Differential interaction of the tyrosine phosphatases PTP-SL, STEP and HePTP with the mitogen-activated protein kinases ERK1/2 and p38α is determined by a kinase specificity sequence and influenced by reducing agents

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The protein tyrosine phosphatases (PTPs) PTP-SL, STEP and HePTP are mitogen-activated protein kinase (MAPK) substrates and regulators that bind to MAPKs through a kinase-interaction motif (KIM) located in their non-catalytic regulatory domains. We have found that the binding of these PTPs to the MAPKs extracellular-signal-regulated kinase 1 and 2 (ERK1/2), and p38α is differentially determined by the KIM-adjacent C-terminal regions of the PTPs, which have been termed kinase-specificity sequences, and is influenced by reducing agents. Under control conditions, PTP-SL bound preferentially to ERK1/2, whereas STEP and HePTP bound preferentially to p38α. Under reducing conditions, the association of p38α with STEP or HePTP was impaired, whereas the association with PTP-SL was unaffected. On the other hand, the association of ERK1/2 with HePTP was increased under reducing conditions, whereas the association with STEP or PTP-SL was unaffected. In intact cells, PTP-SL and STEP distinctively regulated the kinase activity and the nuclear translocation of ERK1/2 and p38α. Our results suggest that intracellular redox conditions could modulate the activity and subcellular location of ERK1/2 and p38α by controlling their association with their regulatory PTPs.

Key words: cell signalling, mitogen-activated protein kinase docking, protein phosphatase.

INTRODUCTION

In mammalian cells, three major mitogen-activated protein kinase (MAPK) subfamilies have been characterized, which are regulated by distinct extracellular signals and show differential substrate specificities; these include the extracellular-signal-regulated kinases (ERK1/2), the c-Jun N-terminal kinases (JNKs)/stress-activated protein kinases and the p38 MAPKs. Overall, JNK and p38 MAPKs account for the cell response to stress and inflammation conditions, whereas ERK1/2 are mainly activated by growth and differentiation factors (reviewed in [1–3]). Additional MAPK family members include the ERK3, ERK5/big MAPK 1 (BMK1), ERK7 and ERK8 MAPKs [4–8]. An important factor in the definition of the signal specificity within each MAPK signalling pathway is the assembly of their components into dynamic multimolecular complexes, that bring together the distinct effectors and regulators of each pathway, in a tightly regulated manner and within specific subcellular compartments. Components of MAPK pathways include the MAPKs themselves, activating MAPK kinases, inactivating phosphatases, MAPK substrates, and regulatory and scaffolding MAPK modulators [1–3,9–13]. The association of substrates and effectors with the MAPKs is facilitated by the existence of a docking groove on the kinases, where the individual interaction sites of those molecules recognize their binding sites [14–16]. Several classes of interaction sites for MAPKs have been defined, which are present in a wide array of MAPK-interacting molecules. These interaction sites include an arginine- and leucine-rich motif [δ domain, D box, kinase-interaction motif (KIM)], as well as a phenylalanine-rich motif (Phe-Xaa-Phe-Pro motif), which are present either alone or in combination in the different MAPK effectors and substrates and confer specificity to recognize the distinct MAPKs [14,17–19].

ERK1/2 associate with PTP-SL [striatal-enriched phosphatase (STEP)-like protein tyrosine phosphatase (PTP)] through the KIM, located in the regulatory non-catalytic domain of the phosphatase; this association leads to phosphorylation/dephosphorylation events between these two enzymes, which have a regulatory role in the signalling through the MAPK pathways [18,20]. Additional MAPK family members that associate physically and functionally with PTP-SL include p38α and ERK5/BMK1 [18,21]. The PTP-SL-related tyrosine phosphatases STEP and HePTP [haematopoietic PTP; also named leucocyte (LC)-PTP] contain a conserved KIM sequence outside of their catalytic domains and are also substrates of MAPKs [20–23]. PTP-SL and STEP are mainly expressed in specialized brain areas [24–29], whereas HePTP is expressed in leucocytes [30–32]. HePTP has been found to suppress the T-cell-antigen-receptor-mediated activation of MAPKs upon KIM-mediated binding

Abbreviations used: BMK1, big MAPK1; DTT, 1,4-dithiothreitol; ERK, extracellular-signal-regulated kinase; EGF, epidermal growth factor; GST, glutathione S-transferase; HA, haemagglutinin; HePTP, haemopoietic PTP; JNK, c-Jun N-terminal kinase; KIM, kinase-interaction motif; KIS, kinase-specificity sequence; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; 2-ME, 2-mercaptoethanol; MKP, MAPK phosphatase; NMDA, *N*-methyl-D-aspartate; PKA, cAMP-dependent protein kinase; PTP, protein tyrosine phosphatase; PTP-SL, STEP-like PTP; STEP, striatal-enriched phosphatase.

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to these enzymes [22,33,34]. Interestingly, the binding of both PTP-SL and HePTP to the MAPKs ERK1/2 and p38α is regulated by the phosphorylation of their KIMs by the cAMP-dependent protein kinase, PKA, which has consequences for the activation of these kinases and their translocation to the nucleus [35,36]. In addition, the activity of STEP has also been reported to be down-regulated by PKA after neuronal cell activation through dopamine receptors [37]. In the present paper, we report the comparative study of the physical and functional association of PTP-SL, STEP and HePTP with the ERK1/2 and p38α MAPKs. We provide evidence that PTP-SL, STEP and HePTP associate differentially with ERK1/2 and p38α, in a process determined by the KIM-adjacent C-terminal regions of these PTPs, and are influenced by the reducing conditions. Furthermore, PTP-SL and STEP have been found to distinctively regulate the activity and the nuclear translocation of ERK1/2 and p38α.

MATERIALS AND METHODS

Plasmid constructions, mutagenesis, chimaeras and purification of glutathione S-transferase (GST) fusion proteins

The PTP-SL, STEP, HePTP, ERK2 and p38α cDNA constructs and primary and secondary antibodies used in this study have been previously described in [18,20,35]. Rabbit polyclonal anti-STEP 1–369 and anti-STEP 1–107 antibodies were obtained by immunization of rabbits with GST–STEP 1–369 or GST–STEP 1–107 respectively. Some cross-reactivity towards PTP-SL was observed with the anti-STEP 1–369 polyclonal serum. Monoclonal anti-GST antibody was obtained in our laboratory. pRK5-GST–PTP-SL 147–549 and pRK5-GST–STEP 1–369 mammalian expression vectors were constructed by PCR with a primer containing a Kozak sequence followed by a start codon and the *Schistosoma japonicum* GST sequence. All point mutations were performed by PCR oligonucleotide site-directed mutagenesis, and mutations were confirmed by DNA sequencing. The chimaera PTP-SL–KIM–STEP [PTP-SL(147–239)–STEP(58–107)] was constructed by PCR using oligonucleotides that were complementary to the regions N-terminal to the KIM of PTP-SL and C-terminal to the KIM of STEP. The chimaera STEP–KIM–PTP-SL [STEP(1–57)–PTP-SL(240–288)] was constructed by PCR using oligonucleotides that were complementary to the regions N-terminal to the KIM of STEP and C-terminal to the KIM of PTP-SL. The chimaeras STEP–PTP-SL [STEP(1–107)–PTP-SL(289–549)] and PTP-SL–STEP [PTP-SL(147–288)–STEP(108–369)] were made using an in frame internal *Eco*RI restriction site located at amino acids 288 of PTP-SL and 107 of STEP. Amino acid numbering of PTP-SL, STEP and HePTP is according to [26], [24] and [31] respectively. Glutathione–Sepharose purification of the GST fusion proteins was performed by standard procedures.

Cell culture, transfection and cell lysis

Human embryonic kidney HEK-293, simian kidney COS-7 and rat fibroblast Rat-1 cell lines were grown in Dulbecco's minimal essential medium containing high glucose (4.5 g/l) supplemented with 5 % (COS-7 cells) or 10 % (HEK-293 and Rat-1 cells) heat-inactivated foetal calf serum. The cells were transfected using the DEAE-dextran method (COS-7 cells) or the calcium phosphate precipitation method (HEK-293 cells) and were harvested 48 h post-transfection. Haemagglutinin (HA)–ERK2 or HA–p38α from transfected COS-7 cells were activated by cell treatment with 50 ng/ml epidermal growth factor (EGF; Invitrogen) for 5 min or 0.5 M sorbitol (Sigma) for 30 min respectively. For association experiments under standard conditions (see Figures 1–4), cells were lysed with lysis buffer A [50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 % (v/v) Igepal CA-630 (Sigma), 1 mM PMSF, 1 μg/ml aprotinin, 100 mM NaF, 2 mM Na_3VO_4 and 20 mM $Na_4P_2O_7$]. For association experiments under different reducing conditions (see Figure 7), cells were lysed in lysis buffer A alone (control conditions) or in lysis buffer A plus 2 mM 1,4-dithiothreitol (DTT; Roche) or 10 mM 2-mercaptoethanol (2-ME; Merck).

Precipitation with GST fusion proteins, immunoprecipitation, immunoblotting, immunofluorescence and *in vitro* kinase assays

For precipitation, cell lysates were incubated with 2 μg of the distinct fusion proteins, or with 30 μl of glutathione–Sepharose in the case of HEK-293 cells transfected with pRK5-GST–PTP-SL 147–549 or pRK5-GST–STEP 1–369. The samples were washed with 20 mM Hepes, pH 7.5, 150 mM NaCl, 10 mM $Na_4P_2O_7$, 0.1 % (v/v) Igepal CA-630 and 10 % (v/v) glycerol. Immunoprecipitation, immunoblotting, and immunofluorescence were performed as described in [18,20]. For *in vitro* kinase assays, cells were lysed in lysis buffer A (see above), and the immune-complex pellets containing EGF-activated HA–ERK2 or sorbitol-activated HA–p38α were incubated at room temperature (20 °C) for 20 min with constant shaking in kinase reaction buffer (20 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, 2 mM Na_3VO_4 and 0.3 μM ATP), in the presence of 2 μg of myelin basic protein (MBP) and 2 μCi of [γ-^{32}P]ATP per sample. The reactions were stopped by adding SDS sample buffer and boiling, followed by SDS/PAGE (15 % gels) under reducing conditions. Gels were processed for quantification of the incorporated radioactivity into the MBP substrate proteins as described in [16].

RESULTS

PTP-SL, STEP and HePTP differentially associate with ERK1/2 and p38α through their N-terminal regulatory domains

PTP-SL, STEP and HePTP tyrosine phosphatases have in common the existence of a highly conserved KIM. Upon binding through the KIM, the MAPKs ERK1/2 phosphorylate PTP-SL, whereas PTP-SL dephosphorylates and inactivates ERK1/2 [20]. To investigate in parallel the association of PTP-SL, STEP and HePTP with ERK1/2 and p38α, Rat-1 cell lysates were precipitated with GST–PTP-SL, GST–STEP or GST–HePTP fusion proteins purified from *Escherichia coli*, followed by immunoblot with anti-ERK1/2 or anti-p38α antibodies. GST–PTP-SL 147–288, GST–STEP 1–107 and GST–HePTP 1–79 fusion proteins, containing the MAPK binding domains (Figure 1A), associated with ERK1/2 and p38α (Figure 1B, lanes 2, 4 and 6). The mutational analysis of the KIM on PTP-SL has shown that either of the two arginine residues within the KIM (Arg^{228} and Arg^{229}) is essential for ERK1/2 binding [18]. Accordingly, amino-acid substitution of the equivalent residues on the KIM of STEP or HePTP [mutants $Arg^{46} \rightarrow$ Ala (R46A)/R47A and R20A/R21A respectively] impaired the association with ERK1/2 and p38α (Figure 1B, lanes 3, 5 and 7). Also, the GST–PTP-SL 289–549 and GST–STEP 108–369 fusion proteins, lacking the MAPK-binding domain of these PTPs, did not associate with ERK1/2 or p38α (Figure 1B, lanes 10 and 12). Interestingly, GST–PTP-SL 147–288 associated more strongly with ERK1/2 than with p38α, whereas GST–STEP 1–107 and GST–HePTP 1–79 associated more strongly with p38α than with ERK1/2 (Figure 1B). Similar patterns of association were found

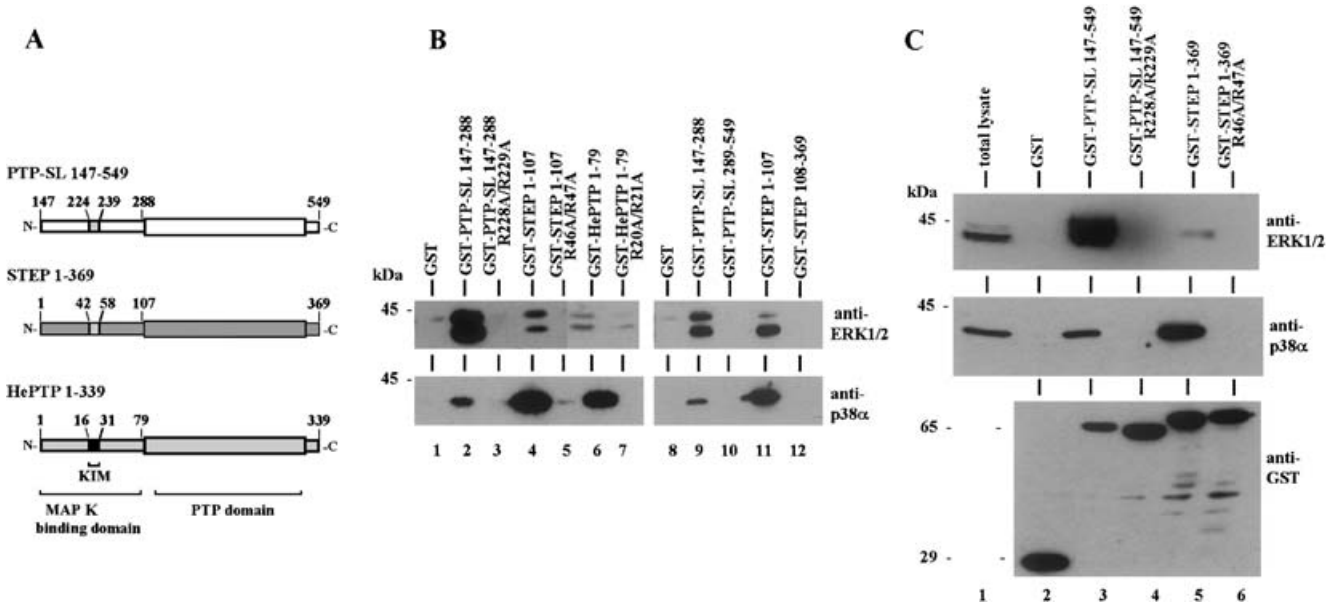

Figure 1 Differential association of PTP-SL, STEP and HePTP with ERK1/2 and p38α

(**A**) Schematic representation of the structure of the PTP-SL, STEP and HePTP proteins used; amino acid numbering is according to [24], [26] and [31] respectively. (**B**) Equal amounts of Rat-1 cell lysates were precipitated with 2 μg of the different GST fusion proteins (purified from *E. coli*) and glutathione–Sepharose, as indicated. Samples were resolved by 10 % SDS/PAGE under reducing conditions, followed by immunoblot analysis with anti-ERK1/2 (upper panels) or anti-p38α (lower panels) antibodies. (**C**) HEK-293 cells were transfected with pRK5-GST (lanes 2), or with pRK5-GST–PTP-SL or pRK5-GST–STEP wild-type or mutants, as indicated, in order to express the fusion proteins ectopically. After 48 h, the GST fusion proteins were precipitated from equal amounts of cell lysates with glutathione–Sepharose, and the co-precipitated endogenous MAPKs were detected by immunoblot analysis with anti-ERK1/2 (upper panel) or anti-p38α (middle panel) antibodies. In lane 1, upper and middle panels, 50 μg of total lysates were loaded. In the lower panel, 50 μg of total lysates were loaded, and analysed by immunoblot with an anti-GST antibody. Experiments were performed at least three times with similar results, and a representative experiment is shown.

upon activation of MAPKs by cell treatment with EGF or sorbitol (results not shown). To corroborate the differential association of PTP-SL and STEP with ERK1/2 and p38α, experiments were performed using GST fusion proteins overexpressed in mammalian cells. GST–PTP-SL 147–549 or GST–STEP 1–369 (that span the complete cytosolic portion of these PTPs; Figure 1A) were ectopically expressed in HEK-293 cells; after transfection and cell lysis, the fusion proteins were precipitated with glutathione–Sepharose and the co-precipitated MAPKs were visualized by immunoblotting (Figure 1C). As shown, GST–PTP-SL preferentially co-precipitated ERK1/2, whereas GST–STEP preferentially co-precipitated p38α.

Chimaeric proteins were designed containing the N-terminal MAPK-binding domain of STEP followed by the PTP domain of PTP-SL (STEP–PTP-SL chimaera), or vice versa (PTP-SL–STEP chimaera; Figure 2A), and their association with the MAPKs was assessed as above. The GST–STEP–PTP-SL chimaeric protein associated with ERK1/2 and p38α in a manner equivalent to that of the STEP wild-type protein (Figure 2B, lanes 4 and 5), whereas the GST–PTP-SL–STEP chimaera associated with these MAPKs in the same way as PTP-SL wild-type (Figure 2B, lanes 3 and 6). Taken together, these results indicate that ERK1/2 and p38α differentially associate with the N-terminal regulatory domains of PTP-SL, STEP and HePTP.

Regions outside the KIM contribute to the differential association of PTP-SL, STEP and HePTP with ERK1/2 and p38α

To test whether the differences in the core KIM amino-acid sequences of PTP-SL and HePTP (Figure 3A) could account for their differential recognition by the MAPKs, amino-acid-substitution mutants were generated that exchanged the core KIM sequences of these two PTPs (mutants PTP-SL T236M and S234A/T236M; and mutants HePTP M28T and A26S/M28T), and their association with ERK1/2 or p38α was analysed using GST fusion proteins and Rat-1 cell lysates, as above. GST–PTP-SL 147–288 T236M or S234A/T236M mutants associated slightly poorer with both ERK1/2 and p38α than GST–PTP-SL 147–288 wild-type did (Figure 3B, lanes 3–5), whereas GST–HePTP 1–79 M28T or A26S/M28T mutants associated with both ERK1/2 and p38α almost in the same manner as GST–HePTP 1–79 wild-type (Figure 3B, lanes 6–8). Thus these KIM mutations did not revert the differential association of GST–PTP-SL and GST–HePTP with ERK1/2 and p38α, but rather slightly diminished or augmented the association of both MAPKs observed with the wild-type PTPs. Next, chimaeric proteins were obtained by swapping the regions adjacent to the KIM in PTP-SL and STEP (Figure 4A), and their association with ERK1/2 and p38α was also analysed. Remarkably, the pattern of association of the chimaera containing the STEP region C-terminal to the KIM (PTP-SL–KIM–STEP) was similar to that shown by wild-type STEP: association with p38α > association with ERK1/2 (Figure 4B, lanes 4 and 6). Conversely, the chimaera containing the PTP-SL region C-terminal to the KIM (STEP–KIM–PTP-SL) associated with the MAPKs in a similar manner to wild-type PTP-SL: association with ERK1/2 > association with p38α (Figure 4B, lanes 3 and 5). These findings indicate that molecular determinants outside the KIM exist that are important for the differential association of PTP-SL and STEP, and probably HePTP with ERK1/2 and p38α, and suggest that the non-catalytic amino-acid regions C-terminal to the KIM on PTP-SL and STEP regulate

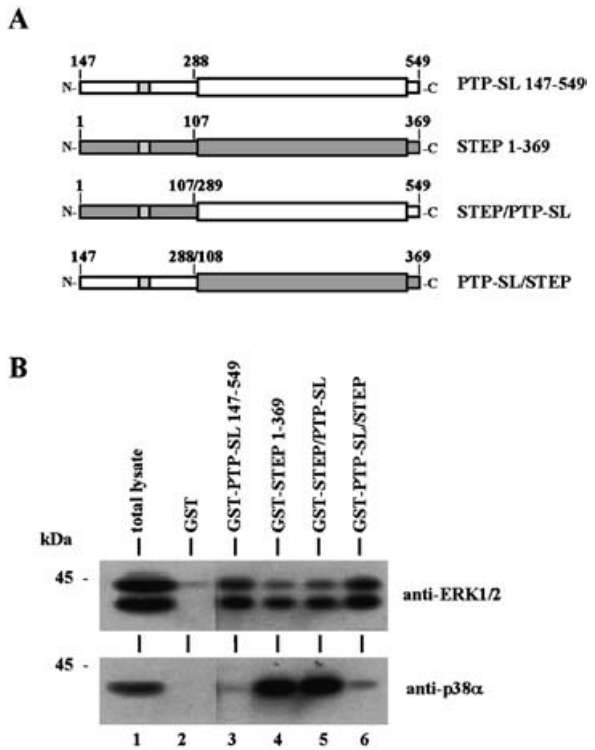

Figure 2 Differential association of PTP-SL and STEP with ERK1/2 and p38α is determined by the MAPK-binding domain, but not by the PTP domain

(**A**) Schematic representation of the chimaeric proteins used. (**B**) Equal amounts of Rat-1 cell lysates were precipitated with 2 μg of the different wild-type or chimaeric GST fusion proteins and glutathione–Sepharose, as indicated. Samples were resolved by SDS/PAGE (10 % gels) under reducing conditions, followed by immunoblot analysis with anti-ERK1/2 (upper panel) or anti-p38α (lower panel) antibodies. In lane 1, 50 μg of total lysate samples were loaded. Experiments were performed at least three times with similar results, and a representative experiment is shown.

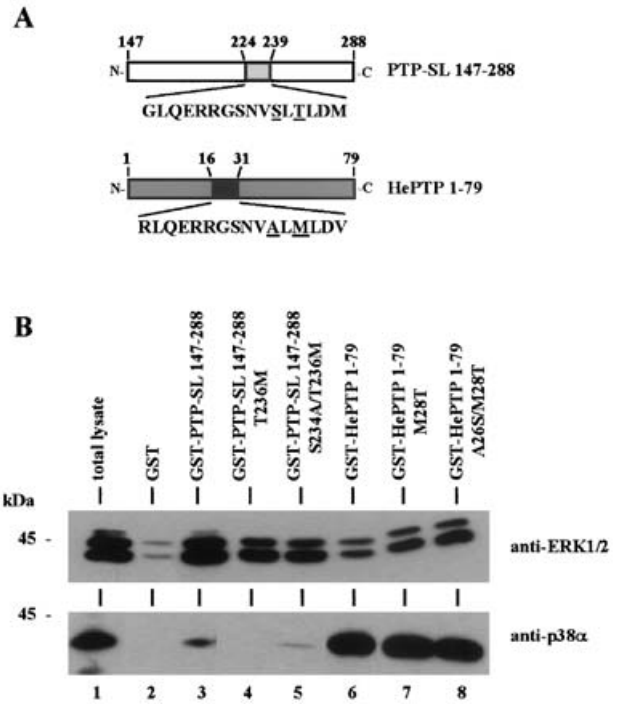

Figure 3 Differential association of PTP-SL and HePTP with ERK1/2 and p38α is not determined by differences in their core KIM amino-acid sequences

(**A**) Schematic representation of the proteins used. The wild-type KIM amino-acid sequences of PTP-SL and HePTP are indicated using the one-letter code. Residues that are different within the two core KIM sequences are underlined; mutants were generated that switched these residues in the two proteins. (**B**) Equal amounts of Rat-1 cell lysates were precipitated with 2 μg of the different wild-type or mutant GST fusion proteins and glutathione–Sepharose, as indicated. Samples were resolved by SDS/PAGE (10 % gels) under reducing conditions, followed by immunoblot analysis with anti-ERK1/2 (upper panel) or anti-p38α (lower panel) antibodies. In lane 1, 50 μg of total lysate samples were loaded. Experiments were performed at least three times with similar results, and a representative experiment is shown.

their selective binding to these MAPKs. We have termed these regions as kinase-specificity sequences (KISs).

Differential effects of PTP-SL and STEP on the function and nuclear translocation of ERK2 and p38α

To test the effect of PTP-SL and STEP on the catalytic activity of ERK2 and p38α in intact cells, COS-7 cells overexpressing the PTPs and the MAPKs were activated with EGF (for HA–ERK2-transfected cells) or osmotic shock (for HA–p38α-transfected cells), followed by immunoprecipitation with the anti-HA 12CA5 monoclonal antibody and *in vitro* kinase assays. As observed, HA–ERK2 and HA–p38α catalytic activities were decreased in cells co-expressing PTP-SL 147–549; however, the catalytic activity of HA–ERK2 was only slightly decreased, and that of HA–p38α was not modified upon co-expression with STEP 1–369 (Figure 5). It should be mentioned that the absence of inhibition of MAPK catalytic activities in these experiments was not due to the lack of catalytic activity on the STEP molecules used in our studies, as monitored by *in vitro* *p*-nitrophenyl phosphate dephosphorylation assays following immunoprecipitation of this PTP (results not shown). The effects of the PTP-SL–STEP and STEP–PTP-SL chimaeras on the catalytic activity of MAPKs in intact cells was also tested. As shown, the PTP-SL–STEP chimaera (containing the MAPK-binding domain of PTP-SL and the catalytic domain of STEP, as depicted in Figure 2) inactivated HA–ERK2 and HA–p38α to a similar extent as that by PTP-SL, whereas the catalytic inactivation of these MAPKs by the STEP–PTP-SL chimaera (containing the MAPK-binding domain of STEP and the catalytic domain of PTP-SL) was weaker (Figure 5C). These results indicate that the MAPK-binding domain of PTP-SL and STEP has an important role in the differential regulation of the catalytic activities of ERK1/2 and p38α by these two PTPs, and suggest the existence of specific mechanisms that control the regulatory effects of PTP-SL and STEP on MAPKs in intact cells.

Since no correlation was observed between the association of STEP with MAPKs, as monitored in the pull-down and co-precipitation assays using GST–STEP fusion proteins, and the catalytic inactivation of MAPKs by STEP, the *in vivo* physical association of STEP with ERK1/2 and p38α was assessed in transfected COS-7 cells by double immunofluorescence analysis, and compared with that observed for PTP-SL. Overexpression of PTP-SL 147–549 retained HA–ERK2 and HA–p38α in the cytoplasm ([35] and Figure 6). However, overexpression of STEP 1–369 resulted in a weak or no cytoplasmic retention of HA–ERK2 or HA–p38α respectively (Figure 6), and did not block the nuclear accumulation of these MAPKs, in correlation with the poor inhibition of their activity by this PTP. Finally, co-localization experiments were performed on COS-7 cells using the STEP–PTP-SL and PTP-SL–STEP chimaeric proteins. As shown,

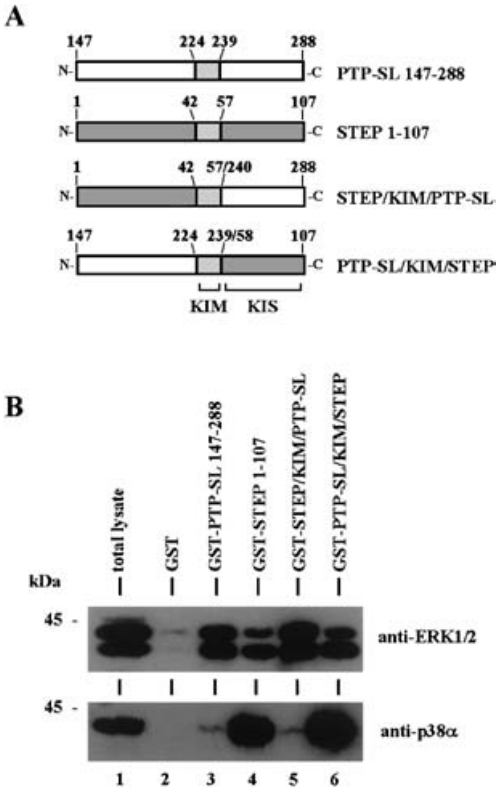

Figure 4 Differential association of PTP-SL and STEP with ERK1/2 and p38α is determined by a KIS located C-terminally to the KIM

(**A**) Schematic representation of the chimaeric proteins used. The KIM and KIS regions are indicated. (**B**) Equal amounts of Rat-1 cell lysates were precipitated with 2 μg of the different wild-type or chimaeric GST fusion proteins and glutathione–Sepharose, as indicated. Samples were resolved by SDS/PAGE (10 % gels) under reducing conditions, followed by immunoblot analysis with anti-ERK1/2 (upper panel) or anti-p38α (lower panel) antibodies. In lane 1, 50 μg of total lysate samples were loaded. Experiments were performed at least three times with similar results, and a representative experiment is shown.

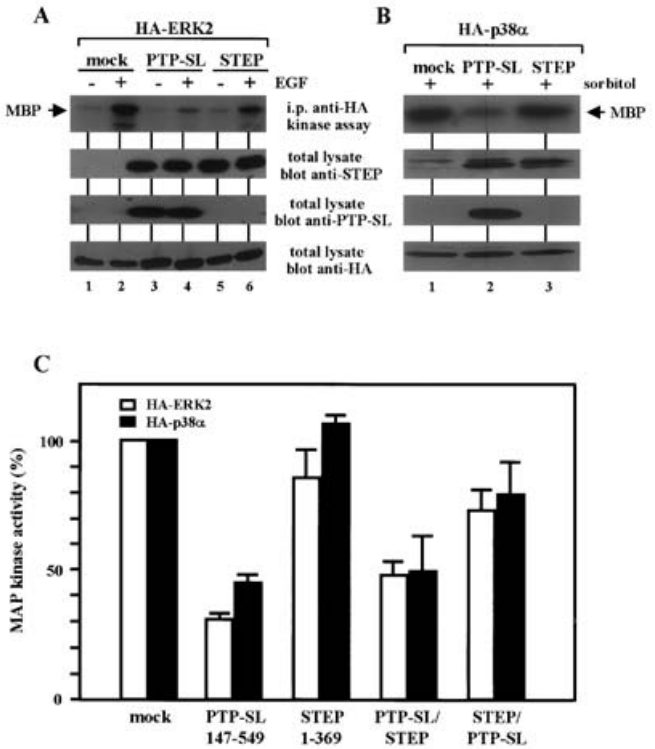

Figure 5 Differential effects of PTP-SL and STEP on the activity of ERK2 and p38α in intact cells

(**A**) COS-7 cells were co-transfected with pCDNA3-HA–ERK2 and pRK5 vector alone (mock) (lanes 1 and 2), pCDNA3-HA–ERK2 plus pRK5-PTP-SL 147–549 (lanes 3 and 4) or pCDNA3-HA–ERK2 plus pRK5-STEP 1–369 (lanes 5 and 6). After 48 h, cells were left untreated ($-$) or were treated for 5 min with 50 ng/ml EGF ($+$). HA–ERK2 was immunoprecipitated (i.p.) with the anti-HA 12CA5 monoclonal antibody, and immune-complex kinase assays were performed in the presence of [γ-^{32}P]ATP and MBP (2 μg) as the substrate (upper panel). In the lower panels, 50 μg of total lysate samples were loaded, and analysed by immunoblot with anti-STEP 1–369, anti-PTP-SL, or anti-HA antibodies, as indicated. (**B**) COS-7 cells were co-transfected with pECE-HA–p38α and pRK5 vector alone (mock) (lane 1), pECE-HA–p38α plus pRK5-PTP-SL 147–549 (lane 2) or pECE-HA–p38α plus pRK5-STEP 1–369 (lane 3). After 48 h, cells were treated for 30 min with 0.5 M sorbitol ($+$), and processed for kinase assays and immunoblot as in (**A**). The PTP-SL signal observed in the anti-STEP immunoblots is due to cross-reaction of the polyclonal anti-STEP 1–369 antibody with PTP-SL. (**C**) COS-7 cells were co-transfected with vectors encoding the MAPKs (pCDNA3-HA–ERK2 or pECE-HA–p38α) and pRK5 vector alone (mock), or with vectors encoding the MAPKs plus pRK5-PTP-SL 147–549, pRK5-STEP 1–369, pRK5-PTP-SL–STEP [PTP-SL(147–288)–STEP(108–369) chimaera; see Figure 2] or pRK5-STEP–PTP-SL [STEP(1–107)–PTP-SL(288–549) chimaera], as indicated. After 48 h, cells were treated for 5 min with 50 ng/ml EGF (for HA–ERK2; white bars) or for 30 min with 0.5 M sorbitol (for HA–p38α; black bars). Cells were lysed and processed for kinase assays as above. Results are percentages of MAPK activity with respect to that shown for mock-transfected cells (100 % activity) and are means $\pm$ S.D. of two separate experiments.

the PTP-SL–STEP chimaera retained HA–ERK2 and HA–p38α in the cytoplasm, as did PTP-SL, whereas the STEP–PTP-SL chimaera did not retain these MAPKs efficiently, unlike STEP (Figure 6). Thus PTP-SL associates in the cytoplasm of intact COS-7 cells with ERK1/2 and p38α, and effectively blocks their activation and entry into the nucleus; however, the co-expression of STEP and these MAPKs in COS-7 cells did not result in their physical association, and no inhibition of MAPK catalytic activity and nuclear translocation was observed.

Reducing conditions influence the association of PTP-SL, STEP and HePTP with MAPKs

To find an explanation for the differences observed in the association of STEP with MAPKs in cell lysates and in intact cells, comparative pull-down assays with GST–STEP 1–107 and GST–PTP-SL 147–288 (lacking the PTP catalytic domains; see Figure 1A) were performed using different conditions of cell lysis, including the presence or absence of reducing agents. As illustrated in Figure 7, the association of GST–STEP with p38α was lost when the assay was performed in the presence of reducing agents, such as DTT or 2-ME (Figure 7A, lower panel, lane 7; Figure 7B, lower panel, lanes 2 and 3). This effect was specific, since the association of GST–STEP with ERK1/2 (Figure 7A, upper panel, lane 7), or of GST–PTP-SL with p38α or ERK1/2 (Figure 7A, lane 3) was not significantly affected by the presence of reducing agents. Furthermore, the binding to p38α of the chimaera containing the KIS of STEP (PTP-SL–KIM–STEP, as depicted in Figure 4A) was also lost upon reducing conditions (Figure 7A, lower panel, lane 8). To rule out the possibility that GST could be involved in the inhibition of the interaction of STEP with p38α upon reducing conditions in the above experiments, co-precipitation experiments were performed using STEP 1–369 and HA–p38α overexpressed in COS-7 cells (Figure 7C). As observed, STEP 1–369 was co-precipitated with HA–p38α under control conditions (Figure 7C, lane 2), but not in the presence of the reducing agent DTT (Figure 7C, lane 3). Finally, the association of GST–HePTP 1–79 with MAPKs was also assessed

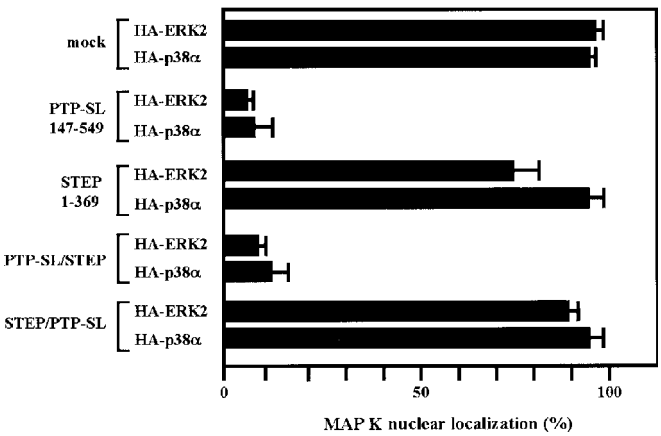

Figure 6 PTP-SL and STEP differentially retain HA–ERK2 and HA–p38α in the cytoplasm

COS-7 cells were co-transfected with pCDNA3-HA–ERK2 or pECE-HA–p38α plus pRK5 vector alone (mock), or plus pRK5-PTP-SL or pRK5-STEP wild-type or chimaeric proteins (as in Figure 5), as indicated. After 48 h, cells were co-stained and analysed by immunofluorescence. HA–ERK2 and HA–p38α were stained with the mouse anti-HA monoclonal antibody 12CA5 plus rhodamine-conjugated goat anti-mouse antibody. PTP-SL, or the chimaeras PTP-SL–STEP or STEP–PTP-SL were stained with the rabbit polyclonal anti-PTP-SL antibody, and STEP was stained with the rabbit polyclonal anti-STEP 1–107 antibody. In both cases, FITC-conjugated goat anti-rabbit was used as a secondary antibody. HA–ERK2 or HA–p38α nuclear localization is scored as the percentage of cells co-expressing the PTPs and HA–ERK2 or HA–p38α that showed the MAPK located into the nucleus. Results are means ± S.D. of at least two separate experiments, with at least 50 double positive cells scored for each bar.

Table 1 Summary of the association of PTP-SL, STEP and HePTP with ERK1/2 and p38α

Estimated association based on pull-down assays, relative to the association of ERK1/2 with PTP-SL under our control experimental conditions: +++, strong association; +, moderate association; −/+, weak association.

	ERK1/2		p38α	
PTP	Control	Reduction	Control	Reduction
PTP-SL	+++	+++	+	+
STEP	+	+	+++	−/+
HePTP	+	+++	+++	−/+

in the presence of reducing agents. Interestingly, a distinct pattern of MAPK association, in comparison with GST–STEP and GST–PTP-SL, was found for GST–HePTP under reducing conditions: the association with ERK1/2 was increased, whereas the association with p38α was decreased (Figure 7D, lane 2). These results are summarized in Table 1. Thus reducing conditions differentially influence the association of PTP-SL, STEP and HePTP with the MAPKs ERK1/2 and p38α, independently of the catalytic domains of the PTPs.

DISCUSSION

Selective recognition of substrates and effectors by MAPKs involves electrostatic and hydrophobic interactions between key residues at the MAPK docking grooves and at the interaction sites of the substrates and effectors. These interactions are thought to provide selectivity for both the differential regulation and signalling outputs of the distinct MAPK pathways upon activation. The tyrosine phosphatases PTP-SL, STEP and HePTP are ERK1/2 and p38α substrates and regulators that possess a conserved interaction site, the KIM, for these MAPKs in their N-terminal regulatory domains [18,20]. In the present study, the regulation of the physical and functional association of PTP-SL, STEP and HePTP with ERK1/2 and p38α has been analysed comparatively. Using GST pull-down and co-precipitation assays, PTP-SL has been found to bind ERK1/2 more efficiently than p38α, whereas STEP and HePTP bind p38α more efficiently than ERK1/2. In addition, we have identified a region adjacent to the KIM of these three PTPs, the KIS (see Figures 4 and 8) that is involved in their differential recognition of ERK1/2 and p38α, as well as in the distinctive effects of reducing agents in their binding capabilities to these MAPKs (summarized in Table 1). The present study and previous studies [18,34] have shown that mutation of the arginine residues within the KIM of PTP-SL, STEP or HePTP abrogate binding to ERK1/2 and p38α, indicating that these motifs are the major molecular determinants on the PTPs that are responsible for binding to MAPKs. However, binding specificity is only achieved in the context of an appropriate KIS, C-terminal to the KIM, which discriminates between ERK1/2 and p38α to differentially target them to PTP-SL, STEP, or HePTP. Thus the combination of a highly conserved KIM with a variable KIS (see Figure 8) allows tight and differential binding of these PTPs to their cognate MAPKs. In this regard, different subregions in the MAPK binding domains of other MAPK effectors or substrates, including MAPK phosphatases (MKPs) and ETS transcription factors, have also been shown to be involved in the differential binding to MAPKs [38,39]. In addition, the association of distinct MAPKs with the different MKPs, as well as with their activating MAPK kinases, has been found to be controlled by specificity mechanisms that involve several parts of these molecules [40–43]. Our results indicate that the differential recognition of MAPKs by PTP-SL, STEP and HePTP involves different regions of their N-terminal MAPK-binding domains, and that the PTP catalytic domains are dispensable for such recognition. The catalytic domain of PTP-SL has been crystallized, together with a partial fragment (helix α0) of the KIS of this PTP; interestingly, extensive intra-molecular interactions are predicted that stabilize the helix α0 of PTP-SL, which is proposed to play a role in the orientation of the phosphatase in a favourable position for interaction with ERK2, underscoring the importance of this region in the regulation of PTP-SL function [44]. In addition, an interaction between the catalytic domain of PTP-SL and ERK2 is expected to exist under physiological conditions to facilitate the dephosphorylation and inactivation of the kinase [44]. In this regard, the inactivation of MAPKs in COS-7 cells by the PTP-SL–STEP and the STEP–PTP-SL chimaeras was partially influenced by their respective PTP domains (Figure 5).

In spite of their identical KIMs and their relatively well conserved KISs (Figure 8 and [45]), the binding patterns of PTP-SL and STEP to ERK1/2 and p38α are different; on the other hand, although STEP and HePTP amino-acid sequences within this region show less conservation, their binding patterns to ERK1/2 and p38α, under control conditions, are similar (Table 1). HePTP is predominantly expressed in leucocytes, where it targets MAPKs and regulates T-cell-antigen-receptor signalling [22,33,34,46]. Interestingly, we show opposite effects of reducing agents in the modulation of the association of HePTP with ERK1/2 and p38α, suggesting that redox conditions could act in leucocytes as a switch to control the interaction of HePTP with each of these MAPKs. Also, these findings could explain some controversial results regarding the specificity of HePTP towards p38α [22,23,46]. The expression of PTP-SL and STEP isoforms is developmentally regulated in the brain [25–29], making possible a specialization in the control of ERK1/2 and p38α functions by these two PTPs in particular brain areas. *In vitro*, STEP binds

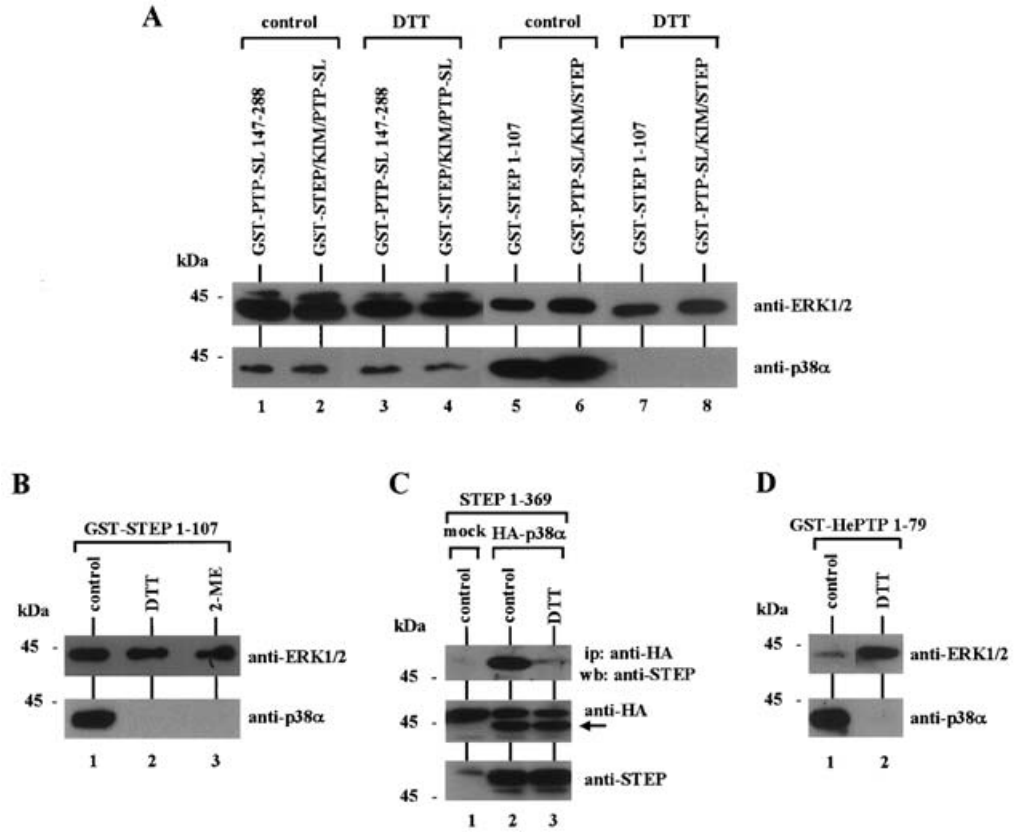

Figure 7 Reducing conditions differentially affect the association of PTP-SL, STEP and HePTP with ERK1/2 and p38α

(**A**) HEK-293 cells were lysed under control or under reducing (2 mM DTT) conditions, as indicated. Lysates were precipitated with 2 μg of the different wild-type or chimaeric GST fusion proteins and glutathione–Sepharose, as indicated. Samples were resolved by SDS/PAGE (10 % gels) under reducing conditions, followed by immunoblot analysis with anti-ERK1/2 (upper panel) or anti-p38α (lower panel) antibodies. (**B**) HEK-293 cells were lysed under different reduction conditions (2 mM DTT or 10 mM 2-ME, as indicated) and lysates were precipitated with 2 μg of GST–STEP 1–107 and processed for immunoblot as in (**A**). (**C**) COS-7 cells were mock-transfected or were transfected with pRK5-STEP 1–369 or with pECE-HA–p38α. After 48 h, cells were lysed under control (lanes 1 and 2) or under reducing (lane 3) conditions, as in (**A**). In the upper panel, lysates containing STEP 1–369 were mixed with the mock-transfected lysates (lane 1) or with the lysates containing HA–p38α (lanes 2 and 3), followed by immunoprecipitation with the anti-HA 12CA5 monoclonal antibody and immunoblot analysis with the anti-STEP 1–107 antibody. In the middle and lower panels, 50 μg of total lysates were loaded, and analysed by immunoblot with the anti-HA or the anti-STEP 1–107 antibodies, as indicated. The arrow in the middle panel indicates the migration of HA–p38α. ip, immunoprecipitation; wb, immunoblot. (**D**) HEK-293 cells were lysed under control (lane 1) or under reducing (lane 2) conditions, as in (**A**), and lysates were precipitated with 2 μg of GST–HePTP and processed for immunoblot as in (**A**). In (**B**), distinct input of cell lysates was used to obtain detectable bands in the control points, making the relative signals of the GST fusion protein with the distinct MAPKs non-comparable. In all cases, when comparing control with the different redox conditions, equal amounts of cell lysates were used. Experiments were performed at least three times with similar results, and a representative experiment is shown.

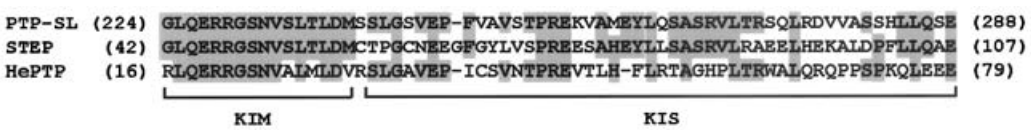

Figure 8 Alignment of the KIM and KIS sequences of PTP-SL (residues 224–288) [26], STEP (residues 42–107) [24] and HePTP (residues 16–79) [31]

Conserved residues are shadowed. Amino acids are indicated using the one-letter code.

very efficiently to p38α under control conditions; however, we have been unable to detect co-localization of these two molecules, or inactivation of p38α by STEP, in intact cells. Our results on the inhibitory effect of reducing conditions on the association of STEP with p38α provides an explanation for this apparent discrepancy. Additional mechanisms may exist that account for the regulation of the binding and the activity of STEP towards MAPKs. For instance, PKA is expected to regulate *in vivo* the functions of STEP by phosphorylation of the KIM in a specific manner [35,37]. Furthermore, different putative phosphorylation sites are present on the KIS of STEP, including the MAPK phosphorylation sites, raising the possibility that phosphorylation of this region could regulate *in vivo* the specificity of the association between STEP and the MAPKs. Our findings that the differential association of STEP with ERK1/2 and p38α is observed using both bacterial- and mammalian-overexpressed recombinant STEP proteins, suggest that this phenomenon is independent of STEP phosphorylation. However, the possibility exists that a protein phosphatase, not inactivated under our cell-lysis conditions, could dephosphorylate STEP in the COS-7 cell

lysates. In addition, an effect of the reducing conditions on the binding properties of p38α towards STEP cannot be excluded. Furthermore, STEP has been found to suffer a calcium-dependent proteolytic cleavage, whose functional consequences are not yet known [47], and a distinctive pattern of STEP isoforms exist in comparison with PTP-SL or HePTP [48]. Finally, the association of STEP with *N*-methyl-D-aspartate (NMDA) receptor complexes and with the Src family kinase, Fyn, has been reported, and a role for STEP in the regulation of NMDA-receptor-channel activity in neurons has been demonstrated [49,50]; the possibility exists that the functional regulation of the NMDA receptor by STEP is linked to the regulated association of this PTP with kinases in neurons, including Fyn and/or MAPKs. Further work will be necessary to elucidate how reducing conditions, or other regulatory mechanisms, may affect the functional properties of STEP in neurons.

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REFERENCES

1 English, J., Pearson, G., Wilsbacher, J., Swantek, J., Karandikar, M., Xu, S. and Cobb, M. H. (1999) New insights into the control of MAP kinase pathways. Exp. Cell Res. **253**, 255–270
2 Chang, L. and Karin, M. (2001) Mammalian MAP kinase signalling cascades. Nature (London) **410**, 37–40
3 Kyriakis, J. M. and Avruch, J. (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol. Rev. **81**, 807–869
4 Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H. and Yancopoulos, G. D. (1991) ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell **65**, 663–675
5 Lee, J. D., Ulevitch, R. J. and Han, J. (1995) Primary structure of BMK1: a new mammalian map kinase. Biochem. Biophys. Res. Commun. **213**, 715–724
6 Zhou, G., Bao, Z. Q. and Dixon, J. E. (1995) Components of a new human protein kinase signal transduction pathway. J. Biol. Chem. **270**, 12665–12669
7 Abe, M. K., Kuo, W.-L., Hershenson, M. B. and Rosner, M. R. (1999) Extracellular signal-regulated kinase 7 (ERK7), a novel ERK with a C-terminal domain that regulates its activity, its cellular localization, and cell growth. Mol. Cell. Biol. **19**, 1301–1312
8 Abe, M. K., Saelzler, M. P., Espinosa, III, R., Kahle, K. T., Hershenson, M. B., Le Beau, M. M. and Rosner, M. R. (2002) ERK8, a new member of the mitogen-activated protein kinase family. J. Biol. Chem. **277**, 16733–16743
9 Garrington, T. P. and Johnson, G. L. (1999) Organization and regulation of mitogen-activated protein kinase signaling pathways. Curr. Opin. Cell Biol. **11**, 211–218
10 Schaeffer, H. J. and Weber, M. J. (1999) Mitogen-activated protein kinases: specific messages from ubiquitous messengers. Mol. Cell. Biol. **19**, 2435–2444
11 Widmann, C., Gibson, S., Jarpe, M. B. and Johnson, G. L. (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. **79**, 143–180
12 Keyse, S. M. (2000) Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. Curr. Opin. Cell Biol. **12**, 186–192
13 Kolch, W. (2000) Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem. J. **351**, 289–305
14 Tanoue, T., Adachi, M., Moriguchi, T. and Nishida, E. (2000) A conserved docking motif in MAP kinases common to substrates, activators and regulators. Nat. Cell Biol. **2**, 110–116
15 Tanoue, T., Maeda, R., Adachi, M. and Nishida, E. (2001) Identification of a docking groove on ERK and p38 MAP kinases that regulates the specificity of docking interactions. EMBO J. **20**, 466–479
16 Tárrega, C., Blanco-Aparicio, C., Muñoz, J. J. and Pulido, R. (2002) Two clusters of residues at the docking groove of mitogen-activated protein kinases differentially mediate their functional interaction with the tyrosine phosphatases PTP-SL and STEP. J. Biol. Chem. **277**, 2629–2636
17 Jacobs, D., Glossip, D., Xing, H., Mulsim, A. J. and Kornfeld, K. (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. Genes Dev. **13**, 163–175
18 Zúñiga, Á., Torres, J., Úbeda, J. and Pulido, R. (1999) 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. J. Biol. Chem. **274**, 21900–21907
19 Sharrocks, A. D., Yang, S. H. and Galanis, A. (2000) Docking domains and substrate-specificity determination for MAP kinases. Trends Biochem. Sci. **25**, 448–453
20 Pulido, R., Zúñiga, Á. and Ullrich, A. (1998) PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. EMBO J. **17**, 7337–7350
21 Buschbeck, M., Eickhoff, J., Sommer, M.N. and Ullrich, A. (2002) Phosphotyrosine-specific phosphatase PTP-SL regulates the ERK5 signaling pathway. J. Biol. Chem. **277**, 29503–29509
22 Saxena, M., Williams, S., Brockdorff, J., Gilman, J. and Mustelin, T. (1999) Inhibition of T cell signaling by mitogen-activated protein kinase-targeted hematopoietic tyrosine phosphatase (HePTP). J. Biol. Chem. **274**, 11693–11700
23 Petifford, S. M. and Herbst, R. (2000) The MAP-kinase ERK2 is a specific substrate of the protein tyrosine phosphatase HePTP. Oncogene **19**, 858–869
24 Lombroso, P. J., Murdoch, G. and Lerner, M (1991) Molecular characterization of a protein-tyrosine-phosphatase enriched in striatum. Proc. Natl. Acad. Sci. U.S.A. **88**, 7242–7246
25 Lombroso, P. J., Naegele, J. R., Sharma, E. and Lerner, M (1993) A protein tyrosine phosphatase expressed within dopaminoceptive neurons of the basal ganglia and related structures. J. Neurosci. **13**, 3064–3074
26 Hendriks, W., Schepens, J., Brugman, C., Zeeuwen, P. and Wieringa, B. (1995) A novel receptor-type protein tyrosine phosphatase with a single catalytic domain is specifically expressed in mouse brain. Biochem. J. **305**, 499–504
27 Raghunathan, A., Matthews, G. A., Lombroso, P. J. and Naegele, J. R. (1996) Transient compartmental expression of a family of protein tyrosine phosphatases in the developing striatum. Dev. Brain Res. **91**, 190–199
28 Watanabe, Y., Shiozuka, K., Ikeda, T., Hoshi, N., Hiraki, H., Suzuki, T., Hashimoto, S. and Kawashima, H. (1998) Cloning of PCPTP1-Ce encoding protein tyrosine phosphatase from the rat cerebellum and its restricted expression in Purkinje cells. Mol. Brain Res. **58**, 83–94
29 van den Maagdenberg, A. M. J. M., Bächner, D., Schepens, J. T. G., Peters, W., Fransen, J. A. M., Wieringa, B. and Hendriks, W. J. A. J. (1999) The mouse *Ptprr* gene encodes two protein tyrosine phosphatases, PTP-SL and PTPBR7, that display distinct patterns of expression during neural development. Eur. J. Neurosc. **11**, 3832–3844
30 Adachi, M., Sekiya, M., Isobe, M., Kumura, Y., Ogita, Z.-I., Hinoda, Y., Imai, K. and Yachi, A. (1992) Molecular cloning and chromosomal mapping of a human protein-tyrosine phosphatase LC-PTP. Biochem. Biophys. Res. Commun. **186**, 1607–1615
31 Zanke, B., Suzuki, H., Kishihara, K., Mizzen, L., Minden, M., Pawson, A. and Mak, T. W. (1992) Cloning and expression of an inducible lymphoid-specific, protein tyrosine phosphatase (HePTPase). Eur. J. Immunol. **22**, 235–239
32 Adachi, M., Sekiya, M., Ishino, M., Sasaki, H., Hinoda, Y., Imai, K. and Yachi, A. (1994) Induction of protein-tyrosine phosphatase LC-PTP by IL-2 in human T cells. LC-PTP is an early response gene. FEBS Lett. **338**, 47–52
33 Saxena, M., Williams, S., Gilman, J. and Mustelin, T. (1998) Negative regulation of T cell antigen receptor signal transduction by hematopoietic tyrosine phosphatase (HePTP). J. Biol. Chem. **273**, 15340–15344
34 Oh-hora, M., Ogata, M., Mori, Y., Adachi, M., Imai, K., Kosugi, A. and Hamaoka, T. (1999) Direct suppression of TCR-mediated activation of extracellular signal-regulated kinase by leukocyte protein tyrosine phosphatase, a tyrosine-specific phosphatase. J. Immunol. **163**, 1282–1288
35 Blanco-Aparicio, C., Torres, J. and Pulido, R. (1999) A novel regulatory mechanism of MAP kinases activation and nuclear translocation mediated by PKA and the PTP-SL tyrosine phosphatase. J. Cell Biol. **147**, 1129–1135
36 Saxena, M., Williams, S., Taskén, K. and Mustelin, T. (1999) Crosstalk between cAMP-dependent kinase and MAP kinase through a protein tyrosine phosphatase. Nat. Cell Biol. **1**, 305–311
37 Paul, S., Snyder, G. L., Yokakura, H., Picciotto, M. R., Nairn, A. C. and Lombroso, P. J. (2000) The Dopamine/D1 receptor mediates the phosphorylation and inactivation of the protein tyrosine phosphatase STEP via a PKA-dependent pathway. J. Neurosc. **20**, 5630–5638
38 Tanoue, T., Yamamoto, T. and Nishida, E. (2002) Modular structure of a docking surface on MAPK phosphatases. J. Biol. Chem. **277**, 22942–22949
39 Barsyte-Lovejoy, D., Galanis, A. and Sharrocks, A. D. (2002) Specificity determinants in MAPK signaling to transcription factors. J. Biol. Chem. **277**, 9896–9903

40 Dang, A., Frost, J. A. and Cobb, M. H. (1998) The MEK1 proline-rich insert is required for efficient activation of the mitogen-activated protein kinases ERK1 and ERK2 in mammalian cells. J. Biol. Chem. **273**, 19909–19913
41 Xu, B., Wilsbacher, J. L., Collisson, T. and Cobb, M. H. (1999) The N-terminal ERK-binding site of MEK1 is required for efficient feedback phosphorylation by ERK2 *in vitro* and ERK activation *in vivo*. J. Biol. Chem. **274**, 34029–34035
42 Slack, D. N., Seternes, O.-M., Gabrielsen, M. and Keyse, S. M. (2001) Distinct binding determinants for ERK2/p38α and JNK MAP kinases mediate catalytic activation and substrate selectivity of MAP kinase phosphatase-1. J. Biol. Chem. **276**, 16491–16500
43 Zhou, B., Wu, L., Shen, K., Zhang, J., Lawrence, D. S. and Zhang, Z.-Y. (2001) Multiple regions of MAP kinase phosphatase 3 are involved in its recognition and activation by ERK2. J. Biol. Chem. **276**, 6506–6515
44 Szedlacsek, S. E., Aricescu, A. R., Fulga, T. A., Renault, L. and Scheidig, A. J. (2001) Crystal structure of PTP-SL/PTPBR7 catalytic domain: implications for MAP kinase regulation. J. Mol. Biol. **311**, 557–568
45 Ogata, M., Sawada, M., Fujino, Y. and Hamaoka, T. (1995) cDNA cloning and characterization of a novel receptor-type protein tyrosine phosphatase expressed predominantly in the brain. J. Biol. Chem. **270**, 2337–2343
46 Gronda, M., Arab, S., Iafrate, B., Suzuki, H. and Zanke, B. W. (2001) Hematopoietic protein tyrosine phosphatase suppresses extracellular stimulus-regulated kinase activation. Mol. Cell. Biol. **21**, 6851–6858
47 Nguyen, T.-H., Paul, S., Xu, Y., Gurd, J. W. and Lombroso, P. J. (1999) Calcium-dependent cleavage of striatal enriched tyrosine phosphatase (STEP). J. Neurochem. **73**, 1995–2001
48 Bult, A., Zhao, F., Dirkx, R., Raghunathan, A., Solimena, M. and Lombroso, P. J. (1997) STEP: a family of brain-enriched PTPs. Alternative splicing produces transmembrane, cytosolic and truncated isoforms. Eur. J. Cell Biol. **72**, 337–344
49 Pelkey, K. A., Askalan, R., Paul, S., Kalia, L. V., Nguyen, T.-H., Pitcher, G. M., Salter, M. W. and Lombroso, P. J. (2002) Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. Neuron **34**, 1227–138
50 Nguyen, T.-H., Liu, J. and Lombroso, P. J. (2002) Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. J. Biol. Chem. **277**, 24274–24279

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