A group of dual specificity protein phosphatases negatively regulates members of the mitogen-activated protein kinase (MAPK) superfamily, which consists of three major subfamilies, MAPK/extracellular signal-regulated kinase (ERK), stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK), and p38. Nine members of this group of dual specificity phosphatases have previously been cloned. They show distinct substrate specificities for MAPKs, different tissue distribution and subcellular localization, and different modes of inducibility of their expression by extracellular stimuli. Here we have cloned and characterized a novel dual specificity phosphatase, which we have designated MKP-5. MKP-5 is a protein of 482 amino acids with a calculated molecular mass of 52.6 kDa and consists of 150 N-terminal amino acids of unknown function, two Cdc25 homology 2 regions in the middle, and a C-terminal catalytic domain. MKP-5 binds to p38 and SAPK/JNK, but not to MAPK/ERK, and inactivates p38 and SAPK/JNK, but not MAPK/ERK. p38 is a preferred substrate. The subcellular localization of MKP-5 is unique; it is present evenly in both the cytoplasm and the nucleus. MKP-5 mRNA is widely expressed in various tissues and organs, and its expression in cultured cells is elevated by stress stimuli. These results suggest that MKP-5 is a novel type of dual specificity phosphatase specific for p38 and SAPK/JNK.

Members of the mitogen-activated protein kinase (MAPK) superfamily are proline-directed serine/threonine protein kinases that play a pivotal role in transducing various extracellular signals to the nucleus. They consist of three major subfamilies, MAPK/ERK, SAPK/JNK, and p38. MAPK/ERK is activated mainly by growth factors and phorbol esters and is associated with cellular proliferation and differentiation. SAPK/JNK and p38 are activated by extracellular stresses, such as UV irradiation and osmotic stress, and by inflammatory cytokines, but are poorly activated by growth factors and phorbol esters. Activation of these protein kinases leads to variable responses, such as gene expression, cell proliferation, differentiation, cell cycle arrest, apoptosis, early development, etc., depending on the cell type (1–8).

The magnitude and duration of activation of the MAPK superfamily are properly regulated and have a great effect on determination of the fates and responses of cells. In the case of PC12 cells, nerve growth factor treatment causes sustained activation of MAPK/ERK and p38 and drives the cells into neuronal differentiation, whereas epidermal growth factor treatment causes transient activation of MAPK/ERK and p38 and induces proliferation of the cells (7, 9–13). MAPK/ERK, SAPK/JNK, and p38 are regulated by dual phosphorylation and dephosphorylation within the motifs TEFY, TPY, and TGY, respectively, by several upstream dual specificity kinases (MAPK kinases) and several types of protein phosphatases.

There is an emerging family of dual specificity phosphatases acting on the MAPK superfamily. Nine members of this group of dual specificity phosphatases have been reported, including VHR (14), CL100 (MKP-1) (15–18), PAC1 (19, 20), MKP-2 (hVH2, TYP-1) (21–23), hVH3 (B23) (24, 25), hVH5 (M3/6) (26, 27), MKP-3 (Pyst1, rVH6) (28–30), Pyst2 (31), and MKP-4 (Pyst3) (31, 32). They share sequence homology, but each has distinct properties concerning substrate specificity, tissue distribution, subcellular localization, and inducibility by extracellular stimuli.

Here we report the cDNA cloning and characterization of a novel member of the dual specificity phosphatase family. We named it MKP-5. We isolated MKP-5 from a human liver cDNA library by yeast two-hybrid screening using p38 as a bait. MKP-5 shares structural features with other members of the dual specificity phosphatase family, including a Cdc25-like domain and a C-terminal phosphatase motif. MKP-5 has a high basal activity in vitro and inactivates both p38 and SAPK/JNK, but not MAPK/ERK. MKP-5 specifically binds to p38 and SAPK/JNK in the yeast two-hybrid system. Intracellular localization of MKP-5 differs from that of other dual specificity phosphatases. The expression of MKP-5 mRNA is elevated by stress stimuli. These results show that MKP-5 is a novel dual specificity phosphatase specific for p38 and SAPK/JNK.

**EXPERIMENTAL PROCEDURES**

**Two-hybrid Screening.—** Yeast two-hybrid screening of the Gal4 system was performed with catalytically inactive p38 (human p38α) as bait. Catalytically inactive p38, in which Lys-53 was replaced by Met, was constructed by PCR-based mutagenesis. The primers used were 5′-ggatggcttgtcgtggtgagctgcagc-3′ and 5′-ggttggagatggcgatgcacaagctact-3′. Full-length p38 in pCR blunt vector (Invitrogen) was used as a template. PCR was performed using Pfu polymerase (Stratagene). A Dpo1 restriction enzyme (Stratagene)-treated PCR product was transformed into Escherichia coli. Positive clones were picked up, and mutagenesis was verified by sequencing. Full-length catalytically inactive p38 was cloned into pBridge Gal4 activation vector (CLONTECH). A human liver cDNA library for two-hybrid screening was purchased from CLONTECH.

5′-Rapid Amplification of cDNA Ends—A SuperScript human fetal brain cDNA library (Life Technologies, Inc.) was used as a template. The vector sequence (5′-caccacaagatgatc-3′) and a gene-specific
primer (5'-cacaagttggtaccttcc-3') were used for the first PCR, and the SP6 primer and a gene-specific primer (5'-ggcacaaggtactcgaac-3') were used for nested PCR. Advantage cDNA polymerase mix (CLONTECH) was used in PCR, and PCR products were subcloned into TOPO TA cloning vector (Invitrogen). Full-length MKP-5 was obtained by the PCR method using the T3 Script human fetal brain library as a template.

Construction of the pBl2AD-MKP-5 Cdc25-like Domain—The Cdc25-like domain of MKP-5 (residues 159–290) was amplified by PCR using full-length MKP-5 in TOPO TA cloning vector. The primers used were 5'-ggggaattcagcacaagctcagac-3' and 5'-ggggaattcgaggggtggattcagac-3'. The fragment was subcloned into pBl2AD vector (CLONTECH) directly.

Bacterial Expression and Purification of Recombinant MKP-5—For bacterial expression, the open reading frame of MKP-5 was amplified by PCR and subcloned into pGEX6P3 (Amer sham Pharmacia Biotech). E. coli cells transformed with pGEX-MKP-5 were grown overnight to saturation in 10 ml of LB medium containing 50 μg/ml ampicillin. The cells were grown in 3 liters of LB medium containing 50 μg/ml ampicillin at 37 °C to reach an optical density of 0.1 at 600 nm. One hour after the temperature shift to 25 °C, isopropyl-β-D-thiogalactoside was added to a final concentration of 50 μM, and the cells were cultured for 9 h. Purification of GST-MKP-5 was performed by the method described previously (33). The yield of GST-MKP-5 was ~18 mg from 3 liters culture.

Phosphatase Assay—The catalytic activity of GST-MKP-5 fusion protein was tested with the substrate 5'-D-[γ-32P]ATP (3000 Ci/mmol) (ICN) as a substrate. The reaction was performed for 15 min in 200 μl of 50 mM imidazole (pH 7.5) containing 10 mM dithiothreitol, 20 mM pNP, and 37 °C to reach A600 = 0.1. Each precipitate was washed three times with 20 mM Tris-HCl (pH 7.5), 1 M NaCl, 1.5 mM MgCl2, 2 mM EDTA, 10 mM NaF, 1 mM sodium vanadate, and 2 mM dithiothreitol, 1 mM sodium vanadate, and 20 μg/ml aprotinin, and the indicated amounts of GST-MKP-5 fusion protein. The reaction was stopped by the addition of 0.1 N NaOH, and the pNP hydrolyzed was measured by absorbance at 405 nm with a microplate reader (Life Technologies, Inc.).

Cell Cultures—NIH3T3, REF, and HeLa cells were cultured in Dulbecco's modified Eagle medium containing 10% fetal calf serum. COS-7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. These cells were maintained in 5% CO2 at 37 °C.

Transfection—Cells were split on 35- or 60-mm dishes at 2 × 105 cells per dish, respectively. After 24 h, the cells were transfected using Lipfectamine Plus reagent (Life Technologies, Inc.) according to the manufacturer's protocol.

Immune Complex Kinase Assays—Myc-tagged MAPK, Myc-tagged SAPK, and HA-tagged p38 were immunoprecipitated from transfected NIH3T3 cells as follows. Cells (two 35-mm dishes in each sample) were lysed in 20 mM Tris-HCl (pH 7.5), 12 mM 2-glycerophosphate, 150 mM NaCl, 1.5 mM MgCl2, 2 mM EDTA, 10 mM NaF, 10 mM NaN3, and 1% Triton X-100 at 4 °C in PBS. The lysates were centrifuged at 105,000 × g at 4 °C and 2 μg/ml aprotinin and 2 mM dithiothreitol, 1 mM sodium vanadate, and 20 μg/ml aprotinin were added. Myc-tagged MAPK, Myc-tagged SAPK, and HA-tagged p38 were immunoprecipitated from cell lysates (~1 × 106 cells in each sample) by incubation with 1 μg of anti-Myc antibody (9E10, Santa Cruz Biotechnology) or 1 μg of anti-HA antibody (12CA5) and protein A-Sepharose beads (15 μl) (Amersham Pharmacia Biotech) for 2 h at 4 °C. Each precipitate was washed three times with 20 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 1.5 mM MgCl2, 2 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol, 1 mM sodium vanadate, and 20 μg/ml aprotinin and then washed twice with 20 mM Tris-HCl (pH 7.5). The washed precipitates were mixed with 3 μg of each substrate (myelin basic protein for MAPK, GST-cJun-(1–79) for SAPK, and His-ATF2 for p38) in kinase reaction buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl2, and 100 μM ATP (2 μCi of [γ-32P]ATP) and incubated for 20 min at 37 °C. The reaction was stopped by the addition of Laemmli sample buffer (34). Substrate phosphorylation was detected by autoradiography and with a molecular imager (Bio-Rad GS-525) after SDS-polyacrylamide gel electrophoresis.

Construction of the Catalytically Inactive Mutant of MKP-5—The catalytically inactive mutant of MKP-5, in which Cys-408 was replaced from brain acetone powder.

Construction of the Catalytically Inactive Mutant of MKP-5—The catalytically inactive mutant of MKP-5, in which Cys-408 was replaced from brain acetone powder.

Phosphoamino Acid Analysis—Phosphorylated p38 was prepared by incubating GST-p38 (20 μg) with His-MKK6 (10 μg) in kinase reaction buffer for 1 h at 37 °C. After precipitation with GSH beads, the sample was divided equally into four aliquots and then incubated with GST-MKP-5 (5 μg each) for the indicated times. The reaction was stopped by the addition of Laemmli sample buffer. GST-p38 was extracted from SDS-polyacrylamide gel after electrophoresis. One-sixth of each sample was spotted on a thin-layer cellulose plate. Extraction of GST-p38 from SDS-polyacrylamide gel and phosphoamino acid analysis were performed as described (35). Phosphoamino acids were detected using a BAS-2500 imager (Fuji Film).

Cell Staining—NIH3T3 and COS-7 cells were transfected with Myc-tagged MKP-5 and Myc-tagged CL100. After 24 h, the cells were fixed with 3.7% formaldehyde in PBS for 10 min at room temperature. After three washes with PBS, the cells were permeabilized in 0.5% Triton X-100 in PBS and washed with PBS three times. The cells were then incubated with anti-Myc antibody (9E10) in 3% bovine serum albumin in PBS for 16 h at 4 °C, washed three times with PBS, and incubated with rhodamine IgG secondary antibody in 3% bovine serum albumin in PBS for 1 h at 37 °C. After three washes with PBS and two washes with Milli-Q water, coverslips were mounted with Mowiol. Fluorescence was viewed with a Zeiss fluorescence microscope.

Northern Blot Analysis—Digoxigenin-conjugated, UTP-labeled MKP-5 riboprobe was generated by T7 RNA polymerase (Takara) transcription of the linearized MKP-5 open reading frame. Southern analysis was performed using a multiple-tissue Northern blot (CLONTECH) according to the manufacturer's protocol.

Quantification of mRNA by Reverse Transcription-PCR—Total RNA was extracted from cells in a 100-mm dish/stimulus using an RNeasy minikit (QiAGEN Inc.) according to the manufacturer's protocol. Total RNA was reverse-transcribed into DNA by the random primer method with Moloney murine leukemia virus reverse transcriptase (Toyobo). PCR was performed using 5'-gagttgtcacagag-3' and 5'-ggagctggagggagttgtcac-3' as 5'- and 3'-primers, respectively. [α-32P]dCTP-incorporated reverse transcription-PCR products were detected by autoradiography (Eastman Kodak x-ray film).

RESULTS

Isolation of MKP-5 cDNA—We sequenced the human liver cDNA library by yeast two-hybrid screening with catalytically inactive p38 as bait. Seven independent positive clones were obtained. Among these, one clone, containing a 1436-base pair cDNA fragment, shares the highest similarity with dual specificity phosphatases. By performing the 5'-rapid amplification of cDNA ends/PCR method in the human fetal brain cDNA library, we obtained a 2061-base pair cDNA fragment. The cDNA contains an open reading frame encoding a protein of 482 amino acids with a calculated molecular mass of 52.6 kDa (Fig. LA). An independently obtained sequence from a human testis cDNA library showed the same amino acid sequence of the open reading frame as this human cDNA sequence (data not shown). Sequence comparison revealed that it is similar to all known dual specificity phosphatases (Fig. 1D). We then designated it MKP-5. MKP-5 contains the active-site sequence LLIHC-QAGVSRSA-(T)IYLM (residues 404–424) at the C-terminal portion (Fig. 1C), which corresponds to, but is slightly different from, the consensus sequence VXXHCXCGXXSRSA(T)IYLM (where X is any amino acid), deduced from known dual specificity phosphatases (36). The two amino acids Cys-408 and Ser-415 in this motif, together with Asp-377, are likely to participate in the catalytic mechanism of dual specificity phosphatase activity (37, 38). The middle portion of MKP-5 contains two regions of amino acid similarity to the Cdc25 phosphatase (Fig. 1B). These two regions are referred to as Cdc25 homology 2 (CH2) domains and are present in all known dual specificity phosphatases (39, 40). Here we tentatively call this portion containing CH2 domains the “Cdc25-like domain.” Recently, it was reported that an N-terminal portion, containing this Cdc25-like domain, of MKP-3/Pyst1/rVH6 and M3/6 (hVH5) determines the binding specificity and substrate specificity (41). The identity sequence similarity to the Cdc25-like domain between MKP-5 and known dual specificity phosphatases is 20–34% (Fig. 1, B and D) and that of the phosphatase catalytic domain is 37–50% (Fig. 1, C and D). MKP-5 has an N-terminal stretch of 150 amino acids (Fig. 1, A and D) that is absent from the other dual specificity phosphatases. This N-terminal region has no homology with other proteins, and no distinct structural motif can be found.
Interaction of MKP-5 with p38 and SAPK/JNK in the Two-Hybrid System—To examine the specificity of the binding between MKP-5 and MAPKs, we performed the semiquantitative yeast two-hybrid interaction assay, which evaluates binding from the level of induction of the β-galactosidase reporter gene. Kinase-negative forms of MAPK/ERK, SAPK/JNK, and p38 were used.
were used. Full-length MKP-5 interacted strongly with p38 and SAPK/JNK and very weakly with MAPK/ERK (Fig. 2A). MKP-5 interacted slightly more strongly with p38 than with SAPK/JNK. It was reported that the Cdc25-like domain of MKP-5 (MKP-5 CH2, residues 159–290) with each of the catalytically inactive MAPKs was examined as described for A.

**Catalytic Activity of MKP-5 in Vitro**—To determine whether MKP-5 has a phosphatase activity, recombinant MKP-5 (as a GST fusion protein) was expressed in E. coli, purified, and assayed for enzymatic activity against pNPP, a well known phosphatase substrate. GST-MKP-5 protein hydrolyzed pNPP (Fig. 3). We then examined whether p38 and SAPK/JNK are directly inactivated by MKP-5. Phosphorylated p38 was prepared by incubating GST-p38 with His-MKK6 in the presence of [γ-32P]ATP, and phosphorylated Myc-tagged SAPK/JNK was isolated by immunoprecipitation from Myc-tagged SAPK/JNK-transfected cells that had been exposed to 0.5 M NaCl osmotic shock. Each sample was incubated with GST-MKP-5 in vitro in the presence or absence of vanadate, and the phosphorylation states of SAPK/JNK and p38 were investigated by immunoblotting with anti-phospho-SAPK/JNK antibody and autoradiography, respectively. The results clearly show that both p38 and SAPK/JNK are direct targets of MKP-5 (Fig. 5A). Phosphorylated Myc-tagged MAPK/ERK could also be dephosphorylated by incubation with GST-MKP-5, but was a poorer substrate than p38 (data not shown).

Next, we examined whether MKP-5 was really a dual specificity phosphatase. Incubation of GST-MKP-5 with MAPKs stimulated moderately the phosphatase activity of MKP-5 for pNPP in vitro, p38 and SAPK/JNK (10 μg each) enhanced the MKP-5 (1 μg) activity up to 1.6-fold and MAPK/ERK (10 μg) 1–2-fold (data not shown). Increasing the amount of MAPKs (up to 16 μg) did not induce further enhancement of the activity (data not shown).

**MKP-5 Inactivates p38 and SAPK/JNK**—Each dual specificity phosphatase has its own substrate specificity for MAPKs (30, 31, 43, 44). To identify the substrate specificity of MKP-5, we tested the activity of MKP-5 toward various MAPKs in cells. NIH3T3 cells were cotransfected with each of the epitope-tagged MAPKs and increasing amounts of a plasmid encoding MKP-5. After stimulation of the cells by appropriate agonists to activate MAPKs (0.5 M NaCl for 20 min for p38 and SAPK/JNK and 50 ng/ml TPA for 15 min for MAPK/ERK), epitope-tagged MAPKs were immunoprecipitated and assayed for their kinase activity. MKP-5 inactivated p38 and SAPK/JNK, but not MAPK/ERK, in a dose-dependent manner (Fig. 4A, first, third, and fifth panels). It was confirmed that nearly the same amounts of MAPKs were precipitated in each lane (Fig. 4A, second, fourth, and sixth panels). Quantification of the data showed clearly that MKP-5 inactivates p38 more strongly than SAPK/JNK (Fig. 4B). It is known that when a Cys residue in the catalytic active site (VXHCGXGXRSRTXXAXYL) is replaced by Ser, dual specificity phosphatases are converted to catalytically inactive forms. We tested whether MKP-5 was made catalytically inactive by such a mutation. As shown in Fig. 4C, the mutant form of MKP-5 (C408S) could not inactivate either p38 or SAPK/JNK.

We then examined whether p38 and SAPK/JNK are directly inactivated by MKP-5. Phosphorylated p38 was prepared by incubating GST-p38 with His-MK6 in the presence of [γ-32P]ATP, and phosphorylated Myc-tagged SAPK/JNK was isolated by immunoprecipitation from Myc-tagged SAPK/JNK-transfected cells that had been exposed to 0.5 M NaCl osmotic shock. Each sample was incubated with GST-MKP-5 in vitro in the presence or absence of vanadate, and the phosphorylation states of SAPK/JNK and p38 were investigated by immunoblotting with anti-phospho-SAPK/JNK antibody and autoradiography, respectively. The results clearly show that both p38 and SAPK/JNK are direct targets of MKP-5 (Fig. 5A). Phosphorylated Myc-tagged MAPK/ERK could also be dephosphorylated by incubation with GST-MKP-5, but was a poorer substrate than p38 (data not shown).

Next, we examined whether MKP-5 was really a dual specificity phosphatase that could dephosphorylate both Thr and Tyr residues. The phosphosamino acid analysis showed that when phosphorylated p38 was incubated with MKP-5 in vitro, both phosphorylated Thr and Tyr residues were dephosphorylated in a time-dependent manner. This result clearly indicates that MKP-5 is indeed a member of the dual specificity phosphatase family.
CL100 in COS-7 and NIH3T3 cells. Indirect immunofluorescence with anti-Myc antibody showed that Myc-MKP-5 localized in both the cytoplasm and the nucleus in both NIH3T3 and COS-7 cells, whereas Myc-CL100 localized in the nucleus, as previously shown (Fig. 6). There was no marked difference in the staining intensity of Myc-MKP-5 between the cytoplasm and the nucleus. Essentially the same result was obtained using green fluorescent protein-MKP-5 (data not shown).

**Distribution of MKP-5 mRNA**—In Northern blot analysis, a 4.6-kilobase mRNA species for MKP-5 was detected in heart, lung, liver, skeletal muscle, and kidney, but was scarcely detected in brain, spleen, or testis (Fig. 7).

**Elevation of MKP-5 mRNA Expression by Stress Stimuli**—Many dual specificity phosphatases are rapidly induced after stress stimuli or serum stimulation (18, 23–26, 30, 40, 43). By the reverse transcription-PCR method, we examined whether or not MKP-5 mRNA was induced by various stimuli. In KB and HeLa cells, the phorbol ester TPA, which is a good agonist for MAPK/ERK, did not induce a marked elevation of MKP-5 mRNA (Fig. 8). In contrast, anisomycin and osmotic
stress (NaCl), which are potent agonists for p38, did induce a marked elevation of MKP-5 mRNA. Tumor necrosis factor-α significantly increased the MKP-5 mRNA level in KB cells, but not in HeLa cells. UV irradiation, which potently activates SAPK/JNK, did not induce an elevation of the mRNA (Fig. 8).

**DISCUSSION**

We have isolated a cDNA clone encoding a novel dual specificity phosphatase, MKP-5, by yeast two-hybrid screening using p38 as bait. All of the amino acids previously shown to be important for the catalytic activity of dual specificity phosphatases are conserved in MKP-5. MKP-5 also has two CH2 domains (the Cdc25-like domain) in its middle portion that are also conserved in dual specificity phosphatases (39, 40). The previously described dual specificity phosphatases, except hVH5 (M3/6), consist of only two regions, the Cdc25-like domain and the phosphatase catalytic domain (Fig. 1D). hVH5 (M3/6) has a long C-terminal stretch whose function is unknown (26, 27). MKP-5 is unique in that it has an additional long N-terminal region, consisting of ~150 amino acids, that is absent from other dual specificity phosphatases (Fig. 1D). This region has no homology with other proteins, and so the role of this N-terminal region is unknown at present. The amino acid sequence identity of the Cdc25-like domain between MKP-5 and other dual specificity phosphatases is 20–34%. hVH5 shows 34% identity. The catalytic domain of MKP-5 shows 37–50% identity to that of other dual specificity phosphatases. From the standpoint of the amino acid sequence, MKP-3, Pyst2, and MKP-4 form one group, and CL100, PAC1, and MKP-2 form another. The identities among MKP-3, Pyst2, and MKP-4 are 42–59% in the Cdc25-like domain and 74–86% in the catalytic domain, and the identities among CL100, PAC1, and MKP-2 are 34–54% in the Cdc25-like domain and 73–81% in the catalytic domain. hVH5 (M3/6) and hVH3/B23 share much lower similarities with either of the two groups and with each other. Because MKP-5 does not share high similarity with either of the two groups, hVH5 (M3/6) or hVH3/B23, MKP-5 may be a distinct member of the dual specificity phosphatase family.

Bacterially expressed GST-MKP-5 possessed a high phosphatase activity for pNPP, which seems relatively strong. For example, under almost the same reaction conditions, incubation with 40 μg of GST-MKP-4/Pyst3 for 60 min (32) is equivalent to incubation with 5 μg of GST-MKP-5 for 15 min.

It has previously been reported that the Cdc25-like domains of MKP-3/Pyst1/rVH6 and hVH5 (M3/6) determine their binding specificity and substrate specificity for MAPKs (39). Using the semiquantitative yeast two-hybrid interaction assay, we tested whether MKP-5 could bind to MAPKs and whether the Cdc25-like domain was responsible for the binding. Full-length MKP-5 could bind to p38 and SAPK/JNK, but only very weakly to MAPK/ERK. The affinity for p38 appeared stronger than that for SAPK/JNK. The Cdc25-like domain of MKP-5 interacted specifically with p38, although the affinity seemed very low. Thereby, the proper binding of MKP-5 to p38 may require not only the Cdc25-like domain, but also other regions. That the binding between MKP-5 and SAPK/JNK is slightly weaker than that between MKP-5 and p38 may be accounted for by the fact that the Cdc25-like domain of MKP-5 has no affinity for SAPK/JNK.

The binding of MAPK/ERK to MKP-3/Pyst1/rVH6, Pyst2, and MKP-4/Pyst3 has been reported to enhance the activities of these phosphatases up to ~20-fold (31, 42). In contrast, incubation with p38 or SAPK/JNK enhanced the catalytic activity of MKP-5 only 1.6-fold. There may be two kinds of dual specificity phosphatases. Members of one subfamily, which comprises MKP-3/Pyst1/rVH6, Pyst2, and MKP-4/Pyst3, may be activated by binding to their physiological substrates, and members of the other subfamily may exist as constitutively active forms. In fact, MKP-5 seems to have a higher basal activity than MKP-3/Pyst1/rVH6, Pyst2, and MKP-4/Pyst3 (see above). It is possible that the N-terminal region of MKP-5 may serve as regulatory sites, i.e., binding sites for inhibitors and/or regulators.

Each dual specificity phosphatase has its own substrate specificity. CL100/MKP-1 inhibits MAPK/ERK, p38, and SAPK/JNK; PAC1 and Pyst2 inhibit MAPK/ERK and p38; MKP-2/hVH2/TYP-1 inhibits MAPK and SAPK/JNK; hVH3/B23, MKP-3/Pyst1/rVH6, and MKP-4/Pyst3 inhibit MAPK/ERK; and hVH5 (M3/6) inhibits p38 and SAPK/JNK (24, 30, 43, 44). We determined the substrate specificity of MKP-5 in cultured cells. When coexpressed, MKP-5 inactivated p38 and SAPK/JNK, but did not inactivate MAPK/ERK. The inactivation of p38 was greater than that of SAPK/JNK. This specificity seems to be consistent with the binding ability of MKP-5 regarding various MAPKs. Among the known dual specificity phosphatases, only M3/6 (hVH5) shows the same or similar substrate specificity (44).

Previous studies showed that CL100/MKP-1, PAC1, MKP-2/hVH2/TYP-1, and hVH3/B23 localize in the nucleus, whereas MKP-3/Pyst1/rVH6, Pyst2, and MKP-4/Pyst3 localize in the cytoplasm (19, 21, 24, 28, 29, 31, 32). MKP-5 is shown here to localize evenly in the cytoplasm and the nucleus. This subel-

![Fig. 5. MKP-5 directly dephosphorylates SAPK/JNK and p38 in vitro.](image-url)
Myc-MKP-5 in cultured cells. NIH3T3 and COS-7 cells were transfected with Myc-MKP-5 or Myc-CL100. Twenty-four hours later, the cells were fixed and stained with anti-Myc antibody (9E10). At the same time, 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining was performed. Bars = 10 μm.

A Novel Dual Specificity Phosphatase

The subcellular localization of MKP-5 is different from that of previously described dual specificity phosphatases. The subcellular localization of MKP-5 is unchanged after stress stimulation (data not shown), p38 and SAPK/JNK, targets of MKP-5, also localize in both the cytoplasm and the nucleus, and their localization does not dramatically change after stress stimulation. In contrast, MAPK/ERK, which is present in the cytoplasm before stimulation, is translocated to the nucleus upon stimulation. The correspondence of the subcellular localization between MKP-5 and its targets, p38 and SAPK/JNK, might have some physiological relevance or may reflect binding between them.

The expression of some dual specificity phosphatases is known to be induced after growth factor stimulation and/or stress stimulation (18, 23–26, 29, 30, 40, 43). The MKP-5 mRNA was elevated significantly by anisomycin treatment, osmotic stress, and tumor necrosis factor-α treatment, which are known to activate p38 strongly. TPA, which is a good agonist for MAPK/ERK, did not induce MKP-5 mRNA; so there may be a correlation between MKP-5 mRNA inducibility and p38 activation in extracellular stimuli. However, UV irradiation, which is able to activate p38, did not increase the mRNA level. The mechanism of elevated expression of MKP-5 mRNA by stress stimuli should be elucidated in future studies.

In summary, we have cloned and characterized a novel dual specificity phosphatase, designated MKP-5. MKP-5 does not seem to belong to known subfamilies of dual specificity phosphatases in terms of structural features. MKP-5 is specific for p38 and SAPK/JNK, and its subcellular localization is unique in that it is present in both the cytoplasm and the nucleus. Thus, MKP-5 may be a novel member of the dual specificity phosphatase family acting on the MAPK family molecules.

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