur within the nucleus (119). These results reveal the essential role of MAP kinases in the transition from quiescence to the DNA replication phase of the eukaryotic cell cycle, and demonstrate a role for MKP-1 in regulating normal cell-cycle progression (118). The overexpression of MKP-1 in NIH 3T3 fibroblasts causes an in-

crease in bi- and multinucleated cells, and an overall negative effect on cell proliferation (103). Recent data also show that atrial natriuretic peptide (ANP) can increase the expression of MKP-1 in glomerular mesangial cells, evoking the possibility that MKP-1 might be the mechanism through which ANP exerts its antiproliferative effects as well (120).

Wild-type MKP-1 is located within the nucleus of the cell (119). Interestingly, when the critical active-site cysteine was mutated to serine, the resulting catalytically inactive protein (MKP-1CS) remained within the cytoplasm. Even in response to serum stimulation, MKP-1CS remained cytoplasmic. The overexpression of MKP-1CS did not affect the cellular localization of MAP kinase under quiescent or stimulated conditions. Since MKP-1 does not contain a nuclear localization sequence (NLS), the mechanism by which MKP-1 becomes nuclear remains unclear. A single point mutation of Cys258 to Ser is sufficient to exclude MKP-1 from the nucleus. This single mutation may be sufficient to modify the tertiary structure of the protein such that it is no longer recognizable by the nuclear translocation machinery. Alternatively, the ability of MKP-1CS to bind either a nuclear or cytoplasmic anchoring protein may be impaired. Thus, MKP-1 dephosphorylation of MAP kinase is solely a nuclear event and may serve to allow MAP kinase to cycle between the nucleus and cytoplasm in a cycle of activation/inactivation (119).

Although much work has been done on the characterization of MKP-1, there is little known about its regulation. One interesting speculation comes from a study done on v-*raf*–transformed macrophages. BAC-1.2F5 macrophages infected with v-*raf* and expressing v-*raf* exhibit suppressed MAP kinase activity in response to growth factor stimulation. Reduced MAP kinase activity is associated with a lack of ERK2 tyrosine phosphorylation and not defective MEK activation. v-*raf*–expressing macrophages have low constitutive levels of MKP-1, while in the nontransformed parental cell line there is no MKP-1 expression unless induced by growth factors. Pretreatment of these macrophages with the potent tyrosine phosphatase inhibitor vanadate restores MAP kinase activity. These data raise the possibility that in macrophages Raf serves to feed back on the MAP kinase pathway by inducing MKP-1 expression (121).

However, a recent report indicates that in fibroblasts, MKP-1 mRNA expression is independent of the MAP kinase pathway. Instead, MKP-1 mRNA induction is dependent on the activation of the c-Jun–N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) pathway (122). Treatment of fibroblasts with the phorbol ester TPA, a selective activator of MAP kinase, failed to induce MKP-1 expression, whereas anisomycin, a genotoxic agent and selective activator of JNK, induced significant MKP-1 mRNA expression (122). Transfection experiments using dominant-negative mutants of Ras, and MEKK1, inhibited growth factor–induced MKP-1 expression. However, transfection of dominant-negative MEK had no affect on MKP-1 expression upon serum stimulation. On the other hand, inducible overexpression of active MEKK1 resulted in a low level of constitutive MKP-1 expression. This evidence reveals that growth factor stimulation of MKP-1 is dependent on Ras activation followed by activation of the JNK–signaling pathway, and uncovers a potential point of crosstalk between the ERK and JNK pathways (122).

Numerous reports emphasize the central role of MAP kinase regulation by MKP-1 for normal mitogenic signaling and eventual cell proliferation (103, 118, 119, 121). Interestingly, the generation of MKP-1–deficient mice resulted in progeny with normal birth rates, normal fertility, and no phenotypic or histologic abnormalities. MAP kinase activity and c-*fos* mRNA induction was unaltered in mouse embryo fibroblasts (MEFs), lacking MKP-1. These MEFs also presented normal growth rates and cell-cycle progression. These mice demonstrate that although MKP-1 may be essential for normal embryonic development, the lack of MKP-1 is compensated for by other phosphatases *in vivo* to maintain normal growth (123).

A *Xenopus laevis* homologue of MKP-1, XCL100, has been cloned (124). Like MKP-1, XCL100 is a dual-specificity phosphatase, which can dephosphorylate both the threonine and tyrosine residues of activated MAP kinase (124). It is primarily localized to the nucleus in transfected COS-1 cells. XCL100 mRNA levels are maintained constitutively in growing Xenopus oocytes and are, in addition, greatly increased at the mid-blastula transition, suggesting that this enzyme may be involved in regulating MAP kinase during early development. In addition to low constitutive levels of expression, XCL100 mRNA is inducible by serum and oxidative/heat stress in a *X. laevis* kidney cell line. In contrast to mammalian fibroblasts, inactivation of MAP kinase in these cells does not require new protein synthesis. These results indicate that the induction of MKP-1–like enzymes may not be required for MAP kinase inactivation in all cell types. XCL100 may only be functionally relevant during embryogenesis (124).

Several related dual-specificity phosphatases have also been identified (125, 126). Recently, two additional members of the MKP-1 family were cloned and named MKP-2 and MKP-3, respectively (125, 126). MKP-2 was originally isolated from PC-12 cells but shows expression over a wide distribution of tissue. Like MKP-1, MKP-2 is inducible by a wide variety of agents including bombesin, EGF, TPA, cyclic-AMP (cAMP), FGF, NGF, and serum (125). However, MKP-2 shows unique expression kinetics. Serum stimulation causes a biphasic response with an initial peak occurring at 1 hr followed by a transient decrease and a subsequent increase in expression by 4 hr. The difference in kinetics implies that MKP-2 might be involved in regulating the more chronic activation of MAP kinase often associated with differentiation, while MKP-1 might specifically regulate acutely activated MAP kinase events, such as proliferation (125). MKP-3 differs from MKP-1 in its tissue distribution and subcellular localization. MKP-3 is not signifi-

cantly expressed in skeletal muscle or testis, and resides in the cytosol. MKP-3 undergoes powerful induction in PC12 cells after 3 hr of NGF treatment but does not appear to respond to either EGF or dibutyryl cAMP (126). MKP-3 may be responsible for the cytosolic regulation of MAP kinase or for blocking nuclear translocation (126).

PAC-1 is an immediate-early mitogen-inducible tyrosine phosphatase that is expressed primarily in the nuclei of T cells and other hematopoietic cells. The constitutive expression of PAC-1 *in vivo* inhibits the activation of MAP kinase induced by EGF, PMA, or T-cell receptor cross-linking (127). Furthermore, activated MAP kinase has been shown to be an *in vivo* substrate of PAC-1, and the inactivation of MAP kinase by PAC-1 results in inhibition of MAP kinase-dependent reporter-gene expression (128).

Studies have shown that the *in vivo* substrate specificities of individual dual-specificity phosphatases are unique (129). PAC-1 specifically recognizes extracellular signal–regulated kinase (ERK) and p38, MKP-2 specifically recognizes ERK and JNK, while MKP-1 recognizes all three substrates (ERK, p38, and JNK). All three phosphatases show reduced sensitivity towards the MAP kinase mutant, ERK2 (D319N), which is analogous to the *Drosophila sevenmaker* (130) gain-of-function mutation. Individual MAP kinase phosphatases can potentially regulate cross-talk between the various MAP kinase pathways (129). The *sevenmaker* gain-of-function mutation results in increased sensitivity to low levels of signaling, without increased basal kinase activity. The elevated responsiveness *in vivo* is believed to be due to the lower sensitivity of MKP-1 towards the *sevenmaker* MAP kinase mutant (131). Pyst1, a recently discovered member of the MKP-1 family, expressed constitutively in human skin fibroblasts, contains even higher substrate specificity (132). In contrast to MKP-1, Pyst1 is not inducible by stress or mitogens, and is localized in the cytoplasm. Pyst1 interacts specifically with activated MAP kinase and shows very low activity towards JNK or p38 (132).

Another dual-specificity phosphatase characterized by nuclear localization and inactivation of MAP kinases, ERK1 and ERK2, is HVH2 (133). It shows considerable sequence homology to the vaccinia virus phosphatase family VH1. The N-terminal region of HVH2 displays sequence identity to the cell-cycle regulator phosphatase, Cdc25, HVH2 mRNA shows an expression pattern distinct from either the mouse or human homologs of MKP-1 or PAC-1, suggesting a unique function in mammalian systems (133).

The molecular diversity seen within the dual-specificity phosphatase family now begins to parallel the number of ERK, SAPK/JNK, p38/HOG1 MAP kinases providing the opportunity for highly specific regulatory control. Mechanisms underlying such specificity remain to be determined, although one could speculate that these might include substrate specificity, time course of stimulus-dependent activation and induction, cell-specific expression, or subcellular compartmentalization.

PTP1B: An Important Regulator of Insulin Signaling

PTP1B was originally purifed as the major PTPase of human placenta (2, 3). Its purification was a major milestone in understanding the physiology of PTPases. Although PTP1B was one of the first PTPases to be identified, cloned, and characterized, only recently has much about its physiological function become known. The overexpression of PTP1B in NIH 3T3 fibroblasts prevented transformation *via* the introduction of the *neu* oncogene (134). It has been found to interact with the EGF receptor and become tyrosine-phosphorylated upon receptor activation (Liu F, Chernoff J, unpublished data). Furthermore, insulin and PMA increase PTP1B mRNA expression, suggesting a role in the feedback regulation of insulin signaling (135). Over the years, our laboratory has been interested in PTP1B and its potential role in insulin and IGF-I signaling.

The direct effects of PTP1B on insulin signaling have been shown by microinjecting purified PTP1B into Xenopus oocytes, which blocked insulin-stimulated S6 peptide phosphorylation and retarded insulin-induced oocyte maturation (136, 137). We have examined the effects of insulin and IGF-I on the activity and expression of PTP1B in various cell lines. PTPase activity was assessed using a synthetic phosphopeptide substrate (IRP), which is identical to the major site of insulin receptor autophosphorylation. In unstimulated L6 myotubes, almost all of the PTPase activity resided in the Triton X-100 soluble fraction (P1). Incubation with either insulin or IGF-I resulted in a dramatic increase in PTPase activity within the P1 fraction over basal levels, and did not result from a redistribution of PTPase activities from other subcellular fractions to P1. Insulin stimulation caused a biphasic increase in PTPase activity, with a small increase seen at 30 min followed by a decline to basal levels within 2 hr, and a subsequent increase in PTPase activity after 4 hr, which gradually reached a maximum at 32 hr. In contrast to insulin, IGF-I–stimulated activity, first detectable at 30 min, gradually increased to a single peak, with its maximum at 32 hr. Immunoprecipitation with anti-PTPase antibody demonstrated that the hormone-stimulated increases in total PTPase activity were mirrored by increased levels of PTP1B-specific activity. PTP1B activity rose concomitantly with increasing PTPase1B protein levels, following increased PTP1B mRNA levels, which were maximal after 12 hr of stimulation with either insulin or IGF-I. Both insulin and IGF-I caused a dose-dependent increase in PTP1B. The low concentrations required to generate these effects suggested that each hormone acted through its own receptor (138).

Based on these previous studies, it became essential to determine whether PTP1B is a positive or negative regulator of insulin action. To determine this, we established clonal cell lines overexpressing wild-type or catalytically inactive PTP1B (C215S) in cells overexpressing insulin (Hirc B) or

IGF-I (CIGFR) receptors. Cysteine 215 is an essential residue within the catalytic domain of PTP1B, and its mutation to serine abolishes the PTPase's enzymatic capability. In parental cells, more than 90% of the PTPase activity was localized to the Triton X-100 soluble particulate (P1) fraction. PTPase activity in cells overexpressing wild-type PTP1B was strikingly higher than that found in parental cells, while in cells expressing C215S it remained unaltered. Insulin-stimulated insulin receptor autophosphorylation was sustained at greater levels in Hirc cells overexpressing C215S mutant PTP1B (Hirc-M) than in Hirc cells overexpressing wild-type PTP1B (Hirc-A). Hirc-A cells also showed strikingly reduced levels of IRS phosphorylation in response to insulin. In CIGFR cells, CIGFR-M (overexpressing inactive C215S PTP1B) displayed increased IRS phosphorylation in comparison with parental CIGFR (CIGFR-P), while CIGFR-A (overexpressing wild-type PTP1B) displayed reduced IRS protein phosphorylation in comparison with CIGFR-P (139).

Basal glucose incorporation rates of Hirc-A and Hirc-M did not significantly differ from that seen in Hirc-P, nor was there any significant difference among CIGFR-M, CIGFR-A, and CIGFR-P. Ligand stimulation increased [^{14}C]glucose incorporation in all cell lines in a dose-dependent manner. However, the relative [^{14}C]glucose incorporation was greatest in cells containing C215S PTP1B, and was lowest in cells overexpressing wild-type PTP1B. Thus, PTP1B could act as a negative regulator of insulin action by dephosphorylating the insulin and/or IGF-I receptors. An excess of PTP1B could completely dephosphorylate and inactivate a large subset of receptors. The remaining pool of fully active receptors may be unable to compensate for the loss, resulting in a reduction of receptor signaling. Another possible scenario is that PTP1B could dephosphorylate only specific, critical receptor tyrosine residues necessary for receptor activation without completely dephosphorylating all tyrosine residues in a non-specific manner. As a final possibility, PTP1B could dephosphorylate both the receptors and IRS proteins, with the IRS proteins as the preferred substrates. The enhanced receptor autophosphorylation and IRS phosphorylation observed during overexpression of C215S PTP1B could be due to binding of the inactive PTPase to phospho-tyrosine residues, thereby protecting them from dephosphorylation by the endogenous PTP1B (139).

The role of PTP1B as a negative regulator of insulin signal transduction pathways has been independently confirmed by other groups (140, 141). Osmotic loading of rat KRC-7 hepatoma cells with affinity-purified neutralizing PTP1B antibodies increased insulin-stimulated DNA synthesis and PI-3 kinase activity. Concurrently, insulin-stimulated receptor autophosphorylation and IRS-1 tyrosine phosphorylation were also significantly augmented, as well as insulin receptor tyrosine kinase activity towards an exogenous peptide substrate. Osmotic loading did not decrease the intracellular content of PTP1B, suggesting that the antibodies blocked PTP1B activity by sterically inhibiting the interaction between PTP1B and its substrates (140). In another study, synthetic tris-sulfotyrosyl dodecapeptide (TRDIY-[S]ETDY[S]Y[S]RK-amide), identical in primary sequence to amino acids 1142–1153 of the insulin proreceptor, inhibited insulin receptor dephosphorylation in solubilized membranes, and digitonin-permealized Chinese hamster ovary cells, expressing high levels of the human insulin receptor (CHO/HIRc) (141). It also inhibited recombinant PTP1B dephosphorylation of a synthetic tyrosine-phosphorylated substrate (141). A *N*-stearyl derivative of the peptide increased insulin-stimulated receptor autophosphorylation in intact CHO/HIRc cells. The peptide showed specificity towards tyrosine-class phosphatases, as alkaline phosphatase and serine/threonine phosphatases were unaffected (141). The sulfotyrosyl peptide functions as a non-hydrolyzable phosphotyrosyl peptide analogue capable of direct interaction with PTP1B's phosphatase domain, which may result in blocking PTP1B activity (141).

Two methods were utilized to investigate whether PTP1B directly interacts with the insulin receptor. The first involved using a PTP1B glutathione S-transferase fusion protein, containing a point mutation in its catalytic domain, which rendered the enzyme inactive (PTP^{C215S}-GST). This mutant retained the phosphotyrosine-binding site, thus providing a PTP1B fusion protein which could bind to, but not dephosphorylate, phosphotyrosine substrates (142). Hirc B whole-cell lysates were isolated and incubated with insulin and PTP^{C215S}-GST. Precipitation with glutathione sepharose pulled down the insulin receptor (IR) as well as an unidentified 120-kDa phosphoprotein (143). In a complementary *in vitro* study, purified insulin receptors were stimulated with insulin and activated in the presence of PTP^{C215S}-GST and recombinant IRS-1. The IR β-subunit, the PTP1B fusion protein, and IRS-1 all showed a marked increase in phosphotyrosine content after hormone stimulation, suggesting that PTP1B becomes tyrosine phosphorylated upon insulin stimulation (143). The interaction between PTP1B and the insulin receptor was also evaluated by incubating the labeled PTP1B fusion protein ([S^{35}]PTP^{C215S}-GST) with autophosphorylated insulin receptor kinase domain peptides immobilized on amylose-agarose beads in the presence or absence of unlabeled fusion protein under equilibrium binding conditions. The radioactive PTP1B fusion protein bound to the activated IR peptides and was displaced in a concentration dependent manner, indicating that the interaction of the two proteins occurs in a specific, competitive manner (143).

To test PTP1B association with the insulin receptor *in vivo,* monolayers of Hirc B cells were stimulated with insulin for varying time intervals. Cell lysates were harvested and immunoprecipitated with anti-insulin receptor antibody, and then immunoblotted with anti-phosphotyrosine (anti-PY) or anti-PTP1B antibody. A 50-kDa protein identified as

PTP1B was precipitated with anti-IR and was tyrosine phosphorylated extremely rapidly after insulin stimulation (143).

In order to localize potential PTP1B binding sites on the insulin receptor, three phosphopeptides were constructed, corresponding to (i) the triple-tyrosine domain containing tyrosines 1150, 1150, and 1151, (ii) the NPXY domain containing tyrosine 960, and (iii) the C-terminal region including tyrosine 1322 (pY1322) (144). An RPXpY peptide was used instead of the NPXpY peptide, since HPLC analysis indicated conformational changes which could have prevented consistent phosphotyrosine binding with the NPXpY motif. The IR was precipitated by increasing concentrations of PTP^{C215S}-GST. IR precipitation was inhibited in the presence of the triple-tyrosine kinase domain phosphopeptide, as well as the RPXpY and pY1322 peptides. Thus, three potential sites of PTP1B interaction with the IR have been identified (143).

To further explore the interaction between PTP1B and the IR, the ability of PTP^{C215S}-GST to precipitate various mutants of the IR missing certain tyrosine residues was examined. ΔCT cells express a truncated insulin receptor missing the C-terminal 43 amino acids, which includes tyrosines 1316 and 1322. The PTP1B fusion protein precipitated ΔCT mutant receptors to an extent comparable to control receptors. Another IR mutant missing the NPEY domain, including tyrosine 960, was also precipitable by the inactive fusion protein. However, PTP^{C215S}-GST was unable to precipitate an AK1018 IR mutant. This variant contains a point mutation (lysine to alanine) within the receptor's ATP-binding site, thus abolishing receptor tyrosine kinase activity while retaining ligand-binding capability. Scatchard analysis provides strong evidence for the existence for more that one binding site. Activation of the receptor's triple tyrosine region in the kinase domain is most likely necessary for the interaction of PTP1B with the insulin receptor. This could be due to the requirement for phosphorylation of the triple tyrosine domain for direct interaction between PTP1B and the insulin receptor, or receptor autophosphorylation may be necessary for tyrosine phosphorylation of other sits. The fact that PTP^{C215S}-GST does not interact with any specific phosphotyrosine motif *in vitro* is consistent with the hypothesis that cellular localization is important for substrate specificity (143).

PTP1B's function may be to dephosphorylate the receptor, thereby preventing receptor association with secondary signaling substrates. To test this hypothesis, partially purified insulin receptors were incubated with p85-GST, increasing concentrations of either the catalytically active PTP1B-GST or inactive PTP1B-GST, and glutathione sepharose. The p85 subunit of PI-3 kinase binds directly to the insulin receptor at tyrosine 1322 and is a major downstream signaling molecule of the IR. p85-GST can precipitate the IR β-subunit in the absence of active PTP1B-GST. As increasing concentrations of PTP1B-GST are added, overall receptor tyrosine phosphorylation is decreased, and the interaction between the receptor and p85-GST is inhibited. The *in vitro* receptor dephosphorylation does not occur when inactive PTP^{C215S}-GST is used, and, remarkably, receptor precipitation is increased. These data suggest that PTP1B may inhibit insulin receptor signal transduction by dephosphorylating phosphotyrosine residues such as tyrosine 1322, thereby preventing receptor interaction with downstream signaling molecules (143).

These biochemical studies demonstrate strong evidence for a direct PTP1BP:insulin receptor interaction and the insulin-stimulated tyrosine phosphorylation of PTP1B. The inability of the inactive PTP1B fusion protein to precipitate IRS-1 could be due to the inaccessibility of phosphotyrosine residues due to binding of SH2-domain proteins such as p85, SHP-2, or Grb2. Tyrosine phosphorylation of PTP1B may serve to either positively or negatively regulate its activity, or to increase substrate specificity by providing another phosphotyrosine "docking" site. Although the precise function of PTP1B in modulating insulin signaling remains elusive, it appears that PTP1B is a potential significant regulator of insulin receptor tyrosine kinase activity.

The second method used to investigate interactions between PTP1B and the insulin receptor employed the overexpression of a catalytically inactive mutant of PTP1B (CS) in Hirc cells (Hirc-M). Lysates from control and insulin treated Hirc-M cells were immunoprecipitated with antibody to PTP1B. Subsequent immunoblotting was performed sequentially using anti-phosphotyrosine, anti-insulin receptor, and anti-PTP1B antibodies. In a parallel experiment, insulin receptors were immunoprecipitated from control and insulin-treated Hirc-M cells. These lysates were also immunoblotted with the same series of antibodies. A 95-kDa protein co-precipitated with anti-PTP1B after insulin stimulation and was identified as the β-subunit of the insulin receptor. Only a residual amount of IR β-subunit was found in precipitates of control cells. Immunoprecipitation with the IR antibody revealed the co-precipitation of a 50-kDa protein identified as PTP1B an insulin-treated cells. Again, little PTP1B was co-precipitated by anti-IR in unstimulated cells. The presence of small quantities of PTP1B in anti-IR immunoprecipitates, and IR in anti-PTP1B immunoprecipitates from unstimulated cells could be due to a low level of insulin receptor tyrosine phosphorylation under basal conditions. These results indicated that PTP1B complexes with the intact insulin receptor in an insulin-dependent manner. The insulin-stimulated interaction between PTP1B-CS and the insulin receptor was accompanied by an increase in PTP1B-CS tyrosine phosphorylation after insulin stimulation. A presently unidentified protein(s) of 180 kDa was also co-precipitated by both PTP1B and insulin receptor antibodies. This protein could represent IRS-1, IRS-2, or a combination of both (144).

To assess the possibility that PTP1B may also interact with other receptor tyrosine kinases, the interaction between PTP1B and the IGF-I receptor was examined. CIGFR-M cells, which overexpress the IGF-I receptor and PTP1B-CS,

were stimulated in the absence and presence of IGF-I. Lysates were immunoprecipitated with anti-PTP1B antibody, and analyzed by sequential immunoblotting with antibodies to phosphotyrosine, the IGF-I receptor and PTP1B. The 105-kDa IGF-I receptor protein co-precipitated with anti-PTP1B in IGF-I–treated cells. In contrast to insulin-stimulated cells, very little tyrosine phosphorylation of PTP1B was detectable in anti-PTP1B precipitates from IGF-I–stimulated cells. This could be due to the lower abundance of phosphorylated PTP1B in IGF-I–treated cells, or it may represent a receptor-specific difference in substrate phosphorylation (144).

Most protein tyrosine kinases phosphorylate substrates a tyrosine residues preceded by acidic amino acids (145). The coding sequence for PTP1B possesses three such candidate sites, Tyr 66 (QEDDNDY) and Tyrs 152 and 153 (EDIKSYY). To determine whether these residues become phosphorylated upon insulin stimulation, either Tyr 66 or Tyrs 152 and 153 were mutated to phenylalanine. Expression vectors containing PTP1B-CS, C215S-Y66F (YF), or C215S-Y152/153F (YYFF) mutants were co-expressed along with wild-type insulin receptor in COS cells. Cells transfected with PTP1B-CS showed increased phosphorylation of PTP1B after ligand stimulation. However, in neither YF nor YYFF transfected cells was PTP1B phosphorylation evident upon insulin treatment, suggesting that tyrosine residues 66, 152, and 153 are all necessary for the insulin-induced phosphorylation of PTP1B. In order to determine if these residues are also essential for the interaction of PTP1B with the activated insulin receptor, PTP1B-CS, YF and YYFF transfectants were treated with insulin and then immunoprecipitated with anti-PTP1B. Compared with PTP1B-CS cells, YF and YYFF cells co-precipitated significantly less of the insulin receptor (144). These data suggest that PTP1B tyrosines 66, 152, and 153 play some role in the interaction with the insulin receptor, although the extent of their importance is unclear.

To identify insulin receptor phospho-tyrosines required for complex formation with PTP1B, several insulin receptor mutants were utilized. ΔCT-1 lacks the C-terminal 30 amino acids, which include tyrosines 1316 and 1322. YFF contains tyrosine to phenylalanine mutations at tyrosines 1150 and 1151. FYY is mutated at tyrosine 1150 and YF′ possesses a point mutation at tyrosine 960. Anti-PTP1B antibody co-precipitated relatively equivalent quantities of insulin receptor from wild-type, ΔCT-1, and YF′ cells after insulin stimulation. On the other hand, very little insulin receptor was seen in anti-PTP1B precipitates from FYY or YFF cells after insulin stimulation. Thus, PTP1B interaction *in vivo* is directly related to the level of insulin receptor autophosphorylation. Insulin receptor tyrosine residues essential for efficient complex formation minimally include residues 1150, 1150, and/or 1151 (144).

The mechanism by which PTP1B tyrosine residues 66, 152, and 153 facilitate the interaction with the IR remains unclear. The sequence surrounding Y66 (pYINA) conforms to the consensus binding site for the SH2 domain of Grb2 (pYXNX). If PTP1B does in fact interact with Grb2 following the phosphorylation of Tyr 66, it is possible that PTP1B could inhibit insulin signaling *via* the Ras/MAP kinase pathway, by competing with IRS-1 and Shc for the limited pool of Grb2.

The requirement for the phosphorylation of insulin receptor tyrosine residues 1150, 1150, and 1151 may be to create a binding site for PTP1B. Alternatively, the phosphorylation of these residues might be necessary for the subsequent tyrosine phosphorylation of alternate PTP1B binding sites, or in order to expose a conformationally dependent binding site. Although, PTP1B does not contain a recognizable SH2 or PTB domain, it does contain the sequence FKVRES, located 19 residues N-terminal of the catalytically essential Cys 215. This sequence is very similar to the highly conserved FLVRES motif of the SH2 domains (146), which has been shown to be essential for the binding of SH2 domains to tyrosine phosphorylated proteins (147). It is possible that PTP1B interacts with the insulin receptor through this FKVRES sequence. The alternative possibility that other accessory proteins indirectly mediate this interaction cannot be ruled out.

An unresolved issue is how PTP1B, which is localized to the endoplasmic reticulum (148), interacts with the insulin and/or IGF-I receptors in the plasma membrane. One possibility is that a fraction of the PTP1B is released into the cytosol (149). However, our studies have shown no observable translocation of PTPase activity between subcellular fractions following hormone stimulation. A fraction of PTP1B might be associated with the plasma membrane instead of the ER, thus bringing the enzyme into a more advantageous location for interaction with its substrates. A final possibility is that activated endosomal receptors could be more relevant in insulin and/or IGF-I signaling than plasma membrane receptors (150, 151). Endosome-associated, activated receptors could be rapidly brought into close proximity with PTP1B at the ER. Subsequent dephosphorylation could inactivate the receptors, preventing further kinase activity (Fig. 2).

Perspectives

For years, the emphasis of studies designed to increase our understanding of growth factor–induced signaling has been primarily on the identification, characterization, and determination of the role of specific protein kinases (PK) and their substrates. The relevance of protein kinases has been underscored by the large number of PK genes discovered in the eukaryotic genome. The most recent estimate places the number of human PKs at as many as 2000 (152).

Only recently has some of the focus shifted to the study of phosphatases and their role in regulating signal transduction pathways, especially *via* the regulation of protein kinase activities. Given the large number of PKs, it is conceivable that a comparable number of protein phosphatases exist. Although the proportion of all protein phosphatases

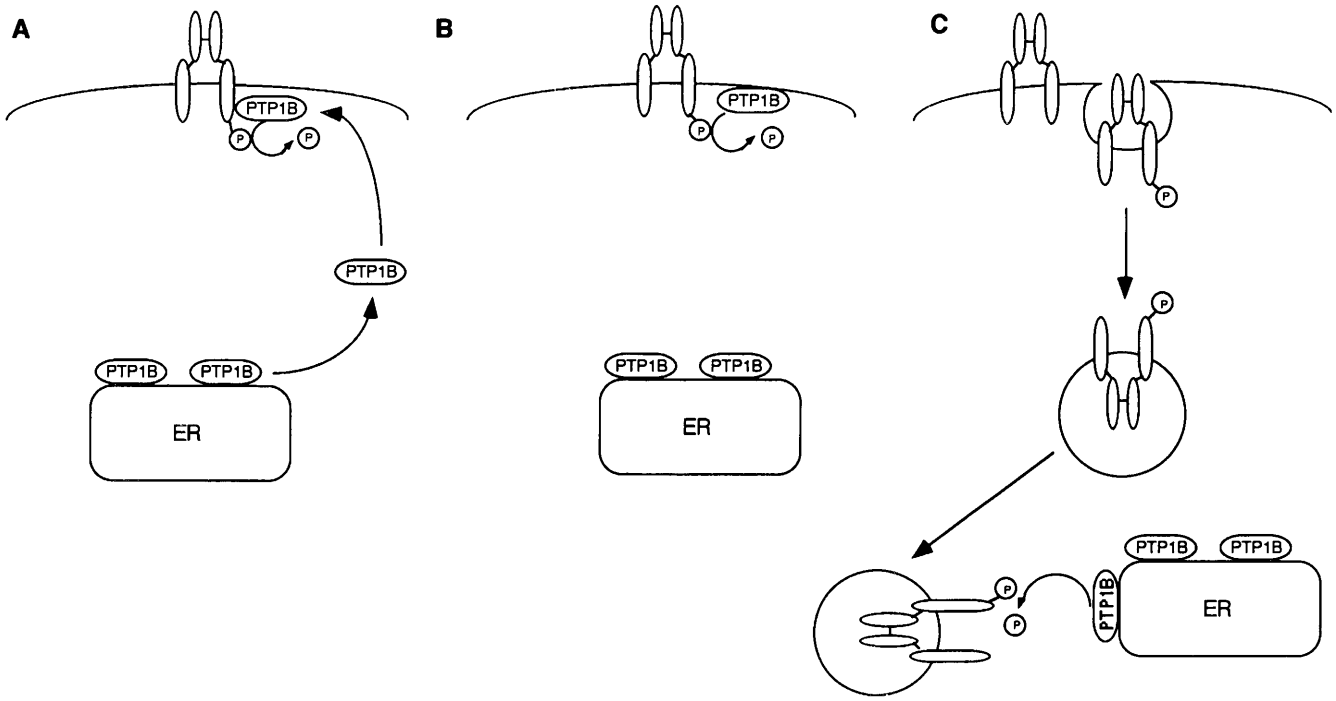

Figure 2. Various models of PTP1B interaction with the insulin receptor. (A) A fraction of the PTP1B normally associated with the endoplasmic reticulum (ER) gets released into the cytosol. (B) There might exist a fraction of the PTP1B population that associates with the plasma membrane, and it is this plasma membrane–associated fraction that is responsible for dephosphorylating the activated insulin receptor. (C) Endosome-associated receptors could be rapidly brought into close proximity with ER-associated PTP1B.

that are protein tyrosine phosphatases (PTPases) is not yet known, given the importance of tyrosine phosphorylation in signaling it is possible that PTPases could make up a significant proportion of this population.

The large body of work accomplished thus far reveals a common theme among the PTPases. Many of these enzymes can act as either positive or negative regulators of signaling, the outcome dependent upon the specific signaling context. Contributing factors may include cell/tissue type and the identity of the receptor activated, and will most probably include subcellular localization. Compartmentalization may be a mechanism for achieving substrate specificity, wherein receptors, receptor targets, and downstream signaling molecules are localized *via* a combination of targeting domains and protein-protein interactions, thus allowing for specific translocation of signaling components either within the cytoplasm or through regulated membrane vesicle movement.

Much work still needs to be done in the field of PTPase research, in particular in the determination of additional substrates, mechanisms of substrate specificity, and mechanisms of PTPase regulation. With the evergrowing complexity of signaling networks, the role of PTPases in regulating the proper transmission of information becomes more prominent. Further investigations into the physiological function of PTPases, and the consequences of their dysregulation or improper function, remain exciting and challenging avenues of exploration.

We apologize to our many colleagues whose contributions were omitted from this review article due to space constraints.

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