Molecular Cloning and Characterization of a Novel Dual Specificity Phosphatase, LMW-DSP2, That Lacks the Cdc25 Homology Domain*

Received for publication, January 17, 2001, and in revised form, April 20, 2001
Published, JBC Papers in Press, May 9, 2001, DOI 10.1074/jbc.M100408200

Koji Aoyama‡, Miyuki Nagata‡, Kenji Oshima, Tsukasa Matsuda, and Naohito Aoki§
From the Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

A novel dual specificity phosphatase (DSP) designated LMW-DSP2 was cloned with a combination of reverse transcription-polymerase chain reaction and cDNA library screening strategies. The LMW-DSP2 open reading frame of 194 amino acids contained a single DSP catalytic domain but lacked the cdc25 homology domain, which is conserved in most known DSPs. Northern blot and reverse transcription-polymerase chain reaction analyses revealed that LMW-DSP2 was specifically expressed in testis. Recombinant LMW-DSP2 protein exhibited phosphatase activity toward an artificial low molecular weight substrate paranitrophenyl phosphate, and the activity was inhibited completely by sodium orthovanadate but not sodium fluoride, pyrophosphate, and okadaic acid. The substitution of critical amino acid residues, aspartic acid and cysteine, completely by sodium orthovanadate but not sodium fluoride. The substitution of a conserved docking motif of p38 and SAPK/JNK was not disrupted by such mutations. Among the DSPs screened, LMW-DSP2 was a member of a distinct class of DSPs and that the mode of its dephosphorylation was different from that of other DSPs reported.

EXPERIMENTAL PROCEDURES

Materials—Human recombinant epidermal growth factor (E9644) and anisomycin (A9789) were obtained from Sigma. Antibodies to HA epitope (Y-11) and Myc epitope (9E10) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to phospho-ERK1/2, phospho-p38, phospho-SAPK/JNK, and phospho-threonine were from New England Biolabs. The anti-phospho-tyrosine (4G10) antibody was purchased from Upstate. Protein A− or G-Sepharose beads were obtained from Amersham Pharmacia Biotech.

PCR Amplification and cDNA Cloning of Mouse LMW-DSP2—Degenerate oligonucleotide sense and antisense primers were based on the consensus sequences for two conserved amino acid stretches within the catalytic domains of low molecular weight type DSPs: ITH(V/I)(L/V/para, which is normally located in the cdc25 homology domain, LMW-DSP2 dephosphorylated and deactivated p38, to a higher extent, and SAPK/JNK, but not ERK1/2, in transfected COS7 cells and in vitro. Further analyses using various mutants suggested that LMW-DSP2 was a member of a distinct class of DSPs and that the mode of its dephosphorylation and deactivation toward MAP kinases was different from that of other DSPs reported.

Experimental procedures

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank† (Accession number(s)) AF237619.

† Both authors contributed equally to this work.
‡ To whom correspondence should be addressed. Tel.: 81-52-789-1431; Fax: 81-52-789-1428; E-mail: naoki@agr.nagoya-u.ac.jp.
§ The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* The abbreviation used are: ERK, extracellular signal-regulated kinase; SAPK, stress-activated protein kinase; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; DSP, dual specificity phosphatase; MKP, MAP kinase phosphatase; MAPK, mitogen-activated protein kinase; RT, reverse transcription; PCR, polymerase chain reaction; HA, hemagglutinin; DN, double negative; GST, glutathione S-transferase; PTP, protein-tyrosine phosphatase.

2 K. Aoyama, M. Nagata, K. Oshima, T. Matsuda, and N. Aoki, manuscript in preparation.
A Novel Dual Specificity Phosphatase, LMW-DSP2

The nucleotide and deduced amino acid sequences of the cDNA encoding LMW-DSP2. Nucleotide and amino acid residues are numbered on the right and left, respectively. The amino acid consensus sequence D(X)/VH-26(V/L)X is any amino acid found in all DSPs is underlined. Starting at ATG, catalytically essential amino acid residues aspartic acid 57 and cysteine 88 are indicated by bold letters. The polyadenylation signal sequence is shown in red. Identical amino acids are shaded. A schematic presentation of the N-terminal phosphatase catalytic domain based on the residue numbers indicated. The values shown are the percentage identities for independent comparisons of the amino acid residues aspartic acid 57 and cysteine 88 are indicated by bold letters.

Expression plasmids for pcDNA1-HA/p44 ERK1, pcDNA3-HA/p38 HOG, pMT2T-HA/p54 SAPK, and pMTSM-MycMKP-4 were kindly provided by Drs. J. Pouyssegur (University of Nice, Nice, France), J. S. Gutkind (National Institutes of Health, Bethesda, MD), J. W. Noggett (Ontario Cancer Institute, Ontario, Canada), and S. Arkinstall (SE-RONO Pharmaceuticals, Tokyo Institute of Technology). The polyadenylation signal sequence is shown in red. The MAP kinase assay was done by using a nonradioactive p44/42 MAP kinase assay kit, SAPK/JNK assay kit, and p38 MAP kinase kit (Cell Signaling Technology).

Subcellular Fractionation and Cell Staining—COS7 cells were transfected with expression plasmids by the modified calcium phosphate precipitation method (37). Prior to stimulation, cells were serum-starved for at least 18 h. The transfected cells were lysed as described (34) and subjected to SDS-polyacrylamide gel electrophoresis (38) followed by immunoblotting onto nitrocellulose membranes (Hybond C ECL, Amersham Pharmacia Biotech). The membranes were probed with the indicated antibodies and visualized with an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech).
A Novel Dual Specificity Phosphatase, LMW-DSP2

**RESULTS**

**Molecular Cloning of LMW-DSP2**—Most of the reported DSPs/MKPs have a single catalytic domain as well as the cdc25 homology domain. Human VHR is also included in the DSP

**Northern Blot and RT-PCR Analyses**—Total RNA was prepared from the indicated tissues of male mice except for the mammary gland from 4-week-old mice. Poly(A) RNA of the indicated tissues under the conditions described above except for annealing temperature (50 °C) and the number of cycles (30). The primer set for mouse GAPDH (GenBankTM accession no. M32599) was 5′-ACA-AAA-TGG-TGA-AGG-TCG-GT-3′ and 5′-CCA-TGA-AAT-TGT-GGA-TAT-GTC-GGG-TCC-GC-3′.

**Phosphatase activity of LMW-DSP2 against sodium pyrophosphate** (pyrophosphatase), or 300 nM okadaic acid, and sodium orthovanadate (vanadate), 10 mM sodium fluoride (NaF), and 0.5% Triton X-100 in PBS for 10 min at room temperature. After PBS washing, the cells were permeabilized in 0.5% Triton X-100 in PBS and fixed with para-nitrophenyl phosphate in the absence or presence of 1 mM para-nitrophenyl phosphate (vanadate), 10 mM sodium pyrophosphate (pyrophosphatase), and 3% bovine serum albumin in PBS. After three washes with PBS and two washes with Milli-Q water, coverslips were mounted. Fluorescence was viewed with an Olympus fluorescence microscope.

**A Novel Dual Specificity Phosphatase, LMW-DSP2**

**Fig. 2 Phosphatase activity**—A, recombinant C88S (C/S) and D57A (D/A) mutant of GST-LMW-DSP2 were assayed for phosphatase activity by the addition of 1 mM NaF, 1 mM sodium orthovanadate (vanadate), 10 mM sodium pyrophosphate (pyrophosphatase), and 3% bovine serum albumin in PBS. Indicated amounts of the indicated tissues of male mice except for the mammary gland from 4-week-old mice. Poly(A) RNA of the indicated tissues under the conditions described above except for annealing temperature (50 °C) and the number of cycles (30). The primer set for mouse GAPDH (GenBankTM accession no. M32599) was 5′-ACA-AAA-TGG-TGA-AGG-TCG-GT-3′ and 5′-CCA-TGA-AAT-TGT-GGA-TAT-GTC-GGG-TCC-GC-3′. Aliquots of the PCR products were separated on a 1.0% agarose gel and stained with ethidium bromide.

**Fig. 3 Expression of LMW-DSP2 in COS7 cells**—COS7 cells that had been transfected with Myc-LMW-DSP2 wild type or empty vector mock were fixed and immunoprobed with an anti-Myc antibody followed by fluorescein isothiocyanate-labeled anti-mouse IgG secondary antibody in PBS containing 1% bovine serum albumin and 0.2% gelatin. 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining was performed. C, COS7 cells that had been transfected with Myc-LMW-DSP2 wild type were fractionated, and equivalent amounts of protein were immunoblotted by an anti-Myc antibody. A, Myc-tagged LMW-DSP2 Cys→Ser was transiently transfected into COS7 cells. Total lysates were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (15%) gel) and Coomassie Brilliant Blue staining. B, COS7 cells that had been transfected with Myc-LMW-DSP2 wild type or empty vector mock were probed with an anti-Myc antibody followed by fluorescein isothiocyanate-labeled anti-mouse IgG secondary antibody in PBS containing 1% bovine serum albumin and 0.2% gelatin. 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining was performed. C, COS7 cells that had been transfected with Myc-LMW-DSP2 wild type were fractionated, and equivalent amounts of protein were immunoblotted by an anti-Myc antibody.

**Fig. 2 Phosphatase activity**

**Fig. 3 Expression of LMW-DSP2 in COS7 cells**
family, but it lacks the cdc25 homology domain and accordingly has a low molecular weight (12), suggesting that a distinct class of the DSP family is present. To look for other members of this low molecular weight-type of DSP family, we searched the data bases and found some sequences of various species that matched the criteria. Alignment of the amino acid sequences of a catalytic domain revealed relatively well conserved amino acid stretches. Degenerate primer sets (see "Experimental Procedures") were then designed, and RT-PCR amplification was performed using poly(A)^+ RNA prepared from various mouse tissues as a template. PCR clone number 2 (LMW-DSP2) exhibited sequence similarities but was not identical to any previously known DSPs or protein-tyrosine phosphatases (PTPs). Using the LMW-DSP2 PCR-generated cDNA fragment, we isolated a full-length cDNA clone from a mouse testis cDNA library and characterized it by sequencing.
FIG. 5. In vitro dephosphorylation of p38 and SAPK/JNK by recombinant GST-LMW-DSP2. A, COS7 cells were transfected with expression plasmids for HA-p38 or HA-SAPK/JNK and stimulated with anisomycin (10 μg/ml) for 30 min after serum starvation. HA-p38 and HA-SAPK/JNK were immunoprecipitated, washed with lysis buffer, and then subjected to an in vitro dephosphorylation assay. After termination of the incubation, proteins were separated by SDS-polyacrylamide gel electrophoresis and analyzed with anti-phospho-p38 or anti-phospho-SAPK/JNK antibody. The same blots were reprobed with an anti-HA antibody after stripping. WT, wild type; C/S, cysteine 88 to serine mutant. B, COS7 cells were transfected with HA-p38 or HA-SAPK/JNK and processed as above. The blots were successively incubated with anti-phospho-tyrosine (top panels), anti-phospho-threonine (middle panels), and anti-HA antibodies (bottom panels).
The cDNA and deduced amino acid sequences of LMW-DSP2 are shown in Fig. 1A. The open reading frame encoded a protein of 184 amino acids with a predicted molecular mass of 21 kDa. The deduced amino acid sequence of LMW-DSP2 had nearly matched the extended active site sequence motif DX26(V/L)(V/I)HCXAG(I/V)SRS(T/A)XXAY(L/I)M (where X is any amino acid) conserved in DSPs (Fig. 1A) but lacked the cdc25 homology domain, which is present in all known DSPs except for human VHR (Fig. 1C). Alignment of the DSP catalytic domain of LMW-DSP2 exhibited 33–41% identity to that of other known DSPs (Fig. 1, B and C). The two amino acids of LMW-DSP2, Asp-57 and Cys-88, are likely to participate in the catalytic mechanism of DSP activity (Fig. 1A).

Catalytic Activity of LMW-DSP2 in Vitro—To examine whether LMW-DSP2 has phosphatase activity, LMW-DSP2 wild-type GST fusion proteins were assayed for enzymatic activity against the well known artificial phosphatase substrate para-nitrophenyl phosphate. GST-LMW-DSP2 wild-type hydrolyzed para-nitrophenyl phosphate in a dose-dependent fashion (Fig. 2A). The substitution of cysteine 88 to serine (LMW-DSP2 C/S) resulted in a complete loss of catalytic activity. Furthermore, substitution of aspartic acid 57 to alanine (LMW-DSP2 D/A) also resulted in a dramatic reduction in phosphatase activity, but slight activity was retained. The catalytic activity of LMW-DSP2 was strongly inhibited by a potent tyrosine phosphatase inhibitor, sodium orthovanadate at the concentration of 1 mM, but not sodium fluoride, pyrophosphate, or okadaic acid (Fig. 2B).

Expression and Subcellular Localization of LMW-DSP2 in COS7 Cells—Wild-type as well as catalytically inactive mutants of Myc-LMW-DSP2 were constructed and transiently transfected into COS7 cells, and then the expression was assessed by Western blotting. LMW-DSP2 was detected as a 21-kDa protein band (Fig. 3A), which was well consistent with the predicted molecular mass. Indirect immunofluorescence cell staining showed that Myc-LMW-DSP2 localized in both the cytosol and the nucleus in COS7 cells and was enriched especially in the perinuclear regions (Fig. 3B). This subcellular localization of the phosphatase was confirmed further by biochemical cell fractionation, which revealed that LMW-DSP2 was distributed in both the cytosol, to a higher extent, and the nucleus (Fig. 3C).

Dephosphorylation and Deactivation of p38 and SAPK/JNK by LMW-DSP2—Individual DSPs have their own substrate specificity for MAP kinases (32). To examine the substrate specificity of LMW-DSP2, we tested the activity of LMW-DSP2 against MAP kinases in cultured cells. COS7 cells were co-

FIG. 6. Mutation in common docking sites did not alter deactivation of p38 and SAPK/JNK by LMW-DSP2. Two mg of wild type or DN mutant of HA-p38 (A) or HA-SAPK/JNK (B) was co-transfected with 2 mg of the wild-type or RRMM mutant of Myc-LMW-DSP2 into COS7 cells as indicated. Cells were lysed with 2 µg/ml for 30 min after serum starvation. The cell lysates were immunoblotted with anti-p38 or anti-SAPK/JNK. The expression of Myc-LMW-DSP2 in total cell lysate (TCL) was assessed by immunoblotting.

FIG. 7. Binding of LMW-DSP2 with p38 and SAPK/JNK was not disrupted by mutation in common docking sites. The wild type or DN mutant of HA-p38 (A) or HA-SAPK/JNK (B) was co-transfected with the wild type or RRMM mutant of Myc-LMW-DSP2 into COS7 cells as indicated. The cells were lysed, and Myc-LMW-DSP2 was immunoprecipitated. The immune complexes were immunoblotted with an anti-HA antibody. The same membranes were reprobed with an anti-Myc antibody after stripping.
transfected with each of the HA-tagged MAP kinases and each of the Myc-LMW-DSP2 wild-type, C88S, or D57A mutants. After stimulation of the cells by the appropriate agonists to activate MAP kinases (50 nm epidermal growth factor for 20 min for MAPK/ERK and 10 μg/ml anisomycin for 30 min for p38 and p54 SAPK/JNK), HA-tagged MAP kinases were immunoprecipitated and subjected to immunoblot analysis using anti-phospho (activated) MAP kinases. LMW-DSP2 wild type dephosphorylated p38 and SAPK/JNK but not MAPK/ERK (Fig. 4A, top panels). On the contrary, the catalytically inactive C88S and D57A mutants exhibited no dephosphorylation activity toward the MAP kinases. It was confirmed that nearly the same amounts of MAP kinases were precipitated in each lane (Fig. 4A, middle panels). Comparable amounts of Myc-LMW-DSP2 were expressed in the transfected COS7 cells (Fig. 4A, bottom panels). Densitometric quantitation of the data clearly showed that LMW-DSP2 dephosphorylated p38 more strongly than SAPK/JNK (Fig. 4B).

To establish whether LMW-DSP2 specifically targets p38 and SAPK/JNK, MAP kinase activity was assayed. To obtain a clear impression of the relative effectiveness of LMW-DSP2 to deactivate each MAP kinase, COS7 cells were transfected with a varied amount of plasmid (0–2 μg). After stimulation, the HA-tagged MAP kinase was immunoprecipitated and activity was assayed in vitro. As clearly shown in Fig. 4, LMW-DSP2 selectively and more than 80% of the MAP kinase activity was lost when the highest amount of plasmid was introduced, to a lesser extent, about 50% of the MAP activity was lost upon the highest expression of LMW-DSP2 on p54 SAPK/JNK than that on the other MAP kinases was dephosphorylated, to a lesser extent, and SAPK/JNK was deactivated, in a dose-dependent manner of the plasmid introduced (Fig. 4E).

We then examined whether p38 and SAPK/JNK were directly dephosphorylated by LMW-DSP2. COS7 cells, which had been transfected with HA-p38 or HA-SAPK/JNK, were stimulated with anisomycin, and then phosphorylated HA-p38 or HA-SAPK/JNK was immunoprecipitated. Each immune complex was incubated with GST-LMW-DSP2 wild type or the C88S mutant, and the phosphorylation levels of p38 and SAPK/JNK were analyzed by immunoblotting with anti-phospho-p38 and anti-phospho-SAPK/JNK, respectively. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).

Next, we studied whether LMW-DSP2 was really a dual specificity phosphatase that could dephosphorylate both Tyr and Thr residues. Phosphorylated p38 and SAPK/JNK were incubated with GST-LMW-DSP2 wild type or the C88S mutant, and the phosphorylation levels of p38 and SAPK/JNK were analyzed by immunoblotting with anti-phospho-p38 and anti-phospho-SAPK/JNK, respectively. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).

Next, we studied whether LMW-DSP2 was really a dual specificity phosphatase that could dephosphorylate both Tyr and Thr residues. Phosphorylated p38 and SAPK/JNK were incubated with GST-LMW-DSP2 and immunoblotted first with an anti-phospho Tyr antibody. The same blots were reprobed with anti-phospho Thr antibodies. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).

We then examined whether p38 and SAPK/JNK were directly dephosphorylated by LMW-DSP2. COS7 cells, which had been transfected with HA-p38 or HA-SAPK/JNK, were stimulated with anisomycin, and then phosphorylated HA-p38 or HA-SAPK/JNK was immunoprecipitated. Each immune complex was incubated with GST-LMW-DSP2 wild type or the C88S mutant, and the phosphorylation levels of p38 and SAPK/JNK were analyzed by immunoblotting with anti-phospho-p38 and anti-phospho-SAPK/JNK, respectively. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).

Next, we studied whether LMW-DSP2 was really a dual specificity phosphatase that could dephosphorylate both Tyr and Thr residues. Phosphorylated p38 and SAPK/JNK were incubated with GST-LMW-DSP2 and immunoblotted first with an anti-phospho Tyr antibody. The same blots were reprobed with anti-phospho Thr antibodies. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).

We then examined whether p38 and SAPK/JNK were directly dephosphorylated by LMW-DSP2. COS7 cells, which had been transfected with HA-p38 or HA-SAPK/JNK, were stimulated with anisomycin, and then phosphorylated HA-p38 or HA-SAPK/JNK was immunoprecipitated. Each immune complex was incubated with GST-LMW-DSP2 wild type or the C88S mutant, and the phosphorylation levels of p38 and SAPK/JNK were analyzed by immunoblotting with anti-phospho-p38 and anti-phospho-SAPK/JNK, respectively. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).

Next, we studied whether LMW-DSP2 was really a dual specificity phosphatase that could dephosphorylate both Tyr and Thr residues. Phosphorylated p38 and SAPK/JNK were incubated with GST-LMW-DSP2 and immunoblotted first with an anti-phospho Tyr antibody. The same blots were reprobed with anti-phospho Thr antibodies. The results clearly showed that both p38 and SAPK/JNK were direct targets of LMW-DSP2 (Fig. 5A). Phosphorylated HA-MAPK/ERK could not be dephosphorylated by incubation with GST-LMW-DSP2 (data not shown).
with an anti-phospho Thr antibody followed by incubation with an anti-HA antibody for normalization. As shown in Fig. 5B, both phosphorylated Tyr and Thr residues on p38 were dephosphorylated efficiently by LMW-DSP2 in vitro. Dephosphorylation of Tyr on SAPK/JNK was also obvious, but that of Thr was indistinguishable as compared with the control experiment. In vitro dephosphorylation experiments also demonstrated that dephosphorylation of p38 by LMW-DSP2 was greater than that of SAPK/JNK. Thus, it was confirmed that LMW-DSP2 is actually a member of the DSP family.

A Conserved Docking Motif in p38 and SAPK/JNK Is Not Essential for Deactivation by and Binding with LMW-DSP2—Recently, Nishida and co-workers (33) have reported that a conserved docking motif in MAP kinases is essential for the binding and biological functions of their substrates, activators, and regulators and that such a docking motif also existed in the substrates, activators, and regulators including MAP kinase phosphatases/DSPs. To test whether such a mechanism is also true to the deactivation of p38 and SAPK/JNK by LMW-DSP2, we constructed docking site mutants of p38 and SAPK/JNK, the aspartic acid stretches of which were substituted with asparagines (referred to as p38 DN and SAPK/JNK DN, respectively). Although LMW-DSP2 does not contain the cdc25 homology domain that has been shown to be involved in interaction with MAP kinases through a cluster of basic amino acids, two repeated arginine residues are located at its C terminus. The two arginines in LMW-DSP2 were also substituted with methionines and are referred to as LMW-DSP2 RRMM. As shown in Fig. 6, mutation in the common docking site for SAPK/JNK and SAPK/JNK revealed apparently no effect on the deactivation efficiency of LMW-DSP2 against the MAP kinases in the cluster of arginines of LMW-DSP2 and no reduction in the phosphatase activity of LMW-DSP2 towards the MAP kinases. Furthermore, co-expression of mutants with the LMW-DSP2 in deactivation of the MAP kinases was also not affected by such mutations in LMW-DSP2 RRMM. This result suggested the binding mechanism is distinct from that in our laboratory.

Distribution of LMW-DSP2 mRNA in the cytosol and nucleus upon stimulation (data not shown). p38 and SAPK/ JNK were also shown to be expressed specifically in testis (Fig. 8, A and B), and p38 and SAPK/JNK were also shown to be expressed in testis (Fig. 8C), suggesting that p38 and SAPK/ JNK are physiological substrates of LMW-DSP2. However, we cannot exclude the possibility that LMW-DSP2 might target other unknown molecules specifically expressed in testis. Substrate-trapping mutants of LMW-DSP2 (D57A and C88S) might be useful in the identification of the other putative substrates.

Several protein phosphatases are reported to be expressed specifically in the testis. Serine/threonine protein phosphatase PP12 is abundant in the testis and localized in the nuclei of late spermatocytes and early spermatids (46). The PTP Typ was also shown to be expressed specifically in testicular germ cells (46). We also observed that cytosolic PTP20 is expressed abundantly in mouse and rat testis, and we showed that one of the splice variants of PTP36 (PTP36-B) is specifically expressed in testis (11). One of the DSPs, TMDP, was also shown

3 K. Aoyama, M. Nagata, K. Oshima, T. Matsuda, and N. Aoki, unpublished observation.

4 K. Aoyama, M. Nagata, K. Oshima, T. Matsuda, and N. Aoki, unpublished data.
to be predominantly expressed in the testis and skeletal muscle (40). Moreover, we have observed that three novel DSP clones are also specifically expressed in testis.4 Testis-specific or predominant expression of many protein phosphatases might suggest involvement in a testis-specific cellular event, particularly in spermatogenesis.

In summary, we have cloned and characterized a novel DSP with low molecular weight designated LMW-DSP2. LMW-DSP2 does not seem to belong to known subfamilies of DSPs with respect to structural features and substrate specificity. LMW-DSP2 is specific for p38 and SAPK/JNK, but the dephosphorylation and deactivation mechanism does not require static interaction between the phosphatase and MAP kinases. Thus, LMW-DSP2 may be a novel member of the DSP family acting on the MAP kinase family.

Acknowledgments—We are grateful to Drs. J. Pouyssegur, J. S. Gutkind, J. Woodgett, and S. Arkinstall for providing pCDN1-HA/p44 ERK1, pCDN3-HA/p38 HOg, pMT2T-HA/p54 SAPKβ, and pMTSM-MycMKP-4, respectively. We also thank Dr. Hagiwara for providing a mouse testis cDNA library.

REFERENCES
1. Ahn, N. G., Seger, R., and Krebs, E. G. (1991) Curr. Opin. Cell Biol. 4, 992–999
2. Sturgill, T. W., and Wu, J. (1991) Biochim. Biophys. Acta 1092, 350–357
3. Nishida, E., and Gotoh, Y. (1993) Trends Biochem. Sci. 18, 128–131
4. Davis, R. J. (1994) Trends Biochem. Sci. 19, 470–473
5. Kyriakis, J. M., and Avruch, J. (1996) Bioessays 18, 567–577
6. Cobb, M. H., and Goldsmith, E. J. (1995) J. Biol. Chem. 270, 14843–14846
7. Marshall, C. J. (1995) Cell 80, 179–185
8. Treisman, R. (1996) Curr. Opin. Cell Biol. 8, 205–215
9. Cahill, M. A., Janknecht, R., and Nordheim, A. (1996) Curr. Biol. 6, 16
10. Cano, E., and Mahadevan, L. C. (1995) Trends Biochem. Sci. 20, 111–114
11. Aoyama, K., Matsuda, T., and Aoki, N. (1999) Biochem. Biophys. Res. Commun. 266, 523–531
12. Ishibashi, T., Bottaro, D. P., Chan, A., Miki, T., and Aaronson, S. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 12170–12174
13. Keyse, S. M., and Emslie, E. A. (1992) Nat. Cell Biol. 644–646
14. Charles, C. H., Abler, A. S., and Lau, L. F. (1992) Nature 350–357
15. Aoki, N., Yamaguchi-Aoki, M., and Ulrich, A. (1996) J. Biol. Chem. 271, 29242–29246
16. Ishibashi, T., Bottaro, D. P., Michieli, P., Kelley, C. A., and Aaronson, S. A. (1994) J. Biol. Chem. 269, 29897–29902
17. Rohan, P. J., Davis, P., Mook, U., and Kelly, K. (1991) Nature 350–357
18. Ward, K., Gupta, S., Nishida, E., and Gotoh, Y. (1993) Nature 364, 819–825
19. Guan, K.-L., and Butch, E. (1995) J. Biol. Chem. 270, 7197–7203
20. Misra-Press, A., Rim, C. S., Yao, H., Roberson, M. S., and Stork, P. J. S. (1995) J. Biol. Chem. 270, 14587–14596
21. King, A. G., O’Farrell, P. H., and Ashworth, A. (1995) Oncogene 11, 2533–2563
22. Kwak, S. P., and Dixon, J. E. (1995) J. Biol. Chem. 270, 1156–1160
23. Ishibashi, T., Bottaro, D. P., Michieli, P., Kelley, C. A., and Aaronson, S. A. (1994) J. Biol. Chem. 269, 29897–29902
24. Martell, K. J., Seasholtz, A. F., Kwak, S. P., Clemens, K. K., and Dixon, J. E. (1995) J. Neurochem. 65, 1823–1833
25. Theodosis, A. M., Rodrigues, N. R., Nesbit, M. A., Ambrose, H. J., Paterson, H., McLellan-Arnold, E., Boyle, Y., Leversha, M. A., Owen, N., Blake, D. J., Ashworth, A., and Davies, R. E. (1996) Hum. Mol. Genet. 6, 675–684
26. Muda, M., Boschert, U., Dickinson, R., Martinou, J.-C., Martinou, I., Camps, M., Schlegel, W., and Arkinstall, S. (1996) J. Biol. Chem. 271, 4319–4326
27. Mourey, R. J., Vega, Q. C., Campbell, J. S., Wenderoth, M. P., Haushka, S. D., Krebs, E. G., and Dixon, J. E. (1996) J. Biol. Chem. 271, 3795–3802
28. Groom, L. A., Sneddon, A. A., Alessi, D. R., Dowd, S., and Keyse, S. M. (1996) EMBO J. 15, 3621–3632
29. Dowd, S., Sneddon, A. A., and Keyse, S. M. (1997) J. Cell Sci. 111, 3389–3399
30. Muda, M., Boschert, U., Smith, A., Antonsson, B., Gillieron, G., Chabert, C., Camps, M., Martinou, I., Ashworth, A., and Arkinstall, S. (1997) J. Biol. Chem. 272, 5141–5151
31. Tanoue, T., Moriguchi, T., and Nishida, E. (1999) J. Biol. Chem. 274, 19949–19956
32. Camps, M., Nichols, A., and Arkinstall, S. (2000) FASEB J. 14, 6–16
33. Tanoue, T., Adachi, M., Moriguchi, T., and Nishida, E. (2000) Nat. Cell Biol. 2, 110–116
34. Aski, N., Yamaguchi-Aoki, M., and Ulrich, A. (1996) J. Biol. Chem. 271, 29242–29246
35. Kinkelin, T. A. (1985) FEBS Lett. 182, 438–442
36. Hagiwara, S., and Terada, M. (1991) Nucleic Acids Res. 19, 2015–2020
37. Chen, C., and Okayama H. (1987) Mol. Cell. Biol. 7, 2745–2752
38. Chabert, C., and Adachi, M. (1993) Oncogene 8, 111–116
39. Ishibashi, T., Bottaro, D. P., Chan, A., Miki, T., and Aaronson, S. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 12170–12174
40. Ishibashi, T., Bottaro, D. P., Chan, A., Miki, T., and Aaronson, S. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 12170–12174
41. Keyse, S. M., and Emslie, E. A. (1992) Nature 350–357
Molecular Cloning and Characterization of a Novel Dual Specificity Phosphatase, LMW-DSP2, That Lacks the Cdc25 Homology Domain
Koji Aoyama, Miyuki Nagata, Kenji Oshima, Tsukasa Matsuda and Naohito Aoki

J. Biol. Chem. 2001, 276:27575-27583.
doi: 10.1074/jbc.M100408200 originally published online May 9, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100408200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 19 of which can be accessed free at http://www.jbc.org/content/276/29/27575.full.html#ref-list-1
Molecular cloning and characterization of a novel dual specificity phosphatase, LMW-DSP2, that lacks the Cdc25 homology domain.

Koji Aoyama, Miyuki Nagata, Kenji Oshima, Tsukasa Matsuda, and Naohito Aoki

This article has been retracted by the publisher.

The Fair Research Committee of Nagoya University has determined that duplicated images were included in Figs. 4, 5, 6, and 7.