A physiologically relevant response to insulin, stimulation of prolactin promoter activity in GH4 pituitary cells, was used as an assay to study the specificity of protein-tyrosine phosphatase function. Receptor-like protein-tyrosine phosphatase α (RPTPα) blocks the effect of insulin to increase prolactin gene expression but potentiates the effects of epidermal growth factor and cAMP on prolactin promoter activity. RPTPα was the only protein-tyrosine phosphatase tested that did this. Thus, the effect of RPTPα on prolactin-chloramphenicol acetyltransferase (CAT) promoter activity is specific by two criteria.

A number of potential RPTPα targets were ruled out by finding (a) that they are not affected or (b) that they are not on the pathway to insulin-increased prolactin-CAT activity. The negative effect of RPTPα on insulin activation of the prolactin promoter is not due to reduced phosphorylation or kinase activity of the insulin receptor or to reduced phosphorylation of insulin receptor substrate-1 or Shc. Inhibitor studies suggest that insulin-increased prolactin gene expression is mediated by a Ras-like GTPase but is not mitogen-activated protein kinase dependent. Experiments with inhibitors of phosphatidylinositol 3-kinase suggest that insulin-increased prolactin-CAT expression is phosphatidylinositol 3-kinase-independent. These results suggest that RPTPα may be a physiological regulator of insulin action.

Prolactin production is abnormal in humans with diabetes (1), and studies with animal and cell culture models of diabetes suggest that a decrease in steady-state prolactin mRNA levels secondary to decreased prolactin gene expression accounts for these findings (2, 3). The GH4 pituitary tumor cells have proven invaluable for studies of prolactin gene regulation by hormones and growth factors. Physiological insulin concentrations increase the level of prolactin and prolactin mRNA production approximately 7–10-fold in GH3 cells (4). Runoff transcription experiments indicate that the predominant effect of insulin is on the transcription of the prolactin gene. The >10-fold insulin stimulation of prolactin promoter/chloramphenicol acetyltransferase (CAT) reporter plasmid expression in GH4 cells confirms this results (5).

The effects of insulin are mediated through the insulin receptor that is a tyrosine kinase. Autophosphorylation of the insulin receptor upon ligand binding activates its kinase activity. The insulin receptor then phosphorylates IRS-1 at multiple sites. This activates IRS-1 for interaction with several signaling systems that have been proposed to mediate the diverse effects of insulin (6). Recently, the adapter protein Shc has also been shown to associate with the insulin receptor through interaction of the PTB domain of Shc with phosphotyrosine 960 of the insulin receptor. Thus, Shc may also act as a mediator of insulin signaling (50).

The Ras/MAP kinase pathway appears to mediate the responses of several growth factors acting through tyrosine kinases, including insulin (7). The phosphorylation of IRS-1 by the insulin receptor promotes the association of Grb2 with IRS-1 (7). This may activate Ras by recruiting SOS, a GTP/GDP exchange protein. Several studies indicate that the activation of Ras is essential for some insulin-mediated effects (8, 9). Activated Ras then recruits Raf to the plasma membrane, apparently in association with other proteins (10). This activates the kinase activity of Raf and the phosphorylation of MAP kinase kinase (MEK-1), MAP kinase, p90rsk, and nuclear transcription factors such as Elk-1 (11). Other studies indicate that phosphorylation of Shc protein and the ensuing Grb2 recruitment may be an important mechanism of Ras activation (12).

A second insulin response pathway is activated by the association of phosphorylated IRS-1 with the SH2 domain of the 85-kDa subunit of PI 3-kinase. This association was shown to activate the enzyme. GTP-Ras was also shown to associate with and activate the catalytic subunit of PI 3-kinase. PI 3-kinase may participate in the increase in glucose transport in response to insulin (13) and in insulin-stimulated mitogenesis (14). However, the mechanism for these effects of PI 3-kinase is unknown.

Since tyrosine phosphorylation is the critical first step in signaling by insulin and other hormones and growth factors, dephosphorylation of phosphotyrosine must be of considerable physiological importance. The protein-tyrosine phosphatases (PTPases) can be divided into two classes: the receptor-like, membrane-spanning PTPases and the cytosolic PTPases. Receptor-like PTPases are characterized by an extracellular domain of variable length, a single membrane-spanning domain, and one or two catalytic domains on the intracellular portion of the molecule (15). Several candidate ligands for receptor-like
PTPases have been defined, although none has so far been shown to modulate PTPase function (16, 17). The cytosolic PTPases have only a single PTPase domain and variable N- and C-terminal extensions. It has been postulated that these sequences target the cytosolic PTPases to specific intracellular locations (18).

These studies demonstrate a specific effect of RPTPα on a physiological response to insulin, activation of prolactin promoter activity. The RPTPα effect is specific by two criteria. First, no other PTPases tested have the same effect. Second, the response to EGFR or elevated cAMP levels is not inhibited (and is actually enhanced) by RPTPα expression.

**EXPERIMENTAL PROCEDURES**

**Materials**—[^2H]ATP (300 Ci/mmol) and [^3H]chloramphenicol (50 mCi/mmol) were obtained from ICN Biochemicals Corp. [^3H]P3PO4 was from DuPont. Fast CAT®, reagents for fluorescent assay of CAT activity were purchased from Molecular Probes (Eugene, OR). Acetyl-CoA, myelin basic protein, and silicone gel plates for thin layer chromatography were obtained from Sigma. Dulbecco’s modified Eagle’s medium containing 4.5 g/liter glucose (DMEM) was from Life Technologies, Inc., and iron supplemented calf serum was obtained from HyClone Laboratories. LY294002 and PD98059 were from Calbiochem. Wortmannin was the gift of T. Payne (Sandoz, Basel, Switzerland). Papainzyme was provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, National Institutes of Health. All other reagents were of the highest purity available and were obtained from Sigma, Pierce, Behring Diagnostics, Bio-Rad, Eastman Kodak Co., Fisher, or Boehringer Mannheim.

**Antibodies**—Antibodies to MAP kinase (anti-Erk-1 and anti-Erk-2), LAR, SHP1, SHP2, and horseradish peroxidase-conjugated goat anti-mouse and donkey anti-goat secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to the T-cell PTPase was purchased from Oncogene Sciences. Antibodies directed against phosphotyrosine and Shc were the generous gift of Dr. J. Schlessinger (New York University Medical Center, New York). Anti-human insulin receptor antibody was supplied by Dr. K. Siddle (Cambridge, United Kingdom). Antibody to IRS-1 was the generous gift of M. White (Brigham and Women’s Hospital, Boston, MA). Antibody against human influenza virus hemagglutinin was purchased from Boehringer Mannheim. Antiserum against RPTPα and RPTPβ were described previously (19, 20).

**Plasmids**—The construction of pPH3CAT plasmids containing −173/+75 of prolactin 5′-flanking DNA was described (4). The human insulin expression vector, pRT31H2, was the gift of Dr. J. Whitaker (Stony Brook, NY). The T-cell PTPase expression plasmids PTP TC and PTP TC-M (21) were generously contributed by Dr. N. Tonks (Cold Spring Brook, NY). The expression plasmids for RPTPα, RPTPβ, and RPTPγ were from DuPont. Fast CAT®, reagents for fluorescent assay of CAT activity were purchased from Molecular Probes (Eugene, OR), and fluorescence intensity was measured using a FluorImager 575 (Molecular Dynamics, Sunnyvale, CA) with ImageQuant software.

Transfection efficiency was controlled for using an RSV-β-galactosidase expression plasmid. Briefly, 2 μg of RSV-β-galactosidase expression plasmid was included in all electroporations. The β-galactosidase activity in the cell lysates was determined using o-nitrophenyl-β-D-galacto-pyranoside. Transfection efficiency did not vary significantly among transfections performed at the same time. The percentage of acetylation was then corrected for minor variations in β-galactosidase activity by converting the percentage of acetylation to percentage of acetylation/A β-galactosidase activity/mg protein. The fold stimulation or inhibition was then determined.

**Immunoprecipitation and Western Immunoblot Analysis**—GH4 cells were harvested in a lysis buffer consisting of 50 mM HEPES, pH 7.5, 1% Triton X-100, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EDTA, 10% glycerol, 1 mM Na₂VO₄, 50 mM NaF, 1 mM 4-(2-aminoethyl)benzenesulfonylfluoride/HCl, and 10 μg/ml aprotinin. Protein was determined using the Bradford reagent (Bio-Rad). Immunoprecipitations were performed for 2–16 h at 4 °C in this buffer using 200 μg of protein. Samples were then incubated for an additional 2 h with protein A-agarose (1.5 mg/immunoprecipitation) and washed extensively. The immunoprecipitates were then dissolved in Laemmli sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis using 10% gels. The proteins were then transferred to nitrocellulose membranes (xon Separations) and immunoblotted using enhanced chemiluminescence (Pierce).

**MAP Kinase Assay**—GH4 cells, transfected with HA-MAP kinase, were treated with hormones for various times or were left untreated as controls. They were then washed and frozen at −70 °C. The kinase assay was performed with HA-MAP kinase immunoprecipitated with 10 μl of anti-HA. The assay was performed in a buffer containing 10 mM HEPES, pH 7.4, 50 μM [^32P]ATP, 25 μM ATP, 10 mM MgCl₂, 1 mM dithiothreitol, and 0.1 μg of myelin basic protein (27). The supernatant was electrophoresed on a 10% SDS-polyacrylamide gel.

[^32P]Labeling and Immunoprecipitation of Insulin Signaling Molecules—GH4 cells in 9-cm² tissue culture wells were incubated for 2 h with phosphate-free DMEM containing 10% dialyzed, charcoal-treated calf serum. They were then washed and incubated in phosphate-free DMEM containing 10% dialyzed, charcoal-treated calf serum with 0.5 mM[^32P]Pi. Insulin was then added, and the incubation was continued for 1 h. The cells were rapidly chilled, washed three times with ice-cold saline, and frozen at −70 °C. The cells were then harvested, and immunoprecipitation was performed as described above.

**Assay of Insulin Receptor Autophosphorylation**—The assay of the autophosphorylation activity of the insulin receptor was performed as described by Wilden et al. (28). GH4 cells were harvested in lysis buffer (see above), and insulin receptor was partially purified by affinity chromatography on wheat germ-agarose. The insulin receptor binding activity in the eluants was then assayed. Kinase assays were performed using equivalent amounts of insulin receptor binding activity. Reactions (50 μl) contained 10 mM HEPES, pH 7.4, 5 mM MnCl₂, 5 mM MgCl₂, and 0.1% Triton X-100. Reactions were incubated with or without insulin for 10 min at 22 °C, and [^32P]ATP (40 μCi/assay) was then added for an additional 20 min. The reaction was stopped by the addition of 2 × Laemmli sample buffer, and the[^32P]-labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis.

**RESULTS**

**Inhibition of Prolactin-CAT Expression by RPTPα**—GH4 cells were transfected with the prolactin-CAT reporter plasmid without or with increasing amounts of an expression vector for RPTPα. The cells were refed after 24 h, and 1 μg/ml insulin, 40 ng/ml EGF, or 0.1 mM cAMP was added to the cultures for an additional 24 h. The cells were harvested, and CAT activity was assayed to determine the effect of RPTPα on hormone-increased prolactin-CAT expression. Insulin increases prolactin-CAT expression almost 20-fold in GH4 cells transfected with only prolactin-CAT and insulin receptor (Fig. 1A). This stimulation is decreased by co-transfection of small amounts of an expression vector for RPTPα. Half-maximal inhibition of insulin-increased prolactin-CAT expression is achieved using only 0.5 μg of expression vector for RPTPα, and maximal inhibition of >90% is achieved with 15 μg or more of RPTPα. Maximal inhibition is achieved without a significant reduction in basal prolactin-CAT expression (1.30 ± 0.5%)
A similar experiment performed with RPTPα (Fig. 1B) shows no significant inhibition of insulin-, EGF- or cAMP-increased prolactin-CAT expression at any concentration of expression vector tested. Insulin increased prolactin-CAT expression 21-fold in control cells and 19-fold in cells cotransfected with 32 μg of expression vector for RPTPα. Likewise, cAMP increased prolactin-CAT expression 17-fold in control cells and 15-fold in cells cotransfected with 32 μg of RPTPα expression vector. Only the effect of EGF was significantly affected by expression of RPTPα. EGF increased prolactin-CAT expression 18-fold in control cells but only 10-fold in cells expressing RPTPα (p < 0.05). The significant reduction in the effect of EGF and data indicating that RPTPα is overexpressed in GH4 cells cotransfected with the RPTPα expression vector suggest that the lack of effect of cotransfection with RPTPα is not due to insufficient expression of RPTPα, but results from the inability of RPTPα to regulate insulin signaling events.

The Effect of RPTPα Is Specific Both for Insulin and for RPTPα—Several other PTPases were found to be without effect on insulin-increased prolactin-CAT expression. The receptor-like PTPases are classified by the structure of their extracellular domain. RPTPα is a type IV PTPase with a short glycosylated extracellular domain. RPTPβ is a receptor-type PTPase of class II and is characterized by one Ig- and several fibronectin type III-like repeats in its extracellular domain. LAR is a receptor-like PTPase of type II that has three Ig-like repeats and nine fibronectin III-like repeats in its extracellular domain (29). PTP TC, PTP TC-M, SHP1, and SHP2 are cytosolic phosphatases. The T cell PTPase PTP-TC is a 48-kDa protein that is found exclusively in the particulate fraction of cellular lysates. Truncation of this PTPase with trypsin yields a 37-kDa protein with constitutive PTPase activity. We have used a plasmid PTP TC-M that has a premature stop codon inserted into the T cell PTPase cDNA so that it produces only this 37-kDa, constitutively active form of T-cell PTPase (21). SHP1 and SHP2 are cytosolic PTPases that have two SH2 domains in their N-terminal regions. These SH2 domains restrict the potential substrates for these phosphatases. The expression of the phosphatases used was checked by Western blotting (Fig. 2A). This technique does not allow the comparison of actual amounts of protein expressed; however, the relative difference in amount of the PTPase in control versus transfected cultures can be determined. All of the phosphatases except the truncated form of the T cell phosphatase, PTP TC-M, were found to be significantly overexpressed in transfected cells, and the level of overexpression was similar in all cases. The truncated T cell PTPase was not detected. This probably results from the removal of the epitope for the monoclonal antibody that was used for detection of this protein, since expression of this protein significantly affected EGF-increased prolactin-CAT expression (see below; Fig. 2B).

In control cells, prolactin-CAT expression was increased 11-fold by insulin, 4-fold by EGF, and 8-fold by cAMP. Co-transfection with RPTPα significantly inhibited the increase in prolactin-CAT expression seen in insulin-treated cells (2-fold control levels). RPTPα significantly enhanced the ability of EGF and cAMP to increase prolactin-CAT expression, consistent with the initial dose-response study (above). EGF increased prolactin-CAT expression almost 25-fold in RPTPα-expressing cells. The increase in prolactin-CAT expression due to EGF or cAMP was not significantly different above that seen in control cultures, even using amounts of RPTPα expression vector that totally abolish the effect of insulin (50 and 150 μg data not shown). Thus, under conditions where identical amounts of enzyme are produced, RPTPα expression specifically inactivates the insulin signaling pathway, while it enhances EGF and cAMP signaling.
The effect of RPTPα to inhibit insulin-increased prolactin-CAT expression is also phosphatase-specific, since none of the other PTPases co-transfected in GH4 cells significantly reduced insulin-increased prolactin-CAT expression. Both insulin and EGF increased prolactin-CAT expression significantly more in cells expressing the T cell PTPase, PTP TC, than in control cells. Expression of the truncated TC-PTPase, PTP TC-M, increased the effect of EGF and cAMP on prolactin-CAT transcription. Finally, expression of SHP1 significantly increased the effect of EGF to activate prolactin-CAT expression. Neither SHP2 nor LAR was seen to have any effect on prolactin-CAT expression or its response to mediators.

These effects were confirmed using cells stably expressing high levels of RPTPα or RPTPκ. GH4 cells were electroporated with vectors expressing the neomycin resistance gene and either RPTPα or RPTPκ expression vectors. Clones that were resistant to G418 were selected. Fig. 3A shows levels of RPTPα and RPTPκ in control cells and in representative RPTPα- and RPTPκ-expressing clones, RPTPα23 and RPTPκ5. RPTPα levels are low in control and in the RPTPκ5 clone (Fig. 3A). High levels of RPTPα are seen only in the clone RPTPα23 that has the stably integrated RPTPα cDNA. Likewise, RPTPκ levels are low in GH4 cells and in RPTPα23 cells, while RPTPκ levels are high in the clone RPTPκ5 that has an incorporated RPTPκ cDNA.

Prolactin-CAT expression in insulin-, EGF- and cAMP-treated control cells and RPTPα- or RPTPκ-overexpressing transformants is shown in Fig. 3B. Insulin treatment results in a 10-fold increase in prolactin-CAT expression in control cells, while insulin-increased prolactin-CAT expression is 15-fold in RPTPκ-overexpressing cells. Insulin-increased prolactin-CAT expression is reduced 75%, to 2.5-fold, in GH4 cells overexpressing RPTPα. In contrast to the observations made using transient expression of RPTPα, experiments with cells stably
overexpressing RPTPa showed no effect of RPTPa on increased prolactin-CAT expression due to EGF or cAMP. This could result from a failure to select cells expressing appropriate levels of RPTPa, since it is clear that the ability of RPTPa to enhance the effects of EGF and cAMP is dose-dependent (see Fig. 1). Alternately, it may result from the long term adaptation of the stably transformed cells. Levels of basal prolactin-CAT expression are also similar in the control (1.03 ± 0.3% acetylation/10 μg of protein) and in RPTPa-expressing clone 23 (1.49 ± 0.3% acetylation/10 μg of protein) and in the RPTPa-expressing clone 5 (0.85 ± 0.15% acetylation/10 μg of protein). Results from the RPTPa-expressing clone a23 and RPTPa-expressing clone k5 are typical. Four other α-expressing clones and three other κ-expressing clones were also tested and gave identical results.

Insulin Receptor Phosphorylation in RPTPa- and RPTPκ-overexpressing Cells—Ligand binding to the insulin receptor results in autophosphorylation of several tyrosines on the insulin receptor β-subunit. Some of these phosphorylations activate the receptor kinase, while others have been shown to be important as binding sites for signaling molecules. It was possible that RPTPa rapidly dephosphorylated the insulin receptor and interfered with its signaling. Experiments done to test this hypothesis are shown in Fig. 4. The magnitude of insulin stimulation of tyrosine phosphorylation of transfected human insulin receptor in control cells and in cells also transduced with RPTPa was determined by immunoprecipitation and Western blotting with antibody to phosphotyrosine and ECL reagents (Pierce). A, control GH4 cells were transfected with 5 µg of pRT3HIR-2 alone (lanes 1 and 2) or RPTPa (lanes 3 and 4). The cells were incubated in hormone-depleted medium for 24 h. Insulin (1 µg/ml) was then added for 6 min. The medium was rapidly removed, and the cultures were washed with normal saline at 4 °C. Lysates were prepared, and immunoprecipitation was performed as described using 200 µg of lysate and a human insulin receptor-specific monoclonal antibody, 83-14 (from K. Siddle, London, UK). The immunoprecipitates were then separated on SDS-polyacrylamide gels and transferred to nitrocellulose. Western analysis was performed using a polyclonal antibody to phosphotyrosine and ECL reagents (Pierce). B, control cells (lanes 1 and 2) and cells stably expressing RPTPa (lanes 3 and 4) or RPTPκ (lanes 5 and 6) were incubated in hormone-depleted medium for 24 h. The cultures were then treated as above except that immunoprecipitation was performed using a polyclonal antibody that recognizes the insulin receptor of the rat. C, the autocatalytic activity of wheat germ agglutinin-purified insulin receptor from control cells (lanes 1 and 2) and cells stably expressing RPTPa (lanes 3 and 4) or RPTPκ (lanes 5 and 6) was determined. After partial purification of the insulin receptors from the various cell types, the insulin binding activity of the eluted insulin receptors was assayed using 125I-iodinated insulin. Equal amounts of binding activity were used for each assay. The reaction was stopped with SDS electrophoresis buffer, and the insulin receptor was resolved by electrophoresis on a 10% SDS-polyacrylamide gel. The position of migration of the insulin receptor is indicated.

**Fig. 3.** Prolactin-CAT expression in GH4 cells stably transfected with RPTPa and RPTPκ. A, cell lysates prepared from control GH4 cells (lanes 1 and 4), RPTPa-transformed GH4 cells (lanes 2 and 5), and RPTPκ-transformed GH4 cells (lanes 3 and 6) were electrophoresed on 10% SDS gels as described. The proteins were transferred to nitrocellulose membranes and Western blotted with antibody to RPTPa (lanes 1–3) or antibody against RPTPκ (lanes 4–6). The position of migration of RPTPa and RPTPκ is indicated. B, GH4 cells stably expressing RPTPa or RPTPκ and control GH4 cells were transfected with 15 µg of Prl(-173/+/75)-CAT and with 5 µg of an expression vector for the human insulin receptor, pRT3HIR2. The cultures were then incubated in hormone-depleted medium for 24 h. The medium was exchanged, and hormones were added for an additional 24 h. The cells were harvested, and CAT enzyme activity was determined. The average percentage of acetylation/10 µg of protein in control and insulin-treated cultures was determined, and the insulin incubations were compared with control levels to determine the -fold stimulation by insulin (Fold-Control). The results are from three separate experiments done in duplicate.

**Fig. 4.** Insulin receptor phosphorylation and autocatalytic activity in cells transfected with PTPases. A, control GH4 cells were transfected with 5 µg of pRT3HIR-2 alone (lanes 1 and 2) or RPTPa (lanes 3 and 4). The cells were incubated in hormone-depleted medium for 24 h. Insulin (1 µg/ml) was then added for 6 min. The medium was rapidly removed, and the cultures were washed with normal saline at 4 °C. Lysates were prepared, and immunoprecipitation was performed as described using 200 µg of lysate and a human insulin receptor-specific monoclonal antibody, 83-14 (from K. Siddle, London, UK). The immunoprecipitates were then separated on SDS-polyacrylamide gels and transferred to nitrocellulose. Western analysis was performed using a polyclonal antibody to phosphotyrosine and ECL reagents (Pierce). B, control cells (lanes 1 and 2) and cells stably expressing RPTPa (lanes 3 and 4) or RPTPκ (lanes 5 and 6) were incubated in hormone-depleted medium for 24 h. The cultures were then treated as above except that immunoprecipitation was performed using a polyclonal antibody that recognizes the insulin receptor of the rat. C, the autocatalytic activity of wheat germ agglutinin-purified insulin receptor from control cells (lanes 1 and 2) and cells stably expressing RPTPa (lanes 3 and 4) or RPTPκ (lanes 5 and 6) was determined. After partial purification of the insulin receptors from the various cell types, the insulin binding activity of the eluted insulin receptors was assayed using 125I-iodinated insulin. Equal amounts of binding activity were used for each assay. The reaction was stopped with SDS electrophoresis buffer, and the insulin receptor was resolved by electrophoresis on a 10% SDS-polyacrylamide gel. The position of migration of the insulin receptor is indicated.
tyrosine kinase cannot be ruled out by these experiments. Therefore, the catalytic activity of the insulin receptor was directly tested. Insulin increases the autokinaytic activity of the insulin receptor 4.2-fold in GH4 cells (Fig. 4C). Insulin increased the kinase activity of its receptor 4.3-fold in cells overexpressing RPTPα and 3.6-fold in cells overexpressing RPTPκ.

**IRS-1 and Shc Phosphorylation in RPTPα- and RPTPκ-overexpressing Cell Lines**—The previous experiments suggest that RPTPα does not decrease levels of insulin receptor kinase activity and that RPTPκ does not reduce the increase in insulin receptor phosphorylation in insulin-treated cells. However, it is possible that RPTPα specifically dephosphorylates a tyrosine on the insulin receptor that impacts its ability to transmit signal to downstream events. Such a condition could occur if dephosphorylation by RPTPα activates a substrate binding site. Therefore, the effect of RPTPα expression on phosphorylation of insulin receptor substrates was examined. Two substrates of the insulin receptor kinase, IRS-1 and Shc, have been shown to be intermediates for some of the actions of insulin (6). Insulin increases the phosphorylation of IRS-1 3 ± 0.25-fold in GH4 cells (Fig. 5A). Levels of IRS-1 phosphorylation in GH4 cells stably expressing RPTPα were increased 3.5 ± 0.4-fold by insulin, while in RPTPκ-overexpressing cells IRS-1 phosphorylation was increased 3.2 ± 0.15-fold by insulin. Thus, IRS-1 phosphorylation does not appear to be affected by overexpression of RPTPα or RPTPκ.

Insulin/growth factor-increased Shc phosphorylation has been linked to recruitment of Grb-2/SOS and Ras activation in some cell lines (12). The extent of Shc phosphorylation in insulin-treated cells (Fig. 5B) was determined. Shc levels are very low in GH4 cells, and only the 46-kDa form of Shc is immunoprecipitated in GH4 cells. In comparison, phosphorylation of all three forms of Shc is seen in Chinese hamster ovary cells (lanes 1–6 versus lanes 7 and 8). Insulin increased the phosphorylation of 46-kDa Shc by only 3-fold in GH4 cells versus 8-fold in Chinese hamster ovary cells. However, no difference is seen between levels of phosphorylation of this Shc isoform in control GH4 cells or in cells overexpressing RPTPκ (lanes 1 and 2 versus lanes 3 and 4).

**Dominant Negative Inhibition of Ras and Raf in GH Cells**—Dominant negative inhibitors of Ras were used to determine whether the Ras/Raf/ERK kinase signaling pathway might be involved in insulin signaling to prolactin-CAT expression. GH4 cells were cotransfected with the prolactin-CAT reporter and the human insulin receptor. Some electroporations also contained either p21S17N-Ras, a dominant negative Ras (30), or Raf-C4, which competes with wild type Raf for binding to Ras and Ras-related proteins and inhibits the downstream effects of these molecules (31). Insulin increased prolactin-CAT expression 10-fold in control cells (Fig. 6). Cotransfection of p21 S17N-Ras had no effect on insulin-increased prolactin gene expression, which was 8–9-fold at 10 μg of cotransfected inhibitor (Fig. 6A). Prolactin-CAT expression increased by cAMP was also not affected. It was 11-fold in control cells and 8–9-fold in p21 S17N-Ras-transfected cells. This does not result from failure of the p21 S17N-Ras to be expressed in GH4 cells, since EGF-increased prolactin-CAT expression was strongly inhibited by p21 S17N-Ras expression. EGF increased prolactin-CAT expression 7-fold in control GH4 cells, whereas in p21 S17N-Ras cotransfected cells EGF did not significantly increase prolactin-CAT expression.

Dominant negative Raf-C4 resulted in the almost complete inhibition of both insulin- and EGF-increased prolactin gene expression (Fig. 6A). Insulin-increased prolactin-CAT expression is reduced from 10- to 3-fold, and the EGF effect is lowered from 7- to 2.5-fold in cells expressing Raf-C4. The effect of cAMP is not diminished in these experiments. Raf-C4 acts by binding to GTPases such as Ras and competing with functional substrate. However, Raf-C4 binds to numerous non-Ras GTPases. These results imply that the effect of insulin to increase prolactin-CAT expression is Ras-independent but that it is dependent on a Ras-related GTPase. The effect of cAMP on prolactin gene expression is independent of Ras, and this agrees with previous studies that suggest that the effect of cAMP is mediated through cAMP-dependent protein kinase A (32, 33).

**Effect of Insulin on MAP Kinase Activity in GH4 Cells Expressed Inhibitors**—Insulin increases MAP kinase activity in a Ras/Raf-dependent manner in several cell lines. MAP kinase activity in cells treated for 6 min with insulin or EGF or left untreated as controls was determined (Fig. 6B). Insulin increased MAP kinase activity 10-fold in control cells. This is not affected by cotransfection with dominant negative Ras. However, MAP kinase activity was increased only 4-fold in insulin-treated cells that were cotransfected with Raf-C4. Thus, dominant negative Raf reduces the effect of insulin by 60%. Cotransfection with RPTPκ reduces the effect of insulin approximately 75%. The effect of EGF is reduced 40% by dominant negative Raf and 33% by dominant negative Ras, but it is not affected by overexpression of RPTPκ.

The effect of inhibitors on insulin- and EGF-increased MAP kinase activity was also tested in control cells cotransfected with p21 S17N-Ras and Raf-C4. Both Raf and Ras were reduced to 33% by cotransfection (30, 31).

**Effect of Insulin on MAP Kinase Activity in GH4 Cells Expressed Inhibitors**—Insulin increases MAP kinase activity in a Ras/Raf-dependent manner in several cell lines. MAP kinase activity in cells treated for 6 min with insulin or EGF or left untreated as controls was determined (Fig. 6B). Insulin increased MAP kinase activity 10-fold in control cells. This is not affected by cotransfection with dominant negative Ras. However, MAP kinase activity was increased only 4-fold in insulin-treated cells that were cotransfected with Raf-C4. Thus, dominant negative Raf reduces the effect of insulin by 60%. Cotransfection with RPTPκ reduces the effect of insulin approximately 75%. The effect of EGF is reduced 40% by dominant negative Raf and 33% by dominant negative Ras, but it is not affected by overexpression of RPTPκ.

**Effect of Insulin on MAP Kinase Activity in GH4 Cells Expressed Inhibitors**—Insulin increases MAP kinase activity in a Ras/Raf-dependent manner in several cell lines. MAP kinase activity in cells treated for 6 min with insulin or EGF or left untreated as controls was determined (Fig. 6B). Insulin increased MAP kinase activity 10-fold in control cells. This is not affected by cotransfection with dominant negative Ras. However, MAP kinase activity was increased only 4-fold in insulin-treated cells that were cotransfected with Raf-C4. Thus, dominant negative Raf reduces the effect of insulin by 60%. Cotransfection with RPTPκ reduces the effect of insulin approximately 75%. The effect of EGF is reduced 40% by dominant negative Raf and 33% by dominant negative Ras, but it is not affected by overexpression of RPTPκ.
kinase activity paralleled effects of the same inhibitors to reduce insulin- and EGF-increased prolactin-CAT expression. However, the magnitude of the changes in early stimulation of MAP kinase was not as great as the inhibition of prolactin-CAT expression. Several studies have indicated that differentiation-enhancing effects of growth factors correlate with the prolonged phase of MAP kinase activation and not with the acute, transient effects (34). Therefore, the effect of insulin or EGF to increase MAP kinase activity was determined after 2 h in control GH4 cells and in GH4 cells co-transfected with dominant negative p21 Ras, Raf-C4, or RPTPα (Fig. 6C). Insulin treatment resulted in a 3.5-fold increase in MAP kinase activity in control cells. This is not significantly different from the 3.1-fold stimulation seen in EGF-treated cultures. Expression of dominant negative Raf prevented both of these effects. MAP kinase activity in cultures expressing dominant negative Ras was increased 2-fold by insulin but was not increased by EGF. MAP kinase activity was not increased by insulin in cells co-transfected with RPTPα. EGF-increased MAP kinase activity was increased in RPTPα-co-transfected cells to 2.8-fold control. Thus, insulin- and EGF-increased steady-state activation of MAP kinase and increased prolactin-CAT expression exhibit identical responses to p21S17N Ras, to Raf-C4, and to RPTPα.

**Inhibition of MEK with PD098059**—These experiments suggested that a Ras-independent activation of MAP kinase by insulin might mediate the increase in prolactin gene expression in insulin-treated cells. The experiment shown in Fig. 7 eliminates this possibility. Incubation of GH4 cells with the inhibitor of MEK, PD098059, for 2 h before the addition of hormones eliminates the ability of insulin and cAMP to activate MAP kinase and reduces the activation of MAP kinase by EGF from 80- to 30-fold (Fig. 7A). Prolactin-CAT expression in response to these hormones is not significantly affected by PD098059 under the same conditions (Fig. 7B). Thus, insulin increased prolactin-CAT expression is MAP kinase-independent. Numerous other negative results support this conclusion. First, Raf kinase assays performed in control GH4 cells, A23 (RPTPα-expressing) cells, and in K5 (RPTPα-expressing) cells show the same level of insulin and EGF activation of both c-Raf-1 and B-Raf kinase (data not shown). Second, a dominant negative MEK and dominant negative MAP kinase do not inhibit prolactin gene transcription (data not shown). Third, constitutively active MEK does not activate prolactin gene transcription (data not shown). Fourth, a MAP kinase phosphatase does not block the action of insulin, and phosphatase-inactive MAP kinase phosphatase does not increase prolactin gene expression (data not shown).

**Effect of Inhibition of PI 3-Kinase and p70S6 Kinase on Prolactin-CAT Expression**—LY294002 and wortmannin inhibit PI 3-kinase and p70S6 kinase activity as described under “Experimental Procedures.” The phosphorylation of myelin basic protein from the different treatments was quantitated with a Molecular Dynamics PhosphorImager using ImageQuant NT software. The results of four separate experiments were averaged to provide these data.
prolactin-CAT expression 11-fold over untreated cells with or without LY294002. However, LY294002 alone causes an increase in prolactin-CAT expression over untreated cells. Thus, it is difficult to determine if the effect of LY294002 is inhibition of the insulin response or whether LY294002 and insulin are affecting the same pathway to increase prolactin-CAT expression. Thus, none of the other phosphatases tested inhibited insulin-increased transcription from the prolactin promoter.

**DISCUSSION**

Stimulation of prolactin gene transcription is a well established and physiologically relevant response to insulin (32, 35). We have used the responsiveness of the prolactin promoter to insulin and other factors as a defined assay to study the effect of PTPases on insulin signaling. The data presented document strikingly specific effects of RPTPα on insulin-dependent gene transcription. First, overexpression of RPTPα inhibits the insulin-mediated increase in prolactin promoter activity. These results are observed both in transiently and stably transfected cells. Second, the overexpression of RPTPα does not reduce either EGF- or cAMP-increased prolactin promoter activity, but, in transient expression experiments, RPTPα expression augmented the increase in prolactin-CAT expression due to EGF and CAMP. This demonstrates that the effect of RPTPα overexpression does not involve a general inhibition of growth factor or second messenger responses. Finally, none of the other phosphatases tested inhibited insulin-increased transcription from the prolactin promoter.

Others have reported studies on the important role of the LAR PTPase as a potential negative regulator of insulin signaling (36). By contrast, we did not observe any effect of LAR expression on the regulation of the prolactin promoter by insulin in GH4 cells (Fig. 2B). Thus, our data may seem to conflict with the reports on the control of insulin signaling by LAR. However, (a) distinct PTPases may regulate different insulin signaling pathways, (b) distinct PTPases may contribute to regulation of insulin signaling in different cell types, (c) more
than one PTPase may act together within the same cell, or (d) different PTPases may control insulin signaling with varying degrees of specificity (see below).

Recently, Moller et al. (37) showed that substratum detachment of insulin-treated, insulin receptor-expressing BHK cells was reduced in cells that also expressed either RPTPα or RPTPε. This was interpreted as evidence that expression of either RPTPα or RPTPε blocked insulin signaling. However, these data might reflect an effect of RPTPα or RPTPε expression on cell adhesion unrelated to a physiological insulin response, since the effect of other factors was not tested. In our assay system, the expression of RPTPα has no effect on basal or on EGF- or cAMP-increased prolactin-CAT transcription. This makes it clear that the effect of RPTPα on prolactin promoter activity is probably mediated through a direct effect on the insulin signaling pathway. This is further supported by the RPTPα inhibition of MAP kinase activation by insulin.

The insulin signaling pathway to gene expression is not currently defined. However, a number of signaling molecules have been identified that may also participate in signaling to gene transcription. Therefore, experiments were performed to determine whether expression of RPTPα alters the response of these molecules to insulin.

Phosphorylation of the insulin receptor was substantially the same in control GH4 cells and in GH4 cells expressing RPTPα, either transiently (Fig. 4A) or stably (Fig. 4B). Previously published studies (37) suggested that insulin receptor was hypophosphorylated in RPTPα-overexpressing cells. However, in that study, both the insulin receptor and RPTPα were highly overexpressed using the 293 expression system. It is possible that, under such conditions, less specific events contribute to the phenomena observed. This is suggested by the fact that transient insulin receptor expression induced ligand-independent phosphorylation of the receptor and that PTPase co-expression also reduced these levels of basal phosphorylation of the receptor. Nonspecificity is also suggested by the fact that, in the latter study, similar degrees of insulin receptor dephosphorylation were induced by CD45 and RPTPα, yet only the RPTPα protein abolished the antiadhesive effect of insulin (37).

Data from in vivo studies suggest that precise control of phosphorylation of the insulin receptor regulatory region is important (38). Since there are multiple sites of insulin receptor phosphorylation, it remains possible that critical tyrosines on the insulin receptor are specifically targeted by RPTPα, whose dephosphorylation might have gone undetected in the experiment shown in Fig. 4, A and B. However, it is unlikely that RPTPα blocks insulin action through selective dephosphorylation of insulin receptor tyrosines, since the kinase activity of the insulin receptor was not significantly changed by the expression of RPTPα (Fig. 4C). Likewise, no effect of RPTPα was seen when insulin-increased phosphorylation of IRS-1 and Shc was examined. However, these studies cannot eliminate an effect on specific phosphorylation sites of these molecules. Further, it is also possible that Shc and IRS-1 are not in the insulin signaling pathway to prolactin. Recently, a new insulin signaling intermediary, IRS-2, was found in IRS-1 knockout mice (39). Thus, it remains possible that this or another, as yet unknown, intermediary mediates insulin signaling to prolactin gene expression.

The process that RPTPα influences probably occurs in association with or close to the cell membrane. The small GTPases such as Ras, Rac, and Rap are membrane-anchored and might be targets for inactivation by RPTPα, or RPTPε might block activation of an insulin-responsive GTPase. This could then result in inactivation of downstream effectors such as Raf/MEK/MAP kinase, PI 3-kinase, or p70S6 kinase. Therefore, experiments were done to determine if any of these signaling pathways were essential for the response to insulin and were altered in RPTPα overexpressing cells.

The experiments using dominant negative Ras and Raf indicate that EGF-increased prolactin-CAT expression is Ras-mediated, while insulin-increased prolactin-CAT expression is dependent on a Ras-like GTPase (Fig. 6). The activity of MAP kinase in hormone-treated GH4 cells expressing these inhibitors paralleled the inhibition of insulin- and EGF-increased prolactin-CAT activity. This suggested that insulin and EGF might increase prolactin-CAT expression in a MAP kinase-dependent manner. However, experiments with PD098059 (Fig. 7) demonstrate that MAP kinase does not mediate the increase in prolactin-CAT expression in insulin-treated cells, and numerous other experiments supported this conclusion.

Ras was also shown to activate PI 3-kinase (40) and, indirectly, p70S6 kinase (41). The experiments with rapamycin (Fig. 8), an inhibitor of p70S6 kinase phosphorylation (42), demonstrate that insulin-increased prolactin-CAT expression is not mediated by p70S6 kinase. However, wortmannin, an irreversible inhibitor of PI 3-kinase, blocked insulin-increased prolactin-CAT expression without affecting basal levels of prolactin-CAT gene transcription or its increase by EGF or cAMP. This could suggest that insulin mediates its effect by activating PI 3-kinase. However, the concentration of wortmannin needed to inhibit insulin-increased prolactin-CAT expression is 100–1000-fold higher than that reported to be necessary for PI 3-kinase inhibition, and lower concentrations of wortmannin do not inhibit insulin-increased prolactin-CAT expression (data not shown). This makes it unlikely that the wortmannin inhibition of insulin-increased prolactin-CAT expression is due to inhibition of PI 3-kinase. The failure of LY294002, a reversible inhibitor of PI 3-kinase (13), to reduce insulin-increased prolactin-CAT expression and other experiments with constitutively activated p110 or with p85 (data not shown) supports this conclusion. Recently, wortmannin was shown to inhibit phosphatidy 4-kinase (43) at concentrations 100–1000-fold higher than necessary to inhibit PI 3-kinase. Experiments are presently focusing on whether insulin increases inositol-4-PO4 and/or inositol-3-PO4 in RPTPα-expressing cell lines.

The identification of PTPases that inactivate insulin signaling will provide important insights into regulation of insulin action and may provide clues for new treatment modalities. PTPases that are potential regulators of insulin responsiveness would be expected to be (a) specific to insulin responses and (b) widely distributed and expressed in insulin-responsive tissues. Experiments in vivo and in vitro have implicated numerous protein tyrosine phosphatases in the control of insulin signaling. These include, in addition to RPTPα, the membrane-spanning PTPases, LAR and CD45, and the cytosolic PTPases, PTP1B, and SHP2 (23, 24, 36, 44–46). However, many of these do not have the characteristics expected of a phosphatase that specifically down-regulates the insulin response pathway. LAR is widely expressed and has been shown to dephosphorylate the insulin receptor in vitro (24), and LAR expression blocks insulin signaling (36). However, these effects are not specific for the insulin receptor (24) and the insulin signaling pathway (47). The transmembrane PTPase CD45 (leukocyte common antigen) was shown to block phosphorylation of the platelet-derived growth factor receptor and the insulin growth factor-1 receptor and to block their downstream effects (23), and it is able to dephosphorylate both the insulin and EGF receptors in vitro (44). But this enzyme is not specific and is confined to cells of hematopoietic origin. PTP1B was shown to dephosphorylate the insulin receptor in vitro, and microinjected PTP1B blocks insulin-induced oocyte maturation (48). However, the
effect of PTP1B was not specific, since it also blocked maturation induced by progesterone and maturation-promoting factor. SHP2 is ubiquitously expressed and dephosphorylates IRS-1, if not the insulin receptor (45), but SHP2 positively regulates the insulin signaling pathways leading to prolactin gene expression (46). By contrast, the data presented here suggest RPTPα may serve as a negative regulator for at least some insulin signaling pathways. It specifically blocks the insulin signaling pathway induced by progesterone and maturation-promoting factor.

Acknowledgments—We thank L. Feig, V. Narayanan, T. Payne, U. Rapp, J. Schlessinger, E. Skolnik, K. Siddle, N. Tonks, M. White, and J. Whittaker for plasmids and antibodies used in these studies.

REFERENCES

1. Iranmanesh, A., Veldhuis, J., Carlsem, E., Vaccaro, V., Booth, J., R. A., Lizaralde, G., Aspin, C., and Evans, W. (1990) J. Clin. Endocrinol. Metab. 71, 73–78
2. Tesone, M., Ladenheim, R., and Charreau, E. (1985) Mol. Cell. Endocrinol. 43, 135–140
3. Prager, D., Yamashita, S., and Melmed, S. (1988) Endocrinology 122, 2946–2952
4. Stanley, F. (1988) J. Biol. Chem. 263, 13444–13448
5. Jacob, K., Ouyang, L., and Stanley, F. (1995) J. Biol. Chem. 270, 27773–27779
6. White, M. F., and Kahn, C. R. (1994) J. Biol. Chem. 269, 463–4640
7. Lowenstein, E., Daly, R., Batzer, A., Li, W., Margolis, B., Lammers, R., Ullrich, J. Biol. Chem. 269, 5699–5704
8. Stokoe, D., Macdonald, S., Cadwalader, K., Symons, M., and Hancock, J. (1994) Science 264, 1463–1467
9. Hill, C., Marais, R., John, S., Wynne, J., Dalton, S., and Treisman, R. (1993) Cell 79, 395–406
10. Prue, W., Yuan, Y.-P., Rose, E., Batzer, A., Harada, N., and Skolnik, E. (1995) Mol. Cell. Biol. 15, 1778–1785
11. Cheatham, B., Vlahos, C., Cheatham, L., Wang, L., Blenis, J., and Kahn, C. (1994) Mol. Cell. Biol. 14, 4902–4911
12. Myers, M. G., Jr., Zhang, Y., Aldaz, G., Grammer, T., Glassbein, E., Yenush, L., Wang, L., Sun, X., Blenis, J., Pieree, J., and White, M. (1996) Mol. Cell. Biol. 16, 4147–4155
13. Walton, K. M., and Dixon, J. E. (1993) Annu. Rev. Biochem. 62, 101–120
14. Sap, J., Jiang, Y.-P., Friedlander, D., Grumet, M., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 1–9
15. Hill, C., Marais, R., John, S., Wynne, J., Dalton, S., and Treisman, R. (1993) Cell 71, 251–260
16. Prue, W., Yuan, Y.-P., Rose, E., Batzer, A., Harada, N., and Skolnik, E. (1995) Mol. Cell. Biol. 15, 1778–1785
17. Peles, E., Nativ, M., Campbell, P. L., Sakurai, T., Martinez, R., Lev, S., Clary, D. O., Schilling, J., Barnea, G., Plowman, G. D., Grumet, M., and Schlessinger, J. (1995) Cell 82, 251–260
18. Mauro, L. J., and Dixon, J. E. (1994) Trends Biochem. Sci. 19, 151–155
19. Su, J., Yang, L.-T., and Sap, J. (1996) J. Biol. Chem. 271, 28086–28089
20. Jiang, Y.-P., Wang, H., D’Eustachio, P., Musacchio, J., Schlessinger, J., and Sap, J. (1993) Mol. Cell. Biol. 13, 2942–2951
21. Lammers, R., Bossemmaier, B., Cool, D. F., Tonks, N. K., Schlessinger, J., Fischer, E. H., and Ullrich, A. (1993) J. Biol. Chem. 268, 22456–22462
22. Su, J., Batzer, A., and Sap, J. (1994) J. Biol. Chem. 269, 18731–18734
23. Bennett, A., Haudsuff, S., O’Reilly, A., Freeman, R., and Neel, B. (1996) Mol. Cell. Biol. 16, 1189–1202
24. Hashimoto, N., Zhang, W.-R., and Goldstein, B. (1992) Biochem. J. 284, 569–576
25. Stanley, F. M. (1989) Mol. Endocrinol. 3, 1627–1633
26. Stanley, F. M. (1992) J. Biol. Chem. 267, 16719–16726
27. Skolnik, K., Batzer, A., Li, N., Lee, C.-H., Lowenstein, E., Mohammadi, M., Margolis, B., and Schlessinger, J. (1993) Science 260, 1953–1955
28. Wilden, P. A., Kahn, C. R., Siddle, K., and White, M. F. (1992) J. Biol. Chem. 267, 16660–16668
29. Charbonneau, H., and Tonks, N. (1992) Annu. Rev. Cell Biol. 8, 463–493
30. Farnsworth, C., and Feig, L. (1991) Mol. Cell. Biol. 11, 4822
31. Bruder, J., Heidecker, G., and Rapp, U. (1992) Gene Dev. 6, 546–556
32. Jacob, K., and Stanley, F. M. (1994) J. Biol. Chem. 269, 25515–25520
33. Liang, J., Kim, K. E., Schoderbek, W. E., and Maurer, R. A. (1992) Mol. Endocrinol. 6, 885–892
34. Traverse, S., Gomez, N., Paterson, H., Marshall, C., and Cohen, P. (1992) Mol. Cell. Biol. 10, 175–181
35. Kulas, D. T., Zhang, W.-R., Goldstein, B. J., Furlanetto, B. R., and Mooney, R. A. (1996) J. Biol. Chem. 271, 2435–2438
36. Meller, N. P., Moller, K. B., Lammers, R., Kharitonenkov, A., Hoppe, E., Wiberg, F., Sures, I., and Ullrich, A. (1995) J. Biol. Chem. 270, 23126–23131
37. Goldstein, B. (1992) J. Cell. Biochem. 48, 33–42
38. Araki, E., Lipes, M., Patti, M., Bruning, J., Haag, B., III, Johnson, R., and Kahn, C. (1994) Nature 372, 186–190
39. Rodriguez-Viciana, P., Warne, P., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M., Waterfield, M., and Downward, J. (1994) Nature 370, 532–537
40. Klippel, A., Reinhardt, C., Kavanagh, M., Apell, G., Eschedo, M.-A., and Williams, L. (1996) Mol. Cell. Biol. 16, 4117–4127
41. Alarcon, C., Cardenas, M., and Heitman, J. (1996) Mol. Cell. Biol. 16, 4117–4127
42. Meyers, R., and Cantley, L. C. (1997) J. Biol. Chem. 272, 4384–4390
43. Tonks, N. K., Diltz, C. D., and Fischer, E. H. (1990) J. Biol. Chem. 265, 10874–10880
44. Clapham, D. E., and Schilling, J. (1996) Mol. Cell. Biol. 16, 6674–6682
45. Kulas, D. T., Goldstein, B. J., and Mooney, R. A. (1996) J. Biol. Chem. 271, 748–754
46. Tonks, N., Ciurel, M., Diltz, C., Krebs, E., and Fischer, E. (1990) Mol. Cell. Biol. 10, 458–463
47. Snipas, J., D’Eustachio, P., Givol, D., and Schlessinger, J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6112–6116
48. Isakoff, S. J., Su, Y. C., Blauke, P., Vajnik, V., Rose, E., Weidner, K. M., Sachs, M., Margolis, B., and Skolnik, E. Y. (1996) J. Biol. Chem. 271, 3959–3962

2 J. Sap, unpublished observations.
Receptor-like Protein-tyrosine Phosphatase α Specifically Inhibits Insulin-increased Prolactin Gene Expression
Kirsten K. Jacob, Jan Sap and Frederick M. Stanley

J. Biol. Chem. 1998, 273:4800-4809.
doi: 10.1074/jbc.273.8.4800

Access the most updated version of this article at http://www.jbc.org/content/273/8/4800

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 34 of which can be accessed free at http://www.jbc.org/content/273/8/4800.full.html#ref-list-1