MOLECULAR AND CELLULAR BIOLOGY, Oct. 2007, p. 7102–7112 Vol. 27, No. 20
0270-7306/07/$08.00+0 doi:10.1128/MCB.02096-06

Association of Tyrosine Phosphatase Epsilon with Microtubules Inhibits Phosphatase Activity and Is Regulated by the Epidermal Growth Factor Receptor[▽]

Tal Sines, Shira Granot-Attas, Sabrina Weisman-Welcher, and Ari Elson*

Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel

Received 9 November 2006/Returned for modification 26 December 2006/Accepted 7 August 2007

Protein tyrosine phosphatases (PTPs) are key mediators that link physiological cues with reversible changes in protein structure and function; nevertheless, significant details concerning their regulation in vivo remain unknown. We demonstrate that PTPε associates with microtubules in vivo and is inhibited by them in a noncompetitive manner. Microtubule-associated proteins, which interact strongly with microtubules in vivo, significantly increase binding of PTPε to tubulin in vitro and further reduce phosphatase activity. Conversely, disruption of microtubule structures in cells reduces their association with PTPε, alters the subcellular localization of the phosphatase, and increases its specific activity. Activation of the epidermal growth factor receptor (EGFR) increases the PTPε-microtubule association in a manner dependent upon EGFR-induced phosphorylation of PTPε at Y638 and upon microtubule integrity. These events are transient and occur with rapid kinetics similar to EGFR autophosphorylation, suggesting that activation of the EGFR transiently down-regulates PTPε activity near the receptor by promoting the PTPε-microtubule association. Tubulin also inhibits the tyrosine phosphatase PTP1B but not receptor-type PTPμ or the unrelated alkaline phosphatase. The data suggest that reversible association with microtubules is a novel, physiologically regulated mechanism for regulation of tyrosine phosphatase activity in cells.

Reversible phosphorylation of tyrosine residues in proteins is a major regulator of protein structure and function. Tyrosine phosphorylation of proteins is regulated in part by the activity of members of the protein tyrosine phosphatase (PTP) superfamily, which currently includes over 100 genes in higher organisms. Of these, 38 genes encode "classical" tyrosine phosphatases (PTPs), which are strictly specific for phosphotyrosine. Products of this gene family all contain one or two copies of the PTP domain and are either receptor-type integral membrane proteins or non-receptor-type proteins (1, 3).

The numbers of known tyrosine kinases and tyrosine phosphatases are similar and small compared to the numbers and complexities of their potential substrates (1). The apparent contradiction between this and the high degree of specificity that exists in signaling processes in vivo has made understanding the mechanisms by which PTP activity is regulated of paramount importance. PTP genes often produce several distinct protein products by alternative splicing or use of alternative promoters (3). At the protein level, PTP activity can be inhibited by dimerization (e.g., see references 22, 23, and 43) or by reversible oxidation of the key cysteine residue in the PTP catalytic domain (9, 44). Proteolysis and phosphorylation, which can affect subcellular localization, conformation, or the ability to bind other proteins can also activate or inhibit PTPs (e.g., see references 6, 13, 17, 48, and 49). Regulation of PTP activity by binding of extracellular ligands to receptor-type PTPs (RPTPs), which inhibits PTP activity and appears to be less common than among tyrosine kinases, has also been described (e.g., see reference 14). Despite these insights, major gaps still exist in our understanding of how PTP activity is regulated in a reversible manner and within the short time periods typical of signal transduction events.

The two major forms of PTPε known today are RPTPε and the non-receptor form (cyt-PTPε), which differ only at their N termini and which are products of distinct promoters of the single *Ptpre* gene (10, 11, 31, 36). Other protein forms of PTPε are p67 PTPε and p65 PTPε, whose production is regulated at the levels of transcription and posttranslational processing, respectively (17, 18). RPTPε dephosphorylates c-Src and the related kinases c-Yes and c-Fyn at their C-terminal inhibitory tyrosine, thereby activating the kinases and assisting mammary tumor cells induced in vivo by Neu to maintain their transformed phenotype (16, 19). RPTPε has also been suggested to downregulate insulin receptor signaling (2, 25, 28, 30). cyt-PTPε is important for proper adhesion of osteoclasts to bone (7) and dephosphorylates and downregulates delayed-rectifier voltage-gated potassium channels in Schwann cells, thereby countering their activation by c-Src and c-Fyn. This effect correlates in vivo with delayed myelination of sciatic nerve axons in young PTPε-deficient mice (33, 40, 41) and with delayed optic nerve myelination in mice expressing a "substrate-trapping" mutant of RPTPε (29). cyt-PTPε can also downregulate signaling mediated by the mitogen-activated protein kinase (42, 45) and JAK-STAT (37–39) pathways and is required for proper functioning of mouse macrophages (35).

In order to better understand at the molecular level the physiological roles and regulation of PTPε activity in vivo, we searched for novel interactors with this phosphatase. We report here that PTPε interacts in vivo with microtubules in a reversible manner. Binding is stabilized by microtubule-associated proteins (MAPs) and is regulated by physiological stim-

* Corresponding author. Mailing address: Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel. Phone: 972-8-934-2331. Fax: 972-8-934-4108. E-mail: ari.elson@weizmann.ac.il.

▽ Published ahead of print on 20 August 2007.

uli, such as specific phosphorylation of PTPε following activation of the epidermal growth factor receptor (EGFR). Binding to tubulin inhibits PTPε catalytic activity, suggesting that physiologically relevant signaling events may down-regulate PTPε activity by promoting its transient association with microtubules. Our data indicate that tubulin also inhibits PTP1B but does not inhibit RPTPμ, suggesting that while association with tubulin is not universal among PTPs, it is not limited to PTPε alone.

MATERIALS AND METHODS

Reagents. The following mouse cDNAs used in this study were described previously: cyt-PTPε (10); RPTPε (11), p67 PTPε (18); S8M-cyt-PTPε, S22M-cyt-PTPε, and Δ11-27-cyt-PTPε (24); D245A cyt-PTPε (33); D302A RPTPε (16); and D1+12 and D2+12 (43). Y638F cyt-PTPε was constructed by PCR-mediated site-directed mutagenesis and sequenced prior to use. The DAP4 cDNA was a gift from Adi Kimchi, The Weizmann Institute; the rat Kv2.1 cDNA was a gift from J. Barhanin and M. Lazdunski, and the EGFR cDNA was a gift from Yosef Yarden, The Weizmann Institute. All cDNAs were cloned in the pcDNA3 expression vector (Invitrogen, Carlsbad, CA) and contained a FLAG tag at their C termini. Cherry-labeled α-tubulin cDNA was a gift of Benjamin Geiger, The Weizmann Institute. The following antibodies were used in this study: monoclonal anti-α-tubulin (clone DM1A; Sigma Chemical Co., St. Louis, MO), monoclonal anti-FLAG M2 (Sigma), monoclonal antiactin (clone AC-40; Sigma), monoclonal antiphosphotyrosine (clone PY99; Santa Cruz Biotechnology, Santa Cruz, CA); polyclonal anti-Kv2.1 (Alomone Laboratories, Jerusalem, Israel); monoclonal anti-PTP1B (BD Transduction Laboratories, Franklin Lakes, NJ); monoclonal anti-LIS1 (gift of Orly Reiner, The Weizmann Institute) (34); and monoclonal anti-Tau (Tau5; gift of Irith Ginzburg, The Weizmann Institute). All other reagents were purchased from Sigma.

Cell culture and fluorescence microscopy. 293 cells, NIH 3T3 cells, and HeLa-JW cells were grown in Dulbecco's modified Eagle's medium (Invitrogen), supplemented with 10% (vol/vol) fetal calf serum (Invitrogen), 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. Jurkat cells were grown in RPMI 1640 medium (Sigma) supplemented with heat-inactivated fetal calf serum, glutamine, and antibiotics as described above. Cells were transfected using the calcium phosphate method (5). For fluorescence studies, cells were washed with phosphate-buffered saline (PBS), fixed in methanol at −20°C for 10 min, washed five times with PBS, and mounted with Fluoromount-G solution (Southern Biotech, Birmingham, AL). Cells were examined using a DeltaVision system (Applied Precision, Inc., Issaquah, WA) and Resolve3D software.

Biochemical purification of tubulin and MAPs. Tubulin was purified from fresh calf brains as described previously (15). Tubulin was purified by three cycles of polymerization/depolymerization, followed by chromatography on a phosphocellulose column to remove MAPs and by a fourth cycle of polymerization/depolymerization. Purified tubulin was resuspended in buffer PM2: 100 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], 2 mM EGTA, 2 mM $MgSO_4$ (pH 6.9). MAPs were eluted from the phosphocellulose column with 1 M KCl and desalted using a Vivaspin 20-ml centrifugal concentrator (Sartorius Vivascience GmbH, Goettingen, Germany). MAPs were diluted in PM2 buffer to a final concentration of 1.5 μg/μl.

Extraction of tubulin from mouse brain. Brains from PTPε-deficient (EKO) (33) or wild-type (WT) mice were homogenized in MES++ buffer (100 mM MES [morpholineethanesulfonic acid], 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol [DTT], 1% Nonidet P-40 [pH 7.4]) supplemented with the following protease inhibitors: 1 mM AEBSF [4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride], 40 μM bestatin, 15 μM E-64, 20 μM leupeptin, and 15 μM pepstatin (Sigma). The extracted material was centrifuged at 30×10^3 *g* and then at 100×10^3 *g* at 4°C to remove polymerized microtubules. In vitro polymerization of microtubules from the supernatant was performed in G-MES++ buffer (MES++ buffer supplemented with 4 M glycerol, 1 mM GTP, 0.4 mM DTT, 5 μM Taxol, and protease inhibitors) for 45 min at 37°C. Following centrifugation at 100×10^3 *g* at 37°C, the pellet containing polymerized microtubules was washed three times in MES++ buffer and was dissolved in 200 μl of buffer A (50 mM Tris-Cl [pH 7.5], 150 mM NaCl, 1% Nonidet P-40). The experiment shown in Fig. 1F was carried out in a similar manner, except that purified cyt-PTPε was added prior to the polymerization step.

Immunoprecipitation and protein blotting. Immunoprecipitation and elution of FLAG-tagged PTPε expressed in 293 cells were carried out as described previously (41). The purity and amounts of eluted material were determined by gel electrophoresis and silver staining. In some studies, endogenous tubulin or PTP1B was precipitated from 1-mg cell lysates prepared in buffer A using the relevant antibodies and protein A/G Sepharose beads (Santa Cruz). Tyrosine-phosphorylated Kv2.1 for use as a substrate of PTPε was purified from 293 cells cotransfected with FLAG-tagged Kv2.1 and activated (Y527F) Src by anti-FLAG immunoprecipitation and elution with FLAG peptide as described previously (41). Sodium dodecyl sulfate (SDS) gel electrophoresis, blotting, and antibody hybridization were done as described previously (18).

Phosphatase and kinase activity assays. Phosphatase activity was assayed in 96-well plates. Each well typically contained 0.1 μg of PTPε in 200 μl of activity assay buffer (50 mM MES, 0.5 mg/ml bovine serum albumin, 0.5 mM DTT, and 10 mM *para*-nitrophenylphosphate [PNPP], pH 7.0). Under these conditions PTPε activity is linear with time and amount of enzyme. Increasing amounts of purified bovine tubulin, bovine serum albumin (BSA; fraction V [MP Biomedicals, Solon, OH]) or actin (Sigma) were added, and activity was measured by monitoring the increase in absorption at 405 nm at room temperature for 1 h. The K_m and V_{max} for PTPε were determined by Lineweaver-Burk analysis by measuring initial PTPε activity in the presence of increasing concentrations of PNPP (0.6 to 15.0 mM) for 5 to 10 min, with or without added tubulin. In some studies, the phosphatase activity of 0.3 μg of calf intestinal alkaline phosphatase (CIP; New England Biolabs, Beverly, MA), 0.6 μg of the cytosolic domain of RPTPμ (Jena Biosciences GmbH, Jena, Germany), or 0.3 μg of endogenous PTP1B precipitated from 293 cells was measured similarly. In other studies, 25 ng of eluted phospho-Kv2.1 was incubated either alone or with 30 ng of purified cyt-PTPε and with 25 to 50 μg purified bovine brain tubulin, actin, or BSA in PTP activity buffer at 32°C for 2 h. The reaction was terminated by addition of SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer and boiling. Phosphorylation of Kv2.1 was analyzed by SDS-PAGE and protein blotting with anti-pTyr antibodies. Src activity assay was performed using enolase according to reference 16.

Interaction between tubulin and PTPε. Eluted cyt-PTPε (0.1 μg) was incubated with 20 μg purified bovine tubulin, either with or without 15.5 μg eluted bovine MAPs, for 1 h at room temperature. PTPε was immunoprecipitated with anti-FLAG antibodies, and associated tubulin was detected by protein blotting. To examine the interaction in cells following EGFR stimulation, endogenous or exogenous EGFR was stimulated in 293 cells with 100 ng/ml of EGF (Sigma) or vehicle (PBS). Cells were lysed in buffer A supplemented with 0.5 mM sodium vanadate at various time points; FLAG-tagged cyt-PTPε was immunoprecipitated and examined by protein blotting. Detection of cyt-PTPε phosphorylation following EGFR stimulation was done in a similar manner using buffer A containing 0.5 mM sodium pervanadate.

RESULTS

PTPε binds tubulin in vivo. In order to identify proteins that interact with PTPε in cells, we expressed the nonreceptor form of the phosphatase, cyt-PTPε, in 293 cells and examined proteins that coprecipitated with it. In this study, we utilized D245A cyt-PTPε, a "substrate-trapping" mutant of PTPε that is virtually inactive but retains the ability to bind substrates and interacting molecules in a cellular context (12). SDS-PAGE analysis of D245A cyt-PTPε immunoprecipitates revealed a coprecipitating protein with a mass of approximately 55 kDa that was subsequently identified by mass spectrometry as α-tubulin (not shown). Further immunoprecipitation experiments confirmed that cyt-PTPε as well as RPTPε bound α-tubulin (Fig. 1A). Both WT and D-to-A trapping mutants of cyt-PTPε and RPTPε bound α-tubulin similarly (Fig. 1A); this result, therefore, does not shed light on whether or not tubulin is a substrate of PTPε, although it does indicate that a complex that contains both molecules exists. Binding was specific since a nonrelated protein, DAP4, did not bind α-tubulin and since actin, a second cytoskeletal protein of high abundance, did not coprecipitate with PTPε (Fig. 1A and B). The association between PTPε and α-tubulin was observed also in HeLa and in A431 cells, as well as when the experiment was performed in reverse by precipitating α-tubulin (see Fig. 3B) (data not

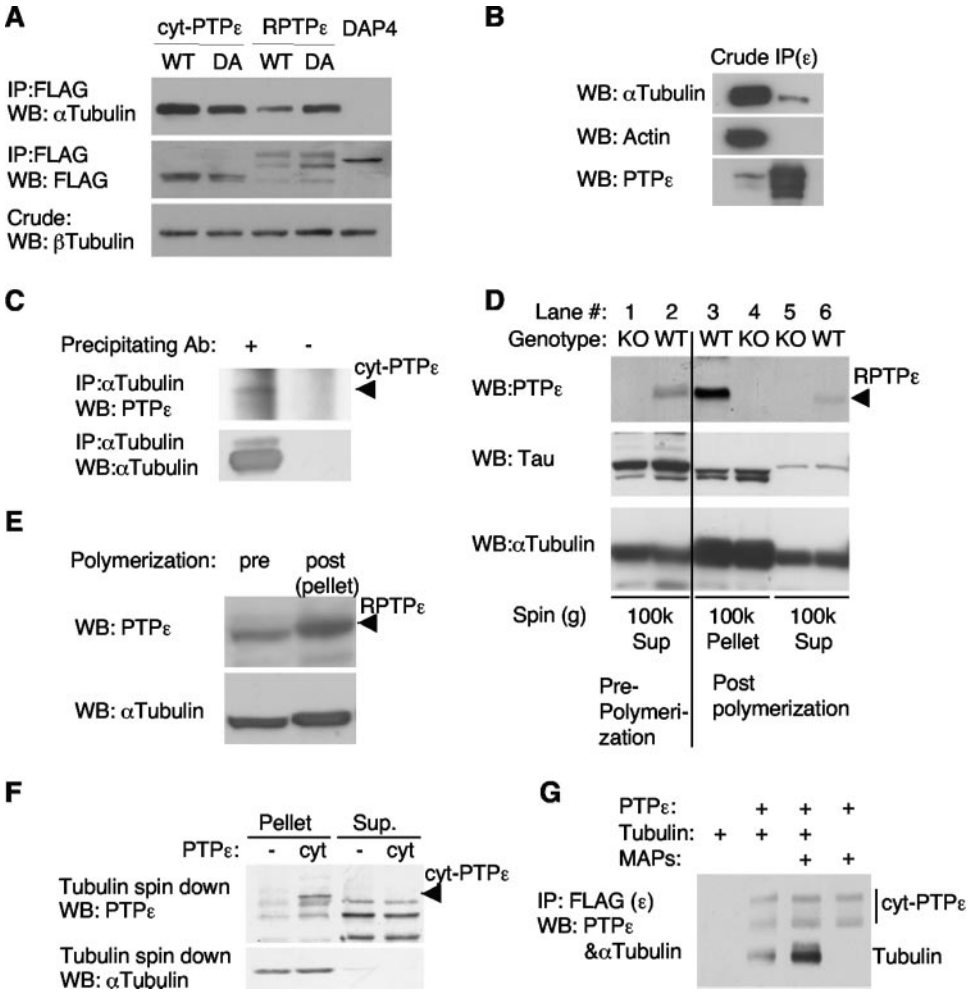

FIG. 1. PTPε binds tubulin in vivo and in vitro. (A) RPTPε, cyt-PTPε, and DAP4 were expressed in 293 cells, precipitated via their FLAG tags, and analyzed by protein blotting. Similar results were obtained with β-tubulin (not shown). IP, immunoprecipitation; WB, Western blotting. (B) cyt-PTPε was expressed in 293 cells and processed as in panel A to show that actin does not bind PTPε. (C) Tubulin was immunoprecipitated from Jurkat cells; protein blotting revealed presence of endogenous cyt-PTPε in the precipitated material. Ab, antibody. (D) Endogenous RPTPε binds tubulin in mouse brain. Polymerized microtubules were removed from brain lysates of PTPε-deficient (EKO) and WT mice by centrifugation at 30,000 × *g* (not shown) and 100,000 × *g* (lanes 1 and 2). Polymerization was induced, and precipitated microtubules (lanes 3 and 4) and unpolymerized material (lanes 5 and 6) were collected by centrifugation at 100,000 × *g*. Samples were analyzed by protein blotting for the presence of RPTPε, Tau, and α-tubulin. Note significant enrichment of endogenous RPTPε and of Tau in the newly polymerized microtubules (lanes 3 and 4). In contrast, actin was not enriched in any of the fractions (not shown). Sup, supernatant. (E) Similar to panel D showing association of RPTPε with tubulin in Neu-induced mouse mammary tumor cells. Shown are the amounts of PTPε and tubulin in the supernatant of the prepolymerized sample (pre) and in the pellet obtained after polymerization and centrifugation of the polymerized material at 100,000 × *g* (post). (F) Recapitulation of the tubulin-PTPε association in vitro. cyt-PTPε purified from expressing cells was added to tubulin obtained from brains of EKO mice. Following polymerization in vitro, microtubules were collected by centrifugation and analyzed for the presence of PTPε by protein blotting. Coomassie blue staining of a similar blot indicated most proteins present in the supernatant (two right lanes) did not sediment with microtubules (two left lanes [not shown]) (G) MAPs increase the tubulin-PTPε association. Purified bovine brain tubulin, bovine brain MAPs, and purified FLAG-tagged cyt-PTPε were incubated together as indicated. cyt-PTPε was precipitated and analyzed by protein blotting. Note that the presence of MAPs increases significantly the amount of tubulin bound to PTPε. The figure is representative of six repeats.

shown). Similar results were obtained also when binding to β-tubulin was examined (not shown); subsequent experiments were therefore performed with antibodies against α-tubulin alone. In separate experiments, comparison of the fraction of FLAG-tagged cyt-PTPε molecules that coprecipitated with a given fraction of precipitated tubulin molecules in 293 cells indicated that 10 to 15% of cyt-PTPε molecules and 0.3 to 0.4% of tubulin molecules participate in the PTPε-tubulin interactions. The last result agrees with the expression level of tubulin in these cells, which is significantly higher than that of cyt-PTPε.

Similar results were obtained when binding between endogenous PTPε and α-tubulin was examined. Accordingly, endogenous cyt-PTPε coprecipitated with α-tubulin from Jurkat T cells (Fig. 1C). In separate experiments, mouse brain lysate, which had been cleared of polymerized microtubules by centrifugation (Fig. 1D, lanes 1 and 2), was incubated at 37°C in the presence of GTP and glycerol to promote assembly of

nonpolymerized tubulin dimers into microtubules. Following their collection by centrifugation, examination of the newly assembled microtubules (Fig. 1D, lanes 3 and 4) revealed that significant amounts of endogenous RPTPε had coassembled and copurified with the precipitated microtubules. Similar results were obtained when binding of endogenous RPTPε to microtubules was examined in mammary tumor cells induced in vivo in mice by expression of an activated Neu transgene (Fig. 1E). Tau, a well-known MAP, was also enriched significantly in the precipitated microtubule fraction (Fig. 1D). Similar binding was observed when exogenous FLAG-tagged cyt-PTPε that had been purified from overexpressing cells was added to depolymerized tubulin obtained from mice genetically lacking PTPε prior to polymerization in vitro (Fig. 1F).

The interaction between PTPε and tubulin was reconstituted in vitro using purified cyt-PTPε and tubulin. These studies used tubulin from bovine brain that had undergone three cycles of assembly/disassembly and was stripped of tightly associated MAPs by phosphocellulose column chromatography. Tubulin was first incubated with cyt-PTPε, cyt-PTPε was then immunoprecipitated via its FLAG tag, and the presence of associated α-tubulin was examined by protein blotting. As seen in Fig. 1G, PTPε-bound α-tubulin was readily detected under these conditions. Interestingly, binding was strengthened significantly when MAPs, which were previously removed from tubulin during its purification, were added to the incubation mixture (Fig. 1G). Neither α-tubulin nor PTPε was detected in the MAP fraction. We conclude, therefore, that the tubulin–cyt-PTPε interaction is stabilized significantly by one or more components of the MAP fraction, which are physiological interactors of microtubules. In all, these results indicate that stable interactions exist between microtubules and the major forms of PTPε, RPTPε and cyt-PTPε.

cyt-PTPε colocalizes with microtubules in cells. The interaction between cyt-PTPε and microtubules was visualized in HeLa cells expressing fluorescently tagged cyt-PTPε and α-tubulin. Cells were fixed and permeabilized in cold methanol, which removes most soluble cytosolic molecules including nonbound cyt-PTPε. cyt-PTPε and tubulin colocalized throughout the cytosol in long fibrous structures (Fig. 2A to C and G to I); cyt-PTPε was detected also in the nucleus, in agreement with previous studies (24). Treatment of cells with nocodazole disrupted the cellular microtubule array and led to a diffuse, punctate cytosolic localization pattern for tubulin (Fig. 2E). Importantly, nocodazole treatment disrupted localization of cyt-PTPε (Fig. 2D and F), confirming that cyt-PTPε was associated with microtubules prior to nocodazole treatment. The qualitative nature of this experiment precluded quantitative conclusions about the effect of nocodazole on the PTPε-microtubule interaction. In order to address the last point, we expressed cyt-PTPε in 293 cells and disrupted their microtubules with nocodazole or colchicine. Coprecipitation of cyt-PTPε with α-tubulin was reduced in treated cells (Fig. 2J), indicating that disruption of the microtubule array reduces binding of PTPε. Stabilization of microtubules with taxol enhanced this interaction in some experiments (e.g., Fig. 2J) but not in others and requires further studies. Disruption of actin cytoskeletal components with cytochalasin D did not affect the PTPε-tubulin interaction. Residual binding of PTPε after disruption of microtubular structures, which is evident by immunofluorescence (Fig. 2D to F) and biochemically (Fig. 2J), may arise from incomplete depolymerization of microtubules or from weaker binding of cyt-PTPε to nonpolymerized tubulin heterodimers. Both explanations are consistent with cyt-PTPε binding preferentially microtubules in cells.

The N terminus and the D2 PTP domains of cyt-PTPε play major roles in binding tubulin. Throughout the above studies, full-length cyt-PTPε consistently bound more α-tubulin than p67 PTPε, a naturally occurring form of cyt-PTPε that lacks the 27 N-terminal residues (18) (Fig. 3A and B). This observation suggested that the N-terminal domain of cyt-PTPε mediates at least part of the ability of this PTP to bind α-tubulin. Further studies indicated that deletion of the first eight amino acids of cyt-PTPε did not affect association between cyt-PTPε and α-tubulin (Fig. 3B). In contrast, deletion of residues 1 to 22 or 11 to 27 or deletion of a larger portion of cyt-PTPε beginning at residue 13 and extending to the C terminus of the first PTP domain (construct D2+12) reduced binding to levels observed with p67 PTPε (Fig. 3B and C). These results suggest that at a minimum, residues between positions 13 and 22 participate in binding of cyt-PTPε to α-tubulin. These residues do not contain sequence motifs known to regulate binding to tubulin heterodimers or to microtubules; they do, however, include several charged residues and a potential phosphorylation site that may regulate binding indirectly.

In order to examine the role of the second, inactive (D2) PTP domain of cyt-PTPε in binding α-tubulin, we examined the tubulin-binding abilities of cyt-PTPε lacking this domain (Fig. 3A). Removal of the D2 PTP domain greatly reduced binding of cyt-PTPε to α-tubulin (Fig. 3C, left panel), attesting to its central role in mediating this interaction. Binding of Y638F cyt-PTPε, which lacks a prominent phosphorylation site at the C terminus of D2, was reduced significantly (Fig. 3D). This indicates that the presence of Y638 plays an important, but not exclusive, role in mediating the contribution of D2 to the PTPε-tubulin interaction. Participation of several domains of PTP molecules, including the D2 domain, in intermolecular associations of PTPs has been noted before (e.g., see reference 22).

Tubulin inhibits PTPε activity in a specific manner. In order to examine in vitro the consequences of the interaction between tubulin and cyt-PTPε we determined the relative amounts of both proteins in cells and tissues. For this purpose, protein lysates from several sources were analyzed by protein blotting. Membranes were probed with antibodies against α-tubulin or PTPε; the absolute amount of either protein in each sample was estimated by comparison to the signal generated by known amounts of purified tubulin or cyt-PTPε that were analyzed in parallel. As expected, a marked excess of α-tubulin was detected in all samples, with the α-tubulin/PTPε molar ratio (calculated per α-tubulin monomer) ranging from approximately 10:1 in Neu-induced mouse mammary tumor cells particularly rich in RPTPε to 1,315:1 in murine embryo fibroblasts (Fig. 4A).

Interactions between microtubules and PTPε may affect either molecule or other molecules that interact with them. In order to begin addressing this issue, we examined the catalytic activity of cyt-PTPε in vitro in the presence of purified tubulin. Addition of tubulin in excesses of 100-, 500-, and 1,000-fold versus cyt-PTPε reduced PTPε activity to 79.9%, 42.2%, and 25.4% of activity in the absence of tubulin, respectively (Fig.

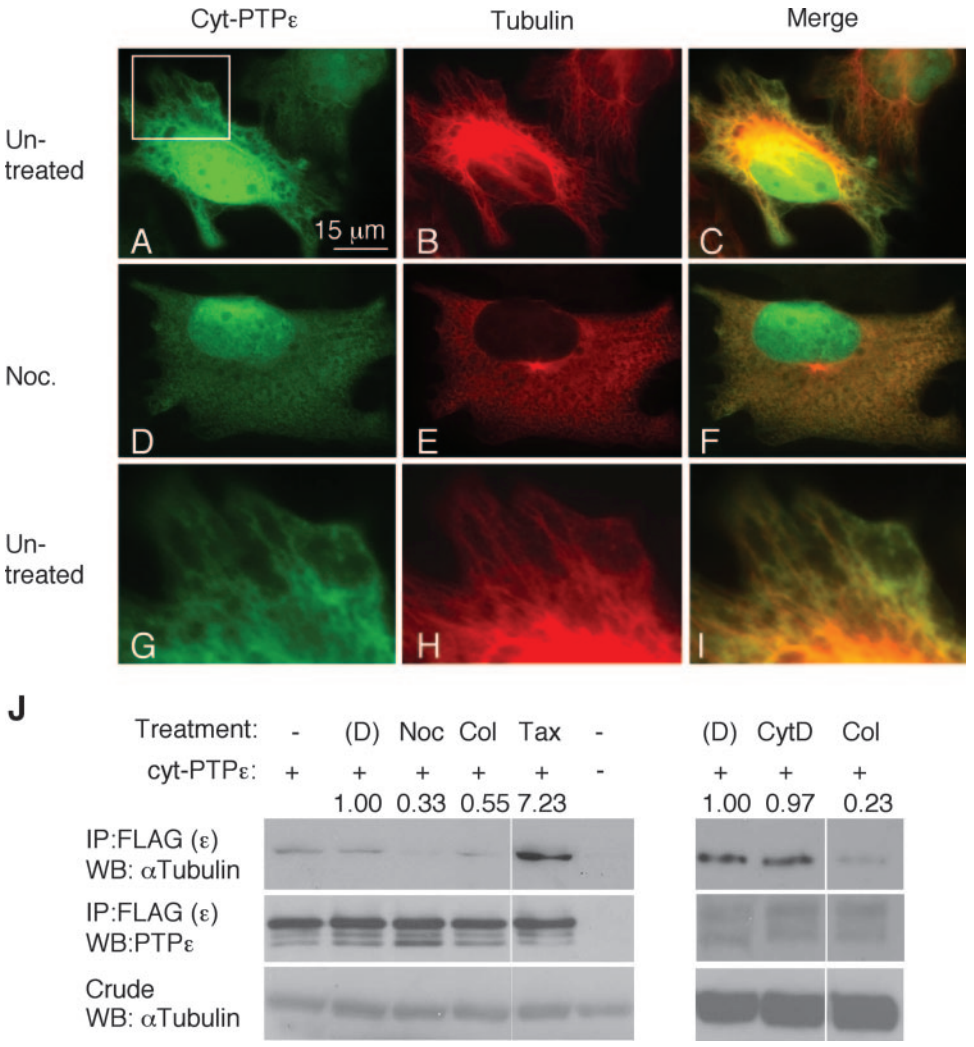

FIG. 2. cyt-PTPε colocalizes with microtubules in cells. (A to I) HeLa-JW cells were cotransfected with cyt-PTPε–green fluorescent protain (GFP) and cherry red-α-tubulin, fixed with methanol at −20°C, and analyzed by fluorescence microscopy. Shown are protein localizations in untreated cells (A to C and G to I) or following treatment with 10 μM nocodazole (Noc. [D to F]). Note similar disruption in localizations of cyt-PTPε and tubulin following nocodazole treatment. The rectangle in panel A outlines the region magnified in panels G to I. (J) 293 cells were transfected with FLAG-tagged cyt-PTPε, followed by treatment for 30 min with 10 μM nocodazole (Noc), 13.5 μM colchicine (Col), 10 μM taxol (Tax), or 10 μM cytochalasin D (CytD). cyt-PTPε was precipitated, and binding to tubulin was analyzed by protein blotting. The figure is representative of three repeats. Lane D contained cells treated with vehicle (dimethyl sulfoxide). Numbers above the top panel show relative intensities of the coprecipitated tubulin. WB, Western blotting; IP, immunoprecipitation.

4B). Heat-treated tubulin did not inhibit cyt-PTPε (Fig. 4B). Inhibition was observed also when purified tubulin was added to purified samples of RPTPε or p67 PTPε (Fig. 4B and results not shown), indicating that tubulin inhibits activity of all the major forms of PTPε in vitro under the conditions used. In agreement with its weak binding to tubulin, activity of cyt-PTPε lacking the D2 domain (construct D1+12 [Fig. 3C]) was only slightly inhibited by tubulin. Kinetic analysis of cyt-PTPε activity in the presence of purified tubulin indicated that tubulin is a noncompetitive inhibitor of the phosphatase. Addition of 40 μg purified tubulin to 0.1 μg purified cyt-PTPε did not affect the K_m of cyt-PTPε for PNPP, which remained at 4.57 ± 0.47 mM, but did reduce the maximal rate of the dephosphorylation reaction from 2.47 ± 0.35 to 0.86 ± 0.13 μmol/min/mg enzyme (mean ± standard error [SE]; $n = 3$; $P = 0.0128$ by Student's *t* test). The ability of tubulin to inhibit PTPε in vivo is likely to be stronger than measured here given the more significant amounts of microtubules and the presence of MAPs in cells, both of which increase the PTPε-tubulin interaction. These results also suggest that tubulin is not a substrate of cyt-PTPε and does not inhibit the phosphatase by competing against other substrates, in agreement with significant involvement of regions outside the D1 PTP domain in binding tubulin (Fig. 3).

Further studies indicated that the effect of tubulin was specific, since addition of 100-, 500-, or 1,000-fold excesses of actin or of BSA did not inhibit cyt-PTPε (Fig. 4C). Addition of actin actually increased cyt-PTPε activity somewhat, possibly due to increased effective concentrations of both cyt-PTPε and PNPP due to molecular exclusion effects of the added actin. Additional studies indicated that a different tyrosine phosphatase,

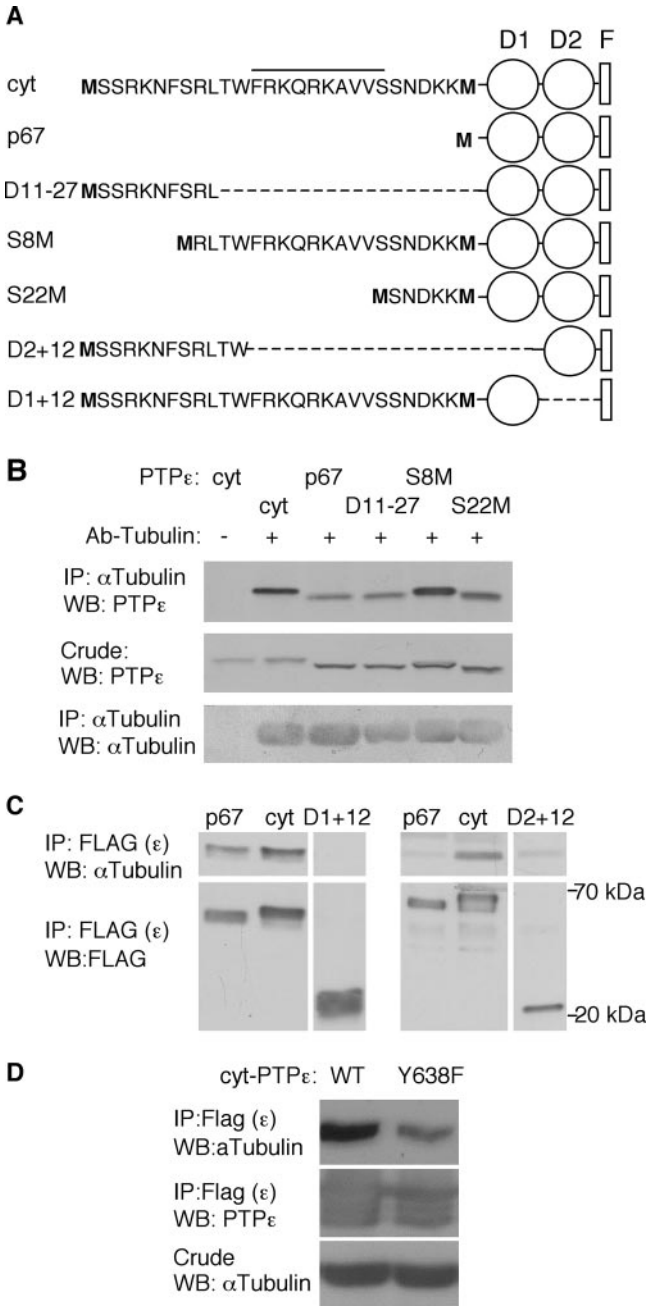

FIG. 3. The D2 domain and the amino terminus of PTPε help mediate the cyt-PTPε–tubulin interaction. (A) Schematic representation of FLAG-tagged cyt-PTPε constructs used in this study. The line above the topmost sequence represents part of the N-terminal region of cyt-PTPε important for binding tubulin. Circles represent PTP domains D1 and D2; rectangles represent the C-terminal FLAG tag. (B) The indicated constructs were expressed in 293 cells. Tubulin was immunoprecipitated (IP) from cell lysates, and associated cyt-PTPε was detected by protein blotting. WB, Western blotting. (C) Deletion of the D2 PTP domain of cyt-PTPε (construct D1+12) significantly reduced the interaction with tubulin, while removal of the D1 PTP domain and part of the N-terminal domain (construct D2+12) reduced binding to a lesser extent. The indicated proteins were expressed in 293 cells and precipitated via their FLAG tags. The presence of associated tubulin (top panel) and precipitated PTPε was determined by protein blotting. Size markers are in kDa. (D) The C-terminal Y638 of cyt-PTPε promotes binding of tubulin; Y638F cyt-PTPε bound less tubulin than WT cyt-PTPε.

PTP1B, is also inhibited in vitro by tubulin, but that a third PTP, the receptor-type RPTPμ, and alkaline phosphatase, which is not a PTP, are not inhibited (Fig. 4C). RPTPm contains a C-terminal tyrosine residue embedded in a sequence distinct from that of PTPε; it does not contain a sequence similar to the N-terminal sequence of cyt-PTPe. Binding of microtubules is therefore not unique to PTPε within the PTP family and needs to be examined for individual PTPs.

We next examined whether modulation of the interaction between cyt-PTPε and microtubules affects phosphatase activity. Treatment of cells with nocodazole reduced binding of cyt-PTPε to tubulin (Fig. 2J), an effect that would be expected to increase cyt-PTPε activity. In order to test this, we treated 293 cells expressing cyt-PTPε with nocodazole, immunoprecipitated the phosphatase, and assayed its activity. cyt-PTPε isolated from cells treated with nocodazole was 20.3% more active than the enzyme from untreated cells (Fig. 5A). Since only a fraction of cyt-PTPε molecules bind tubulin, the data in Fig. 5A, which were obtained using total cellular cyt-PTPε, most likely underestimate the effect of nocodazole on cyt-PTPε activity in cells. Importantly, this experiment demonstrates that association with microtubules can affect the activity of cyt-PTPε isolated from cells and that its effect is not limited to experiments in which purified components are combined in vitro.

Addition of MAPs increased binding of purified cyt-PTPε and tubulin in vitro (Fig. 1G), suggesting that MAPs may further reduce cyt-PTPε activity by stabilizing the PTPε-tubulin interaction. In agreement, addition of 25 μg purified tubulin to 0.1 μg purified cyt-PTPε reduced cyt-PTPε activity to 85.1% of cyt-PTPε activity in the absence of tubulin, while the presence of MAPs decreased activity further to 72.3% (Fig. 5B). In the absence of tubulin, MAPs did not inhibit cyt-PTPε activity (not shown), consistent with MAPs reducing cyt-PTPε activity by promoting its interaction with tubulin.

We next examined the effect of purified tubulin on the in vitro activity of cyt-PTPε towards a physiological substrate—the voltage-gated potassium channel Kv2.1. Activation of Kv2.1 by depolarization of the cell membrane can be increased by phosphorylation at Y124 by c-Src or c-Fyn; cyt-PTPε counters this effect by dephosphorylating Kv2.1 mainly at Y124 (33, 40, 41). Kv2.1 was coexpressed with activated (Y527F) Src in 293 cells, and phosphorylated Kv2.1 (pKv2.1) was immunoprecipitated and purified. Equal amounts of pKv2.1 were incubated with cyt-PTPε in the presence or absence of a 250- or 500-fold molar excess of purified tubulin, actin, or BSA. The remaining pKv2.1 was detected by blotting the purified Kv2.1 with antiphosphotyrosine antibodies. In agreement with the data obtained using PNPP (Fig. 4), the presence of tubulin, but not of actin or BSA, inhibited dephosphorylation of pKv2.1 by cyt-PTPε (Fig. 5C). Finally, cyt-PTPε activates Src (16), suggesting that disrupting the PTPε-microtubule interaction may further increase Src activity in cells. Treatment of 293 cells expressing WT Src with nocodazole reduced Src activity to 55.2%±13.3% of Src activity in nontreated cells (mean ± SE; $P = 0.0266$ by paired t test; $n = 5$ to 6). Expression of cyt-PTPε increased Src activity slightly to 110.2% ± 18.6%, while treatment of cyt-PTPε-expressing cells with nocodazole increased Src activity further to 160.3% ± 31.7% (mean ± SE; $P = 0.0318$ by paired t test; $n = 6$ [Fig. 5D]). Increased Src activity

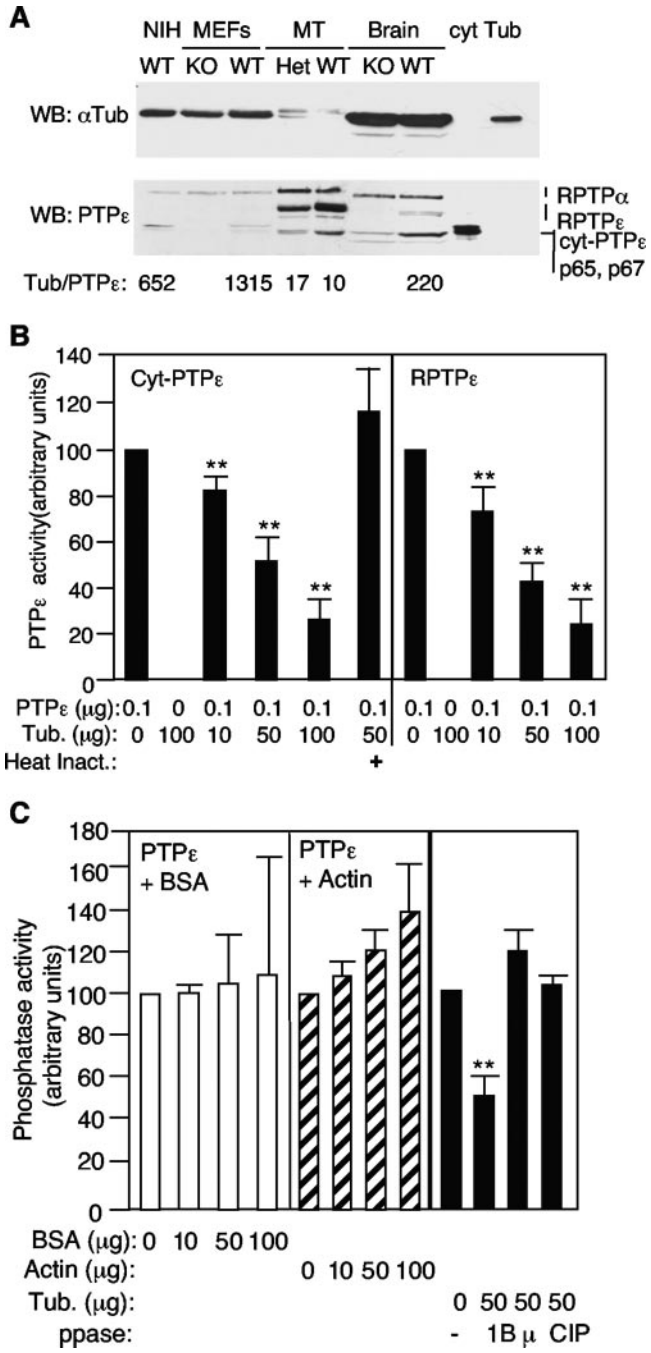

FIG. 4. Tubulin inhibits PTPε activity in vitro in a specific manner. (A) Amounts of endogenous PTPε and tubulin (Tub) in various cells and tissues. Extracts from NIH 3T3 cells (NIH), EKO and WT embryo fibroblasts (MEFs), mouse mammary tumor cells induced by Neu in female mice carrying two (WT) or one (Het) active *Ptpre* allele (MT) (16), and mouse brain, as well as 0.02 μg purified cyt-PTPε and 0.2 μg purified tubulin, were analyzed by protein blotting. The antibody used cross-reacts with RPTPα, which was not included in the calculation of the tubulin/PTPε ratio. WB, Western blotting. (B) Tubulin inhibits activities of cyt-PTPε (left panel) and RPTPε (right panel) in vitro. PTPε activity in the presence of 100-, 500-, or 1,000-fold excess of purified tubulin was reduced to 79.9% ± 3.9%, 42.2% ± 8.3%, and 25.4% ± 8.2% of cyt-PTPε activity in the absence of tubulin, respectively (mean ± SE; $n = 5$ or 6; $P \leq 0.0043$ by the Mann-Whitney test). Heat-treated (Heat inact.) tubulin did not inhibit cyt-PTPε (left panel). (C) Activity of 0.1 μg purified cyt-PTPε towards PNPP was not inhibited in the presence of similar amounts of actin or BSA (left and middle panels, respectively). Purified tubulin inhibits activity of 0.1 μg PTP1B (1B) but not that of the receptor type RPTPμ (μ) or of the unrelated CIP (right panel).

in the presence of PTPε in cells following microtubule disruption is consistent with microtubules inhibiting cyt-PTPε in vivo, although further studies are required to determine whether this is caused exclusively by breakdown of the PTPε-microtubule interaction.

EGFR-mediated phosphorylation at Y638 drives cyt-PTPε to bind tubulin in vivo. Previous studies have shown that activation of the EGFR results in phosphorylation of cyt-PTPε within minutes in a temporal pattern that follows closely autophosphorylation of the EGFR itself. This phenomenon occurs when either exogenous (41) or endogenous (Fig. 6A) EGFR is examined. Interestingly, EGFR signaling also resulted in increased association between cyt-PTPε and α-tubulin that followed a very similar time course (Fig. 6B). Basal association between cyt-PTPε and tubulin was detected in the cells studied (not visible in the exposure shown in Fig. 6B). Association between tubulin and cyt-PTPε increased significantly as early as 1 min following addition of EGF to the cells; the association peaked at 2.5 and 5 min and receded afterwards. Disruption of microtubules with colchicine reduced basal association between cyt-PTPε and tubulin and prevented its increase following stimulation with EGF (Fig. 6C).

The temporal correlation between EGFR activation, cyt-PTPε phosphorylation, and association between α-tubulin and cyt-PTPε suggests that phosphorylation of cyt-PTPε may regulate its ability to bind tubulin. A prime candidate site for EGFR-mediated phosphorylation of cyt-PTPε is its C-terminal Y638, whose presence supports the PTPε-tubulin interaction (Fig. 3D). Y638 is analogous to Y695 of RPTPε and to Y789 of the related RPTPα, whose phosphorylation is crucial for PTP-mediated activation of c-Src (4, 49). In agreement, Y638F cyt-PTPε was not phosphorylated even following activation of exogenous EGFR, which phosphorylates WT cyt-PTPε and promotes its association with microtubules more strongly than the endogenous EGFR (Fig. 7A) (data not shown). This result indicates that EGFR-mediated phosphorylation of cyt-PTPε is highly specific and affects this single tyrosine from among the 22 tyrosine residues present in this phosphatase.

In order to determine whether EGFR-mediated phosphorylation of cyt-PTPε at Y638 is important for cyt-PTPε-tubulin binding, we examined this association in 293 cells following stimulation of their endogenous EGFR. As in previous experiments, the association between WT cyt-PTPε and α-tubulin increased transiently during the first few minutes following EGFR stimulation. In contrast, no increase in binding of α-tubulin was observed in cells expressing Y638F cyt-PTPε (Fig. 7B). cyt-PTPε autodephosphorylates strongly (4), a finding that precluded isolation of cyt-PTPε and measuring its activity while still phosphorylated and associated with microtubules immediately following EGFR activation. Nevertheless, results presented in this study indicate that cyt-PTPε is inhibited by associating with microtubules in cells and that this association is influenced by transient phosphorylation of cyt-PTPε at Y638

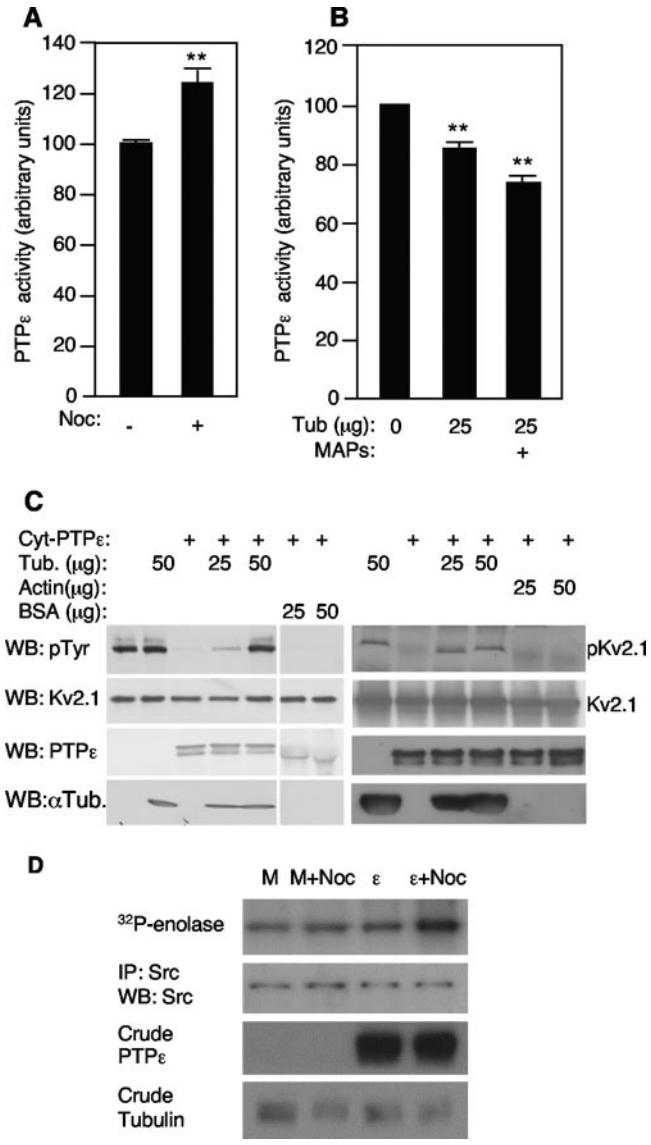

FIG. 5. Regulation of tubulin binding to cyt-PTPε affects phosphatase activity. (A) Disruption of microtubules increases cyt-PTPε activity. 293 cells expressing FLAG-tagged cyt-PTPε were treated with 10 μM nocodazole (Noc) or vehicle (dimethyl sulfoxide [DMSO]) for 2 h, and lysed, and activity of immunoprecipitated cyt-PTPε towards PNPP was measured. Nocodazole treatment increased cyt-PTPε activity by 20.3% ± 5.6% (mean ± SE; $n = 7$; $P = 0.0003$ by the Mann-Whitney test). (B) Activity of 0.1 μg purified cyt-PTPε towards PNPP was assayed in the presence or absence of 25 μg purified tubulin and 15.5 μg MAPs. Addition of tubulin reduced cyt-PTPε activity to 85.1% ± 2.7%; addition of MAPs decreased the activity further to 72.3% ± 2.7% (mean ± SE; $n = 5$ to 6, $P < 0.0159$ by the Mann-Whitney test). (C) Tubulin inhibits activity of cyt-PTPε towards Kv2.1. Purified tubulin, actin, or BSA was incubated together with 0.03 μg purified cyt-PTPε and 0.025 μg purified phospho-Kv2.1, as indicated in the figure, at 30°C for 2 h. Proteins were analyzed by Western blotting (WB) for phospho-Kv2.1 (top panel), as well as the presence of Kv2.1, cyt-PTPε, and α-tubulin (second to fourth panels, respectively). BSA comigrates with cyt-PTPε and distorts the cyt-PTPε bands when added. (D) Inhibition of the PTPε-tubulin interaction increases the ability of PTPε to activate Src in cells. 293 cells expressing FLAG-tagged cyt-PTPε and Src were treated with 10 μM nocodazole or vehicle (DMSO) for 45 min and lysed. Src was immunoprecipitated (IP), and its activity (ability to phosphorylate enolase) was measured. Shown are phosphorylated enolase (top panel), Src present in precipitates (second panel), and amounts of cyt-PTPε and endogenous tubulin in the crude lysates (lower two panels). Shown is an experiment representative of five repeats.

that occurs in vivo following physiological activation of the EGFR.

DISCUSSION

In this study, we show that microtubules are physiological interactors of PTPε and that this association inhibits activity of PTPε. The association between microtubules and the two major forms of PTPε, RPTPε and cyt-PTPε, can be detected biochemically when the endogenous proteins are examined as well as when PTPε is expressed in a variety of cells. Furthermore, tubulin and cyt-PTPε colocalize in microtubule structures, and the organizations of both proteins are affected strongly and similarly when microtubules are disrupted. Importantly, actin, another major cytoskeletal protein, does not associate with PTPε under the same conditions, attesting to the specificity of this interaction.

The tubulin-PTPε interaction can also be recapitulated in vitro using purified proteins. A striking finding here is that the interaction is strengthened significantly in the presence of MAPs (Fig. 1G). The finding that a third protein component that is known to bind microtubules physiologically stabilizes the PTPε-tubulin interaction further supports its physiological context. This finding also suggests that the interaction between PTPε and microtubules may be indirect, accounting in part for how PTPε and PTP1B, which are structurally distinct, can both be influenced by microtubules. Finally, the requirement for MAPs and absence of PTPε from the MAP fraction itself indicate that the phosphatase is not among the proteins that interact most tightly with microtubules, further suggesting that this interaction may be subject to dynamic regulation by physiological processes.

Analysis of the structural requirements of this interaction sheds light on its regulation and further supports its physiological relevance. The role of the D2 PTP domain is crucial, since deleting it significantly reduces the interaction. Phosphorylation of Y638 in the D2 domain of cyt-PTPε (Y695 in RPTPε) plays a significant, but not exclusive, role in supporting the interactions mediated by D2. The role of the short N-terminal region of cyt-PTPε is also striking, since removal of residues 13 to 22 of this domain decreases binding by about 50%. Several tubulin-binding sequence motifs have been described to date, such as the imperfect 18-residue repeats of MAP2 and Tau (21, 27), multiple copies of the short KKEE/I/V motif found in MAP1B (32), or the PX_6EX_4L motif found in dynein heavy chain, kinesin, and related molecules (46). None of these motifs are present in cyt-PTPε, suggesting that cyt-PTPε binds tubulin via an uncharacterized domain or that binding is indirect. As indicated above, the latter possibility is consistent with enhancement of the cyt-PTPε–tubulin interaction by MAPs. The first 27 residues of cyt-PTPε contain 9 positively charged residues and 6 serine/threonine potential phosphorylation sites; of these, 4 charged residues and 1 serine are located between positions 13 and 22. Further study is

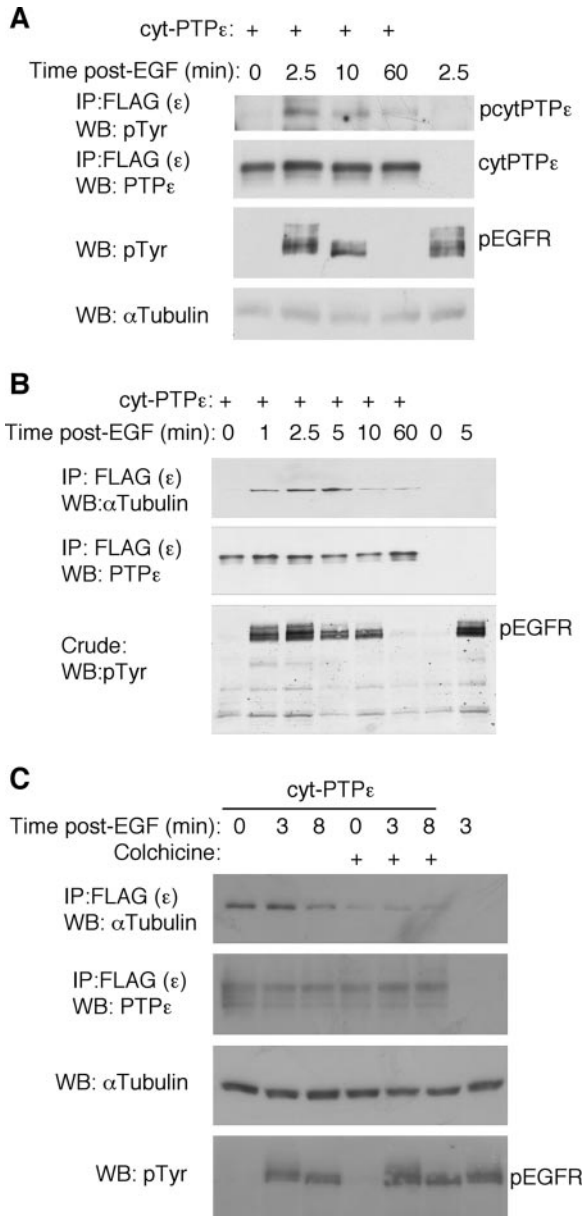

FIG. 6. EGFR activation drives cyt-PTPε to bind tubulin in vivo. (A) Activation of the endogenous EGFR causes phosphorylation of cyt-PTPε. 293 cells transfected with cyt-PTPε were activated with 100 ng/ml EGF. Cells were lysed at 0, 2.5, 10, and 60 min after EGF stimulation, and phosphorylation of precipitated cyt-PTPε was analyzed. Phosphorylation of PTPε peaked 2.5 min after stimulation with EGF (top panel), coinciding with the peak of EGFR autophosphorylation (third panel). Also shown are precipitated cyt-PTPε (second panel) and tubulin in crude lysates as a loading control. Panel A represents three repeats. IP, immunoprecipitation; WB, Western blotting. (B) EGFR stimulation drives cyt-PTPε to associate with tubulin. The experiment in panel A was repeated, assaying for tubulin that coprecipitated with cyt-PTPε following EGFR activation. Again, the time course of the cyt-PTPε interaction broadly agrees with that of EGFR autophosphorylation. Panel B is representative of six repeats. (C) Disruption of microtubules reduces the cyt-PTPε–tubulin association and prevents its increase following EGFR activation. 293 cells expressing cyt-PTPε were treated with 10 μM colchicine or dimethyl sulfoxide for 45 min and then activated with EGF. Cells were lysed at the indicated time points, and PTPε was precipitated.

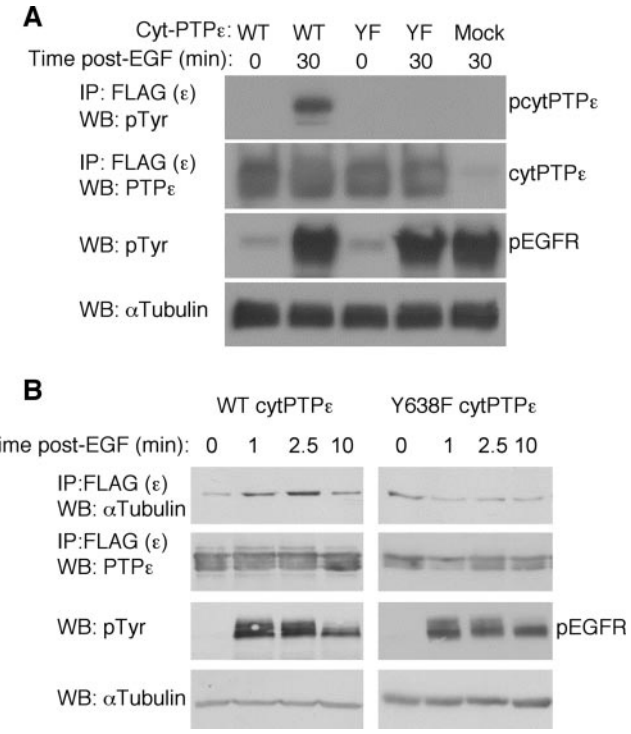

FIG. 7. EGFR-mediated phosphorylation of cyt-PTPε at Y638 drives cyt-PTPε to bind tubulin in vivo. (A) EGFR activation induces phosphorylation of cyt-PTPε specifically at Y638. 293 cells were cotransfected with EGFR and either WT or Y638F cyt-PTPε. Following stimulation with EGF for 30 min, phosphorylation of precipitated cyt-PTPε was analyzed by blotting (top two panels). Also shown are autophosphorylation of the EGFR (third panel) and a loading control (fourth panel). Panel A represents six repeats. IP, immunoprecipitation; WB, Western blotting. (B) Phosphorylation at Y638 is required for cyt-PTPε to associate with tubulin following EGFR activation. Activation of the endogenous EGFR increased transiently the basal association between tubulin and WT, but not Y638F, cyt-PTPε (top two panels). Also shown are EGFR autophosphorylation and a loading control (bottom two panels). Panel B is representative of five repeats.

required to determine if any of these residues participate in indirect binding of tubulin. The precise molecular mechanism by which tubulin binding inhibits PTPε is not clear at the present time. The noncompetitive nature of this inhibition suggests that bound tubulin or MAPs may alter the conformation of PTPε, thus reducing its activity. Complete accounting of this effect will be aided by identification of the relevant MAP(s) and determination of the structure of the PTPε-MAP/tubulin complex.

Binding of PTPε to tubulin may affect the phosphatase, microtubules, or other proteins that associate with microtubules. Limited available information indicates that tubulin is phosphorylated in B lymphocytes by the tyrosine kinase Syk and following stimulation of Jurkat T cells with alpha interferon (47). In other studies, c-Fes phosphorylated tubulin and promoted tubulin polymerization in vitro (26). The tyrosine phosphatase PTP-BL localizes to centrosomes during interphase and metaphase and to the spindle midzone during anaphase, and elevated PTP-BL expression leads to defects in cytokinesis (20). CDC14B, a dual-specificity phosphatase, binds and stabilizes microtubules in vitro and in interphase

cells (8). We detected basal phosphorylation of tubulin, although this was not affected by expression of PTPε (not shown).

In this study, we focused on the effect of binding on PTPε activity, showing that presence of tubulin inhibits cyt-PTPε activity in vitro when both proteins are present in ratios in the range found in cells and tissues. Importantly, tubulin inhibited PTPε activity also towards Kv2.1 and towards Src, both of which are physiological substrates of the phosphatase. The finding that tubulin inhibits PTPε activity while similar amounts of actin or BSA do not do so argues strongly that tubulin has a unique effect on the phosphatase. Similarly, the ability of tubulin to inhibit the tyrosine phosphatase PTP1B but not RPTPm or the unrelated alkaline phosphatase suggests that the effect of tubulin is not common to all types of phosphatases and should be examined on a case-by-case basis within the PTP family. Of note, PTP1B does not contain the N-terminal and D2 domains of cyt-PTPε, which play important roles in binding tubulin. This suggests that the mechanisms by which both PTPs interact with tubulin are distinct.

In support of the above results, treatments that affect binding of cyt-PTPε to microtubules cause the expected changes in cyt-PTPε activity. Accordingly, addition of MAPs purified from bovine brain, which increase binding of cyt-PTPε to tubulin, significantly increased the inhibitory effect of tubulin on activity of purified cyt-PTPε (Fig. 5B). These results indicate that interactions with other proteins may modify further the effect of tubulin on PTPε activity in vivo. In other experiments, disruption of the cellular microtubule network by nocodazole treatment increased activity of cyt-PTPε purified from these cells (Fig. 5A) and increased activation of Src by cyt-PTPε in the cells (Fig. 5D). Importantly, the last results show that PTPε is inhibited by its interaction with tubulin not only in vitro but also in intact cells. We note that nocodazole-induced mitotic arrest of cells increased activity of the related RPTPα and increased its ability to activate Src (50) in a manner suggested to be linked with phosphorylation of the juxtamembrane residues S180 and S204 of RPTPα (48). Although the effect of nocodazole on RPTPα activity is consistent with results presented in the present study, the experimental systems are not identical. In particular, nocodazole-induced mitotic arrest requires significantly longer treatment of cells with nocodazole (10 h [50] versus 45 min in the present study); furthermore, S180 is not conserved in PTPε, and replacement of the residue in PTPε analogous to S204 with an aspartic acid residue did not affect PTPε activity towards Src (4). It remains, therefore, to be determined whether the molecular mechanisms that regulate phosphatase activity are the same in both cases.

Binding of cyt-PTPε to tubulin is affected by physiological signals in a reversible manner and with rapid kinetics typical of signaling processes. Activation of the EGFR results in phosphorylation of cyt-PTPε specifically at its C-terminal Y638, an event that is required for increased binding of the phosphatase to tubulin in this context. Increased binding and phosphorylation of cyt-PTPε occur rapidly after receptor activation and follow closely the time course of receptor autophosphorylation. Since association with microtubules reduces activity of cyt-PTPε in cells (Fig. 5A), this result makes it tempting to speculate that increased binding of cyt-PTPε to microtubules during the initial stages of EGFR activation reduces phosphatase activity and removes it from the immediate vicinity of the receptor. The dependence on specific phosphorylation of cyt-PTPε downstream of the EGFR should ensure that cyt-PTPε binds microtubules in concert with a true physiological signal and in a manner most likely limited to the vicinity of the activated receptor. At later time points, phosphorylation of cyt-PTPε and its binding to microtubules are reduced, restoring the conditions that existed prior to EGFR stimulation. This latter process is most likely driven by the normal down-regulation of EGFR signaling and by auto- and *trans*-dephosphorylation of cyt-PTPε (4). We note that the receptor type of PTPε, RPTPε, binds microtubules constitutively in mammary tumor cells induced in mice by Neu. RPTPε is constitutively phosphorylated at Y695 (Y638 of cyt-PTPε) in these cells (4), providing another correlation between phosphorylation of PTPε at its C-terminal tyrosine downstream of a (constitutively active) growth factor receptor and increased binding of microtubules. RPTPε activates Src and supports the transformed phenotype of these tumor cells (16), suggesting that the fraction of RPTPε molecules that bind microtubules in this system may perform a distinct role. The precise mechanism by which phosphorylation of PTPε promotes its binding to microtubules is unknown at present. It is possible that phosphorylation promotes association of cyt-PTPε with additional proteins that are needed to stabilize the interaction with microtubules, in agreement with promotion of this interaction by MAPs. Further studies are required to address this issue.

In summary, this study suggests that association of PTPε with microtubules is a novel mechanism for regulation of PTPε activity that is regulated by physiological signals in a dynamic and reversible manner. We note that our data do not rule out other possible roles for the tubulin-PTPε association, such as regulating microtubule dynamics or providing access for PTPε to other molecules that also bind microtubules. The data suggest that the effect of microtubules is not limited to PTPε but may affect other tyrosine phosphatases, thereby providing an additional mechanism for physiological regulation of phosphatase activity.

ACKNOWLEDGMENTS

We thank the colleagues mentioned in the text for generous gifts of reagents used in this study. We also thank Orly Reiner of The Weizmann Institute for critical reading of the manuscript.

This study was supported by a grant from the Israel Science Foundation, founded by the Israel Academy of Sciences and Humanities.

REFERENCES

1. **Alonso, A., J. Sasin, N. Bottini, I. Friedberg, A. Osterman, A. Godzik, T. Hunter, J. Dixon, and T. Mustelin.** 2004. Protein tyrosine phosphatases in the human genome. Cell **117:**699–711.
2. **Andersen, J. N., A. Elson, R. Lammers, J. Romer, J. T. Clausen, K. B. Moller, and N. P. Moller.** 2001. Comparative study of protein tyrosine phosphatase-epsilon isoforms: membrane localization confers specificity in cellular signalling. Biochem. J. **354:**581–590.
3. **Andersen, J. N., P. G. Jansen, S. M. Echwald, O. H. Mortensen, T. Fukada, R. Del Vecchio, N. K. Tonks, and N. P. Moller.** 2004. A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. FASEB J. **18:**8–30.
4. **Berman-Golan, D., and A. Elson.** 2007. Neu-mediated phosphorylation of protein tyrosine phosphatase epsilon is critical for activation of Src in mammary tumor cells. Oncogene [E-pub ahead of print.] doi:10.1038/sj.onc.1210505.
5. **Chen, C. A., and H. Okayama.** 1988. Calcium phosphate-mediated gene transfer: a highly efficient transfection system for stably transforming cells with plasmid DNA. BioTechniques **6:**632–638.
6. **Chen, M., S. C. Chen, and C. J. Pallen.** 2006. Integrin-induced tyrosine phosphorylation of protein-tyrosine phosphatase-alpha is required for cytoskeletal reorganization and cell migration. J. Biol. Chem. **281:**11972–11980.

7. **Chiusaroli, R., H. Knobler, C. Luxenburg, A. Sanjay, S. Granot-Attas, Z. Tiran, T. Miyazaki, A. Harmelin, R. Baron, and A. Elson.** 2004. Tyrosine phosphatase epsilon is a positive regulator of osteoclast function in vitro and in vivo. Mol. Biol. Cell **15:**234–244.
8. **Cho, H. P., Y. Liu, M. Gomez, J. Dunlap, M. Tyers, and Y. Wang.** 2005. The dual-specificity phosphatase CDC14B bundles and stabilizes microtubules. Mol. Cell. Biol. **25:**4541–4551.
9. **den Hertog, J., A. Groen, and T. van der Wijk.** 2005. Redox regulation of protein-tyrosine phosphatases. Arch. Biochem. Biophys. **434:**11–15.
10. **Elson, A., and P. Leder.** 1995. Identification of a cytoplasmic, phorbol ester-inducible isoform of protein tyrosine phosphatase epsilon. Proc. Natl. Acad. Sci. USA **92:**12235–12239.
11. **Elson, A., and P. Leder.** 1995. Protein-tyrosine phosphatase epsilon. An isoform specifically expressed in mouse mammary tumors initiated by v-Ha-ras OR neu. J. Biol. Chem. **270:**26116–26122.
12. **Flint, A. J., T. Tiganis, D. Barford, and N. K. Tonks.** 1997. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc. Natl. Acad. Sci. USA **94:**1680–1685.
13. **Frangioni, J. V., A. Oda, M. Smith, E. W. Salzman, and B. G. Neel.** 1993. Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP-1B) in human platelets. EMBO J. **12:**4843–4856.
14. **Fujikawa, A., D. Shirasaka, S. Yamamoto, H. Ota, K. Yahiro, M. Fukada, T. Shintani, A. Wada, N. Aoyama, T. Hirayama, H. Fukamachi, and M. Noda.** 2003. Mice deficient in protein tyrosine phosphatase receptor type Z are resistant to gastric ulcer induction by VacA of Helicobacter pylori. Nat. Genet. **33:**375–381.
15. **Fygenson, D. K., E. Braun, and A. Libchaber.** 1994. Phase diagram of microtubules. Phys. Rev. E **50:**1579–1588.
16. **Gil-Henn, H., and A. Elson.** 2003. Tyrosine phosphatase-epsilon activates Src and supports the transformed phenotype of Neu-induced mammary tumor cells. J. Biol. Chem. **278:**15579–15586.
17. **Gil-Henn, H., G. Volohonsky, and A. Elson.** 2001. Regulation of protein-tyrosine phosphatases alpha and epsilon by calpain-mediated proteolytic cleavage. J. Biol. Chem. **276:**31772–31779.
18. **Gil-Henn, H., G. Volohonsky, H. Toledano-Katchalski, S. Gandre, and A. Elson.** 2000. Generation of novel cytoplasmic forms of protein tyrosine phosphatase epsilon by proteolytic processing and translational control. Oncogene **19:**4375–4384.
19. **Granot-Attas, S., and A. Elson.** 2004. Protein tyrosine phosphatase epsilon activates Yes and Fyn in Neu-induced mammary tumor cells. Exp. Cell Res. **294:**236–243.
20. **Herrmann, L., T. Dittmar, and K. S. Erdmann.** 2003. The protein tyrosine phosphatase PTP-BL associates with the midbody and is involved in the regulation of cytokinesis. Mol. Biol. Cell **14:**230–240.
21. **Himmler, A., D. Drechsel, M. W. Kirschner, and D. W. Martin, Jr.** 1989. Tau consists of a set of proteins with repeated C-terminal microtubule-binding domains and variable N-terminal domains. Mol. Cell. Biol. **9:**1381–1388.
22. **Jiang, G., J. den Hertog, and T. Hunter.** 2000. Receptor-like protein tyrosine phosphatase α homodimerizes on the cell surface. Mol. Cell. Biol. **20:**5917–5929.
23. **Jiang, G., J. den Hertog, J. Su, J. Noel, J. Sap, and T. Hunter.** 1999. Dimerization inhibits the activity of receptor-like protein-tyrosine phosphatase-alpha. Nature **401:**606–610.
24. **Kraut, J., G. Volohonsky, H. Toledano-Katchalski, and A. Elson.** 2002. Nuclear localization of non-receptor protein tyrosine phosphatase epsilon is regulated by its unique N-terminal domain. Exp. Cell Res. **281:**182–189.
25. **Lacasa, D., N. Boute, and T. Issad.** 2005. Interaction of the insulin receptor with the receptor-like protein tyrosine phosphatases PTPalpha and PTPepsilon in living cells. Mol. Pharmacol. **67:**1206–1213.
26. **Laurent, C. E., F. J. Delfino, H. Y. Cheng, and T. E. Smithgall.** 2004. The human c-Fes tyrosine kinase binds tubulin and microtubules through separate domains and promotes microtubule assembly. Mol. Cell. Biol. **24:**9351–9358.
27. **Lewis, S. A., D. H. Wang, and N. J. Cowan.** 1988. Microtubule-associated protein MAP2 shares a microtubule binding motif with tau protein. Science **242:**936–939.
28. **Moller, N. P., K. B. Moller, R. Lammers, A. Kharitonenkov, E. Hoppe, F. C. Wiberg, I. Sures, and A. Ullrich.** 1995. Selective down-regulation of the insulin receptor signal by protein-tyrosine phosphatases alpha and epsilon. J. Biol. Chem. **270:**23126–23131.
29. **Muja, N., G. Lovas, E. Romm, D. Machleder, M. Ranjan, V. Gallo, and L. D. Hudson.** 2004. Expression of a catalytically inactive transmembrane protein tyrosine phosphatase epsilon (tm-PTP epsilon) delays optic nerve myelination. Glia **48:**278–297.
30. **Nakagawa, Y., N. Aoki, K. Aoyama, H. Shimizu, H. Shimano, N. Yamada, and H. Miyazaki.** 2005. Receptor-type protein tyrosine phosphatase epsilon (PTPepsilonM) is a negative regulator of insulin signaling in primary hepatocytes and liver. Zool. Sci. **22:**169–175.
31. **Nakamura, K., Y. Mizuno, and K. Kikuchi.** 1996. Molecular cloning of a novel cytoplasmic protein tyrosine phosphatase PTP epsilon. Biochem. Biophys. Res. Commun. **218:**726–732.
32. **Noble, M., S. A. Lewis, and N. J. Cowan.** 1989. The microtubule binding domain of microtubule-associated protein MAP1B contains a repeated sequence motif unrelated to that of MAP2 and tau. J. Cell Biol. **109:**3367–3376.
33. **Peretz, A., H. Gil-Henn, A. Sobko, V. Shinder, B. Attali, and A. Elson.** 2000. Hypomyelination and increased activity of voltage-gated K(+) channels in mice lacking protein tyrosine phosphatase epsilon. EMBO J. **19:**4036–4045.
34. **Sapir, T., M. Elbaum, and O. Reiner.** 1997. Reduction of microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. EMBO J. **16:**6977–6984.
35. **Sully, V., S. Pownall, E. Vincan, S. Bassal, A. H. Borowski, P. H. Hart, S. P. Rockman, and W. A. Phillips.** 2001. Functional abnormalities in protein tyrosine phosphatase epsilon-deficient macrophages. Biochem. Biophys. Res. Commun. **286:**184–188.
36. **Tanuma, N., K. Nakamura, and K. Kikuchi.** 1999. Distinct promoters control transmembrane and cytosolic protein tyrosine phosphatase epsilon expression during macrophage differentiation. Eur. J. Biochem. **259:**46–54.
37. **Tanuma, N., K. Nakamura, H. Shima, and K. Kikuchi.** 2000. Protein-tyrosine phosphatase PTPepsilon C inhibits Jak-STAT signaling and differentiation induced by interleukin-6 and leukemia inhibitory factor in M1 leukemia cells. J. Biol. Chem. **275:**28216–28221.
38. **Tanuma, N., H. Shima, K. Nakamura, and K. Kikuchi.** 2001. Protein tyrosine phosphatase epsilonC selectively inhibits interleukin-6- and interleukin-10-induced JAK-STAT signaling. Blood **98:**3030–3034.
39. **Tanuma, N., H. Shima, S. Shimada, and K. Kikuchi.** 2003. Reduced tumorigenicity of murine leukemia cells expressing protein-tyrosine phosphatase, PTPepsilon C. Oncogene **22:**1758–1762.
40. **Tiran, Z., A. Peretz, B. Attali, and A. Elson.** 2003. Phosphorylation-dependent regulation of Kv2.1 channel activity at tyrosine 124 by Src and by protein-tyrosine phosphatase epsilon. J. Biol. Chem. **278:**17509–17514.
41. **Tiran, Z., A. Peretz, T. Sines, V. Shinder, J. Sap, B. Attali, and A. Elson.** 2006. Tyrosine phosphatases epsilon and alpha perform specific and overlapping functions in regulation of voltage-gated potassium channels in Schwann cells. Mol. Biol. Cell **17:**4330–4342.
42. **Toledano-Katchalski, H., J. Kraut, T. Sines, S. Granot-Attas, G. Shohat, H. Gil-Henn, Y. Yung, and A. Elson.** 2003. Protein tyrosine phosphatase epsilon inhibits signaling by mitogen-activated protein kinases. Mol. Cancer Res. **1:**541–550.
43. **Toledano-Katchalski, H., Z. Tiran, T. Sines, G. Shani, S. Granot-Attas, J. den Hertog, and A. Elson.** 2003. Dimerization in vivo and inhibition of the nonreceptor form of protein tyrosine phosphatase epsilon. Mol. Cell. Biol. **23:**5460–5471.
44. **Tonks, N. K.** 2005. Redox redux: revisiting PTPs and the control of cell signaling. Cell **121:**667–670.
45. **Wabakken, T., H. Hauge, E. F. Finne, A. Wiedlocha, and H. Aasheim.** 2002. Expression of human protein tyrosine phosphatase epsilon in leucocytes: a potential ERK pathway-regulating phosphatase. Scand. J. Immunol. **56:**195–203.
46. **Wilkerson, C. G., S. M. King, and G. B. Witman.** 1994. Molecular analysis of the gamma heavy chain of Chlamydomonas flagellar outer-arm dynein. J. Cell Sci. **107:**497–506.
47. **Zheng, H., P. Hu, D. F. Quinn, and Y. K. Wang.** 2005. Phosphotyrosine proteomic study of interferon alpha signaling pathway using a combination of immunoprecipitation and immobilized metal affinity chromatography. Mol. Cell Proteomics **4:**721–730.
48. **Zheng, X. M., R. J. Resnick, and D. Shalloway.** 2002. Mitotic activation of protein-tyrosine phosphatase alpha and regulation of its Src-mediated transforming activity by its sites of protein kinase C phosphorylation. J. Biol. Chem. **277:**21922–21929.
49. **Zheng, X. M., R. J. Resnick, and D. Shalloway.** 2000. A phosphotyrosine displacement mechanism for activation of Src by PTPalpha. EMBO J. **19:**964–978.
50. **Zheng, X. M., and D. Shalloway.** 2001. Two mechanisms activate PTPalpha during mitosis. EMBO J. **20:**6037–6049.