Nonreceptor Tyrosine Phosphatases in Cellular Signaling: Regulation of Mitogen-Activated Protein Kinases

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I. Introduction

Cells continuously monitor changes in their extracellular and intracellular environments and elicit appropriate adaptive responses. Nearly every aspect of cell life is controlled by signal transduction processes, by which extracellular perturbations are perceived, converted into intracellular signals, and conveyed to effectors, whose activities are necessary to generate cellular responses. Many of these intracellular processes are controlled by signal transduction pathways that regulate protein phosphorylation. In particular, reversible tyrosine phosphorylation is one of the fundamental mechanisms for controlling cell proliferation, differentiation, and development. The extent of tyrosine phosphorylation is determined by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases).[1−4] During the past two decades, protein kinases have taken the center stage as tyrosine phosphorylation emerged as a central theme in the field of signal transduction. Studies into tyrosine dephosphorylation were relegated to the "back seat" until the first protein tyrosine phosphatase PTP1B was biochemically characterized.[5] In the past decade, much progress has been made toward understanding the catalytic mechanism, cellular regulation, and physiological functions of PTPases. A large number of nonreceptor tyrosine phosphatases have been shown to specifically regulate mitogen-activated protein kinase (MAPK) signaling. Moreover, recent evidence has demonstrated that PTPases themselves, like many other critical components in cellular signaling, are controlled by diverse mechanisms. This review will first provide an overview of MAPK signaling and then discuss the functions of intracellular PTPases, particularly the functions of PTPases in MAPK regulation.

II. MAP Kinase Signaling Transduction Pathways

The MAP kinase cascade is one of the best-characterized signaling pathways, initiated by a wide variety of extracellular stimuli. Through a series of protein−protein interactions and phosphorylation, a protein kinase cascade consisting of at least three kinases, MAPK, MKK (MAP kinase kinase), and MEKK (MAP kinase kinase kinase), is activated. A sequential phosphorylation mechanism by which one kinase phosphorylates and activates a downstream kinase ultimately results in MAPK activation. Once activated, MAPKs phosphorylate numerous cellular substrates and elicit specific responses. These kinases, which constitute the MAP kinase modules, have been adapted in diverse signal transduction

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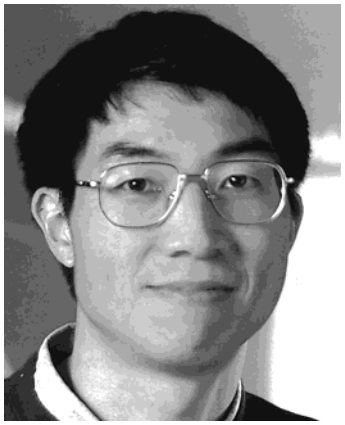
pathways. Currently there are at least three well characterized pathways utilizing MAPK modules in mammalian systems (Figure 1), five pathways in *S. cerevisiae* (Figure 2), and several in other organisms.

A. Mammalian MAP Kinase Pathways

Molecular cloning of ERKs, MKKs, and their homologous genes have identified 11 distinct MAPKs and 7 MKK genes in mammals.[6,7] Members of MAPK family include (I) ERK1 and ERK2,[8,9] (II) Jun NH2-terminal kinases/stress-activated protein kinase (JNK/SAPK) isoforms α, β, and γ,[10,11] and (III) p38 MAPK isoforms α, β, γ, and δ.[12−17] Biochemical analyses



Kun-Liang Guan is currently a professor of Biological Chemistry at the University of Michigan. He was born in China 1963 and received his B.S. degree (1982) from Hangzhou University, China, and his Ph.D. degree (1989) from Purdue University working with Dr. Henry Weiner. He did his postdoctoral work on protein tyrosine phosphatases with Dr. Jack Dixon at Purdue University and joined the faculty at University of Michigan in 1992. He has been the recipient of numerous awards including the MacArthur Fellowship and the Sherling-Plaugh Award from the American Society for Biochemistry and Molecular Biology. His current research activities focus on protein phosphorylation in regulation of cell growth and differentiation.

have demonstrated that each of these subfamilies is selectively phosphorylated and activated by upstream MAP kinase kinases: (I) MEK1 and 2, (II) MKK4 and MKK7, and (III) MKK3 and MKK6, respectively. Less well-characterized MAPKs include ERK4 and ERK7, for which upstream regulators have yet to be identified, and ERK5, which associates with MKK5. Contemporaneous work in other eukaryotic systems, ranging from fungi, plants, nematode, fly, to frog, have also identified orthologous kinase genes and MAPK pathways. In nearly every case there is a kinase cascade that consists of a MAPK, a MKK, and an upstream activating kinase MEKK. This review will highlight some background information of these pathways for the discussion of MAPK regulation by PTPases.

The ERK1/2 pathway is the best understood MAPK pathway in mammalian systems due to its involvement in mediating growth factor actions and cancer-related cellular transformation.[18] Interaction of growth factors with their receptors triggers multimerization and *trans*-phosphorylation of receptor protein tyrosine kinases (RPTK).[19] Adapter proteins containing Src-homology domain 2 (SH2) or phospho-tyrosine binding (PTB) domains, such as Grb2 and Shc, are then recruited to the tyrosine-phosphorylated receptors. In turn, these adapter molecules bring Ras guanine nucleotide exchange factor, SOS, to the plasma membrane where it can stimulate the exchange of GDP for GTP on Ras. Once GTP-bound, Ras protein is active and recruits Raf kinase to the plasma membrane, thereby allowing Raf to be activated by a yet poorly understood mechanism. The activated Raf phosphorylates and activates the dual-specificity protein kinases, MEK1/2, which in turn activate ERK1/2 MAP kinase by phosphorylating both tyrosine and threonine residues in the conserved Thr-Glu-Tyr (TxY) motif on ERK1/2. Dual phosphorylation is required for full activation of ERK1/2.[18,20] Following activation, MAPKs can phosphor-

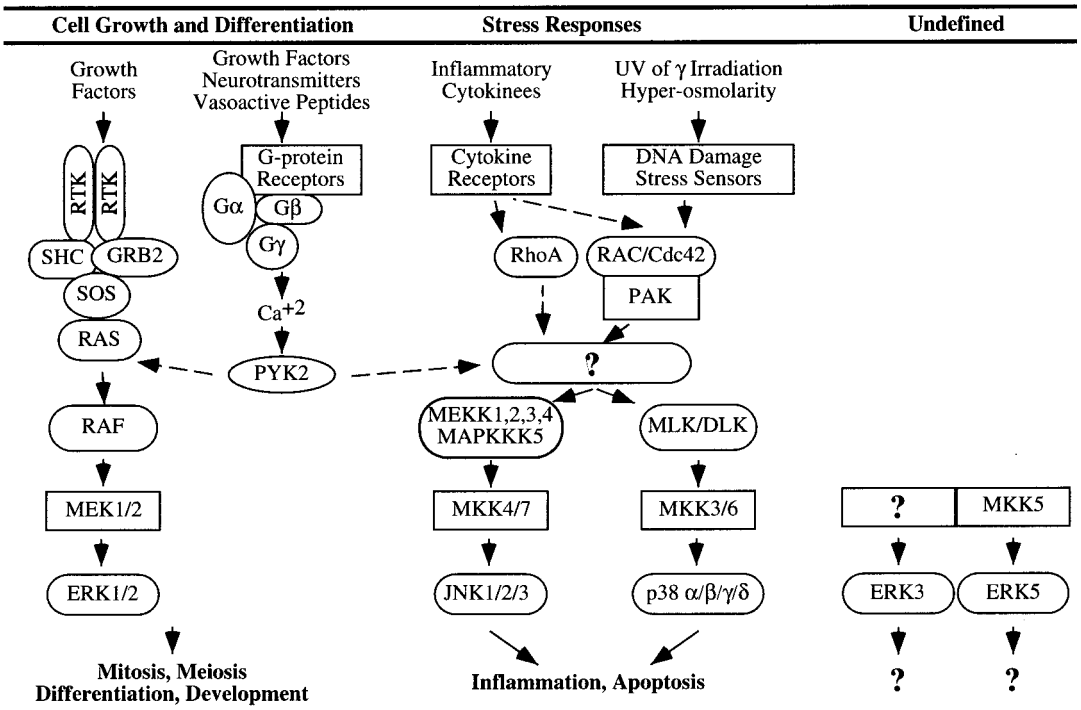


Figure 1. Mammalian MAPK pathways. (Adapted from ref 6.)

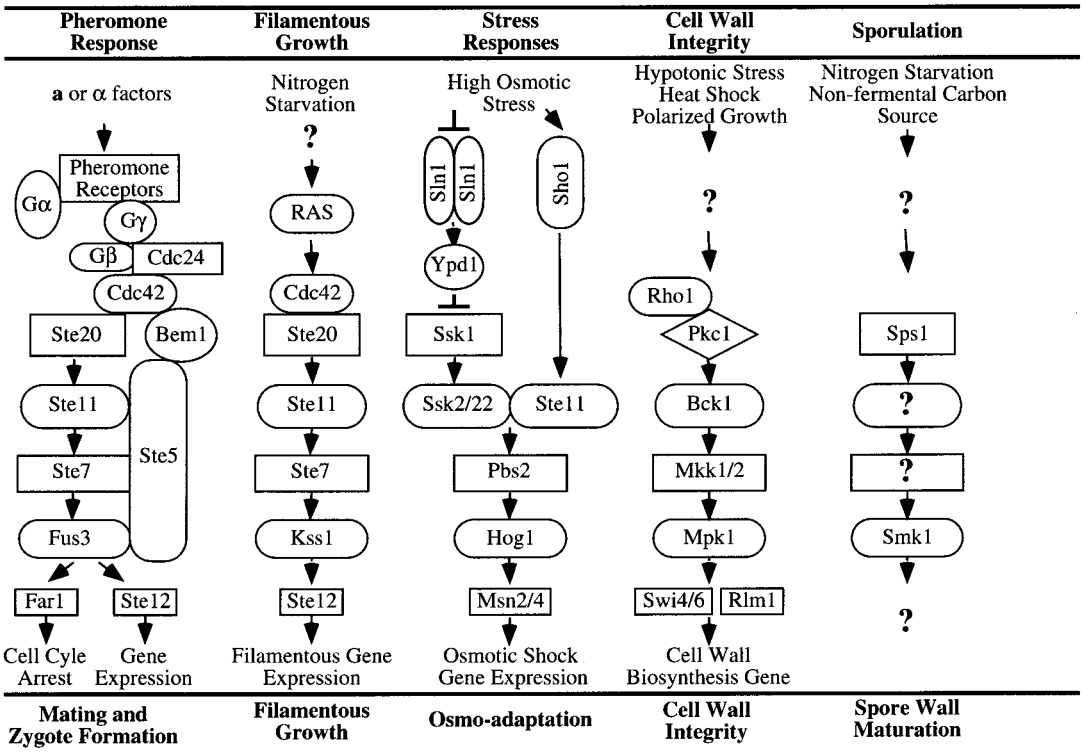


Figure 2. MAPK pathways in *S. cerevisiae.*

ylate a variety of substrates, including Rsk, MAPKAP kinase 2, and cytoskeletal proteins. Active ERK1/2 are also capable of translocating into the nucleus, where they phosphorylate various transcription factors, such as Elk-1. In addition to ERK1/2 activation by receptor tyrosine kinases, this class of MAPKs are also activated by cytokines, lymphocyte antigen stimulation, integrin binding, as well as of G-protein-coupled receptors. Therefore, engagement of a wide array of extracellular ligands with their receptors at the cell surface transduces a signal into cytosol and nucleus by way of a MAPK cascade, thereby regulating cellular responses (Figure 1).

Although architecturally homologous to the ERK1/2 pathways, the JNK/SAPK and p38 MAPKs are activated primarily by inflammatory cytokines and cellular stresses, including ionizing or ultraviolet radiation, protein synthesis inhibition, temperature shock, mechanical stress, oxidative stress, or hyperosmotic stress.[7,10,11,21,22] JNK/SAPK and p38 pathways control growth arrest, apoptosis, and activation of immune and reticuloendothelial cells in response

to stresses in a manner distinct from cell growth and transformation responses controlled by the ERK1/2 pathway. Despite their distinct activating stimuli, JNK/SAPK is activated by phosphorylation of its Thr-Pro-Tyr motif by MKK4 and MKK7 whereas MKK3 and MKK6 phosphorylate p38 MAPK at the Thr-Gly-Tyr motif. Although their individual activators have been characterized, less is known about the mechanisms for sensing many different types of cellular insults and how these signals are integrated at the level of JNK/SAPK and p38.

B. Yeast MAP Kinases Pathways

The yeast *Saccharomyces cerevisiae* possesses a diverse array of signal transduction pathways. These pathways allow yeast cells to quickly adapt to their changing environment and physiological conditions. Investigations in the signaling pathways in *S. cerevisiae* have provided a wealth of information on MAPK signaling. The MAPK, Fus3, was first discovered by genetic studies of the pheromone-regulated mating response in *S. cerevisiae*.[23] Extensive genetic and biochemical analyses have provided a more comprehensive understanding of *S. cerevisiae* MAPK pathways than those of other organisms. There are striking similarities between yeast and metazoan MAPK signaling. For instance, the biochemical mechanisms of MAPK regulation are highly conserved. Many observations made in yeast cells are readily translated into higher eukaryotic systems. In addition, extensive sequence homology exists between yeast and metazoan MAPK components, and many new mammalian homologues of yeast MAPK components are likely to be found. There are a total six MAPKs existing in the yeast genome, and five physiologically distinct MAPK pathways have been characterized. These five pathways participate in diverse processes from mating and zygote formation, filamentous growth triggered by nutritional starvation, high osmotic-stress response, maintenance of cell wall integrity, to spore wall formation. In all of these pathways except spore wall formation, a MAPK module containing three protein kinases (MAPK, MKK, and MEKK) has been identified (Figure 2).[24−26]

The mating-pheromone response pathway represents one of the best-characterized MAPK pathways of all eukaryotic systems. Yeast cells can exist as either **a** or α haploid cells, and each cell type secrets **a** or α peptide mating pheromone, respectively. Haploid cells of opposite type mate with each other, form zygotes, and eventually become **a**/α diploid cells. This mating process is triggered by the secreted pheromones and controlled by a Fus3 MAPK pathway. Binding of pheromone to a heterotrimeric G-protein-coupled receptor on the surface of cells with the opposite mating type promotes the dissociation of G$\beta\gamma$ subunit from Gα subunit. The unleashed G$\beta\gamma$ transmits signals to the downstream kinase, Ste20, whose mammalian homologue is $p65^{PAK}$. Ste20 is activated by a poorly understood mechanism to transduce the activating signal into a MAPK module consisting of Ste11 MEKK, Ste7 MKK, and Fus3 MAPK. Activation of the Fus3 MAPK pathway triggers cell cycle arrest at the G1-stage, expression of pheromone-inducible genes, and morphological changes, which collectively prepare haploid cells for mating.

Yeast cells respond to high osmotic conditions by increasing intracellular Osmolite concentration and decreasing membrane permeability. This adaptation is driven by a stress-activated Hog1 MAPK signaling pathway,[27] which is homologous to mammalian p38 MAPK pathways. The evolutionary conservation of the mammalian p38 and yeast Hog1 pathways is bolstered by observations that p38 MAPK α and β can complement *hog1*Δ^- deletion in yeast.[28,29] Two distinct cell surface receptors, Sln1 and Sho1, act as osmosensors that regulate the downstream Hog1 MAPK module. Sln1 functions through Ypd1 and Ssk1, and these three proteins are structurally and functionally similar to regulatory proteins that form two-component or three-component phospho-relay systems in bacteria and plants.[30] This three-component system regulates the activity of two closely related and partially redundant MEKKs, Ssk2 and Ssk22.[31,32] On another branch, the putative transmembrane protein, Sho1, regulates Ste11 MEKK.[32,33] Therefore, three MEKKs, Ssk2, Ssk22, and Ste11, receive inputs from two osmosensing branches and activate a single downstream MKK, Pbs2, that in turn activates a single MAPK, Hog1. Once activated, the Hog1 pathway regulates the expression of genes necessary for countering the elevated extracellular osmolarity.

In addition to these evolutionarily conserved components in signaling pathways, the mating-pheromone response pathway also utilizes Ste5 as a scaffold protein that organizes components into a preassembled signaling complex to enhance the efficiency and specificity of signal transduction.[34−36] Although a Ste5 homologue has not been identified in mammals, recent evidence supports the existence of scaffold proteins in mammalian ERK1/2[37] and JNK/SAPK pathways.[38,39]

The diversity of cellular processes in which MAPK pathways participate is remarkable. Model organisms have revealed some fundamental principles underlying signaling. For example, homologous kinase modules are utilized in parallel pathways where individual MAPKs generate distinct physiological outputs in response to diverse stimuli. Components can be shared among MAPK pathways, and specificity is achieved by organizing proteins into signaling complexes through scaffold proteins. It has recently been observed that there is crosstalk and coordinated mutual regulation among MAPK pathways.[40] Despite this rapid progress, we are just beginning to understand how these signals are integrated to the specific activation of a core MAPK pathway to elicit proper cellular responses. Future research requires the integration of genetic and biochemical approaches to dissect functions and regulations of MAPK signaling pathways.[7] One important challenge is to determine the molecular details by which an individual pathway is activated in response to stimulation and how activation of these pathways is coordinated to elicit complex responses. Another significant issue of MAPK regulation is how MAPK pathway activity is down-

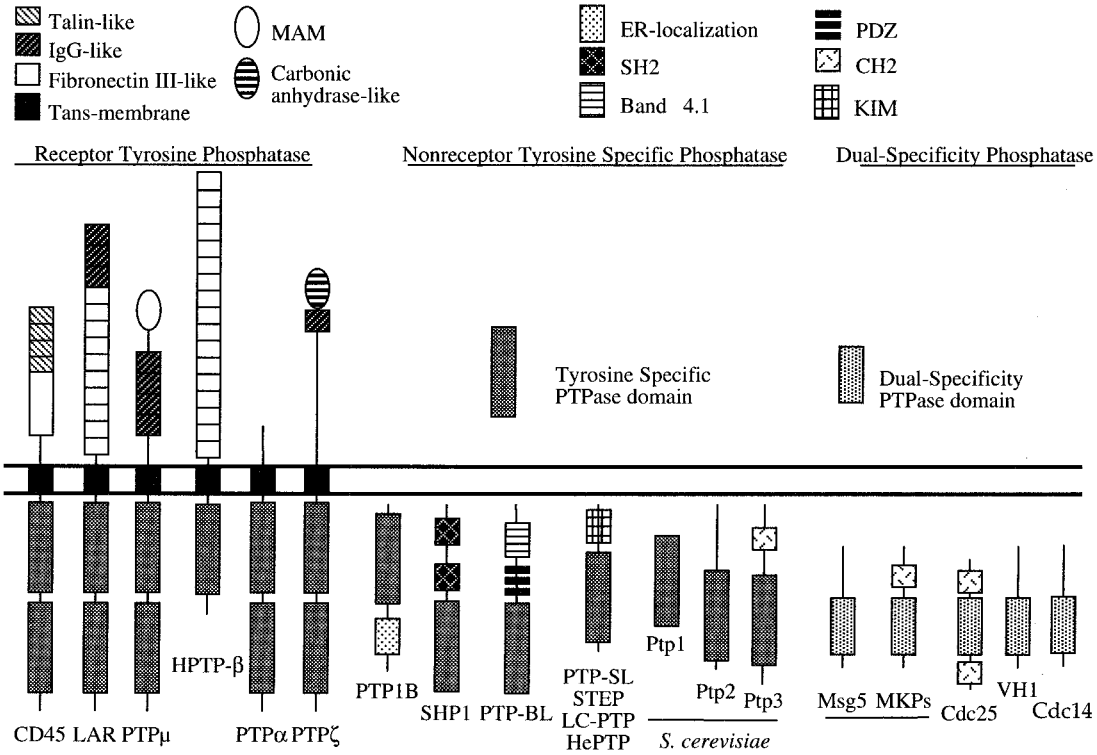


Figure 3. Protein tyrosine phosphatases.

regulated, allowing a cell to tailor the exact level of MAPK activity in response to various levels of stimulation. Since MAPK activation requires phosphorylation on both tyrosine and threonine residues, dephosphorylation of either residue by protein phosphatases is sufficient to inactivate MAPK. The investigation of the negative regulation of MAPK pathways, especially by protein phosphatases, represents a key step in understanding cellular signaling.

III. Protein Tyrosine Phosphatases

PTPases were originally identified by purifying an enzyme with catalytic specificity toward tyrosine-phosphorylated proteins.[5] Characterization of the first purified PTPase, PTP1B, revealed that it had no similarity to known serine/threonine phosphatases.[5] Instead, PTP1B defined a new family of phosphatases, and currently approximately 80 PTPases have been identified. PTPases are characterized by the presence of a conserved catalytic domain of 200∼250 residues, containing the active site signature motif, CxxxxxR. PTPases are categorized into subfamilies depending on the three-dimensional X-ray crystal structure, subcellular localization, and substrate specificity (Figure 3): (1a) phosphotyrosine-specific receptor PTPase, (1b) phosphotyrosine-specific nonreceptor intracellular PTPases, (2) dual-specificity PTPases, (3) Cdc25-like, and (4) low molecular weight PTPases.[41−43] Although PTPases constitute a diverse family of enzymes, our understanding of the physiological functions of PTPases in signaling is limited.

Receptor-like PTPases (RPTPs) usually consist of a variable extracellular domain, a single transmembrane domain, and one or two intracellular PTPase domains.[44] RPTPs possess the potential to relay extracellular signals by modulating tyrosine dephosphorylation within cells. The function and regulatory mechanism for RPTPs are the subject of other reviews in this issue.

A. Intracellular PTPases

The intracellular PTPases contain one catalytic domain that is often flanked by a noncatalytic sequence. Such domains may function in regulating PTPase catalytic activity as well as in protein−protein interactions and subcellular localization. One subtype of intracellular PTPases identified from *C. elegans*, *Drosophila*, and mammals contains Src homology 2 (SH2) domains[45−49] (reviewed by Feng and Pawson and Neel and Tonks).[3,50] SH2 domains direct the association of signaling proteins with tyrosine-phosphorylated residues. The SH2 domain containing SHP-1 is expressed at its highest levels in hematopoietic cells where it negatively controls hematopoietic cell proliferation by regulating signaling pathways downstream of cytokine receptors, antigen receptors, and receptor tyrosine kinases (RTKs).[51,52] In contrast, the related PTPase, SHP-2, and its *Drosophila* homologue *corkscrew* (*CSW*) are expressed ubiquitously and play positive functions in receptor tyrosine kinase signaling.[46,47,53] In addition to SH2 domains, other protein domains including band 4.1, PDZ, and PEST domains have also been found in intracellular PTPases.[3,4,42] The function of these PTPases and their noncatalytic domains remain to be elucidated.

Several PTPases including the RPTP LAR (leukocyte antigen-related), the cytosolic PTPase SHP2, and PTP1B have been implicated in insulin receptor tyrosine dephosphorylation.[54] There is compelling evidence that PTP1B is a key physiological regulator

of insulin signaling. First, overexpression of PTP1B in mammalian cells suppresses insulin signals[55−57] whereas inhibition of PTP1B enhances insulin signals.[58,59] Furthermore, injection of PTP1B into *Xenopus* oocytes impedes insulin-stimulated maturation.[60] Third, PTP1B binds to the insulin receptor and efficiently dephosphorylates it in vitro.[55,61,62] Finally, deletion of the PTP1B gene in mice causes marked insulin sensitivity and prolonged insulin receptor autophosphorylation.[63] These results demonstrate that PTP1B plays an important role in down-regulation of insulin signaling.

Additional targets of PTP1B have also been identified, including p210 bcr-abl,[64] c-Src,[65] and STAT5a and STAT5b.[66] PTP1B can dephosphorylate and antagonize oncoprotein p210 bcr-abl signaling in vivo.[64] PTP1B is also able to specifically dephosphorylate STAT5a and STAT5b in transfected cells and in vitro. Overexpression of PTP1B inhibits nuclear translocation of STAT5a and STAT5b and Prolactin (PRL)-dependent transcriptional activation, suggesting that PTP1B can negatively regulate the PRL and tyrosine kinase JAK (janus kinase) 2 signaling pathway.[66] In contrast, PTP1B seems to activate c-Src by dephosphorylating the inhibitory phosphorylation on c-Src kinase. In several human breast cancer cell lines, biochemical purification has identified PTP1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src. In these cell lines, PTP1B protein levels are elevated, correlating to the elevated c-Src specific kinase activity.[65] These observations suggest that PTP1B functions in multiple signaling pathways.

In *S. cerevisiae* there are a total of three PTPases: Ptp1,[67] Ptp2,[68−70] and Ptp3.[71] Ptp1 only contains a catalytic domain. Although a possible substrate for Ptp1 has been reported in yeast, its physiological function has yet to be assigned.[72] It is likely that Ptp1 plays a general housekeeping function. In contrast, Ptp2 and Ptp3 are involved in negative regulation of Hog1 and Fus3 MAPK.[71,73−75] In *S. pombe*, pyp1 and pyp2 tyrosine phosphatases have been shown to dephosphorylate and inactivate spc1/sty1 MAPK, a *S. pombe* homologue of Hog1.[76,77] Therefore, it appears that most yeast intracellular PTPases regulate MAPKs. Recent evidence has suggested that mammalian and *Drosophila* MAPKs are also regulated by PTPases. The functions of tyrosine-specific phosphatases in MAPK regulation are discussed in detail in a later section of this review.

B. Dual-Specificity Phosphatases

The second subfamily of tyrosine phosphatases is termed dual-specificity phosphatases (DSP). In contrast to receptor and intracellular PTPases, which are selective toward phosphotyrosine, DSPs display catalytic activity toward phosphorylated tyrosine, serine, threonine, RNA, or lipid substrates in vitro. The prototype of this subfamily is VH1, identified in the double-stranded DNA virus *vaccinia*.[78] Since then, increasing numbers of PTPases containing a VH1-like catalytic domain have been identified in yeast and mammals. Comparison of the crystal structure of human VH1-related dual-specificity phosphatase, VHR, to the tyrosine-specific phosphatase, PTP1B, revealed that the relatively shallow substrate recognition pocket of VHR accommodates both phosphotyrosine and phosphoserine/threonine while the pocket depth of PTP1B favors phosphotyrosine as a substrate.[79−83] Therefore, on the basis of their amino acid sequence homology and distinct enzymatic activity, the dual-specificity phosphatases (DSPs) comprise a distinct subfamily of tyrosine phosphatases.

Another distinct subfamily of phosphatase is represented by Cdc25, which regulates cyclin-dependent kinases (CDKs) in cell cycle progression (reviewed in refs 84−87). Activation of cyclin-dependent kinases in higher eukaryotic cells can be achieved through dephosphorylation by members of the Cdc25 phosphatase family, Cdc25A, Cdc25B, and Cdc25C. Cdc25 plays an important role at the G1/S-phase transition. Cdc25B undergoes activation during the S-phase and plays a role in activating the mitotic kinase Cdk1/cyclin B in the cytoplasm. Active Cdk1/cyclin B then phosphorylates and activates Cdc25C, leading to a positive feedback mechanism that initiates the G2/M cell cycle transition. Cdc25A and B are potential human oncogenes.[88] In addition, Cdc25 is a main player of the G2 arrest caused by DNA damage or in the presence of unreplicated DNA.[84−87] Another major subgroup of dual-specific phosphatase is termed MAP kinase phosphatase (MKP).[89−93] The function of dual-specificity phosphatases in MAPK regulation will be discussed in detail in the next section.

The full genomic sequence of *S. cerevisiae* encodes 12 potential phosphatases with homology to dual-specificity phosphatases.[94] Three of them, Cdc14, Yvh1, and Msg5, have been identified previously. The mammalian homologues of all three proteins have been isolated. Cdc14 is involved in cell cycle regulation and chromosome segregation.[95−99] The yeast Cdc14 DSP[100] has been shown to interact and dephosphorylate proteins phosphorylated by the cyclin-dependent kinase Cdc28/clb, including Cdc15, Sic1, and Swi5, to promote mitotic exit.[95,96,98,99,101,102] Two human orthologs of the yeast CDC14, termed hCdc14A and B, have been identified. The hCdc14A and hCdc14B proteins may interact with the tumor suppressor protein p53 both in vitro and in vivo. This interaction is dependent on the N-terminus of the hCdc14 proteins and the C-terminus of p53. Furthermore, the hCdc14 phosphatases were found to dephosphorylate p53 specifically at the p34(Cdc2)/cyclin B phosphorylation site (Ser315).[103] These findings suggest conserved functions of the Cdc14 subclass of dual-specificity phosphatase in cell cycle control.

The yeast DSP, Yvh1 has a cysteine-rich COOH-terminal domain capable of coordinating Zn, thus defining it as a novel zinc finger domain.[104] Disruption of *YVH1* gene results in slow growth[105] and defects in sporulation in *S. cerevisiae*.[106−108] A human ortholog of the yeast Yvh1 has been cloned and is able to rescue the slow growth phenotype of yeast cells with *YVH1* deletion.[104] The human YVH1 homologue is located on chromosome 1q21-q22, which falls in a region amplified in human liposarcomas.[104] Thus, Yvh1 constitutes a distinct subgroup of DSPs that contains a novel zinc finger domain.

Another yeast DSP, Msg5, has been suggested to inactivate Fus3 MAPK in mating pheromone response pathway.[109] Similar to the function of Msg5 in *S. cerevisiae*, the majority of known mammalian DSPs appear to inactivate MAPK by catalyzing the dephosphorylation on both tyrosine and threonine residues. The roles of phosphatases in MAPK signaling are extensively studied and are further discussed in this review.

In light of the essential role of PTKs and tyrosine phosphorylation in transformation and tumorgenesis, it has been speculated that tyrosine phosphatases may act as tumor suppressors by decreasing tyrosine phosphorylation levels of cellular proteins. The loss of heterozygosity for a novel tyrosine phosphatase gene, PTEN/MMAC1, occurs at high frequency in various forms of carcinomas, suggesting that PTEN may function as a tumor suppressor gene.[110−113] Despite having a catalytic domain homologous to dual-specificity phosphatases, PTEN dephosphorylates specific inositol phospholipids instead of protein phosphotyrosine.[114−116] The *C. elegans daf-18* gene encodes a PTEN homologue that acts in the insulin receptor-like metabolic signaling pathway to limit AKT-1 and AKT-2 activation by decreasing PI(3,4,5)P_3 levels.[117,118] Another second DSP, myotubularin, has recently been shown to exhibit substrate specificity toward PI(3)P in vivo,[119] and myotubularin may represent novel subfamilies of PTPases, which are active toward unique substrates, such as phospholipid.[115,116,120−122]

IV. Regulation of MAP Kinases by Dual-Specificity Protein Phosphatases

As a key component in signal transduction, the timing and duration of MAPK activity is critical for proper signaling. For instance, when PC-12 cells are treated with EGF or NGF, ERK1/2 display transient or sustained activation, respectively, correlating to the phosphorylation status of the TxY motif. It is believed that the different temporal patterns of MAPK activation stimulated by EGF or NGF underlie their differential effects on cellular proliferation and differentiation.[123] In some instances, failure to exert temporal control of MAPK activity has a pronounced effect on cells. For example, constitutive activation of mammalian ERK1/2 MAPK can lead to oncogenic transformation.[124,125] In *S. cerevisiae*, unregulated activation of Fus3 or Hog1 MAPK pathways results in lethality.[126−128] While research has mainly focused on activation mechanisms of MAPKs, an equally critical aspect of MAPK regulation is how MAPKs are inactivated. Because both threonine and tyrosine on TxY motif must be phosphorylated for MAPK activation, dephosphorylation of either residue by protein phosphatases is sufficient to inactivate the MAPKs, implying that different protein phosphatases may inactivate MAPKs.

A. The MKP Family

In 1993, molecular cloning of growth factor or stress-induced immediate-early genes identified the human CL100/hVH1 gene[129−131] and mouse homologue, MKP1/3CH134/ERP,[132,133] which encode proteins with homology to the VH1 dual-specificity phosphatase. In fibroblasts, the time course of induction of this phosphatase correlates with the time course of ERK inactivation, indicating a possible role in ERK regulation. Purified CL100/3CH134 protein inactivates ERK2 MAPK in vitro by concomitant dephosphorylation of both tyrosine and threonine residues of ERK2. CL100/3CH134 displays substrate selectivity toward MAPK in vitro over a number of artificial substrates and unrelated protein kinases. Furthermore, expression of 3CH134/CL100 blocks activation of p42MAPK induced by serum, oncogenic Ras, or activated Raf.[129,132,134] Therefore, CL100/3CH134 has been renamed as MAPK-specific phosphatase-1 (MKP-1) to reflect its function in MAPK regulation. The expression of the MKP-1 is induced by mitogen, growth factor, oxidative, and thermal stress. The protein is localized to the nucleus, thereby providing a possible negative regulation of nuclear MAPKs.[129,131,134] MKP-1 represents a subgroup of VH1-like dual-specificity phosphatases that are specific toward MAPK family members.[89,92,93] Despite these early studies that link MKP-1 to MAPK regulation, MKP-1 may not be the physiological regulator of ERK activity but may rather act on JNK and p38 MAPKs. For example, the activity of ERK is not affected in MKP-1-deficient fibroblasts.[135] MKP-1 has been shown to more effectively inactivate JNK1/SAPK and p38 MAPK than ERK.[136,137] In addition, newly identified dual-specificity phosphatases appear to be highly selective for ERK and thus may represent more specific ERK1/2 phosphatases.

In addition to MKP-1, at least nine dual-specificity phosphatase genes have been identified in mammals.[89,91,93] These include PAC1,[138,139] hVH2/TYP-1/MKP-2,[140−142] hVH-3/B23,[143,144] hVH-5/M3/6,[145,146] PYST1/MKP-3/rVH6,[147−149] PYST2/B59/MKP-X,[148−150] MKP-4,[151] MKP-5,[152,153] and VHR.[154] The highest degree of sequence similarity surrounds the catalytic active site motif **D**x_{26}(V/L)x(V/I)H**C**xAG(I/V)S**R**SxT-(I/V)xxAY(L/I)M (residues critical for catalysis are in bold). All mammalian dual-specificity phosphatases, except VHR,[154] contain two intriguing features termed CH2A and CH2B motifs (Cdc25 homology), which display significant homology to Cdc25 phosphatase.[145,148,151,155,156] Interestingly, the CH2A and CH2B motifs flank the Cdc25 phosphatase domain whereas in MKPs they are present at the amino terminus of the VH1-like dual-specificity phosphatase domain. It is now known that the domains containing CH2A and CH2B motifs are important for targeting MKPs to specific MAPKs. VHR is unique in that it contains the VH1-like dual-specificity phosphatase domain but lacks the amino-terminal extension and the CH2A and CH2B motifs found in the MKPs. Recently, VHR has been shown to selectively regulate ERK1/2 by dephosphorylating the tyrosine within the MAPK TxY motif.[157] Therefore, VHR may represent a distinct subclass of dual-specificity phosphatases that regulate MAPKs. Some of the general features of the identified MKPs are summarized in Table 1 (also refer to a recent review for mammalian MKPs by Camps et al).[89]

Table 1. Mammalian Dual-Specificity Phosphatases (Adapted from refs 89 and 93)

human gene	homologue	subcellular localization	transcriptional induction	substrate selectivity
CL100/hVH1	MKP-1/3C134 ERP (mouse)	nuclear	mitogen/growth factor oxidative stress/heat shock brain ischemia and seizure	p38, JNK/SAPK > ERK1/2
PAC1		nuclear	mitogen brain ischemia and seizure	ERK1/2 > p38 > JNK/SAPK
hVH-2/TYP-1	MKP-2 (rat)	nuclear	mitogen	ERK1/2, JNK/SAPK > p38
hVH3/B23		nuclear	mitogen thermal stress	n.d.
VHR		nuclear	constitutive expression	ERK1/2 > p38, JNK/SAPK
hVH5	M3/6 (mouse)	nuclear and cytosolic	growth factors	JNK/SAPK > p38 >ERK1/2
PYST1	MKP-3/rVH6 (rat)	cytosolic	NGF, bFGF, retinoic acid, nitric oxide, brain seizure	ERK1/2 ≫ JNK/SAPK, p38
PYST2/B59	MKP-X (rat)	cytosolic	n.d.	n.d.
MKP-4		cytosolic, punctuated pattern in nucleus	phorbol esters, mitogen oxidative and osmotic stress	ERK1/2 > JNK/SAPK, p38
MKP-5		nuclear and cytosolic	n.d.	p38, JNK/SAPK > ERK1/2

B. Substrate Selectivity and Direct Binding

The existence of a family of dual-specificity phosphatases that can dephosphorylate MAPKs imparts considerable complexity in MAPK regulation. One significant advancement has come with the discovery that specific MKPs display substrate selectivity toward different subclasses of MAPKs. Experiments with purified recombinant proteins and transfection in cultured cells have demonstrated that Pyst1/MKP-3 is selective toward ERK1/2.[145,158] For example, expression of MKP-3 blocks epidermal growth factor or oncogenic Ras (G12V)-stimulated ERK1 activation, whereas stress-induced activation of p54 SAPKβ and p38 MAP kinases is only partially inhibited under identical conditions. Moreover, recombinant Pyst1 protein is approximately 100-fold more efficient at dephosphorylating and inactivating ERK2 than p38. Therefore, MKP-3 may represent a specific phosphatase for ERK1/2. MKP-4, which is most similar to MKP-X/Pyst2 (61% identity) and MKP-3/Pyst1 (57% identity), has also been found to exhibit a similar substrate preference for ERK1/2.[151]

In contrast to MKP-3/Pyst1, mouse M3/6 has been shown to be selective toward JNK/SAPK and p38 MAPK.[145] Remarkably, when M3/6 is transfected into COS-7 cells, stress-induced activation of p54 SAPKβ, p46 SAPKγ (JNK1), and p38 MAP kinases is abolished whereas epidermal growth factor-stimulation of ERK1 is unaffected. The substrate selectivity of other MKPs have been similarly determined: PAC1, MKP-2/hVH-2/TYP-1 can inactivate ERK1/2, JNK1/SAPK, and p38 MAPKs but are less active toward JNK/SAPK and p38,[136] whereas MKP1 preferentially inhibits p38 and SAPKs.[136,137]

Although VHR lacks the amino-terminal domain found in all known MKPs, it has recently been found to be an ERK1/2 regulator.[157] ERK1 and ERK2 bind to a substrate-trapping mutant of VHR protein. Kinetic analyses and transfection studies have demonstrated that VHR specifically dephosphorylates and inactivates ERK1 and ERK2 but not p38 and JNK in vitro and in vivo. Moreover, immunodepletion of endogenous VHR attenuates dephosphorylation of cellular ERK. In contrast to reported MKPs, which can rapidly hydrolyzed both phosphoamino acids on the TxY motif of ERK, VHR specifically hydrolyzes phospho-Tyr but not Thr.[157] VHR is constitutively expressed and localized to the nucleus; therefore, it may contribute to the maintenance of ERK in an inactive state in quiescent cells and to the rapid inactivation of ERK following stimulation in the nucleus.

It should be emphasized that the substrate specificity of these enzymes is not absolute. Investigation of substrate specificity often utilizes purified recombinant proteins for in vitro assay or relies on transfection of phosphatases in cultured cells. Such approaches may sometimes create artifacts. For example, early evidence suggested that MKP-1 was able to down-regulate ERKs. Despite these important early works that have established the involvement of MKPs in MAPK regulation, it has been demonstrated that by titrating the levels of MKP-1 expression from the human metallothionein IIa promoter, MKP-1 is more effective in inactivating p38 MAPK and SAPK than ERK2.[137] Therefore, the relative substrate specificity might represent a significant mechanism to differentially control MAPKs by MPKs under physiological conditions. Furthermore, recent findings have pointed to the significant functions of the amino-terminal noncatalytic domain in determining substrate specificity.

While studying the regulation of ERK2 by Pyst1/MKP-3, Groom et al. observed that the phosphatase and kinase form a tight protein complex.[158] Surprisingly, this interaction was directly mediated by the noncatalytic amino-terminal rather than the carboxyl-terminal phosphatase domain of MKP-3.[159] In addition, the binding specificity of MKP-3 appears to correlate its substrate selectivity since the amino-terminal domain specifically interacted with ERK1/2 but not with JNK2/3 nor p38.[89,159] A similar correlation between substrate binding and specificity was also found between MKP-5 and p38 and SAPK/JNK. The CH2-containing domains in the amino terminus of MKP-5 are sufficient for p38 binding.[152] Since the CH2 domain is a general feature of MKPs, it has been postulated that CH2 motifs function as MAPK-targeting sequences. In support of this notion, a chimera consisting of the N-terminal domain of MKP-3 and the C-terminal catalytic domain of M3/6 binds tightly to ERK1 but not to JNK3/SAPKβ.[159] When the N-terminal domain of MKP-3 is deleted, the MKP-3ΔN protein binds less efficiently than full length MKP-3. Moreover, site-directed mutagenesis

of a basic region adjacent to the N-terminal CH2 motif of MKP-3 selectively diminishes high-affinity binding to ERK2 and p38 but not JNK1.[160] Together these observations suggest that the physiological specificity for inactivation of MAPKs by MKPs reflects tight substrate binding by MKP-specific N-terminal domains. A definitive demonstration of the function of CH2 domain in MAPK targeting has come from studying the substrate selectivity of yeast Ptp3 toward Fus3 (below).

C. Activation of MKP Enzymatic Activity by MAP Kinase

In an unexpected and exciting development, it was found that ERK2 binding leads to catalytic activation of MKP-3.[161] In the presence of purified ERK2, the activity of MKP-3 increases more than 40-fold toward artificial substrates in vitro. This activation was independent of ERK protein kinase activity but required binding of ERK2 to the noncatalytic amino terminus of MKP-3. Neither the *Sevenmaker* gain of function ERK2 mutant (D319N) nor JNK/SAPK or p38 MAP kinases bound MKP-3 and activated the phosphatase.

Thus, activation of the phosphatases by association with substrate kinases provides an elegant feedback mechanism for signal modulation and down-regulation. MKP-3 gene expression is induced by ERK activation. The newly synthesized MKP-3 phosphatase localizes in the cytoplasm, forms a complex with ERK via its amino terminal domain, and inactivates the ERK kinase with its carboxyl-terminal phosphatase domain. Similarly, it has been shown that MKP-1 associates directly with p38 MAP kinase both in vivo and in vitro and that this interaction enhances the catalytic activity of MKP-1.[160,162] Although it remains to be tested whether all MKPs can be catalytically activated by MAPK binding, the observation that a relatively nonselective phosphatase, MKP-4, can be bound and activated by ERK2, JNK/SAPK, and p38 MAP kinases suggests that this may be a general mechanism for activating MKPs[161] and targeted inactivation of MAPKs.

D. Mechanism for Catalytic Activation

What is the biochemical mechanism responsible for catalytic activation of MKPs by ERK binding? The catalysis carried out by tyrosine phosphatases proceeds through a two-step general acid/general base-catalyzed reaction.[42,163] Step 1: substrate binding induces a dramatic conformational change of the flexible loop approximately 30−40 residues amino terminal to the active site cysteine. The nucleophilic cysteine thiolate anion within the PTPase active site (**C**x_5R) attacks the substrate phosphoryl group to form a thiol−phosphate intermediate. The three nonbridging oxygens of phosphoryl group are coordinated by hydrogen bonds with the guandinium group of the catalytic arginine and by the backbone amide N−H groups of the active site loop. The movement of the flexible loop also brings a conserved aspartic acid into the active site where it acts as a general acid by protonating the tyrosine leaving group to facilitate its release (Figure 4). Step 2: the catalytic aspartic acid acts as a general base by abstracting a proton to activate a water molecule, which in turn attacks the thiol−phosphate intermediate. Hydrolysis of the intermediate results in the release of free phosphate and regeneration of the enzyme.

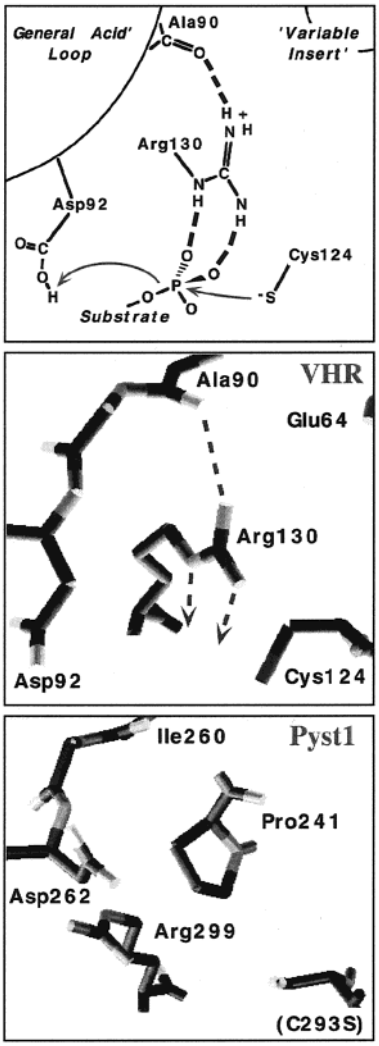


Figure 4. Catalytic mechanism for dual-specificity phosphatases and comparison of active site geometry of VHR and Pyst1. (top) Catalytic mechanism of enzyme−substrate [E−S] complex formation for dsPTPs. Amino acid numbering and structural labels correspond to human, VHR. Hydrogen bonds are indicated by dashed lines. (middle and bottom) Active site geometry of the DSPs, VHR (middle) and Pyst1 (bottom), based on crystal structure data (Brookhaven PDB: 1VHRa and 1MKP, respectively). Stick representations of side chains involved in [E−S] complex formation were generated using Swiss-Pdb Viewer.[253] Hydrogen bonds are indicated by dashed lines. The structure of Pyst1 was solved with a serine for cysteine substitution (C293S) at the active site.

Comparison of the crystal structure of the catalytic domains of Pyst1/MKP-3 and VHR yields clues about the mechanism of catalytic activation of MKPs by ERK binding (Figure 4). The Pyst1 structure adopts a typical protein tyrosine phosphatase (PTPase) fold with similarity to the dual-specificity phosphatase VHR.[82,83] However, the most striking feature of the structure is that it displays a distorted geometry in the absence of its substrate. First, the catalytic

aspartic acid residue (Asp262 in Pyst1) is not well positioned for coordinating the substrate phosphoryl group. Second, the catalytically essential aspartic acid (Asp262 in Pyst1) is displaced by almost 5.5 Å compared to VHR, indicating that it is unlikely to act as a general acid/base at this distance. Despite this structural disparity, the Asp262 residue in MKP-3 is likely involved in catalysis. The pH activity profiles of ERK-activated MKP-3 indicate the involvement of general acid catalysis.[164] In addition, mutation of Asp 262, located 5.5 Å distal to the active site, demonstrates that it is essential for the high-activity ERK2-dependent conformation of Pyst1 but not for the low-activity ERK2-independent form.[83] Thus, it appears that ERK binding induces a conformational change in the catalytic Asp general acid loop and stabilizes MKP-3 in its active conformation. This mechanism is supported by several lines of evidence. For instance, ERK-activated MKP-3 catalyzes substrate hydrolysis 100-fold more efficiently than MKP-3 alone and displays an enhanced affinity for substrate and oxyanions. Consistent with ERK stabilizing the active conformation of MKP-3, the chemical chaperone dimethyl sulfoxide was able to mimic this activation.[164,165] These biochemical analyses have suggested that ERK activates MKP3 by inducing closure of the catalytic "general acid" loop and the stabilizing of the active phosphatase conformation. In the active conformation, the loop residue participates efficiently in general acid/base catalysis, substrate binding, and transition-state stabilization.[166]

E. Relief of Autoinhibition vs Allosteric Activation Mechanism

Using NMR titration analysis, a specific but weak interaction (~20-fold less than that of N-terminal domain of MKP-3 and ERK2) between the N-terminal domain of MKP-3 and the C-terminal catalytic domain of PAC-1 (74% identical to MKP-3 catalytic domain) has been detected.[168] Solution structure and biochemical analysis show that the MKP-3 N-terminal domain essential for ERK2 binding partly overlaps with the region responsible for the interaction with its own C-terminal phosphatase domain. In SHP-2 tyrosine phosphatases, the N-terminal domain displays autoinhibitory function because it interacts intramolecularly with the C-terminal PTPase domain. Binding of the SH2 domain to phospho-Tyr-containing proteins or deletion of the N-terminal domain can relieve the autoinhibition in SHP-2.[167] Does the N-terminal domain of MKP have autoinhibitory functions? Structural and biochemical studies argue that the role of the N-terminal domain is not simply to suppress the activity of the catalytic domain via intramolecular interaction. In contrast to SHP-2, the interaction between the N-terminal domain and ERK2 may allosterically induce a conformational change of the C-terminal catalytic domain, resulting in the enzymatic activation of MKP-3.[168] Demonstration of such an allosteric activation mechanism for MKPs will require three-dimensional structures of the full-length MKP-3 both free and in complex with ERK2.

F. Physiological Functions of MKPs

With the accumulating evidence for the roles of MKPs in regulating MAPKs in vivo, one major direction for future study is to investigate how the functions of MKPs are integrated during cellular proliferation, differentiation, and signal transduction. One approach is to disrupt MKP genes in model organisms. In the mating pheromone response of *S. cerevisiae*, Fus3 MAPK appears to be negatively regulated by a the dual-specificity phosphatase, Msg5.[109] Overexpression of Msg5 suppresses the lethality of yeast *gpa1*Δ deletion, which constitutively activated the mating-pheromone response pathway. Consistent with its function in MAPK inactivation, deletion of *MSG5* results in elevated Fus3 kinase activity and compromises the ability of cells to recover from pheromone stimulation. Similar to mammalian MKPs, the expression of *MSG5* is transcriptionally induced upon stimulation. In addition to the regulation of the Fus3 MAPK pathway, Msg5 has also been implicated in the negative regulation of the Mpk1 pathway, which functions in maintaining cell wall integrity and hypotonic stress response.[169,170] Therefore, MKPs may provide an evolutionarily conserved negative feedback mechanism for MAPK down-regulation from *S. cerevisiae* to mammals.

In *S. pombe*, pmk1, a homologue of *S. cerevisiae* Mpk1, regulates cell wall integrity.[171] This novel MAPK pathway is also involved in cytokinesis, resistance to Cl^- ion, and possibly cell volume control. A dual-specificity phosphatase, *pmp1*, has been identified to regulate this pathway by directly dephosphorylating and inactivating the pmk1 MAPK, providing the first evidence for a dual-specificity phosphoatase in MAPK regulation in *S. pombe*.[172]

An interesting function has been assigned to Cpp1p DSP, a homologue of Msg5, to regulate the virulence of *Candida albicans*, an opportunistic pathogenic fungus.[173] Transition from the yeast form (blastospores) to the hyphal form is induced by environmental factors and contributes to early steps in invasion of epithelial tissues.[174] The developmental transition is regulated by a MAPK pathway, and related signaling cascades have been identified to play an analogous role in regulating mating and virulence in the plant fungal pathogen *Ustilago maydis* and the human fungal pathogen *Cryptococcus neoformans* (reviewed in ref 175). Although a direct demonstration of Cpp1 inactivating the Cek1p MAPK has not been established, genetic evidence suggests that the Cpp1 DSP represses the hyphal transition in *Candida albicans*, presumably by inactivating MAPK.[173] Interestingly, in an animal model, *cpp1* null mutant *C. albicans* showed a dramatic reduction of virulence in addition to the repression of hyphal growth.[173] It is currently unclear what is the molecular mechanism for the requirement of Cpp1 for the virulence. YopH tyrosine phosphatase has been shown to play a role in the pathogenesis of the bacterial genus *Yersinia*. These observations indicate that tyrosine phosphatase could be a potential target for treatment of infectious bacterial and fungal diseases.

The *Drosophila* gene, *puckered*, was identified as a negative regulator in the fly JNK pathway.[176−178]

It encodes a new member of the MKP family.[176−178] Mutations in this gene lead to cytoskeletal defects that result in a failure in dorsal closure, a phenotype which is related to those associated with mutations in *basket*, the Drosophila JNK homologue.[179,180] Basket is hyperactivated in *puckered* mutants, and conversely, gain-of-function of *puc* mimics *basket* loss of function mutant phenotypes. These observations demonstrated that the *puckered* phosphatase is likely to be a direct inactivator of the *basket* JNK. In addition, the *puckered* gene expression itself is upregulated as a consequence of the activation of the JNK pathway, thereby providing negative feedback control in a fashion similar to mammalian MKPs.

The effect of MKP-1 gene disruption has been studied by Dorfman et al.[135] MKP-1-deficient mice are born at normal frequency, are fertile, and present no phenotypic or histological abnormalities. Mouse embryonic fibroblasts lacking MKP-1 protein display normal ERK activity and induction of c-fos mRNA, indicating no alteration of the MAP kinase pathway. In addition, MKP-1-deficient MEFs grow and enter DNA synthesis at the same rate as control cells.[135] These results demonstrate that MKP-1 is not essential for embryonic development, and the lack of MKP-1 activity can be compensated for by other phosphatases in vivo. However, given the later observation that MKP-1 preferentially inactivates JNK/SAPK and MKP-1 gene expression is induced by oxidative stress or heat shock, it might be informative to examine whether the MKP-1-deficient mouse displays any abnormality in cellular stress response.

G. New Twist: Function of Styx Proteins

The phosphatases involved in MAPK dephosphorylation include a diverse collection of tyrosine-specific and dual-specificity phosphatases. Remarkably, despite the limited sequence identity within the entire PTPase superfamily, all PTPases contain elements of a conserved catalytic core structure[181] and also share a catalytic mechanism involving the active site motif CxxxxxR (Cx_5R) and a variably positioned aspartic acid residue.[182] As mentioned above, a cysteine thiolate makes the nucleophilic attack on phosphate that leads to its hydrolysis,[182] whereas the Arg and Asp play direct roles in the formation, stabilization, and breakdown of catalytic intermediates.[182] Structural determinants for targeting and substrate specificity reside outside of these residues since single mutations of the catalytic Cys, Arg, or Asp do not abolish PTPase:substrate interaction.[76,80,132,183−186] In fact, mutation of the catalytic Cys or Asp is often used to stabilize PTPase:substrate interactions in vivo[76,80,132,183−186] and forms the basis of PTPase:substrate trapping strategies.[185,186]

With these in vitro substitutions in mind, it was intriguing to find a naturally occurring Cys substitution in the active site of a mouse phosphatase-like protein, Styx.[187] In contrast to all PTPases, Styx contains a Gly in place of the active site Cys that renders it catalytically inactive as a phosphatase.[187] Conversion of this Gly to Cys (G/C), which structurally mimics the DSP active site, confers phosphatase activity to this molecule such that Styx(G/C) hydrolyzes artificial substrate phosphotyrosine with comparable activity to native DSPs.[187] Moreover, Styx(G/C) dephosphorylates both phosphotyrosine and phosphothreonine from biologically relevant diphosphorylated (pT-x-pY) peptides from MAPKs. Thus, native Styx possesses all the structural components necessary for phosphorylated substrate interaction but not substrate hydrolysis. Since no active paralogue exists, Styx has the distinction of being the first example of a naturally occurring binding/interaction protein structurally related to DSPs.[187,188]

To demonstrate the physiological importance of Styx, gene targeting was used to create to a loss of Styx protein function in mouse (Wishart and Dixon, manuscript in preparation). Male mice homozygous for a disrupted *Styx* allele are infertile, being devoid of normal epididymal sperm, and exhibit a derangement of the orderly differentiation of round spermatids into spermatozoa. Co-immunoprecipitation of Styx with a unique RNA-binding protein suggests that together they may regulate a translational checkpoint governing this process. Collectively these findings both identify *Styx* as a candidate fertility gene in men and fundamentally establish STYX/like-phosphatase domains as important components of biological systems.

Interestingly, the single modular domain structure of Styx suggests that the STYX/pseudo-phosphatase domain could be the functional prototype for non-catalytic regions of other proteins within the PTPase superfamily. This potential has been realized in an assortment of noncatalytic Styx-like domains in organisms as diverse as flies, worms, yeast, and viruses. Database searching has revealed a unique family of Styx-like proteins in human, mouse, *xenopus*, and zebrafish that are similar to the MKPs. The N-terminus of MAPK-directed Styx (MK−Styx) contains conserved CH2 regions; however, a Ser for Cys substitution in its active site suggests that MK−Styx is not catalytically active. Exon mapping in humans, mice, and zebrafish demonstrates that MK−STYX shares a common gene structure with MKPs. Although the function of MK−Styx is currently under investigation, it interacts with MAPKs in vitro in a manner analogous to MKPs (Wishart and Dixon, personal communication).

V. Regulation of MAP Kinases by Tyrosine-Specific PTPases

There is an increasing amount of evidence suggesting that the dual-specificity MKPs are not the only physiologically relevant phosphatases for MAPK inactivation. In several cell lines, such as PC-12, PAE, or 3T3-L1 cells, there is a discrepancy between the kinetics of ERK inactivation and the time course of MKP induction, suggesting the existence of additional MAPK-directed phosphatases.[189,190] In these cells the serine/threonine protein phosphatase 2A (PP2A) and an unidentified tyrosine-specific phosphatase function coordinately in the rapid inactivation of ERK.[190−192] The existence of multiple inactivation mechanisms might be necessary for the elaborate regulation of MAPKs, yet the identities of

tyrosine-specific PTPases involved in MAPK regulation are not fully resolved.

A. PTPases in the Regulation of Stress-Activated MAP Kinases in Yeast

The first evidence that intracellular phosphatases regulate MAPK pathways came from genetic analysis of *S. cerevisiae* high-osmotic-stress response pathway. Double deletion of a PP2C serine/threonine phosphatase, *PTC1*, and an intracellular tyrosine phosphatase, *PTP2*, results in lethality of the yeast cells. The lethality of *ptc1*Δ*ptp2*Δ double deletion cells can be rescued by deleting components in the Hog1 MAPK pathway,[31,73] implying that in the double deletion cells the Hog1 pathway is constitutively activated. Subsequent genetic and biochemical studies[70,75] have revealed that Hog1 is regulated by Ptp2 and its closely related homologue, Ptp3. Of these two enzymes, Ptp2 is the major regulator of Hog1 while Ptp3 plays a more limited function (Figure 5).

In searching for additional substrates of Ptp2 and Ptp3, it was observed that Ptp2 and Ptp3 could inactivate another yeast MAPK, Mpk1, in the cell wall integrity pathway.[193] In vivo and in vitro evidence indicates that Ptp2 is the more effective negative regulator. For example, growth defects due to constitutive activation of this pathway can be suppressed by overexpression of *PTP2* but not *PTP3* while deletion of *PTP2* but not *PTP3* exacerbated the growth defects. In addition, purified Ptp2 was more efficient than Ptp3 at binding and dephosphorylating Mpk1 in vitro. Moreover, *PTP2* transcription but not *PTP3* increased in response to heat shock in a Mpk1-dependent manner, suggesting that Ptp2 acts in a negative feedback loop to inactivate Mpk1.[193]

In *S. pombe*, Sty1/Spc1, the functional equivalent of the *S. cerevisiae* Hog1 and mammalian SAPK2, is activated by high osmotic-stress, oxidative stress, heat shock, and UV irradiation. Activation of the Spc1 pathway is crucial for survival under various

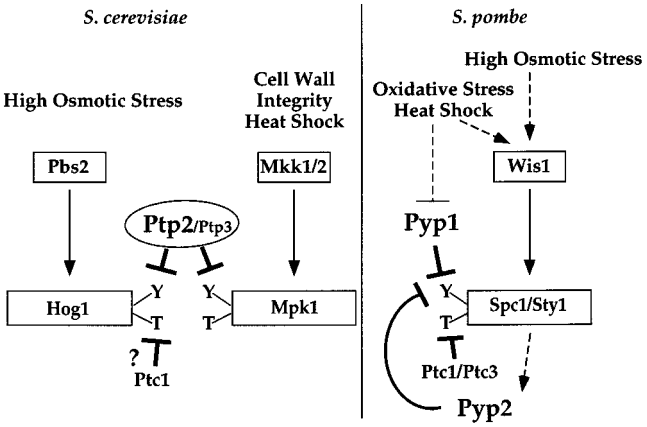


Figure 5. Model for the regulation of Hog1 and Mpk1 in *S. cerevisiae* and Spc1/Sty1 in *S. pombe* by protein phosphatases. Ptp2 plays major role in Hog1 and Mpk1 regulation in *S. cerevisiae*. A Ser/Thr phosphatase of PP2C family, Ptc1, may also regulate Hog1. In *S. pombe* stress response pathway, the tyrosine phosphorylation of Spc1/Sty1 is regulated by a constitutively expressed tyrosine-specific phosphatase Pyp1 and an inducible Pyp2. Ptc1 and Ptc3, two members of PP2C family, dephosphorylate Thr residue and negatively regulate Spc1/Sty1.

forms of stress.[194] Disruption of pyp1 and pyp2 results in cell lethality.[195] Isolation of spontaneous recessive mutations that bypass the requirement for pyp1 and pyp2 have identified sty1/spc1 SAPK and sty2/wis1 MKK.[77] Genetic and biochemical evidence have shown that Pyp1 and Pyp2 dephosphorylate and inactivate Sty1/Spc1.[77] The expression of *pyp1* is constitutive, while *pyp2* transcription is up-regulated in response to stress, and transcriptional induction is dependent on the activation of Spc1 pathway. Thus, similar to Hog1, Sty1/Spc1 is negatively regulated by tyrosine-specific phosphatases, Pyp1 and Pyp2. Recent studies have suggested that the Sty1 may be activated through a different mechanism in response to osmotic stress and heat/oxidative stress. The phosphorylation of Wis1 (MKK for Sty1/Spc1) and presumably the activation of Wis1 is essential for activation of Sty1/Spc1 by osmotic stress, whereas it is dispensable for heat shock and oxidative stress response. The later stimulation seems to inhibit the binding of Pyp1 to Sty1/Spc1, thus leading to activation.[196−199] However, it is unclear whether the inhibition of Pyp1 is solely responsible for Sty1/Spc1 activation and what modification prevents the interaction between Pyp1 and Sty1/Spc1.

B. Inactivation of Fus3 by Ptp3 and Msg5

Studies in the yeast pheromone response pathway provided the first evidence that there is an intricate MAPK regulatory network comprised of both subfamilies of PTPases. The dual-specificity phosphatase Msg5 was originally identified as an inactivating phosphatase for Fus3 MAPK in this pathway.[109] However, in yeast cells lacking the *MSG5* gene, inactivation of Fus3 MAPK in the mating pheromone pathway is impaired but not abolished, suggesting that an additional phosphatase may also be involved in the down-regulation of this pathway. Genetic screens for negative regulators of Fus3 pheromone response pathway have identified the tyrosine-specific phosphatase Ptp3 as a Fus3 regulator.[71] When both *PTP3* and *PTP2* genes are disrupted, cells display a defect in recovery from pheromone stimulation. The triple disruption of *PTP2*, *PTP3*, and *MSG5* results in a profound defect in Fus3 inactivation, suggesting overlapping roles of these PTPases in Fus3 regulation.[71] However, biochemical and genetic analyses indicate that these phosphatases make distinct contributions toward Fus3 inactivation. Constitutively expressed Ptp3 is responsible for dephosphorylation of Fus3 to prevent ectopic activation of the kinase in the absence of pheromone. The function of Ptp3 is shared partially by Ptp2. In contrast, the pheromone-induced Msg5 expression contributes to the inactivation of Fus3 postpheromone stimulation, thus providing negative feedback regulation that is analogous to mammalian MKPs.[71] These studies demonstrate the intricacy of coordinated regulation of MAPK function by both tyrosine-specific and dual-specificity phosphatases, each exerting differential regulation through a similar mechanism: dephosphorylation of Fus3 (Figure. 6).

It is interesting that Ptp2 and Ptp3 display a substrate preference for Hog1 in the osmotic-stress

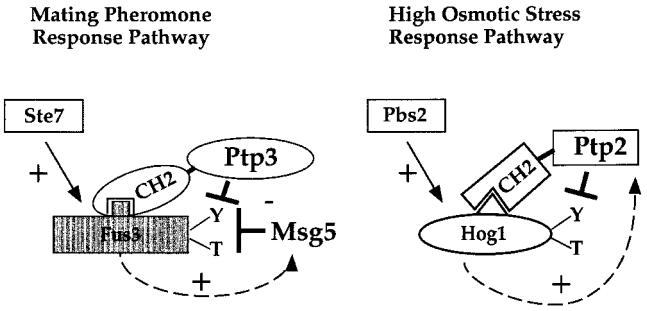


Figure 6. Model for the regulation of Fus3 and Hog1 by protein phosphatases in *S. cerevisiae* and determination of substrate selectivity. The Fus3 MAPK is regulated by the coordinated actions of tyrosine-specific PTPases Ptp3 and dual-specificity phosphatase Msg5. Ptp3 plays more important roles in the regulation of Fus3 Tyr phosphorylation than Ptp2. Ptp3 regulates tyrosine phosphorylation levels of Fus3p both in the absence of and following pheromone stimulation, while Msg5 is only induced following pheromone stimulation. Ptp2 plays a major role in Hog1 and Mpk1 regulation. Ptp3 specifically interacts with its physiological substrate, Fus3, via CH2 domains at the amino terminus. Such mechanism may also be employed by Ptp2. The formation of PTPases and MAPKs complex provides a molecular basis for substrate selectivity.

response pathway and Fus3 in the pheromone response pathway, respectively. In a structure−function study of Ptp3, a cryptic CH2 domain was found in the amino-terminal noncatalytic domain.[200] It is the CH2 domain, rather that the tyrosine phosphatase domain, that directly interacts with Fus3. Through genetic and biochemical analysis, it has been established that formation of a complex between Ptp3 and Fus3 is essential for Fus3 MAPK regulation. Point mutations in the conserved residues within the CH2 motif of Ptp3 or mutation in a highly conserved Asp residue in Fus3 can abolish this interaction and result in dysregulation of Fus3 MAPK tyrosine phosphorylation and activity. In addition, swapping the N-terminal domains of Ptp2 and Ptp3 alters their substrate preference in vivo.[200] Therefore, the specific targeting of a tyrosine phosphatase Ptp3 to its physiological substrate, Fus3 MAPK, via the conserved N-terminal CH2 domain is required for MAPK regulation and determination of phosphatase substrate specificity in vivo (Figure 6).

C. Roles of PTPases in ERK Regulation

The first evidence that mammalian tyrosine-specific PTPases, STEP (striatal-enriched phosphatase),[201] and PTP−SL (STEP-like phosphatase)[202−204] were involved in MAPK regulation was reported in 1998[205,206] (reviewed in ref 207). PTP−SL and STEP are two related, nonnuclear PTPases which exist in transmembrane and cytosolic forms and are mainly expressed in neuronal cells. These PTPases dephosphorylate the regulatory phosphotyrosine residues of ERK1/2 and inactivate them in vitro and in vivo. Similar results were obtained for PTPBR7, a closely related phosphatase.[208] A segment of 16 amino acids in PTP−SL is necessary and sufficient for ERK1/2 binding and has been termed the kinase interaction motif (KIM) (residues 224−239). Upon binding, the N-terminal domains of PTP−SL and STEP can be phosphorylated by ERK1/2 at Thr253. The physiological significance of this phosphorylation is unclear, but it is possible that phosphorylation can modulate the affinity of KIM for ERK.[205,206] When the KIM is deleted, the ability of PTP−SLΔKIM to bind and dephosphorylate ERK is impaired.

The function of PTP−SL/STEP in ERK regulation seems to be evolutionarily conserved. A *Drosophila* homologue, protein tyrosine phosphatase-ERK/enhancer of Ras1 (PTP-ER), has been isolated as a negative regulator of Ras1 signaling in R7 photoreceptor cell differentiation.[209,210] PTP-ER mutants produce extra R7 cells and enhance activated Ras1 signaling, and ectopic expression of PTP-ER dramatically inhibits RAS1/Rolled MAPK signaling. PTP-ER localizes to the cytoplasm. It can bind to and inactivate *Drosophila* ERK encoded by the *rolled* gene; however, PTP-ER is unable to dephosphorylate and down-regulate the gain-of-function mutant Rolled MAPK (encoded by *Sevenmaker* allele).[209]

Two related lymphoid-specific PTPases, HePTP (hematopoietic PTP) and LC−PTP (leukocyte PTP), are strongly expressed in activated T-cells. It has been demonstrated that transfection of HePTP impairs TCR-mediated activation of the MAPK family members ERK2 and p38 but not JNK.[211−213] Catalytically inactive HePTP mutants (trapping mutants) binds ERK2 in a tyrosine phosphorylation-dependent manner, indicating that HePTP is specifically targeted to activated ERK2. Similar to STEP/PTP−SL, KIM sequences reside at the N-terminus and are also required for substrate interaction in addition to the PTPase domain. Deletion or point mutations that abolish ERK binding also result in impaired ERK inactivation. HePTP gene is mapped to human chromosome 1q32.1, where abnormalities are frequently found in hematopoietic malignancies. HePTP is highly expressed in acute myeloid leukemia,[214,215] suggesting a role for HePTP/LC−PTP in the growth control and differentiation of lymphocytes.[216]

D. Utilization of Multiple MAPK Interaction Motifs by Tyrosine Phosphatases

Collectively, studies from yeast, fly, and mammalian culture systems have demonstrated roles for tyrosine-specific PTPases in MAPK regulation. A common theme among tyrosine-specific PTPases is that they utilize binding motifs outside the catalytic domain to specifically interact with their kinase substrates. The formation of PTPase/kinase complex contributes to the specificity and efficiency of the PTPases in MAPK regulation. It is becoming an emerging paradigm that both dual-specificity MKPs and tyrosine-specific PTPase use separate domains, catalytic phosphatase domain and MAPK targeting domains, to achieve substrate specificity toward different MAPKs. It is intriguing that several different MAPK binding motifs are utilized by PTPases. Yeast Ptp2 and Ptp3 use the CH2 domain,[200] which is found in mammalian dual-specificity MKPs, while the mammalian STEP/PTP−SL/PTP−ER/HePTP family utilizes the KIM motifs. It is tempting to speculate

that these PTPases have evolved in a modular fashion, in which a variety of MAPK binding motifs combine with a core PTPase catalytic domain to create enzymes specific for MAPKs

Despite no similarity between CH2 and KIM at the primary sequence level, they may recognize the same key sites on MAPKs. Point mutations on a highly conserved Asp residue (Asp319 on ERK2) MAPKs, including ERK1/2,[136,161] fly *rolled* MAPK(*sevenmaker* allele),[209] p38,[162] and Fus3,[200] abolished binding to either CH2 or KIM, consequently, rendering MAPKs insensitive to dephosphorylation by PTPases. Using a series of p38/ERK chimeric molecules, ERK kinase subdomains V-XI was identified as the region that is necessary and sufficient for binding and catalytic activation of MKP-3.[217] These domains constitute the major COOH-terminal structural lobe of ERK, and two aspartic acids (Asp319 and 322 in ERK2) in this domain are essential for docking. This docking domain serves as a common site for recognition of their activators, substrates, and regulators, including MEK1, the MAPK-activated protein kinase MNK1, and the MKP-3.[152] A corresponding domain in the p38 and JNK/SAPK also serves as a common docking site for their MEKs, MAPK-activated protein kinases, and MKPs.[152,217]

In an interesting twist, examination of the MKP-3 primary amino acid sequence reveals that a pentapeptide sequence IMLRR (amino acids 61−65) localized within the CH2 domain is loosely related to the KIM motif, which includes within its core the sequence LQERR in repeats.[217] Mutation of the arginine residues within this motif abolishes binding of MKP-3 to ERK.[218] Consistent with this, the MKP-3 mutant (R65A) and particularly MKP-3 (R64A, R65A) are insensitive to catalytic activation by ERK2.[217] In a detailed biochemical analysis of the molecular basis for the specific ERK2 recognition by MKP-3 and the ERK2-induced MKP-3 activation, it has been shown that specific ERK2 recognition by MKP3 involves multiple regions of MKP3.[219] The KIM sequence (residues 61−75) in MKP-3 and a unique sequence (residues 161−177 in MKP3) conserved in cytosolic MKPs (including MKP-3, 4 and MKP-X) are important for the high-affinity ERK2 binding. Similar mutation of the positively charged residues within the KIM motif in MKP-1 abolishes only the binding and activation of MKP-1 by ERK2 and p38a but not JNK, indicating that this is one of the distinct binding determinants for these MAP kinase isoforms.[160] However, these two regions are not essential for ERK2-induced MKP3 activation. Instead, ERK2-induced MKP3 activation requires a third ERK2 binding site (a DEF motif) localized in the C-terminus of MKP-3 (residues 348−381).[219] These studies suggest that multiple protein−protein interaction motifs such as CH2, KIM, and DEF may be woven into a common interface on DSPs and tyrosine phosphatases to contact for the ERK COOH-terminal lobe. These motifs contribute to the specific MAPK recognitions and/or couple the MAPK binding to the catalytic activation of corresponding phosphatases. Structural studies of MAP kinases and PTPases complex are now eagerly awaited to provide further clarification on the molecular basis for tight and specific interaction between these two important families of proteins.

VI. Regulation of Phosphatases

In the past, the relative numbers of identified kinases and phosphatases have lead to the view that phosphatases may act constitutively in a hit-and-run fashion, with little regulation of catalytic activity or specificity for distinct substrates. As described in this review, it is now better appreciated that some dual-specificity and tyrosine-specific phosphatases are highly specific for different members of MAPK family. One of the mechanisms to achieve the specificity is by selective interactions between PTPases and their substrates. In the case of some MKPs, such interaction also stabilizes MKPs in an active conformation and results in their catalytic activation. There is also an increasing amount of evidence that phosphatases are also regulated at both transcriptional and post-translational levels, including phosphorylation, protein degradation, and reversible oxidation of active site cysteine (Figure 7).

A. Transcriptional Regulation

Many members of MKPs display a restricted pattern of tissue expression. For instance, hVH-5 is expressed predominantly in brain, heart, and skeletal muscle. MKP-4 mRNA is detected only in the placenta, kidney, and embryonic liver. PAC1 is enriched in hematopoietic cells, while MKP-3 has broad tissue distribution (summarized in Table 1). In addition, even within the same tissue, detailed analysis of the expression patterns of MKP-1, MKP-X, MKP-3/PYST1, and hVH-3/B23 in rat brain by in situ hybridization revealed that these MKPs exhibit different temporal expression patterns.[220] Thus, tissue-specific expression may play a critical role in fine-tuning MAPK activity in a cell-type-specific manner.

In addition to the tissue-specific expression, MKPs are also under tight transcriptional control. In fact, CL100/MKP-1 and PAC1 were initially identified as immediate early gene induced in cultured cells by mitogens, thermal, or oxidative stress.[130,139] Some MKPs and tyrosine-specific phosphatases are induced by the same stimuli that activate MAPK. For example, MKP-3 is induced by NGF in PC-12 cells and HePTP expression is up-regulated by TCR activation. In some cases, the induction of MKPs requires activation of a specific MAPK pathway. For instance, mating pheromone triggers activation of Fus3 MAPK in *S. cerevisiae*. As a consequence of Fus3 activation, Msg5 expression is induced, which can inactivate Fus3.[109] Similarly, the transcriptional induction of *PTP2* in response to heat shock is dependent on Mpk1.[193] Another example is in the *Drosophila* Basket (JNK) pathway, which regulates the expression of Puckered MKP.[221] In cultured mammalian cells, the induction of CL100/MKP-1, hVH2/MKP-2, and PAC-1 has been reported to be dependent, at least in part, on the activation of MAPK pathway.[222−224] Thus, one aspect of the transcriptional regulation of these phosphatases is to provide nega-

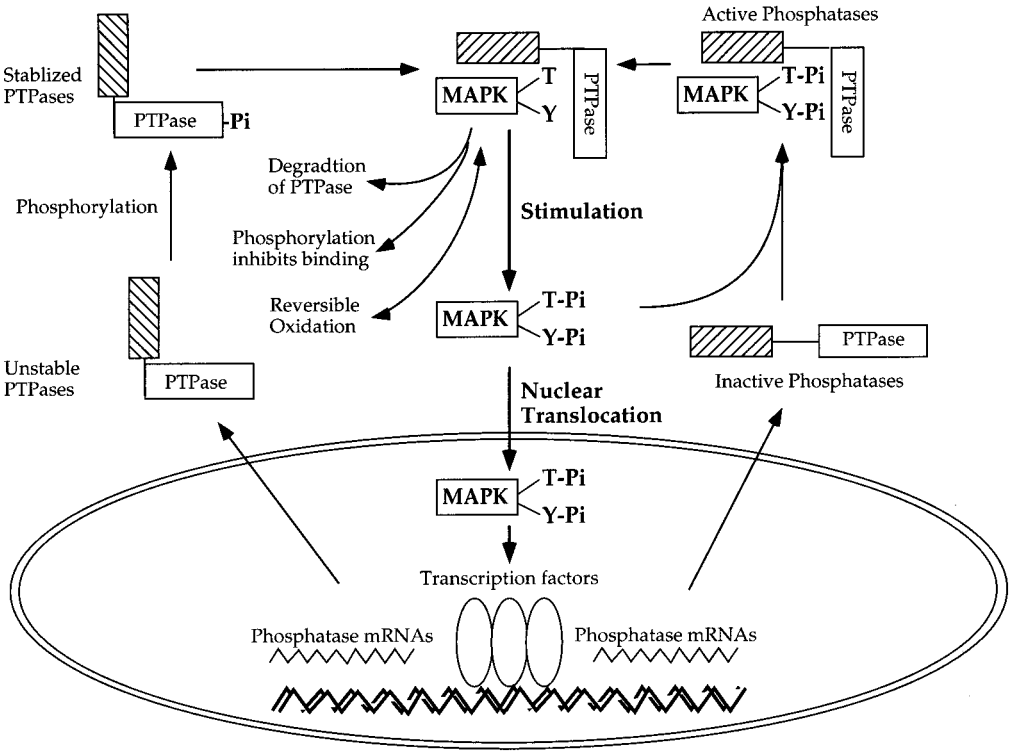


Figure 7. Diverse regulations of PTPases. Protein tyrosine phosphatases involved in MAPK regulation are controlled by diverse mechanisms. At the transcriptional level, the expression of a subset of PTPases is induced by a variety of stimuli. PTPases are also regulated at the posttranslational level. PTPases can form complexes with MAPK, and association with MAPK can lead to catalytic activation. PTPases also can be phosphorylated by PKA or MAPK. Phosphorylation can either result in inhibition of MAPK binding or stabilization of PTPases. Last, PTPases can be reversibly inactivated by oxidation of active site Cys residue according to the cellular redox state.

tive feedback controls to restrict the time course of MAPK activity. The mechanisms of transcriptional control of MKPs are likely to be complex. Upon seizure, expression of MKP-1, MKP-X, MKP-3/PYST1, and hVH-3/B23 are differentially up-regulated at distinct brain areas.[220,225] A potential physiological significance of transcriptional control is that induction of a subset of phosphatases by one particular signaling pathway including the MAPK pathway may, in turn, down-regulate parallel MAPK pathways, thus preventing undesirable cross-talk. During hypertrophic growth of cardiac myocytes, it has been shown that calcineurin can enhance MKP-1 expression and p38 MAPK inactivation.[226] It will be interesting to determine whether such PTPases-mediated mechanisms exist as a paradigm to achieve selective activation of specific MAPKs.

B. Intracellular Localization

It has been well documented that activated MAPKs can translocate from the cytosol to the nucleus. MKPs and tyrosine-specific phosphatases also display distinct subcellular localization. CL100/MKP-1, PAC1, hVH-2/MKP-2, hVH3, and VHR are localized in the nucleus, whereas MKP-3/PYST1 appears to be exclusively localized in the cytosol. Putative nuclear localization motifs have been implicated within the amino terminus of CL100/MKP-1, hVH-2/MKP-2, and hVH-3/B23.[143] MKP-3/PYST1 and PYST2/B59 carry potential nuclear export motifs.[147,150] Other members of MKPs can be present in both the nucleus and cytosol. Interestingly, PTP−SL and STEP can exist in both transmembrane and cytosolic forms through alternative splicing of exons encoding a single transmembrane domain.[227] Both the membrane-bound and cytosolic forms can down-regulate ERK2 activity, suggesting that these enzymes might have access to distinct pools of ERK2 within the cell. In *S. cerevisiae*, Ptp2 tyrosine-specific phosphatase is found in the nucleus whereas Ptp3 is localized in cytosol.[193,228] The existence of PTPases in distinct subcellular compartments may provide a mechanism to differentially inactivate MAPKs within these subcellular compartments, therefore ensuring specific spatial regulation of MAPKs.

Recent studies have suggested that PTPases may contribute to the regulation of subcellular localization of MAPK. It has been shown that expression of a catalytically inactive form of cytoplasmic MAP kinase phosphatase (MKP-3/Pyst-1) sequesters ERK1/2 in the cytoplasm.[229] Coexpression of PTP−SL and ERK2 in COS-7 cells can also result in the retention of ERK2 in the cytoplasm in a KIM-dependent manner.[205] In *S. cerevisiae*, Hog1 MAPK transiently accumulates in the nucleus upon activation.[228,230] Unexpectedly, when the nuclear protein tyrosine phosphatase, Ptp2, is deleted, Hog1 nuclear accumulation is decreased. In contrast, a strain lacking the cytoplasmic Ptp3 showed prolonged Hog1 nuclear accumulation, suggesting that Ptp2 may act as a nuclear tether for Hog1, with Ptp3 acting as a cytoplasmic anchor. Consistent with this notion, *PTP2* overexpression sequestered Hog1 in the nucleus while *PTP3* overexpression restricted Hog1 to the

cytoplasm.[228] These results indicate that PTPase can regulate MAPK localization via direct protein associations. Since MAPKs phosphorylate cytosolic and nuclear substrates, modulating MAPK nuclear translocation constitutes an important regulatory step in MAPK signaling.[229]

C. Posttranslational Regulation, Phosphorylation, and Degradation

Studies of HePTP and PTP−SL regulation of ERK function support the notion that tyrosine phosphatases regulate MAPK localization. PTP−SL/STEP and HePTP binds to ERK1/2 and p38 through a KIM sequence in its noncatalytic amino terminus, and the their association maintains the kinases in an inactive dephosphorylated state. It has been demonstrated that a conserved serine residue with KIM (Ser231 in PTP−SL and Ser23 in HePTP) is phosphorylated by the cyclic-AMP-dependent protein kinase (PKA) in vitro and in intact cells. Phosphorylation of this Ser residue impairs its interaction with kinases, thereby allowing ERK1/2 and p38α to become Tyr-phosphorylated and capable of phosphorylating nuclear targets.[231,232] Consistent with the PKA-mediated release of MAPK from phosphatase, treatment of COS-7 cells with PKA activators or overexpression of the Cα catalytic subunit of PKA inhibited the cytoplasmic retention of ERK2 and p38α by wild-type PTP−SL but not by an unphosphorylatable PTP-SL S231A mutant.[232] These findings support the existence of a novel mechanism for tyrosine phosphatases to not only regulate the activity of MAP kinases, but also mediate crosstalk between the cAMP system and the MAPK cascade by controlling the localization of MAPK.

In addition to being phosphorylated by PKA, PTP−SL/STEP, PTP-ER, and HePTP are also phosphorylated by ERK. The phosphorylation site has been mapped to Thr253 on PTP−SL. The amino terminus of *Drosophila* PTP-ER contains additional MAPK interaction motifs, termed docking sites for ERK, FxFP motif (DEF). Such motifs are often found in close proximity to ERK phosphorylation sites in ERK substrate proteins and represent another evolutionarily conserved docking site that mediates high-affinity interactions between ERK and substrates.[233] Several MAPK phosphorylation consensus sites (PxS/TP sequence) are located around the DEF and KIM motifs in the N-terminus of PTP-ER.[209] The close proximity of these potential phosphorylation sites to the KIM motif implies that phosphorylation may modulate the interaction between PTP-ER and ERK.

Consensus MAPK phosphorylation sites are also present in MKPs. Remarkably, the phosphorylation of MKPs by MAPK seems to regulate the stability of MKP proteins. CL100/MKP-1 has short half-life.[133,134] MKP-1 is a labile protein in CCL39 hamster fibroblasts, where it is ubiquitinated and targeted to proteasome for degradation. MKP-1 can be phosphorylated in vivo and in vitro by p42/p44 ERK1/2 on two carboxyl-terminal serine residues, serine 359 and 364. This phosphorylation does not modify the MKP-1 intrinsic ability to dephosphorylate p44 ERK1; however, MAPK activation and increased MKP-1 protein stability was observed, suggesting that phosphorylation by ERK1/2 may stabilize MKP-1.[234] These findings illustrate an additional layer of complexity to shape the time course of MAPK activity by regulating MKP-1 degradation.

D. Reversible Oxidation of Active Site Cysteine Residue in PTPases

Numerous reports have shown that cellular redox status plays an important role in tyrosine phosphorylation-dependent signal transduction pathways. For instance, H_2O_2 and superoxide radical anion are transiently generated during growth factor stimulation and H_2O_2 production is concomitant with elevated tyrosine phosphorylation. Biochemical analysis has shown that hydrogen peroxide can rapidly inactivate several PTPases, including PTP1B, LAR (leukocyte antigen-related), and VHR, by selectively oxidating the catalytic cysteine thiolate of PTPases. This oxidation is specific to PTPases, and H_2O_2 displays no apparent inhibitory effect on serine/threonine phosphatases.[235,236] PTP1B can also be specifically and efficiently inactivated by the superoxide radical anion. The initial oxidative product, the Cys-215 sulfenic derivative, can easily be oxidized further to its irreversible sulfinic and sulfonic derivatives. This step is prevented by glutathionylation of the sulfenic derivative to form a *S*-glutathionylated PTP-1B, which can be reactivated by dithiothreitol or thioltransferase. Oxidation of the catalytic cystenine and glutathionylation of the cysteine sulfenic derivative constitute an efficient reversible regulatory mechanism for PTPases.[237,238] Since the balance of cellular tyrosine phosphorylation is determined by tyrosine kinases and tyrosine phosphatases, reversible inactivation of PTPases can shift the state of tyrosine phosphorylation. All PTPases utilize a catalytic active site Cys residue, and inactivation by reversible oxidation of Cys potentially represents a universal mechanism for PTPases regulation, including those involved in MAPK inactivation. Future analysis is necessary to determine whether such a mechanism operates under physiological conditions.

VII. Conclusions

In contrast to other protein kinases, MAPKs are unique in that catalytic activation and subcellular translocation require phosphorylation of both a threonine and a tyrosine residue within the activation loop TxY motif. Thus, MAPKs can be inactivated through dephosphorylation of one or both phosphorylated residues by a diverse collection of serine/threonine-specific, tyrosine-specific, or dual-specificity phosphatases. A large number of phosphatases have been identified that regulate MAPK. Although the details of orchestrating these events are only partially understood, various patterns of MAPK inactivation likely reflect differences in the temporal, spatial, and cell-specific activity of these phosphatases.

On the basis of a large body of work, a highly sophisticated network of MAPK regulation emerges which invokes distinct subgroups of phosphatases that act sequentially during different stages of MAPK

inactivation. In the absence of stimulation, MAPKs are bound with constitutively expressed cytoplasmic PTPases, which repress the basal activity of MAPKs. It is known that MAPKs can autophosphorylate within the TxY motif, and autophosphorylation can lead to some degree of activation. Kinetic analysis also has shown that Tyr-phosphorylated MAPK serves as a better substrate for MEK, so another potential functional consequence of autophosphorylation is that it may set the threshold for MAPK activation. Therefore, repressing basal phosphorylation via the PTPases constitutes an important step in MAPK regulation.

Upon stimulation, MAPK is phosphorylated and activated by MEK. Sustained activation requires dissociation of phosphatase from active MAPK. Phosphorylating MAPK binding sites on PTPases may be one mechanism to inhibit MAPK binding. Following stimulation, dephosphorylation and inactivation of MAPKs occurs within time frames spanning minutes to hours depending on the cell type and activating stimulus. Such differential temporal patterns of controlling MAPK activity are achieved by the combinatorial actions of distinct phosphatases: constitutive PTPases are responsible for the rapid inactivation phase, while inducible PTPases contribute to delayed inactivation. In pheochromocytoma PC12 cells, for example, ERK activation is attenuated within minutes of EGF stimulation whereas exposure to NGF leads to prolonged ERK activity.[190] Rapid ERK inactivation in PC12 cells has been attributed to threonine dephosphorylation by cytoplasmic PP2A and tyrosine dephosphorylation via an unidentified PTP.[190] Also, constitutively expressed forms of the tyrosine-specific HePTP/STEP/PTP−SL family have been shown to rapidly dephosphorylate and inactivate cytoplasmic ERKs.[206,213] Therefore, concerted actions of multiple PTPases specific for MAPKs are necessary to ensure a temporal regulation of MAPK activity. Since it is imperative to tightly regulate MAPK activity, MAPK regulation involves additional protein phosphatases. There is some evidence that serine/threonine-specific protein phosphatases, especially members from the PP2A[190,239−241] and PP2C family,[199,242−245] can dephosphorylate and inactivate MAPK. However, compared to tyrosine phosphatases for MAPKs, these enzymes are mainly cytosolic and constitutively expressed and generally do not appear to exclusively regulate MAPKs.

Despite an array of inactivating cytoplasmic phosphatases in the cytoplasm, under most conditions a significant fraction of activated MAPKs translocate to the nucleus. Thus, extracellular stimulation leads to phosphorylation of nuclear MAPK substrates and transcription of MAPK-inducible genes, including a subset of MKPs. Upon translation of their respective mRNAs, catalytically inactive MKP proteins translocate to subcellular locations in either the nucleus or cytoplasm where they regulate MAPKs as a form of negative feedback control. The constitutively expressed dual-specificity phosphatase, VHR, can also contribute to tyrosine-specific dephosphorylation and inactivation of ERK1/2 in the nucleus. Differential localization of phosphoatases provides a mechanism

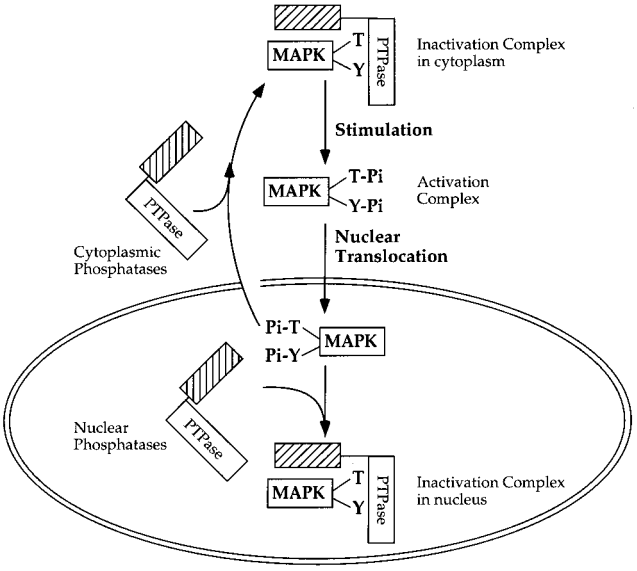


Figure 8. Models for MAPK regulation by PTPases. In resting cells, constitutively expressed cytoplasmic PTPases anchor MAPKs in the cytoplasm and repress the basal activity of MAPKs. Upon stimulation, MAPKs dissociate from PTPases and become phosphorylated and activated. A fraction of active MAPKs translocate into the nucleus to phosphorylate nuclear substrates. Following stimulation, inactivation of MAPKs occurs both in the cytoplasm and nucleus by discreetly compartmentalized PTPases.

for compartment-specific regulation of MAPKs. In addition to regulating the phosphorylation state of MAPKs, recent reports also suggest that tyrosine-specific phosphatases can also modulate the intracellular localization of MAPKs (Figure 8).

It has been demonstrated that phosphorylation-dependent homodimerization plays an important role in mediation nuclear translocation of ERK2. Given the high affinity of dual-phosphorylated ERK2 (K_d = ~7.5 nM), it is possible that all activated ERK2 will be dimerized.[246] It has been shown that the dual-phosphorylation of MAPKs is not necessary for between MAPKs:phosphatases interactions. Structural analyses suggest that the MAPK dimerization motif and phosphatase binding motifs are distributed on a distinct surface on ERK2,[168,246] arguing that dimerization might not affect MAPK and phosphatase interaction. One interesting implication is that an MKP might bind to an activated MAPK dimer and dephosphorylate its dimerization partner in trans. In addition, since divergent PTPases functioning in MAPK regulation share several common MAPK recognition motifs, it is tempting to speculate that upon formation of MAPK dimer, different PTPases might simultaneously associate with each MAPK in the dimer and exert dephosphorylation. Perhaps such convergent dephosphorylation mechanisms by divergent PTPases provide a combinatorial regulatory mechanism to the fine-tuning of MAPK activity.

In the past few years, formation of high-order protein−protein complexes during signal transduction has been increasingly appreciated. The existence of such complexes in MAPK signaling was first demonstrated in the yeast mating pheromone response pathway, in which a Ste5 scaffold protein tethers multiple components into a complex.[228,230]

Although no direct homologue of Ste5 has been identified in other model organisms, mammalian MAPK are similarly organized into various complexes through the function of potential scaffold proteins, such as MP1,[37] JIP1,[39] KSR,[247−250] SUR-8,[247−250] and β-arrestins.[251,252] The formation of higher order complexes or so-called "signalingsomes" may play a significant role in defining the specificity and ensuring signaling efficiency. Many PTPases involved in MAPK regulation can associate with their substrates. It is evident that disruption of PTPase:kinase interactions can result in ectopic activation of MAPKs, and there is also evidence that activation of MAPK by some stimuli is mediated by inhibition of PTPases.[196−199,232] Perhaps in vivo MAPKs exist either in an activation complex, which organizes components necessary for MAPK activation, and/or in an inactivation complex, which includes PTPases. Stimulation triggers activation of MAPK and dynamic redistribution of MAPK among different complexes. An emerging theme is that MAPK activity is tightly regulated by both positive (phosphorylation by MKK) and negative (dephosphorylation by PTPases) actions. Activated MAPK induces specific cellular responses and concomitantly activates a negative feedback loop through induction, activation, and stabilization of phosphatases. Therefore, a specific physiological response is achieved via the highly orchestrated actions of MAPKs and PTPases.

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