Protein-tyrosine phosphatases (PTPs) are involved in the regulation of diverse cellular processes and may function as positive effectors as well as negative regulators of intracellular signaling. Recent data demonstrate that malignant transformation of cells is frequently associated with changes in PTP expression or activity. Our analysis of PTP expression in mammary carcinoma cell lines resulted in the molecular cloning of a receptor-like PTP, also known as DEP-1. DEP-1 was found to be expressed at varying levels in mammary carcinoma cell lines and A431 cells. In all tumor cell lines analyzed, DEP-1 was constitutively phosphorylated on tyrosine residues. Phosphorylation of DEP-1 increased significantly after treatment of cells with the PTP inhibitor pervanadate. In A431 cells, tyrosine phosphorylation of DEP-1 was constitutively phosphorylated on tyrosine residues. Phosphorylation of DEP-1 increased significantly after treatment of cells with the PTP inhibitor pervanadate. In A431 cells, tyrosine phosphorylation of DEP-1 was also observed after stimulation with epidermal growth factor, however, only after prolonged exposure of the cells to the ligand, suggesting an indirect mechanism of phosphorylation. In addition, DEP-1 co-precipitated with several tyrosine-phosphorylated proteins from pervanadate-treated cells. In vitro binding experiments using a glutathione S-transferase fusion protein containing the catalytically inactive PTP domain of DEP-1 (Gst-DEP-1-C/S) identify these proteins as potential substrates of DEP-1. In addition, we found a 64-kDa serine/threonine kinase to be constitutively associated with DEP-1 in all tumor cell lines tested. The 64-kDa kinase forms a stable complex with DEP-1 and phosphorylates DEP-1 and DEP-1-interacting proteins in vitro. These data suggest a possible mechanism of DEP-1 regulation in tumor cell lines involving serine/threonine and/or tyrosine phosphorylation.

Phosphorylation of proteins on tyrosine residues is a key mechanism in the regulation of cell growth and differentiation and is controlled by two families of enzymes: protein-tyrosine kinases (PTKs)\(^1\) (1) and protein-tyrosine phosphatases (PTPs) (2, 3). In recent years, considerable progress has been made in the analysis of PTK structure and function, whereas still relatively little is known about the biological role and regulation of PTPs.

Similar to PTKs, PTPs can be structurally subdivided into two classes: transmembrane receptor-like molecules (RPTPs) and cytosolic enzymes. All PTP domains contain a conserved 11-residue sequence motif that specifies the active site of the phosphatase. The conserved cysteine and arginine residues within this motif are essential for catalytic activity (4). Interestingly, most of the RPTPs identified to date contain two tandem PTP domains, with the membrane proximal domain accounting for most of the activity (5, 6).

Within their extracellular domains, RPTPs frequently contain multiple fibronectin type III and immunoglobulin-like repeats, reminiscent of cell adhesion molecules such as neural cell adhesion molecules and fasciclin (7, 8), suggesting that these enzymes might be regulated by cell-to-cell contact. Consistent with this structural characteristic, homophilic binding of the extracellular domains has been demonstrated for RPTP-\(\mu\) and RPTP-\(\kappa\) (9–12), although these interactions did not result in changes of the activity of the respective PTPs.

Initial studies on the function of PTPs suggested that this class of enzymes is largely involved in negative regulation of cell growth and is needed for the termination of growth-promoting signals, i.e. after activation of receptor tyrosine kinases (RTKs). Experiments with PTP inhibitors supported this idea. For example, incubation of NRK cells with vanadate results in increased tyrosine phosphorylation of cellular proteins and cell transformation (13).

Interestingly, an increasing number of reports describe elevated expression of PTPs in tumor cell lines and tissues. For example, PTP1B was found to be highly expressed in breast cancer (14). The RPTP SAP-1, which is related to HPTP-\(\beta\), is expressed at high levels in pancreatic and colorectal cancer cells, whereas its expression could not be detected in normal pancreas and colon tissue (15). Another example is PRL-1, a mitogen-induced PTP that is predominantly located in the nucleus. Transfection of PRL-1 into tissue culture cells results in cell transformation and altered growth characteristics (16). These data demonstrate that the function of PTPs in cell growth and development is far more complex than previously anticipated, and that, depending on the individual phosphatase and the cellular context, PTPs may function in either a signal-promoting or inhibiting manner.

We used a polymerase chain reaction (PCR)-based approach to identify PTPs that are expressed in mammary carcinoma cell lines. This study led to the molecular cloning of a RPTP, which is characterized by eight fibronectin type III repeats within the extracellular domain, a transmembrane domain, and a single catalytic domain. Further analysis revealed its identity with the previously described RPTP DEP-1 (17). Here we demon-
strate that DEP-1 is expressed in various mammary carcinoma cell lines and in A431 cells. Using coprecipitation and in vitro binding experiments, we found several cellular proteins associated with DEP-1. Coprecipitating proteins were detected due to their tyrosine phosphorylation in cells treated with pervanadate or, in the case of A431 cells, with epidermal growth factor (EGF). Further analysis revealed that one of the DEP-1-associated proteins is a 64-kDa serine/threonine kinase, which specifically associates with the C terminus of the phosphatase and phosphorylates DEP-1 in vitro.

MATERIALS AND METHODS

cDNA Cloning and Plasmid Construction—Degenerate oligonucleotide primers corresponding to the highly conserved amino acid sequence motifs KCAQYWP and HCSAGIG within PTP domains were used in a PCR with single-stranded cDNA, derived from mRNA isolated from the human mammary carcinoma cell line BT474 as template. An amplified ~200-base pair PCR fragment was used to screen a human mammary cDNA library (CLONTECH). cDNA clones encoding DEP-1 were isolated using standard techniques (18) and sequenced using the dideoxy chain termination method (19). For expression in tissue culture cells, the entire DEP-1 cDNA was subcloned as an EcoRI fragment into the expression vector pCMV, resulting in the plasmid pCMV-DEP-1.

For the construction of glutathione S-transferase (GST) fusion proteins, the entire cytoplasmic domain (amino acid residues 997 to 1337), the juxtamembrane domain (amino acid residues 997 to 1059), and the C-terminal domain (amino acid residues 1297 to 1337), respectively, were amplified by PCR. PCR products of the expected size were digested with EcoRI and BamHI and subcloned in frame into the expression vector pGEX2T*, a derivative of pGEX2T with a modified polylinker (details of the vector are available upon request). All PCR products were verified by sequence analysis. The purification of GST fusion proteins was done essentially as described (20).

Antibodies and Reagents—Polyclonal rabbit antibodies (anti-DEP-1 antisera 42) were generated against a peptide corresponding to 15 amino acids of the juxtamembrane region of DEP-1 (QPKYAEELAN-RRK) and affinity purified using the same peptide. Anti-phosphotyrosine antibodies (RC20) were purchased from Transduction Laboratories.

Cell Lines and Transfection—All cell lines used were purchased from American Type Culture Collection and cultured in media as recommended. For transient expression studies, 293 human embryonic kidney fibroblasts were transfected with CsCl gradient-purified plasmid DNA according to the protocol of Chen and Okayama (21).

Cell Lysis and Immunoprecipitation—Cells were lysed in 1% Triton X-100-containing lysis buffer as described previously (22). Immunoprecipitations, SDS-PAGE, and immunoblotting procedures were carried out essentially as described previously (22). Briefly, lysates were cleared by centrifugation at 14,000 rpm for 10 min at 4 °C. For immunoprecipitations, ~10 μg of anti-DEP-1 antibodies or 10 μl of preimmune serum together with 30 μl of protein A-Sepharose slurry were added to the cleared lysates and incubated for 3 h at 4 °C with gentle agitation. Precipitates were washed with 3 × 1 ml HNTG buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 0.1% Triton X-100, and 1 mM EDTA), and the proteins were separated by 7.5% SDS-PAGE and transferred onto nitrocellulose. The blots were incubated with the primary antibodies followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit antibodies. Immunoreactive proteins were visualized using a chemiluminescence detection system (ECL; American Corp.).

In Vitro Kinase Assay—For in vitro kinase assays, immunoprecipitates were washed three times with HNTG, once with Trits buffer (50 mM Tris-HCl, pH 7.5, 10 mM MnCl₂, and 10 mM MgCl₂) and incubated with 10 μl of Trits buffer containing 5 μCi of [γ-32P]ATP (3000 Ci/mmol; DuPont NEN) for 10 min at 23 °C. The samples were diluted with HNTG, washed once, and then mixed with SDS sample buffer. The samples were resolved on 5.5% SDS-PAGE, and phosphorylated proteins were visualized by autoradiography. Where indicated, myelin basic protein (MBP) was used as exogenous substrate for in vitro phosphorylation, 10 μg of MBP was added to the kinase buffer, and the reaction was carried out as described above, omitting the washing step.

In-gel Kinase Assay—For the detection of protein kinase activity in in-gel kinase assays, the method described by Kameshita and Fujisawa (23) was used with minor modifications. Briefly, immunoprecipitates were washed in HNTG buffer and fractionated on 10% SDS-PAGE, with
tested. Given the previous report on density-dependent expression of DEP-1 in WI-38 and AG1518 cells (17), we analyzed the influence of cell density on the expression of DEP-1 in A431 cells and HBL100 mammary carcinoma cells. For both cell lines, cell density had no significant influence on DEP-1 protein levels, as detected by immunoblot analysis (data not shown).

Detection of DEP-1-interacting Proteins—As a first step to further analyze DEP-1 on a molecular level, we asked if DEP-1 was associated with other cellular proteins. The identification of proteins interacting with DEP-1 or of potential substrates could give further clues regarding the function and regulation of this phosphatase.

We tried to detect proteins that could be directly coprecipitated with DEP-1 from different tumor cell lines. As a means to visualize DEP-1 coprecipitating proteins, we incubated the cells with pervanadate prior to lysis. Pervanadate is a potent membrane-permeable PTP inhibitor (26, 27). Incubation of cells with pervanadate results in high level phosphorylation of proteins on tyrosine residues and allows the detection of proteins by anti-phosphotyrosine immunoblot, even if their tyrosine phosphorylation is subject to rapid turnover under normal growth conditions (28).

A431, DU4475, and MDA-MB231 cells were incubated with 100 mM pervanadate prior to lysis, followed by immunoprecipitation with the anti-DEP-1 antisera 42 and then by immunoblot analysis with anti-phosphotyrosine antibodies. As shown in Fig. 2A (upper panel), a tyrosine-phosphorylated protein of 190 kDa was specifically detected in anti-DEP-1 immunoprecipitates from pervanadate-treated cells. This protein was not detected when preimmune serum (NS) was used for immunoprecipitation. Similarly, this protein was absent when anti-DEP-1 antibodies were preincubated with the peptide antigen before addition to the cell lysate (data not shown). Reprobing the same blot with anti-DEP-1 antibodies further confirmed the identity of p190 (Fig. 2A, lower panel).

We consistently observed the presence of other tyrosine-phosphorylated proteins in anti-DEP-1 immunoprecipitates from pervanadate-treated cells (Fig. 2A). As for DEP-1, these proteins could not be detected when preimmune serum was used for the immunoprecipitations. For A431 cells, tyrosine-phosphorylated proteins with apparent molecular weights of M , 58,000–60,000, 60,000–63,000, 120,000 and 170,000 were detected. With the exception of the 170-kDa protein, the pattern observed for MDA-MB231 cells was very similar, although the signal intensities for these proteins in anti-phosphotyrosine immunoblots were much weaker. In anti-DEP-1 precipitates from lysates of DU4475 cells, all of the above proteins plus an additional phosphotyrosine-containing protein of 105 kDa were detected. When the proteins in the lysates of pervanadate-treated cells were denatured by boiling in the presence of SDS prior to immunoprecipitation with 42 antibodies, only tyrosine-phosphorylated DEP-1 could be detected in anti-phosphotyrosine immunoblots of anti-DEP-1 immunoprecipitates. This indicates that the anti-DEP-1 antibody has no cross-reactivity with the DEP-1-associated proteins (data not shown).

When pervanadate treatment was omitted, constitutive tyrosine phosphorylation of DEP-1 was observed in all cell lines used for this study (data not shown and Fig. 3A). However, no other phosphotyrosine-containing proteins were detected in anti-DEP-1 immunoprecipitates from lysates of untreated cells.

To confirm these results and to address the question of whether tyrosine phosphorylation of these proteins is required for the interaction with DEP-1, we performed in vitro binding experiments using DEP-1 fusion proteins with Gst as affinity matrix. For this analysis, we used fusion proteins containing the complete intracellular domain of DEP-1 (Gst-DEP-1). In Gst-DEP-1/C/S, the active site cysteine residue had been exchanged for serine, resulting in an enzymatically inactive phosphatase. When lysates of pervanadate-treated DU4475 cells were incubated with the fusion proteins, only Gst-DEP-1/C/S precipitated tyrosine-phosphorylated proteins (Fig. 2B). As judged by molecular weight, the profile of precipitated proteins detected in anti-phosphotyrosine immunoblots was essentially identical to the one observed previously in anti-DEP-1 immunoprecipitates from DU4475 cell lysates (compare Fig. 2A). However, because antibodies to these proteins are not yet available, we cannot directly test for the identity of the proteins detected in these two assays. The specific detection of the tyrosine-phosphorylated versions of DEP-1-associated proteins in Gst-DEP-1/C/S but not in Gst-DEP-1 precipitates suggests that these proteins are potential substrates of DEP-1. When...
the enzymatic activity of the wild-type fusion protein, Gst-DEP-1, was blocked by incubation with vanadate before addition to the cell lysate, two of the observed tyrosine-phosphorylated proteins, p120 and p72, were coprecipitated. Vanadate exerts its inhibiting effect on PTPs through interacting with the active site of the protein tyrosine phosphatase (28). Consequently, the detection of tyrosine-phosphorylated p120 and p72 in precipitates of vanadate-treated Gst-DEP-1 is most likely the result of constitutive binding of these proteins to sites outside the active center of the phosphatase and inhibition of DEP-1 catalytic activity by vanadate. The failure to detect tyrosine-phosphorylated p105, p62, and p50 under these conditions suggests that these proteins primarily interact with DEP-1 through binding of phosphorylated tyrosine residues with the active site, which would be blocked in the presence of vanadate. Because vanadate specifically interacts with the active site cysteine residue, preincubation of Gst-DEP-1-C/S with vanadate has no significant influence on the binding of tyrosine-phosphorylated substrates (Fig. 2B).

**DEP-1 Is Tyrosine Phosphorylated after EGF Stimulation**—In all tumor cell lines used in this study, we found DEP-1 to be constitutively phosphorylated on tyrosine residues. Mammary carcinomas and mammary carcinoma cell lines frequently exhibit high levels of expression of RTKs such as epidermal growth factor receptor (EGF-R) or p185HER2/neu (c-erbB2) (29–32). We, therefore, tested whether DEP-1 was a direct or indirect target of RTKs.

Because the highest levels of constitutive tyrosine phosphorylation were observed in A431 cells, which overexpress the EGF-R, we investigated whether DEP-1 was a substrate for this RTK. We analyzed the phosphorylation state of DEP-1 in this cell line after stimulation with EGF. As shown in Fig. 3A, increased tyrosine phosphorylation of DEP-1 as well as associated proteins was observed only after stimulation of A431 cells with EGF for 30 min. Shorter periods of stimulation had no significant effect on DEP-1 tyrosine phosphorylation. After incubation of cells for 1 h with EGF, tyrosine phosphorylation of DEP-1 returned back to the basal level (data not shown). The kinetics of EGF-dependent tyrosine phosphorylation suggested that DEP-1 was not directly phosphorylated by the EGF-R, which was confirmed by coexpression experiments in 293 human embryonic kidney fibroblasts. 293 cells also express endogenous EGF-R as well as low but detectable levels of DEP-1, which makes this cell line suitable for this analysis. EGF stimulation of cells cotransfected with DEP-1 and EGF-R had no detectable effect on DEP-1 tyrosine phosphorylation, whereas pervanadate treatment of DEP-1-overexpressing cells resulted in significant phosphorylation of DEP-1 on tyrosine residues (Fig. 3B, upper panel). Reprobing the blot with anti-DEP-1 antibodies confirmed expression in all samples (Fig. 3B, lower panel). This result suggests that A431 cells, and possibly other cell lines used in this study, express a protein-tyrosine kinase the activity of which may be stimulated by RTKs and which is able to phosphorylate DEP-1, and that this kinase may not be expressed to significant levels in 293 cells.

**DEP-1 Stably Associates with a Protein Kinase**—To further elucidate the nature of the DEP-1-associated proteins, we tested if one of these was a PTK itself. To this end, DEP-1 was immunoprecipitated from the lysates of different tumor cell lines and tested if one of these was a PTK itself. EGF-R, which was confirmed by coexpression experiments in 293 human embryonic kidney fibroblasts. Before treatment with EGF, the cells were lysed and subjected to immunoprecipitation with anti-DEP-1 antibodies. Tyrosine phosphorylation of precipitated proteins was analyzed by anti-phosphotyrosine immunoblot. Cells treated with pervanadate (Van.; 100 μM for 15 min.) and the resulting lysates precipitated in the presence (peptide) or absence of competing antigen were used as control. EGF-dependent tyrosine phosphorylation of DEP-1 and coprecipitating proteins was maximal after 30 min of EGF treatment. After 60 min of EGFR stimulation, tyrosine phosphorylation of DEP-1 and coprecipitating proteins returned to the basal level (data not shown).

**DEP-1 Phosphorylation and Complex Formation**—To further elucidate the nature of the DEP-1-associated proteins, we tested if one of these was a PTK itself. To this end, DEP-1 was immunoprecipitated from the lysates of different tumor cell lines and tested if one of these was a PTK itself. EGF-R, which was confirmed by coexpression experiments in 293 human embryonic kidney fibroblasts. Before treatment with EGF, the cells were lysed and subjected to immunoprecipitation with anti-DEP-1 antibody 42. Tyrosine phosphorylation of precipitated proteins was analyzed by anti-phosphotyrosine immunoblot (α PY; upper panel). The blot was then stripped and reprobed with anti-DEP-1 antibody 42 (lower panel).

![Fig. 3. EGF-dependent tyrosine phosphorylation of DEP-1 in A431 cells.](image)

**Fig. 3.** EGF-dependent tyrosine phosphorylation of DEP-1 in A431 cells. A, A431 cells were starved in media containing 0.5% fetal calf serum for 24 h and then stimulated with EGF (100 ng/ml) as indicated. The cells were lysed and subjected to immunoprecipitation with anti-DEP-1 antibodies. Tyrosine phosphorylation of precipitated proteins was analyzed by anti-phosphotyrosine immunoblot. Cells treated with pervanadate (Van.; 100 μM for 15 min.) and the resulting lysates precipitated in the presence (peptide) or absence of competing antigen were used as control. EGF-dependent tyrosine phosphorylation of DEP-1 and coprecipitating proteins was maximal after 30 min of EGF treatment. After 60 min of EGFR stimulation, tyrosine phosphorylation of DEP-1 and coprecipitating proteins returned to the basal level (data not shown). B, coexpression of DEP-1 with EGF-R in 293 cells. DEP-1 and human EGF-R were either expressed alone or cotransfected into 293 human embryonic kidney fibroblasts. Before treatment with EGFR or pervanadate, the cells were starved for 24 h in media containing 0.5% fetal calf serum. After stimulation, the cells were lysed and subjected to immunoprecipitation with anti-DEP-1 antibody 42. Tyrosine phosphorylation of precipitated proteins was analyzed by anti-phosphotyrosine immunoblot (α PY; upper panel). The blot was then stripped and reprobed with anti-DEP-1 antibody 42 (lower panel).

![Image](image)

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2 J. Zachwieja and B. Jallal, unpublished observation.
DEP-1 Phosphorylation and Complex Formation

and in vitro kinase assays were performed (Fig. 5, upper panel). Following SDS-PAGE and autoradiography, the band corresponding to MBP was excised from the gel, eluted, and subjected to phosphoamino acid analysis (Fig. 5, lower panel). Phosphorylation was found in serine and threonine residues, whereas no phosphorylation of tyrosine residues was detected. In similar experiments, we performed phosphoamino acid analysis on individual DEP-1-associated proteins from DU4475 cells after in vitro kinase reactions and SDS-PAGE. For all proteins analyzed, including DEP-1, only serine and threonine phosphorylation was detected (data not shown).

The 64-kDa Serine/Threonine Kinase Specifically Binds to the C-terminal Tail of DEP-1.—To identify the binding site of the DEP-1-associated kinase, we used Gst-fusion proteins containing the juxtamembrane and the C-terminal domain of DEP-1, respectively, as affinity matrices. Binding of the 64-kDa serine/threonine kinase to DEP-1 or DEP-1-Gst fusion proteins was detected by an in-gel kinase assay (Fig. 6). As shown in Fig. 6, kinase activity was recovered in anti-DEP-1 immunoprecipitates as well as in precipitates with the DEP-1 C-terminal Gst-fusion protein. No binding of the kinase to either Gst alone or the fusion protein containing the juxtamembrane domain of DEP-1 was detected. These data demonstrate that the C-terminal tail is sufficient for binding of the 64-kDa serine/threonine kinase to DEP-1.

**DISCUSSION**

PTKs and PTPs play critical roles in the regulation of cell growth and differentiation, and changes in the activity of both classes of enzymes may contribute to malignant transformation of cells and the development of cancer. A possible involvement of PTPs in contact inhibition was proposed by Pallan and Tong (33), who observed that the PTP activity in the membranes of contact-inhibited Swiss 3T3 cells was increased 8-fold compared with proliferating cells.

We analyzed the expression of DEP-1 in various mammary carcinoma cell lines as well as in A431 cells. In all cell lines tested, DEP-1 expression was readily detectable by immuno- blot analysis. We also observed that expression of DEP-1 is not up-regulated in dense versus sparse cultures of A431 and HBL100 cells. Keane et al. (34) recently analyzed the expression of DEP-1 in breast cancer cell lines undergoing sodium butyrate-induced differentiation. Although the expression of DEP-1 was up-regulated in differentiating ZR75–1 and SKBr-3 cells, no significant influence of cell density on DEP-1 expression was observed (34). To further address the involvement of DEP-1 in contact inhibition, density-dependent expression of DEP-1 must be studied in more detail in a larger number of different cell types.

In all tumor cell lines analyzed, we found DEP-1 to be constitutively phosphorylated on tyrosine residues. This phosphorylation increased significantly after incubation of the cells with pervanadate or, in A431 cells, after stimulation with EGF. However, two lines of evidence suggest that DEP-1 is not a direct target for phosphorylation by the EGF-R: (a) the relatively long-time A431 cells had to be stimulated with EGF to increase DEP-1 tyrosine phosphorylation and is indicative of a secondary response; and (b) when DEP-1 and EGF-R were coexpressed in 293 fibroblasts, EGF-dependent phosphorylation of the phosphatase could not be detected. Thus, the EGF-dependent phosphorylation of DEP-1 in A431 cells is presumably mediated by a PTK distinct from the EGF-R, but one that
can be activated in response to EGF stimulation. We do not yet know if the observed constitutive phosphorylation is mediated by the same or a second kinase.

We demonstrate that the RPTP DEP-1 is associated with several cellular proteins. Initially, we detected DEP-1-associated proteins due to their tyrosine phosphorylation in pervanadate-treated cells and their specific coprecipitation with DEP-1. The increase in protein-tyrosine phosphorylation in pervanadate-treated cells is a direct result of the active PTKs present in a given cell line and the simultaneous inhibition of cellular PTPs (28). Thus, the differences observed in the pattern of tyrosine-phosphorylated proteins coprecipitating with DEP-1 from different cell lines could reflect either differences in the expression of these proteins or differences in the repertoire of PTKs expressed in the cell lines tested. The analysis of DEP-1-interacting proteins from DU4475 cells using Gst-DEP-1 fusion proteins as affinity matrix resulted in the recovery of tyrosine-phosphorylated proteins of comparable molecular weight as the ones observed in anti-DEP-1 immunoprecipitates. The specific interaction with the catalytically inactive Gst-DEP-1-C/S suggests that these proteins are potential targets for dephosphorylation by DEP-1. Although tyrosine-phosphorylated p105, p62, and p50 most likely bind to the active site of the phosphatase, consistent with the idea of being substrates of DEP-1, we could demonstrate that DEP-1 also engages in protein-protein interactions that require binding determinants other than the catalytic center. For proteins from DU4475 cells, this is the case for p120, p72, and the 64-kDa serine/threonine kinase. For the 64-kDa kinase (see below), we could localize the binding site to the C-terminal tail of DEP-1. The mode of interaction of these proteins with DEP-1 could indicate that they are involved in the regulation of DEP-1 activity or function.

We attempted to identify the tyrosine kinase that phosphorylates DEP-1 by analyzing if this kinase was one of the proteins coprecipitating with DEP-1. However, no tyrosine kinase activity could be detected in anti-DEP-1 immunoprecipitates. Instead, we found that DEP-1 is constitutively associated with a serine/threonine kinase (p64). Further analysis revealed that the 64-kDa kinase is capable of phosphorylating DEP-1 as well as associated proteins in vitro. Interestingly, anti-DEP-1 immunoprecipitates from A431 and DU4475 cells contain a 62-kDa protein that is highly phosphorylated on tyrosine residues in pervanadate cells but only weakly phosphorylated in vitro kinase assays. Because of the lack of specific antibodies, we presently cannot verify the identity of this protein with the 64-kDa serine/threonine kinase.

Phosphorylation of PTPs on serine and/or tyrosine residues has been described, but in only a few cases could these phosphorylation events be correlated with changes in the catalytic activity of the respective phosphatase. For example, the cytosolic enzyme PTP-PEST is negatively regulated by serine phosphorylation, which results in reduced substrate affinity (35). Yamada et al. (36) have shown that CD45 phosphorylation in response to PKC activation results in down-regulation of PTP activity. Conversely, phosphorylation of CD45 on a C-terminal tyrosine residue by p60
cs resulted in activation of the phosphatase and created a binding site for p60
cs (37). Stower and Walsh (38) analyzed the influence of phosphorylation on CD45 activity in vitro and found that sequential phosphorylation on tyrosine and serine residues is necessary for activation.

It is tempting to speculate that also the activity of DEP-1 can be modulated by serine/threonine and/or tyrosine phosphorylation. It has been reported that ectopic overexpression of DEP-1 can inhibit the growth of breast cancer cell lines (34). Although all cell lines used in our study show significant levels of DEP-1 protein, these cell lines are characterized by a transformed phenotype and are not contact inhibited. It is possible that inhibition of DEP-1 by phosphorylation is one of the steps leading to cell transformation. This will have to be tested in future experiments. Of special interest in this respect will be the identification of the DEP-1-associated serine/threonine kinase. The molecular cloning of this kinase will allow a direct analysis of its influence on DEP-1 activity.

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REFERENCES

1. Hunter, T., and Cooper, J. A. (1985) Annu. Rev. Biochem. 54, 897–930
2. Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991) Science 253, 401–406
3. Walton, K. M., and Dixon, J. E. (1993) Annu. Rev. Biochem. 62, 101–120
4. Stone, R. L., and Dixon, J. E. (1994) J. Biol. Chem. 269, 31233–31236
5. Streuli, M., Krueger, N. X., and Saito, H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8698–8702
6. Loz, K.-H. W., and Baylink, D. J. (1993) Crit. Rev. Oncog. 4, 452–471
7. Saito, H., and Streuli, M. (1991) Cell Growth & Differentiation 2, 59–65
8. Brady-Kalnay, S. M., and Tonks, N. K. (1995) Curr. Opin. Cell Biol. 7, 650–657
9. Brady-Kalnay, S. M., Flint, A. J., and Tonks, N. K. (1993) J. Cell Biol. 121, 962–971
10. Gebben, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., van Rijen, L., and Moelenaar, W. H. (1993) J. Biol. Chem. 268, 16101–16104
11. Sap, J., Jiang, Y. P., Friedlander, D., Grumet, M., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 1–9
12. Zondag, G. C., Koningstein, G. M., Jiang, Y.-P., Sap, J., Moelenaar, W. H., and Gebben, M. F. (1995) J. Biol. Chem. 270, 14247–14250
13. Klarlund, J. K. (1985) Cell 41, 707–717
14. Wiener, J. R., Kerns, B. J., Harvey, E. L., Cinaway, M. R., Iglehart, J. D., van Etten, I., and Moolenaar, W. H. (1993) J. Natl. Cancer Inst. 86, 374–378
15. Motozaki, T., Suzuki, T., Uchida, T., Inawaa, J., Ariyama, T., Mitsuda, K., Horita, K., Noguchi, H., Mizuno, H., Sakamoto, C., and Kusaga, M. (1994) J. Biol. Chem. 269, 2075–2081
16. Diamond, R. H., Cressman, D. E., Lazar, T. M., Abrams, C. S., and Taub, R. (1994) Mol. Cell. Biol. 14, 3752–3762
17. O’Riordan, A. J., Sutherland, T. M., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
19. Frangioni, J. V., and Neel, B. G. (1993) Anal. Biochem. 210, 179–187
20. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
21. Jallal, B., Schlessinger, J., and Ullrich, A. (1992) J. Biol. Chem. 267, 4357–4363
22. Kameshita, I., and Fujisawa, H. (1989) Anal. Biochem. 183, 139–143
23. Cooper, J. A., Sefton, B. M., and Hunter, T. (1983) Methods Enzymol. 99, 397–402
24. Zhang, Z.-Y., and Dixon, J. E. (1994) Adv. Enzymol. 68, 1–36
25. Fantus, I. G., Kadota, S., Deragon, G., Foster, B., and Posner, B. I. (1989) Biochemistry 28, 8864–8871
26. Heffetz, D., Buskbin, I., Dror, R., and Zick, Y. (1990) J. Biol. Chem. 265, 10906–10902
27. Garton, A. J., and Tong, P. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4608–4618
28. Yamada, A., Streuli, M., Rothstein, D. M., Schlossman, S. F., and Morimoto, C. (1990) Biochim. Biophys. Acta 1072, 707–712
29. van den Vijver, M. J., and Nusse, R. (1991) Biochim. Biophys. Acta 1072, 33–50
30. Nak, C. J., and Tong, P. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6996–7000
31. Keane, M. M., Lowery, G. A., et al. (1996) Cancer Res. 56, 4258–4263
32. Garton, A. J., and Tonks, N. K. (1994) EMBO J. 13, 3763–3771
33. Yamada, A., Streuli, M., Saito, H., Rothstein, D. M., Schlossman, S. F., and Morimoto, C. (1990) Eur. J. Immunol. 20, 1655–1660
34. Auerer, M., Saharinen, J., Pessa-Morikawa, T., znajdu-Rothbell, M., Oetken, C., Gassmann, M., Bergman, M., Altalba, K., Burn, P., Gahmberg, C. G., and Mustelin, T. (1994) Mol. Cell. Biol. 14, 1308–1321
35. Stower, D. R., and Walsh, K. A. (1994) Mol. Cell. Biol. 14, 5523–5532
The Receptor-like Protein-tyrosine Phosphatase DEP-1 Is Constitutively Associated with a 64-kDa Protein Serine/Threonine Kinase
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