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Review

Mitogen-activated protein kinase phosphatase: a negative regulator of the mitogen-activated protein kinase cascade

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Abstract

Mitogen-activated protein kinases (MAPKs) are activated by various stimuli, such as growth factors, cytokines, or stress, and are considered to be important mediators in intracellular signal transduction networks. The dual-specificity kinases, MAPK kinases (MKKs), which phosphorylate the TXY motif in the catalytic domain of MAPKs, can cause the activation of MAPKs. Recently, a family of dual-specificity phosphatases has been identified, members of which are able to dephosphorylate and inactivate MAPKs. The studies cited in this review have revealed that these MAPK phosphatases might play an important role in various cellular functions by downregulating the MAPK cascade.

Keywords: Signal transduction; (Intracellular); MAPK (mitogen-activated protein kinase); Dual-specificity phosphatase; MAPK phosphatase

1. Introduction

Activation of the mitogen-activated protein kinase (MAPK) cascade is considered to play a key role in signal transduction pathways activated by various stimuli including growth factors, vasoactive peptides and cytokines. Three major subclasses of MAPKs, namely, extracellular signal-regulated kinase (ERK), c-*jun* NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK), and homologue of budding yeast HOG1 (p38 MAPK), have been identified. Full activation of these MAPKs requires the phosphorylation of both the threonine and tyrosine residues of the TXY motif in the catalytic domain by upstream dual-specificity kinases, termed MAPK kinases (MKK-1/2, MKK-3/6, and MKK-4 for ERK, p38 MAPK, and JNK/SAPK, respectively) (Guan, 1994; Seger and Krebs, 1995; Denhard, 1996; Kyriakis and Avruch, 1996; Robinson and Cobb, 1997). In order to understand the importance of MAPKs in subsequent cellular functions, it is necessary to clarify the mechanisms responsible for the activation and inactivation of MAPKs. For example, in PC12 cells, transient activation of ERK was found to cause cell proliferation, whereas sustained activation of ERK was shown to induce cell differentiation (Marshall, 1995), indicating the importance of the inactivation of ERK in the regulation of cellular functions. Recently, a family of dual-specificity protein phosphatases, which can dephosphorylate both phospho-threonine and phospho-tyrosine residues, has been identified and termed MAPK phosphatases (Guan, 1994; Keyse, 1995). In this review, we will discuss the general biochemical characters and physiological significance of these phosphatases.

2. Identification of a family of MAPK phosphatases

A highly conserved sequence, (I/V)HCXAGXXR(S/T)G, in a family of protein tyrosine phosphatase (PTPase) has been found to be essential for phosphatase activity (Denu et al., 1996) and is considered to determine the specificity of PTPase for the phospho-tyrosine residue in substrates. In 1991, Guan et al. used the conserved active site sequence of PTPase to search a database and cloned a new gene from vaccinia virus, vaccinia virus H1 phosphatase (VH1), which encodes a 20-kDa PTPase (Guan et al., 1991). VH1 does not show significant sequence homology to members of the PTPase family except for this conserved phosphatase active site. The recombinant protein of VH1 is able to dephosphorylate proteins containing

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phospho-serine, phospho-threonine, and phospho-tyrosine residues. Thus, VH1 is the first identified dual-specificity protein phosphatase which can dephosphorylate both phospho-tyrosine and phospho-serine/threonine residues.

Subsequently, Keyse and Emslie identified CL100 cDNA as an oxidative stress-inducible gene from the cDNA library of human skin fibroblasts (Keyse and Emslie, 1992). CL100 cDNA encodes a PTPase domain which has sequence homology to VH1. The amino acid sequence of CL100 is 96.5% identical to a murine protein (3CH134), which was originally identified as an immediate early gene induced by growth factors (Charles et al., 1992). The recombinant protein of CL100 was able to hydrolyze *p*-nitrophenyl phosphate, a chromogenic substrate structurally related to phospho-tyrosine, however, it could not dephosphorylate phosphorylated casein or myelin basic protein, which could be dephosphorylated by VH1. The only substrate of CL100 identified at that time was phosphorylated recombinant ERK2, which suggested that CL100 might be a phosphatase specific to MAPK in vitro (Alessi et al., 1993). Similar results were obtained from experiments in vitro with 3CH134 recombinant protein (Charles et al., 1993). Overexpression of 3CH134 was found to block oncogenic *ras*-induced activation of ERK in rat embryonic fibroblasts, REF-52 cells (Sun et al., 1994). Pretreatment with the protein synthesis inhibitor, cycloheximide, to block the de novo expression of 3CH134 proteins, was found to induce sustained ERK activation in NIH3T3 fibroblasts (Sun et al., 1993). The catalytically inactive mutant 3CH134, made by the replacement of cysteine by serine in the active site of phosphatase, was able to bind to ERK in transformed African green monkey kidney cells (COS cells) (known as a substrate-trapping phenomenon, Tonks and Neel, 1996), suggesting that 3CH134 (CL100) also functions as a MAPK phosphatase in vivo. On the basis of these results, 3CH134 (CL100) was renamed MAPK phosphatase-1 (MKP-1, Sun et al., 1993).

At the same time as when MKP-1/CL100 was identified, a mitogen-induced nuclear PTPase was isolated from human T cells (Rohman et al., 1993). This gene, PAC-1, has sequence homology to MKP-1 and the product is able to dephosphorylate ERK in vitro and in vivo (Ward et al., 1994). However, the pattern of mRNA expression of PAC-1 is unique. MKP-1 is widely expressed in various tissues and cell lines, whereas the distribution of PAC-1 mRNA is restricted to haematopoietic cells. These findings suggest the existence of a family of MAPK phosphatases. Currently, up to nine distinct mammalian MAPK phosphatases have been identified by polymerase chain reaction (PCR)-oriented cloning or by searching gene databases. These phosphatases are considered to be under tight transcriptional regulation and to be localized in distinct tissues, cells, or subcellular compartment (Table 1 of Noguchi et al., 1993; Zheng and Guan, 1993; Ishibashi et al., 1994; Guan and Butch, 1995; King et al., 1995 Kwak et al., 1995; Martell et al., 1995; Misra-Press et al., 1995; Groom et al., 1996; Mourey et al., 1996; Muda et al., 1996a; Theodosiou et al., 1996; Muda et al., 1997; Shin et al., 1997).

3. Substrate specificity of MAPK phosphatases

In order to understand the role of MAPK phosphatases in various cellular functions, it is necessary to clarify the substrate specificity of these MAPK phosphatases for members of the MAPK family, namely, ERK, JNK/SAPK, or p38 MAPK. The recombinant phosphatases show low substrate specificity for MAPKs in vitro, except for MKP-3/pyst1/rVH6 and hVH-5/M3-6, which are highly specific in their inactivation of ERK or JNK/SAPK and p38

Table 1
The characterization of members of the mammalian MAP kinase phospatase family

Gene	Substrate specificity	Intracellular localization	Remarks
MKP-1/hVH1/3CH134/CL100/erp	ERK = JNK/SAPK = p38MAPK	Nucleus	Prototype of MAP kinase phospatase family
PAC-1	ERK = p38MAPK > JNK/SAPK	Nucleus	Hematopoetic cell-specific phospatase
MKP-2/hVH-2/TYP-1	ERK = JNK/SAPK > p38MAPK	Nucleus	
hVH3/B23	N.D.	Nucleus	
MKP-3/rVH6/PYST1	ERk ≫ JNK/SAPK = p38MAPK	Cytosol	Cytoplasmic MAP kinase phospatase highly specific for ERK, binding with ERK
hVH5/M3-6	JNK/SAPK = p38MAPK ≫ ERK	Cytosol nucleus	Containing the proline-rich sequence, expression mainly in adult brain, heart, and skeletal muscle
MKP-4	ERK > JNK > SAPK = p38MAPK	Cytosol > nucleus	Binding with ERK, JNK/SAPK and p38MAPK, expression only in placenta, kidney and embryonic liver
B59	N.D.	N.D.	Inhibition of *ras* or v-*raf* induced transformation in NIH3T3
MKP-X/PYST2	N.D.	N.D.	only partial cDNA is cloned

N.D. = not determined.

MAPK, respectively (Chu et al., 1996; Groom et al., 1996; Muda et al., 1996a,b) (Table 1). Recently, VH-1-like phosphatase was identified from *Drosophila* and was found to show high specificity for *Drosophila* and mammalian JNK/SAPK in vitro (Martin-Blanco et al., 1998). To evaluate substrate specificity in vivo, Franklin et al. established the U937 human leukemia cell line that conditionally expresses MKP-1 from the human metallotionein IIa promoter. The MKP-1 expressed in this cell line preferentially inhibits p38 MAPK and JNK/SAPK relative to ERK (Franklin and Kraft, 1997). They also showed that MKP-1 has cytoprotective effects against UV-induced apoptosis by inhibiting UV-induced JNK/SAPK activation (Franklin et al., 1998). Other groups have also reported that MKP-1 inactivates JNK/SAPK rather than ERK in UV-stimulated PC12 cells (Hirsch and Stork, 1997). However, the substrate specificity of MAPK phosphatases is still controversial.

4. Mechanism of the inactivation of ERK by MAPK phosphatases and other phosphatases

Upon activation, ERK translocates to the nucleus (Chen et al., 1992; Gonzalez et al., 1993; Lenormand et al., 1993; Zheng and Guan, 1994), where it can phosphorylate transcriptional factors, such as Elk-1, and induce the expression of various genes (Gille et al., 1992; Marais et al., 1993; Nakajima et al., 1993; Gille et al., 1995; Hill and Treisman, 1995; Sugimoto et al., 1997). Since MKP-1 is reported to be mainly expressed in the nucleus, a simple model is evoked by which the growth factor-induced activation and nuclear translocation of ERK can induce MKP-1 expression in the nucleus, and then MKP-1 can inactivate ERK, forming a negative feedback loop in the ERK signalling pathway (Fig. 1). This model is supported by results obtained with serum-stimulated mouse 3T3 fibroblasts, in which the kinetics of ERK inactivation coincided with the synthesis of MKP-1 protein and the inhibition of protein synthesis resulted in sustained activation of ERK (Sun et al., 1993). In vascular smooth muscle cells, actinomycin D and specific antisense oligonucleotides for MKP-1, both of which inhibit the expression of MKP-1 mRNA, were able to induce sustained activation of ERK (Duff et al., 1995). However, the importance of phosphatases other than MKP-1 in the inactivation of ERK was suggested by the results obtained with PC12 cells. In PC12 cells, Epidermal growth factor (EGF) induced MKP-1 mRNA expression, but the inactivation of ERK was shown to occur before the induction of MKP-1, which suggests that the inactivation of ERK could be independent of the induction of MKP-1 in EGF-stimulated PC12 cells (Wu et al., 1994). Other phosphatases, such as PP2A or an as yet unidentified vanadate-sensitive PTPase, may be responsible for this rapid inactivation of ERK in PC12 cells (Alessi et al., 1995). These data suggest that the induction of MKP-1 is not always involved in the inactivation of ERK and that the mechanism of ERK inactivation may differ according to the type of cell and the type of stimulation.

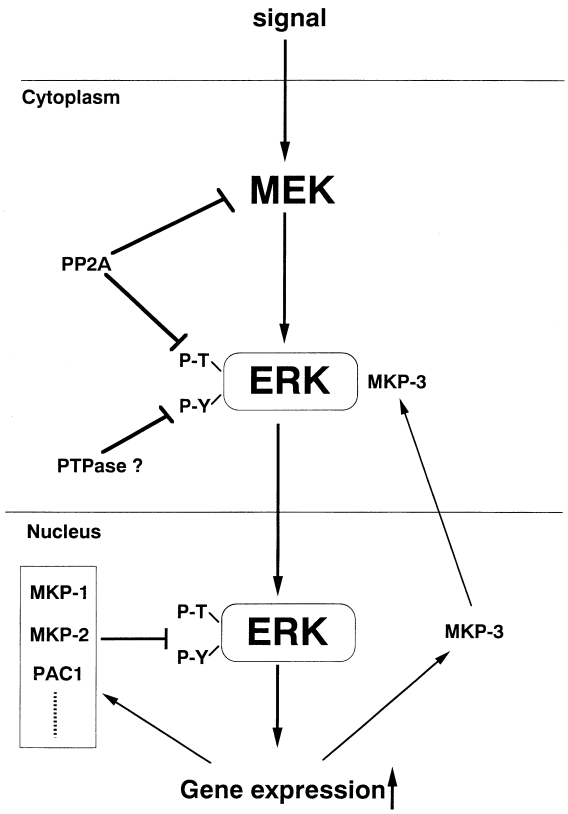

Fig. 1. A model for the inactivation of ERK. When cells are stimulated, ERK is phosphorylated and activated by MEK. Activated ERK translocates to the nucleus and then turns on the expression of various genes including those of the MAPK phosphatase family. MAPK phosphatases, such as MKP-1/2 or PAC-1, dephosphorylate ERK in the nucleus. In contrast, MKP-3 can bind to ERK and inactivate ERK in the cytosol. Under certain conditions, other types of phosphatases, such as PP2A or unidentified PTPase, are also responsible for the inactivation of ERK.

Recently, Camps et al. proposed a novel way to determine the substrate specificity of MAPK phosphatases against MAPKs and the mechanism for the inactivation of ERK (Camps et al., 1998; Muda et al., 1998). As mentioned above, MKP-3 has unique specificity for ERK and cannot dephosphorylate JNK/SAPK or p38 MAPK. It has also been reported that MKP-3 expression is induced by ERK activation and MKP-3 protein is located in the cytoplasm (Groom et al., 1996; Muda et al., 1996a). Camps et al. demonstrated that MKP-3 could bind to ERK via its amino-terminal domain, but not to JNK/SAPK or P38 MAPK. The association of MKP-3 with ERK leads to an about 30-fold stimulation of MKP-3 phosphatase activity for ERK. A gain-of-function mutant, D319N ERK2, which was genetically identified from *Drosophila* (Brunner et al., 1994), is resistant to MKP-3 because MKP-3 cannot bind this mutant form of ERK. These data indicate a new

mechanism for the regulation of ERK signal transduction pathways. The activation of ERK can induce MAPK phosphatase gene expression and the newly synthesized MKP-3 can inactivate ERK in the cytoplasm. Other MAPK phosphatases expressed in the nucleus, such as MKP-1, may dephosphorylate the active ERK after it has translocated to the nucleus (Fig. 1). The catalytic activation of MKP-3 by binding with ERK raises the possibility that the activity of MAPK phosphatases is also regulated in a manner independent of gene induction.

5. Mechanism of the induction and physiological significance of MAPK phosphatases

As discussed above, MKP-1 has been identified as an immediate early gene (Charles et al., 1992). Various stimuli, such as growth factors, stress, phorbor ester, and vasoactive peptides, have been reported to induce mRNA expression (Charles et al., 1992; Keyse and Emslie, 1992; Duff et al., 1993; Zheng and Guan, 1993; Sugimoto et al., 1997). The promoter sequence of the MKP-1 gene contains the AP-1 and CRE sites (Kwak et al., 1994), which respond to protein kinase C and Ca^{2+}/cAMP signalling pathways. Indeed, stimuli which activate protein kinase C, increase the intracellular cAMP content or raise the concentrations of Ca^{2+}, also increase the expression of mRNA for MKP-1 (Charles et al., 1992; Duff et al., 1993; Pelvin et al., 1997; Scimeca et al., 1997; Togawa et al., 1997). Although there is no obvious serum response element in the MKP-1 promoter, several groups have reported that the activation of ERK is necessary for the stimulation of MKP-1 mRNA expression, as determined in studies using MEK1/2 inhibitor or overexpression of the dominant negative form of MEK1 (Brondello et al., 1997; Cook et al., 1997). A recent study of the promoter of PAC-1 indicates that the E-box and AP-2 motifs may respond to activation of the ERK cascade (Grumont et al., 1996). These motifs are also conserved in the MKP-1 promoter, suggesting that the activation of ERK can induce MKP-1 mRNA transcription via the E-box and AP-2 in the promoter of MKP-1. However, another group has reported conflicting results by showing that MKP-1 transcription is mediated by the JNK/SAPK pathway rather than by the ERK pathway in NIH3T3 fibroblasts (Bokemeyer et al., 1996). Further experiments are necessary to identify the precise regulation of MKP-1 mRNA induction by members of the MAPK family.

The physiological role of MAPK phosphatases has been examined in various tissues and cells. Most of the studies are related to cell proliferation, hypertrophy, and differentiation. In cultured myoblast cells, growth factor deprivation leads to an inhibition of cell proliferation and induces cell differentiation. In C2C12 cells, the activity of ERK was decreased during differentiation evoked by serum starvation. The overexpression of MKP-1 was shown to inhibit ERK activity and to induce muscle-specific gene expression. However, endogenous MKP-1 expression decreased in the late phase of mitogenesis and overexpression of MKP-1 prevented myotube formation (Bennett and Tonks, 1997). In another muscle cell line, MM14, rVH6/MKP-3 mRNA was found to be highly expressed during cell proliferation and to decline rapidly during myogenesis (Mourey et al., 1996). These reports indicate that MAPK phosphatases play important roles in muscle differentiation.

In the cardiovascular and renal systems, MKP-1 is reported to play a role in cell proliferation and hypertrophy. In cardiac myocytes, the transcriptional responses accompanying cardiac myocyte hypertrophy were found to be inhibited by the overexpression of MKP-1 (Fuller et al., 1997). MKP-1 was also shown to inhibit arterial smooth muscle cell proliferation after balloon injury of the rat carotid artery (Lai et al., 1996; Koyama et al., 1998). We have demonstrated that agents that increase levels of cAMP or cGMP are able to induce the expression of MKP-1 and to inhibit the proliferation of glomerular mesangial cells, a prominent feature of proliferative glomerulonephritis (Sugimoto et al., 1996; Togawa et al., 1997). These results suggest that the induction of MAPK phosphatase might inhibit cell proliferation and hypertrophy and facilitate the differentiation of cells. Thus the pharmacological induction of MAPK phosphatases might be useful in the treatment of diseases characterized by marked cell proliferation.

In situ hybridization studies have revealed that the MAPK phosphatases have distinct expression patterns in the brain: MKP-1 mRNA is mainly expressed in the cortex and thalamus, MKP-2 mRNA in the dentate gyrus, piriform cortex and suprachiasmatic nucleus, and MKP-3 mRNA in the hippocampus. In contrasts, hVH-5 is expressed in many brain areas (Martell et al., 1995; Misra-Press et al., 1995; Muda et al., 1996a). It has been reported that drug induced-epilepsy can increase MKP-1 and MKP-3 mRNA expression in the brain (Gass et al., 1996; Muda et al., 1996a), suggesting that these phosphatase isoforms have important and specific roles in brain function through the regulation of MAP kinase cascades.

The most useful method to evaluate the physiological function of a certain gene is gene targeting. MKP-1 knock-out mice have been already established (Dorfman et al., 1996). These MKP-1 knock-out mice are born normally and do not have any phenotypic or histological abnormalities. Fibroblasts isolated from the embryos of these mice have normal MAPK activity and c-*fos* gene expression. These data indicate that MKP-1 is not essential for embryo development. However, interpretation of the results obtained with knock-out mice is difficult, because there may be functional overlap between MAPK phosphatase family members. Indeed, little is known about whether other members of the MAPK phosphatase family compensate for the missing activity in knock-out mice.

6. Concluding remarks

We have reviewed recent progress in research into the MAPK phosphatases, which dephosphorylate and inactivate members of the MAPK family. MAPK phosphatases have been identified in species ranging from yeast (Doi et al., 1994) to humans, which suggests that the mechanisms for inactivation of MAPKs are highly conserved, as are the mechanisms for their activation. However, there are still questions to be answered, namely, (1) the precise mechanisms of the transcriptional activation of MAPK phosphatases by various stimuli, (2) the molecular mechanism underlying the specificity of MAPK phosphatase isoforms for the members of MAPK family including ERK, JNK/SAPK, and p38 MAPK, and (3) the functions of MAPK phosphatases in development, cell and organ physiology, and pathological states. Further experiments are required to answer these questions.

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