THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 31, Issue of July 30, pp. 21900–21907, 1999

Printed in U.S.A.

Interaction of Mitogen-activated Protein Kinases with the Kinase Interaction Motif of the Tyrosine Phosphatase PTP-SL Provides Substrate Specificity and Retains ERK2 in the Cytoplasm*

(Received for publication, February 26, 1999, and in revised form, April 30, 1999)

Ángel Zúñiga‡, Josema Torres§, Josefa Úbeda¶, and Rafael Pulido‖

From the Instituto de Investigaciones Citológicas, Amadeo de Saboya 4, 46010 Valencia, Spain

ERK1 and ERK2 associate with the tyrosine phosphatase PTP-SL through a kinase interaction motif (KIM) located in the juxtamembrane region of PTP-SL. A glutathione *S*-transferase (GST)-PTP-SL fusion protein containing the KIM associated with ERK1 and ERK2 as well as with p38/HOG, but not with the related JNK1 kinase or with protein kinase A or C. Accordingly, ERK2 showed *in vitro* substrate specificity to phosphorylate GST-PTP-SL in comparison with GST-c-Jun. Furthermore, tyrosine dephosphorylation of ERK2 by the PTP-SLΔKIM mutant was impaired. The *in vitro* association of ERK1/2 with GST-PTP-SL was highly stable; however, low concentrations of nucleotides partially dissociated the ERK1/2·PTP-SL complex. Partial deletions of the KIM abrogated the association of PTP-SL with ERK1/2, indicating that KIM integrity is required for interaction. Amino acid substitution analysis revealed that Arg and Leu residues within the KIM are essential for the interaction and suggested a regulatory role for Ser^{231}. Finally, coexpression of PTP-SL and ERK2 in COS-7 cells resulted in the retention of ERK2 in the cytoplasm in a KIM-dependent manner. Our results demonstrate that the noncatalytic region of PTP-SL associates with mitogen-activated protein kinases with high affinity and specificity, providing a mechanism for substrate specificity, and suggest a role for PTP-SL in the regulation of mitogen-activated protein kinase translocation to the nucleus upon activation.

Extracellular signal-regulated kinases (ERKs)[1] are serine/threonine kinases of the MAP kinase family that are activated by a variety of growth and differentiation factors (1–4). ERK1/2-related kinases include members of the stress- and inflammation-activated MAP kinase subgroups, JNK/stress-activated protein kinase and p38/HOG (5–8). Differences in the response of ERK1/2 to activation agents have been postulated to account for the cell type- and stimulus-specific adaptive responses mediated by these kinases (9). For instance, inhibition of ERK1/2 function prevents proliferation of Chinese hamster ovary fibroblasts in response to growth-stimulating agents (10), and constitutive activation of the ERK1/2 pathway induces transformation of 3T3 fibroblasts (11, 12). On the other hand, ERK1 and ERK2 also mediate the inhibition of cell growth arrest induced by nerve growth factor in 3T3 cells (13), and the differentiation of neuronal PC12 or erythroleukemia K562 cells is blocked by inhibition of the ERK1/2 pathway (11, 14, 15). Cytoplasmic activation of ERK1/2 requires the phosphorylation of both tyrosine and threonine regulatory residues, which is accomplished by the dual-specificity kinases MEK1 and MEK2 (16). Upon activation, ERK1 and ERK2 phosphorylate cytosolic and membrane-bound proteins, including signal transduction molecules and cytoskeletal components (1, 2). In addition, after stimulus-induced translocation to the nucleus, ERK1 and ERK2 phosphorylate and regulate the function of several transcription factors, thereby controlling the expression of specific responsive genes (17, 18). However, the mechanism of ERK1/2 nuclear translocation is not well understood. In this regard, the physical interaction of ERK1/2 with cytosolic and nuclear substrates has been reported (19, 20), suggesting that tight binding to their substrates may be a mechanism that regulates the subcellular location of these kinases as well as their enzymatic specificity. The inactivation of ERK1/2 in the nucleus is achieved by specific dual-specificity phosphatases (MKPs), which are induced following ERK1/2 activation (21). In addition, a cytosolic MKP, MKP-3/Pyst1/rVH6, has been found to inactivate ERK1/2 (22–24).

Protein-tyrosine phosphatases (PTPs) inactivate ERK1 and ERK2 by dephosphorylating their tyrosine regulatory residue (25). Several PTPs have been shown to tyrosine-dephosphorylate and to inactivate ERK1/2 *in vitro* (26–28), and vanadate-sensitive PTPs inactivate ERK1/2 in a constitutively manner in intact cells (29–31). In this context, we have recently shown that the tyrosine phosphatase PTP-SL dephosphorylates and inactivates ERK1/2 by association through a kinase interaction motif (KIM) and that such association leads to the phosphorylation of PTP-SL at a Thr residue downstream of the KIM (32). Here, we present a mutational analysis of the KIM of PTP-SL and characterize the binding requirements and the substrate specificity of MAP kinases for this phosphatase. We also show that binding to PTP-SL through the KIM retains ERK2 in the cytoplasm, blocking its translocation to the nucleus. A major checkpoint role in the MAP kinase signaling pathways is proposed for the physical and functional association between MAP kinases and PTP-SL.

* This work was supported in part by Ministerio de Educación y Cultura Grant PB96-0278 and Generalitat Valenciana Grant GV-C-VS-20-047-96. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of a postdoctoral fellowship from Bancaja.

§ Supported in part by a fellowship from the Fundación Científica de la Asociación Española Contra el Cáncer.

¶ Recipient of a predoctoral fellowship from the Instituto de Investigaciones Citológicas.

‖ To whom correspondence should be addressed. Tel.: 96-3391256; Fax: 96-3601453; E-mail: rpulido@ochoa.fib.es.

[1] The abbreviations used are: ERKs, extracellular signal-regulated kinase; MAP, mitogen-activated protein; MAPKK, MAP kinase kinase; JNK, c-Jun N-terminal kinase; MEK, MAP kinase/ERK kinase; MKP, MAP kinase phosphatase; PTP, protein-tyrosine phosphatase; KIM, kinase interaction motif; GST, glutathione *S*-transferase; PKC, protein kinase C, PKA, protein kinase A; HA, hemagglutinin; mAb, monoclonal antibody; pNPP, *p*-nitrophenyl phosphate; EGF, epidermal growth factor; PAGE, polyacrylamide gel electrophoresis; AMP-PNP, adenosine 5′-(β,γ-iminotriphosphate); RSK, ribosomal S6 protein kinase.

EXPERIMENTAL PROCEDURES

Plasmid Construction, Mutagenesis, and Purification of GST Fusion Proteins—Mouse PTP-SL and ERK2 cDNA constructs have been previously described (32). Bovine PKCα (33) and JNK1 (amplified by reverse transcription-polymerase chain reaction from a human placenta cDNA library) were subcloned into the expression vectors pRK5 and pcDNA3, respectively. pcDNA-I-NEO-HA-p44^{mapk}/ERK1 (hamster sequence) (34) was provided by J. Pouysségur. pCEV29-HA-p38/HOG was provided by S. Gutkind. PTP-SL mutations were performed by polymerase chain reaction oligonucleotide site-directed mutagenesis, and mutations were confirmed by DNA sequencing. For the construction of plasmids encoding GST fusion proteins, cDNAs were subcloned into the expression vector pGEX-5X. pGEX-5X-c-Jun-(1–96) was obtained by subcloning of a cDNA fragment (obtained by reverse transcription-polymerase chain reaction from a human placenta cDNA library) encoding the N-terminal portion of c-Jun (amino acids 1–96). pGEX-ATF2 encodes amino acids 1–254 from ATF2. Purification of the GST fusion proteins was done by standard procedures. GST-PTP-SL-(147–288) was covalently coupled to CNBr-activated Sepharose (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom) following the manufacturer's instructions. The apparent molecular masses of all purified GST fusion proteins on SDS-polyacrylamide gels were in accordance with their predicted sizes, as follows: GST, 29 kDa; GST-PTP-SL-(147–549), 74 kDa; GST-PTP-SL-(147–288), 45 kDa; GST-ATF2, 55 kDa; and GST-c-Jun-(1–96), 42 kDa.

Antibodies and Reagents—Rabbit polyclonal anti-PTP-SL, anti-ERK1 (C-16), and anti-ERK2 (C-14) antibodies and the 12CA5 anti-HA and 4G10 anti-phosphotyrosine mAbs have been described (32). The anti-PKC mAb MC5 was from Sigma, and the anti-PKA catalytic subunit antibody was from Transduction Laboratories. Horseradish peroxidase-conjugated goat anti-rabbit and anti-mouse secondary antibodies were from Oncogene Research Products (Cambridge, MA) and Promega (Madison, WI). Fluorescein isothiocyanate- and rhodamine-conjugated goat anti-rabbit and anti-mouse secondary antibodies, as well as pNPP and nucleotides, were from Sigma. EGF was from Life Technologies, Inc. and was used at a final concentration of 50 ng/ml. Glutathione-Sepharose, protein A-Sepharose, [γ-^{32}P]ATP, and polyvinylidene difluoride filters were from Amersham Pharmacia Biotech.

Cell Culture, Transfections, Precipitation with GST Fusion Proteins, Immunoprecipitation, and Immunoblotting—Rat fibroblastic Rat-1, human embryonic kidney 293, and simian COS-7 cell lines (American Type Culture Collection) were grown in Dulbecco's minimal essential medium (Life Technologies, Inc.) containing high glucose (4.5 g/liter) supplemented with 5% (for COS-7 cells) or 10% heat-inactivated fetal calf serum (Life Technologies, Inc.). COS-7 cells were transfected using the DEAE-dextran method, and 293 cells were transfected by the calcium phosphate precipitation method; in both cases, cells were harvested after 48–72 h of culture. Precipitation and immunoblotting were carried out as described (32). Briefly, cells were washed with ice-cold phosphate-buffered saline and lysed in lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 100 mM NaF, 2 mM Na_3VO_4, and 20 mM $Na_4P_2O_7$). Lysates were centrifuged for 10 min at 14,000 rpm, and post-nuclear supernatants were precleared with glutathione-Sepharose or protein A-Sepharose. For GST fusion protein precipitation, 1 μg of GST fusion protein was added per sample, followed by glutathione-Sepharose. In samples precipitated with GST-PTP-SL-(147–288) coupled to Sepharose, addition of glutathione-Sepharose was omitted. For immunoprecipitation, mAb 12CA5 was added, followed by protein A-Sepharose. Precipitates were washed with HNTG buffer (20 mM HEPES (pH 7.5), 150 mM NaCl, 0.1% Triton X-100, and 10% glycerol) and resolved by SDS-PAGE, followed by immunoblotting and chemiluminescence analysis. Dilution of antibodies and washing of polyvinylidene difluoride filters for immunoblotting were done in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.05% Triton X-100, and 0.25% gelatin.

In Vitro Kinase and Phosphatase Assays—For *in vitro* kinase assays, pellets obtained after precipitation with mAb 12CA5 and washing with HNTG buffer were washed once with kinase reaction buffer (20 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, 2 mM Na_3VO_4, and 0.3 μM ATP); pellets were then resuspended in 20 μl of kinase reaction buffer containing the GST fusion proteins as substrate(s) and 0.5–2 μCi of [γ-^{32}P]ATP and incubated at room temperature for 15 min under constant shaking. Reactions were stopped by adding SDS sample buffer and boiling. The reaction mixture was loaded and resolved on SDS-polyacrylamide gels, and radioactivity incorporated into the GST fusion proteins was visualized by autoradiography. *In vitro* phosphatase assays were performed in phosphatase reaction buffer (25 mM HEPES (pH 7.3), 5 mM EDTA, and 10 mM dithiothreitol). For dephosphorylation of HA-ERK2, wild-type or mutant GST-PTP-SL-(147–549) fusion proteins were mixed with pellets containing phosphorylated HA-ERK2 and incubated at room temperature for 45 min. Reactions were stopped; samples were resolved as described above; and HA-ERK2 phosphotyrosine content was determined by immunoblotting with anti-phosphotyrosine mAb 4G10. For pNPP hydrolysis, GST-PTP-SL fusion proteins were incubated at 37 °C for 30 min in phosphatase reaction buffer containing 20 mM pNPP (200-μl final volume). Reactions were stopped by adding 800 μl of 0.2 N NaOH, and pNPP hydrolysis was measured by absorbance at 410 nm.

Immunofluorescence—COS-7 cells were seeded at 1×10^4 cells/cm^2 onto glass coverslips and cultured for 24 h; the cells were then transfected as described above and processed for immunofluorescence after 36–48 h of culture. Cells were rinsed once with IPBS buffer (1.5 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 0.7 mM $CaCl_2$, and 0.5 mM $MgCl_2$ (pH 7.4)) and then fixed with methanol at −20 °C for 5 min. Samples were incubated in blocking solution (IPBS buffer containing 3% bovine serum) at room temperature for 15 min, followed by incubation at 37°C for 90–120 min with a mixture of the anti-HA and anti-PTP-SL primary antibodies. After washing with IPBS buffer, cells were incubated for 1 h at room temperature with a mixture of the fluorescein isothiocyanate- and tetramethylrhodamine B isothiocyanate-conjugated secondary antibodies, followed by washing with IPBS buffer and mounting.

RESULTS

Interaction of MAP Kinases with PTP-SL through the KIM Provides Substrate Specificity—We have shown that PTP-SL associates with ERK1/2 through a KIM (residues 224–239; amino acid numbering is according to Ref. 35) located in the cytosolic juxtamembrane region of this PTP (32). To study the specificity of this association, the interaction of PTP-SL with other kinases was investigated. COS-7 cells were transfected with HA-tagged ERK1, ERK2, JNK1, or p38/HOG kinase, and cell lysates were precipitated with a GST-PTP-SL fusion protein containing the KIM (GST-PTP-SL-(147–288)), followed by immunoblotting with anti-HA mAb 12CA5 (Fig. 1*A*). GST-PTP-SL-(147–288) precipitated both recombinant HA-ERK1 and HA-ERK2 (Fig. 1*A*, *lanes 3* in the *first* and *second panels*, respectively), whereas a mutant fusion protein lacking the KIM (GST-PTP-SL-(147–288)ΔKIM) did not associate with the kinases (*lanes 4*). Interestingly, HA-p38/HOG, but not HA-JNK1, was also precipitated by GST-PTP-SL-(147–288) in a KIM-dependent manner (Fig. 1*A*, *third* and *fourth panels*, respectively). The expression of the distinct kinases as controls in the transfected cells is shown (Fig. 1*A*, *lanes 1*). Since the juxtamembrane region of PTP-SL possesses several phosphorylation consensus sites for PKA and PKC, the possibility was also tested whether PTP-SL associates with either of these kinases. Cell lysates from Rat-1 cells or PKCα-transfected 293 cells were precipitated with GST-PTP-SL-(147–288), followed by immunoblotting with anti-PKA or anti-PKC antibodies, respectively. No association of GST-PTP-SL with PKA or PKC was observed under these conditions (Fig. 1*B*, *lanes 3* in the *upper* and *lower panels*, respectively). Thus, PTP-SL specifically associates through the KIM with ERK1, ERK2, and p38/HOG.

Binding of PTP-SL to ERK1 and ERK2 results in phosphorylation of the PTP by these kinases at Thr^{253} (Ref. 32; see also Fig. 6). Next, the phosphorylation of PTP-SL by ERK2, JNK1, and p38/HOG was compared. Cell lysates from activated COS-7 cells transfected with HA-ERK2, HA-JNK1, or HA-p38/HOG were immunoprecipitated with anti-HA mAb 12CA5; *in vitro* kinase assays were then performed on the immune complexes using GST-PTP-SL-(147–288) as the substrate in combination with JNK- and p38/HOG-specific substrates, *i.e.* GST-c-Jun and GST-ATF2 (Fig. 1*C*). As shown, ERK2 specifically phosphorylated GST-PTP-SL-(147–288) (Fig. 1*C*, *lanes 1* and *2*), whereas this substrate was poorly phosphorylated by JNK1 as compared with the extent of phosphorylation of GST-c-Jun

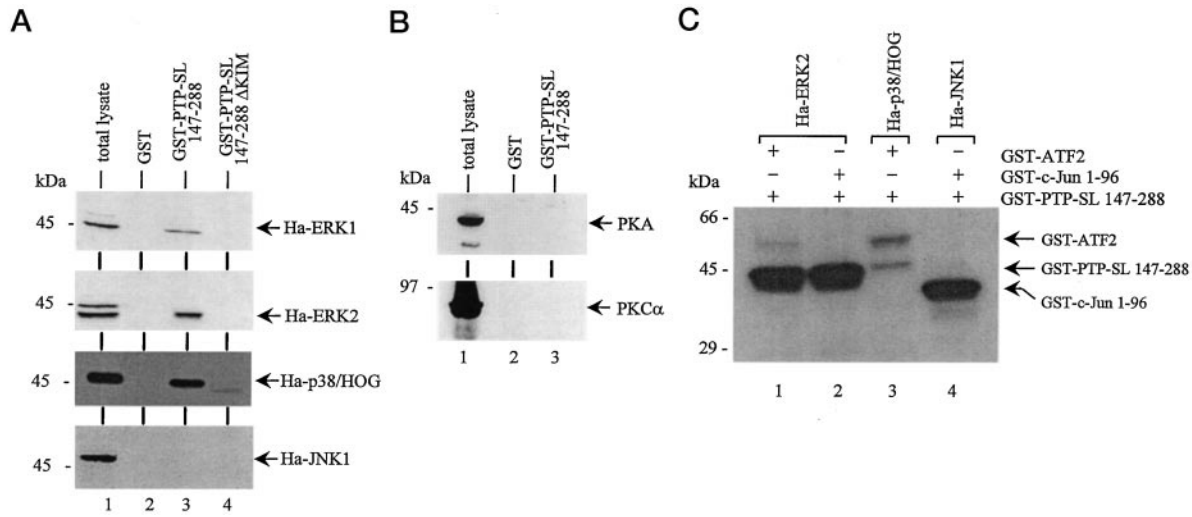

FIG. 1. *A* and *B*, PTP-SL associates with ERK1, ERK2, and p38/HOG. In *A*, COS-7 cells were transfected with pcDNA-I-NEO-HA-ERK1 (*first panel*), pcDNA3-HA-ERK2 (*second panel*), pCEV29-HA-p38/HOG (*third panel*), or pcDNA3-HA-JNK1 (*fourth panel*). In *B* (*lower panel*), 293 cells were transfected with pRK5-PKCα. After 48 h, cells were lysed and precipitated with 1 μg of GST fusion protein as indicated. In *B* (*upper panel*), Rat-1 cell lysates were used as a source of endogenous PKA catalytic subunit. In *lanes 1*, total lysate samples were loaded. All samples were resolved by 10% SDS-PAGE under reducing conditions, followed by immunoblot analysis with anti-HA mAb 12CA5 (*A*), the anti-PKA catalytic subunit antibody (*B*, *upper panel*), or anti-PKC mAb MC5 (*B*, *lower panel*). Reactive bands were visualized by chemiluminescence. *C*, substrate specificity of MAP kinases for PTP-SL. COS-7 cells were transfected with pcDNA3-HA-ERK2 (*lanes 1* and *2*), pCEV29-HA-p38/HOG (*lane 3*), or pcDNA3-HA-JNK1 (*lane 4*). After 48 h, HA-ERK2-transfected cells were activated with EGF (50 ng/ml, 5 min), and HA-p38/HOG- and HA-JNK1-transfected cells were activated with sorbitol (0.5 M, 15 min). The distinct kinases were precipitated from the cell lysates using anti-HA mAb 12CA5, and immune complex kinase assays were performed in the presence of a combination of equimolar amounts (1 μM) of the GST fusion proteins used as substrates as indicated. *Arrows* indicate the migration of the distinct kinases or GST fusion proteins. Molecular mass standards are shown on the left of each panel.

(*lane 4*). p38/HOG phosphorylated both GST-ATF2 and GST-PTP-SL-(147–288) (Fig. 1*C*, *lane 3*), although the levels of phosphorylation under our experimental conditions were weaker than those observed for ERK2 and JNK1 with their substrates.

Finally, the *in vitro* dephosphorylation of ERK2 by wild-type PTP-SL and PTP-SLΔKIM was compared. Phosphorylated HA-ERK2 was immunoprecipitated from EGF-activated COS-7 cells transfected with HA-ERK2, and immune complex pellets were subjected to phosphatase assays in the presence of wild-type GST-PTP-SL-(147–549) or mutant forms. Tyrosine dephosphorylation of ERK2 was efficiently achieved by wild-type PTP-SL (Fig. 2*A*, *lanes 2* and *3*), but not by PTP-SLΔKIM (*lanes 4* and *5*). As a control, the lack of ERK2 tyrosine dephosphorylation upon incubation with the catalytically inactive PTP-SL C480S mutant is shown (Fig. 2*A*, *lanes 6* and *7*). *In vitro* phosphatase assays were performed in parallel with GST-PTP-SL-(147–549) and GST-PTP-SL-(147–549)ΔKIM using the nonspecific substrate pNPP (Fig. 2*B*). No differences were observed between the activities for pNPP in wild-type PTP-SL and the ΔKIM mutant, indicating that the deletion of the KIM did not affect the PTP-SL phosphatase activity itself, but rather the recognition of the ERK2-specific substrate.

ERK1 and ERK2 Bind Tightly to PTP-SL, but Are Dissociated by ATP—To examine the stability of the ERK1/2·PTP-SL complex, Rat-1 cell lysates were incubated with GST-PTP-SL-(147–288) coupled to Sepharose; after washing, pellets containing GST-PTP-SL-(147–288) and bound ERK1/2 were incubated separately in HEPES buffer at distinct pH values. The eluted samples and the corresponding pellets were resolved by SDS-PAGE, and ERK1 and ERK2 were detected by immunoblotting with anti-ERK1/2 antibodies (Fig. 3*A*). ERK1 and ERK2 dissociated from PTP-SL only under extreme pH conditions (Fig. 3*A*, *lanes 1* and *5* and *lanes 6* and *10*). The effect of distinct ionic strength or denaturing conditions on the stability of the complex was also analyzed (Fig. 3*B*). In these experiments, the GST-PTP-SL-(147–288)-Sepharose pellets containing ERK1/2 were eluted sequentially with increasing concentrations of NaCl, urea, or SDS, and ERK1 and ERK2 were detected in the eluted supernatants and in the final pellets (obtained after the last extraction). Upon elution with NaCl or urea up to 4 M, only a small fraction of ERK1/2 was eluted (Fig. 3*B*, *upper panel*, *lanes 3–5* and *lanes 8–10*), and most of the kinase remained bound to the beads (*lanes 6* and *11*). Elution with SDS was achieved at SDS concentrations of 0.1–0.5% (Fig. 3*B*, *lower panel*, *lanes 3–7*), although a significant amount of ERK1/2 was still detected in the beads (*lane 8*).

Since kinases bind ATP to phosphorylate their substrates, the effect of nucleotides on the dissociation of the ERK1/2·PTP-SL complex was investigated (Fig. 4). Rat-1 cells were left untreated or were treated with EGF to activate ERK1/2, and cell lysates were precipitated with GST-PTP-SL-(147–288). Under these conditions, no difference is observed in the amount of ERK1/2 precipitated by GST-PTP-SL-(147–288) from untreated or EGF-treated cells (32). Pellets containing ERK1/2 were then sequentially incubated in HEPES buffer containing increasing concentrations of nucleotides, and the eluted supernatants and final pellets were processed for ERK1/2 detection. Low concentrations of ATP dissociated the ERK1/2·PTP-SL complex in the pellets from untreated cells (Fig. 4*A*, *lanes 2–5*), whereas in those from EGF-treated cells, the complex was more resistant to dissociation (*lanes 8–11*). Similar elution profiles were achieved with the nonhydrolyzable ATP analog AMP-PNP; and other nucleotides, including ADP, AMP, and GTP, also partially eluted ERK1/2 from GST-PTP-SL-(147–288) beads, although at a lesser extent than ATP (Fig. 4*B*; data not shown).

Mutational Analysis of the KIM Sequence on PTP-SL—The contribution of the amino acids within the PTP-SL KIM sequence to the interaction with ERK1/2 was investigated. Site-directed mutagenesis of the KIM was performed, and the corresponding mutant GST-PTP-SL-(147–288) fusion proteins were obtained and used to precipitate ERK1/2 from Rat-1 cell lysates (Fig. 5). Deletion of the N-terminal (Δ222–231, Δ224–

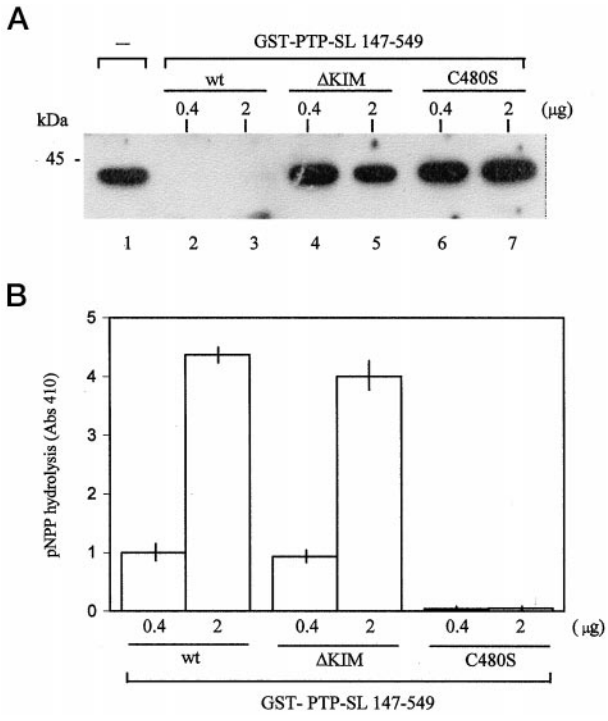

FIG. 2. **PTP-SL dephosphorylates ERK2 in a KIM-dependent manner.** *A*, tyrosine-phosphorylated HA-ERK2 was precipitated from lysates from EGF-activated COS-7 cells transfected with pcDNA3-HA-ERK2. Immune complex pellets were subjected to *in vitro* phosphatase assays in the presence of the indicated amounts of the distinct GST fusion proteins. In *lane 1*, no GST fusion protein was added. Samples were resolved by 10% SDS-PAGE, followed by immunoblot analysis with anti-phosphotyrosine mAb 4G10. The molecular mass standard is shown on the left. *B*, pNPP *in vitro* phosphatase assays were performed with distinct amounts of the GST fusion proteins as indicated. pNPP hydrolysis was measured by absorbance (*Abs*) at 410 nm. *wt*, wild-type.

233) or C-terminal (Δ232–240) portions of the KIM abrogated the association with ERK1/2 (Fig. 5*A*, *lanes 5–7*), indicating that integrity of the motif is required for the kinase recognition. Single amino acid substitutions were then generated within the KIM to identify the residues necessary for the interaction (Fig. 5*B*). Substitution of Leu^{225}, Arg^{228}, Arg^{229}, Leu^{235}, or Leu^{237} with Ala (mutations L225A, R228A, R229A, R228A/R229A, L235A, and L237A) abolished the recognition of ERK1/2 (Fig. 5*B*, *lanes 5*, *8–10*, *16*, and *18*). Furthermore, conservative substitution of Arg^{228} or Arg^{229} with Lys or Leu^{235} with Ile (mutations R228K, R229K, and L235I) also abrogated the association with ERK1/2 (Fig. 5*C*, *lanes 5*, *6*, and *8*). However, substitution of Leu^{225} or Leu^{237} with Ile (mutations L225I and L237I) did not affect such an association (Fig. 5*C*, *lanes 7* and *9*). Finally, Ala substitution of other KIM residues, including Gly^{230}, Asn^{232}, and Val^{233} (mutations G230A, N232A, and V233A), partially affected the binding to ERK1/2 (Fig. 5*B*, *lanes 11*, *13*, and *14*), whereas Ala substitution of the rest of the KIM amino acids (mutations Q226A, E227A, S231A, S234A, T236A, and D238A) did not affect the binding (*lanes 6*, *7*, *12*, *15*, *17*, and *19*). The Ser^{231} KIM residue is located within a phosphorylation consensus sequence for PKA. To test the putative effect of phosphorylation of this residue on the association with ERK1/2, Ser^{231} was substituted with Glu (mutant S231E) (Fig. 5*D*). Interestingly, the GST-PTP-SL-(147–288) S231E fusion protein was unable to precipitate ERK1/2 (Fig. 5*D*, *lane 6*), suggesting that phosphorylation of Ser^{231} could play a negative role in the regulation of the interaction of PTP-SL with ERK1/2.

In vitro kinase assays with EGF-activated HA-ERK2, using the GST-PTP-SL KIM mutants as substrates, showed that all

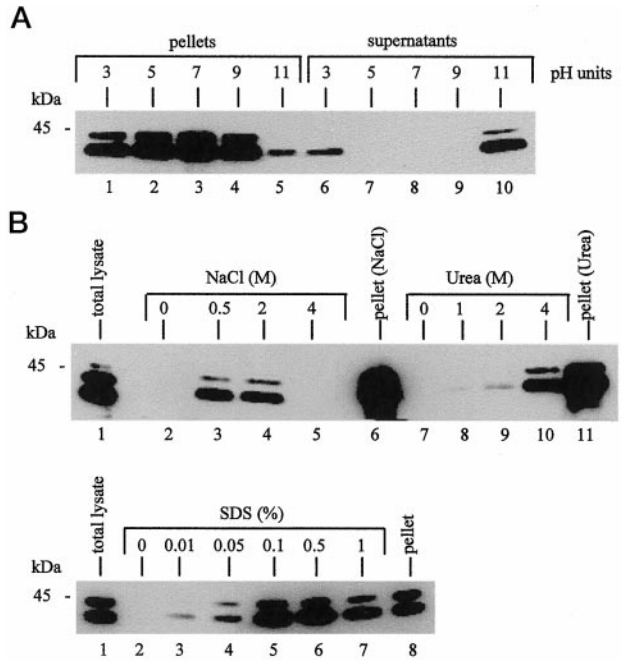

FIG. 3. **Stability of the ERK1/2·GST-PTP-SL-(147–288) complex.** *A*, effect of pH on stability of the complex. Rat-1 cell lysates were precipitated with GST-PTP-SL-(147–288)-Sepharose; after washing, the beads were divided, and samples were separately incubated at room temperature for 10 min in 20 mM HEPES buffer at distinct pH as indicated. Supernatants and pellets from each fraction were resolved by 10% SDS-PAGE and analyzed for the presence of ERK1/2 by immunoblot analysis with anti-ERK1/2 antibodies. *B*, effect of ionic strength and denaturing agents on stability of the complex. Rat-1 cell lysates were incubated with GST-PTP-SL-(147–288)-Sepharose; after washing, the beads were sequentially extracted with 20 mM HEPES buffer (pH 7.5) containing increasing concentrations of NaCl (*upper panel*, *lanes 2–5*), urea (*upper panel*, *lanes 7–10*), or SDS (*lower panel*, *lanes 2–7*). Supernatants of each fraction, as well as the final pellets remaining after the last extraction (*upper panel*, *lanes 6* and *11*; and *lower panel*, *lane 8*), were resolved by 10% SDS-PAGE and analyzed for the presence of ERK1/2 by immunoblot analysis with anti-ERK1/2 antibodies. In *B* (*lanes 1*), 10 μg of total lysate samples were loaded. Molecular mass standards are shown on the left of each panel.

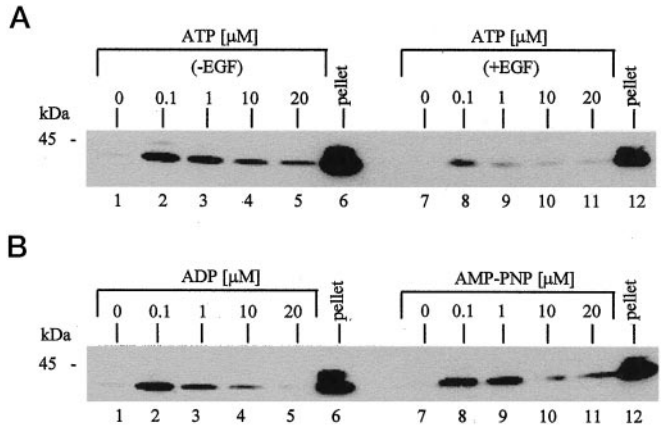

FIG. 4. **Effect of nucleotides on the stability of the ERK1/2·GST-PTP-SL-(147–288) complex.** Rat-1 cell lysates were incubated with GST-PTP-SL-(147–288)-Sepharose; after washing, the beads were sequentially extracted with 20 mM HEPES buffer (pH 7.5) containing increasing concentrations of ATP (*A*), ADP (*B*, *lanes 1–6*), or AMP-PNP (*B*, *lanes 7–12*) as indicated. Supernatants of each fraction, as well as final pellets (*lanes 6* and *12*), were resolved by 10% SDS-PAGE and analyzed for the presence of ERK1/2 by immunoblot analysis with anti-ERK1/2 antibodies. In *A* (*lanes 7–12*), Rat-1 cells were stimulated with 50 ng/ml EGF for 5 min before lysis. Molecular mass standards are shown on the left of each panel.

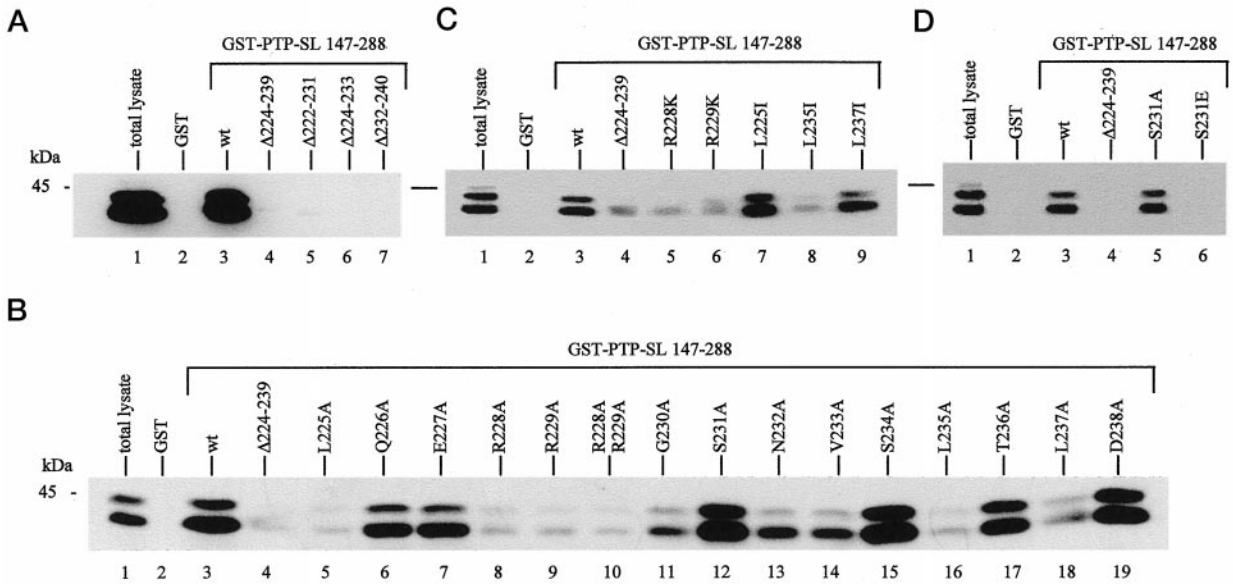

FIG. 5. **Association of ERK1/2 with PTP-SL KIM mutants.** Rat-1 cell lysates were precipitated with 1 μg of GST or wild-type (*wt*) or mutant GST-PTP-SL-(147–288) protein as indicated. In *lanes 1*, 10 μg of total lysates were loaded. Samples were resolved by 10% SDS-PAGE and analyzed for the presence of ERK1/2 by immunoblot analysis with anti-ERK1/2 antibodies. Molecular mass standards are shown on the left of each panel.

the mutants that failed to associate with ERK1/2 were poorly phosphorylated by the kinase at Thr^{253} (Fig. 6; data not shown), confirming our previous finding that docking through the KIM is a prerequisite for ERK1/2 to phosphorylate PTP-SL. A summary of the association of ERK1/2 with the panel of PTP-SL KIM mutants is shown in Fig. 7*A*.

PTP-SL Retains ERK2 in the Cytoplasm in a KIM-dependent Manner—ERK1 and ERK2 translocate to the nucleus upon cell stimulation with growth factors. To study the subcellular location of ERK2 in the presence of PTP-SL, COS-7 cells were cotransfected with HA-ERK2 and PTP-SL, followed by two-color immunofluorescence analysis. Under the normal growing conditions of COS-7 cells (5% fetal calf serum), HA-ERK2 was distributed in both the nucleus and the perinuclear area (Fig. 8*A*, *inset*). Upon coexpression with cytosolic, non-transmembrane PTP-SL (PTP-SL-(147–549)), HA-ERK2 was retained in the cytoplasm, being excluded from the nucleus (Fig. 8, *A* and *C*). An identical effect was observed upon coexpression with the PTP-SL-(147–549) C480S catalytically inactive mutant (Fig. 8, *G* and *I*), indicating that cytoplasmic retention of ERK2 is independent of the catalytic activity of PTP-SL. Remarkably, coexpression with the PTP-SL mutant lacking the KIM (PTP-SL-(147–549)ΔKIM) did not affect the nuclear location of HA-ERK2 (Fig. 8, *D* and *F*). Coexpression of HA-ERK2 with transmembrane PTP-SL (PTP-SL-(1–549)) also resulted in exclusion of HA-ERK2 from the nucleus and its accumulation in perinuclear areas in the cytoplasm, where PTP-SL-(1–549) is mainly located, likely as a result of its accumulation during biosynthesis (data not shown). Our results demonstrate that the *in vivo* association of PTP-SL with ERK2 through the KIM retains this kinase in the cytoplasm and blocks its entry into the nucleus.

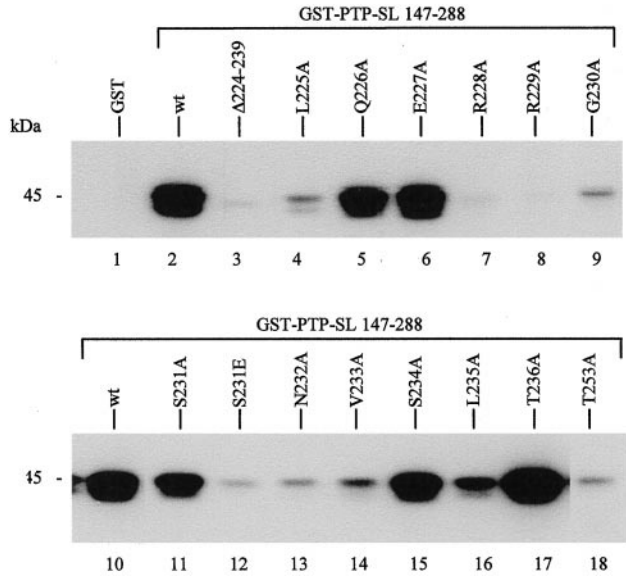

FIG. 6. **Phosphorylation of PTP-SL KIM mutants by ERK2.** HA-ERK2 was immunoprecipitated with anti-HA mAb 12CA5 from EGF-activated COS-7 cells transfected with pcDNA3-HA-ERK2, and immune complex kinase assays were performed using the wild-type (*wt*) or mutant GST-PTP-SL-(147–288) proteins (1 μg) as substrates as indicated. Molecular mass standards are shown on the left of each panel.

DISCUSSION

Cell type- and stimulus-specific MAP kinase activation is tightly regulated *in vivo*. Thus, in mammalian cells, differential activation of MAP kinases commits the cells to proliferation, growth arrest, and differentiation or apoptotic death (8, 9). Specific regulation of the MAP kinase signaling cascades is achieved by the physical and functional assembly of regulatory and effector molecules, including cell-surface receptors, GTP-binding proteins, adapter proteins, kinases, and phosphatases (36, 37). As a result, the distinct signals are transduced from the plasma membrane receptors to the MAP kinase effector molecules, leading to the activation of nuclear transcription factors and the selective expression of genes (17, 18). In this report, we present evidence that the association of MAP kinases with the KIM of the tyrosine phosphatase PTP-SL provides the molecular basis for specific interaction and substrate specificity between these enzymes. Several findings support such a conclusion. 1) PTP-SL associated with ERK1 and ERK2, but not with the related JNK1 kinase or with PKA or PKC. 2) PTP-SL was selectively phosphorylated by ERK2, but not by JNK1, as compared with the phosphorylation of c-Jun by this kinase. 3) Both phosphorylation of PTP-SL by ERK2 and dephosphorylation of ERK1/2 by PTP-SL were dependent on docking through the PTP-SL KIM sequence. 4) PTP-SL retained ERK2 in the cytoplasm in a KIM-dependent manner.

A

PTP-SL mutant	Association with ERK1/2
wild type	+++
Δ208–223	+++
Δ224–239	–
Δ238–255	+++
Δ222–231	–
Δ224–233	–
Δ232–240	–
L225A	–
Q226A	+++
E227A	+++
R228A	–
R229A	–
G230A	+
S231A	+++
N232A	+
V233A	+
S234A	+++
L235A	–
T236A	+++
L237A	–
D238A	+++
R228K	–
R229K	–
L225I	+++
L235I	–
L237I	+++
S231E	–

B

```
                 *  **     * *
PTP-SL   (224)GLQERRGSNVSLTLDM (239)
RSK1     (710)ILAQRRV--RKLPSTT (723)
Elk1     (310)PQKGRKP--RDLELPL (323)
MEK1       (1) MPKKKP--TPIQLNP (13)
```

FIG. 7. *A*, association of PTP-SL KIM mutants with ERK1/2. Association is indicated as an estimation of anti-ERK1/2 reactive bands after precipitation with the wild-type or mutant GST-PTP-SL-(147–288) fusion proteins (see Fig. 5). Association with the Δ208–223 and Δ238–255 mutants has been previously reported (32). +++, 90–100% association; +, 20–40% association; –, 0–10% association. *B*, alignment of the ERK1/2 interaction motifs of PTP-SL (residues 224–239) (35), RSK1 (residues 710–723) (45), Elk1 (residues 310–323) (46), and MEK1 (residues 1–13) (47). Amino acids are indicated using the one-letter code. Residues required for association of PTP-SL with ERK1/2 are denoted by *asterisks*. The conserved basic and Leu/Ile residues are *boxed*.

Interestingly, p38/HOG was also found to associate with PTP-SL in a KIM-dependent manner, indicating that our findings on the regulation of ERK2 activity and subcellular location by PTP-SL could be extended to the p38/HOG MAP kinase. In this context, other tyrosine and dual-specificity phosphatases show specificity for members of the MAP kinase family (38–41), suggesting that the diversity of MAP kinases could have a counterbalance in a wide number of specific phosphatases.

Our mutational analysis revealed that integrity of the KIM is required for ERK1/2 binding and PTP-SL phosphorylation. Thus, conservative and nonconservative substitutions of Arg^{228}, Arg^{229}, and Leu^{235} within the KIM abrogated the interaction with ERK1/2. The contribution of local positive charge due to Arg^{228} and Arg^{229} is likely to be important for the recognition and/or stabilization of the ERK1/2-binding site; however, additional structural factors seem to be crucial since substitutions of any of the Arg residues with Lys also inhibited the binding. It should also be noted that all the hydrophobic Leu residues along the KIM (Leu^{225}, Leu^{235}, and Leu^{237}) are important for the association with ERK1/2, suggesting that both polar and nonpolar interactions contribute to the stabilization of the ERK1/2·PTP-SL complex. Remarkably, all the KIM residues required for the association of PTP-SL with ERK1/2 are conserved in the related tyrosine phosphatases PTP-SL, STEP, and LC-PTP/HePTP, favoring the hypothesis that these three PTPs play similar roles in the regulation of ERK1/2 activities in the distinct cell types where they are expressed (32, 42). In addition, ERK1 and ERK2 also interact with the docking site of non-phosphatase molecules, including the ERK1/2 nuclear substrate Elk1 (20), the ERK1/2 regulatory kinase MEK1/MAPKK/Ste7 (19, 43), and the ribosomal S6 protein kinase (RSK) (44). Interestingly, the ERK1/2 interaction motifs assigned to Elk1, MEK1/MAPKK/Ste7, and RSK correspond to the KIM sequence on PTP-SL (Fig. 7*B*); in particular, a stretch of basic residues followed by a motif including one or two Leu/Ile residue(s) (48) is conserved in all these molecules. Remarkably, these conserved amino acids are crucial for ERK1/2 association with both Elk1 (20) and PTP-SL (this study), suggesting similar mechanisms of recognition by ERK1/2. An attractive hypothesis is that substrates, as well as positive and negative regulators of ERK1/2, could compete for efficient binding to these kinases. Thus, phosphorylation and dephosphorylation events regulating ERK1/2 catalysis could be finely tuned *in vivo* by competition between kinases and phosphatases for overlapping ERK1/2-binding sites. Also, tight interaction with cytoplasmic or nuclear proteins could account for the differential subcellular location shown by ERK1/2 during cell activation (see also below).

We have found that the association of ERK1/2 with our GST-PTP-SL fusion proteins is highly stable *in vitro* and that dissociation of the complex between these two molecules is achieved only under strong pH or denaturing conditions. However, low concentrations of ATP (0.1 μM) were able to release part of the ERK1/2 bound to the GST-PTP-SL beads, suggesting that the ATP-binding site in the catalytic groove of the kinase is close to the KIM-binding site. In this regard, Thr^{253} on PTP-SL is located next to the KIM and is strongly phosphorylated by ERK1/2 (32). Thus, a high affinity interaction of ERK1/2 with PTP-SL through the KIM would provide both substrate specificity and optimal conditions for catalysis. Accordingly, an analogous mechanism has been proposed for the association between JNK and its specific substrate, c-Jun (49). On the other hand, our observations suggest the existence of physiological mechanisms that regulate *in vivo* the interaction of PTP-SL with ERK1/2. First, the effect of the ATP on the dissociation of the ERK1/2·PTP-SL complex *in vitro* was less prominent when ERK1/2 had been previously activated by EGF cell treatment, making it possible that conformational changes due to kinase activation could stabilize the complex. Also, substitution of the PKA phosphorylation consensus site Ser^{231} on the KIM with Glu, but not with Ala, abrogated the association with ERK1/2, suggesting that phosphorylation of Ser^{231} could play a negative regulatory role in the interaction of PTP-SL with ERK1/2. In this regard, the involvement of PKA in the regulation of ERK1/2 activities has been widely documented (50–54). Our preliminary observations indicate that PKA phosphorylates PTP-SL, both *in vitro* and *in vivo*. Since PKA was not found to stably associate with our GST-PTP-SL fusion proteins (Fig. 1*B*), different mechanisms of specificity are likely to exist for the phosphorylation of PTP-SL by ERK1/2 and PKA.

The association of ERK1/2 with the dual-specificity phosphatase MKP-3/Pyst1/rVH6 has been recently reported (22, 55), and such an association was found to cause catalytic activation of this phosphatase, indicating a model of phosphatase catalytic regulation by substrate binding (56); however, we have not detected changes in the *in vitro* tyrosine phosphatase activity of PTP-SL for pNPP upon binding to purified recombinant

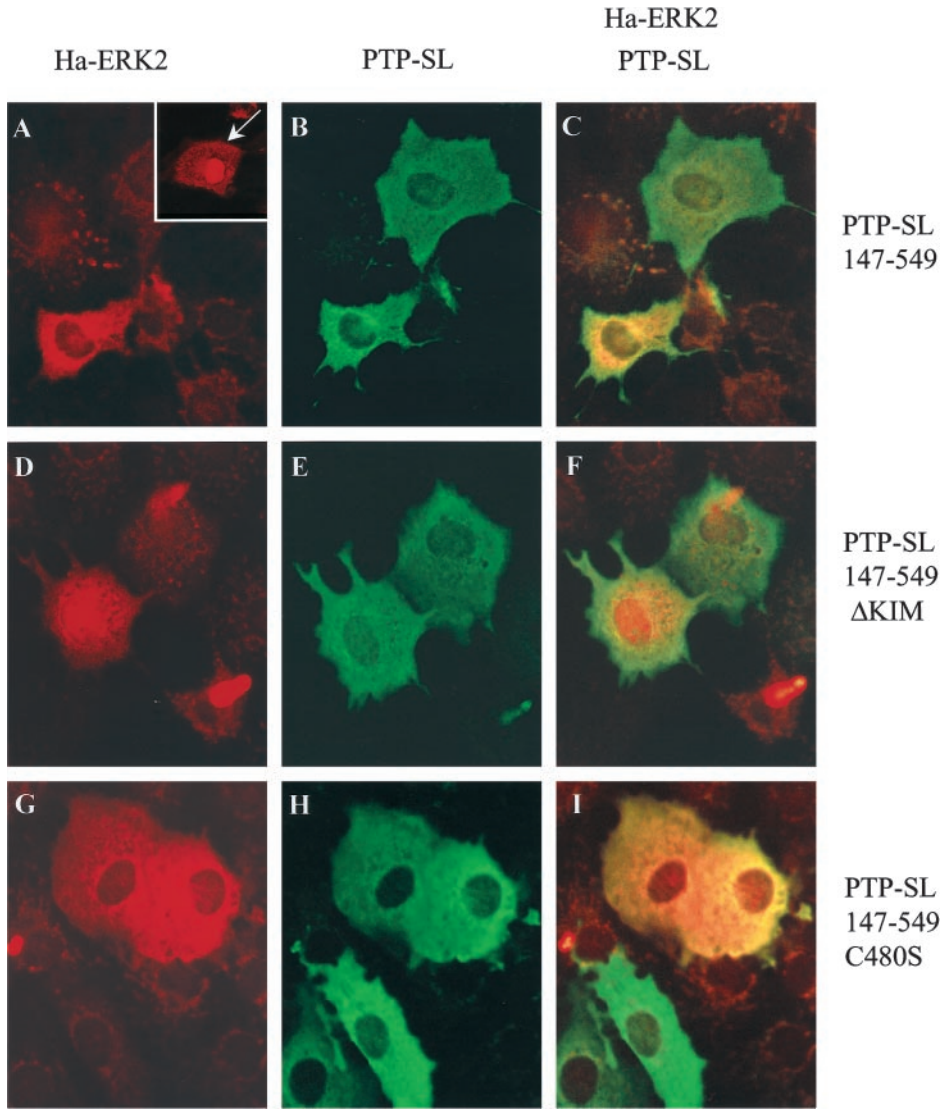

FIG. 8. **PTP-SL retains ERK2 in the cytoplasm.** COS-7 cells were cotransfected with pcDNA3-HA-ERK2 plus wild-type and mutant pRK5-PTP-SL-(147–549) forms, and 48 h after transfection, cells were co-stained and analyzed by immunofluorescence. HA-ERK2 was stained with mouse anti-HA mAb 12CA5 plus rhodamine-conjugated goat anti-mouse antibodies (*A*, *D*, and *G*; *red*). As a control of HA-ERK2 location in the absence of PTP-SL, a cell transfected only with pcDNA3-HA-ERK2 and stained with the anti-HA mAb is shown by an *arrow* in the *inset* in *A*. PTP-SL was stained with rabbit polyclonal anti-PTP-SL antibody plus fluorescein isothiocyanate-conjugated goat anti-rabbit antibody (*B*, *E*, and *H*; *green*). In *C*, *F*, and *I*, double-color staining is shown; *yellow* areas correspond to co-localization of HA-ERK2 and PTP-SL.

ERK2,[2] suggesting that a different regulatory mechanism than interaction with the substrate could operate in the catalytic regulation of PTP-SL. Nevertheless, the possibility of an *in vivo* regulation of PTP-SL activity upon binding to ERK1/2 cannot be excluded. It is likely that different levels of catalytic regulation of ERK1/2-specific phosphatases could exist, depending on the enzyme-substrate specificities within the distinct ERK1/2·phosphatase complexes.

In cells subjected to mitogenic or differentiating stimuli, ERK1 and ERK2 are activated and translocated to the nucleus, where these kinases phosphorylate their nuclear targets (57–60). Several processes have been proposed to regulate this translocation, including phosphorylation and homodimerization of ERK1/2 (61); association with nuclear anchor proteins (62); and association in the cytoplasm with their upstream kinase activators, MEK1 and MEK2 (43), or with other cytoplasmic anchor proteins (63). Our results showing that coexpression of PTP-SL and ERK2 in COS-7 cells results in ERK2 retention in the cytoplasm favors the hypothesis of cytoplasmic retention as a major regulatory mechanism of ERK1/2 nuclear translocation. Furthermore, the possibility exists that subcellular distribution of ERK1/2 within the cytoplasm could be controlled by the selective expression of PTP-SL isoforms or other KIM-containing PTPs. Interestingly, MKP-3/Pyst1/rVH6 phosphatase has also been shown to retain ERK1 and ERK2 in the cytoplasm and to inhibit their entry into the nucleus (64), suggesting that distinct negative regulators of ERK1/2 activity could use similar mechanisms to prevent ERK1/2 nuclear translocation. The results presented here, together with our finding that PTP-SL dephosphorylates and inactivates ERK1/2 in a KIM-dependent manner, support the notion that adjustable binding through the KIM could operate as a mechanism of specificity for PTP-SL and related PTPs to maintain ERK1/2 and p38/HOG inactive in the cytoplasm. Further work will be necessary to elucidate how the binding of PTP-SL to ERK1/2 and to p38/HOG and the PTP-SL enzymatic activity are regulated *in vivo* upon cell stimulation, allowing the rapid activation of these MAP kinases and their translocation to the nucleus.

Acknowledgments—We are indebted to A. Ullrich for support at the beginning of this project and for generously providing valuable reagents. We thank J. Pouysségur and S. Gutkind for gifts of plasmids, I. Roglá and C. Tormo for technical assistance, and E. Knecht for critical reading of the manuscript.

REFERENCES

1. Cobb, M. H., Boulton, T. G., and Robbins, D. J. (1991) *Cell Regul.* **2,** 965–978
2. Davis, R. J. (1993) *J. Biol. Chem.* **268,** 14553–14556
3. Cobb, M. H., and Goldsmith, E. J. (1995) *J. Biol. Chem.* **270,** 14843–14846
4. Cobb, M. H., Xu, S., Cheng, M., Ebert, D., Robbins, D., Goldsmith, E., and

[2] Á. Zúñiga, J. Torres, J. Úbeda, and R. Pulido, unpublished observations.

Robinson, M. (1996) *Adv. Pharmacol.* **36,** 49–65
5. Davis, R. J. (1994) *Trends Biochem. Sci.* **19,** 470–473
6. Kyriakis, J. M., and Avruch, J. (1996) *J. Biol. Chem.* **271,** 24313–24316
7. Cohen, P. (1996) *Adv. Pharmacol.* **36,** 15–27
8. Ip, Y. T., and Davis, R. J. (1998) *Curr. Opin. Cell Biol.* **10,** 205–219
9. Marshall, C. J. (1995) *Cell* **80,** 179–185
10. Pagès, G., Lenormand, P., L'Allemain, G., Chambard, J.-C., Meloche, S., and Pouysségur, J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 8319–8323
11. Cowley, S., Paterson, H., Kemp, P., and Marshall, C. J. (1994) *Cell* **77,** 841–852
12. Mansour, S. J., Matten, W. T., Hermann, A. S., Candia, J. M., Rong, S., Fukasawa, K., Vande Woude, G. F., and Ahn, N. G. (1994) *Science* **265,** 966–970
13. Pumiglia, K. M., and Decker, S. J. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 443–452
14. Racke, F. K., Lewandowska, K., Goueli, S., and Goldfarb, A. N. (1997) *J. Biol. Chem.* **272,** 23366–23370
15. Whalen, A. M., Galasinski, S. C., Shapiro, P. S., Stines Nahreini, T., and Ahn, N. G. (1997) *Mol. Cell. Biol.* **17,** 1947–1958
16. Marshall, C. J. (1994) *Curr. Opin. Genet. Dev.* **4,** 82–89
17. Karin, M. (1995) *J. Biol. Chem.* **270,** 16483–16486
18. Treisman, R. (1996) *Curr. Opin. Cell Biol.* **8,** 205–215
19. Bardwell, L., Cook, J. G., Chang, E. C., Cairns, B. R., and Thorner, J. (1996) *Mol. Cell. Biol.* **16,** 3637–3650
20. Yang, S.-H., Yates, P. R., Whitmarsh, A. J., Davis, R. J., and Sharrocks, A. D. (1998) *Mol. Cell. Biol.* **18,** 710–720
21. Keyse, S. M. (1995) *Biochim. Biophys. Acta* **1265,** 145–160
22. Groom, L. A., Sneddon, A. A., Alessi, D. R., Dowd, S., and Keyse, S. M. (1996) *EMBO J.* **15,** 3621–3632
23. Muda, M., Boscher, U., Dickinson, R., Martinou, J.-C., Martinou, I., Camps, M., Schlegel, W., and Arkinstall, S. (1996) *J. Biol. Chem.* **271,** 4319–4326
24. Mourey, R. J., Vega, Q. C., Campbell, J. S., Wenderoth, M. P., Hauschka, S. D., Krebs, E. G., and Dixon, J. E. (1996) *J. Biol. Chem.* **271,** 3795–3802
25. Tonks, N. K., and Neel, B. G. (1996) *Cell* **87,** 365–368
26. Anderson, N. G., Maller, J. L., Tonks, N. K., and Sturgill, T. W. (1990) *Nature* **343,** 651–653
27. Gómez, N., Tonks, N. K., Morrison, C., Harmar, T., and Cohen, P. (1990) *FEBS Lett.* **271,** 119–122
28. Sarcevic, B., Erikson, E., and Maller, J. L. (1993) *J. Biol. Chem.* **268,** 25075–25083
29. Alessi, D. R., Gómez, N., Moorhead, G., Lewis, T., Keyse, S. M., and Cohen, P. (1995) *Curr. Biol.* **5,** 283–295
30. Zhao, Z., Tan, Z., Diltz, C. D., You, M., and Fischer, E. H. (1996) *J. Biol. Chem.* **271,** 22251–22255
31. Gophalbai, K., and Meloche, S. (1997) *J. Cell. Physiol.* **174,** 35–47
32. Pulido, R., Zúñiga, Á., and Ullrich, A. (1998) *EMBO J.* **17,** 7337–7350
33. Coussens, L., Parker, P. J., Rhee, L., Yang-Feng, T. L., Chen, E., Waterfield, M. D., Francke, U., and Ullrich, A. (1986) *Science* **233,** 859–866
34. Meloche, S., Pagès, G., and Pouysségur, J. (1992) *Mol. Biol. Cell* **3,** 63–71
35. Hendriks, W., Schepens, J., Brugman, C., Zeeuwen, P., and Wieringa, B. (1995) *Biochem. J.* **305,** 499–504
36. Robinson, M. J., and Cobb, M. H. (1997) *Curr. Opin. Cell Biol.* **9,** 180–186
37. Whitmarsh, A. J., and Davis, R. J. (1998) *Trends Biochem. Sci.* **23,** 481–485
38. Millar, J. B., Buck, V., and Wilkinson, M. G. (1995) *Genes Dev.* **9,** 2117–2130
39. Cavigelli, M., Li, W. W., Lin, A., Su, B., Yoshioka, K., and Karin, M. (1996) *EMBO J.* **15,** 6269–6279
40. Muda, M., Theodosiou, A., Rodrigues, N., Boschert, U., Camps, M., Gillieron, C., Davies, K., Ashworth, A., and Arkinstall, S. (1996) *J. Biol. Chem.* **271,** 27205–27208
41. Wurgler-Murphy, S. M., Maeda, T., Witten, E. A., and Saito, H. (1997) *Mol. Cell. Biol.* **17,** 1289–1297
42. Saxena, M., Williams, S., Gilman, J., and Mustelin, T. (1998) *J. Biol. Chem.* **273,** 15340–15344
43. Fukuda, M., Gotoh, Y., and Nishida, E. (1997) *EMBO J.* **16,** 1901–1908
44. Smith, J. A., Poteet-Smith, C. E., Malarkey, K., and Sturgill, T. W. (1999) *J. Biol. Chem.* **274,** 2893–2898
45. Alcorta, D. A., Crews, C. M., Sweet, L. J., Bankston, L., Jones, S. W., and Erikson, R. L. (1989) *Mol. Cell. Biol.* **9,** 3850–3859
46. Rao, V. N., Huebner, K., Isobe, M., ar-Rushdi, A., Croce, C. M., and Reddy, E. S. P. (1989) *Science* **244,** 66–70
47. Zheng, C. F., and Guan, K. (1993) *J. Biol. Chem.* **268,** 11435–11439
48. Bardwell, L., and Thorner, J. (1996) *Trends Biochem. Sci.* **21,** 373–374
49. Hibi, M., Lin, A., Smeal, T., Minden, A., and Karin, M. (1993) *Genes Dev.* **7,** 2135–2148
50. Burgerin, B. M. T., Pronk, G. J., van Weeren, P. C., Chardin, P., and Bos, J. L. (1993) *EMBO J.* **12,** 4211–4220
51. Cook, S. J., and McCormick, F. (1993) *Science* **262,** 1069–1072
52. Graves, L. M., Bornfeldt, K. E., Raines, E. W., Potts, B. C., Macdonald, S. G., Ross, R., and Krebs, E. G. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 10300–10304
53. Sevetson, B. R., Kong, X., and Lawrence, J. C. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 10305–10309
54. Wu, J., Dent, P., Jelinek, T., Wolfman, A., Weber, M. J., and Sturgill, T. W. (1993) *Science* **262,** 1065–1069
55. Muda, M., Theodosiou, A., Gillieron, C., Smith, A., Chabert, C., Camps, M., Boschert, U., Rodrigues, N., Davies, K., Ashworth, A., and Arkinstall, S. (1998) *J. Biol. Chem.* **273,** 9323–9329
56. Camps, M., Nichols, A., Gillieron, C., Atonsson, B., Muda, M., Chabert, C., Boschert, U., and Arkinstall, S. (1998) *Science* **280,** 1262–1265
57. Chen, R.-H., Sarnecki, C., and Blenis, J. (1992) *Mol. Cell. Biol.* **12,** 915–927
58. Seth, A., Gonzalez, F. A., Gupta, S., Raden, D. L., and Davis, R. J. (1992) *J. Biol. Chem.* **267,** 24796–24804
59. González, F. A., Seth, A., Raden, D. L., Bowman, D. S., Fay, F. S., and Davis, R. J. (1993) *J. Cell Biol.* **122,** 1089–1101
60. Lenormand, P., Sardet, C., Pagès, G., L'Allemain, G., Brunet, A., and Pouysségur, J. (1993) *J. Cell Biol.* **122,** 1079–1088
61. Khokhlatchev, A. V., Canagarajah, B., Wilsbacher, J., Robinson, M., Atkinson, M., Goldsmith, E., and Cobb, M. H. (1998) *Cell* **93,** 605–615
62. Lenormand, P., Brondello, J.-M., Brunet, A., and Pouysségur, J. (1998) *J. Cell Biol.* **142,** 625–633
63. Meili, R., and Ballmer-Hofer, K. (1996) *FEBS Lett.* **394,** 34–38
64. Brunet, A., Roux, D., Lenormand, P., Dowd, S., Keyse, S., and Pouysségur, J. (1999) *EMBO J.* **18,** 664–674

CELL BIOLOGY AND METABOLISM:

Interaction of Mitogen-activated Protein Kinases with the Kinase Interaction Motif of the Tyrosine Phosphatase PTP-SL Provides Substrate Specificity and Retains ERK2 in the Cytoplasm

Ángel Zúñiga, Josema Torres, Josefa Úbeda and Rafael Pulido

J. Biol. Chem. 1999, 274:21900-21907.
doi: 10.1074/jbc.274.31.21900