The protein-tyrosine phosphatases PTP1B and Syp have both been implicated as modulators of the mitogenic actions of insulin. However, the roles of these protein-tyrosine phosphatases in the metabolic actions of insulin are not well characterized. In this study, we directly assessed the ability of PTP1B and Syp to modulate insulin-stimulated translocation of the insulin-responsive glucose transporter GLUT4 in a physiologically relevant insulin target cell. Primary cultures of rat adipose cells were transiently transfected with either wild-type PTP1B (PTP1B-WT), wild-type Syp (Syp-WT), or the catalytically inactive mutant PTP1B-C/S or Syp-C/S. The effects of overexpression of these constructs on insulin-stimulated translocation of a co-transfected epitope-tagged GLUT4 were studied. Cells overexpressing either PTP1B-C/S or Syp-WT had insulin dose-response curves similar to those obtained with control cells expressing only epitope-tagged GLUT4. In contrast, for cells overexpressing PTP1B-WT the level of GLUT4 on the cell surface at each insulin dose (ranging from 0 to 60 nM) was significantly lower than that observed in the control cells. Interestingly, cells overexpressing the dominant inhibitory mutant Syp-C/S also had a small but statistically significant impairment in insulin responsiveness. At a maximally stimulating concentration of insulin (60 nM), cell surface epitope-tagged GLUT4 was approximately 20% less than that of the control cells. It is possible that effects from high level overexpression of Syp and PTP1B constructs may not reflect what occurs under physiological conditions. Nevertheless, our data raise the possibility that PTP1B may be a negative regulator of insulin-stimulated glucose transport, while Syp may have a small role as a positive modulator of the metabolic actions of insulin.

Insulin is an important regulator of growth and metabolism. The pleiotropic actions of insulin are initiated by the binding of insulin to its receptor and the resultant activation of intrinsic receptor tyrosine kinase activity (1). Because tyrosine kinase activity is central to insulin signaling, protein-tyrosine phosphatases (PTPases) may be important for modulating insulin signal transduction pathways (2). Although there is good evidence that PTPases regulate mitogenic actions of insulin, the roles of various PTPases in metabolic actions of insulin are not well characterized.

The ubiquitously expressed prototype nontransmembrane PTPase PTP1B was among the first PTPases to be identified, cloned, and characterized (3–8). PTP1B dephosphorylates the insulin receptor both in vitro and in intact cells (9–11). In addition, PTP1B regulates the mitogenic actions of insulin (12, 13). Interestingly, in tissue culture models an increase in the level and activity of PTP1B has been associated with insulin resistance induced by exposure to high glucose levels. In addition, the level and activity of PTP1B in human skeletal muscle is positively correlated with in vivo measures of insulin sensitivity (14–16).

Syp (also known as SH-PTP2, PTP1D, SHPTP3, or PTP2C) is a cytosolic PTPase containing two SH2 domains in addition to a catalytic phosphatase domain (17). Binding of the SH2 domains of Syp to phosphotyrosine motifs on either the insulin receptor or insulin receptor substrate-1 (IRS-1) results in activation of Syp PTPase activity (18, 19). Recently, a number of studies have shown that Syp participates in Ras and mitogen-activated protein kinase-dependent pathways as a positive modulator of mitogenic actions of insulin and other growth factors (20–23). In addition, Hausdorff et al. (24) have investigated the role of Syp in differentiated 3T3-L1 cells (tissue culture cells capable of differentiating into an adipocyte-like phenotype under appropriate conditions). They report that microinjection of either the SH2 domains of Syp or anti-Syp antibodies interfered with the mitogenic actions of insulin, but had no detectable effect on the insulin-stimulated translocation of the insulin-responsive glucose transporter GLUT4 (24).

One of the most important metabolic actions of insulin is to increase glucose transport in tissues such as muscle and fat by recruiting GLUT4 to the cell surface. Previously, we used a transient transfection system for rat adipose cells in primary culture to demonstrate roles for the insulin receptor tyrosine kinase, IRS-1, and phosphatidylinositol 3-kinase in the insulin-stimulated translocation of GLUT4 (25–28). In the present study, we used a similar approach to overexpress wild-type or catalytically inactive mutant forms of PTP1B or Syp to directly test the roles of these PTPases in modulating insulin-stimulated translocation of GLUT4 in a physiologically relevant insulin target cell. Our data suggest that PTP1B may function as a negative regulator of the metabolic actions of insulin, while Syp may mediate a small positive effect on the ability of insulin to recruit GLUT4 to the cell surface.

This paper is available on line at http://www-jbc.stanford.edu/jbc/
The amount of plasmid DNA (µg/cuvette) and the number of cuvettes used for each of the transfection experiments is shown. The experimental group co-transfected with GLUT4-HA and either PTP1B-WT, PTP1B-CS, Syp-WT, or Syp-CS was compared with the control group co-transfected with pCIS2 and GLUT4-HA. A group transfected with pCIS2 alone was used in each experiment to determine the nonspecific signal. Each individual experiment was performed on pools of cells obtained by combining the contents of the cuvettes from each group. All cells were exposed to a total DNA concentration of 6 µg/cuvette.

| Group          | Experimental constructs versus control | Number of cuvettes | GLUT4-HA | Experimental pCIS2 | µg/cuvette |
|----------------|----------------------------------------|--------------------|----------|--------------------|------------|
| Experimental   |                                        | 20                 | 2        | 4                  |            |
| Control        |                                        | 20                 | 2        | 4                  |            |
| Nonspecific    |                                        | 10                 | 6        |                    |            |

**MATERIALS AND METHODS**

**DNA Vector Constructions**—An expression vector (pCIS2) that generates high expression levels in transfected rat adipose cells (25) was used as the parent vector for subsequent constructions. The DNA coding for human GLUT4 with the influenza hemagglutinin epitope (HA1) inserted in the first exofacial loop of GLUT4 was subcloned into pCIS2 (GLUT4-HA). An XbaI/SmaI fragment containing the cDNA for human PTP1B (generous gift from Dr. Jonathan Chernoff) was ligated into XbaI/HpaI sites in the multiple cloning region of pCIS2 (PTP1B-WT).

An XbaI/SmaI fragment containing the cDNA for a catalytically inactive mutant PTP1B with a cysteine to serine substitution at position 215 (generous gift from Dr. Jonathan Chernoff) was ligated into XhoI/HpaI sites in the multiple cloning region of pCIS2 (PTP1B-CS). An XbaI/XhoI fragment containing the cDNA for human Syp (generous gift from Dr. Benjamin Neel) was ligated into the multiple cloning region of pCIS2 (Syp-WT).

An XhoI/DraI fragment containing the cDNA for a catalytically inactive mutant Syp with a cysteine to serine substitution at position 459 (generous gift from Dr. Benjamin Neel) was ligated into XhoI/HpaI sites in the multiple cloning region of pCIS2 (Syp-CS).

Milligram quantities of the plasmid DNA vectors described above were obtained using a Magic Megaprep kit (Promega). The wild-type and mutant sequences in the catalytic domain of the respective PTP1B and Syp constructs were confirmed by direct sequencing.

**Isolated Rat Adipose Cell Preparation**—Isolated adipose cells were prepared from the epididymal fat pads of male rats (170–200 g, CD strain, Charles River Breeding Laboratories, Wilmington, MA) by collagenase digestion as described (25,29).

**Electroporation**—Isolated adipose cells were transfected by electroporation as described (25–28). Cells from multiple cuvettes were pooled to obtain the necessary volume of cells for each experiment. Table 1 shows the combinations and concentrations of plasmid DNA as well as the number of cuvettes used for each of the insulin dose-response experiments.

**Assay for Cell Surface Epitope-tagged GLUT4**—20 h after electroporation, adipose cells were processed as described (26–28) and treated with insulin at final concentrations of 0, 0.024, 0.072, 0.3, or 60 nM at 37 °C for 30 min. Cell surface epitope-tagged GLUT4 was determined by using the anti-HA1 mouse monoclonal antibody 12CA5 (Boehringer Mannheim) in conjunction with [3H]-labeled sheep anti-mouse IgG as described (26–28). Cells transfected with the empty expression vector pCIS2 were used to determine nonspecific binding of the antibodies. Typically, the nonspecific binding was ~30% of the total binding to cells transfected with GLUT4-HA and maximally stimulated with insulin (26). The actual specific counts were comparable from experiment to experiment (see figure legends). The lipid weight from a 200-µl aliquot of cells was determined as described (30) and used to normalize the data for each sample.

**Immunoblotting of PTP1B, Syp, and GLUT4-HA**—Expression of recombinant PTP1B, Syp, or GLUT4-HA was confirmed by immunoblotting extracts of cells that were prepared at the same time and had undergone transfection in parallel with the cells used for the translocation assay described above. Whole cell homogenates were prepared from cells co-transfected with GLUT4-HA (2 µg/cuvette) and either pCIS2, PTP1B-WT, PTP1B-CS, Syp-WT, or Syp-CS (4 µg/cuvette). Cells from 15 cuvettes were pooled for each group. After electroporation and overnight incubation, the cells were washed once and resuspended in 3 ml of TES buffer (20 mM Tris, 1 mM EDTA, 8.73% sucrose, 10 µg/ml leupeptin, 10 µg/ml aprotinin, 10 µg/ml soybean trypsin inhibitor, 10 µg/ml bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, pH 7.4, 18 °C). The cells were homogenized by being passed through a 25-gauge needle three times and then were centrifuged for 10 min at 400 × g, 4 °C, to pellet nucleic acids and lipid material that may have been released. For detection of PTP1B and GLUT4-HA, the total membrane fraction was isolated from the whole cell homogenate by centrifuging for 30 min at 400,000 × g, 4 °C. The pellet containing the total membrane fraction was resuspended in 600 µl of TES buffer and stored at −70 °C until further processing.

**Immunodetection of PTP1B constructs**—The specific bands of the cytoplasmic protein PTP1B were detected with a polyclonal anti-PTP1B antibody (Upstate Biotechnology, Inc., Lake Placid, NY) and visualized using an antibody against rabbit IgG in conjunction with an enhanced chemiluminescent detection system (ECL, Amersham).

**Statistical Analysis**—Insulin dose-response curves were compared using multivariate analysis of variance. Paired t tests were used to compare individual points where appropriate. p values of less than 0.05 were considered statistically significant. The insulin dose-response curves were fit to the equation $y = a + b \times (x/k + h)$ using a Marquardt-Levenberg nonlinear least squares algorithm. When plotted on linear log axes, this equation gives a sigmoidal curve where the parameters are associated with the following properties: $a$ = basal response, $a + b$ = maximal response, $k$ = half-maximal dose (ED50), and $h$ = concentration of insulin.

**RESULTS**

**Effects of Overexpression of PTP1B-WT or PTP1B-CS**—To directly evaluate the role of PTP1B in insulin-stimulated translocation of GLUT4, we overexpressed either wild-type or cata-

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**Table 1**

| Group          | Experimental constructs versus control | Number of cuvettes | GLUT4-HA | Experimental pCIS2 | µg/cuvette |
|----------------|----------------------------------------|--------------------|----------|--------------------|------------|
| Experimental   |                                        | 20                 | 2        | 4                  |            |
| Control        |                                        | 20                 | 2        | 4                  |            |
| Nonspecific    |                                        | 10                 | 6        |                    |            |
nM insulin, the maximal insulin response for cells overexpressing PTP1B-WT was approximately 3-fold higher than that of the control cells transfected with the empty expression vector pCIS2. In lanes 2 and 3, comparable overexpression of the recombinant PTP1B constructs can be seen in membrane fractions from cells transfected with PTP1B-WT or PTP1B-C/S. A representative blot is shown from an experiment that was repeated independently three times.

When analyzed by multivariate analysis of variance, the difference in the two curves is statistically significant by multivariate analysis of variance (P = 72, p < 1 × 10⁻⁴). B, recruitment of epitope-tagged GLUT4 to the cell surface of cells co-transfected with either PTP1B-WT/GLUT4-HA (▲) or pCIS2/GLUT4-HA (○). Results are the means ± S.E. of six independent experiments. The actual value for the specific cell-associated radioactivity for the control group at 60 nM insulin was 1095 ± 89 cpm. The best fit curve generated from the mean data for the control group had an ED₅₀ of 0.06 nM. The best fit curve generated from the mean data for the control group had an ED₅₀ of 0.06 nM. The best fit curve generated from the mean data for the control group had an ED₅₀ of 0.14 nM. The difference in the two curves is statistically significant by multivariate analysis of variance (P = 72, p < 1 × 10⁻⁴). B, recruitment of epitope-tagged GLUT4 to the cell surface of cells co-transfected with either PTP1B-WT/GLUT4-HA (▲) or pCIS2/GLUT4-HA (○). Results are the means ± S.E. of six independent experiments. The actual value for the specific cell-associated radioactivity for the control group at 60 nM insulin was 1206 ± 230 cpm. The best fit curve generated from the mean data for the control group had an ED₅₀ of 0.06 nM. The two curves are not statistically different by multivariate analysis of variance (p > 0.14).

Effects of Overexpression of Syp-WT or Syp-C/S—We confirmed overexpression of Syp-WT and Syp-C/S by using a human-specific polyclonal anti-Syp antibody to immunoprecipitate whole cell homogenates of cells transfected with either the empty expression vector pCIS2, Syp-WT, or Syp-C/S in conjunction with immunoblotting with a monoclonal anti-Syp antibody (Fig. 4). The lanes containing extracts from cells transfected with Syp-WT or Syp-C/S when compared with the control cells (Fig. 2B).

It is possible that the lower level of cell surface GLUT4-HA we observed in cells overexpressing PTP1B-WT is due to an inhibitory effect on insulin signal transduction pathways related to GLUT4 translocation.
Cells overexpressing Syp-WT had an insulin dose-response that was repeated independently twice. GLUT4 antibody without prior immunoprecipitation with the anti-HA transfected with pCIS2 alone were immunoblotted with the anti-control for immunoblotting GLUT4, total membrane fractions from cells units were 1527, 1302, 859, 1226, and 1319 respectively). As a positive control for immunoblotting GLUT4, total membrane fractions from cells transfected with pCIS2 alone were immunoblotted with the anti-GLUT4 antibody. Cells transfected with pCIS2 alone represent a negative control for immunoblotting GLUT4-HA.

GLUT4-HA had a 3-fold increase in cell surface GLUT-HA upon maximal insulin stimulation with an ED50 of 0.06 nM. Control cells co-transfected with GLUT4-HA and either pCIS2, PTP1B-WT, PTP1B-C/S, Syp-WT, or Syp-C/S were immunoprecipitated (ippt) with the anti-HA antibody. Cells transfected with pCIS2 alone represent a negative control for immunoprecipitation since these cells do not express GLUT4-HA (lane 1). Roughly comparable levels of GLUT4-HA are seen for cells co-transfected with GLUT4-HA and either pCIS2, PTP1B-WT, PTP1B-C/S, Syp-WT, or Syp-C/S (lanes 2–6, density measurements in arbitrary units were 1527, 1302, 859, 1226, and 1319 respectively). As a positive control for immunoblotting GLUT4, total membrane fractions from cells transfected with pCIS2 alone were immunoblotted with the anti-GLUT4 antibody without prior immunoprecipitation with the anti-HA antibody (lane 7). A representative blot is shown from an experiment that was repeated independently twice.

Overexpression of recombinant Syp constructs in adipose cells. Whole cell homogenates made from cells transfected with either pCIS2, Syp-WT, or Syp-C/S were immunoprecipitated with a human-specific polyclonal anti-Syp antibody, followed by immunoblotting with a monoclonal anti-Syp antibody. A specific band (~66 kDa) representing comparable overexpression of recombinant Syp is seen in extracts of cells transfected with either Syp-WT or Syp-C/S. Endogenous rat Syp is not seen in the first lane (cells transfected with pCIS2) because the samples were immunoprecipitated with a human-specific anti-Syp antibody. A representative blot is shown from an experiment that was repeated independently twice.

To gain insight into the ability of Syp to modulate insulin-stimulated translocation of GLUT4, we tested the effects of overexpressing either Syp-WT or Syp-C/S on the ability of insulin to recruit co-transfected GLUT4-HA to the cell surface (Fig. 5). Control cells co-transfected with pCIS2 and GLUT4-HA had a 3-fold increase in cell surface GLUT-HA upon maximal insulin stimulation with an ED50 of 0.06 nM. Cells overexpressing Syp-WT had an insulin dose-response curve that was not significantly different from that of the control cells (p = 0.43) (Fig. 5A). In contrast, cells overexpressing Syp-C/S had decreased insulin responsiveness when compared with control cells (Fig. 5B). The level of cell surface GLUT4-HA for cells overexpressing Syp-C/S at the highest insulin concentration (60 nM) was approximately 20% lower than that of the control cells (p < 0.02). The actual value for the specific cell-associated radioactivity for the control group at 60 nM insulin was 966 ± 123 cpm. The level of cell surface GLUT4-HA for cells co-transfected with the Syp-C/S mutant and GLUT4-HA (Fig. 5B) was significantly different from that of the control cells (by multivariate analysis of variance, p < 0.01). In particular, the level of cell surface GLUT4-HA for cells overexpressing Syp-C/S at the highest insulin concentration (60 nM) was approximately 20% lower than that of the control cells (p < 0.02). The actual value for the specific cell-associated radioactivity for the control group at 60 nM insulin was 966 ± 123 cpm.
of overexpressing Syp-WT or Syp-C/S on the total level of GLUT4-HA in transfected cells. Thus, the difference in the insulin dose-response curve of cells overexpressing Syp-C/S is most likely due to an effect on signal transduction pathways related to GLUT4 translocation. Since Syp-C/S is known to behave in a dominant inhibitory manner, our results suggest that Syp may play a small positive role in mediating insulin-stimulated translocation of GLUT4.

**DISCUSSION**

A large family of PTPases is thought to be involved in modulating signal transduction pathways initiated by receptor tyrosine kinases such as the insulin receptor (31, 32). All known PTPases have a conserved phosphatase domain containing a cysteine residue that is critical for catalytic activity. Substitution of serine for this critical cysteine results in a catalytically inactive molecule. Particular PTPases appear to show specificity for certain receptor tyrosine kinases (11). A subgroup of PTPases (of which Syp is a member) achieve specificity via SH2 domains that interact with phosphotyrosine motifs on various signaling proteins (32). Other PTPases such as PTP1B (which is ubiquitously expressed and localized to the endoplasmic reticulum) appear to have less specificity in their interactions with various receptor tyrosine kinase signaling pathways (6, 8). Syp, PTP1B, LAR, and PTPα are among the PTPases that have been implicated as modulators of insulin action (2, 33). However, few studies have directly tested the ability of particular PTPases to modulate metabolic actions of insulin.

**Effects of Overexpression of PTP1B—**PTP1B is a good candidate to test for a role in modulating metabolic actions of insulin because it is known to dephosphorylate the insulin receptor in intact cells (9–11). In addition, using a neutralizing antibody against PTP1B, Ahmad *et al.* (13) have recently shown that PTP1B can influence insulin-stimulated phosphatidylinositol 3-kinase activity in rat hepatoma cells (13). Phosphatidylinositol 3-kinase is a necessary component of the insulin signal transduction pathway leading to the translocation of GLUT4 in adipose cells (28). Therefore, the ability of PTP1B to decrease insulin-stimulated phosphatidylinositol 3-kinase activity suggests that PTP1B may be involved with some of the metabolic actions of insulin. Furthermore, differences in the level and activity of PTP1B have been correlated with differences in both in vitro and in vivo insulin sensitivity with respect to glucose uptake (14–16).

Although only 5% of the cells are transfected in our transient system (26), we are able to study GLUT4 translocation exclusively in the transfected cells by using a co-transfected epitope-tagged GLUT4 (GLUT4-HA) as a reporter. That is, for the purpose of studying GLUT4 translocation, the tagged GLUT4 allows us to distinguish and study transfected cells without interference from non-transfected cells. Because total levels of GLUT4-HA are comparable for all groups of cells, any differences we observe in the insulin dose-response curves presumably reflect alterations in the insulin signaling pathway leading to translocation of GLUT4. In our co-transfection experiments, we used twice as much DNA for the PTPase constructs as we did for GLUT4-HA to increase the likelihood that cells transfected with GLUT4-HA were also transfected with the vector of interest. If some fraction of cells were transfected only with GLUT4-HA, our results would underestimate the differences between control and experimental groups. Based on previous studies (using an identical protocol) where we demonstrated nearly complete inhibition of insulin-stimulated translocation of GLUT4-HA by co-expressing a dominant inhibitory mutant of phosphatidylinositol 3-kinase, we believe that at least 95% of cells expressing GLUT4-HA also express the co-transfected second plasmid under our experimental conditions (28). Unfortunately, the 5% transfection efficiency of our system limits our ability to study the effects of overexpression of PTP1B-WT on functions other than GLUT4 translocation (e.g. the tyrosine phosphorylation state of the insulin receptor, IRS-1, or other substrates). However, we did demonstrate that the overexpressed PTP1B-WT is capable of dephosphorylating the exogenous substrate pNPP.

The levels of overexpression we achieved for the PTP1B constructs in transfected adipose cells are similar to what we have previously observed with other recombinant genes in our system (26, 28, 34). Overexpression of wild-type PTP1B (PTP1B-WT) had the striking effect of decreasing both the insulin sensitivity and responsiveness of insulin-stimulated translocation of GLUT4 when compared with control cells. Interestingly, overexpression of PTP1B-WT caused a decrease in the level of cell surface GLUT4 even in the absence of insulin. Previously, we presented evidence consistent with the possibility that the insulin receptor exhibits a low level of intrinsic tyrosine kinase activity even in the absence of a ligand that is capable of signaling recruitment of GLUT4 in adipose cells (26). Thus, in addition to decreasing signaling in the presence of insulin, it is possible that overexpression of PTP1B-WT is attenuating the small signal generated by uncoupled insulin receptors.

Others have shown that the catalytically inactive mutant PTP1B-C/S can still bind with high affinity to its substrates (8). However, overexpression of PTP1B-C/S in adipose cells did not significantly affect insulin-stimulated translocation of GLUT4. The effect of overexpression of PTP1B-WT on decreasing insulin-stimulated translocation of GLUT4 appears to depend specifically on the presence of PTPase activity (in addition to the ability to bind particular substrates). If endogenous PTP1B is helping to regulate GLUT4 translocation, one might expect that overexpression of PTP1B-C/S would cause an increase in cell surface GLUT4. Our observation that PTP1B-C/S had no effect on insulin-stimulated translocation of GLUT4 may be due to low levels of endogenous PTP1B in adipose cells or the existence of redundant pathways. Taken together, our data suggest that overexpressed PTP1B is capable of functioning as a negative regulator of insulin-stimulated translocation of GLUT4 in the physiologically relevant adipose cell. It is possible that the contribution of PTP1B is relatively small under normal conditions, but that under pathological conditions where levels of PTP1B are increased (14–16), PTP1B may contribute significantly to insulin-resistant states.

**Effects of Overexpression of Syp—**Although PTPases are usually predicted to be negative regulators of tyrosine kinase-dependent pathways, there is strong evidence that Syp functions as a positive mediator of the mitogenic actions of insulin and other hormones (20–23, 35). The SH2 domains of Syp interact directly with phosphotyrosine motifs on both the insulin receptor and IRS-1, resulting in activation of Syp PTPase activity. However, the phosphorylated insulin receptor does not seem to be a substrate for the Syp PTPase. The positive effects of Syp on insulin signaling are presumably due to dephosphorylation of other signaling molecules that have an inhibitory role when they are tyrosine-phosphorylated. As with the PTP1B-WT construct, we were able to demonstrate that the overexpressed Syp-WT construct was capable of dephosphorylating the substrate pNPP after insulin stimulation.

In contrast to the PTP1B-WT construct, overexpression of wild-type Syp (Syp-WT) in adipose cells did not significantly affect the ability of insulin to recruit GLUT4 to the cell surface. However, overexpression of the catalytically inactive mutant Syp-C/S resulted in a small but statistically significant impairment in insulin-stimulated translocation of GLUT4 at high
insulin concentrations. Both Syp-WT and Syp-C/S contain identical SH2 domains, and both constructs were expressed at comparable levels. Therefore, the ability of the wild-type and mutant Syp constructs to interact with phosphotyrosine motifs on the insulin receptor, IRS-1 and other proteins is presumably the same. As with PTP1B, the effects we observe on insulin-stimulated translocation of GLUT4 most likely reflect differences in the PTPase activity of the two constructs. Syp-C/S has been shown to function in a dominant inhibitory fashion for the mitogenic actions of insulin in other cell types (20–23, 35). That is, overexpression of Syp-C/S inhibits the functioning of endogenous Syp. Therefore, the decreased insulin responsiveness with respect to GLUT4 translocation observed in adipose cells overexpressing Syp-C/S is consistent with a role for Syp as a positive mediator of metabolic actions of insulin. Thus, in physiological conditions. Nevertheless, our data raise the possibility that PTP1B may be a negative regulator of insulin-stimulated glucose transport, while Syp may have a small role as a positive mediator of metabolic actions of insulin. Thus, in addition to contributing to mitogenic actions of insulin, these PTPases (and others) may play a role in insulin signal transduction pathways related to metabolic functions.

Acknowledgments—We thank Dr. G. I. Bell for supplying the human GLUT4 cDNA, Dr. C. Gorman for the pCI82 expression vector, Dr. J. Chernoff for the human PTP1B cDNA, and Dr. B. Neel for the human Syp cDNA. We also thank Dr. S. I. Taylor for thoughtful reading of this manuscript.

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