To understand the physiological role of protein-tyrosine phosphatase 1B (PTPase 1B) in insulin and insulin-like growth factor-I (IGF-I) signaling, we established clonal cell lines overexpressing wild type or inactive mutant (C215S) PTPase 1B in cells overexpressing insulin (Hirc) or IGF-I (CIGFR) receptors. PTPase 1B overexpression in transfected cells was verified by immunoblot analysis with a monoclonal PTPase 1B antibody. Subfractionation of parental cells demonstrated that greater than 90% of PTPase 1B activity was localized in the Triton X-100-soluble particulate (P1) cell fraction. PTPase activity in the P1 fraction of cells overexpressing wild type PTPase 1B was 3-6-fold higher than parental cells but was unaltered in all fractions from C215S PTPase 1B-containing cells. The overexpression of wild type and C215S PTPase 1B had no effects on intrinsic receptor kinase activity, growth rate, or general cell morphology. The effects of PTPase 1B overexpression on cellular protein tyrosine phosphorylation were examined by anti-phosphotyrosine immunoblot analysis. No differences were apparent under basal conditions, but hormone-stimulated receptor autophosphorylation and/or insulin receptor substrate tyrosine phosphorylation were inhibited in cells overexpressing wild type PTPase 1B and increased in cells expressing mutant PTPase 1B, in comparison with parental cells. Metabolic signaling, assessed by ligand-stimulated [3H]glucose incorporation into glycogen, was also inhibited in cells overexpressing active PTPase 1B and enhanced in cells containing C215S PTPase 1B. These data strongly suggest that PTPase 1B acts as a negative regulator of insulin and IGF-I signaling.

Insulin and insulin-like growth factor-I (IGF-I) receptors belong to the type II family of receptors, which are heterotetrameric ligand-stimulated tyrosine kinases (1–3). Ligand binding results in the autophosphorylation of tyrosine residues within the β subunit of the receptor, activating receptor tyrosine kinase activity toward endogenous substrates (4). These are believed to be the crucial first steps of the signaling processes that culminate in the pleiotropic biological responses of these two associated hormones.

The reversible nature of tyrosine phosphorylation enables cells to respond rapidly to hormonal cues and, in the context of an entire organism, to maintain glycemic control in an ever-changing environment. This adaptability requires an efficient and properly controlled system of protein tyrosine dephosphorylation, in addition to well regulated tyrosine kinase activities. Within this system, the role of specific protein-tyrosine phosphatases (PTPases) is tantamount to that of protein-tyrosine kinases, yet very little is known so far about the identities of the PTases involved, their regulation, or their specific functions within these signaling cascades.

Several PTPases have been identified in major insulin-sensitive tissues, including skeletal muscle, liver, and adipose tissue. These include LAR, Syp (SHPTP-2/PTP2C), leukocyte common antigen-related phosphatase/RPTP-α, PTPase 1C, and PTPase 1B (for review see Ref. 5 and also see Refs. 6–11). For each of these PTPases additional evidence supports some involvement in insulin and/or IGF-I signaling. The transmembrane PTPase 1B has been implicated as a negative regulator of insulin action through antisense inhibition of endogenous LAR expression, which increases insulin receptor tyrosine kinase activity and insulin-dependent phosphatidylinositol 3-kinase activity in rat hepatoma cells (12). Several lines of evidence suggest that PTPase 1B acts as a positive mediator of insulin/IGF-I action. Syp and a GST-SH2 fusion protein of Syp have been shown to associate with IRS-1 in vitro (13, 14). Microinjection of an interfering Syp antibody or a Syp-GST-SH2 fusion protein into mammalian fibroblasts dramatically inhibited stimulation of DNA synthesis by insulin and IGF-I (15). In addition, the overexpression of catalytically inactive Syp in several cell lines expressing human insulin receptors inhibited multiple insulin signaling events, including c-fos reporter gene expression, and the activation of Ras and mitogen-activated protein kinases, Erk1 and Erk2 (16, 17, 18). Tyrosine-phosphorylated leukocyte common antigen-related phosphatase (RPTP-α) has been shown to associate with Grab2 in vitro and in transiently transfected cells, through a Grab2-SH2 domain (19). Grab2 is an important mediator of Ras activation in insulin-stimulated cells and, hence, could link leukocyte common antigen-related phosphatase to insulin action (20). PTPase 1C associates with autophosphorylated insulin receptors. Its subsequent phosphorylation is accompanied by increased phosphatase activity, suggesting that it might also be involved in insulin action (10).

PTPase 1B was one of the earliest PTPases identified and associated with insulin signaling. In initial studies, microinjection of Xenopus oocytes with purified placental PTPase 1B...
blocked insulin-induced S6 peptide phosphorylation and inhibited insulin-induced oocyte maturation (21, 22). Subsequent studies demonstrated that PTPase 1B is expressed at relatively high levels in insulin-sensitive tissues (5–7). In clinical studies, we recently demonstrated that skeletal muscle biopsies from patients with impaired insulin action contained decreased PTPase 1B protein in comparison with control subjects (8). We subsequently demonstrated in the rat L6 myotube cell culture system that both insulin and IGF-I increased total cellular PTPase activity in a time- and dose-dependent manner. Increased activity was due mainly, if not entirely, to increased PTPase 1B activity, following increased PTPase 1B mRNA and protein expression (23). The results of these studies led us to conclude that insulin and IGF-I regulate PTPase 1B activity and strongly suggested that PTPase 1B plays an important role in insulin and IGF-I signaling. PTPase 1B could act in a negative-feedback loop to down-regulate insulin or IGF-I signaling, or it could act as a positive signaling intermediate within the insulin and IGF-I signaling pathways.

In order to determine the nature of the regulatory role played by PTPase 1B within these signaling cascades, we developed cell lines overexpressing active PTPase 1B or an inactive mutant derivative (C215S) of PTPase 1B. We then analyzed the effects of the overexpression of these two proteins on early signaling events and on ligand-stimulated glucose incorporation into glycogen, a distal biological response. The overexpression of PTPase 1B inhibited ligand-stimulated receptor autophosphorylation, the phosphorylation of IRS proteins, and glucose incorporation into glycogen, whereas the overexpression of C215S PTPase 1B enhanced these signaling events.

**EXPERIMENTAL PROCEDURES**

**Materials—**Expression plasmids containing either wild type or C215S PTPase 1B under the control of a cytomegalovirus promoter were graciously provided by Dr. Jack Dixon (University of Michigan Medical School, Department of Biological Chemistry, Ann Arbor, MI). Monodiorinated A-14 125I-insulin, 125I-IGF-I, porcine insulin, IGF-I, and des-IGF-I were kindly provided by Lilly. U-13C6-glucose and [γ-32P]ATP (600 Ci/mmole) were obtained from DuPont NEN. Synthetic insulin receptor peptide IRP, corresponding to the major autophosphorylation sites of the human insulin receptor, 32P-TIDYET-DYRYK (numbering system of Ullrich et al. (24)), was synthesized at the Peptide Synthesis Core Facility at the University of California, San Diego, CA. Fetal calf serum (FCS), cell culture media, phosphate-buffered saline (PBS), penicillin, glutamaze, and Lipofectamine were purchased from Life Technologies, Inc. Custom ATV solution was from Irvine Scientific (Santa Ana, CA). Bovine serum albumin was from Boehringer Mannheim. Methotrexate and hygromycin B were purchased from Calbiochem. Mouse monodonal anti-PTPase 1B antibody (FG6–1G) and mouse monodonal anti-IGF-I receptor antibody were from Oncogene Science (Uniondale, NY). Mouse monoclonal anti-phosphotyrosine antibody (PY-20) was from Transduction Laboratories (Lexington, KY). Tween-20, protein molecular weight standards, AG 1-X2, acrylamide, and TEMED were purchased from Bio-Rad. Non-fat dry milk was from Nestle Foods Co. (Glendale, CA). Anti-mouse IgG, conjugated to horseradish peroxidase, and ECL horseradish peroxidase reaction products were from Amersham Life Sciences. Wheat germ agglutinin coupled to agarose was from Vector Laboratories, Inc. (Burlingame, CA). Silicotungstic acid was from J.T. Baker Chemical Co. Sep Pak C18 cartridges were from Waters Associates (Milford, MA). Nitrocellulose membrane was from Schleicher & Schuell. All other reagents were purchased from Sigma.

**Transfection and Selection—**Rat1 fibroblasts overexpressing human insulin receptors at a level of 2 x 106 receptors/cell (Hir-c) and derivative cell lines were propagated in Dulbecco's minimal media (F12) containing 10% FCS, 50 μg/ml gentamicin, and 500 μM methotrexate. Chinese hamster ovary cells overexpressing IGF-I receptors at a level of 5 x 106 receptors/cell (CIGFR-P) and derivative cell lines were propagated in Ham's F12 containing 10% FCS, 50 μg/ml gentamicin, and 400 μg/ml methotrexate.

Lipofectamine was used to transfect the DNA constructs into host cells, along with the selective marker for hygromycin resistance. Transfected cells were selected using hygromycin (400 μg/ml). After transfection and selection, derivative cell lines were established by clonal propagation of cells expressing high levels of wild type or C215S PTPase 1B, respectively. To retain high expression levels of the transfected proteins, derivative cell lines were propagated in the continual presence of hygromycin (400 μg/ml), unless otherwise specified. The designations given to each of the cell lines are as follows: Hir-c-P, parental Hir-c cell line; Hir-c-A, Hir-c cells overexpressing wild type PTPase 1B; Hir-c-M, Hir-c cells expressing C215S PTPase 1B; CIGFR-P, parental CIGFR cells; CIGFR-A, CIGFR cells overexpressing wild type PTPase 1B; CIGFR-M cells, CIGFR cells expressing C215S PTPase 1B.

**Measurement of Cell Growth Rate—**Cells were plated in 35-mm wells in growth media without hygromycin at a seeding density of 1 x 104 cells/well. At various times after plating, duplicate wells were trypsinized in a final volume of 1.0 ml and counted in a hemocytometer.

Establishment of Cell Growth Rates—Cells were plated in 35-mm wells in growth media without hygromycin at a seeding density of 1 x 104 cells/well. At various times after plating, duplicate wells were trypsinized in a final volume of 1.0 ml and counted in a hemocytometer.

Preparation of Whole Cell Homogenates and Subcellular Fractions—Cells were grown to confluence in growth media without hygromycin and then harvested as whole cell homogenates and fractionated into subcellular fractions as described previously (25).

**Quantitation of PTPase Activity—**In vitro PTPase activity was measured using 32P-IRP as described previously (23). Briefly, wheat germ agglutinin-purified insulin receptors (25) were preincubated with 1 μM insulin for 1 h and then overnight at 4 °C in the presence of 25 μM [γ-32P]ATP (specific activity 56 Ci/mmol). Five μM Mn2+, 2 mM IRP, and 32P-IRP (approximately 5 μCi/mmol) were added to labeled IRP was purified by sequential chromatography through AG1-X2 acetate (Bio-Rad) (26) and a C-18 Sep-Pak cartridge (27). The specific activity of the radiolabeled IRP was 7 x 106 cpm/mg IRP.

**For the dephosphorylation reaction, subcellular protein fractions (50 μg/ml) were combined with 2 μM 32P-labeled IRP in low salt buffer and incubated for 6 min at 30 °C (23). The reaction was terminated by adding a 3-fold excess volume of ice-cold 10% trichloroacetic acid. 32P released from the labeled substrate was measured after organic extraction by the method of Shacter (28). The reaction was linear with respect to time and amount of PTPase activity present until at least 40% of the 32P, had been released. All determinations were performed within the linear range. Protein concentrations were determined by the method of Bradford (29).

**Insulin Receptor Kinase Activity—**Kinase activity associated with the insulin and IGF-I receptors was determined in vitro as described previously (30). Briefly, wheat germ agglutinin-enriched receptors were quantified by insulin or IGF-I binding analysis. After overnight preincubation at 4 °C in the absence or presence of 500 ng/ml unlabeled ligand, 20 fmol of receptors were used to phosphorylate 2 mg/ml poly-(Glu-Tyr) (43) in 5 mM MnCl2, 10 mM MgCl2, 500 μM CTP, and 50 μM [γ-32P]ATP (approximately 5 μCi/mmol). The reaction was terminated by adding 50 μM ATP. 32P-labeled Glu-Tyr was quantitated by filter paper analysis. Background levels of radioactivity were assessed in the absence of peptide and were generally less than 10% of the basal activity measured in the presence of peptide.

**Cell Solubilization, Gel Electrophoresis, and Immunoblotting—**Cells were solubilized in Laemmli sample buffer (31). Insoluble particulate matter was removed by centrifugation at 14,000 x g for 5 min. Solubi- lized proteins were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and reducing agents (SDS- PAGE) and transferred to nitrocellulose.

**For immunoblot analysis, the nitrocellulose blots were immersed in Tris-buffered saline (TBS), pH 7.5, containing 5% non-fat dry milk, and 0.01% Tween 20 and then immunoblotted in the same solution containing FG6-1G (anti-PTPase 1B) or PY-20 (anti-phosphotyrosine) monoclonal antibodies, as indicated in figure legends. The blots were then rinsed and incubated in TBS, 0.01% Tween 20, containing anti-mouse IgG, conjugated to horseradish peroxidase. Immunoreactive proteins were visualized using the ECL detection system (Amersham Corp.). Blots were then stained with the entire antibody via a radiographic film detection system. Quanti- tation was performed by scanning densitometry analysis using Stratagene with Gelquest software (Strategene, San Diego, CA).

**Glucose Incorporation into Glycogen—**Cells were seeded and sus- tained in growth media without hygromycin to 60–80% confluence and then incubated overnight in media containing 0.05% FCS. Cells were
cells were comparable to those in Hirc-M and CIGFR-M cells, PTPase 1B protein expression levels in Hirc-A and CIGFR-A cell lines. Moreover, densitometry also indicated that associated with each cell fraction was then quantitated using from each of the six cell lines were fractionated into supernatant and -insoluble (P2) proteins. PTPase activity in the P1 fraction from Hirc-A cells was 3-fold higher than in Hirc-P or Hirc-M cells (p < 0.005), and in the P1 fraction from CIGFR-A cells it was 6-fold higher than in CIGFR-P or CIGFR-M cells (p < 0.005). The increases in PTPase activity in cells overexpressing wild type PTPase 1B correlated with PTPase 1B protein overexpression detected in whole cell lysates from the same cells by Western analysis (see Fig. 1). In addition these data suggested that the cellular localization of the overexpressed PTPase 1B protein is similar to that of the endogenous protein, as described in a variety of cell types (8, 23, 35, 36).

Cell Growth Rates—Cell growth rates were measured as a means of detecting any general effects of the transfection procedure or of the transfected PTPase 1B protein on cell function and viability. Growth rates were unaffected by the presence of wild type or C215S PTPase 1B protein. The doubling times were 20–24 h for all cell lines (data not shown). The cell lines also remained comparable in cell morphology and protein content (data not shown).

Receptor Number and Intrinsic Receptor Kinase Activity—The cells chosen for overexpression of wild type and C215S PTPase 1B already overexpressed human insulin (Hirc-P) or IGF-I (CIGFR-P) receptors. In order to ensure that the receptor number in each of the derivative cell lines remained comparable with that of the parental cell lines, we performed receptor binding studies. Hirc-P, Hirc-A, and Hirc-M cells all expressed approximately 200,000 receptors per cell, with affinities of 0.6, 0.9, and 0.2 nM, respectively (data not shown). The CIGFR-P, CIGFR-A, and CIGFR-M cell lines all expressed approximately 50,000 receptors per cell with receptor binding affinities of approximately 0.4 nM (data not shown).

We partially purified insulin and IGF-I receptors from each of the cell lines and measured receptor kinase activity against

Fig. 1. PTPase 1B expression in parental and derivative cell lines. Cells were grown to near-confluence and then solubilized in Laemmli's sample buffer. Cell homogenates were segregated by SDS-PAGE and immunoblotted with FG6–1G (2 μg/ml) as described under "Experimental Procedures." Arrows indicate PTPase 1B protein. Lanes: 1, Hirc-P; 2, Hirc-A; 3, Hirc-M; 4, CIGFR-P; 5, CIGFR-A; 6, CIGFR-M. This is a representative experiment, independently performed four times.

Fig. 2. PTPase activity in subcellular fractions from parental and derivative cell lines. Cells were grown to near-confluence in growth media and then homogenized and centrifuged at 14,000 × g into supernatant (S) and pellet fractions. Pellets were resolubilized in the presence of 2% Triton X-100 and fractionated into Triton X-100-soluble (P1) and -insoluble (P2) proteins. PTPase activity was measured in vitro using 32P-labeled IRP as described under "Experimental Procedures." This figure provides the mean and standard error for three experiments. *, p < 0.05 in comparison with Hirc-P and Hirc-M. **, p < 0.05 in comparison with CIGFR-P and CIGFR-M.

RESULTS

Two cell lines were chosen to assess the role of PTPase 1B: Hirc, a rat 1 fibroblast cell line overexpressing human insulin receptors at a level of 2 × 10^6/cell, and CIGFR, a Chinese hamster ovary cell line overexpressing human IGF-I receptors at a level of 5 × 10^6/cell. Effects of insulin and IGF-I are well defined in both cell lines (32, 33). Expression plasmids containing the coding sequence for either wild type PTPase 1B or an inactive mutant, in which the critical active site cysteine residue had been mutated to serine (C215S), were transfected into each of the parental cell lines. Clonal selection of hygromycin-resistant cells resulted in the establishment of cell lines overexpressing active PTPase 1B or C215S PTPase 1B, respectively.

PTPase 1B Expression—To verify the overexpression of active or C215S PTPase 1B and to choose clones with comparable PTPase 1B expression for subsequent studies, we used the monoclonal PTPase 1B antibody, FG6–1G, for Western analysis of whole cell lysates from each of the parental and derivative cell lines. As shown in Fig. 1, a 50-kDa protein, the size expected for PTPase 1B (34), was recognized by FG6–1G in both parental cell lines, Hirc-P (lane 1) and CIGFR-P (lane 4). Scanning densitometry of these data (not shown) confirmed that lysates from cells overexpressing either wild type (lanes 2 and 5) or mutant (lanes 3 and 6) PTPase 1B expressed significantly (p < 0.005) more PTPase 1B than the respective parental cell lines. Moreover, densitometry also indicated that PTPase 1B protein expression levels in Hirc-A and CIGFR-A cells were comparable to those in Hirc-M and CIGFR-M cells, respectively.

PTPase Activity—To ensure the correct subcellular localization of the overexpressed PTPase 1B, whole cell homogenates from each of the six cell lines were fractionated into supernatant (S), Triton X-100 soluble particulate (P1), and Triton X-100-insoluble particulate (P2) fractions. The PTPase activity associated with each cell fraction was then quantitated using 32P-labeled IRP.

As shown in Fig. 2, at least 90% of the total cellular PTPase activity of the two parental cell lines (Hirc-P and CIGFR-P) was associated with the Triton X-100-soluble P1 fraction. PTPase activity in all fractions from Hirc-M and CIGFR-M cells remained comparable with parental cells. For Hirc-A and CIGFR-A cells, essentially all of the overexpression of PTPase 1B activity was contained in the P1 fraction. PTPase activity in the P1 fraction from Hirc-A cells was 3-fold higher than in Hirc-P or Hirc-M cells (p < 0.005), and in the P1 fraction from CIGFR-A cells it was 6-fold higher than in CIGFR-P or CIGFR-M cells (p < 0.005). The increases in PTPase activity in cells overexpressing wild type PTPase 1B correlated with PTPase 1B protein overexpression detected in whole cell lysates from the same cells by Western analysis (see Fig. 1). In addition these data suggested that the cellular localization of the overexpressed PTPase 1B protein is similar to that of the endogenous protein, as described in a variety of cell types (8, 23, 35, 36).

Cell Growth Rates—Cell growth rates were measured as a means of detecting any general effects of the transfection procedure or of the transfected PTPase 1B protein on cell function and viability. Growth rates were unaffected by the presence of wild type or C215S PTPase 1B protein. The doubling times were 20–24 h for all cell lines (data not shown). The cell lines also remained comparable in cell morphology and protein content (data not shown).

Receptor Number and Intrinsic Receptor Kinase Activity—The cells chosen for overexpression of wild type and C215S PTPase 1B already overexpressed human insulin (Hirc-P) or IGF-I (CIGFR-P) receptors. In order to ensure that the receptor number in each of the derivative cell lines remained comparable with that of the parental cell lines, we performed receptor binding studies. Hirc-P, Hirc-A, and Hirc-M cells all expressed approximately 200,000 receptors per cell, with affinities of 0.6, 0.9, and 0.2 nM, respectively (data not shown). The CIGFR-P, CIGFR-A, and CIGFR-M cell lines all expressed approximately 50,000 receptors per cell with receptor binding affinities of approximately 0.4 nM (data not shown).

We partially purified insulin and IGF-I receptors from each of the cell lines and measured receptor kinase activity against
Protein-tyrosine Phosphatase 1B Inhibits Insulin/IGF-I Signaling

The extent and duration of insulin-stimulated receptor autophosphorylation and the phosphorylation of IRS proteins were altered in cells overexpressing C215S or wild type PTPase 1B, in comparison with the parental cell lines. Relative levels of protein tyrosine phosphorylation were quantitated densitometrically from the blots, and the results were expressed relative to the amount of phosphorylation detected under unstimulated conditions. In control experiments Hirc-P cells were stimulated with insulin for 5 min, and phosphotyrosine immunoreactivity associated with the insulin receptor β-subunit was quantitated over a range of increasing amounts of whole cell lysate protein. Band intensity increased linearly with increasing protein content for quantities of 5-200 μg of whole cell lysate per lane (linear correlation (r) = 0.942, data not shown). Receptor autophosphorylation in Hirc-P cells was sustained at 4-fold basal levels throughout the 150-min time course. Auto-phosphorylation was sustained at greater levels in Hirc-M cells (5-6-fold basal, p < 0.001 at 3 min, in comparison with Hirc-P) and was reduced in Hirc-A cells, reaching only 3-fold basal at 3 min (p < 0.05, in comparison with Hirc-P cells), and thereafter gradually decreasing.

The effects of wild type PTPase 1B overexpression on the tyrosine phosphorylation of IRS proteins were more pronounced. Hirc-A cells exhibited strikingly reduced IRS phosphorylation in response to insulin stimulation. In contrast the tyrosine phosphorylation of IRS proteins in Hirc-P and Hirc-M cells was sustained at high levels throughout the 150-min time course. In CIGFR-P cell stimulation of IRS proteins was maximal after 3 min and was retained to a lesser extent up to 150 min (Fig. 3B). Maximal stimulation in CIGFR-A cells was reduced in comparison with parental cells (p > 0.005) and returned to near basal levels within 30 min. In contrast, the phosphorylation of IRS proteins in CIGFR-M cells exceeded that of CIGFR-P cells (p < 0.01 at 3 min) and was retained longer than in either of the other two cell lines. The IGF-I-stimulated tyrosine phosphorylation of the IGF-I receptor could not be assessed in whole cell homogenates, due to the presence of co-migrating tyrosine-phosphorylated proteins. The relative increases in receptor and IRS protein tyrosine phosphorylation in response to increasing concentrations of ligand confirmed the inhibitory effect of wild type PTPase 1B overexpression (Fig. 4). Insulin-stimulated receptor autophosphorylation in Hirc-A cells was lower than in either the Hirc-P or Hirc-M cells at each insulin concentration used (Fig. 4A). The insulin-stimulated tyrosine phosphorylation of IRS pro-
Protein-tyrosine Phosphatase 1B Inhibits Insulin/IGF-I Signaling

Results

Glucose Incorporation into Glycogen—Cells were serum-deprived and then incubated 2 h in the presence of [U-14C]D-glucose and increasing concentrations of insulin (A) or IGF-I (B). After solubilization, [14C]glycogen was precipitated in 66% ethanol containing 10 mg/ml unlabeled glycogen. Radioactivity was quantitated in a beta counter. Data shown are the average and standard error for 5 (A) or 4 (B) experiments performed in duplicate. *, p < 0.05 in comparison with Hirc-P and **, p < 0.05 in comparison with CIGFR-P.

The observed decreases in ligand-stimulated receptor auto-phosphorylation and IRS protein tyrosine phosphorylation in cells overexpressing wild type PTPase 1B were not due to an irreversible alteration in intrinsic receptor tyrosine kinase activity. When insulin and IGF-I receptors from control and PTPase 1B overexpressing cell lines (Figs. 3 and 4), suggesting that PTPase 1B acts in a specific manner within the insulin and IGF-I signaling pathways. This selective antagonism of a specific subset of phosphotyrosine containing proteins may indicate an inability of PTPase 1B to gain access to other phosphoproteins. Alternatively, PTPase 1B may possess a substrate specificity that includes a select subset of tyrosine-phosphorylated proteins, including the insulin and IGF-I receptors, and/or IRS proteins. Additional tyrosine-phosphorylated signaling intermediates may also be regulated by PTPase 1B and may contribute to the effect of wild type or C215S PTPase 1B on hormone-stimulated glycogen synthesis. Future studies of specific signaling components will be required to determine the scope of PTPase 1B regulatory activities in these pathways.

Several possible mechanisms could explain the reduced ligand-stimulated receptor autophosphorylation and even more pronounced decrease in the phosphorylation of IRS proteins in cells overexpressing wild type PTPase 1B. PTPase 1B could dephosphorylate insulin and/or IGF-I receptors. The receptors each have several sites of tyrosine phosphorylation important for kinase activity, most notably, three tyrosine residues within the kinase domain of the β-subunit (40). In the presence of
excess PTPase 1B, a large subset of receptors could be completely dephosphorylated and inactivated. The remaining pool of fully active receptors may be unable to compensate for the loss, resulting in a reduction in receptor signaling. As a consequence, the ligand-stimulated phosphorylation of IRS proteins would be inhibited more fully than that of receptors. Alternatively, PTPase 1B could dephosphorylate only specific, critical receptor tyrosine residues, resulting in receptor inactivation without complete receptor dephosphorylation. The apparent effect on receptor substrate phosphorylation would be greater than on the receptor itself, since receptor kinase activity would be absent, even though some receptor phosphorylation would remain. The observed preferential dephosphorylation of insulin receptor tyrosine residues 1150 and 1151 (24) favors this hypothesis (41). As a final possibility, the overexpression of wild type PTPase 1B could result in more rapid dephosphorylation of both the hormone receptors and IRS proteins, with IRS proteins as the preferred substrates. However, this final possibility is less likely in light of recent findings by Seely et al. (2): an inactive PTPase 1B-glutathione S-transferase fusion protein precipitated activated insulin receptors but failed to precipitate IRS-1 from whole cell homogenates.

The overexpression of catalytically inactive PTPase 1B in cells results in enhanced ligand-stimulated tyrosine phosphorylation of the insulin receptor and IRS proteins. The precise mechanism for the increased tyrosine phosphorylation is not clear. C215S PTPase 1B could bind to tyrosine-phosphorylated insulin receptors and/or IRS proteins, protecting them from dephosphorylation by the endogenous PTPase 1B. Recently, Milarski et al. (42) have shown in vitro high affinity association of a glutathione S-transferase fusion protein containing C215S PTPase 1B with specific phosphotyrosine residues on the epidermal growth factor receptor and suggested that the FKVREGS sequence, a conserved Src homology 2 (SH2) domain-like sequence located 19 amino acids NH2-terminal to the catalytic site of PTPase 1B and several other mammalian PTPases, might be involved in phosphotyrosine recognition (42–44). In addition Jin et al. (45) have analyzed the crystal structure of C215S PTPase 1B complexed with high affinity peptide substrates corresponding to an autophosphorylation site of the epidermal growth factor receptor and demonstrated the structural basis for substrate recognition within the catalytic pocket of PTPase 1B (45). Amino acids surrounding the catalytic cysteine (C215) confer specificity for phosphotyrosine residues, with a preference for peptide substrates containing acidic amino acids in the P-1 to P-4 positions NH2-terminal to the phosphotyrosine residue. Both the human insulin receptor and rat liver IRS-1 contain multiple sites that would conform to this preference (24, 46).

An unresolved issue is how PTPase 1B, which is localized to the endoplasmic reticulum (36), might interact with insulin and/or IGF-I receptors in the plasma membrane or with their substrates. As one possibility, a fraction of PTPase 1B might be released into the cytosol (47). However, in our study, subcellular fractionation of total cellular PTPase activity did not concur with this possibility (see Fig. 2). Very little PTPase activity was associated with the supernatant fractions, containing cytosolic proteins, and the activity in these fractions remained virtually unchanged after overexpression of active PTPase 1B. As another possibility, a fraction of PTPase 1B might associate with cell membranes other than the endoplasmic reticulum, such as the plasma membrane, bringing the PTPase into a more advantageous location for interaction with its endogenous sub-

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