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Mitochondrial AKAP121 Binds and Targets Protein Tyrosine Phosphatase D1, a Novel Positive Regulator of src Signaling†

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A-kinase anchor protein 121 (AKAP121) and its spliced isoform AKAP84 anchor protein kinase A (PKA) to the outer membrane of mitochondria, focusing and enhancing cyclic AMP signal transduction to the organelle. We find that AKAP121/84 also binds PTPD1, a src-associated protein tyrosine phosphatase. A signaling complex containing AKAP121, PKA, PTPD1, and src is assembled in vivo. PTPD1 activates src tyrosine kinase and increases the magnitude and duration of epidermal growth factor (EGF) signaling. EGF receptor phosphorylation and downstream activation of ERK 1/2 and Elk1-dependent gene transcription are enhanced by PTPD1. Expression of a PTPD1 mutant lacking catalytic activity inhibits src and downregulates ERK 1/2 but does not affect the activity of c-Jun N-terminal kinase 1/2 and p38α mitogen-activated protein kinase. AKAP121 binds to and redistributes PTPD1 from the cytoplasm to mitochondria and inhibits EGF signaling. Our findings indicate that PTPD1 is a novel positive regulator of src signaling and a key component of the EGF transduction pathway. By binding and/or targeting the phosphatase on mitochondria, AKAP121 modulates the amplitude and persistence of src-dependent EGF transduction pathway. This represents the first example of physical and functional interaction between AKAPs and a protein tyrosine phosphatase.

G-protein-coupled receptor-mediated activation of adenylate cyclase increases cyclic AMP (cAMP) levels at discrete points along the membrane. cAMP binds the regulatory (RI and RII) subunits of protein kinase A (PKA), dissociating the holoenzyme and releasing the free active catalytic subunit (C-PKA). Phosphorylation of nuclear and cytoplasmic substrates by PKA plays an essential role in the regulation of multiple cell functions (23, 33, 36, 45).

PKA holoenzyme is anchored to discrete cellular compartments by A-kinase anchor proteins (AKAPs) (15, 20, 34, 39). AKAPs possess a targeting domain, which serves as scaffold and membrane anchor, and a tethering or R domain, consisting of an amphipathic helix that interacts with PKA regulatory subunits. AKAPs act as localized multifunctional protein complexes that may contain several protein kinases, serine/threonine phosphatase, and phosphodiesterase. These signaling complexes ensure efficient reception, integration, and propagation of distinct signals generated inside the cell (12, 14, 18, 32, 43, 44).

AKAP84 and AKAP121 (also called D-AKAP1) arise from a single gene by alternative pre-mRNA splicing (10, 16, 25, 26, 30, 47). Both are widely expressed in different tissues and cell types. The two proteins carry the same NH_2-terminal segment, which includes the anchoring and RII-binding domains, but diverge significantly at the C terminus. AKAP84 and AKAP121 tether PKA to the mitochondrial outer surface. This localization is mediated through the interaction of AKAP121/84 with β-tubulin, an integral component of the mitochondrial outer membrane (9). Anchoring of PKA to mitochondria supports cAMP signaling and suppresses apoptosis (2, 22). AKAP84 associates with AMY-1, a c-*myc*-binding protein abundantly expressed in testes. AMY-1 may impede cAMP signal transduction to mitochondria, since the AMY-1/AKAP84/R complex binds C-PKA poorly (17). Recent studies reveal that the C-terminal KH domain of AKAP121 binds the 3′ untranslated regions of transcripts encoding the Fo-f subunit of mitochondrial ATP synthase and manganese superoxide dismutase (MnSOD) in a PKA-dependent manner. Anchoring of PKA and transcripts may link cAMP signal transduction to cotranslational events on mitochondria (19).

Protein tyrosine phosphatase D1 (PTPD1) is a widely expressed cytosolic nonreceptor tyrosine phosphatase. PTPD1 interacts with and is phosphorylated by src tyrosine kinase (29, 35). It has been implicated in the regulation of the Tec family members of membrane receptors (28). PTPD1 cDNA encodes a protein of 1,174 amino acids with N-terminal sequence ho-

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† We dedicate this paper to the memory of Stelio Varrone, an everlasting friend and remarkable mentor.

mology to the ezrin-radixin-moesin (ERM) protein family, which also includes PTPH1 and PTPMEG1 (3, 29, 35, 37, 38, 46). The catalytic PTP domain is positioned at the extreme C terminus of PTPD1. An intervening sequence of about 580 residues without apparent homology to known proteins separates the ezrin-like and the PTP domains. Furthermore, PTPD1 contains SH2 and SH3 binding domains that may subserve as a molecular platform for several signal transduction molecules. PTPD1 associates with KIF1C, a tyrosine-phosphorylated kinesin-like protein that regulates retrograde transport of vesicles from the Golgi apparatus to the endoplasmic reticulum (13). These distinct features of PTPD1 suggest that it might play a role in anchoring the cytoskeleton to signaling molecules at the cell membrane.

Here we show that PTPD1 binds to and activates src. The PTPD1/src complex up-regulates epidermal growth factor (EGF) receptor (EGFR) phosphorylation and increases ERK 1/2 signaling in response to EGF. Inhibition of src or expression of a catalytically defective PTPD1 mutant downregulate EGF signaling. AKAP121 inhibits PTPD1-dependent signaling by binding and sequestering the phosphatase to mitochondria. The dynamic interaction between AKAP121 and PTPD1 may, therefore, promote cross talk between the cAMP and tyrosine phosphatase pathways.

MATERIALS AND METHODS

Cell lines. The human embryonic kidney cell line HEK293 was cultured in Dulbecco modified Eagle's medium containing 10% fetal calf serum in an atmosphere of 10% CO_2. Where indicated, HEK293 cells were transfected with a cytomegalovirus (CMV) G418^r vector expressing either wild-type or mutant PTPD1 and subjected to selection for 4 weeks in medium containing G418 (800 μg/ml). Resistant clones were isolated, screened for expression of the transgene, and pooled (11 independent positive clones for PTPD1, 13 clones for PTPD1C1108S, and 15 clones for the CMV vector). Pools were expanded and grown in medium supplemented with 200 μg of G418/ml. GC2 cells were derived from primary mouse preleptotene spermatocytes by stable cotransfection with transgenes encoding simian virus 40 large-T antigen and a temperature-sensitive variant of the p53 transcriptional regulator protein (49). GC2 cells were grown at 37°C in Dulbecco modified Eagle's medium supplemented with 10% fetal calf serum. NIH 3T3 and COS-1 cells were propagated in Dulbecco modified Eagle's medium containing 10% calf serum in an atmosphere of 10% CO_2.

Antibodies. Anti-AKAP121 polyclonal antibody was raised as previously described (10). The antibody recognizes AKAP121 as well as AKAP84. Anti-PTPD1 polyclonal antibody was produced as previously described (35). Anti-RII antibody was purchased from Santa Cruz. Anti-RII, anti-AKAP121, and anti-PTPD1 antibodies were used for immunoprecipitation (dilution, 1/200) and immunoblot (dilution, 1/1,000) analyses. Antibodies against the following proteins were used: phospho$_{ser383}$Elk1, phospho$_{ser73}$-c-Jun, c-Jun, phospho$_{thr183/tyr185}$-JNK, JNK, glutathione *S*-transferase (GST)–ATF2, and GST–c-Jun recombinant proteins (Cell Signaling, Inc.); Flag (monoclonal; Sigma); phospho$_{Y204}$-ERK 1/2 and -ERK 2, phospho-p38α$_{MAPK}$, and p38α$_{MAPK}$ (Santa Cruz Biotechnology, Inc.); EGFR and phosphoY (Upstate, Inc.); hemagglutinin (HA) epitope (HA.11, monoclonal; Covance); src (Oncogene Research Products) and phospho$_{Y416\ and\ Y527}$ src (Cell Signaling).

Plasmids and transfection. A fusion between full-length AKAP84 and green fluorescent protein (GFP) was generated by subcloning rat AKAP84 cDNA in frame with GFP in the pEGFP-N1 vector (Clontech) (9). Mouse pCEP4-AKAP121 cDNA was a gift of C. Rubin (Albert Einstein College of Medicine, New York, N.Y.). An AKAP121 mutant carrying a deletion of residues 35 to 110 was generated by PCR with the following oligonucleotide primers: fragment 1 (encoding residues 1 to 35 of AKAP121) forward (5′-tgtaagcttATGGCAATCC AGTTGCGTTCG) (HindIII in lowercase type) and reverse (5′-gctggctagCCG ATCTTTTTTACG) (NheI in lowercase type); fragment 2 (encoding residues 111 to 857 of AKAP121) forward (5′-gctggctagCGGACACGAGGTCGCAG CCA) (NheI in lowercase type) and reverse (5′-actggatccTCAGAGGCTGGCA TAGTAGCT) (BamHI in lowercase type).

PCR fragments (1 and 2) were sequentially cloned and ligated in frame into the pCEP4 vector. For HA epitope, PTPD1 cDNA was excised from pBKS (35) and subcloned into the pcDNA 3.1 vector (Invitrogen). The HA epitope was placed at the extreme NH_2 terminus of PTPD1 by PCR with the following primers: forward, 5′-cccaagcttCCCATGTATGATGTTCCTGATTATGCTAG CCTCCCA CTGCCATTTGGGTTGAAA CTG-3′ (HindIII in lowercase type); reverse, 5′-cccaagcttGTCTGAGAGTGACAGTAGTATCCGTTC (HindIII in lowercase type).

The pcDNA3.1-PTPD1 vector was digested with the HindIII restriction enzyme. The digestion released the 5′ end of PTPD1 (nucleotides [nt] 1 to 1128), leaving intact the 3′ end of PTPD1 (nt 1129 to 3525). The digested plasmid containing the 3′ end of PTPD1 was purified from an agarose gel and ligated with the PCR fragment coding the NH_2 terminus of PTPD1 fused to the HA epitope. The PTPD1$_{\Delta 921\text{-}1150}$ mutant was generated by PCR with specific oligonucleotides. The HA-PTPD1$_{1\text{-}376}$ mutant was generated by digesting HA-PTPD1 cDNA with HindIII restriction enzyme. The HindIII-HindIII PTPD1 fragment (nt 1 to 1128) containing the HA epitope sequence was ligated in Rc/CMV vector that was predigested with the same restriction enzyme. A clone with correct orientation was used for further analysis. All plasmids were purified by using QIAGEN (Chatsworth, Calif.) tip columns and sequenced with the CEQ2000 DNA analysis system and a Beckman automated sequencer. Vectors encoding wild-type and dominant negative src (src$^-$) were kindly provided by A. Migliaccio (Second University of Naples, Naples, Italy); vector encoding dominant negative ras (ras$^-$) (H17) was a gift of C. Marshall (Institute of Cancer Research, London, United Kingdom).

Kinase assays. Cell lysates were immunoprecipitated by anti-p38α, anti-JNK 1/2, or anti-ERK 1/2. The immunoprecipitates were washed three times with lysis buffer and once with kinase buffer. The immunoprecipitates were incubated with 3 μg of GST–ATF-2 (residues 1 to 109), 3 μg of GST–c-Jun (residues 1 to 169), or 3 μg of myelin basic protein, respectively, in 25 μl of kinase buffer (25 mM HEPES, 20 mM MgCl, 20 mM glycerol 2-phosphate, 2 mM dithiothreitol, 0.1 mM sodium orthovanadate) and 10 μM [^{32}P]ATP (10 μCi; Amersham, Arlington Heights, Ill.) at 30°C for 20 min.

CAT assays. For chloramphenicol acetyltransferase (CAT) experiments, 2×10^6 cells were plated 12 h prior to transfection. Plasmid cDNAs were used at the indicated concentrations. The pGAL-CAT reporter gene and pBD-Elk1 plasmids were purchased from Stratagene. Cell extracts were prepared 48 h after transfection. CAT and β-galactosidase activities were determined as described previously (2). The total amount of transfected DNA was adjusted to 40 μg/ml with salmon sperm DNA. The pSV-LacZ (2.5 μg/ml) reporter plasmid was included to normalize for transfection efficiency. CAT activities were quantified by beta-scope and normalized to β-galactosidase activities. All transfections in mammalian cells were performed by using FuGENE 6 transfection Reagent (Roche) according to the manufacturer's instructions.

Expression of recombinant proteins in *Escherichia coli*. His$_6$-tagged AKAP84$_{1\text{-}400}$, AKAP84$_{31\text{-}100}$, and AKAP84$_{111\text{-}400}$ polypeptides were generated by PCR with the following oligonucleotide primers: AKAP121$_{1\text{-}400}$ forward, 5′-cccaagcttATGGCAATCCAGTTCCGCTCACTC (HindIII in lowercase type); AKAP121$_{31\text{-}400}$ forward, 5′-cccaagcttCGGCTCAGCAGCAATGG CAAG (HindIII in lowercase type); AKAP121$_{111\text{-}400}$ forward, 5′-cccaagcttGAC ACAAGGTTGCAGGCAGGA (HindIII in lowercase type); reverse, 5′-cccaag cttGGGGTCCTTGGTGGGGCCTGACAG (HindIII in lowercase type).

PCR products were digested with HindIII and cloned into pET-22b (Novagen) in frame with the hexahistidine tag. Retention of the correct coding frame was confirmed by DNA sequencing. To generate a GST fusion protein, rat PTPD1 cDNA (also referred to as PTP2E) (nt 1628 to 3793; GenBank accession no. NM133545) (29) was excised from the pGAD10 yeast vector with EcoRI and SalI and cloned into the pGEX-4T-3 vector in frame with GST polypeptide (Amersham). For GST-PTPD1$_{914\text{-}1174}$ (nt 2783 to 3793), PCR was performed on pGAD-PTP2E cDNA with the following primers: forward, 5′-ccggaattcCCG GATGAAAAAGACTCGAGCAGAT-3′ (EcoRI in lowercase type); reverse, 5′-acgcgtcgacGATGAGCCTAGAGCTCTTCA-3′ (SalI in lowercase type).

PCR products were digested with EcoRI and SalI and cloned in the pGEX-4T-3 vector. Hexahistidine (His$_6$)- and GST-tagged recombinant proteins were expressed in *E. coli* BL-21(DE3) and purified as previously described (9).

GST pull-down experiments. GST-PTPD1 beads (20 μl) were incubated with 1 μg of recombinant His$_6$-tagged AKAP121 polypeptides in 200 μl of 1× phosphate-buffered saline (PBS) containing 0.5% Triton X-100 in rotation at 4°C for 3 h. The pellets were washed four times in binding buffer and eluted in Laemmli buffer. The eluted samples were resolved on an 8% polyacrylamide gel electrophoresis (PAGE) gel, transferred to polyvinylidene difluoride membranes, and immunoblotted with anti-GST or anti-AKAP84 antibodies. ^{35}S-labeled AKAP84 was synthesized in vitro with the TNT quick-coupled transcription/translation system (Promega) in the presence of 45 μCi of [^{35}S]methionine.

Immunoprecipitation and immunoblot analysis. Cells or rat tissues were homogenized in lysis buffer (20 mM Tris-HCl [pH 7.4], 0.15 M NaCl, 10 mM EDTA, 0.25% Triton X-100, 0.05% Tween 20, 0.02% sodium azide) containing aprotinin (5 μg/ml), leupeptin (10 μg/ml), pepstatin (2 μg/ml), and 0.5 mM phenylmethylsulfonyl fluoride. The lysates were cleared by centrifugation at 15,000 × *g* for 15 min. Cell lysates (2 mg) were immunoprecipitated with the indicated antibodies. An aliquot of cell lysate (100 μg) or immunoprecipitates were resolved on a sodium dodecyl sulfate (SDS)-PAGE gel and transferred to an Immobilon P membrane. The immunoblot analysis was performed as previously described (2). Chemiluminescent signals were quantified by scanning densitometry (Molecular Dynamics). Highly purified mitochondria and the supernatant fraction were isolated as described previously (24).

Immunofluorescence analysis. GC2 cells and transiently transfected COS-1 cells were rinsed with PBS and fixed in 3.7% formaldehyde for 20 min. After permeabilization with 0.5% Triton X-100 in PBS for 5 min, the cells were incubated with 1× PBS and 0.1-mg/ml bovine serum albumin for 1 h at room temperature. Double immunofluorescence was carried out with the following antibodies: anti-SOD monoclonal (1/200), anti-AKAP121 goat polyclonal (1/200) (sc-6439; Santa Cruz), anti-PTPD1 rabbit polyclonal (1/200), anti-AKAP121/84 rabbit polyclonal (1/100) (10), anti-HA monoclonal (1/100). Fluorescein- or rhodamine-tagged anti-rabbit and anti-mouse immunoglobulin G (Technogenetics) secondary antibodies were used. Coverslips were analyzed by confocal microscopy.

DNA synthesis was evaluated by a short pulse of 5-bromo-2-deoxyuridine (BrdU) as follows. HEK293 cells were transiently cotransfected with GFP and CMV or $PTPD1_{C1108S}$ vectors. Twenty-four hours after transfection, cells were replated, serum deprived overnight, and stimulated with serum for the indicated times. Three hours before harvesting, the cells were labeled with 100 μM BrdU (Roche Diagnostics). Labeled cells were fixed, permeabilized, and immunostained for BrdU incorporation according to the instruction manual. Briefly, the coverslips were treated for 10 min with HCl (1.5 M in PBS), followed by three washes in PBS, incubated with diluted (1:1 in PBS) fluorescein-conjugated mouse anti-BrdU monoclonal antibodies (Boehringer Mannheim Co.), and washed three times with PBS. Mouse antibody was detected by using Texas red-conjugated goat anti-mouse antibodies (Calbiochem). The BrdU-labeled nuclei (red) of GFP-positive cells (green) were visualized by fluorescence microscopy and counted.

RESULTS

PTPD1 associates with AKAP121/AKAP84. We sought to isolate gene products that interact with AKAP84/AKAP121. AKAP121 and AKAP84 share the same amino terminus, including the region between the mitochondrial targeting domain and the RII-binding site (Fig. 1A) (10, 16, 25, 30). A cDNA encoding the NH_2 terminus of rat AKAP84 (residues 1 to 117) was inserted into the yeast bait plasmid pBD-Gal4. The GBD-A84 fusion (Fig. 1A) was used to screen a rat thyroid cDNA library in a yeast two-hybrid system. The screen yielded multiple isolates of two cDNAs. One clone was identified as β-tubulin cDNA (9). A second cDNA clone (pGAD-PTPD1) encoded a polypeptide with homology to the carboxy terminus of PTPD1 (residues 584 to 1174), a src-associated PTP (Fig. 1B) (29, 35). Cotransfection of pGAD-PTPD1 and pGBD-$A84_{1-117}$ strongly activated transcription of *his* and *lacZ* reporter genes in the yeast YRG2 strain (data not shown).

We measured in vitro binding between AKAP84 and PTPD1. First, we incubated GST-tagged $PTPD1_{584-1174}$ and in vitro-translated ^{35}S-labeled AKAP84. Figure 1C shows that AKAP84 bound GST-$PTPD1_{584-1174}$. We then repeated the assay with purified recombinant proteins. Purified His_6-tagged $AKAP84_{1-400}$ polypeptide directly bound recombinant GST-$PTPD1_{584-1174}$ (Fig. 1D). An AKAP84 mutant ($AKAP84_{31-400}$) lacking the mitochondrial targeting motif efficiently bound PTPD1. The deletion of residues 1 to 110 ($AKAP84_{111-400}$), however, nearly abolished interaction with the phosphatase (Fig. 1D). Finally, we performed in vitro pull-down experiments on rat testis extracts, which contain high levels of endogenous AKAP84 (9, 30). GST-$PTPD1_{584-1174}$ bound endogenous AKAP84 (Fig. 2A). Taken together, the data demonstrate that PTPD1 binds to the amino terminus of AKAP84 between the mitochondrial anchoring and RII binding domains.

AKAP121, PTPD1, and PKA form a stable complex in vivo. To determine whether PTPD1 was bound to AKAP84 in vivo, membrane proteins were solubilized from rat testis extracts by using Triton X-100 and subjected to immunoprecipitation with specific antibodies directed against PTPD1 or AKAP84. Anti-PTPD1, anti-RII, and anti-AKAP84 immunoprecipitates contain significant amounts of endogenous PTPD1 (Fig. 2B). AKAP121 shares the same NH_2 terminus as AKAP84 (Fig. 1A) and also binds PTPD1 in mammalian cells. This was demonstrated as follows. HEK293 cells were transfected with HA-PTPD1 and AKAP121 vectors, harvested, and lysed. Cell lysates were subjected to immunoprecipitation and immunoblot analysis with anti-HA or anti-AKAP121 antibodies. Figure 2C shows that AKAP121 and HA-PTPD1 formed a stable complex in cell extracts. To identify the domain of PTPD1 that mediates interaction with AKAP121, we performed deletion mutagenesis on PTPD1. HA-tagged PTPD1 mutants lacking the catalytic domain ($PTPD1_{\Delta 921-1150}$) or coding for the NH2 terminus of the phosphatase ($PTPD1_{\Delta 377-1174}$) were coexpressed with AKAP121 in HEK293 cells. Cell lysates were immunoprecipitated with anti-HA antibody and immunoblotted with anti-AKAP121 antibody. As shown in Fig. 2D, Wild-type PTPD1 and $PTPD1_{\Delta 921-1150}$ associated with AKAP121, whereas $PTPD1_{\Delta 377-1174}$ did not. The expression levels of both mutants were comparable. Altogether, the data indicate that residues 584 to 921 on PTPD1 mediate the interaction with the NH_2 terminus of AKAP121/84.

PTPD1 was originally described as a src-binding protein (35). We confirmed this observation and found that PTPD1 and src form a complex with AKAP121. Protein extracts from cells transiently transfected with PTPD1 and src were immunoprecipitated with anti-src antibodies. The immunoprecipitates were size fractionated on SDS-PAGE and immunoblotted with anti-HA or anti-src antibody. Figure 2E shows that src and PTPD1 formed a stable complex in vivo. To determine whether src was present in PTPD1/AKAP121 complexes, we transiently transfected HEK293 cells with plasmids expressing src, PTPD1, and AKAP121. The cells were lysed, and the extracts were treated with anti-src antibody. Figure 2F shows that anti-src antibody immunoprecipitated PTPD1 and AKAP121. Complementary experiments in which we immunoprecipitated AKAP121 or PTPD1 also revealed a src-AKAP121-PTPD1 complex (data not shown).

We then determined the intracellular localization of AKAP121 and PTPD1 in intact cells. We used primary mouse preleptotene spermatocytes (GC2) (49), which express high levels of endogenous AKAP121 (9, 16). The cells were subjected to double immunostaining with anti-AKAP121 or anti-PTPD1 and anti-MnSOD antibody. The signals were collected and analyzed by confocal microscopy. As previously shown, AKAP121 selectively localizes on mitochondria. Thus, the AKAP121 signal colocalized with MnSOD, a protein that resides in the mitochondrial matrix (Fig. 3A, a to c) (9, 10, 25).

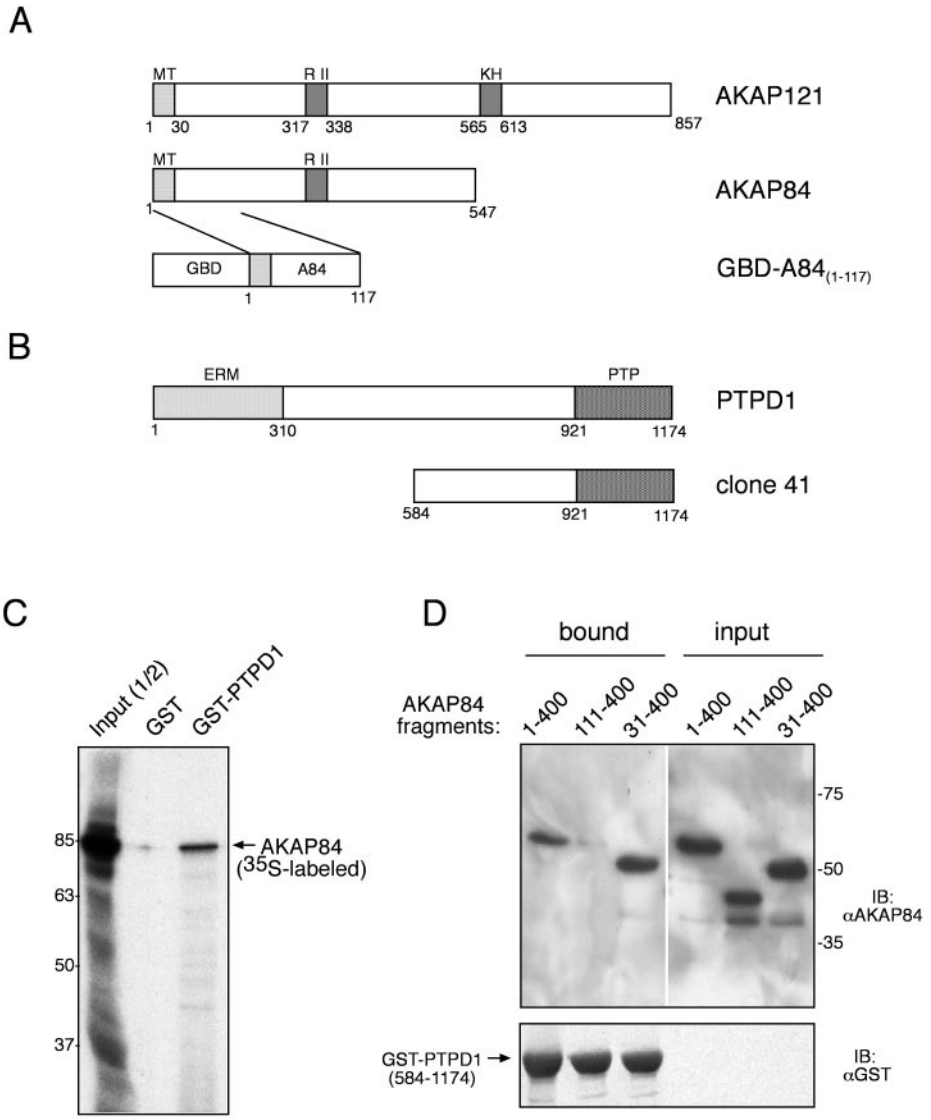


FIG. 1. The amino terminus of AKAP121 interacts with tyrosine phosphatase PTPD1. (A) Schematic representation of AKAP84 and AKAP121 proteins. The mitochondrial targeting motif (MT), PKA binding domain (RII), and RNA-binding motif (KH) are boxed. GBD-A84$_{1–117}$ is the recombinant chimera (pBD-A121N) containing the DNA-binding domain of GAL4 (GAL4-BD) fused to the NH_2 terminus (residues 1 to 117) of AKAP121/84. (B) Schematic representation of the clone (clone no. 41) isolated by a yeast two-hybrid assay and its sequence homology with human PTPD1. The ERM and catalytic domain (PTP) are indicated. (C) In vitro-translated ^{35}S-labeled AKAP84 was subjected to pull-down experiments with GST-PTPD1$_{584–1174}$. The input (1/2) and bound fractions were resolved on an SDS–10% PAGE gel. The gel was fixed, dried, and exposed to X-ray film. (D) His$_6$-tagged AKAP84$_{1–400}$ polypeptide was incubated with purified GST-PTPD1$_{584–1174}$, GST polypeptide, or Sepharose beads. Deletion mutants (AKAP84$_{31–400}$ and AKAP84$_{111–400}$) were also used. The bound and input fractions were immunoblotted (IB) with anti-AKAP84 (αAKAP84) (upper panel) or anti-GST (αGST) (lower panel) antibodies.

PTPD1 staining partly overlaps with MnSOD (Fig. 3A, d to f). We could also demonstrate that some PTPD1 was associated with AKAP121 at mitochondria (Fig. 3A, g to i). Note, however, that PTPD1 staining is also evident at the cell periphery (membrane), where no mitochondria or AKAP121 were detectable, suggesting that PTPD1 may have additional cellular partners. To demonstrate that AKAP121 binding was required for localization of PTPD1 on mitochondria, we generated an AKAP121 mutant carrying a deletion of the PTPD1 binding domain (residues 35 to 110). Thus, PTPD1, when coexpressed with AKAP121, accumulates in part on mitochondria, as shown by merging double labeling with anti-AKAP121 and anti-HA antibodies (Fig. 3A, l to n). In contrast, in cells expressing mutant AKAP121$_{\Delta35\text{-}110}$, the PTPD1 staining is quite distinct from that of AKAP121$_{\Delta35\text{-}110}$ (Fig. 3A, o to q). Mutant AKAP121 selectively localizes on mitochondria as wild-type protein, as demonstrated by double labeling with MnSOD (data not shown).

To confirm PTPD1 localization on mitochondria, organelles were isolated from HEK293 cells transiently transfected with PTPD1 or AKAP121 or both. Mitochondrial and supernatant fractions were immunoblotted with the indicated antibodies.

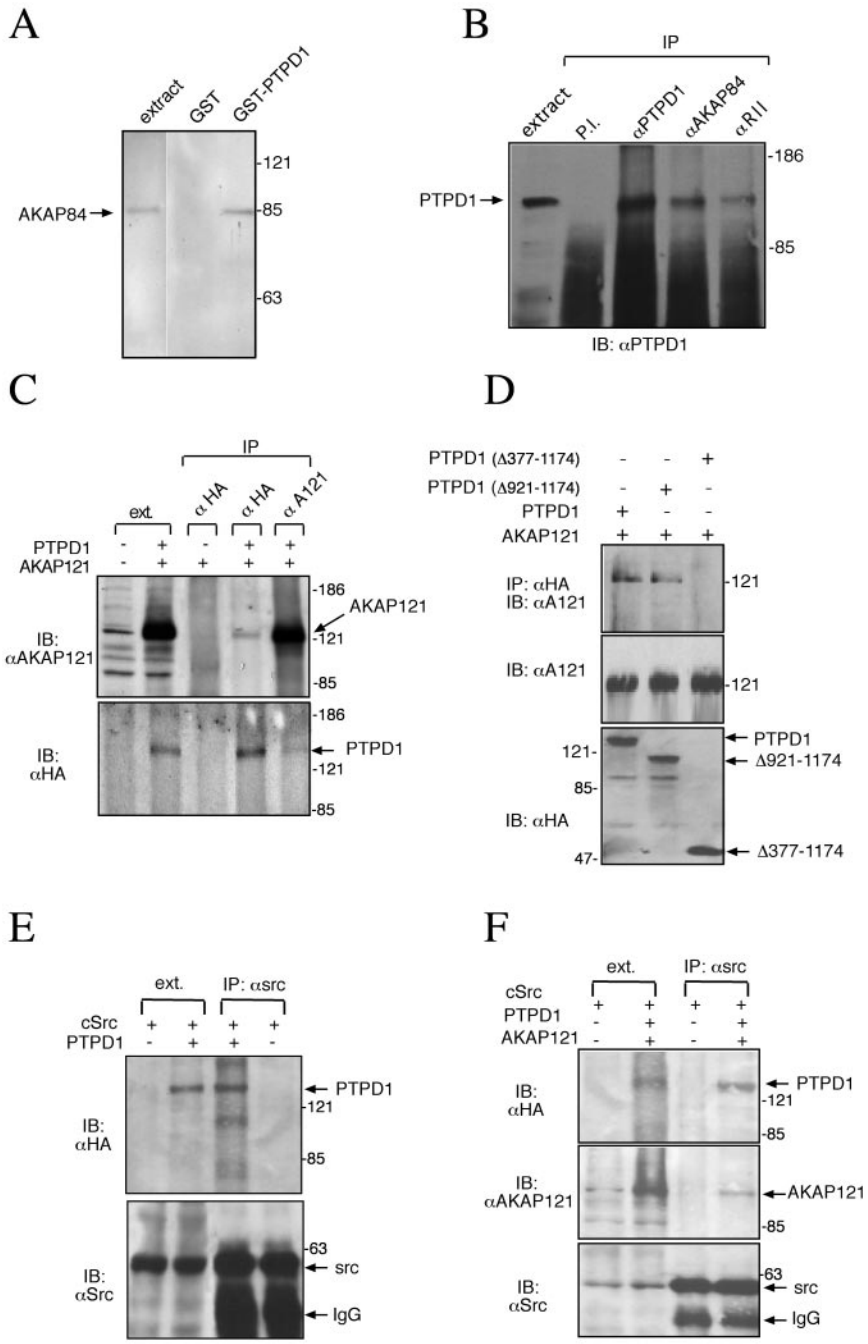
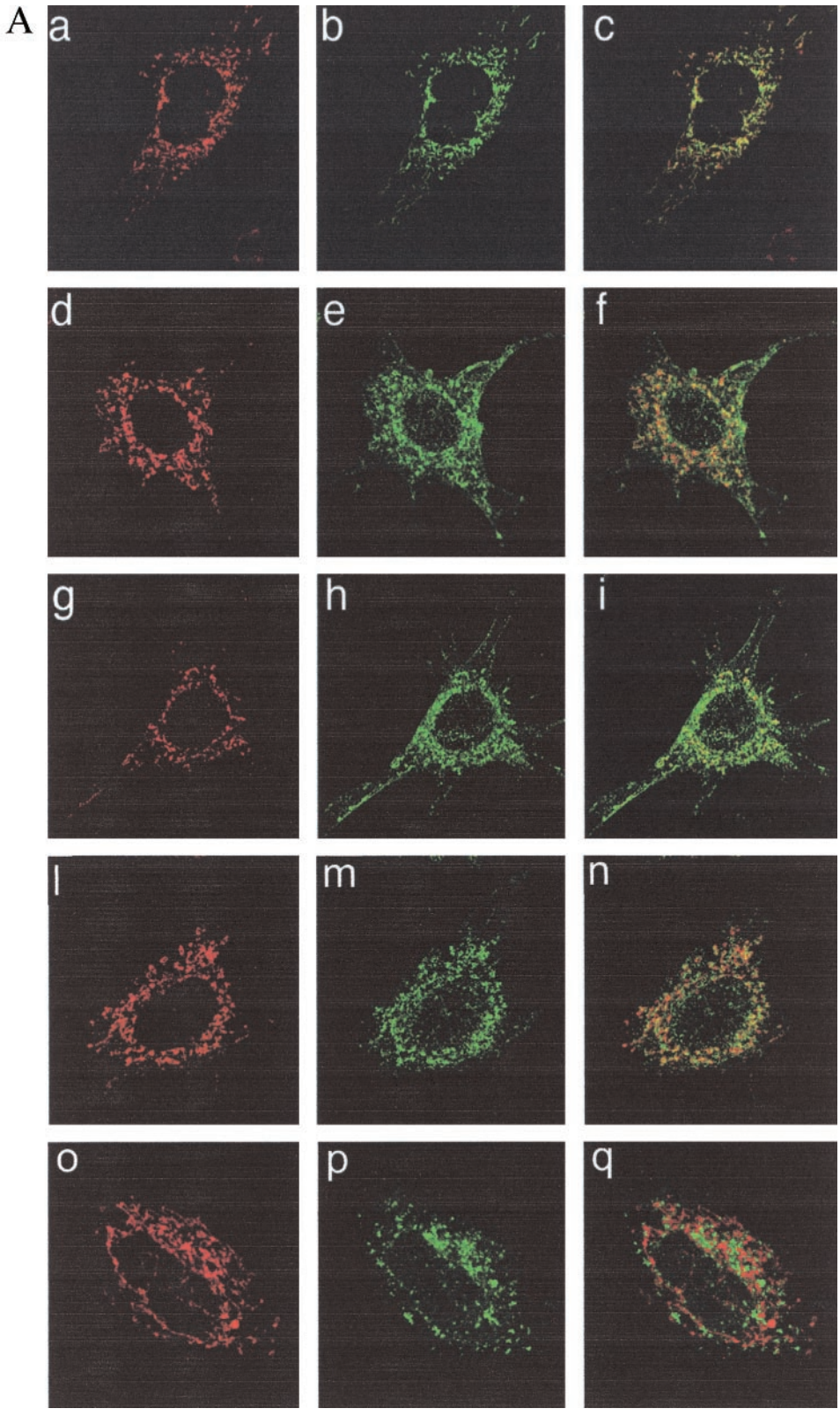


FIG. 2. AKAP121 interacts with PTPD1. (A) Rat testis extracts (2 mg) were subjected to pull-down assays with GST-PTPD1$_{584-1174}$ or GST. The bound fraction and 100 μg of testis extract were immunoblotted with anti-AKAP84 antibody. (B) A protein extract (4 mg) from rat testes was immunoprecipitated with the indicated antibodies. Preimmune serum (P.I.) was used as the negative control. The immunoprecipitates (IP) and 100 μg of testis extract (ext) were immunoblotted with anti-PTPD1 antibody. The specificity of the signal seen in the testes was verified by preadsorbing the antibody with a purified recombinant fragment of PTPD1 (data not shown). (C) Protein extracts from control or HA-PTPD1/AKAP121-expressing cells were immunoprecipitated with anti-HA or anti-AKAP121 antibody. The immunoprecipitates and a sample (100 μg) of protein extracts (ext) were immunoblotted with anti-HA (lower panel) or anti-AKAP121 (upper panel) antibody. (D) HEK293 cells were transiently cotransfected with expression vectors coding for AKAP121 and HA-PTPD1, AKAP121 and HA-PTPD1$_{\Delta 921-1174}$, or AKAP121 and HA-PTPD1$_{\Delta 377-1174}$. Cell lysates were immunoprecipitated with anti-HA antibody and immunoblotted with anti-AKAP121 antibody (upper panel). Similar amounts of AKAP121 (middle panel) and HA-PTPD1 proteins (lower panel) were expressed in the three lysates. (E) Protein extracts from cells transiently transfected with src or HA-PTPD1/src expression vectors were immunoprecipitated with anti-src antibody. The immunoprecipitates and a sample (100 μg) of protein extracts (ext) were immunoblotted with anti-HA (upper panel) or anti-src (lower panel) antibody. (F) HEK293 cells were singly or triply transfected with expression vectors for src or AKAP121/HA-PTPD1/src, respectively. Cell lysates were immunoprecipitated with anti-src antibody. The immunoprecipitates were immunoblotted with anti-HA (upper panel), anti-AKAP121 (middle panel), or anti-src (lower panel) antibody. α, anti.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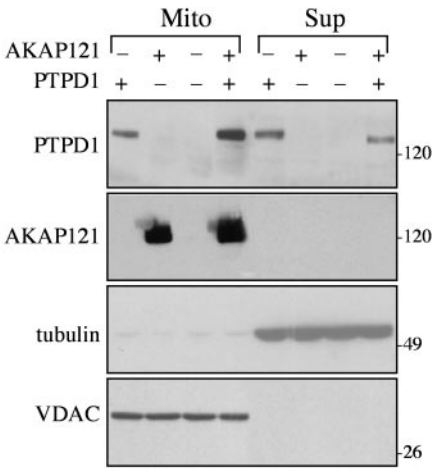

FIG. 3. AKAP121 and PTPD1 colocalize on mitochondria. (A) Growing GC2 cells were subjected to double immunostaining with anti-AKAP121 or anti-PTPD1 and anti-MnSOD. The images were collected and analyzed by confocal microscopy. The following antibodies were used: (a to c) anti-AKAP121 (green) and anti-MnSOD (red, labeled with Texas Red); (d to f) anti-PTPD1 (green) and anti-MnSOD (red); (g to i) anti-PTPD1 (green) and anti-AKAP121 (red). (l to q) COS-1 cells were transiently cotransfected with HA-PTPD1 and AKAP121 (panels l to n) or HA-PTPD1 and $AKAP121_{\Delta 35\text{-}110}$ panels (o to q) and immunostained with anti-HA mouse (green) and anti-AKAP121 rabbit (red) antibodies. The right panel of each set of the images is a merged composite of both signals (green and red). (B) HEK293 cells were transiently transfected with AKAP121 or HA-PTPD1 or both expression vectors and fractionated. Mitochondrial (Mito) and supernatant (Sup) fractions, prepared as described previously (24), were immunoblotted with the following antibodies: anti-voltage-dependent anion channel (VDAC), anti-tubulin, anti-HA (for PTPD1), and anti-AKAP121. A representative set of autoradiograms is presented.

As shown in Fig. 3B, AKAP121 accumulates in the mitochondrial fraction, as does the mitochondrial voltage-dependent anion channel. About 30% of the PTPD1 copurified with the mitochondrial fraction. The remainder codistributed with tubulin, which is abundant in the cytosolic fraction. Coexpression of AKAP121 significantly redistributed PTPD1 from the supernatant to mitochondria; about 70% of PTPD1 was now recovered in the mitochondrial fraction (Fig. 3B, compare columns 1, 4, 5, and 8). Collectively, these findings indicate that AKAP121 binds and sequesters PTPD1 on mitochondria.

PTPD1 amplifies EGF signaling. EGFR belongs to the family of tyrosine kinase membrane receptors (8, 11, 27, 41, 51). Upon binding of its ligand (EGF), EGFR undergoes dimerization and transautophosphorylation at its cytoplasmic tail. This leads to the recruitment of the Sos guanine nucleotide exchange factor to the plasma membrane via the Grb2 adapter protein. Sos activates ras, which then stimulates raf1-MEK–mitogen-activate protein kinase (MAPK) (ERK) and other signaling pathways.

Src is both an effector and upstream regulator of the EGFR (1, 4, 5, 7, 51). Given the ability of PTPD1 to bind src, we suspected that PTPD1 could modulate the EGF pathway. We therefore evaluated EGF-dependent phosphorylation of transcription factor Elk1, a substrate of ERK 1/2. HEK293 cells were transiently cotransfected with vectors encoding Flag-tagged Elk1 and HA-PTPD1. Twenty-four hours posttransfection, the cells were serum deprived overnight, stimulated with EGF (100 ng/ml), and harvested at the indicated times. In control cells, EGF stimulation induced a time-dependent increase of Elk1 phosphorylation (Fig. 4A and C). Phosphorylation peaked about 20 min after EGF treatment and sharply declined over the next 10 min. Cotransfection with PTPD1 enhanced and prolonged responsiveness to EGF. We then sought to determine whether PTPD1 amplification of EGF-stimulated ERK1/2 required ras or src activity (21, 27, 41). Cotransfection with a ras^- or src^- greatly reduced EGF-induced Elk1 phosphorylation, both in control cells and in cells cotransfected with PTPD1 (Fig. 4A to C). These findings indicate that PTPD1 upregulates the src-ras-raf-MEK-MAPK-Elk1 signal transduction pathway.

To determine whether the catalytic activity of PTPD1 was required for src activation, we generated a PTPD1 mutant carrying a Cys_{1108}-to-serine substitution ($PTPD1_{C1108S}$). The Cys_{1108} residue, located within the PTP domain, is highly conserved among distinct families of tyrosine phosphatases. Mutations in the residue block the catalytic activity of these enzymes (3, 28). HEK293 cells were transiently cotransfected with Elk1-Flag and wild-type or $PTPD1_{C1108S}$ vectors, serum deprived, and stimulated with EGF. Cell lysates were immunoblotted with anti-phosphoElk1 and anti-Flag antibody. As seen above, transfection with wild-type PTD1 strongly stimulated Elk1 phosphorylation. In contrast, expression of $PTPD1_{C1108S}$ partially inhibited EGF induction of Elk1 phosphorylation (Fig. 5A). PTPD1 stimulation of EGF signaling was replicated in NIH 3T3 cells, which express low levels of endogenous EGFR and are less responsive to EGF stimulation. We coexpressed EGFR by transiently transfecting the cells with EGFR cDNA. Again, wild-type PTPD1 stimulated and $PTPD1_{C1108S}$ inhibited EGF-induced phosphorylation of Elk1 (Fig. 5B). The finding that the $PTPD1_{C1108S}$ mutant acted in a dominant negative manner toward Erk 1/2 signaling, both in NIH 3T3 and HEK293 cells, implies a critical role of the endogenous phosphatase in the EGF transduction pathway.

We also investigated the effect of PTPD1 expression on ERK 1/2 phosphorylation. HEK293 cells were transiently transfected with Elk1-Flag and PTPD1 or $PTPD1_{C1108S}$ vectors, serum deprived, and stimulated with EGF. Cell lysates were immunoblotted with an antibody that recognizes ERK 1/2 phosphorylated at tyrosine 204. In control cells, phosphorylation of ERK 1/2 peaked 10 to 20 min after EGF stimulation and sharply declined over the next 10 min (Fig. 5C and D). Expression of PTPD1 enhanced ERK 1/2 phosphorylation, whereas $PTPD1_{C1108S}$ was inhibitory. Collectively, these data indicate that PTPD1 plays a major positive role in the regulation of EGF signaling.

Indeed, expression of the PTPD1 mutant interferes with cell growth. HEK293 cells were transiently transfected with $PTPD1_{C1108S}$ or CMV vector. Twenty-four hours after transfection, cells were replated, harvested at the indicated times, and counted. As shown in Fig. 5E, the $PTPD1_{C1108S}$ mutant significantly impaired cell growth. Moreover, expression of $PTPD1_{C1108S}$ mutant significantly decreased serum-dependent

DNA synthesis of HEK293 cells, as shown by BrdU labeling experiments (Fig. 5F).

To confirm that PTPD1 selectively up-regulates ERK 1/2, we stably expressed wild-type PTPD1 or $PTPD1_{C1108S}$ in HEK293 cells and evaluated both the phosphorylation and activity of endogenous MAPKs in response to EGF. Figure 6A and B shows that PTPD1 significantly increased and sustained both the phosphorylation and activity of ERK 1/2 compared to control cells. Expression of $PTPD1_{C1108S}$ drastically decreased both the level and persistence of ERK 1/2 phosphorylation and activity. We also analyzed two related MAPKs, p38α and c-Jun N-terminal kinases (JNK 1/2), in these cell lines. Cell lysates were immunoblotted with antibodies specific for Thr/Tyr dual-phosphorylated p38α and JNK 1/2 or immunoprecipitated and assayed for kinase activity on specific substrates. In control cells, EGF treatment slightly increased phosphorylation of JNK 1-p46. However, neither PTPD1 nor $PTPD1_{C1108S}$ affected the phosphorylation or activity of JNK 1/2 (Fig. 6C and D) or p38α (Fig. 6E and F) kinases.

PTPD1 increases src activity and enhances phosphorylation of EGFR. We have demonstrated that PTPD1 selectively increases ERK 1/2 signaling and that this requires src activity (Fig. 4). Accordingly, we now sought to determine whether PTPD1 modulates src kinase activity. HEK293 cells stably expressing PTPD1 or $PTPD1_{C1108S}$ were serum deprived overnight and exposed to EGF for various times. Src protein was immunoprecipitated from cell lysates, and its kinase activity was evaluated in vitro with enolase as the substrate. In control cells, EGF stimulation increased src activity ~2.5-fold 15 to 30 min following exposure. Under the same conditions, expression of PTPD1 stimulated src activity ~4-fold, whereas expression of $PTPD1_{C1108S}$ inhibited src activity ~2-fold relative to controls (Fig. 7A).

Activation of src requires dephosphorylation of src residue Y527. This promotes displacement of the SH2 domain from this residue and subsequent autophosphorylation of residue Y416 within the activation loop (1, 40). Since PTPD1 was isolated in complex with src and stimulated a src-dependent pathway, we sought to determine whether PTPD1 enhanced Y527 dephosphorylation. HEK293 cells stably expressing either PTPD1 or $PTPD1_{C1108S}$ were serum deprived overnight, stimulated with EGF, harvested, and lysed at the indicated time points. Cell lysates were immunoblotted with specific antibody directed against phospho-Y527. In control cells, dephosphorylation of Y527 was observed 15 min after EGF stimulation and persisted for at least the next 45 min. Dephosphorylation of Y527 was more pronounced in cells expressing PTPD1 at each time point tested. $PTPD1_{C1108S}$ drastically inhibited Y527 dephosphorylation (Fig. 7B and C)

Src and EGFR participate in a positive regulatory loop. EGF stimulation activates src, which in turn phosphorylates EGFR at several tyrosine residues (1, 4, 6, 7, 42, 48, 50, 51). We therefore sought to determine whether PTPD1 enhances the phosphorylation of EGFR. Cell lysates from serum-deprived or EGF-treated cells were immunoprecipitated with anti-EGFR antibody and sequentially immunoblotted with anti-phosphotyrosine and anti-EGFR antibody. EGF stimulation of control cells induced phosphorylation and turnover of EGFR. EGFR phosphorylation was increased in cells expressing PTPD1, although the amount of the receptor was unaffected (Fig. 7D).

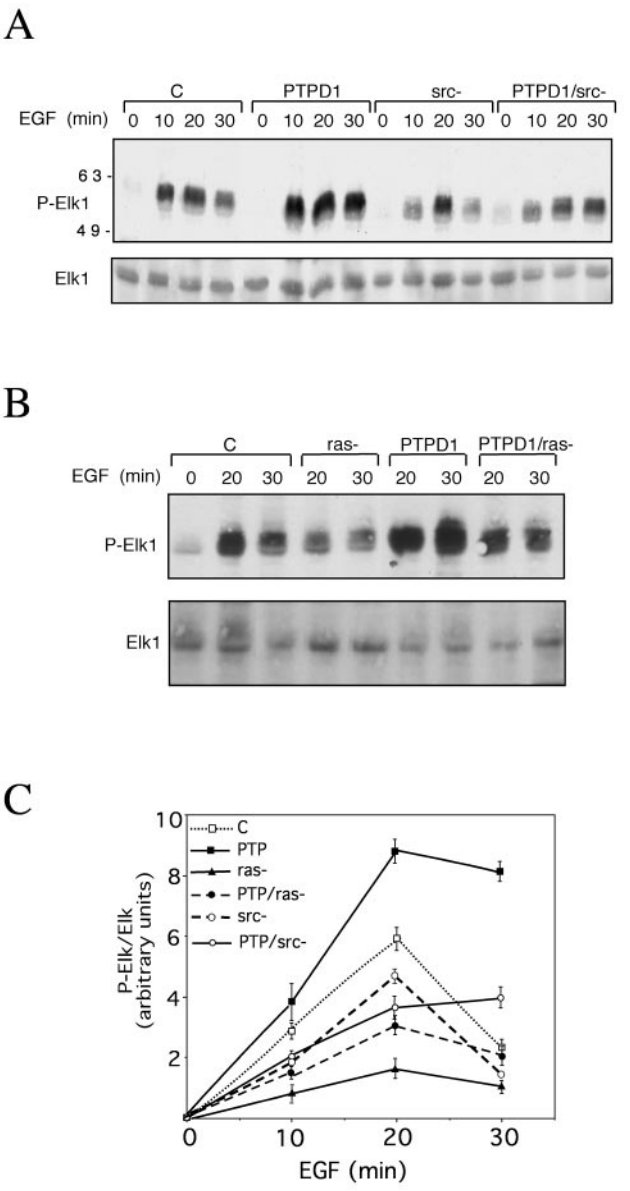

FIG. 4. PTPD1 increases ERK 1/2 signaling. HEK293 cells were transiently cotransfected with expression vectors for Elk1-Flag and HA-PTPD1 in either the presence or absence of cDNAs encoding dominant negative derivatives of src (src^-) (A) or ras (ras^-) (B). Twenty-four hours following transfection, cells were deprived of serum overnight and stimulated with EGF (100 ng/ml) for the indicated times. Cells were harvested and lysed. Cell lysates were immunoblotted with anti-phospho-Elk1 or anti-Flag antibody. The antibody recognizes Elk1 phosphorylated at Ser_{136}. Similar amounts of HA-PTPD1 were expressed in the three lysates tested. (C) Cumulative data are expressed as means ± standard errors of the results from four to six independent experiments.

AKAP121 binds PTPD1 and inhibits EGF signaling. We demonstrated that AKAP121 binds to PTPD1 and colocalizes it to mitochondria. We also showed that PTPD1 amplifies EGF signaling via src activation. We now sought to determine whether binding of AKAP121 to PTPD1 interferes with EGF

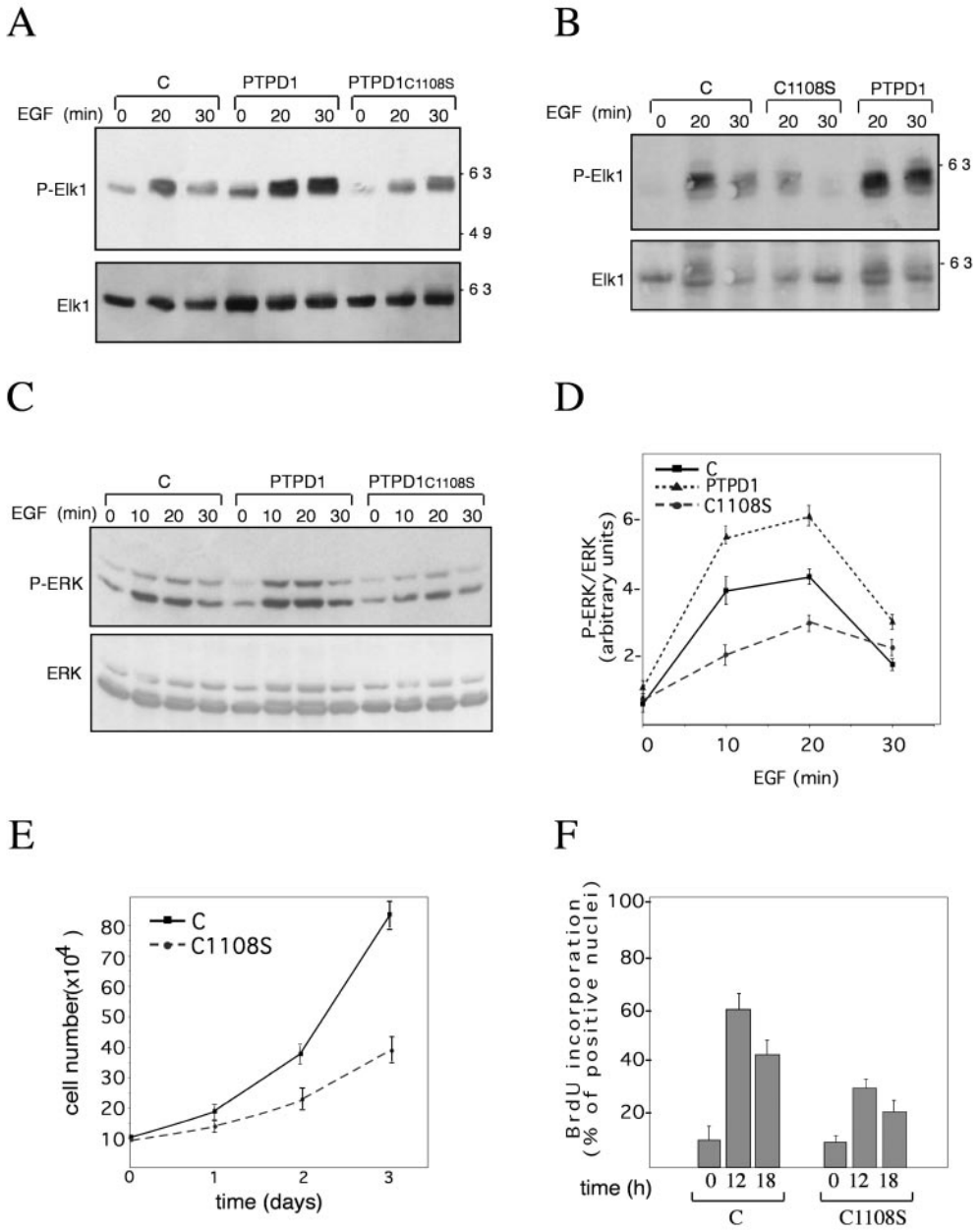

FIG. 5. Catalytically inactive mutant of PTPD1 inhibits ERK 1/2 signaling. HEK293 (A) or NIH 3T3 (B) cells were transiently cotransfected with expression vectors for Elk1-Flag, Elk1-Flag and PTPD1, or Elk1-Flag and $PTPD1_{C1108S}$. For NIH 3T3 cells, a vector expressing human EGFR was included. Stimulation with EGF was performed as described above (see the legend to Fig. 4). Cell lysates were immunoblotted with anti-phospho-Elk1 and anti-Flag antibody. (C) Cell lysates from serum-deprived or EGF-stimulated HEK293 cells transiently transfected with HA-PTPD1 or HA-$PTPD1_{C1108S}$ cDNAs were immunoblotted with anti-ERK 1/2 or anti-phospho-Y204 ERK 1/2 antibody. (D) Cumulative data are expressed as means ± standard errors of the results from three independent experiments. Under these conditions, similar amounts of PTPD1 or HA-$PTPD1_{C1108S}$ were expressed. (E) Growth curve of HEK293 cells transiently transfected with either CMV or HA-$PTPD1_{C1108S}$ expression vector. Twenty-four hours following transfection, cells were replated in multiple wells (10^5 cells/well), harvested at the indicated days, and counted. The data are expressed as means ± standard errors of the results from three independent experiments made in triplicate. (F) HEK293 cells were transiently cotransfected with GFP and CMV or $PTPD1_{C1108S}$ vectors. Twenty-four hours after transfection, cells were replated, serum deprived overnight, and stimulated with serum for the indicated times. Three hours before harvesting, the cells were labeled with 100 μM BrdU, fixed, immunostained for BrdU incorporation, and analyzed by fluorescent microscopy. The data are expressed as percentages of BrdU-labeled nuclei/GFP-positive cells and are expressed as means ± standard errors of the results from two independent experiments made in triplicate. Similar data were obtained by treating the transfected and serum-deprived cells with EGF (data not shown).

signaling. Figure 8A and B shows that this is indeed the case. Cotransfection of HEK293 cells with AKAP121 eliminated PTPD1 stimulation of Elk1 phosphorylation. Furthermore, AKAP121 alone inhibited EGF-induced Elk1 phosphorylation in a dose-dependent manner (Fig. 8C and D). To demonstrate that AKAP121 binding to PTPD1 was required for the inhibition of EGF signaling, we analyzed Elk phosphorylation in the presence of the AKAP121 mutant lacking the PTPD1 binding

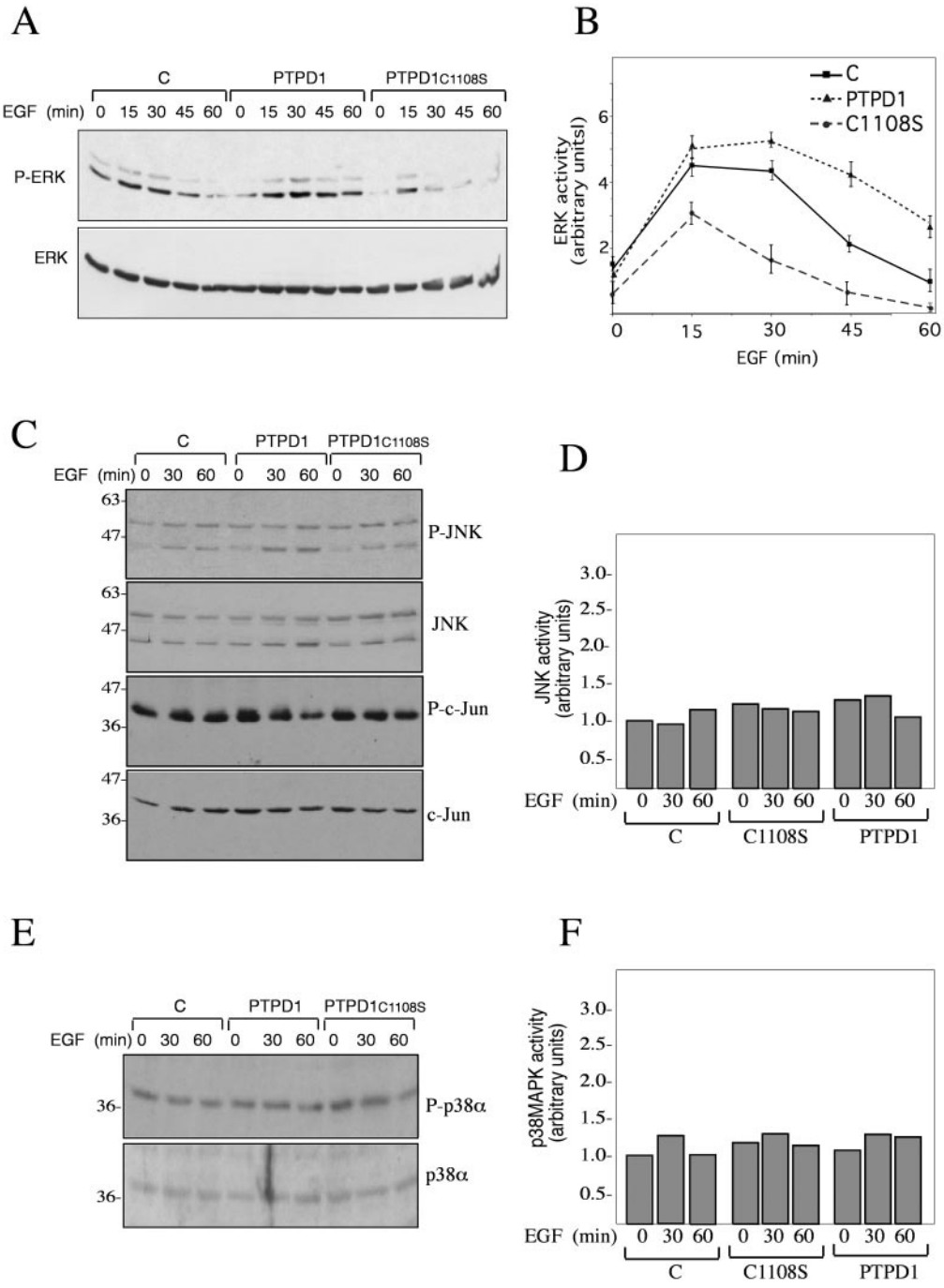

FIG. 6. PTPD1 selectively increases ERK 1/2 activity. HEK293 cells stably expressing similar amounts of HA-PTPD1 and HA-PTPD1$_{C1108S}$ were serum deprived overnight, stimulated with EGF, and lysed. Cells stably transfected with CMV vector were used as controls (c). Cell lysates were sequentially immunoblotted with anti-phospho-ERK 1/2 and anti-ERK 2 (A) or immunoprecipitated with anti-ERK 2 antibody and analyzed by in vitro kinase assay on myelin basic protein as the substrate. (B) Data are presented as means ± standard errors of the results from three independent experiments. The value from unstimulated control cells was set as 1. (C) Cell lysates (as described for panel A) were sequentially immunoblotted with anti-phospho$_{thr183/tyr185}$-JNK and anti-JNK or anti-phospho$_{ser63}$-c-Jun and anti-c-Jun antibodies. Note that phospho-JNK gel was overexposed to enhanced chemiluminescence reagents (30 min) compared to the JNK gel (15 s). (D) Cell lysates were immunoprecipitated with anti-JNK 1/2 antibody and subjected to in vitro kinase assay on GST–c-Jun as the substrate. (E) Cell lysates (as described for panel A) were sequentially immunoblotted with anti-phospho-p38α MAPK and anti-p38α MAPK. (F) Cell lysates were immunoprecipitated with anti-p38α MAPK antibody and analyzed by in vitro kinase assay on GST-ATF2 as the substrate. Data shown in panels D and F are expressed means of the results from two independent experiments that gave similar results and represent changes over the value from unstimulated control cells (set as 1).

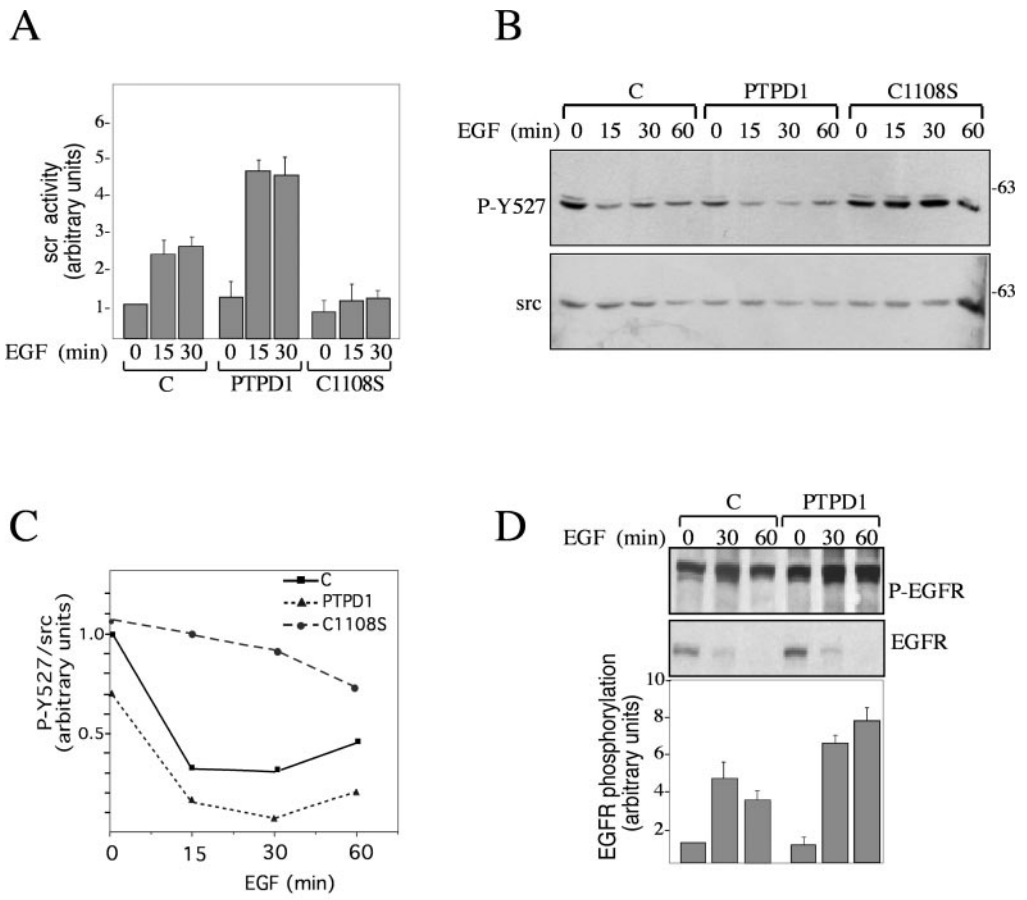

FIG. 7. PTPD1 activates src and increases tyrosine phosphorylation of EGFR. (A) Control (c) and HA-PTPD1- or HA-PTPD1$_{C1108S}$-expressing cells were serum deprived overnight and stimulated with EGF (100 ng/ml). Cell lysates were immunoprecipitated with anti-src antibody (10 μg/ml). The immunoprecipitates were analyzed by in vitro kinase assay with enolase as the substrate. The data are expressed as means ± standard errors of the results from three independent experiments that gave similar results. Values from basal, unstimulated control cells were set as 1. (B) Cell lysates from control and HA-PTPD1- and HA-PTPD1$_{C1108S}$-expressing cells were sequentially immunoblotted with anti-phospho-Y527 and anti-src antibodies. (C) Cumulative data are expressed as means of the results from two independent experiments that gave similar results. (D) Cell lysates from panel B were immunoprecipitated with anti-EGFR antibody and immunoblotted with anti-phosphotyrosine and anti-EGFR antibody. A representative set of three independent experiments is shown. Quantitative analysis (mean ± standard error) is presented.

domain (AKAP121$_{\Delta 35\text{-}110}$). Thus, expression of AKAP121$_{\Delta 35\text{-}110}$ did not inhibit Elk phosphorylation (Fig. 8E).

We also determined the effect of PTPD1 on ERK 1/2-dependent gene transcription in HEK293 cells. HEK293 cells were transiently cotransfected with the pBD-Elk1 plasmid and a pGAL-CAT reporter gene (Fig. 8F) (see Materials and Methods). pBD-Elk1 is a fusion between the activation domain of Elk1 and the DNA binding domain of GAL4. pGAL-CAT carries the CAT gene downstream to a GAL4 synthetic promoter. In this system, Elk1 phosphorylation by ERK 1/2 increases transcription of the CAT reporter gene (2). EGF stimulated CAT activity ~3-fold in control cells and ~5-fold in cells cotransfected with PTPD1. PTPD1 stimulation of CAT transcription was eliminated by AKAP121 or by a dominant negative src. Cotransfection of PTPD1 with a dominant-negative ras blocked both basal and EGF-induced CAT expression. The level of PTPD1 expression was similar in all cases (data not shown). These data confirm that PTPD1 amplifies EGF stimulation of src-ras-ERK 1/2 signaling and that this amplification is blocked when PTPD1 binds to AKAP121.

DISCUSSION

We found that AKAP121, which anchors PKA to mitochondria, binds to the ERM PTPD1 in vitro and in intact cells. This observation led us to explore the role of PTPD1 in vivo and how AKAP121 modulates PTPD1 activity.

Our results demonstrate that PTPD1 enhances EGF signaling by activating src. Src, in turn, increases tyrosine phosphorylation of EGFR, augmenting responsiveness to EGF. Stimulation of the EGF pathway by PTPD1/src led to sustained activation of ERK 1/2 signaling after EGF stimulation, as shown by Elk1 phosphorylation and Elk1-dependent gene transcription. PTPD1$_{C1108S}$, a catalytically inactive PTPD1 mutant, inhibited src activation and downregulated ERK 1/2 signaling, possibly by competing with endogenous PTPD1. Binding of AKAP121 to PTPD1 downregulated the ERK 1/2 signal transduction pathway.

Src tyrosine kinase is both an effector and upstream regulator of EGFR (1, 4, 7, 48). EGF stimulation leads to activation of src which, in turn, phosphorylates EGFR at several sites (6,

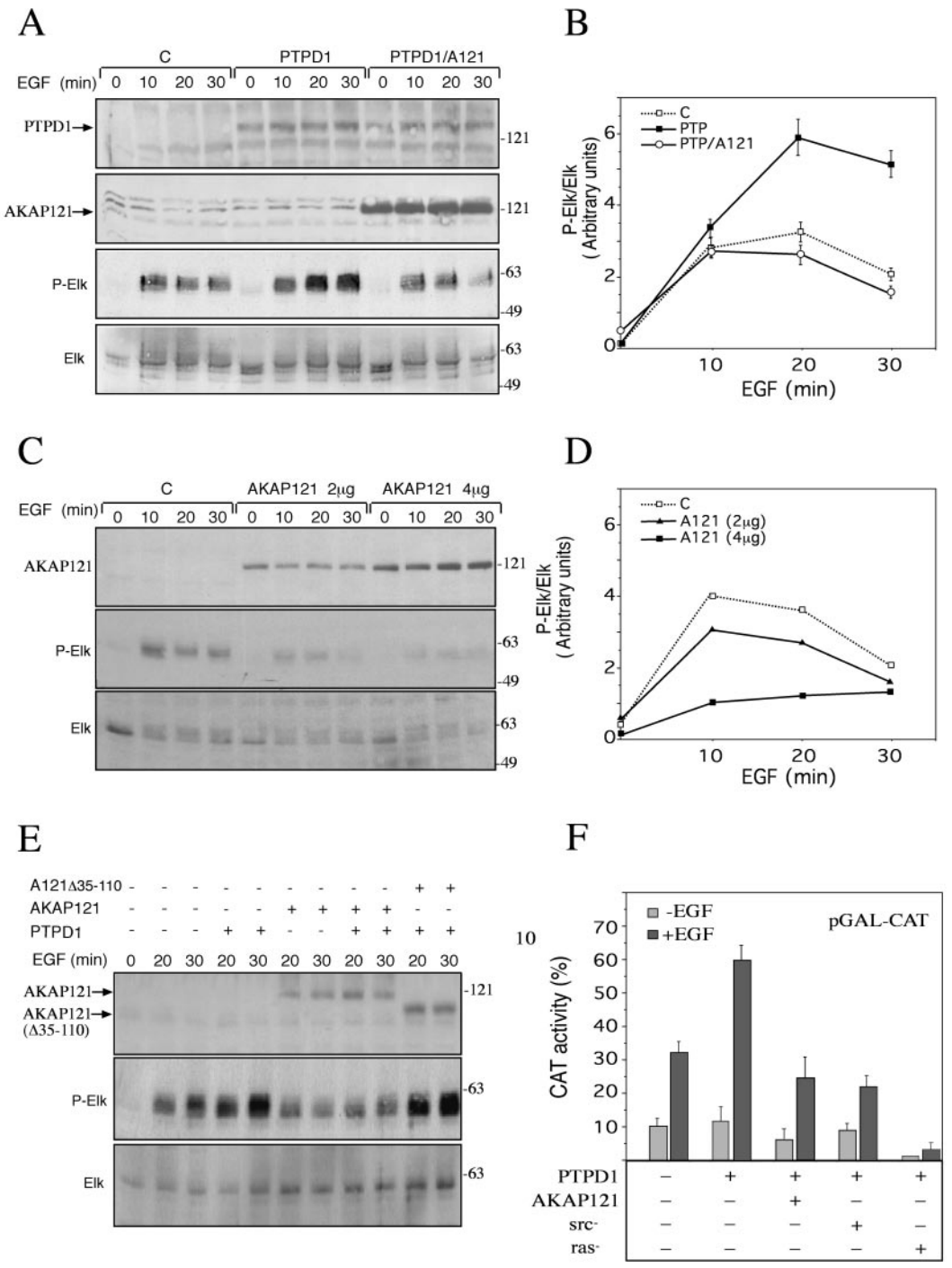

FIG. 8. AKAP121 inhibits ERK 1/2 signaling. (A) HEK293 cells were transiently transfected with HA-PTPD1, with or without AKAP121, serum deprived overnight, and stimulated with EGF. Cell lysates were subjected to immunoblot analysis with the indicated antibodies. (B) Cumulative data are expressed as means of the results from four independent experiments that gave similar results. (C) Cells were transiently transfected with Elk1-Flag vector in either the presence or absence of increasing concentrations (2 and 4 μg) of AKAP121 cDNA vector. Cells were serum starved overnight and stimulated with EGF as described above. Cell lysates were immunoblotted with the indicated antibodies. (D) Densitometric analysis of the experimental results shown in panel C. This is representative of two independent experiments. (E) Cells were transiently cotransfected with the indicated vectors. EGF stimulation was performed as described above. Cell lysates were immunoblotted with the anti-phospho-Elk1 and anti-Flag antibodies. Results are representative of four independent experiments. A similar amount of PTPD1 was expressed in PTPD1-transfected cells (data not shown). (F) HEK293 cells were transiently transfected with pGal-CAT and pBD-Elk1 in the presence or absence of the PTPD1 expression vector. Where indicated, pCEP-AKAP121, CMV-ras$^-$, and CMV-src$^-$ expression vectors were included in the transfection mixture. Twenty-four hours after transfection, cells were serum deprived overnight and stimulated with EGF for 4 h. Results are expressed as percentages of CAT activity and represent the means ± standard errors of the results from three independent experiments.

51). The positive regulatory loop generated by src contributes to the robustness and persistence of EGF signaling and mediates, at least in part, the effects of EGF on cell shape, proliferation, and gene transcription (1, 4, 51). Although PTPD1 was recognized to bind to and to be phosphorylated by src in vivo (35), the biological relevance of this interaction was unknown. Here we show that PTPD1 stimulates src activity by promoting dephosphorylation of src residue Y527. Preliminary data indicate that recombinant PTPD1 incubated in vitro with immunoprecipitated src dephosphorylates Y527 (data not shown). This suggests that src may be a direct substrate of PTPD1, although further analysis is required to resolve this issue. The ERK 1/2 pathway is critical for cell viability (27, 31). To further demonstrate the role of PTPD1 in the pathway, we expressed $PTPD1_{C1108S}$ in HEK293 and in MCF-7 cells (Fig. 5E and F and data not shown). $PTPD1_{C1108S}$ significantly impaired DNA synthesis and cell growth. Collectively, these findings disclose a previously unrecognized role of PTPD1 in src activation and highlight the importance of the phosphatase in ligand-induced activation of EGF transduction pathway.

AKAPs were originally identified on the basis of their interaction with PKA. Further analysis disclosed a more complex role for these proteins in signal transduction. Several AKAPs also associate with signaling molecules such as Ser/Thr phosphatases, protein kinase C, and phosphodiesterases. AKAPs thus form a node at which signal transduction pathways converge and focus (15, 20, 23, 34, 39). This is the first report of physical and functional interaction between AKAP and a PTP. A domain at the NH_2 terminus of AKAP84/121 mediates binding to PTPD1 (residues 35 to 110). This domain is distinct from those that interact with PKA, mitochondria, or in the case of AKAP121, mRNA. The interacting region of PTPD1 lies in the center of the protein (residues 584 to 921) and does not include the catalytic domain. Coexpression of AKAP121 blocked PTPD1 stimulation of the EGF pathway. To inhibit EGF, AKAP121 must bind to PTPD1. Thus, an AKAP121 mutant with a deletion of the PTPD1 binding domain did not prevent PTPD1 activation of the EGF response. Coexpression of AKAP121 also increased targeting of the phosphatase to mitochondria. The data suggest that AKAP121 may alter the intracellular localization of PTPD1 and thus can affect the generation and propagation of signaling pathway(s) regulated by PTPD1.

The interaction between AKAP121 and PTPD1 may provide a molecular framework to selectively modulate signaling pathways directed to distinct subcellular compartments. For example, AKAP121 binds and targets PKA to mitochondria, amplifying cAMP signaling to these organelles. This significantly suppresses apoptosis (2). Here we show that AKAP121 can also induce long distance effects on cell signaling. By binding and/or sequestering PTPD1 to mitochondria, AKAP121 inhibits EGF signaling generated at the cell membrane. Physiologically, expression and accumulation of AKAP121 is induced by hormones that activate adenylate cyclase (16). Exposure to hormones may, therefore, alter the subcellular localization of AKAP121-associated kinase(s)/phosphatase(s), thus exerting important biological effects on cell signaling.

Intracellular localization of a PTP represents a novel mechanism by which AKAPs may shift the focus of tyrosine kinase/phosphatase pathway(s) from cytoplasm to distal organelles. Specifically, our model predicts that AKAP121, by binding PTPD1/src complex, may shift src signaling from the plasma membrane to mitochondria. In this context, our preliminary data suggest that AKAP121 increases src-dependent phosphorylation of mitochondrial substrates. Our aim now is to determine the role of PTPD1 in mitochondrial physiology, to characterize the signals that regulate the formation of AKAP121-PTPD1 complex, and to identify the specific targets of this complex.

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