The focal adhesion kinase (FAK) is a key regulator of cell migration. Phosphorylation at Tyr-397 activates FAK and creates a binding site for Src family kinases. FAK phosphorylates the cytoskeletal protein α-actinin at Tyr-12. Here we report that protein-tyrosine phosphatase 1B (PTP 1B) is an α-actinin phosphatase. PTP 1B-dependent dephosphorylation of α-actinin was seen in COS-7 cells and PTP 1B-null fibroblasts reconstituted with PTP 1B. Furthermore, we show that coexpression of wild-type α-actinin and PTP 1B causes dephosphorylation at Tyr-397 in FAK. No dephosphorylation was observed in cells coexpressing the α-actinin phosphorylation mutant Y12F and PTP 1B. Furthermore, the phosphorylation at four other sites in FAK was not altered by PTP 1B. In addition, we found that phosphorylated α-actinin bound to Src and reduced the binding of FAK to Src. The dephosphorylation at Tyr-397 in FAK triggered by wild-type α-actinin and PTP 1B caused a significant increase in cell migration. We propose that phosphorylated α-actinin disrupts the FAK-Src complex exposing Tyr-397 in FAK to PTP 1B. These findings uncover a novel feedback loop involving phosphorylated α-actinin and PTP 1B that regulates FAK-Src interaction and cell migration.

Cell migration is an integrated and dynamic process requiring integrin receptors, multiprotein signaling complexes localized at integrin-extracellular matrix adhesion sites, and structural proteins that link these complexes to the actin skeleton (1–5). The focal adhesion kinase (FAK) is among the first proteins recruited by ligand-occupied integrin receptors, multiprotein signaling complexes localized at integrin-extracellular matrix adhesion sites, and structural proteins that link these complexes to the actin skeleton (1–5). The focal adhesion kinase (FAK)3 is among the first proteins recruited by ligand-occupied integrins to nascent adhesions (6, 7). The focal adhesion kinase (FAK) is a key regulator of cell migration. Phosphorylation at Tyr-397 activates FAK and facilitates the recruitment of integrins to nascent adhesions (6, 7). An auto- and/or transphosphorylation site for Src family kinases. FAK phosphorylates the cytoskeletal protein phosphatase 1B (PTP 1B) is an α-actinin phosphatase. PTP 1B-de-...
alters the focal adhesion dynamics (42, 43). These observations support the possibility that the phosphorylation of α-actinin may serve to modulate the coupling/uncoupling of integrins to the cytoskeleton.

Recently we found that α-actinin is dephosphorylated by the hematopoietic PTP SHP-1 (44). Here we show that α-actinin is also dephosphorylated by the ubiquitously expressed phosphatase, PTP 1B. Unexpectedly, we found that coexpression of PTP 1B with wild-type α-actinin in either COS-7 cells or in PTP 1B-null fibroblasts triggered dephosphorylation of FAK at Tyr-397, the primary Src binding site. The sequence that surrounds the site of phosphorylation in α-actinin, Tyr-12, is similar to the one surrounding Tyr-397 in FAK. This raises the exciting possibility that phosphorylated α-actinin may function as a “FAK decoy” and, as such, may regulate the interaction between FAK and Src (41). The results presented support this possibility and establish that phosphorylated α-actinin binds Src and reduces the binding of Src to FAK. We propose that the disruption of the FAK-Src complex enables the dephosphorylation of FAK at Tyr-397 by PTP 1B. Furthermore, we show that the dephosphorylation of Tyr-397 in FAK triggered by PTP 1B and wild-type α-actinin caused a significant increase in the rate of cell migration. These data establish a novel role for α-actinin in regulating FAK-Src interaction and suggest the existence of an α-actinin-dependent feedback loop that regulates cell migration.

**Experimental Procedures**

**Cell Lines and Antibodies—**COS-7 cells were from the American Tissue Type Culture Collection (Manassas, VA). The PTP 1B-null cell line was described (45, 46). Polyclonal antibodies against FAK, Src and HA, and mAbs to PTP 1B were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phospho-site-specific antibodies to FAK (Tyr-397, Tyr-407, Tyr-576, Tyr-577, and Tyr-861) and Src (Tyr-418 and Tyr-529) were from BIOSOURCE International (Hopkinton, MA). The mAbs to phospho-tyrosine (4G10) and to Src were from Upstate Biotechnology (Lake Placid, NY). The mAb against 6-His was purchased from Qiagen (Valencia, CA). A polyclonal antibody against α-actinin, 579, generated by us was described (Izaguirre et al. (48)).

**Molecular Cloning and Constructs—**The complete cDNA sequences encoding for wild-type PTP 1B and D181A PTP 1B were subcloned between the BamHI and EcoRI sites of pcDNA 3.1 (+) vector (Invitrogen) for mammalian cell expression. cDNAs encoding for a constitutively active c-Src (Y529F) and a HA-tagged FAK were kindly provided from Dr. David Schlaepfer (The Scripps Research Institute, La Jolla, CA). The pPUR vector encoding a puromycin-resistant gene was from BD Biosciences Clontech (Palo Alto, CA). Wild-type and Y12F His-tagged α-actinin constructs were described before (41).

**Isolation of an α-Actinin Phosphatase from Thrombin-stimulated Platelets—**Human platelets were isolated by gel filtration from either fresh or outdated platelet units as previously described (44). The platelets were stimulated for 10 min with thrombin (1 unit/ml, Sigma-Aldrich). The resulting platelet aggregates were concentrated by centrifugation at 800 × g for 5 min, resuspended and lysed by sonication in 30 ml of buffer A (20 mM Tris, pH 8.0, 5 mM EDTA, 1 mM dithiothreitol, and 1 mM PMSF). This and all subsequent steps were carried out at 4 °C. The platelet lysate was centrifuged at 17,000 × g for 20 min, and the resulting pellet was resuspended and sonicated in buffer A supplemented with 1% Triton X-100. Insoluble material was removed by centrifugation at 17,000 × g for 20 min. The supernatant was mixed for 1 h with 50 ml of Reactive Green dye 19 resin cross-linked to agarose beads (Sigma-Aldrich). The resin was washed with 100 ml of buffer C (buffer A supplemented with 0.2% Triton X-100). The proteins were eluted in 100-ml fractions with a step gradient of 0.2, 0.4, 0.6, and 0.8 M KCl prepared in buffer C. After analysis of the fractions for phosphatase activity (using the assay described below), the fractions eluted with 0.4 and 0.6 M KCl were combined, concentrated, and dialyzed against buffer containing 20 mM Tris, pH 8.0, 5 mM EDTA, 1 mM dithiothreitol, 0.2% Triton X-100, and 1 mM PMSF using Vivascience, Edgewood, NY. The resulting sample was loaded onto two interconnected, 5-ml Hitrap™ columns (total volume, 10 ml, Amersham Biosciences). The columns were washed with 50 ml of buffer C. Bound proteins were eluted in 10-ml fractions with a linear gradient of 0 to 400 mM KCl prepared in buffer C. Fractions eluted with 180–300 mM KCl contained the phosphatase activity. These fractions were combined and concentrated by dialyzing as described above. The resulting sample was loaded onto a 5-ml heparin column (Amersham Biosciences). The column was washed with 20 ml of buffer C, and bound proteins were eluted in 5-ml fractions with a linear gradient of 0 to 400 mM KCl prepared in buffer C. The active fractions (270–300 mM KCl) were collected and then were resolved by Sephacryl S200 HR column (Amersham Biosciences) with buffer A. The fractions were assayed for phosphatase activity as described below. The final active fraction was analyzed by in gel-phosphatase assay and silver staining exactly as described (44).

**Phosphatase Assay—**Phosphorylated recombinant α-actinin was isolated from transfected COS-7 cells as previously described (41). The purified protein (1 μg) was mixed with phosphatase assay buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM dithiothreitol, 0.1% β-mercaptoethanol, and 1 mM PMSF in a final volume of 35 μl. The reaction mix was incubated for 30 min at 37 °C. The phosphorylation of α-actinin was analyzed by Western blotting with the antibody to phosphorytine (pTyr), 4G10.

**Protein Identification by Tandem Mass Spectrometry—**A fraction representing the pooled sample eluted off the Sephacryl S200 HR column was resolved by gel electrophoresis. The gel was stained with Coomassie Blue. A gel slice containing the region immediately above the 37-kDa marker was sliced out and submitted to analysis at the Harvard Microchemistry Facility. The protein was digested and analyzed by microcapillary reverse-phase high pressure liquid chromatography nanoelectrospray tandem mass spectrometry on Finnigan LCQ DECA XP quadrupole ion trap mass spectrometer.

**Transfection and Expression of Recombinant Proteins in COS-7 and PTP 1B Null Cells—**COS-7 cells were transfected using Lipofectamine Plus reagents (Invitrogen) as described (41). Where indicated, cells were transfected with 2 μg of either wild-type- or Y12F-α-actinin cDNAs plus 1 μg of each of the remaining cDNA to a total of 4 μg of cDNA per 10-cm dish. When necessary, an empty vector was used to bring the total cDNA amount to 4 μg. Vanadate (0.5 mM) prepared as described (41) was added to the culture medium 48 h after transfection. Unless otherwise indicated, the cells were cultured in the presence of vanadate for 24 h prior to analysis. In experiments in which the cells were not treated with vanadate, the cells were detached with trypsin and replated onto fibronectin-coated dishes (10 μg/ml, Sigma-Aldrich) for 1 h prior to lysis. The PTP-1B-null cells were transfected as described. In some experiments the cDNAs mix (total of 4 μg per 10-cm dish) contained 0.1 μg of cDNA encoding for the puromycin-resistant gene (pPUR, BD Biosciences Clontech). The cells were treated with puromycin at a concentration of 2 μg/ml starting at 24 h post-transfection. The puromycin-resistant cultures were propagated and expanded for ~2 weeks Irrespective of whether the cells were selected with puromycin or not, on the day of the experiment the cells were detached with trypsin and replated onto a fibronectin-coated surface for 1 h as described above.

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Immunoprecipitation and Western Blotting—Cells were washed with ice-cold phosphate-buffered saline containing vanadate and lysed in modified radioimmunoprecipitation assay buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 1.5 mM MgCl2, 1 mM EGTA, 1 mM sodium deoxycholate, 1 mM sodium vanadate, 10 mM sodium pyrophosphate, 100 mM NaF, 1% Triton X-100, 0.1% SDS, 1 mM PMSF). The lysates were passed several times through a 27-gauge one-half needle to disrupt the cells and to shear the DNA. The samples were normalized for protein content (1 mg/ml) and preabsorbed for 1 h with 30 μl of protein A/G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA). The lysates (0.5–1 mg of protein per sample) were then incubated for 2 h with the specified antibodies. Antibody-antigen complexes were precipitated with 30 μl of protein A/G-agarose beads, washed three times with lysis buffer, and eluted with Laemmli sample buffer. The proteins were resolved by SDS-PAGE and blotted onto a polyvinylidene difluoride membrane. The membranes were probed with primary antibodies. Secondary antibody was either horseradish peroxidase-conjugated goat anti-rabbit or rabbit anti-mouse (Bio-Rad). Immunoreactive bands were visualized by chemiluminescence using ECL reagents (PerkinElmer Life Sciences).

Pull-down Assay—Two biotin-tagged 20-mer peptides designed based on the N-terminal end of human α-actinin (biotin-DHYDSQQT-NDYMQPEEDWDR) were synthesized at the W.C. Keck Biotechnology Resource Center (New Haven, CT). One of the peptides (phosphopeptide) contained a phosphotyrosine residue at position 12. Stock solutions (1 mM) were prepared in buffer containing 25 mM HEPES, pH 7.5, 25 mM NaCl, 5 mM MgCl2, and 1 mM dithiothreitol. The peptides (10 μl) were immobilized onto 90 μl of streptavidin-conjugated agarose beads (Pierce). The volume was brought up to 200 μl with phosphate-buffered saline. Immobilized peptides were incubated with lysates from COS-7 cells transfected with c-Src cDNA (500 μg/ml) for 1 h at 4°C. The beads were washed three times with buffer containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% CHAPS, and 1 mM PMSF, prior to elution and analysis by Western blotting and silver staining.

Motility Assay—Cell migration was determined using the protocol described by Yu et al. (28). Cells were trypsinized and washed once with Dulbecco’s modified Eagle’s medium containing 0.2% soybean trypsin inhibitor (Sigma-Aldrich) and then twice with Dulbecco’s modified Eagle’s medium only. Cells (1 × 105 cells/well) were added to the upper chambers of Transwells containing poly-carbonate membranes (tissue culture inserts, 6.5-mm diameter, 8-micron pores, Costar, Cambridge, MA) precoated with fibronectin (10 μg/ml, Sigma-Aldrich) for 1 h at 37°C. The lower chamber was filled with Dulbecco’s modified Eagle’s medium containing 4 μg/ml fibronectin. After incubation for 16 h at 37°C, the membrane was fixed in methanol, and cells on the upper surface were mechanically removed. The 16-h time point was chosen because in preliminary experiments we found that the total number of cells that migrated at earlier time points was extremely small. Migrated cells on the lower side of membranes were stained using the Diff-Quik (Dade International, Miami, FL) and enumerated under the microscope at ×200 magnification. Five random microscopic fields were counted per well, and all experiments were performed at least three times in duplicates.

RESULTS

PTP 1B Is an α-Actinin Phosphatase—Recently, we reported that the protein-tyrosine phosphatase (PTP) SHP-1 is an α-actinin phosphatase (44). However, because the expression of SHP-1 is confined to cells of a hematopoietic origin, we reasoned that α-actinin must be dephosphorylated by an additional PTP that is more ubiquitously expressed. In the course of the purification of SHP-1 from unstimulated platelet lysates, we observed a second phosphatase activity against α-actinin in lysates of thrombin-stimulated platelets. The phosphatase was purified using a previously described approach (44). The phosphatase activity against α-actinin was partially purified using four sequential chromatography steps that included a reactive Green 19 resin, an ion exchange Hitrap Q column, a Heparin column, and an S200 HR gel exclusion column (not shown). A peak fractions from the first and last purification steps were examined using an in-gel phosphatase assay with γ-32P-labeled random copolymer poly(Glu,Tyr) (41) and autoradiography. The peak fraction recovered off the last chromatography step (lane 2) contained a single phosphatase activity band of about 45 kDa that was identified as PTP 1B by tandem mass spectrometry (Fig. 1A) and found to contain a single phosphatase activity band of ~45 kDa. The protein was identified as PTP 1B by tandem mass spectrometry. Purified PTP 1B dephosphorylated α-actinin in vitro (Fig. 1B), but because PTP 1B can dephosphorylate a number of phosphoproteins in vitro, the specificity of PTP 1B toward α-actinin had to be confirmed in vivo.

Because platelets are not amenable to genetic manipulation, we used two different cell systems to ask whether PTP 1B is an α-actinin phosphatase. Although the phosphorylation of α-actinin is difficult to detect in many cell types, robust phosphorylation of α-actinin was observed in vanadate-treated COS-7 cells (Fig. 2A) (41). Vanadate is a reversible inhibitor of PTPs and as such it reduces, but does not completely inhibit, the activity of PTPs (47). Hence, although vanadate causes the accumulation of multiple tyrosine-phosphorylated species, over time (in our experiments a 24-h time period), a relevant phosphatase that is overexpressed in these cells can trigger significant substrate-specific dephosphorylation (e.g. Ref. 44). FAK, wild-type α-actinin (His-tagged), and wild-type PTP 1B or the PTP 1B mutant D181A (D181A PTP 1B) cDNAs were co-expressed in COS-7 cells. Western blotting analysis of whole cell lysates was used to monitor the expression of the endogenous and the recombinant proteins. As shown in Fig. 2B, the level of PTP 1B and α-actinin expressed in the transfected COS-7 cells was high. In contrast, because the cells were transfected with a small concentration of FAK cDNA, the level of total FAK (total- and recombinant-FAK) were detected, respectively, with anti-FAK and anti-HA) expressed in the transfected cells was not significantly different from that expressed in untransfected cells. Because available antibodies failed to immunoprecipitate endogenous phosphorylated α-actinin, the analysis of α-actinin tyrosine phosphorylation is currently limited to epitope-tagged recombinant α-actinin (42, 48). In this study, α-actinin was immunoprecipitated with an antibody to His. α-Actinin was tyrosine-phosphorylated in cells expressing no recombinant PTP 1B and in cells expressing...
Phosphorylation of α-actinin was next examined in PTP 1B-null fibroblasts. The experiments described with COS-7 cells were repeated in the PTP 1B-null cells with several modifications. First, the cells were transfected with cDNAs encoding for recombinant α-actinin and PTP 1B but not with FAK. Second, integrin-dependent signaling events were initiated by adherence of the cells to a fibronectin-coated surface for 1 h. Third, the PTP 1B-null cells were not treated with vanadate. Western blotting analysis of whole cell lysates (Fig. 2D) revealed that in this cell system the level of total α-actinin (detected with anti-α-actinin) was not significantly altered following the expression of recombinant α-actinin (detected with anti-His). The level of recombinant PTP 1B expressed in the transfected cells was similarly low. The low expression levels should alleviate the concern that the expressed recombinant proteins affect signaling events in a nonphysiologic manner. α-Actinin immunoprecipitated from transfected PTP 1B-null cells was tyrosine-phosphorylated (Fig. 2E). Furthermore, the phosphorylation of α-actinin was abolished in PTP 1B-null cells reconstituted with wild-type PTP 1B (Fig. 2E, lane 4). These data established that α-actinin is dephosphorylated in both COS-7 cells and PTP 1B-null cells reconstituted with PTP 1B.

Tyrosine phosphorylation at Tyr-397 in FAK is decreased in cells coexpressing wild-type α-actinin and PTP 1B—Even though the results provided a strong indication that PTP 1B dephosphorylates α-actinin in vivo, they did not exclude the possibility that the drop in the phosphorylation of α-actinin noted in the presence of wild-type PTP 1B was caused, at least in part, by FAK inactivation. To determine whether PTP 1B affected FAK phosphorylation, transfected COS-7 cells were lysed and subjected to immunoprecipitation with an antibody to FAK. Analysis of the immunoprecipitates with a pan antibody to phosphotyrosine (mAb 4G10, pTyr) failed to reveal a difference in the level of FAK phosphorylation among the various groups (Fig. 3A). Surprisingly, an analysis of parallel FAK immunoprecipitates with a phospho-site-specific antibody to Tyr-397 in FAK revealed a significant variability in the level of phosphorylation at this site (Fig. 3B). Specifically, compared with the control group, cells transfected with recombinant FAK, with or without α-actinin, exhibited an increase of ~3–4-fold in the level of Tyr-397 phosphorylation. Coexpression of PTP 1B in these cells triggered a dramatic decrease in the level of Tyr-397 phosphorylation (Fig. 3B, lane 3), reducing it to the level detected in the control group. Western blotting of whole cell lysates with the phospho-site-specific antibody to Tyr-397 in FAK confirmed these findings and went on to show that, while Tyr-397 in FAK was dephosphorylated in cells coexpressing wild-type PTP 1B, the site was phosphorylated in cells expressing D181A PTP 1B (Fig. 3C).
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FIGURE 3. Analysis of FAK dephosphorylation in COS-7 cells and PTP 1B-null fibroblasts. COS-7 cells (A–D) were either untransfected (lane 1 in all panels) or were transiently transfected with the indicated cDNAs. The cultures shown in panels A–C were treated with vanadate and were plated onto fibronectin-coated dishes for 1 h prior to analysis. FAK immunoprecipitates were analyzed by Western blotting with (A) a mAb to pTyr (4G10) and a pan FAK antiserum or (B) phospho-site-specific antibodies to Tyr-397 in FAK (p397 FAK) and a pan FAK antiserum. C and D, whole cell lysates were analyzed by Western blotting with phospho-site-specific antibodies to Tyr-397 in FAK (p397 FAK) and a pan FAK antiserum. WT, wild-type FAK; C, and D, whole cell lysates were analyzed by Western blotting with phospho-site-specific antibodies to Tyr-397 in FAK (p397 FAK) and a pan FAK antiserum. PTP 1B-null fibroblasts (E and F) were transfected with the indicated cDNAs. Whole cell lysates were analyzed by Western blotting with the indicated antibodies.

FIGURE 4. Selective dephosphorylation of Tyr-397 in FAK in cells that coexpress wild-type PTP 1B and wild-type α-actinin but not Y12F α-actinin. COS-7 cells were either untransfected (lane 1) or were transiently transfected with the indicated cDNAs. A, whole cell lysates were analyzed by Western blotting with phospho-site-specific antibodies to the indicated tyrosine residues in FAK or α, with antibodies to FAK, α-actinin, and PTP 1B, to confirm equal recombinant proteins expression levels.

At Tyr-397 was caused by the overexpression of α-actinin irrespective of its state of phosphorylation. To this end, COS-7 cells (Figs. 3 and 4) were cotransfected with either wild-type α-actinin or the α-actinin phosphomutant Y12F (Y12F α-actinin), which is no longer phosphorylated (41). Coexpression of Y12F α-actinin with PTP 1B in COS-7 cells did not disrupt the phosphorylation of FAK (Fig. 4A, lane 4). The phosphorylation of FAK at Tyr-397 was similarly not affected by re-expression of Y12F α-actinin and wild-type PTP 1B in PTP 1B-null cells (Fig. 3F, lane 4) suggesting that the dephosphorylation was linked to the expression a phosphorylation-competent α-actinin protein.

Src Activity (Src pTyr-418/Src pTyr-529) Is Not Altered in PTP 1B-null Cells Reconstituted with PTP 1B—PTP 1B activates Src in 293 cells, L cells, and platelets but not in murine fibroblasts (52, 55–57). The phosphorylation of Src at Tyr-529 promotes an intramolecular interaction between the carboxyl-terminal end and the SH2 domain, which inactivates the enzyme (58). Dephosphorylation of Tyr-529 activates Src (52). Although the phosphorylation of Src at Tyr-529 was somewhat elevated in PTP 1B-null transformed fibroblasts held in suspension, no difference in Src phosphorylation was seen between the PTP 1B-null and wild-type cells upon adhesion to fibronectin (52). If PTP 1B activates Src, overexpression of PTP 1B should increase FAK phosphorylation, and consequently, α-actinin phosphorylation. Although the results presented thus far were inconsistent with such a scenario, we next examined the state of Src activation, as detected by the relative phosphorylation at Tyr-418 and Tyr-529 in untransfected and transfected PTP 1B-null cells. As shown in Fig. 5, Src immunoprecipitates probed with phospho-site-specific antibodies to pTyr-529 and pTyr-418 showed no evidence for a PTP 1B-dependent Src activation (i.e. no decrease in pTyr-529 phosphorylation and no increase in pTyr-418 phosphorylation) in the transfected cells. Experiments carried out in the COS-7 cell system yielded identical results (data not shown). Thus, as...
reported by others (52), it appears that PTP 1B does not significantly alter the activity of Src in either the murine fibroblasts or COS-7 cells.

Phosphorylated α-Actinin Binds Src and Reduces the Binding of Src to FAK—The observation that phosphorylated α-actinin caused a selective dephosphorylation of FAK at the Src binding site raised the possibility that phosphorylated α-actinin may disrupt the FAK/Src complex by competing with FAK for binding to Src. The sequence similarity between the amino acid residues that surround Tyr-12 in α-actinin and those that surround Tyr-397 in FAK, highlighted in Fig. 6A, prompted us to ask whether an α-actinin model peptide could bind Src. To test this possibility, we synthesized two biotin-tagged 20-mer peptides based on residues 1–20 in human α-actinin. One peptide was modified by incorporation of a phosphorylated tyrosine residue at position 12 (phosphopeptide). The peptides were incubated with lysates of COS-7 cells that overexpress Src. As shown in Fig. 6B, the phosphorylated α-actinin model peptide, but not the unphosphorylated peptide, captured Src, strongly suggesting that phosphorylation at Tyr-12 in α-actinin creates a Src binding site.

The binding of phosphorylated FAK to Src was documented in chicken embryo fibroblasts superinfected with a retroviral vector encoding an oncogenically activated variant of Src (8, 10). Building on this experimental approach, we transfected COS-7 cells with the indicated cDNAs plus a constitutively active Src cDNA. Cell lysates were subjected to immunoprecipitation with an antibody to Src and probed with antibodies to FAK or to α-actinin. As expected, FAK coimmunoprecipitated with Src from lysates of cells that were not transfected with α-actinin cDNAs or that were transfected with the Y12F α-actinin cDNA (Fig. 6C). Strikingly, in cells expressing wild-type α-actinin, α-actinin coimmunoprecipitated with Src but FAK was no longer detected in the Src immunoprecipitates. Probing of whole cell lysates with the respective antibodies confirmed equal recombinant proteins expression levels (Fig. 6D). Because Src preferentially binds to phosphorylated α-actinin (Fig. 6B) but the bulk of α-actinin is dephosphorylated by PTP 1B (Fig. 2), we speculate that the binding of α-actinin to Src takes place immediately after the phosphorylation of α-actinin by FAK.

To examine whether phosphorylated α-actinin disrupted the interaction between FAK and Src in the PTP 1B-null cell system, lysates of cells transfected with the indicated cDNAs were immunoprecipitated with an antibody to Src and probed with antibodies to FAK and to α-actinin. Consistent with the results obtained with COS-7 cells, coexpression of wild-type α-actinin with PTP 1B in PTP 1B-null cells resulted in Src/α-actinin binding and limited the interaction between FAK and Src (Fig. 6E).

Coexpression of α-Actinin and PTP 1B in PTP 1B-null Cells Enhances Cell Migration—Deficiencies in several PTPs involved in the regulation of the integrin-dependent FAK/Src pathway reduce cell migration (24–26). To determine whether the PTP 1B-dependent dephosphorylation of FAK at Tyr-397 and/or α-actinin affects cell migration, the migratory behavior of untransfected PTP 1B-null cells and cells reconstituted with PTP 1B alone, or in combination with α-actinin cDNAs, were compared. Expression of PTP 1B alone enhanced cell migration by 2-fold as compared with untransfected cells, suggesting that the PTP 1B deficiency affects cell migration (Fig. 7). Coexpression of wild-type α-actinin in combination with PTP 1B enhanced cell migration by an additional 2-fold; FAK phosphorylation at Tyr-397 was no longer detected in these cells (Fig. 3E). Importantly, coexpression of Y12F α-actinin with PTP 1B did not alter the migration rate as compared with cells transfected with PTP 1B alone. These observations established that the
dephosphorylation of FAK at Tyr-397 triggered by wild-type α-actinin and PTP 1B is associated with a significant increase in cell migration.

**DISCUSSION**

We have shown that α-actinin regulates cell migration by triggering disassembly of the FAK-Src complex and by enabling a PTP 1B-mediated dephosphorylation of FAK at Tyr-397. Emerging data suggested that an increase in the phosphorylation/dephosphorylation cycles of FAK, rather than the FAK phosphorylation level per se, is the important determinant of cell migration (60). Our results support this view and highlight a novel role for phosphorylated α-actinin in regulating these cycles in concert with PTP 1B. A working model of the molecular mechanism by which α-actinin and PTP 1B coregulate the turnover of the FAK-Src complex is presented in Fig. 8. We propose that, immediately following the phosphorylation of α-actinin by FAK, α-actinin competes for the binding of Src to FAK, freeing FAK while forming Src-α-actinin complexes. The exposed phosphorylated residue Tyr-397 in FAK is then dephosphorylated by PTP 1B. Intriguingly, because Tyr-397 was the only site dephosphorylated by PTP 1B, it is possible that molecular interactions requiring the phosphorylation of FAK at other sites remain intact. Phosphorylated α-actinin exhibits a reduced affinity for actin and fails to localize to focal complexes in SHP-2−/− fibroblasts that express a hyperphosphorylated FAK; normal α-actinin distribution is restored in these cells by FAK inhibition (41, 42). These observations raise the possibility that the bulk of the α-actinin population that is phosphorylated is removed from the plaques and dephosphorylated. Re-phosphorylation of FAK at Tyr 397 by either auto- and/or trans-phosphorylation, and the dissociation of the low affinity Src-α-actinin complex are likely to trigger a repeat of the cycle. This model predicts that the availability of phosphorylated α-actinin and PTP 1B within focal adhesions determines the rate at which the Src-FAK complex turns over.

The strengthening and/or maintenance of integrin-cytoskeletal linkages requires the localization of α-actinin to focal complexes, supporting the notion that α-actinin regulates the mechanical stability and the maturation of these sites (40, 42, 43). This study uncovered an additional signaling-regulatory function for α-actinin at adhesion sites. We propose that phosphorylated α-actinin acts as a “FAK decoy” due to the sequence similarity between the amino acid residues that surround Tyr-12 in α-actinin and Tyr-397 in FAK. Earlier studies revealed that Src family kinases preferentially interact with a pTyr-Glu-Glu-Ile sequence or pTyr-hydrophilic-hydrophilic-Ile motif (61). The Src binding motif in FAK, pTyr-397-hydrophobic-hydrophilic-Ile, bears similarity to the motif pTyr-12-hydrophobic-hydrophilic-Pro in α-actinin. Our data established that the sequence similarity is functionally significant, in that phosphorylated α-actinin binds to Src in vitro, and furthermore, is able to compete for the binding of Src to FAK in vivo. Cobb et al. (8) proposed more than a decade ago that Src protects FAK from the activity of a phosphatase. Our results build on this notion and suggest that phosphorylated α-actinin has the capacity to disassemble the FAK-Src complex leaving FAK “exposed” to the phosphatase activity of PTP 1B. Importantly, PTP 1B was detected in focal adhesions where phosphorylated FAK is localized (56, 62).

The role of PTP 1B relative to integrin signaling is controversial. Overexpression of PTP 1B in 3Y1 cells transformed with v-crk caused p130CAS dephosphorylation (63). Similarly, overexpression of PTP 1B in Rat-1 fibroblasts impaired cell spreading and migration on fibronectin and inhibited the phosphorylation of p130CAS and FAK, leading these investigators to propose that PTP 1B is a negative regulator of integrin signaling (64). In contrast, expression of a catalytically inactive PTP 1B mutant in L cells, or treatment of these cells with a PTP 1B inhibitor (65), decreased fibronectin-mediated cell spreading and FAK phosphorylation, whereas overexpression of wild-type PTP 1B had no effect (56). Furthermore, in the L cell system, PTP 1B triggered Src activation, raising the possibility that PTP 1B is a positive regulator of integrin-dependent migratory pathways (56). Similar results were also obtained in 293 cells overexpressing wild-type PTP 1B (55). PTP 1B and αIIbβ3 integrin-co-dependent activation of c-Src was most recently demonstrated in platelets (57). In contrast, no change in Src activity was observed in primary PTP 1B-null fibroblasts or in fibronectin-adherent PTP 1B−/− fibroblasts transformed with SV-40 Large T-antigen (52). Consistent with these data (52), we also found no evidence that re-expression of PTP 1B in the PTP 1B fibroblasts cells affects Src activity. Thus, although our data support the view that PTP 1B is a positive regulator of integrin-signaling, the PTP 1B targets identified here, and hence the mechanism by which PTP 1B may affect the FAK-Src pathway, are novel. Compared with the parental PTP 1B-null cells, re-expression of PTP 1B in the PTP 1B−/− fibroblasts enhanced cell migration by 2-fold. Furthermore, coexpression of wild-type α-actinin and PTP 1B in these cells enhanced cell migration by 4-fold and obliterated the phosphorylation of FAK at Tyr-397. Importantly, the α-actinin phosphorylation mutant Y12F failed to reproduce these effects. These findings demonstrated that the increase in cell migration inversely correlates with the decrease in FAK phosphorylation at Tyr-397. We propose that α-actinin and PTP 1B act in concert to dephosphorylate FAK. Building on prior studies, we also suggest that the dephosphorylation of α-actinin by PTP 1B facilitates the recruitment and/or accumulation of α-actinin in focal adhesions, and consequently, increases their stability. Assuming that FAK maintains the capacity to auto-phosphorylate and/or transphosphorylate for as long as it is in contact with integrins, the dephosphorylation and/or rephosphorylation of FAK at Tyr-397 would reiterate the Src binding site triggering rapid reassembly of the FAK-Src complex, as depicted in the model shown in Fig. 8. This sequence of events would assure that the number of α-actinin molecules and their state of phosphorylation within focal adhesions is optimal for migration.
and explains why PTP 1B-null cells reconstituted with PTP 1B and wild-type α-actinin migrate faster than the untransfected, parental cells.

One of the striking observations highlighted by this study is the remarkable site-specific activity of PTP 1B, in that Tyr-397 in FAK was dephosphorylated while the phosphorylation at four other sites remained intact. This, and other examples, speak for a great degree of selectivity in phosphatase-substrate interaction that often cannot be predicted based on *in vitro* phosphatase assays (49, 66, 67). Furthermore, the selective dephosphorylation at Tyr-397 suggests that PTP 1B and α-actinin can rapidly and effectively enhance the turnover of the FAK/Src complex without disturbing other molecular interactions built around the FAK scaffold. Recent studies suggested that adhesions at the leading edge that turnover rapidly contain FAK that is phosphorylated at Tyr-397 but lack α-actinin (27). In contrast, adhesions containing organized α-actinin do not turnover and tend to slide toward the cell body (59). It is therefore possible that phosphorylated α-actinin and PTP 1B coregulate the undisrupted and organized movement of these adhesion complexes, with an overall positive impact on cell migration. Although most models suggest that FAK/Src play a significant role in rear retraction, the mechanism by which adhesions are disassembled at the rear are currently poorly understood (4, 59). It is intriguing to speculate that the phosphorylation of α-actinin at the rear will have a dual function: weakening the linkages of integrins with the cytoskeleton and triggering a rapid disassembly of the FAK/Src complex. Both of these activities are expected to facilitate rear retraction and, consequently, accelerate cell migration.

Acknowledgment—We thank David D. Schlaepfer (The Scripps Research Institute) for FAK and Src cDNAs.

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