Inhibition of T Cell Signaling by Mitogen-activated Protein Kinase-targeted Hematopoietic Tyrosine Phosphatase (HePTP)*

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Activation of T lymphocytes to produce cytokines is regulated by the counterbalance of protein-tyrosine kinases and protein-tyrosine phosphatases, many of which have a high degree of substrate specificity because of physical association with their targets. Overexpression of hematopoietic protein-tyrosine phosphatase (HePTP) results in suppression of T lymphocyte activation as measured by T cell antigen receptor-induced activation of transcription factors binding to the 5′ promoter of the interleukin-2 gene. Efforts to pinpoint the exact site of action and specificity of HePTP in the signaling cascade revealed that HePTP acts directly on the mitogen-activated protein (MAP) kinases Erk1 and 2 and consequently reduces the magnitude and duration of their catalytic activation in intact T cells. In contrast, HePTP had no effects on N-terminal c-Jun kinase or on events upstream of the MAP kinases. The specificity of HePTP correlated with its physical association through its noncatalytic N terminus with Erk and another MAP kinase, p38, but not Jnk or other proteins. We propose that HePTP plays a negative role in antigen receptor signaling by specifically regulating MAP kinases in the cytosol and at early time points of T cell activation before the activation-induced expression of nuclear dual-specific MAP kinase phosphatases.

Phosphorylation of proteins on tyrosyl residues is an important mechanism for many signal transduction pathways controlling cell growth, differentiation, and development (1–3). Although the phosphotyrosine (Tyr(P)) content of cellular proteins is the net result of the opposing effects of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPases),1 most investigators have concentrated on the protein-tyrosine kinases, and considerably less is currently known about the PTPases. The hematopoietic protein-tyrosine phosphatase (HePTP) was cloned from human T lymphocytes (4, 5), and it is expressed in thymus, spleen, and in most leukemic cell lines examined, including Jurkat T leukemia cells (6). HePTP belongs to a subgroup of PTPases with two other members, STEP (7) and PCPTP1 (8, 9). In contrast to HePTP, the other two enzymes are not expressed in hematopoietic cells but mainly in the central nervous system: STEP mainly in striatum (7), and PCPTP1 particularly in cerebellum (8). Like PCPTP1 and the 46-kDa isoform of STEP, HePTP consists of a single PTPase domain that occupies the C-terminal 3/4 of the enzyme and is preceded by an ~80-amino-acid noncatalytic N terminus. As might be expected from the lack of putative transmembrane sequences or other recognizable targeting motifs, immunofluorescence microscopy indicates that HePTP is located exclusively in the cytosol in RBL mast cells (10) and Jurkat T cells.2

The biological function of HePTP has remained elusive. A potential role in cell proliferation or differentiation was suggested by the finding that the HePTP gene is located at 1q32 (11) on the long arm of chromosome 1, which is often found in extra copies (trisomy) in bone marrow cells from patients with myelodysplastic syndrome (12, 13), a disease characterized by reduced hematopoiesis. In contrast, deletions of 1q32 have been reported in non-Hodkin lymphomas and chronic lymphoproliferative disorders (14). Thus, these findings suggest that excess HePTP may correlate with reduced proliferation (in myelodysplasia) and loss of HePTP with increased cell proliferation and/or survival. Amplification and overexpression of HePTP has also been reported in a case of myelogenous leukemia (11). A connection with lymphoid proliferation is also supported by the finding that the HePTP gene is transcriptionally activated in T cells treated with interleukin-2 (15). Although mRNA levels increased severalfold upon stimulation of normal mouse lymphocytes with phytohemagglutinin, lipopolysaccharide, concanavalin A, or anti-CD3 (4), the HePTP protein was present in resting cells, and its amount increased only moderately.

Finally, HePTP has been reported to become phosphorylated on tyrosine in RBL-2H3 mast cells stimulated through their FceRI (10).

We recently reported that transient expression of HePTP in T cells caused a clear reduction in antigen receptor-induced transcriptional activation of a reporter gene driven by a nuclear factor of activated T cells (NFAT)/activator protein-1 (AP-1) element taken from the interleukin-2 gene promoter (6). In contrast, a catalytically inactive C270S mutant of HePTP had no effect, suggesting that the PTPase activity of HePTP was required for inhibition. We have continued this work and have found that HePTP inhibits NFAT/AP-1 activation, and thereby the entire 5′ interleukin-2 promoter, by dephosphorylating the Erk MAP kinase. Specificity for this substrate is the result of a physical association between HePTP and Erk mediated by the noncatalytic N terminus of HePTP. This region also bound the p38 MAP kinase but not the N-terminal c-Jun ki-

1 The abbreviations used are: PTPase, protein-tyrosine phosphatase; HePTP, hematopoietic protein-tyrosine phosphatase; NFAT, nuclear factor of activated T cells; AP-1, activator protein-1; MAP, mitogen-activated protein; GST, glutathione S-transferase; TPCK, l-tosylamido-2-phenylethyl chloromethyl ketone; MBP, myelin basic protein; HA, hemagglutinin; mAb, monoclonal antibody.

2 M. Saxena, S. Williams, and T. Mustelin, unpublished observation.
nases Jnk1 and Jnk2 or other signaling proteins. We also show that HePTP is a substrate for Erk and p38, and we identify the sites of phosphorylation and effects of phosphorylation on the association between HePTP and these kinases.

MATERIALS AND METHODS

Reagents—Most antibodies and plasmids were as described before (6). The HePTP fragments ΔN (amino acids 92–339), N ter (amino acids 1–92), N1 (amino acids 1–40), N2 (amino acids 10–55), and N3 (amino acids 92–167) were expressed in Escherichia coli, purified, and used as indicated. The HePTP fragment NN (amino acids 92–339) was expressed in mammalian cells and used as indicated.
FIG. 2. Direct inhibition of Erk by HePTP. a, anti-Tyr(P) (anti-P-Tyr) immunoblot of kinase-inactive GST-Erk1 phosphorylated by active Mek in the presence of 1 mM ATP for 10 min and then incubated with active HePTP for the indicated times. PSer, Ser(P). b, phosphoamino acid analysis (PAA) of kinase-inactive GST-Erk1 phosphorylated by Mek in the presence of $[^32P]ATP$ for 10 min and then incubated with active HePTP for the indicated times. c, phosphorylation of MBP during a 30-min assay by 10 ng of recombinant Erk2 in the presence of 100 ng of GST-HePTP, GST, GST-HePTP-C270S, or GST-SHP2. d, similar assay in the presence of the indicated amounts of GST-HePTP or controls. Similar results were obtained in at least three independent experiments each.

RESULTS

HePTP Reduces AP-1-dependent Gene Activation—A crucial step in the initiation of an immune response is the production of cytokines by T cells challenged with properly presented antigen (22). T cell antigen receptor-induced activation of the interleukin-2 gene is the result of the coordinated action of several transcription factors (23, 24), including a trimeric complex consisting of a NFAT family protein (25) and an AP-1 dimer (26, 27) of Fos and Jun family proteins, with some assistance from octamer-binding proteins (Oct) and NF-kB. Although these proteins operate in a synergistic manner, they can be measured separately using their target DNA elements coupled to a reporter gene.

When HePTP was expressed in Jurkat T cells together with a luciferase reporter gene under the control of the 5′ interleukin-2 promoter, the activation of this reporter was reduced to 58.1 ± 9.3% (n = 9). In contrast, expression of the catalytically inactive mutant HePTP-C270S or two other PTPases, SHP2 and TCPTP, did not affect the activation of the reporter gene although being expressed at similar levels. The inhibition by HePTP was not as pronounced as its effect on a reporter gene driven by a subregion of the interleukin-2 promoter, one of the NFAT/AP-1 response elements, which was inhibited by more than 80% (6). This discrepancy is probably explained by the minimal effects of HePTP on luciferase reporter constructs driven by Oct or NF-kB (not shown). Together, these results further support the notion that HePTP may play a role in antigen-induced T cell activation and suggests that HePTP dephosphorylates some signaling molecule in the pathways that lead from the antigen receptor to the activation of the NFAT/AP-1 response elements in the interleukin-2 gene. Therefore, we decided to examine several receptor-proximal signaling steps upstream of this transcription factor complex.

HePTP Inhibits Erk but Not Jnk—First, we measured the two types of MAP kinases known to be involved in AP-1 activation in T cells, Erk and Jnk (27, 28). Jurkat T cells were transiently co-transfected with epitope-tagged Erk2 together with HePTP, HePTP-C270S, TCPTP, or SHP2. After stimulation of the cells, the catalytic activity of the kinases was measured. These experiments consistently revealed that the antigen receptor-induced activation of Erk was significantly reduced by HePTP. In cells stimulated for different periods of time with anti-CD3ε (Fig. 1a), the inhibition by HePTP was seen at all time points but most clearly at 2–5 min, coinciding with the peak of Erk activity. Similar results were also obtained in Zap-70-deficient P116 cells (18) co-transfected with Zap-70, except that the effects of both wild-type HePTP and HePTP-C270S were even stronger (Fig. 1b), presumably because of the lower levels of endogenous HePTP in these cells.2 HePTP also
blotted interleukin-2 promoter activation more efficiently in these cells (not shown). Thus, the effect of transfected HePTP correlates inversely with the amount of endogenous HePTP, suggesting that the observed inhibition of MAP kinase represents the normal function of HePTP.

In agreement with the notion that HePTP inhibits the activation of the interleukin-2 gene by reducing the magnitude and duration of Erk activation, we observed that expression of Erk2 plus an activated mutant (29) of Mek, the upstream activator of Erk, augmented interleukin-2 promoter activation and increased its sensitivity to HePTP (Fig. 1e). As a control, coexpression of activated Mkk6 plus p38 kinases did not affect the interleukin-2 reporter or its inhibition by HePTP (not shown). Furthermore, the activation of another gene known to be up-regulated, in part, through Erk-mediated phosphorylation of the Elk-1 transcription factor (30), c-fos, was also increased in T cells overexpressing HePTP but not in cells expressing the inactive HePTP-C270S (not shown). Together, all these results indicate that HePTP reduces the activation of Erk in vivo. This notion is supported by the opposite effect of catalytically inactive HePTP-C270S in the same assays.

**HePTP Inhibits Endogenous Erk but Not Upstream Events**—To ascertain that the observed inhibition by HePTP was not limited to exogenous transfected MAP kinase, we next utilized the JCaM1 cell line, which is unresponsive to T cell antigen receptor stimulation because of lack of Lck kinase (17). Transient expression of Lck (or Syk) restores responsiveness (20). JCaM1 cells were transfected with Lck together with HePTP, HePTP-C270S, or control PTPases and used for analysis of antigen receptor-induced appearance of activated and phosphorylated MAP kinases by immunoblotting with activation-specific antibodies. Fig. 1d shows that HePTP reduced the appearance of phospho-Erk1 and -2, while at the same time and in the same cells, not having any effects on activation of Mek or the receptor-induced tyrosine phosphorylation of cellular proteins (Fig. 1e), including the ζ chain of the T cell antigen receptor and Zap-70 (verified by immunoprecipitation). Additional immunoblots of the same samples showed that the expression of endogenous Erk and Mek, as well as transfected Lck and PTPases, was equal in all samples. On longer exposures, it was also noted that the catalytically inactive HePTP-C270S elevated the amount of phospho-Erk in the resting cells. Thus, HePTP readily inhibits endogenous MAP kinase, and the catalytically inactive HePTP-C270S acts as a dominant negative reducing the action of endogenous HePTP.

**HePTP Also Inhibits Phorbol Ester-induced Erk Activation**—The MAP kinase pathway can also be efficiently activated by phorbol esters, which bypass all receptor-induced proximal tyrosine phosphorylation events by activating the Raf kinase through protein kinase C (31, 32). When tagged Erk2 was expressed in JCaM1 cells together with HePTP and the cells were stimulated with 20 nM phorbol myristate acetate, the activation of the MAP kinase was profoundly inhibited compared with cells expressing Erk2 alone (Fig. 1f). In contrast to cells stimulated by anti-CD3 (lanes 2, 4, and 6), phorbol ester-induced MAP kinase activation did not require expression of Lck. Control blots confirmed that the immunoprecipitates contained equal amounts of Erk2 and that equal amounts of HePTP and Lck were expressed in the transfectants (Fig. 1f, lower panels). Because HePTP is specific for Tyr(P), this result supports the conclusion that HePTP does not block MAP kinase activation by dephosphorylating receptor-proximal tyrosine
phosphorylation events. Rather, these data suggest a more direct effect on Erk.

Direct Effect of HePTP on Erk—Having found that HePTP had no effects on the phosphorylation of Mek in the same cells where Erk phosphorylation was reduced, we asked if HePTP acts directly on phospho-Erk. First, we phosphorylated recombinant kinase-inactive Erk1 at the activation loop threonine and tyrosine residues using active recombinant Mek and treated the resulting phospho-Erk with recombinant HePTP at 37 °C. As shown in Fig. 2a, the Tyr(P) content of Erk decreased detectably within 10–30 s and was very low by 1–5 min. Phosphoamino acid analysis of similarly treated Erk1 phosphorylated by Mek in the presence of [γ-32P]ATP revealed that HePTP caused a rapid loss of phosphate from tyrosine without hydrolyzing phosphothreonine (Fig. 2b). Furthermore, a brief incubation at 37 °C of active recombinant Erk with HePTP resulted in a total loss of its kinase activity (Fig. 2c). In contrast, GST, HePTP-C270S, or SHP2 had no effects. Using 10 ng (15 fmol) of HePTP had insignificant (<10%) effects even during a 30-min assay (Fig. 2d). This result suggests that HePTP acts on Erk at a 1:1 stoichiometry, perhaps by binding and primarily dephosphorylating only the bound kinase molecules.

Physical Association between HePTP and MAP Kinase—To directly test the possibility that HePTP binds Erk, we used GST fusion proteins of HePTP, HePTP-C270S, or SHP2 and found that both HePTP and HePTP-C270S readily bound Erk (Fig. 3a) in lysates of resting T cells or cells treated with pervanadate (to maximize tyrosine phosphorylation (33)). The precipitates also reacted strongly with antibodies to another MAP kinase, p38. In contrast, neither Jnk1 nor Jnk2 bound, and control GST, GST-SHP2 (Fig. 3a), or GST-TCPTP (not shown) did not precipitate any of these kinases. Immunoblotting with antibodies to the 36–38-kDa LAT (34) or to other signaling molecules gave negative results. The Erk and p38 that bound active HePTP did not contain Tyr(P) and were catalytically inactive. In contrast, Erk and p38 bound to inactive HePTP-C270S from pervanadate-treated cells were phosphorylated on tyrosine and enzymatically highly active against myelin basic protein or GST-ATF2, a preferred substrate of HA-tagged p38 kinase (Fig. 3b) and primarily dephosphorylating only the bound kinase molecules.

HePTP Also Inhibits Activation of Jnk—The finding that HePTP associates with both Erk and p38, but not with Jnk, prompted us to study the direct effects of HePTP on the kinase activity of p38 in vitro and on the activation of p38. p38 was not detected in intact cells. First, we used 10 ng of recombinant active p38 kinase, added 100 ng of GST-HePTP, GST-HePTP-C270S, control GST, or GST-SHP2, and incubated the samples with [γ-32P]ATP for 30 min. As shown in Fig. 4a, active HePTP inhibited p38 profoundly, whereas the inactive C270S mutant, GST, and the control SHP2 PTase lacked effects. Next, we co-transfected HA-tagged p38 or Jnk2 with empty vector, with HePTP, or with HePTP-C270S. Two days later, the cells were stimulated with a combination of anti-CD3 and anti-CD28 mAbs (as neither mAb alone activates these kinases). After 15–20 min at 37 °C, the cells were lysed, the tagged kinases were immunoprecipitated, and their activity was measured with GST-ATF2 or GST-c-Jun-N as substrates. These assays revealed that HePTP reduced both the basal activity of p38 and its further activation (Fig. 4b) but not the activation of Jnk2 (Fig. 4c). The same result was obtained in several independent experiments. We conclude that HePTP inhibits p38, but not Jnk, in intact cells.

Mapping of the Binding Site in HePTP—The region of HePTP that binds Erk and p38 was first mapped to its non-catalytic N-terminal 92 amino acids. A GST fusion protein of GST-HePTP lacking this region (GST-DN) failed to bind any Tyr(P)-containing proteins, Erk, or p38 (Fig. 5a) in lysates of Jurkat T cells. In contrast, a GST fusion protein containing only the N-terminal 92 amino acids of HePTP (GST-N ter) bound both Erk and p38 (Fig. 5b). To determine the binding region more precisely, we made three smaller constructs encompassing amino acids 1–40, 10–55, 30–80, respectively. These three GST fusion proteins were incubated with cell lysates, washed extensively, and immunoblotted for the presence of Erk and...
Phosphorylation of the N terminus of HePTP by MAP Kinase—This region also contains two potential phosphorylation sites for a proline-directed kinase (such as Erk), Ser-72, and Thr-45. Because HePTP was readily phosphorylated on both serine and threonine by the bound Erk and p38 (Fig. 3b) and by recombinant Erk2 (Fig. 6a) or p38 (not shown), we mutated these residues to alanines and examined their phosphorylation by tryptic peptide mapping, which revealed that both peptides containing Ser(P) (peptides 1 and 2) contained Ser-72, whereas Thr-45 was the phosphorylated residue in peptides 3–5 (Fig. 6b). These peptides were also seen in tryptic peptide maps of HePTP from metabolically 32P-labeled T cells but were missing in a HePTP-T45A/S72A mutant (not shown). Thus, the non-catalytic N terminus of HePTP binds Erk and p38 and is phosphorylated at Ser-72 and Thr-45 by these kinases.

Possible Role of Phosphorylation at Thr-45 and Ser-72—The phosphorylation of the N terminus of HePTP by the Erk or p38 kinases introduces the possibility of a regulatory role of this event. Measurement of the catalytic activity of HePTP revealed a small decrease in activity upon phosphorylation by Erk (not shown). This decrease is presently of questionable significance. Instead, we addressed the possibility that phosphorylation regulates the physical association between HePTP and Erk or p38. Because a GST-N ter protein having both the T45A and S72A mutations still bound Erk and p38 as readily as the wild-type GST-N ter protein (Fig. 5b), it seems that the hydroxyl groups of Thr-45 and Ser-72 or their phosphorylation are not required for binding of Erk or p38. To test the opposite, namely that phosphorylation is involved in dissociation of the kinases, we incubated HePTP-Erk complexes in the presence of ATP and Mg2+ at 37°C and measured the release of HePTP. These experiments utilized catalytically active GST-Erk2 adsorbed onto glutathione-Sepharose with bound HA-tagged HePTP-C270S from transfected Jurkat T cells and resulted in a time-dependent dissociation of HePTP from the beads (Fig. 6c). Thus, the N terminus of HePTP binds Erk and p38 but may release them upon phosphorylation. Curiously, both phosphorylation sites are outside the minimal necessary binding site for Erk and p38, but their phosphorylation could influence the conformation of the binding site.

**Elimination of the Phosphorylation Sites Augments Inhibition of MAP Kinase by HePTP**—To study the impact of phosphorylation of HePTP at Thr-45 and Ser-72 in intact cells, we generated a mutant in which both residues were replaced by alanine residues. The mutant, HePTP-T45A/S72A, was included in a co-transfection experiment similar to that in Fig. 1f using JCaM1 cells. As shown in Fig. 6d, wild-type HePTP reduced the anti-CD3-induced activity of Erk2 to about half, whereas HePTP-C270S augmented it. In contrast, HePTP-T45A/S72A was more efficient than wild-type HePTP and essentially eliminated any increase in MAP kinase activation in the anti-CD3 stimulated cells. Thus, it is clear that phosphorylation of HePTP at Thr-45 and/or Ser-72 is not required for inhibition of MAP kinase. Rather, it seems that phosphorylation has the opposite effect, namely to lessen the inhibitory effect of HePTP. This fits our hypothesis that phosphorylation causes a dissociation of Erk from HePTP; the T45A/S72A mutant would not allow bound Erk to escape, and the inhibition of Erk activation would be stronger.

**DISCUSSION**

Taken together, our findings show that HePTP, a strictly Tyr(P)-specific protein phosphatase, forms a physical complex through a small region in its unique N terminus with the Erk and p38 MAP kinases. Because HePTP very efficiently dephosphorylates these kinases and inactivates them in vitro, it seems that the physical association serves to position HePTP correctly for this catalysis. The small amount of HePTP required for MAP kinase inactivation in vitro and the reduction in Erk phosphorylation and activity in intact T cells transfected with HePTP indicate that this function of HePTP is physiologically significant. This conclusion is also supported by the finding that HePTP is readily phosphorylated by Erk and p38 at Thr-45 and Ser-72, both of which are phosphorylated in intact cells. Finally, very similar conclusions were recently drawn using mast cells from mice deficient in HePTP. Together these observations indicate that the role of HePTP is to negatively regulate the Erk and p38 MAP kinases in hematopoietic cells. Whether the related PTPases STEP and PTP1C carry out this task in other cell types remains to be determined. Both share an N-terminal sequence with a high degree of homology to the

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2 B. Zanke, personal communication.
HePTP Binds and Dephosphorylates MAP Kinase

FIG. 6. Mapping of phosphorylation sites in N terminus of HePTP. a, first panel, autoradiogram of GST-HePTP-C270S incubated with a lysate from untreated (lane 1) or pervanadate-treated cells (lane 2), washed extensively, and then incubated with [γ-32P]ATP for 5 min. Second panel, tryptic peptide map of the band in lane 2 of the first panel. Third panel, phosphoamino acid analysis (PAA) of peptides 1, 2, 4, and 5 from the same tryptic peptide map. PSer, Ser(P); PTyr, Tyr(P). b, tryptic peptide maps of GST-HePTP, GST-HePTP-S72A, and GST-HePTP-T45A phosphorylated by recombinant Erk2 in vitro in the presence of [γ-32P]ATP, wt, wild type. c, anti-HA immunoblots of supernatants (upper panel; soluble) or pellets (lower panel; bound) of glutathione-Sepharose beads preadsorbed with 0.5 μg of active GST-Erk2 incubated in a lysate of T cells transfected with HA-tagged HePTP-C270S, washed extensively, and then incubated with 10 μM ATP at 37 °C for the indicated time. Note the increase in soluble HePTP and the decrease in bound HePTP. Similar results were obtained in at least one other experiment for each panel. d, in vitro kinase assay of myc-tagged Erk2 MAP kinase expressed in JCaM1 cells alone or together with the indicated plasmids and either left unstimulated or treated with anti-CD3ε mAbs for 5 min. The upper panel shows the autoradiogram of the kinase assay. The amount of Erk2 in the precipitates was visualized by immunoblotting (second panel) and the expression of HePTP by anti-HA tag immunoblotting of the lysates (third panel). The same result was obtained in another independent experiment.

Erk/p38 binding region of HePTP.

It is well established that MAP kinases are the targets for dual-specificity phosphatases (35–37) in T cells; particularly, the Pac1 phosphatase (36). In contrast to HePTP (4, 6), however, these enzymes are not present in resting T cells but are induced and synthesized some 30–60 min after receptor ligation (35, 36). Thus, they are unlikely to be responsible for suppression of MAP kinases in resting T lymphocytes or during early time points of T cell activation. It has been shown also in other cell types that the inactivation of MAP kinases precedes the induction of dual-specificity phosphatases and that a cytosolic PTPase is involved (38, 39). In the budding yeast, Saccharomyces cerevisiae, the mating pheromone-induced activation of the MAP kinases Fus3p and Kss1p is tightly regulated by the concerted action of the dual-specificity protein phosphatase Msg5p and the conventional PTPases Ptp2p and Ptp3p (40). In this system, the latter are responsible for the basal suppression of the MAP kinases and for terminating their ligand-induced activation. In contrast, the dual-specificity phosphatase is encoded by an inducible gene, and the role of the enzyme is primarily to dephosphorylate the MAP kinases at later time points (“recovery”). Our findings suggest that a mammalian PTPase, HePTP, is a functional homologue of Ptp2p and Ptp3p and is responsible for basal dephosphorylation and control at early time points.

Lymphocyte activation is initiated by the action of several protein-tyrosine kinases (41, 42) and is very likely to be negatively regulated by a number of PTPases (43). Only one such PTPase is currently known, namely SHP1 (44, 45), which dephosphorylates receptor-associated signaling molecules. Here we show that HePTP functions at a distinct step to negatively regulate lymphocyte activation. In contrast to SHP1, HePTP has a high degree of specificity for Erk and perhaps p38 MAP kinases and does not affect the tyrosine phosphorylation events upstream of these kinases. This specificity is due, in part, to a strong physical association of HePTP with Erk and p38, an interaction that is constitutive and occurs through the unphosphorylated N terminus of HePTP. We suggest that upon activation of Erk by Mek, the activated Erk either rapidly phosphorylates Thr-45 and Ser-72, dissociates (perhaps also because of other mechanisms), and escapes into the nucleus to phosphorylate its nuclear substrates. A fraction of the activated Erk molecules, however, are rapidly inactivated by the bound HePTP. As determined by immunofluorescence, HePTP appears to remain exclusively cytosolic (Ref. 10) and, thus, unable to inactivate MAP kinase molecules in the nucleus. Instead, these are later inactivated by the nuclear dual-specificity phosphatases, which are absent in resting T cells but are induced within 30–60 min of T cell activation. This sequential phosphatase model (Fig. 7) provides a flexible mechanism for the regulation and fine-tuning of MAP kinases, which function as crucial signal integration and decision points in T cell activation as well as in growth and stress responses in many other cell types. Our model predicts a potential role for HePTP in positive selection of T cells in the thymus (46), cytokine pro-
duction (27, 47), T cell proliferation (48), or anergy (49) and potentially in other MAP kinase-dependent aspects of T cell differentiation and activation.

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