Stimulation of Receptor Protein-Tyrosine Phosphatase α Activity and Phosphorylation by Phorbol Ester

Jeroen den Hertog,[1,2] Jan Sap, Cornelieke E. G. M. Pals, Joseph Schlessinger, and Wiebe Kruijer[3]

Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands [J. d. H., C. E. G. M. P., W. K.], and New York University Medical Center, Department of Pharmacology, New York, NY 10016 [J. S., J. S.]

Abstract

Receptor Protein-Tyrosine Phosphatase α (RPTPα) is a transmembrane protein with two cytoplasmic catalytic protein-tyrosine phosphatase (PTP) domains and a relatively short (123 amino acids) extracellular domain. Here we report that treatment of transfected cells that express RPTPα with the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate, a direct activator of protein kinase C, induced a rapid, transient increase in RPTPα activity due to a 2- to 3-fold increase in substrate affinity. A transient increase in RPTPα serine phosphorylation was concomitant with the enhanced activity. Tryptic phosphopeptide mapping of RPTPα demonstrated that phosphorylation of three tryptic peptides was enhanced in response to phorbol ester. *In vitro* dephosphorylation of RPTPα from phorbol ester-treated cells reduced RPTPα activity to prestimulation levels, indicating that enhanced serine phosphorylation directly accounted for the increase in activity. Our results demonstrate that serine phosphorylation may play a key role in the regulation of the activity of transmembrane PTPs.

Introduction

Protein phosphorylation on tyrosine residues, one of the main cell signaling mechanisms, is of profound importance for cellular processes such as proliferation and differentiation. Cellular P.Tyr[4] levels are regulated by the PTKs and PTPs. The PTKs have been studied extensively in the past decade, providing insight into the regulation of PTK enzymatic activity and in the role of PTKs in cell signal transduction (1, 2). Relatively little is known about the role of PTPs in cell signal transduction or about regulation of PTP activity.

In recent years, more than 40 PTPs have been cloned, based on sequence homology in the catalytic PTP domain (3–5). RPTPα (also known as LRP; Ref. 6) is a transmembrane member of the PTP family with a relatively short extracellular domain (7) that is highly glycosylated (8). Like most transmembrane PTPs, RPTPα has two homologous cytoplasmic catalytic PTP domains. The domain closest to the membrane exhibits the majority of the enzymatic activity, while the second domain has low but detectable activity (9, 10). Specific physiological substrates have not been identified for RPTPα, although it has been demonstrated that overexpression of RPTPα leads to specific dephosphorylation of the cytoplasmic PTK c-Src (11, 12). RPTPα may play an important role in neuronal differentiation, since RPTPα mRNA expression is enhanced during neuronal differentiation of three distinct cell lines. Moreover, overexpression of RPTPα in P19 EC cells dramatically alters the differentiation fate of these cells in favor of neuronal differentiation (12).

Serine phosphorylation has been implicated in the regulation of PTP enzymatic activity in several studies. Activation of PKC or cyclic AMP-dependent kinase leads to an increase in cellular PTP activity through an ill-understood phosphorylation event (13). In addition, several PTPs have been found to be phosphoproteins, including the transmembrane PTP CD45 and the cytoplasmic PTPs PTP1B and PTP-PEST (14–17). CD45 serine phosphorylation is reduced in response to treatment of cells with the calcium ionophore ionomycin, which is concomitant with a reduction in CD45 PTP activity (15). Phorbol ester treatment of cells activates PKC (18) and leads to an increase in CD45 serine phosphorylation (14). However, PKC-mediated serine phosphorylation of CD45 does not modulate CD45 activity significantly (19). Three major serine phosphorylation sites have been identified in the cytoplasmic PTP1B. Ser378 and Ser386 are substrates of PKC and p34CDC2, respectively, while the kinase for Ser352 is not known. No significant modulation of PTP1B activity could be detected upon phosphorylation of these sites (16). PTP-PEST is phosphorylated on Ser39 and Ser435 following stimulation of PKC or cAMP-dependent protein kinase. Phosphorylation of Ser39 results in a decrease in PTP-PEST activity, while Ser435 phosphorylation does not modulate PTP-PEST activity directly (17).

PTPs have been found to be phosphorylated, not only on serine but also on tyrosine. CD45 is phosphorylated on tyrosine following stimulation of T cells after pretreatment with the PTP inhibitor phenylarsene oxide (20, 21). In addition, the cytoplasmic Syp (also known as PTP1D) is phosphorylated on tyrosine upon stimulation of cells with growth factors and in v-Src-transformed cells (22, 23). It remains to be determined definitively whether tyrosine phosphorylation affects Syp activity. Recently, we have demonstrated that RPTPα is constitutively phosphorylated on tyrosine and serine in NIH 3T3 cells (10). The tyrosine phosphorylation site is located at Tyr789, five residues from the COOH terminus. Phosphorylation of Tyr789 may have a negative effect on RPTPα activity, but since RPTPα has autodephosphorylation activity, it is difficult to assay these effects accurately.

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[1] J.d.H. is the recipient of a Dutch Cancer Society Fellowship.

[2] To whom requests for reprints should be addressed.

[3] Present address: Rijksuniversiteit Groningen, Department of Genetics, Kerklaan 30, 9750 AA Haren, the Netherlands.

[4] The abbreviations used are: P.Tyr, phosphotyrosine; PTK, protein-tyrosine kinase; PTP, protein-tyrosine phosphatase; RPTPα, receptor protein-tyrosine phosphatase α; EC, embryonal carcinoma; PKC, protein kinase C; SH2, Src homology 2; MBP, myelin basic protein; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; AP, alkaline phosphatase.

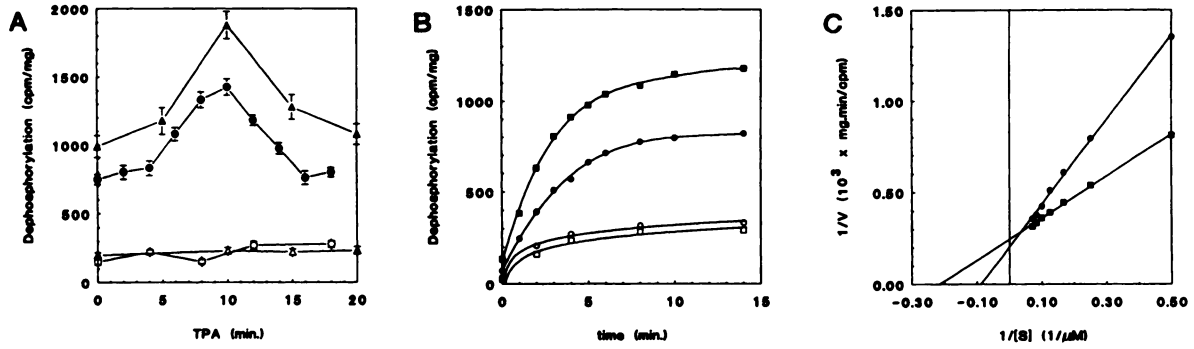

Fig. 1. Enhanced RPTPα activity in response to TPA. *A*, PTP activity of RPTPα immune complexes from stably transfected retinoic acid-treated P19-N-2 cells (○), P19-RPTPα-75 cells (●), or transiently transfected human embryonic kidney 293 cells after treatment with TPA (100 ng/ml) for the times indicated. 293 cells were transiently transfected with vector alone (pSG5, △) or with pSG-RPTPα (▲). RPTPα immune complexes were incubated with ^{32}P.Tyr containing MBP, and PTP activity is expressed as free phosphate released from the substrate in cpm/mg protein that was used for the immunoprecipitations. *B*, time curve of RPTPα PTP activity in immune complexes from control (○) and TPA-stimulated P19-N-2 cells (100 ng/ml for 10 min, □) or control (●) and TPA-stimulated P19-RPTPα-75 cells (■). *C*, Lineweaver-Burk plot of RPTPα activity from control (●) or 10-min TPA-treated (100 ng/ml, ■) P19-RPTPα-75 cells. Reciprocal substrate concentrations ($1/[S]$) is plotted against reciprocal velocity of the reaction ($1/V$). All assays were performed at least three times in duplicate with similar results. Representative experiments are depicted as the mean from duplicate experiments, and the SE (*bars*) as depicted in (*A*) was less than 10% for all points.

Tyrosine phosphorylation of PTPs may not only directly affect PTP activity but also provide binding sites for SH2-domain containing proteins, since SH2 domains bind to short P.Tyr-containing sequences in specific phosphoproteins (24). Conditions that induce tyrosine phosphorylation of CD45 lead to binding of the cytoplasmic PTK Lck through its SH2 domain (21). In addition, tyrosine-phosphorylated Syp (PTP1D) binds to the SH3-SH2-SH3 adapter protein GRB2 in an SH2-dependent manner (25). RPTPα is phosphorylated on Tyr789, a consensus binding site for GRB2 (10). We and others have shown that GRB2 binds to RPTPα *in vitro* and *in vivo* (10, 26) and that phosphorylation of Tyr789 is essential for binding.

Here we report that phorbol ester-mediated activation of PKC induces a transient increase in RPTPα serine phosphorylation, concomitant with a transient increase in RPTPα activity. Enhanced phosphorylation is essential for the increase in activity, since dephosphorylation of RPTPα from stimulated cells *in vitro* reduces its activity to prestimulation levels.

Results and Discussion

The effect of TPA-mediated activation of PKC on RPTPα activity and phosphorylation was investigated in two distinct cell systems, stably transfected P19 EC cells and transiently transfected human embryonic kidney 293 cells. P19 EC cells expressing RPTPα and control cells were derived by stable transfection of pSG-RPTPα (P19-RPTPα-75) or vector alone (P19-N-2) (12). Undifferentiated P19 EC cells are not responsive to TPA. However, retinoic acid (10^{-6} M)-induced differentiation for 5 days renders P19 cells fully responsive to TPA, such that, for instance, early-response gene expression can be detected within 15 min of TPA treatment (27).

RPTPα activity was assayed using RPTPα immune complexes from 5-day retinoic acid-treated P19-RPTPα-75 cells or P19-N-2 cells with ^{32}P.Tyr-containing MBP as a substrate. RPTPα immune complexes from P19-N-2 cells showed only background levels of PTP activity that were not influenced by treatment with TPA for up to 20 min (Fig. 1, *A* and *B*). Dephosphorylation by RPTPα immune complexes from P19-RPTPα-75 cells, treated with TPA for different periods of time, demonstrated that RPTPα activity is enhanced transiently with maximal levels of 1.5- to 2-fold over control levels after TPA treatment for 10 min (Fig. 1*A*). Similar activation ratios (1.5- to 2-fold) were observed in transiently transfected 293 cells following treatment with TPA for 10 min (Fig. 1*A*).

Time courses of RPTPα-mediated dephosphorylation were determined by allowing the reaction to proceed for 1–14 min. As depicted in Fig. 1*B*, initial velocities of MBP dephosphorylation are linear for at least 5 min for both control as well as 10-min TPA-treated P19-RPTPα-75 cells.

RPTPα immune complexes from P19-RPTPα-75 cells were incubated with different concentrations of substrate to determine the influence of TPA treatment on the enzymatic parameters K_m and V_{max}. As can be deduced from the Lineweaver-Burk plot in Fig. 1*C*, V_{max} did not change significantly by treatment of the cells with TPA for 10 min. The K_m of control RPTPα-mediated MBP dephosphorylation (K_m ~12 μM) is in the same range as the K_m of purified human RPTPα (K_m ~10 μM; Ref. 28). Following stimulation of the cells with TPA for 10 min, the K_m was reduced 2- to 3-fold (K_m ~5 μM), indicating that enhanced RPTPα activity was caused by an increase in substrate affinity.

The phosphorylation state of RPTPα was investigated by immunoprecipitation from [^{32}P]P_i-labeled transiently transfected 293 cells. Fig. 2*A* shows that RPTPα is a phosphoprotein. Treatment of the cells with TPA led to a transient increase in RPTPα phosphorylation with maximal levels after 10 min. As a control, RPTPα was immunoprecipitated from [^{35}S]methionine and [^{35}S]cysteine-labeled cells that were cultured in parallel. Treatment of these cells with TPA did not induce large modulations in the amount of RPTPα that was immunoprecipitated (Fig. 2*B*). The stoichiometry of phosphorylation of RPTPα in transiently transfected 293 cells was determined by parallel ^{32}P- and ^{35}S-labeling and immunoprecipitation of RPTPα. RPTPα in control cells contained 0.37 mol phosphate/mol and 1.26 mol phosphate/mol in 10-min, TPA-treated cells.

Phosphoamino acid analysis demonstrated that RPTPα was exclusively phosphorylated on serine in resting cells.

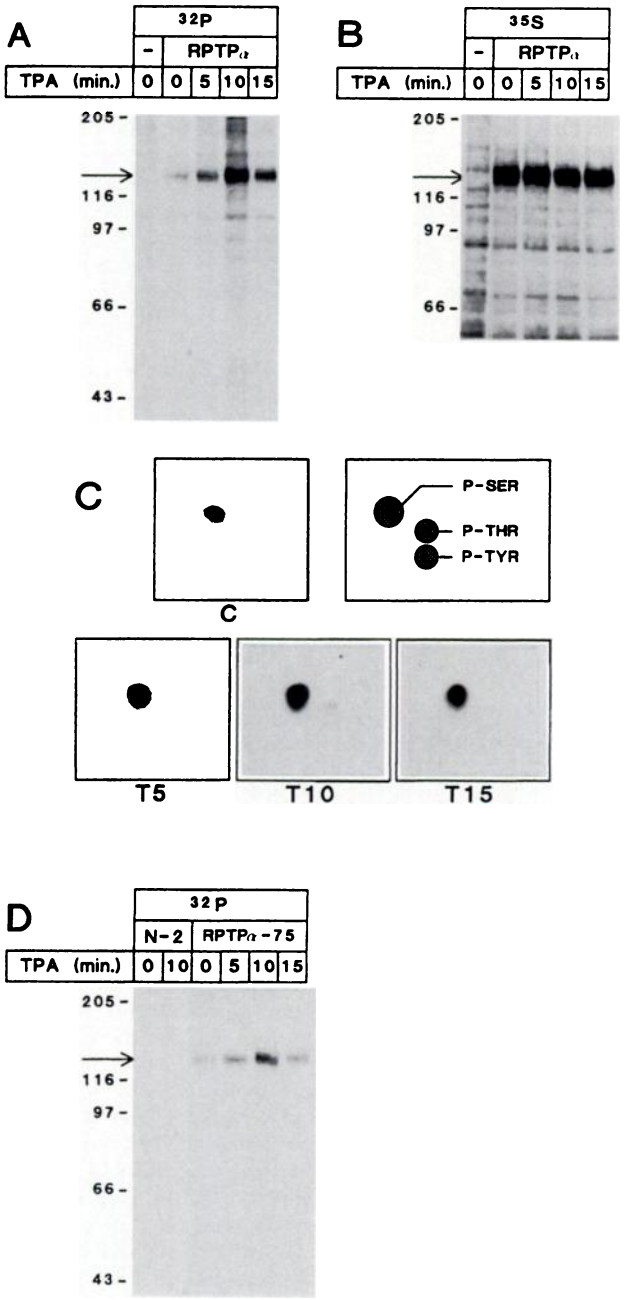

Fig. 2. Transient phosphorylation of RPTPα in response to TPA *in vivo*. *A*, immunoprecipitation of RPTPα from ^{32}P-labeled 293 cells transiently transfected with pSG5 (–) or pSG-RPTPα (*RPTPα*) following stimulation with TPA (100 ng/ml) for the times indicated. *B*, immunoprecipitation of RPTPα from [^{35}S]methionine- and [^{35}S]cysteine-labeled 293 cells transfected and stimulated as under (*A*). *C*, phosphoamino acid analysis of ^{32}P-labeled RPTPα depicted in (*A*). Immunoprecipitated RPTPα from control (*C*) and TPA-treated cells (*T5*, 5 min; *T10*, 10 min; *T15*, 15 min) were analyzed. The positions of P.Tyr, P.Thr, and P.Ser markers that were coelectrophoresed with the samples are indicated. *D*, immunoprecipitation of RPTPα from ^{32}P-labeled stably transfected control (P19-N-2) and P19-RPTPα-75 cells following treatment with TPA (100 ng/ml) for the times indicated. Molecular weights of marker proteins that were coelectrophoresed with the samples are indicated on the *left* (in thousands). *Arrow*, position of RPTPα.

TPA treatment led to an increase in serine phosphorylation and very low levels of threonine phosphorylation (Fig. 2*C*).

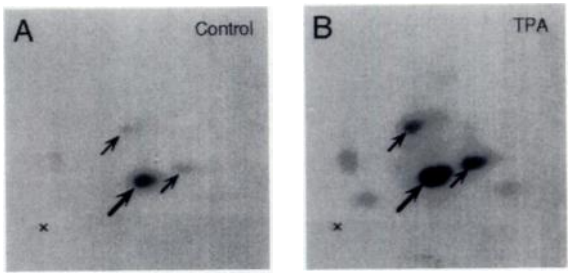

Fig. 3. Tryptic phosphopeptide mapping of RPTPα. RPTPα was isolated from ^{32}P-labeled transiently transfected 293 cells by immunoprecipitation, and tryptic phosphopeptide mapping was done as described in "Materials and Methods." The samples were electrophoresed in pH 1.9 buffer, followed by ascending chromatography. The anode was at *left*, and chromatography was done in the vertical direction. *A*, RPTPα from control cells. *B*, RPTPα from TPA-treated cells (100 ng/ml for 10 min). *Arrows*, the three major tryptic phosphopeptides. The origin is marked with an *X*.

In these experiments, we did not detect any P.Tyr in RPTPα, although we have reported earlier that RPTPα is constitutively phosphorylated on tyrosine (10). The data presented here reflect the autodephosphorylation activity of RPTPα, since RPTPα phosphorylation and activity were studied under exactly the same experimental conditions, *i.e.*, in the absence of PTP inhibitors. In addition, the antiserum that was used in this study was raised against a COOH-terminal peptide. This peptide encompasses Tyr789, the tyrosine phosphorylation site of RPTPα, and phosphorylation of this site may impair binding of the antibody to RPTPα. We have repeated the experiments depicted in Fig. 1*A* with an antibody raised against the complete cytoplasmic domain of RPTPα with similar results (data not shown), indicating that the observed effect is not an artifact of the anti-peptide antibody that was used in these studies.

Immunoprecipitation of RPTPα from stably transfected P19-RPTPα-75 cells illustrated a similar phosphorylation pattern as in transiently transfected 293 cells, with maximal levels of phosphorylation after 10-min TPA treatment (Fig. 2*D*).

Tryptic phosphopeptide mapping experiments using *in vivo*-labeled RPTPα resulted in three major phosphopeptides in unstimulated, transiently transfected 293 cells (Fig. 3*A*). TPA treatment enhanced phosphorylation of these three phosphopeptides, and traces of novel phosphopeptides could be detected (Fig. 3*B*). Quantitation of the tryptic peptides in Fig. 3 using an AMBIS scanner indicated that phosphorylation of the major peptide in control cells (Fig. 3, *thick arrow*) was enhanced 2.5-fold in 10-min, TPA-treated cells, while phosphorylation of the two minor peptides (Fig. 3, *thin arrows*) was enhanced 5-fold. The serine phosphorylation sites of RPTPα have been mapped recently.[5] The major peptide (Fig. 3, *thick arrow*) contained P.Ser204, while the minor peptides (Fig. 3, *thin arrows*) both contained P.Ser180. In addition, both Ser180 as well as Ser204 could be phosphorylated by PKC *in vitro*, indicating that these sites may be direct substrates of PKC *in vivo* as well. It is noteworthy that both Ser180 as well as Ser204 are located in the juxtamembrane domain of RPTPα. To date, no serine phosphorylation sites have been found in the catalytic domain of PTPs. Ser352, Ser378, and Ser386 in

[5] S. Tracy, P. van der Geer, and T. Hunter, manuscript in preparation.

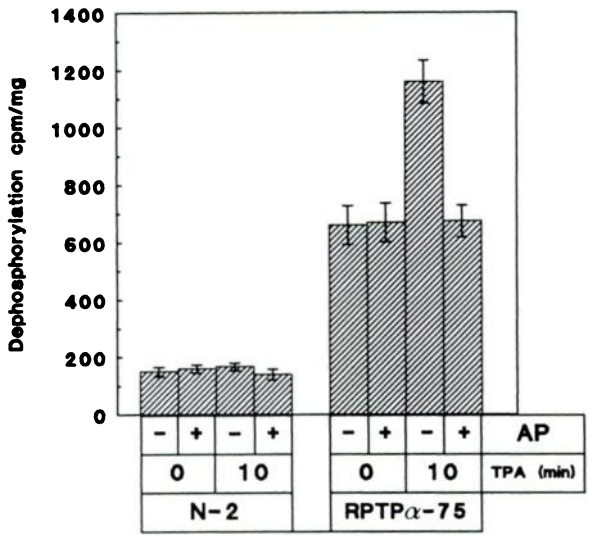

Fig. 4. Serine phosphorylation regulates RPTPα activity directly. RPTPα was immunoprecipitated from control (*N-2*) cells or P19-RPTPα-75 (*RPTPα-75*) cells treated with TPA (100 ng/ml) for 0 and 10 min. Following immunoprecipitation, the Sepharose beads were incubated with AP or with buffer alone. Subsequently, the RPTPα immune complexes were washed extensively with M6 PTP assay buffer and incubated with ^{32}P.Tyr containing MBP for 2.5 min. Dephosphorylation was assayed as described in "Materials and Methods" and is given as free phosphate released from the substrate in cpm/mg protein that was initially used for the immunoprecipitations.

PTP1B are located to the COOH-terminal side of the PTP domain (16). The two major sites in PTP-PEST are located at Ser39 and Ser435, to the NH_2-terminal and COOH-terminal side of the PTP domain, respectively (17). It is interesting that serine phosphorylation outside the catalytic PTP domain regulates RPTPα and PTP-PEST activity, which may indicate that serine phosphorylation of these PTPs modulates substrate recognition.

In order to determine the effect of serine phosphorylation on RPTPα activity directly, we phosphorylated bacterially expressed RPTPα with PKC *in vitro*. However, the stoichiometry of *in vitro* phosphorylation of RPTPα was too low (approximately 0.1 mol phosphate/mol) to measure a significant increase of RPTPα activity (data not shown). Therefore, we dephosphorylated RPTPα immune complexes *in vitro* using AP to establish whether phosphorylation of RPTPα directly causes the increase in activity. AP treatment of RPTPα immune complexes from *in vivo* ^{32}P-labeled P19-RPTPα-75 reduced phosphorylation of RPTPα to undetectable levels, indicating that AP efficiently dephosphorylated RPTPα (data not shown). Fig. 4 demonstrates that RPTPα activity from TPA-treated (10-min) cells was reduced to prestimulation levels following AP-treatment of the immune complexes. AP treatment of RPTPα immune complexes from control cells did not affect RPTPα activity significantly. Therefore, it appears that the basal level phosphorylation of RPTPα does not play a role in regulation of RPTPα activity. The reduction in RPTPα activity from TPA-treated cells by *in vitro* dephosphorylation demonstrates that enhanced serine phosphorylation of RPTPα is directly responsible for the increase in activity.

We demonstrate here that the activity of the transmembrane RPTPα can be regulated by serine phosphorylation in transiently and stably transfected cells. Phosphorylation of endogenous RPTPα in unstimulated NIH 3T3 cells is qualitatively very similar to phosphorylation of RPTPα in transfected 293 cells (10). However, TPA stimulation of NIH 3T3 cells leads to only a small increase in RPTPα phosphorylation (data not shown). This may indicate that the observed effects are cell type specific, and analysis of endogenous RPTPα phosphorylation in other cell lines will establish whether enhanced RPTPα serine phosphorylation and activity in response to activation of PKC is a general phenomenon. The role of RPTPα activation by serine phosphorylation in cell signaling can only be determined upon identification of physiological substrates of RPTPα. It will be interesting to see whether PKC-mediated activation of RPTPα leads to (more rapid) dephosphorylation of those substrates.

Materials and Methods

Cells and Plasmids. Human embryonic kidney 293 cells (29) were cultured in DMEM supplemented with 10% bovine calf serum. P19 EC cells (30) were cultured in DMEM supplemented with 7.5% fetal calf serum. Stably transfected derivatives of P19 EC cells, either control (P19-N-2) or expressing RPTPα at high levels (P19-RPTPα-75), have been described (12) and were cultured like wild-type P19 EC cells.

The SV40 promoter-driven expression vectors pSG5 (31) and pSG-RPTPα (12) were used for transient transfection of 293 cells by calcium phosphate precipitation. Briefly, 10 μg plasmid DNA was mixed with 200 μl 2 × HBSP [42 mM HEPES, 275 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, and 10 mM dextrose (pH 7.05)] and 200 μl 250 mM $CaCl_2$ and left for 25 min at room temperature. The precipitate was resuspended and added directly to the medium on the cells. After overnight incubation, fresh medium was added to the cells, and another 24 h later, the cells were harvested.

Metabolic Labeling and Immunoprecipitation. Cells were labeled to equilibrium in phosphate-free medium supplemented with [^{32}P]P_i (0.5 mCi/ml; ICN Radiochemicals) for 2 h. Alternatively, cells were incubated in cysteine- and methionine-free medium supplemented with [^{35}S]cysteine and [^{35}S]methionine (25 μCi/ml; ICN Radiochemicals) for 14 h. The cells were lysed in nondenaturing cell lysis buffer [50 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 1% Triton X-100, 10% glycerol, 10 u/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 100 μM NaF, 30 mM *p*-nitrophenylphosphate, and 10 mM PP_i]. RPTPα was immunoprecipitated using anti-RPTPα antiserum 2A (7) bound to protein A-Sepharose (Pharmacia). The beads were washed four times in HNTG [20 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, and 0.1% Triton X-100) and used for *in vitro* PTP assays or resuspended in Laemmli sample buffer and fractionated on SDS polyacrylamide gels.

PTP Assays. For the *in vitro* PTP assays, MBP (Sigma Chemical Co.) was phosphorylated on tyrosine using immunoprecipitated human epidermal growth factor receptor and [γ-^{32}P]ATP. ^{32}P-labeled MBP was separated from free [γ-^{32}P]ATP by precipitation with 25% trichloroacetic acid. Using this protocol, typically 80–90% of the phosphate was incorporated in P.Tyr in MBP, as determined by phosphoamino acid analysis (data not shown).

Immune complex PTP assays were performed using RPTPα immunoprecipitated from cell lysates (1–3 mg protein/sample) as described above. RPTPα immunoprecipitates were washed four times with HNTG and twice in PTP

assay buffer without β-mercaptoethanol [M6 buffer: 20 mM MES (pH 6.0), 5% glycerol, and 0.1% Triton X-100; Ref. 28]. Dephosphorylation reactions were done in 50 μl (final volume) M6 buffer containing 0.1% β-mercaptoethanol with 4 μM ^{32}P-labeled MBP (80–200 × 10^3 cpm/nmol) for 2.5 min at 37°C. Dephosphorylation reactions were terminated by the addition of 750 μl stop buffer (0.9 M HCl, 90 mM PP_i, 2 mM Na_2HPO_4, and 4% activated charcoal; Norit A; Ref. 32). Free [^{32}P]phosphate in the supernatant was determined by Cerenkov counting.

RPTPα immune complexes were dephosphorylated by AP (Boehringer-Mannheim) for 20 min at 37°C, exactly as described by the manufacturer. AP-treated immune complexes were washed five times in M6 buffer without β-mercaptoethanol, prior to the assay of PTP activity as described above.

Tryptic Phosphopeptide Mapping and Phosphoamino Acid Analysis. Tryptic phosphopeptide mapping and phosphoamino acid analysis was done exactly as described by Boyle *et al.* (33). ^{32}P-labeled immunoprecipitated RPTPα was eluted from the dried gel, precipitated with trichloroacetic acid, oxidized using performic acid, and digested with trypsin. The samples were loaded on TLC plates and fractionated by electrophoresis in pH 1.9 buffer, 1 kV for 25 min (first dimension) and ascending chromatography in P.Chromo buffer (second dimension).

Phosphoamino acid analysis was done by acid hydrolysis of the samples (6 M HCl at 110°C for 1 h). The phosphoamino acids were separated by electrophoresis in pH 1.9 buffer (1.5 kV for 25 min) and pH 3.5 buffer (1.3 kV for 16 min) for the first and second dimensions, respectively.

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References

1. Hunter, T., and Cooper, J. A. Protein tyrosine kinases. Annu. Rev. Biochem., *54:* 897–930, 1985.

2. Schlessinger, J., and Ullrich, A. Growth factor signalling by receptor tyrosine kinases. Neuron, *9:* 383–391, 1992.

3. Fischer, E. H., Charbonneau, H., and Tonks, N. K. Protein tyrosine phosphatases—a diverse family of intracellular and transmembrane enzymes. Science (Washington DC), *253:* 401–406, 1991.

4. Charbonneau, H., and Tonks, N. K. 1002 protein phosphatases. Annu. Rev. Cell Biol., *8:* 463–493, 1992.

5. Walton, K. M., and Dixon, J. E. Protein tyrosine phosphatases. Annu. Rev. Biochem., *62:* 101–120, 1993.

6. Matthews, R. J., Cahir, E. D., and Thomas, M. L. Identification of an additional member of the protein-tyrosine phosphatase family—evidence for alternative splicing in the tyrosine phosphatase domain. Proc. Natl. Acad. Sci. USA, *87:* 4444–4448, 1990.

7. Sap, J., D'Eustachio, P., Givol, D., and Schlessinger, J. Cloning and expression of a widely expressed receptor tyrosine phosphatase. Proc. Natl. Acad. Sci. USA, *87:* 6112–6116, 1990.

8. Daum, G., Regenass, S., Sap, J., Schlessinger, J., and Fischer, E. H. Multiple forms of the human tyrosine phosphatase RPTPα—isozymes and differences in glycosylation. J. Biol. Chem., *269:* 10524–10528, 1994.

9. Wang, Y., and Pallen, C. J. The receptor-like protein tyrosine phosphatase hPTPα has two active catalytic domains with distinct substrate specificities. EMBO J., *10:* 3231–3237, 1991.

10. den Hertog, J., Tracy, S., and Hunter, T. Phosphorylation of receptor protein-tyrosine phosphatase α on Tyr789, a binding site for the SH3-SH2-SH3 adaptor protein GRB2 *in vivo*. EMBO J., *13:* 3020–3032, 1994.

11. Zheng, X. M., Wang, Y., and Pallen, C. J. Cell transformation and activation of pp60$^{c\text{-}src}$ by overexpression of a protein tyrosine phosphatase. Nature (Lond.), *359:* 336–339, 1992.

12. den Hertog, J., Pals, C. E. G. M., Peppelenbosch, M. P., Tertoolen, L. G. J., de Laat, S. W., and Kruijer, W. Receptor protein-tyrosine phosphatase α activates pp60^{csrc} and is involved in neuronal differentiation. EMBO J., *12:* 3789–3798, 1993.

13. Brautigan, D. L., and Pinault, F. M. Activation of membrane protein-tyrosine phosphatase involving cAMP-dependent and Ca^{2+}/phospholipid-dependent protein kinases. Proc. Natl. Acad. Sci. USA, *88:* 6696–6700, 1991.

14. Yamada, A., Streuli, M., Saito, H., Rothstein, D. M., Schlossman, S. F., and Morimoto, C. Effect of activation of Protein kinase C on CD45 isoform expression and CD45 protein-tyrosine phosphatase activity in T-cells. Eur. J. Immunol., *20:* 1655–1660, 1989.

15. Ostergaard, H. L., and Trowbridge, I. S. Negative regulation of CD45 protein tyrosine phosphatase activity by ionomycin in T-cells. Science (Washington DC), *253:* 1423–1425, 1991.

16. Flint, A. J., Gebbink, M. F. B. G., Franza, B. R., Hill, D. E., and Tonks, N. K. Multi-site phosphorylation of the protein tyrosine phosphatase, PTP1B—identification of cell cycle regulated and phorbol ester stimulated sites of phosphorylation. EMBO J., *12:* 1937–1946, 1993.

17. Garton, A. J., and Tonks, N. K. PTP-PEST: a protein tyrosine phosphatase regulated by serine phosphorylation. EMBO J., *13:* 3763–3771, 1994.

18. Nishizuka, Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature (Lond.), *334:* 661–665, 1984.

19. Tonks, N. K., Diltz, C. D., and Fischer, E. H. CD45, an integral membrane protein tyrosine phosphatase—characterization of enzyme activity. J. Biol. Chem., *265:* 10674–10680, 1990.

20. Stover, D. R., Charbonneau, H., Tonks, N. K., and Walsh, K. A. Protein tyrosine phosphatase CD45 is phosphorylated transiently on tyrosine upon activation of Jurkat T-cells. Proc. Natl. Acad. Sci. USA, *88:* 7704–7707, 1991.

21. Autero, M., Saharinen, J., Pessamorikawa, T., Soularothut, M., Oetken, C., Gassmann, M., Bergman, M., Alitalo, K., Burn, P., Gahmberg, C. G., and Mustelin, T. Tyrosine phosphorylation of CD45 phosphotyrosine phosphatase by p50^{csk} kinase creates a binding site for p56^{lck} tyrosine kinase and activates the phosphatase. Mol. Cell. Biol., *14:* 1308–1321, 1994.

22. Feng, G. S., Hui, C. C., and Pawson, T. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science (Washington DC), *259:* 1607–1611, 1993.

23. Vogel, W., Lammers, R., Huang, J. T., and Ullrich, A. Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. Science (Washington DC), *259:* 1611–1614, 1993.

24. Pawson, T., and Gish, G. D. SH2 and SH3 domains—from structure to function. Cell, *71:* 359–362, 1992.

25. Li, W., Nishimura, R., Kahishian, A., Batzer, A. G., Kim, W. J. H., Cooper, J. A., and Schlessinger, J. A new function for a phosphotyrosine phosphatase—linking GRB2-Sos to a receptor tyrosine kinase. Mol. Cell. Biol., *14:* 509–517, 1994.

26. Su, J., Batzer, A., and Sap, J. Receptor tyrosine phosphatase R-PTP-α is tyrosine phosphorylated and associated with the adaptor protein GRB2. J. Biol. Chem., *269:* 18731–18734, 1994.

27. de Groot, R. P., Schoorlemmer, J., van Genesen, S., and Kruijer, W. Differential expression of *jun* and *fos* genes during differentiation of mouse P19 embryonal carcinoma cells. Nucleic Acids Res., *18:* 3195–3202, 1990.

28. Daum, G., Zander, N. F., Morse, B., Hurwitz, D., Schlessinger, J., and Fischer, E. H. Characterization of a human recombinant receptor-linked protein tyrosine phosphatase. J. Biol. Chem., *266:* 12211–12215, 1991.

29. Graham, C. F. Teratocarcinoma cells and normal mouse embryogenesis. *In:* M. I. Sherman and C. F. Graham (eds.), Concepts in Mammalian Embryogenesis, pp. 315–394. Cambridge, MA: MIT Press, 1977.

30. McBurney, M. W., and Rogers, B. J. Isolation of male embryonal carcinoma cells and their chromosome replication patterns. Dev. Biol., *89:* 503–508, 1982.

31. Green, S., Isseman, I., and Sheer, E. A versatile *in vivo* and *in vitro* eukaryotic expression vector for protein engineering. Nucleic Acids Res., *16:* 369, 1988.

32. Streuli, M., Krueger, N. X., Thai, T., Tang, M., and Saito, H. Distinct functional roles of the 2 intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatases LCA and LAR. EMBO J., *9:* 2399–2407, 1990.

33. Boyle, W. J., van der Geer, P., and Hunter, T. Phosphopeptide mapping and phosphoamino acid analysis by 2-dimensional separation on thin-layer cellulose plates. Methods Enzymol., *201:* 110–149, 1991.