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*

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Regulated function of mitogen-activated protein (MAP) kinases involves their selective association through docking sites with both activating MAP kinase kinases and inactivating phosphatases, including dual specificity and protein-tyrosine phosphatases (PTP). Site-directed mutagenesis on the mammalian MAP kinases ERK2 and p38α identified within their C-terminal docking grooves two clusters of residues important for association with their regulatory PTPs, PTP-SL and STEP. ERK2 and p38α mutations that resembled the sevenmaker gain-of-function mutation in the Rolled D. melanogaster ERK2 homologue failed to associate with PTP-SL, were not retained in the cytosol, and were poorly inactivated by this PTP. Additional ERK2 mutations at the docking groove showed deficient association and dephosphorylation by PTP-SL, although their cytosolic retention was unaffected. Other ERK2 mutations, resembling gain-of-function mutations in the FUS3 yeast ERK2 homologue, associated to PTP-SL and were inactivated normally by this PTP. Our results demonstrate that mutations at distinct regions of the docking groove of ERK2 and p38α differentially affect their association and regulation by the PTP-SL and STEP PTPs.

The MAP1 kinase signaling pathways are highly conserved among eukaryotes and play essential roles in the regulation of the cell adaptative responses to environmental conditions and to growth and development stimuli. Regulated assembly and sequential phosphorylation of their components transduce signals through these pathways, leading to the phosphorylation, activation, and translocation to the nucleus of the effector MAP kinases, whose major examples in mammals are the ERK1/2, p38α, and c-Jun N-terminal kinase MAP kinases (1–5). The genetic analysis of nonmammalian organisms has been instrumental in the definition of the role of MAP kinases in cell development. For example, in the budding yeast Saccharomyces cerevisiae, two major MAP kinases, FUS3 and HOG1 (homologous of mammalian ERK2 and p38α, respectively), regulate mating and the response to osmotic stress, respectively (6, 7). On the other hand, the D. melanogaster Rolled MAP kinase (homologous to mammalian ERK2) is required for the correct determination of the R7 photoreceptor cell fate in the eye disc, and D-p38 MAP kinases have been involved in wing and egg development, as well as on the stress and antimicrobial responses of the fly (8–10). Genetic screenings have been performed in S. cerevisiae and D. melanogaster to identify gain-of-function FUS3 and rolled alleles on the basis of hypersensitivity to mating pheromone in the yeast or overabundance of R7-like cells in the fly. From these studies, conserved gain-of-function mutations of these MAP kinases have been identified, D317N (or D317G) in Saccharomyces and the analogous D334N (sevenmaker) in Drosophila, which result in hyperactive MAP kinases (11–14). An analogous gain-of-function mutation, D319N (mouse sequence) has also been defined in mammalian ERK2 and found to be insensitive to inactivation by phosphatases (15, 16). More recently, a second gain-of-function, sevenmaker-like mutation (D175N), has also been identified in Drosophila (14).

A major checkpoint to control the signaling through the MAP kinase pathways involves the direct regulation of both the catalytic activity and the subcellular location of the effector MAP kinases. Thus, although the enzymatic activity and the nucleocytoplasm transport of MAP kinases are independent processes, their regulation occurs simultaneously under physiological circumstances. For example, the catalytic activity of ERK2 is up-regulated by phosphorylation of their threonine and tyrosine regulatory residues by the MEK1/2 MAP kinase phosphatases (15, 16). More recently, a second gain-of-function, sevenmaker-like mutation (D175N), has also been identified in Drosophila (14).

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1 The abbreviations used are: MAP, mitogen-activated protein; ERKs, extracellular signal-regulated kinase; EGF, epidermal growth factor; GST, glutathione S-transferase; HA, hemagglutinin; KIM, kinase interaction motif; mAb, monoclonal antibody; MBP, myelin basic protein; MEK, MAP kinase/ERK kinase; MKP, MAP kinase phosphatase; PTP, protein-tyrosine phosphatase.
N-terminal region in ERK2 has also been involved in the binding to MEK1/2 (33). Remarkably, the gain-of-function mutations of ERK2 identified in yeast and Drosophila target the C-terminal docking motifs of the molecule. In addition, conserved docking sites for ERK1/2 and p38α have been identified in the noncatalytic domains of MAP kinase kinases, MKPs, and the tyrosine phosphatases PTP-1B, STEP, and HePTP/LC-PTP (29, 30, 34–38). It is postulated that the selective docking of MAP kinases with their effectors through these conserved motifs may play a major role in the definition of the specificity of the MAP kinases signal outputs (1–5). In this report, we have identified the molecular determinants at the docking grooves of ERK2 and p38α that are important for their association with the PTP-1B and STEP tyrosine phosphatases. In addition, we have investigated the biological properties of putative gain-of-function-like mutations of mammalian ERK2 and p38α and their physical and functional association with PTP-1B and STEP. Our results provide an insight into the molecular basis of the physiological regulation of the MAP kinases functions by their physical association to effectors.

MATERIALS AND METHODS

Reagents, Mutagenesis, and Purification of GST Fusion Proteins—PTP-1B, STEP, ERK2, and p38α cDNA constructs and primary and secondary antibodies used in this study have been previously described (25, 29, 34). Anti-phospho-active ERK1/2 antibody was from New England Biolabs Inc., and anti-phosphotyrosine was a mixture of 4G10 (Upstate Biotechnology Inc.) and PY20 (Santa Cruz Biotechnology Inc.) mAb. ERK2 and p38α mutations were performed by polymerase chain reaction oligonucleotide site-directed mutagenesis, and mutations were confirmed by DNA sequencing. Glutathione-Sepharose purification of the GST fusion proteins was done by standard procedures.

Cell Culture, Transfections, Preparation with GST Fusion Proteins, Immunoprecipitation, Immunoblotting, and Immunofluorescence—Human embryonic kidney 293 and simian kidney COS-7 cell lines were grown in Dulbecco’s modified Eagle’s medium containing high glucose supplemented with 5% (for COS-7 cells) or 10% (for 293 cells) heat-inactivated fetal calf serum. The cells were transfected using the DEAE-dextran method (COS-7 cells) or the calcium phosphate precipitation method (293 cells) and were harvested after 48 h of culture. HA-ERK2 or HA-p38α from transfected COS-7 or 293 cells were activated by cell treatment with EGF (Invitrogen) (5 or 30 min, 50 ng/ml) and sorbitol (Sigma) (30 or 120 min, 0.5 M), respectively. Cell lysis was performed in 50 mM Tris-Cl (pH 7.5) 150 mM NaCl, 1% Nonidet P-40, 1 mM PMSF, 1 mM dithiothreitol, 2 mM Na3VO4, and 0.3 mM NaF, 2 mM NaVO4 and 20 mM Na3PO4. For precipitation, the lysates were incubated in the presence of 1–3 μg of the distinct fusion proteins, followed by washing with 20 mM HEPES (pH 7.5), 150 mM NaCl, 10 mM Na3PO4, 0.1% Triton X-100, and 10% glycerol. Immunoprecipitation, immunoblotting, and immunofluorescence were done as described (29, 34).

In Vitro Kinase and Phosphatase Assays—For in vitro kinase assays, MBP (2 μg) was mixed with immune complex pellets containing EGF-activated HA-ERK2 or sorbitol-activated HA-p38α as described (29) and were incubated at room temperature for 20 min with constant shaking in kinase reaction buffer (20 mM HEPES, pH 7.5, 10 mM MgCl2, 1 mM dithiothreitol, 2 mM Na3VO4, and 0.3 μM ATP) in the presence of 2 μCi of [γ-32P]ATP. The reactions were stopped by adding SDS sample buffer and boiling, followed by SDS-PAGE and autoradiography. For quantification, the MBP substrate proteins were excised from the gel and counted in a scintillation counter. Statistics for Figs. 2 and 6 were determined using the unpaired Student’s t test. In vitro phosphatase assays were performed using activated HA-ERK2 or HA-p38α pellets, as above. The reactions were performed by adding the indicated amounts of GST-PTP-P-SL to the pellets, followed by incubation at 37 °C, during 30 min, in phosphatase reaction buffer (25 mM HEPES, pH 7.3, 5 mM EDTA, and 10 mM dithiothreitol) (final volume, 40 μl). The reactions were stopped by adding SDS sample buffer and boiling, followed by SDS-PAGE and immunoblot with anti-phospho ERK1/2 (for HA-ERK2) or anti-phosphotyrosine (for HA-p38α) antibodies.

RESULTS

Identification of Gain-of-Function Mutations in Mammalian ERK2—Several gain-of-function mutations have been described in the genes encoding the D. melanogaster Rolled protein and the S. cerevisiae FUS3 protein that are homologous to ERK2. To test the effect of these mutations on the mammalian ERK2 protein, analogous mutations were generated on the background of the mouse ERK2 cDNA (14, 39); these mutations included the amino acid substitutions E58Q, D160N, V171L, H230N, and D319N (amino acid numbering is according to Ref. 40; Table I). As controls, two loss-of-function mutations were also generated on ERK2, namely the amino acid substitutions K52A, which affects the ATP binding site of the kinase, and T183E, which affects the regulatory phospho-sites of the kinase. The activation properties of the distinct ERK2 mutations were determined on EGF-activated 293 cells by the degree of phosphorylation of their regulatory Thr183 and Tyr185 residues, as monitored by immunoblot using a phospho-specific anti-ERK1/2 mAb (Fig. 1), as well as by in vitro kinase activity toward MBP after ERK2 immunoprecipitation (Fig. 2). As shown in Figs. 1A and 2A, the mutations E58Q, D160N, and H230N were very similar in these assays to the wild type ERK2. On the other hand, the V171L mutation, like the kinase-dead K52A mutation, displayed very low kinase activity, although its capability to be phosphorylated at Thr183 and Tyr185 upon activation was unaffected. The mutation T183E was defective in phosphorylation at the regulatory sites and also showed low kinase activity. Interestingly, the only mutation that conferred an activated phenotype to mouse ERK2, in both terms of kinase activity and hyperphosphorylation of the regulatory sites, was the mutation D319N. Thus, in mammalian ERK2, the D319N mutation, but not the mutations E58Q, D160N, V171L, or H230N, seems to confer a gain-of-function-like phenotype to this molecule.

The Asp160 and Asp160c ERK2 residues lie within the conserved docking motifs of the kinase, which mediates binding of ERK2 to their effectors (Refs. 30 and 31; see a depiction of the motifs in Fig. 7). Next, additional amino acid substitutions of residues contained in these docking motifs were generated, and mutations were also tested for their response to activation. Asp319 was substituted with Glu (D319E) and with Ala (D319A); and substitutions of Asp319E with Asn (D319N), Pro321E with Ala (P321A), Glu324E with Gln (E324Q), and Pro326E with Ala (P326A) were also made. As shown in Figs. 1B and 2B, the mutations P317A, P321A, E324Q, and P326A did not significantly change the phosphorylation of the regulatory sites or the kinase activity of ERK2 upon activation. On the other hand, the mutations D316N, D319E, and D319A showed an increase in their phospho-active content (Fig. 1B) and were found to be more sensitive to activation than the ERK2 wild type (Fig. 2B). Additional substitutions to Ala and Glu of the Asp160 residue were also made (mutations D160A and D160E), as well as substitutions of Leu161 with Ala (L161A) and Asp165 with Ala (D165A), and tested for their phospho-active content upon EGF activation. As illustrated in Fig. 1B and Table II, the phospho-active content of these mutations was indistinguishable with that...
shown by the wild type ERK2. The mutation D165A, as expected from the role of Asp 165 as a catalytic residue (43), rendered a catalytically inactive enzyme (Fig. 2 and Table II). Finally, a p38 mutation, D316N (mouse sequence; Ref. 44; see Fig. 7), analogous to the ERK2 D319N mutation, was generated and tested on sorbitol-activated COS-7 cells for phosphotyrosine content and enzymatic activity. As shown, the phosphotyrosine content and kinase activity of the p38 D316N mutation was similar to that displayed by the wild type p38 (Fig. 3). Together, these results indicate that the Asp319 and, in a lesser extent, the Asp 316, are residues of importance for the gain-of-function-like phenotype of ERK2; however, the alteration of the p38 Asp316 residue is not sufficient to increase the activation of this MAP kinase.

Mutations at the Docking Groove of MAP Kinases, Including Gain-of-Function Mutations, Fail to Interact with the Tyrosine Phosphatases PTP-SL and STEP—PTP-SL associates with ERK2 and p38/H9251 through a kinase interaction motif (KIM) and retains these kinases in the cytoplasm in an inactive state by dephosphorylating their tyrosine regulatory residues (25, 29, 34). To test the effect of the ERK2 mutations on the association with PTP-SL, the distinct mutations were overexpressed on 293 cells and precipitated using a GST-PTP-SL fusion protein containing the KIM. The association of PTP-SL with the mutations K52A, E58Q, D160N, D160A, E324Q, and P326A was indistinguishable with that shown toward the wild type ERK2 (Fig. 4A). On the other hand, the association of PTP-SL with the mutations D160N, D160A,
D319N, D319E, and D319A was severely impaired, whereas the association with the mutations D160E, L161A, D316N, and P321A was consistently reduced (Fig. 4A). The PTP-SL-related PTP, STEP, possesses a conserved KIM motif and also binds to ERK2 (34). Hence, the association of STEP with ERK2 mutations was also assessed. As shown, the ERK2 D160N and D139N mutations also bound deficiently to a GST-STEP fusion protein (Fig. 4B). Finally, the p38α D316N mutation (analogous to the ERK2 D319N mutation) was tested for association with PTP-SL and STEP. Consistent with the above results, the p38α D316N mutation also resulted in the impairment of the association with both PTP-SL and STEP (Fig. 4C).

To test the capability of PTP-SL to retain the distinct MAP kinases mutations outside of the nucleus, immunofluorescence analysis was performed on COS-7 cells expressing the MAP kinases mutations alone or in the presence of cytosolic PTP-SL. Upon coexpression with PTP-SL, the Asp319 ERK2 gain-of-function-like mutations, as well as the p38α D316N mutation, were retained in the cytosol by PTP-SL and excluded from the nucleus (summarized in Table II). Thus, the Asp319 ERK2 gain-of-function-like mutations, as well as the p38α D316N mutation, fail to be retained in the cytosol by PTP-SL.

Dephosphorylation and Inactivation of MAP Kinase Gain-of-Function-like Mutations by PTP-SL Is Impaired—The effect of
the PTP-SL tyrosine phosphatase activity on the inactivation of the gain-of-function-like MAP kinases mutations was investigated by in vitro phosphatase assays using GST-PTP-SL fusion proteins and activated ERK2 or p38α wild type and mutations, followed by SDS-PAGE analysis and immunoblot with anti-phospho-active ERK1/2 or anti-phosphotyrosine mAb (Fig. 5). As shown, GST-PTP-SL dephosphorylated efficiently both ERK2 and p38α wild type, whereas the dephosphorylation of ERK2 D316N mutations was impaired, indicating that the defective binding to these MAP kinases hampers their dephosphorylation by PTP-SL. The in vivo inactivation of ERK2 wild type and ERK2 mutations by PTP-SL was also assessed by coexpression experiments in EGF-activated 293 cells, followed by immunoprecipitation of the recombinant ERK2 molecules and analysis of their in vitro kinase activity toward MBP (Fig. 6). In keeping with the results obtained in the in vitro phosphatase assays, the inactivation of the D319N gain-of-function-like mutation in intact cells by cytosolic PTP-SL (residues 147–549) was less pronounced than the inactivation shown by the ERK2 wild type molecule (56% inhibition versus 81% inhibition) or other ERK2 mutation, such as H230N (Fig. 6). Together, these results indicate that the defective association of the tyrosine phosphatase PTP-SL with ERK2 D319N and p38α D316N mutations impairs the retention and inactivation in the cytosol of these MAP kinases by PTP-SL.

**DISCUSSION**

Constitutively active forms of signal-regulated protein kinases may play a determinant role in the unbalancing of pivotal cell signaling pathways, leading to abnormal cell growth- and development-related diseases. Although constitutively active variants of the MAP kinases ERK1/2 have not been identified, gain-of-function mutations have been defined on the *S. cerevisiae FUS3* gene, homologous to the mammalian ERK2 gene, that confer hypersensitivity to the yeast mating pheromone that activates the FUS3 pathway. FUS3 gain-of-function dominant alleles include the mutations D48N, I161L, D227N, or p38α D316N mutations was impaired, indicating that the defective binding to these MAP kinases hampers their dephosphorylation by PTP-SL. The in vivo inactivation of ERK2 wild type and ERK2 mutations by PTP-SL was assessed by coexpression experiments in EGF-activated 293 cells, followed by immunoprecipitation of the recombinant ERK2 molecules and analysis of their in vitro kinase activity toward MBP (Fig. 6). In keeping with the results obtained in the in vitro phosphatase assays, the inactivation of the D319N gain-of-function-like mutation in intact cells by cytosolic PTP-SL (residues 147–549) was less pronounced than the inactivation shown by the ERK2 wild type molecule (56% inhibition versus 81% inhibition) or other ERK2 mutation, such as H230N (Fig. 6). Together, these results indicate that the defective association of the tyrosine phosphatase PTP-SL with ERK2 D319N and p38α D316N mutations impairs the retention and inactivation in the cytosol of these MAP kinases by PTP-SL.

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studies. Furthermore, the ERK2 mutation V171L, although properly phosphorylated in the ERK2 regulatory residues, displayed very low kinase activity, indicating that this mutation behaves as a loss-of-function ERK2 allele in mammals, likely by influencing the folding of the nearby activation loop, as has been proposed for other ERK2 loss-of-function mutations (46).

Variations on the physiological regulation of the MAP kinases activities may exist between yeast and mammalian cells that account for these differences in gain-of-function-related mutations. As previously reported by others (15, 16), the mouse ERK2 D319N mutation (analogous to the D334N sevenmaker mutation of the fly) conferred a gain-of-function-like phenotype to the molecule. In addition, our mutagenesis analysis revealed that other amino acid substitutions of the Asp319 ERK2 residue, such as the conservative and nonconservative mutations D319E and D319A, as well as an amino acid substitution of the Asp316 residue (D316N mutation), also rendered a modest gain-of-function-like phenotype.

A common docking site has been identified in the C-terminal domain of MAP kinases that binds substrates, activators, and regulators (Ref. 30 and Fig. 7). This docking motif includes the Asp316 and Asp319 residues important for the gain-of-function ERK2 phenotype, and mutations at these residues abrogate the association of ERK2 with its effectors. We have found that the Asp319 residue of ERK2 (and to a lesser extent, the Asp316 and the Pro 321 ERK2 residues), as well as the Asp 316 residue of p38/H9251, is important for binding to the tyrosine phosphatases PTP-SL and STEP. Interestingly, both conservative and non-conservative substitutions at the Asp319 residue of ERK2 abrogated PTP-SL binding, indicating that this residue is necessary for binding to PTP-SL. In addition, we have identified the Asp160, as well as the Leu161, as important residues mediating ERK2 binding to PTP-SL and STEP. The alignment of the amino acid sequences of MAP kinase family members, adjacent to the Asp319 and Asp160 residues (Fig. 7) shows that the Asp319 residue is conserved on several MAP kinases, including those (ERK2 and p38α) known to bind to PTP-SL but not on those (c-Jun N-terminal kinase isoforms) that do not associate with this PTP. On the other hand, the ERK2 Asp160 residue is substituted by Glu 163 in p38/H9251, and we show that the ERK2 D160E mutation is permissive for the association with PTP-SL.

**FIG. 5.** Dephosphorylation of MAP kinases wild type and mutations by PTP-SL in vitro. A, 293 cells were transfected with pCDNA3-HA-ERK2 wild type (wt) or D160N or D319N mutations. After 48 h, the cells were activated with 50 ng/ml EGF for 5 min, and HA-ERK2 was immunoprecipitated (i.p.) from cell lysates with the anti-HA 12CA5 mAb. The immune complexes were subjected to in vitro phosphatase assays in the presence of increasing amounts of GST-PTP-SL 147–549, as indicated. The samples were resolved on 10% SDS-PAGE, and the phospho-active content of HA-ERK2 was measured by immunoblot with an anti-phospho-active-ERK1/2 antibody. B, COS-7 cells were transfected with pECE-HA-p38MAPK (p38α) wild type (wt) or D316N mutation. After 48 h, the cells were activated with 0.5 M sorbitol for 30 min, and HA-p38α was immunoprecipitated from cell lysates with the anti-HA 12CA5 mAb. Immune complexes were subjected to in vitro phosphatase assays as in A. The samples were resolved on 10% SDS-PAGE, and the phospho-active-content of HA-p38α was measured by immunoblot with anti-phosphotyrosine antibodies. In the right panels, the expression of the distinct MAP kinases is shown, as monitored by immunoblot analysis of total lysate samples with the anti-HA mAb.

**FIG. 6.** Kinase activity of ERK2 wild type and D319N mutation in intact cells in the presence of PTP-SL. A and B, 293 cells were transfected with pCDNA3-HA-ERK2 wild type (wt) or H230N or D319N mutation plus empty vector (mock) or plus pRK5-PTP-SL 147–549. After 48 h, the cells were lysed and treated with 50 ng/ml EGF for 5 min, followed by cell lysis, HA-ERK2 immunoprecipitation with anti-HA mAb, and in vitro kinase assays in the presence of MBP, as in Fig. 2. The data are presented as the percentages of HA-ERK2 activity with respect to that shown by cells transfected with pCDNA3-HA-ERK2 wild type plus empty vector (mock; 100%). The values represent the means of four separate experiments ± S.D. Significant differences with respect to wild type are denoted with an asterisk. *, p < 0.05. In all of the experiments, the controls of expression of PTP-SL and ERK2 wild type mutations were run in parallel.
MAP Kinase Molecular Determinants for Association to PTP-SL

Fig. 7. Alignment of the docking motifs important for the association with PTP-SL and/or STEP on ERK2 and p38α, together with the equivalent residues of c-Jun N-terminal kinase 1 (JNK1). The ERK2 and p38α residues important for the association with PTP-SL and/or STEP are underlined. Amino acids are indicated using one-letter codes. The numbers in brackets correspond to the amino acid numbering of the MAP kinases (Refs. 40, 44, and 47). It is conceivable that modifications of the Asp319 residue affect more dramatically the association of ERK2 with some of its effectors than modifications of the Asp160 residue, which could serve as a secondary docking element conferring additional specificity to the interaction. These could explain the milder gain-of-function phenotype observed in the Drosophila D175N mutation, compared with the D334N mutation (14), as well as the lack of hyperactivity observed in the mouse D160N mutation (this study; see also below). In this regard, we have observed that the effect of the D160N ERK2 mutation on the association of ERK2 with PTP-SL and STEP is consistently less strong than that of the D319N mutation (Fig. 4B). A recent report has identified p38α residues (ED motif), adjacent to the Glu163 residue that are involved in the docking interaction between p38α and its upstream activator MAPKAPK-2/3/3pk; together with the C-terminal common docking motif, these residues would generate the major determinants of specificity that are present in the MAP kinase common docking groove (31). Our results are in concordance with this model and further emphasize the importance of at least two clusters of residues at the MAP kinases docking grooves in the specific binding to both activators and inactivators. Deficient PTP-SL binding to the Asp319 ERK2 mutations impaired tyrosine dephosphorylation and cytosolic retention and inactivation of ERK2 by PTP-SL, and a correlation was found between the degree of the gain-of-function phenotype in the distinct Asp319 ERK2 mutations and the lack of association with PTP-SL; however, the Asp160 ERK2 mutations, as well as the p38α D316N mutation, associated poorly to both PTP-SL and STEP and were weakly inactivated by PTP-SL but did not show a gain-of-function-like phenotype (Table II). Thus, other MAP kinases-specific factors, in addition to the impairment of their association with inactivating phosphatases, might exist that account for the final gain-of-function phenotypes of MAP kinases mutations. In this regard, hyperactive HOG1 mutations have been identified that affect residues close to the ED and C-terminal common docking motifs (48), and a discordance between the binding affinity of distinct MAP kinases to MKP-2 and MAP kinase inactivation has been documented (49).

The interaction of PTP-SL with ERK1/2 is mediated, at the level of PTP-SL, through a KIM located in the regulatory domain of this PTP, and major determinants for ERK1/2 recognition of the KIM include two positively charged Arg residues (29). Thus, electrostatic interactions between the acidic Asp residues at the docking groove of ERK2 (Asp160, Asp166, and Asp319 in the mouse sequence) and basic residues at the KIM of PTP-SL (Arg292 and Arg328 in the mouse sequence), as well as hydrophobic interactions between these regions (Refs. 29 and 32 and this report), are likely to stabilize the ERK1/2-PTP-SL complexes in vivo. The crystal structure of the catalytic domain of PTP-SL (lacking the MAP kinase interaction domain) has been recently solved (50); further insight into the characterization of the ERK1/2-PTP-SL interaction will have to wait until the solving of the crystal structure of the ERK1/2-PTP-SL complexes. Remarkably, phosphorylation of the KIMs of PTP-SL and HePTP/LC-PTP by the cAMP-dependent protein kinase disrupts the association of these tyrosine phosphatases with ERK1/2 and p38α (25, 28), raising the possibility that cAMP-dependent protein kinase-mediated hyperphosphorylation of these KIMs could indirectly confer a gain-of-function-like phenotype to ERK1/2 and p38α in certain cell types. Whether some post-transcriptional or post-translational regulatory modification of the MAP kinases could affect the capability of their docking motifs to discriminate between distinct effectors remains uncertain. In this regard, an alternatively spliced variant of ERK1 has been identified that contains an insert that disrupts the ERK1 C-terminal common docking motif, hampering the association of ERK1 with both MEK1 and PTP-SL (51, 52). Also, the phosphorylation of the ERK2 activation loop has been shown to alter the conformation of the ERK2 C-terminal extension that contains the common docking motif (53), and a tyrosine phosphorylation-dependent association of ERK2 to the tyrosine phosphatase HePTP/LC-PTP has been documented (54). It is likely that distinct levels of regulation exist that modulate the affinity of the different MAP kinases and their isoforms toward their cognate activating and inactivating effectors.

The expression of PTP-SL and STEP is developmentally regulated in the brain (55–59), and PTP-SL-isoform variants are also detected in the uterus, intestine, and placenta (60). In addition, the PTP-SL-related tyrosine phosphatase HePTP/LC-PTP is expressed on cells of hematopoietic lineage (61, 62). Thus, in tissues that express these PTPs, the impairment of their association with ERK2 could confer an altered phenotype associated with hyperactivity of the ERK1/2 MAP kinase pathway. For example, a PTP-SL-related tyrosine phosphatase from D. melanogaster, PTE-ER, is involved in the R7 photoreceptor differentiation of the fly, and the deficient association between PTP-ER and Rolled partially accounts for the Drosophila sevenmaker mutant phenotype (63). Also, thymocyte developing is affected in transgenic mice expressing the ERK2 D319N gain-of-function mutation (64), likely because of the impairment of ERK2 regulation by HePTP/LC-PTP or other effectors (35). Additional work, including specific targeting of the PTP-SL and STEP genes on mice, will be necessary to ascertain the role of these PTPs in mammalian tissue development. No homologous of PTP-SL are found on the yeast S. cerevisiae; instead, two distantly related tyrosine phosphatases, PTP2 and PTP3, down-regulate the activity of HOG1 (homologous of mammalian p38α) and FUS3 in yeast (65–67). In addition, MAP kinase-specific dual specificity phosphatases are also major regulators of mammalian MAP kinases activities (22). Interestingly, the D317G and D317N gain-of-function FUS3 mutations also fail to interact with PTP3 (68), and the D316N and D319N mammalian MAP kinases gain-of-function mutations bind defectively to the dual specificity phosphatases MKP-1 and MKP-3 (26, 30, 69, 70). Thus, the recognition of a common motif on the MAP kinases has been preserved along evolution, albeit with the divergence of effectors that bind to the motif. An important consequence of the docking-dependent binding of MAP kinases to activators and inactivators is their confinement to specific subcellular compartments, where the effectors are located. In particular, PTP-SL and HePTP/LC-PTP retain to ERK1/2 and p38α in the cytoplasm and prevent their translocation to the nucleus (25, 28, 29). Also, PTP2 and PTP3 differentially modulate the subcellular localization of HOG1 by direct binding to the kinase (71). Interestingly, the cytosolic retention mediated by PTP-SL was prevented in the Asp319 ERK2 mutations and in the p38α D316N mutation but not in all of the other mutations tested in this study, including the Asp160 mutations, suggesting that distinct regions at the docking groove of the MAP kinases are differentially involved.
in distinct MAP kinases functions. Also, the possibility exists that major modifications in the avidity toward PTP-SL are necessary to bypass the cytosolic retention of MAP kinases by this phosphatase. Our results suggest that the selective recognition of different motifs at the MAP kinases docking grooves by the distinct MAP kinases effectors might be determinant to finely tune the adaptive cell responses under the control of the MAP kinase signaling pathways.

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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

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