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Protein-Tyrosine Phosphatases and the Regulation of Insulin Action

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Abstract Protein-tyrosine phosphatases (PTPases) play an important role in the regulation of insulin action by dephosphorylating the active (autophosphorylated) form of the insulin receptor and attenuating its tyrosine kinase activity. PTPases can also modulate post-receptor signalling by catalyzing the dephosphorylation of cellular substrates of the insulin receptor kinase. Dramatic advances have recently been made in our understanding of PTPases as an extensive family of transmembrane and intracellular proteins that are involved in a number of pathways of cellular signal transduction. Identification of the PTPase(s) which act on various components of the insulin action cascade will not only enhance our understanding of insulin signalling but will also clarify the potential involvement of PTPases in the pathophysiology of insulin-resistant disease states. This brief review provides a summary of reversible tyrosine phosphorlyation events in insulin action and available data on candidate PTPases in liver and skeletal muscle that may be involved in the regulation of insulin action.

Key words: protein-tyrosine phosphatases, phosphoprotein phosphatases, protein phosphorylation, insulin receptor, insulin resistance

Post-translational modification of proteins by phosphorylation on tyrosine residues is an essential regulatory mechanism for the control of a variety of specialized cellular functions. Over the past decade, there has been extensive molecular and biochemical characterization of a number of tyrosine kinases which include the receptors for insulin and other growth factors [1,2]. More recently, increasing attention has been given to protein-tyrosine phosphatases (PTPases) that reverse the phosphorylation of specific tyrosyl residues and contribute to the overall control of signal transduction through these pathways. This brief review will summarize current work on PTPases in insulin-sensitive tissues and the role they play in regulating insulin action.

INSULIN ACTION AND TYROSINE PHOSPHORYLATION

Insulin initiates its pleiotropic effects on cellular growth and metabolism by binding to a specific tetrameric plasma membrane receptor which encodes a tyrosine-specific protein kinase [3]. Insulin binds to the receptor α-subunit which elicits a rapid autophosphorylation of specific tyrosine residues in the cytoplasmic domain of the β-subunit [4–7]. This is followed by activation the receptor kinase activity which has been shown to play a key role in signal transduction by insulin [3,4].

Insulin-stimulated autophosphorylation of its receptor occurs as a sequential cascade involving both the receptor kinase domain and the C-terminus [5–8]. Phosphorylation of two tyrosines in the kinase domain, involving tyr-1146[1] and either tyr-1150 or 1151 occurs first; this is rapidly followed by modification of the third tyrosyl residue in this region as well as the two tyrosines at C-terminal positions 1316 and 1322. Additional residues at the juxtamembrane region may also be slowly phosphorylated [8,9]. The 2Tyr(P) form of the receptor that forms initially is associated with minimal activation of

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[1]Amino acids are numbered according to the human insulin proreceptor cDNA sequence published in *Nature* 313:756–761, 1985.

its kinase activity, whereas phosphorylation to the 3Tyr(P) form of this "regulatory domain" leads to full activation of the insulin receptor kinase towards exogenous substrates (Fig. 1A) [5,7]. While the phosphorylation of tyrosines in the C-terminal region does not influence activation of the receptor kinase, modification of this domain may be involved in the regulation of certain distal effects of insulin [10–13].

A number of cellular proteins have now been shown to be potential substrates for tyrosine phosphorylation by the insulin receptor kinase in vivo that have a potential role in distal signalling [see 9 for a review]. The recent cloning of one of these substrates, IRS-1 (pp185), has provided a paradigm for transmission of a post-receptor insulin signal by reversible tyrosine phosphorylation [14,15]. IRS-1 contains over ten potential sites of tyrosine phosphorylation, six of which are in a Y-M-X-M motif. Although IRS-1 apparently lacks intrinsic enzymatic activity, in response to insulin stimulation of cultured cells, IRS-1 is phosphorylated on tyrosine residues and becomes physically associated with cellular phosphatidylinositol 3'-kinase (PtdIns-3k) activity. This occurs through binding of phosphotyrosine motifs in IRS-1 with *src* homology 2 or 3 (SH2/SH3) domains of the noncatalytic subunit of the PtdIns-3k, which has been described for other signalling systems [16–18]. These interactions also lead to activation of PtdIns-3K enzyme activity, which may be a factor in the regulation of cell growth by insulin [16,19]. A number of additional signal transduction proteins with SH2/SH3 domains, such as phospholipase C-γ and the *ras*-GTPase activating protein (GAP), will noncovalently associate with phosphorylated Y-M-X-M motifs on other proteins. Thus, following rapid phosphorylation by the insulin receptor kinase, IRS-1 may act as an

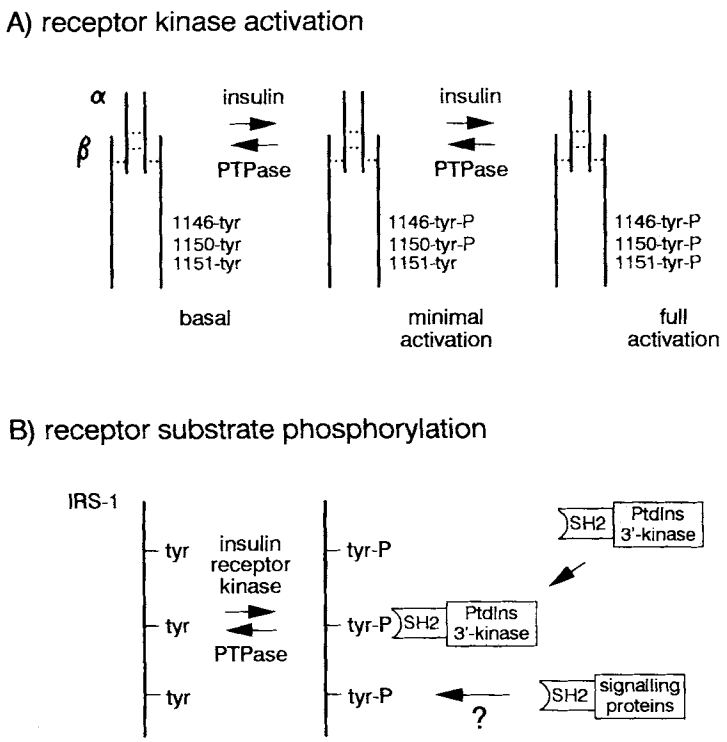

Fig. 1. Regulation of insulin signal transduction by reversible tyrosine phosphorylation: sites of action of cellular PTPases. **A:** Receptor kinase activation. Insulin-stimulated receptor autophosphorylation of one or two tyrosines in the regulatory domain (tyr-1146 and either tyr-1150 or 1151) is associated with minimal activation of the receptor kinase; modification of the third tyrosine residue in this region leads to full activation of the receptor kinase towards exogenous substrates [5,7]. The steady-state phosphorylation of these residues and the activation state of the receptor kinase are regulated by a balance between insulin-stimulated receptor autophosphorylation and receptor dephosphorylation catalyzed by PTPases. **B:** Receptor substrate phosphorylation. The phosphorylation state of specific tyrosine residues of the insulin receptor substrate IRS-1, for example, is determined by a balance between the kinase activity of the receptor and the action of cellular PTPases. These modifications regulate the association between IRS-1 and the phosphatidylinositol (PtdIns) 3'-kinase enzyme and possibly between IRS-1 and other cellular proteins, which leads to their activation and involvement in signalling to distal pathways in the cell.

adapter or "docking" protein for binding and activating a variety of SH2/SH3 domain-containing signalling proteins (Fig. 1B). This model provides some insight into how phosphotyrosine signals from the insulin receptor might diverge even from a single substrate protein to multiple pathways that ultimately lead to its pleiotropic effects on cellular metabolism, although additional substrates are most likely involved in mediating insulin effects in various cell types.

This scheme of insulin receptor and substrate protein activation provides specific potential sites of action for cellular PTPases (Fig. 1). Enzymes that reverse the insulin-stimulated autophosphorylation of specific tyrosines of the insulin receptor, especially those in the regulatory domain, will closely balance activation of the receptor kinase and play a key role in modulating insulin signalling to post-receptor pathways. The interaction of the receptor with other cytoplasmic proteins, induced by conformational changes in the autophosphorylated receptor, will also be affected by the action of PTPases. Furthermore, reversal of substrate protein tyrosine phosphorylation by PTPases will regulate their activation of distal signalling molecules.

DEPHOSPHORYLATION IS AN ESSENTIAL COMPONENT OF THE REGULATION OF INSULIN SIGNALLING

Close regulation of insulin receptor autophosphorylation in vivo has been demonstrated in intact cells, where, after stimulation with insulin, the receptor regulatory domain is frequently found in the mono(P) or 2tyr(P) form [5,20,21]. This is in contrast to stimulation of purified receptors in vitro where autophosphorylation proceeds to the 3tyr(P) form of the regulatory domain and the receptors become fully activated [5,20]. These data suggest that the 3tyr(P) regulatory region might be a preferential substrate for dephosphorylation in intact cells and that the action of one or more PTPases might be to limit progression of the receptor autophosphorylation cascade and attenuate full activation of the receptor kinase. Consistent with this hypothesis, the 3tyr(P) form of the regulatory region of isolated insulin receptors was shown to be dephosphorylated three to ten times more rapidly than the 2tyr(P) form by PTPase activity in an extract of rat liver [22]. The initial dephosphorylation, which involved either tyr-1150 or 1151 in the 3tyr(P) regulatory domain, was also closely correlated with deactivation of the receptor kinase activity [23], providing further evidence that this discrete dephosphorylation event may have a pivotal role in the regulation of receptor activation.

Dephosphorylation of insulin receptor substrate proteins is also highly regulated. In liver, for example, pp185 protein is phosphorylated within seconds following stimulation of the receptor by insulin. This phosphorylation is transient, however, and the dephosphorylation of pp185 also occurs rapidly despite the continued presence of insulin and receptor activation [14,24]. Recently, two PTPases have been characterized in 3T3-L1 cells that catalyze the dephosphorylation of pp15, a putative mediator of insulin signalling that also has a rapid turnover of its tyrosine phosphorylation [25]. Cellular PTPase activity against pp185 has not yet been described.

Following insulin stimulation in vitro, highly purified insulin receptors retain their autophosphorylation state and the kinase remains activated even if insulin is removed from the ligand-binding site, indicating that the receptor phosphatase activity is extrinsic to the receptor itself [26–29]. Under some conditions, however, PTPases may co-purify with isolated insulin receptors [30,31]. In studies with cultured cells [28] or a permeabilized adipocyte model [32], dissociation of insulin from the receptor is followed by a rapid dephosphorylation of the β-subunit and deactivation of the receptor kinase, indicating that the cellular PTPase activity is closely associated with the receptor in situ.

Direct effects of excessive PTPase activity on insulin signalling in intact cells has also been demonstrated by microinjection of purified placental cytosolic PTPase (PTPase 1B) into Xenopus oocytes, which blocked insulin-stimulated S6 peptide phosphorylation and retarded insulin-induced oocyte maturation [33,34]. Conversely, the potentiation of insulin action by vanadate or pervanadate compounds, both potent PTPase inhibitors, suggests that enhancement of the phosphorylation state of the insulin receptor, or of its effector proteins, can serve to augment insulin signalling [35–37]. These studies have focused attention on identifying and characterizing the cellular PTPases that have a physiological role in regulating the insulin action pathway.

PROTEIN-TYROSINE PHOSPHATASES

Over the past few years, dramatic advances have been made in our understanding of

PTPases as an extensive family of proteins that exert both positive and negative influences on a number of pathways of cellular signal transduction and metabolism [for recent reviews see 38, 39]. PTPases were first described in 1981 [40,41] as enzymes distinct from other types of protein phosphatases by their specificity and high activity for protein phosphotyrosyl residues, as well as additional properties that include a neutral pH optimum, resistance to EDTA, and, with a few exceptions, unique inhibition by low concentrations of zinc or vanadate [42]. Early studies provided a glimpse into the complex nature of this enzyme family, demonstrating that multiple forms of PTPases occurred in both cytosolic and particulate fractions of cells [43]. A lack of *uniformity* in phosphorylated substrates used to characterize PTPases has also made it difficult to compare the results of some studies.

A major breakthrough in this field was made possible by purification and protein sequence analysis of the cytosolic placental PTPase 1B and the finding that it had high sequence homology to the tandem intracellular domains of CD45 (LCA, Leukocyte Common Antigen) [44,45]. CD45 is an abundant transmembrane protein in hematopoietic cells that had previously been cloned, but was not recognized to be a PTPase [46]. The availability of these sequences has enabled several laboratories to identify a number of additional PTPase homologs from a variety of species [39].

The catalytic domain of proteins in the mammalian PTPase superfamily consists of single or tandemly duplicated segments of approximately 260 amino acids that contains a series of highly conserved residues [47]. The cloned enzymes may be divided into two broad categories: receptor-type, which are similar to CD45 and have a general structure like a membrane receptor with a glycosylated extracellular domain, a single transmembrane segment, and one or two conserved PTPase domains; and nonreceptor-type, which are similar to PTPase 1B in that they lack a transmembrane segment and have a single PTPase domain (Fig. 2).

The family of receptor-type PTPases with tandem cytoplasmic domains now includes, in addition to CD45, LAR (for leukocyte antigen–related) and LRP (for LCA-related phosphatase; also called RPTP-α), both of which have a wide tissue distribution [47–52]. An expanding list of additional homologs that are in the early stages of characterization have also been reported [53–55]. One unique receptor-type enzyme designated PTP-β by Krueger et al. [47] has a large extracellular domain and only a single cytoplasmic PTPase repeat. The receptor-like structure of these enzymes suggests that their cytoplasmic PTPase activity may be modulated by as yet undiscovered circulating ligands. The exposed portion of several of these enzymes has structural similarity to fibronectin type III repeats and with other cell adhesion motifs, raising the possibility that their extracellular domains may have a role in homotypic cell-cell interactions or with signals derived from the extracellular matrix [39,47].

To date, the nonreceptor-type PTPases incorporates three subgroups: one consists of PTPase 1B [56,57] and a closely related enzyme isolated from a T-cell cDNA library [58]; another subgroup is comprises two enzymes named PTPH1 and MEG [59,60]; the third subgroup contains a unique enzyme termed PTP1C [61]. Interestingly, the full-length forms of PTPase 1B and the T-cell PTPase have a C-terminal segment, downstream from the PTPase domain, that apparently directs the association of the native proteins with the particulate fraction of cells either through a hydrophobic domain or by attachment to a noncatalytic subunit [62–65]. During the isolation of these enzymes by subcellular fractionation, or after mild trypsin treatment of a particulate fraction, a truncated, soluble form can be released that has enhanced PTPase activity and altered specificity for artificial substrates [62,66]. It is still unresolved whether proteolytic cleavage and potential activation of a soluble catalytic domain of PTPase 1B and the T-cell PTPase is an artifact of tissue disruption or a physiological regulatory event.

The other nonreceptor PTPases also have fascinating structural properties. PTPH1 and MEG have a unique segment, located toward the N-terminus from the single PTPase domain, that has homology to proteins associated with the cytoskeleton, such as band 4.1 and ezrin [59,60]. Analogous to the C-terminal domains of the T-cell PTPase and PTPase 1B, the unique domains of PTPH1 and MEG may be important in their subcellular localization to certain intracellular compartments involved in phosphorylation events that influence cytoskeletal organization. The recently cloned PTP1C enzyme has two SH2 domains located toward the N-terminus from the single PTPase domain that may direct its association with certain phosphoty-

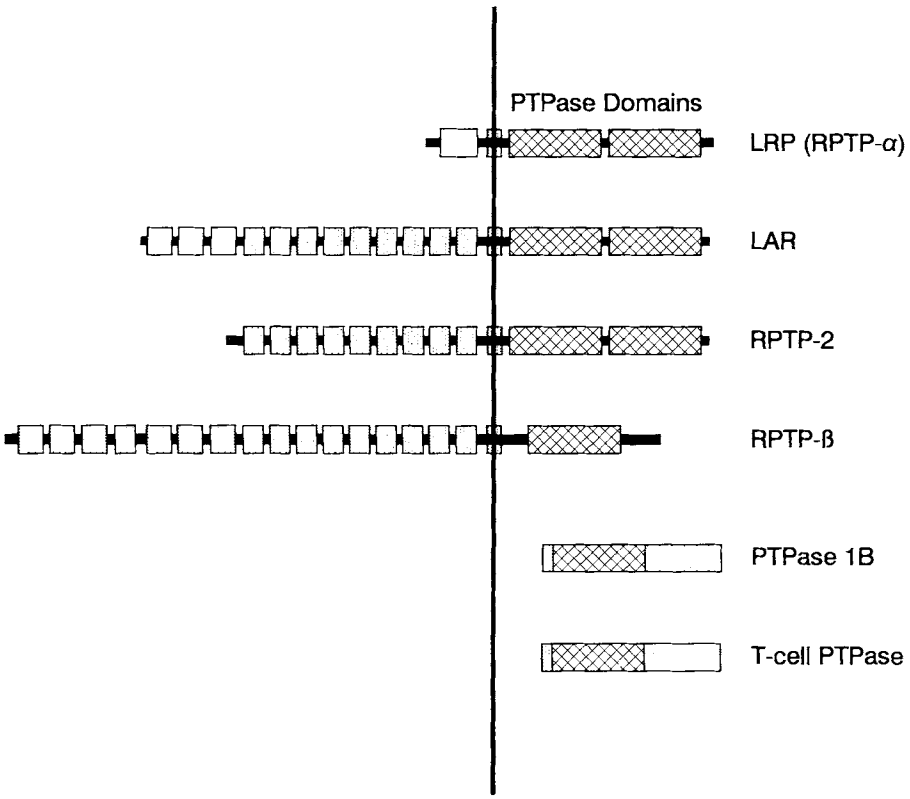

Fig. 2. Schematic structures of PTPase homologs that have been found to be expressed in normal liver and skeletal muscle. The plasma membrane is indicated by the vertical line. Single or tandem PTPase catalytic domains are shown by the cross-hatched segments in the intracellular region; the stippled extracellular segments indicate domains with homology to cell adhesion motifs that are found in several transmembrane PTPases.

rosine-containing proteins in the cell and modulate its substrate specificity [61].

IDENTIFICATION OF PTPASES THAT POTENTIALLY REGULATE INSULIN ACTION

The challenge in this area of research is to determine which PTPase enzymes might have a role in the regulation of reversible tyrosine phosphorylation events in the insulin action pathway. From the available data, several factors to be considered would include the potential specificity of individual PTPases for the autophosphorylated insulin receptor or its cellular substrate proteins, expression of the PTPases in insulin-sensitive tissues, and subcellular localization of the enzymes, which may affect their access to particular substrates.

Substrate Specificity

Characterization of the enzymes that dephosphorylate the insulin receptor in extracts of a number of tissues has shown that they are most likely members of the newly described PTPase family, since they are specific for protein-tyrosine residues and are biochemically distinct from other protein phosphatases including acid, alkaline, or calmodulin-activated protein phosphatase [67–70]. The potential role of other types of multifunctional (serine/tyrosine) phosphatases in insulin receptor dephosphorylation remains to be determined [71,72].

Since it has been difficult to purify PTPases from tissue extracts to homogeneity, recent studies have used cDNA expression in heterologous systems to characterize the potential substrate preferences of individual cloned enzymes. One of the major findings from these studies is that while maintaining specificity for protein-tyrosine moieties, in general, these enzymes exhibit only relative substrate preferences and in vitro will dephosphorylate a number of protein and peptide substrates, albeit with different kinetic paramemters [47,66,73–77]. Several PTPases have been found to be active against

the autophosphorylated insulin receptor in vitro, including purified CD45 and PTPase 1B [73,76] and the catalytic domains of several PTPases expressed in a recombinant bacterial system, including CD45, LAR, and RPTP-2, a transmembrane PTPase closely related to LAR and human RPTP-δ [51,78,79]. Since many of these PTPases will also dephosphorylate EGF receptors and other substrates, however, the insulin receptor interaction is not specific [51,73,77,79]. Any of these PTPase homologs thus fulfills the necessary criterion of being able to dephosphorylate the insulin receptor in vitro, and might potentially act as an insulin receptor PTPase in intact cells.

The lack of substrate specificity that PTPases exhibit in vitro, however, may well be a consequence of the type of experimental system used in these studies. This is reminiscent of the ability of protein kinases to phosphorylate a variety of substrates in vitro that may not represent physiological substrates in living cells, where the individual kinases phosphorylate a limited set of characteristic substrates. PTPases might act in a similar fashion and exhibit substrate specificity in vivo that can be determined by factors such as steric considerations, subcellular localization, or the action of inhibitors or other associated regulatory proteins. For those enzymes in which only the catalytic domain has been studied, it is possible that the transmembrane and extracellular segments of the holoenzymes influence conformation and substrate preferences of the cytoplasmic PTPase domain.

Potential substrate specificity for PTPases in vivo has been suggested by a few reports in the literature. The best studied example is that of CD45, which, in contrast to its relative lack of specificity in vitro, has been implicated in the activation of the $p56^{lck}$ kinase by the specific dephosphorylation of tyr-505 in vivo [80,81]. Coclustering of CD45 and CD4 with monoclonal antibodies also leads to dephosphorylation and activation of $p56^{lck}$ in intact cells [82]. However, in similar experiments, CD45 did not promote dephosphorylation of EGF receptors after antibody-induced cross-linking at the cell membrane [83]. In other studies, potential substrate specificity was exhibited by the T-cell PTPase after transfection into baby hamster kidney cells where it appeared to accelerate the dephosphorylation of endogenous substrates of the PDGF receptor without affecting the autophosphorylation of the receptor itself [62].

As an alternative hypothesis to a close coupling between individual PTPases and specific substrates in vivo, the presence of multiple PTPase enzymes with broad-based substrate preferences might provide the cell with redundant PTPase activity. This would doubly insure that reversible protein-tyrosine phosphorylations in the cell that critically influence cellular growth and metabolism are rapidly regulated. The occurrence of multiple PTPases with similar structural features in a single tissue, for example LAR and RPTP-2 in liver [78], or PTPase 1B and the T-cell PTPase in several tissues [51,56–58], suggests that reduplicative phosphatase activity may indeed occur.

In vivo expression studies of full-length PTPase cDNAs in appropriate cell types may be the best way to more directly assess their role in specific regulatory pathways and will provide important information regarding their potential substrate specificity in intact cells. Clearly, the more specific the interaction is between an individual PTPase and its physiological substrate(s), the more amenable it will be to successful pharmacological intervention for a particular signalling pathway.

Tissue Expression of PTPases

The recent cloning of a host of PTPase cDNA sequences has outpaced investigations into their potential physiological roles, especially in tissues responsible for normal metabolic homeostasis. Since defective postprandial glucose disposal in skeletal muscle and increased glucose output from the liver are fundamental to the pathophysiology of insulin resistance in noninsulin–dependent diabetes mellitus [84], these two tissues are the most appropriate candidates to investigate cellular mechanisms that regulate insulin action. The tissue distribution of some PTPases, such as CD45, is restricted to certain cell types and this may be an important factor that, in part, determines their specialized physiological roles. Other PTPases appear to have a wider tissue distribution and may be involved in regulating "housekeeping" functions that are essential in many cell types. At this early stage, however, the relative expression of many of the recently cloned mammalian PTPases in a panel of normal tissues has not been reported. Identification of which PTPase homologs are expressed in liver and muscle is necessary to pro-

vide at least circumstantial evidence for a potential role in insulin action.

Liver tissue is a rich source of PTPase activity and multiple enzymes with M_r 25–400 kDa have been described in particulate and cytosolic liver fractions that will dephosphorylate the insulin receptor [67–69,85]. PTPase 1B was recently identified among these enzymes in the membrane fraction of liver by immunoblotting with a specific anti-peptide antibody [67]. Molecular studies have provided some insight into which PTPase homologs might be present in liver. Messenger RNAs for several widely expressed PTPases, including PTPase 1B, LAR, and LRP (RPTP-α), have been found in normal liver by Northern analysis and cDNA library screening [49–51,57,86]. The abundance of cDNA inserts for LAR and the insulin receptor was identical in multiple screenings of a rat liver cDNA library (seven per million), suggesting that the encoded proteins might have a similar abundance in this tissue [51]. We have also found that two additional PTPases, the T-cell PTPase and RPTP-2, are also expressed in liver, although they are less abundant than the enzymes mentioned above [51,78]. In agreement with these findings, polymerase chain reaction (PCR) amplification of human liver cDNA with primers derived from conserved residues in the PTPase domain also revealed sequences corresponding to LRP, LAR, PTPase 1B, and the T-cell PTPase [54]. The PCR studies identified cDNAs encoding RPTP-β, CD45, and four novel putative PTPase sequences. Further studies are needed to confirm and quantitate the abundance of these latter PTPases in liver. Especially in the case of CD45, the sensitivity of cDNA amplification may have detected its mRNA from lymphocytes in the hepatic blood pool, rather than from expression in the hepatocytes themselves [52].

Studies on PTPase homologs in skeletal muscle are limited. When compared to liver, muscle extracts have been reported to have one-tenth the PTPase activity against the autophosphorylated insulin receptor [69]. By a combination of molecular cloning techniques, we recently demonstrated that LAR and LRP (RPTP-α) are expressed in rat skeletal muscle [52]. Northern blot analysis with appropriate cDNA probes has also demonstrated the expression of mRNA for both PTPase 1B (unpublished observations) and, at a lower abundance, the T-cell PTPase in skeletal muscle [51].

The common expression of specific PTPases in liver and muscle has identified candidate enzymes that may ultimately be shown to play a role in insulin signalling (Table I). Further work will require characterizing additional PTPases that occur in these tissues, either from the group of PTPases that have already been cloned or perhaps through the discovery of novel enzymes. In addition, the availability of antibodies to specific PTPases will help identify the enzymes in these tissues and provide supporting data for the molecular studies.

Subcellular Localization

In fractionated liver tissue, the bulk of PTPase activity toward insulin receptor dephosphorylation is recovered in a particulate fraction [68] with the highest specific PTPase activity present in a glycoprotein extract of solubilized rat liver membranes [51]. In permeabilized adipocytes, the in situ insulin receptor is rapidly dephosphorylated, also suggesting that the receptor PTPase activity is either an integral membrane protein or is tightly associated with a membrane or cytoskeletal compartment in the cells [32]. As discussed above, however, the actual subcellular localization of many of the cloned PTPases may be difficult to predict from structural data alone. Furthermore, many transmembrane proteins, including the insulin receptor and CD45, are found in intracellular organelles as well as the plasma membrane of the cell. The distribution of intracellular CD45, which represents ~30% of the total in the cell, also appears to be highly regulated and is rapidly cleared from the Golgi fraction to a separate particulate

TABLE I. Messenger RNA Expression of Mammalian PTPase Homologs in Insulin-Sensitive Tissues*

Liver	Skeletal Muscle
LAR	LAR
LRP (RPTP-α)	LRP (RPTP-α)
PTPase 1B	PTPase 1B
RPTP-β	
[RPTP-2][a]	[T-cell PTPase]
[T-cell PTPase]	

*For references, see text.

[a]Enzymes shown in brackets are expressed at a relatively lower abundance compared to the other PTPases listed for that tissue.

compartment during activation of a T-cell hybridoma [87]. This dynamic redistribution may enable it to have access to additional substrates that are involved in regulation of the T-cell activation cascade. The subcellular localization of the nonreceptor-type enzymes is apparently directed by unique protein segments flanking the PTPase catalytic domain, as discussed above. Both the receptor-type and the nonreceptor-type enzymes, therefore, appear to have a complex intracellular itinerary that may be important in determining their activity and proximity to intracellular substrates, including the insulin receptor. Further studies are needed to characterize the intracellular localization of PTPase homologs in vivo and define more clearly their potential role in the regulation of specific signalling pathways.

Given the data available to date regarding enzyme activity, tissue expression and subcellular localization, PTPase 1B, LAR, and LRP are potential candidates for insulin receptor dephosphorylation at the plasma membrane. After insulin binding, as the receptor is internalized to other sites in intact cells, it is possible that cytosolic PTPases or PTPases associated with membranous organelles or cytoskeletal structures come into play. The same or additional intracellular PTPases may also dephosphorylate cytosolic protein substrates of the insulin receptor kinase, such as IRS-1, and modulate distal elements of the insulin action cascade.

REGULATION OF PTPASE ACTIVITIES IN INSULIN-RESISTANT STATES

Although the specific PTPases that regulate insulin action have not yet been identified, a few studies have examined alterations in tissue PTPases in insulin-resistant disease states where defects in the insulin receptor kinase activity have been observed. In two rat models of insulin-deficient diabetes, streptozotocin-diabetes and the genetically diabetic BB rat, PTPase activity in liver cytosol against a phosphopeptide derived from the insulin receptor regulatory region was increased ~twofold compared to control animals [85]. The increased PTPase activity could also be detected using phosphorylated holoreceptors and was associated with reduced receptor autophosphorylation in the streptozotocin-treated animals. Insulin treatment, however, did not affect the increased PTPase activity in the liver cytosol. A related study using alloxan-diabetic rats demonstrated that the diabetic state did not affect tissue PTPase activities measured with artificial substrates [88]. In the particulate fraction of liver, however, a modest decrease in insulin receptor PTPase activity was observed in all three diabetic rat models, although after different durations of diabetes [85,88].

In a recent study, insulin infusion rapidly produced a 25% decrease in cytosolic PTPase activity against an artificial substrate in serial skeletal muscle biopsies from insulin-sensitive human subjects, whereas insulin-resistant (but nondiabetic) subjects had a markedly blunted response [89]. The basal PTPase activity in the particulate fraction of muscle was also noted to be significantly higher in the insulin-resistant subjects. It is tempting to speculate that these interesting findings may be related to the pathogenesis of insulin resistance in human diabetes. The interpretation of these data, as well as the rodent studies described above, however, should be tempered by our poor understanding of the physiological role of the multiple PTPase activities measured in various tissue fractions, especially with artificial substrates. As we learn more about specific PTPase enzymes in liver and skeletal muscle, more precise tools will be developed that will enable us to evaluate their potential role in insulin action and insulin-resistant disease states.

PROSPECTS

PTPases catalyze the reversible tyrosine phosphorylation of the insulin receptor and its cellular substrate proteins and play a central role in the regulation of insulin action. Identification of the specific PTPase(s) that act on the various components of the insulin action pathway will enhance our understanding of normal insulin signalling and clarify the potential involvement of PTPases in the pathophysiology of insulin-resistant disease states. The recent profusion of information on PTPases has revealed that mammalian cells possess a large family of enzymes with PTPase activity that have fascinating structural features and complex physiological itineraries. The available data has suggested a few candidate enzymes that are expressed in normal liver and muscle and may ultimately be shown to be involved in the regulation of insulin action. Since studies of PTPases in vitro have, in general, provided little insight into the physiological role of individual enzymes, experiments using transfection of PTPase cDNAs, or genetic manipulations such as homologous recombination, will

likely be necessary to provide important data regarding their involvement in specific pathways of signal transduction. After a more complete understanding of these enzymes is achieved, modulating the interaction of the relevant PTPases with particular substrates in target cells might eventually provide a novel therapeutic approach to enhancing insulin action in common forms of human diabetes with insulin resistance.

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