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A Growing Family of Dual Specificity Phosphatases with Low Molecular Masses[1]

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Five putative dual specificity protein phosphatases (DSPs), designated LMW-DSP1, -DSP4, -DSP6, -DSP10, and -DSP11, were cloned with a combination of RT-PCR and cDNA library screening strategies. Sequencing analysis revealed that all lacked the cdc25 homology domain that is conserved in most known DSPs/MAP kinase phosphatases (MKPs). LMW-DSP1 exhibited the highest similarity to plant DSPs. LMW-DSP4 exhibited the highest similarity to human YVH1 and rat GKAP, but its C-terminal region was much shorter than that of the human and rat clones. LMW-DSP6 was found to be identical to recently cloned TMDP, and LMW-DSP11 seemed to be a mouse ortholog of human VHR. LMW-DSP10 was found to have a DSP catalytic-like domain, but the critical cysteine residue for catalytic activity was missing. Recombinant LMW-DSP1, -DSP6, and -DSP11 exhibited obvious and strong activity against an artificial low molecular substrate, *para*-nitrophenyl phosphate (pNPP). Recombinant LMW-DSP4 exhibited slight but significant activity, whereas no activity was detected for LMW-DSP10. The phosphatase activity of the recombinant LMW-DSPs was inhibited by orthovanadate but not sodium fluoride. However, none of the DSPs could dephosphorylate MAP kinases such as ERK1, p38, and SAPK/JNK in transiently transfected COS7 cells under the conditions used. Northern blot analysis revealed that LMW-DSP1, -DSP6, -DSP10, and -DSP11 were specifically expressed in testis, while LMW-DSP4 was broadly expressed. The testis-specific expression and apparent absence of dephosphorylation action on MAP kinases suggest that LMW-DSP1, -DSP6, -DSP10, and -DSP11 play specific roles in testis. Taken together, it is conceivable that a distinct class of low molecular mass DSPs is present and plays a role in dephosphorylating unknown molecules other than MAP kinases.

Key words: dual specificity phosphatase, MAP kinase, protein dephosphorylation, testis.

Extracellular signal–regulated kinase (ERK), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and p38/RK/CSBP (p38) are distinct classes of mitogen-activated protein (MAP) kinase. 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. The activation of these protein kinases leads to a variety of responses, such as gene expression, cell proliferation, differentiation, cell cycle arrest, apoptosis, early development, *etc.*, depending on the cell type (*1–4*).

Full MAP kinase activation requires phosphorylation on both tyrosine and threonine residues by selective upstream dual specificity kinases (*3*). Because MAP kinase activation is a reversible process, protein phosphatases play critical regulatory roles. Recently, there has been an emerging information about a family of dual specificity phosphatases (DSPs)/MAP kinase phosphatases (MKPs) that act on the MAP kinase superfamily. Ten members of this group of DSPs/MKPs have been reported, including VHR (*5*), CL100 (MKP-1) (*6, 7*), PAC1 (*8, 9*), MKP-2 (hVH2, TYP-1) (*10–12*), hVH3 (B23) (*13, 14*), hVH5 (M3/6) (*15, 16*), MKP-3 (Pyst1, rVH6) (*17–19*), Pyst2 (*20*), MKP-4 (Pyst3) (*20, 21*), and MKP-5 (*22*). They share sequence homology, but each has distinct properties concerning substrate specificity, tissue distribution, subcellular localization, and inducibility by extracellular stimuli.

As a part of an investigation of DSP diversity and cellular function, we have tried to clone novel DSPs. Amino acid sequences of putative low molecular mass–type dual specificity phosphatases (LMW-DSPs) that lack the cdc25 homology sequences in the databases were aligned and relatively well-conserved amino acid stretches were found. RT-PCR with degenerate primer sets corresponding to these se-

[1] The nucleotide sequences reported in this paper have been submitted to the GenBank with Accession Numbers AF237618 (LMW-DSP1), AF280811 (LMW-DSP4), AF237620 (LMW-DSP6), AF-357887 (LMW-DSP10), and AF280809 (LMW-DSP11).

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Abbreviations: DSP(s), dual specificity phosphatase(s); MAP, mitogen-activated protein; MKP(s), MAP kinase phosphatase(s); ERK, extracellular signal-regulated kinase; SAPK, stress-activated protein kinase; JNK, c-Jun N-terminal kinase; RT-PCR, reverse transcription–polymerase chain reaction; pNPP, *para*-nitrophenyl phosphate; ECL, enhanced chemiluminescence; HA, hemagglutinin.

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quences and nucleotide sequence analysis revealed four sequences and full-length cDNA clones designated LMW-DSP1, -DSP6, -DSP10, and -DSP11 obtained by screening a mouse testis cDNA library. Another clone, designated LMW-DSP4, was also cloned in the course of cDNA library screening. All the DSPs obtained contained a single catalytic domain but lacked the cdc25 homology domain that is conserved in known DSPs, and exhibited varying phosphatase activity towards an artificial substrate, *para*-nitrophenyl phosphate (pNPP), except for LMW-DSP10, which lacks a critical cysteine residue. Unexpectedly, none of the DSPs could dephosphorylate MAP kinases such as ERK1, p38, and SAPK/JNK in transfected COS7 cells under the conditions used. Northern blot analysis revealed that LMW-DSP1, -DSP6, -DSP10, and -DSP11 are specifically expressed in testis, while LMW-DSP4 is broadly expressed. Testis-specific expression and the apparent absence of dephosphorylation action on MAP kinases suggest that LMW-DSP1, -DSP6, -DSP10, and -DSP11 play specific roles in testis. Taken together, it is conceivable that a distinct class of DSPs with low molecular masses is present and plays a role by dephosphorylating molecules other than MAP kinases.

MATERIALS AND METHODS

Materials—Human recombinant EGF and anisomycin were obtained from Sigma. Antibodies to HA epitope (Y-11), Myc epitope (9E10), ERK1/2, and p38 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to phospho-ERK1/2, phospho-p38, phospho-p54, and SAPK/p54 were from New England Biolabs (MA, USA). Protein A- and Protein G-Sepharose beads were obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK).

PCR Amplification and cDNA Cloning of Mouse LMW-DSPs—Degenerate oligonucleotide sense and antisense primers were based on consensus sequences for two conserved amino acid stretches within the catalytic domains of small DSPs: ITH(V/I)(L/V/I)NA and IXPNXGF (Fig. 1). Random-primed cDNA (up to 100 ng) from poly(A)$^+$ RNA of various mouse tissues was used as a template for PCR. Both sense and antisense primers were added to a 100-μl reaction mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% bovine serum albumin, all four dNTPs (each at 200 μM), 1 unit of *ExTaq* polymerase (TaKaRa, Kyoto), and template cDNA. Thirty-five cycles were carried out on a thermal cycler; each cycle involved incubation at 94°C for 1 min, at 42°C for 1 min, and 72°C for 1 min. The PCR products were separated in 1.5% agarose gels. Fragments of about 350 bp were excised, subcloned into pTargeT (Promega) and sequenced. Full-length cDNA clones for the respective LMW-DSPs were cloned by screening a mouse testis cDNA library (generously provided by Dr. Hagiwara, Tokyo Institute of Technology) as described (*23*).

Plasmid Construction—Myc-tagging to LMW-DSP1, -DSP4, -DSP6, -DSP10, and -DSP11 at their N-termini was done by PCR amplification using primer sets of 5′-CCA-CCA-TGG-AAC-AAA-AAC-TTA-TTT-CTG-AAG-AAG-ATC-TGC-ACT-CCC-TGA-ACC-AAG-AAA-TC-3′ and 5′-GAG-T-AC-AGA-TCA-CAG-ACC-3′, 5′-CCA-CCA-TGG-AAC-AAA-AAC-TTA-TTT-CTG-AAG-AAG-ATC-TCT-TGG-AAG-CGC-AGG-GTT-CTA-AC-3′ and 5′-GGT-CCT-AAG-TTC-ACA-G-CT-TC-3′, 5′-CCA-CCA-TGG-AAC-AAA-AAC-TTA-TTT-CT-G-AAG-AAG-ATC-TGG-ACTCGC-TAC-AGA-AGC-AGG-AA-3′ and 5′-CCT-AAT-TCA-GAG-TCT-TCC-TG-3′, 5′-CCA-CCA-TGG-AAC-AAA-AAC-TTA-TTT-CTG-AAG-AAG-ATC-TGG-GGA-ATG-GCA-TGA-CCA-AGG-TA-3′ and 5′-ACGG-ACTAAGGCACCAGGTG-3′, and 5′-CCA-CCA-TGG-AAC-AAA-AAC-TTA-TTT-CTG-AAG-AAG-ATC-TGT-CCA-GCT-CGT-TCG-AAC-TCT-CG-3′ and 5′-TGT-GGG-CAC-CCT-A-GA-GTT-TC-3′, respectively. All PCR products were cloned into a mammalian expression vector, pTargeT vector (Promega) and confirmed by sequencing on both strands.

The Myc-tagged LMW-DSP1 mutants containing a cysteine to serine alteration at position 149 and an aspartic acid to alanine at position 118 were generated using oligonucleotide primers, 5′-ACC-TGC-ATT-ACT-GTG-CAC-GA-G-3′ and 5′-TTC-AGG-CAC-AGC-CAG-TAT-AGA-3′, respectively, according to the protocol of Kunkel (*24*). The Myc-tagged LMW-DSP4 mutants containing a cysteine to serine alteration at position 114 and an aspartic acid to alanine at position 83 were generated using oligonucleotide primers, 5′-TCC-TGC-ATG-ACT-GTG-CAC-CAA-3′ and 5′-CTC-GG-G-TTT-GGC-CAG-CGC-CGG-3′, respectively. The Myc-tagged LMW-DSP6 mutants containing a cysteine to serine alteration at position 138 and an aspartic acid to alanine at position 106 were generated using oligonucleotide primers, 5′-CCC-CAT-AGC-GCT-GTG-GAC-CAG-3′ and 5′-GAA-GG-G-GTT-GGC-ATC-AGC-CTC-3′, respectively. The Myc-tagged LMW-DSP11 mutants containing a cysteine to serine alteration at position 124 and an aspartic acid to alanine at position 92 were generated using oligonucleotide primers, 5′-GCC-CTC-GCG-GCT-ATG-GAC-AAG-3′ and 5′-CTC-CT-G-CGT-AGC-ATT-GGC-CTT-3′, respectively. The mutations were confirmed by DNA sequencing.

Expression plasmids for pMYSM-MycMKP4, pcDNA1-HA/p44 ERK1, pcDNA3-HA/p38 HOG, and pMT2T-HA/p54 SAPKβ were kindly provided by Drs. S. Arkinstall (Serono Pharmaceutical Research Institute, Geneva, Switzerland), J. Pouyssegur (University of Nice, France), J.S. Gutkind (NIDCR/OPCB, USA), and J. Woodgett (Ontario Cancer Institute, Canada), respectively. hVH5 (NM_004420) was amplified by RT-PCR and Myc-tagging at its N-terminus was done by PCR using 5′-CCA-CCA-TGG-AAC-AAA-AAC-TTA-TTT-CTG-AAG-AAG-ATC-TGG-CTG-GGG-ACC-GG-C-TCC-CGA-GG-3′ and 5′-TCA-GGA-CAC-CTC-GAT-GAC-CTC-CAC-3′.

In Vitro Dephosphorylation Assay—GST fusion proteins containing full-length LMW-DSP1, -DSP4, -DSP6, -DSP10, and -DSP11 were purified on glutathione-Sepharose beads and eluted with 10 mM glutathione. The enzymatic activities of the GST fusion proteins were determined using pNPP, as described previously (*23*).

Cell Culture and Transfection—COS7 cells were inoculated at a density of 2 × 10^5 cells/6 cm dish and grown overnight in DMEM containing 10% FCS. Expression plasmids were transfected into the cells by the modified calcium phosphate precipitation method. After incubation under 3% CO_2/97% air for 18 h, the transfected cells were washed twice with PBS and cultured in fresh DMEM containing 10% FCS for another 48 h under humidified 5% CO_2 and 95% air. Prior to stimulation, the cells were serum starved for at least 18 h.

Cell Lysis and Western Blotting—The transfected cells were lysed with "lysis buffer" comprising 50 mM Tris-HCl

(pH 7.5), 5 mM EDTA, 150 mM NaCl, 10 mM sodium phosphate, 10 mM sodium fluoride, 1 mM sodium orthovanadate, 1% Triton X-100, 1 mM PMSF, and 10 μg/ml leupeptin. Proteins in the cell lysates were separated by SDS-PAGE under reducing conditions followed by blotting onto nitrocellulose membranes (Hybond C$^+$, Amersham Pharmacia Biotech). The membranes were blocked in NET buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.05% Triton X-100) containing 1% gelatin and then incubated sequentially with the respective antibodies and peroxidase-conjugated goat anti-rabbit or -mouse IgG (Bio-Rad). The protein bands were visualized with an enhanced chemiluminescence (ECL) detection kit (Amersham Pharmacia Biotech).

Northern Blot Analysis—Digoxigenin-conjugated, dUTP-labeled LMW-DSP1, -DSP4, -DSP6, -DSP10, and -DSP11 riboprobes were generated by T7 RNA polymerase (Roche) transcription of the open reading frames of the linearized LMW-DSPs. All procedures were based on the attached protocols.

RESULTS

Molecular Cloning of LMW-DSPs—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 family but lacks the Cdc25-like domain and, accordingly, has a low molecular mass (*5*). By screening databases, we found several sequences of human, mouse, and *Caenorhabditis elegans* proteins, which seem to be related to human VHR. Alignment of the amino acid sequences of a catalytic do-main revealed relatively well-conserved amino acid stretches (Fig. 1A), suggesting the presence of a distinct class of the DSP family. To search for other members of this low-molecular-mass DSP family, degenerate primer sets were designed and RT-PCR amplification was performed using poly(A)$^+$ RNA prepared from various mouse tissues as a template. This reaction gave specific products of about 350 bp, where a much thicker band was observed in testis sample as compared to other tissue samples (Fig. 1B). The band amplified from testis sample was isolated, cloned and sequenced. Sequencing analyses revealed the presence of novel cDNA clones that exhibited sequence similarity to DSPs but not PTPs (January 7, 2000). Based on this novelty, 4 clones were selected and subjected to further cDNA cloning. By screening a mouse testis cDNA library, full-length cDNA clones designated LMW-DSP1, -DSP6, -DSP10, and -DSP11 were obtained. An another clone, designated LMW-DSP4, was also obtained in the course of cDNA library screening. All the cloned LMW-DSPs contained a single catalytic domain but lacked the cdc25 homology domain. In the course of this project, LMW-DSP6 turned out to be identical to the recently cloned TMDP (*25*). The deduced amino acid sequences of the LMW-DSPs nearly matched the extended active site sequence motif Dx26(V/L)x(V/I)HCxAG(I/V)SRSxT(I/V)xxAY(L/I)M (where x is any amino acid) conserved in dual specificity phosphatases, but lacked the cdc25 homology domain, which is present in all known DSPs with the exception of human VHR (Fig. 2A).

The open reading frame of LMW-DSP1 encoded a protein of 220 amino acids with a predicted molecular mass of approximately 24 kDa, and showed the highest similarity to two plant proteins, DsPTP1 and arabidopsis DSP (Fig. 2B). The open reading frame of LMW-DSP4 encoded protein of 163 amino acids with a predicted molecular mass of approximately 17 kDa (Fig. 2C). LMW-DSP4 exhibited highest homology to human YVH1 (83.5%, Ref. *26*) and rat GKAP (90.2%, Ref. *27*), but its C-terminal region was much shorter than that of the human and rat proteins. The open

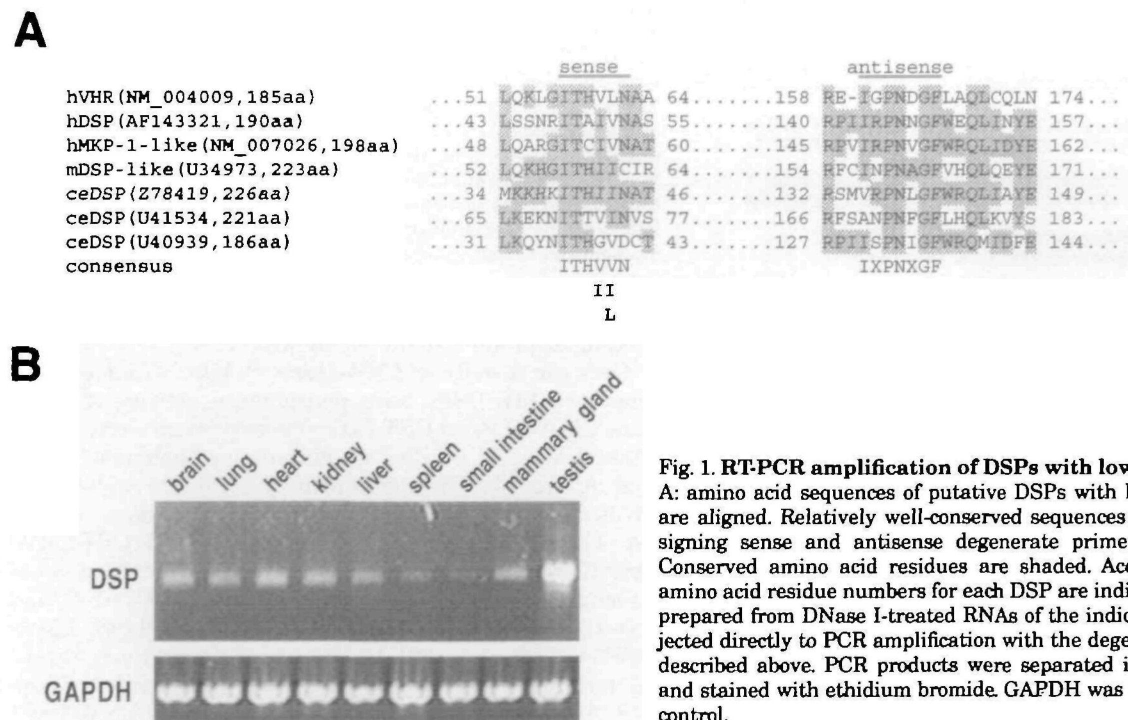

Fig. 1. **RT-PCR amplification of DSPs with low molecular masses.** A: amino acid sequences of putative DSPs with low molecular masses are aligned. Relatively well-conserved sequences were selected for designing sense and antisense degenerate primers (double-overlined). Conserved amino acid residues are shaded. Accession numbers and amino acid residue numbers for each DSP are indicated. B: cDNAs were prepared from DNase I-treated RNAs of the indicated tissues and subjected directly to PCR amplification with the degenerate primer sets as described above. PCR products were separated in a 1.0% agarose gel and stained with ethidium bromide. GAPDH was included as a positive control.

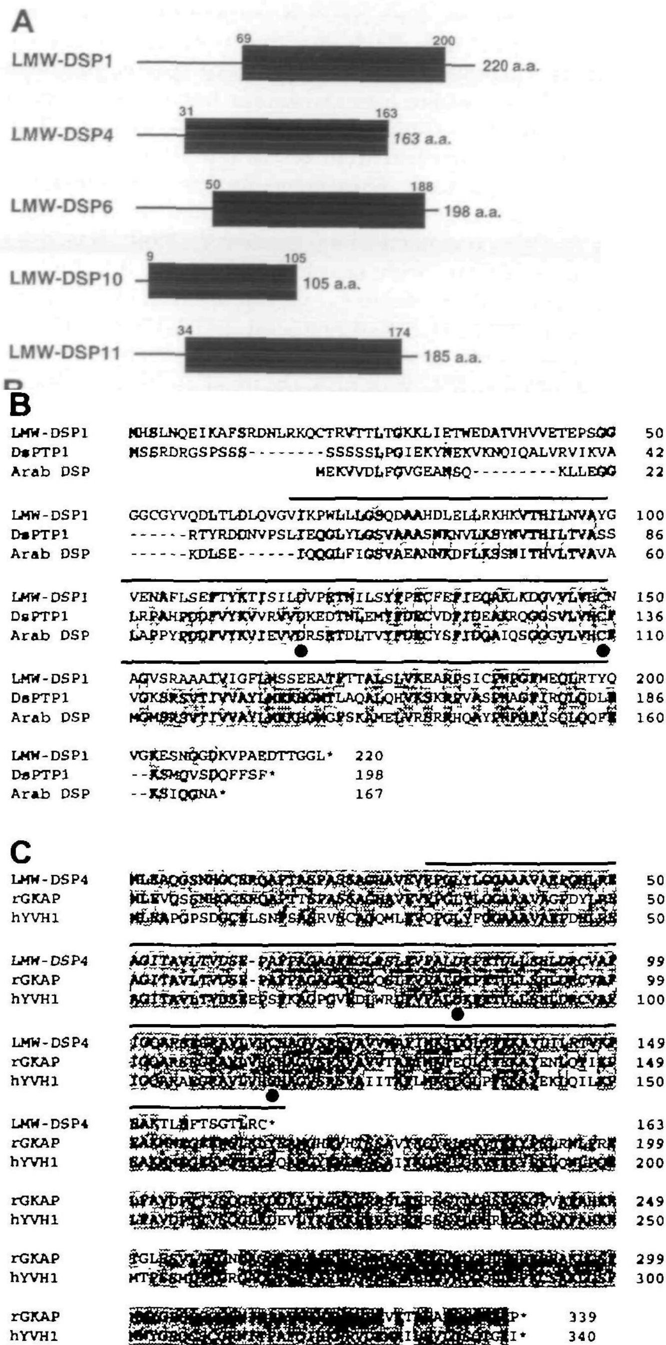

Fig. 2. **Molecular cloning of LMW-DSPs.** A: schematic drawing of cloned LMW-DSPs. DSP catalytic domains are indicated by closed bars. B: LMW-DSP1, DsPTP1 (Y18620), and Arabidopsis DSP (AC018907) were aligned with the aid of Mac-DNASIS (ver. 3.0) software and identical amino acid residues are shaded. C: LMW-DSP4, YVH1 (NM_022248), and rat GKAP (AF119226) were aligned as above. D: LMW-DSP10 and human MKP-X (NM_020185) were aligned as above. *Note that a critical cysteine residue is missing* from the sequence of LMW-DSP10. E: LMW-DSP11 was aligned with human VHR (NM_004090) as above. For panels B, C, D, and E, catalytic domains are double-overlined and closed circles indicate critical cysteine and aspartic acid residues for catalytic activity.

reading frame of LMW-DSP10 encoded protein of 105 amino acids with a predicted molecular mass of approximately 12 kDa. Interestingly, LMW-DSP10 also contained the nearly matched extended active site sequence motif, but the critical cysteine residue for catalytic activity was missing (Fig. 2D). Sequence alignment showed the highest similarity of LMW-DSP10 to human MKP-X. The open reading frame of LMW-DSP11 encoded protein of 185 amino acids with a predicted molecular mass of approximately 20.5 kDa. LMW-DSP11 showed 92% sequence identity to human VHR (*5*), suggesting that LMW-DSP11 is a putative mouse ortholog of VHR (Fig. 2E). Hydropathy analysis indicated that none of the LMW-DSPs contained hydrophobic segments and, therefore, were most likely intracellular proteins (data not shown).

Catalytic Activity of LMW-DSPs In Vitro—To determine whether LMW-DSPs have phosphatase activity, recombinant LMW-DSPs as GST fusion proteins were expressed in *Escherichia coli*, purified on glutathione-Sepharose beads, and assayed for enzymatic activity against a well-known artificial phosphatase substrate, pNPP. As shown in Fig. 3A, GST-LMW-DSP1, GST-LMW-DSP6, and GST-LMW-DSP11 wild-type strongly hydrolyzed pNPP in a dose-dependent fashion. Substitution of Cys-149, Cys-138, and Cys-124 by Ser to generate LMW-DSP1 C149S, LMW-DSP6 C138S, and LMW-DSP11 C124S, respectively, resulted in complete loss of catalytic activity. Substitution of Asp-118, Asp-106, and Asp-92 by Ala to generate LMW-DSP1

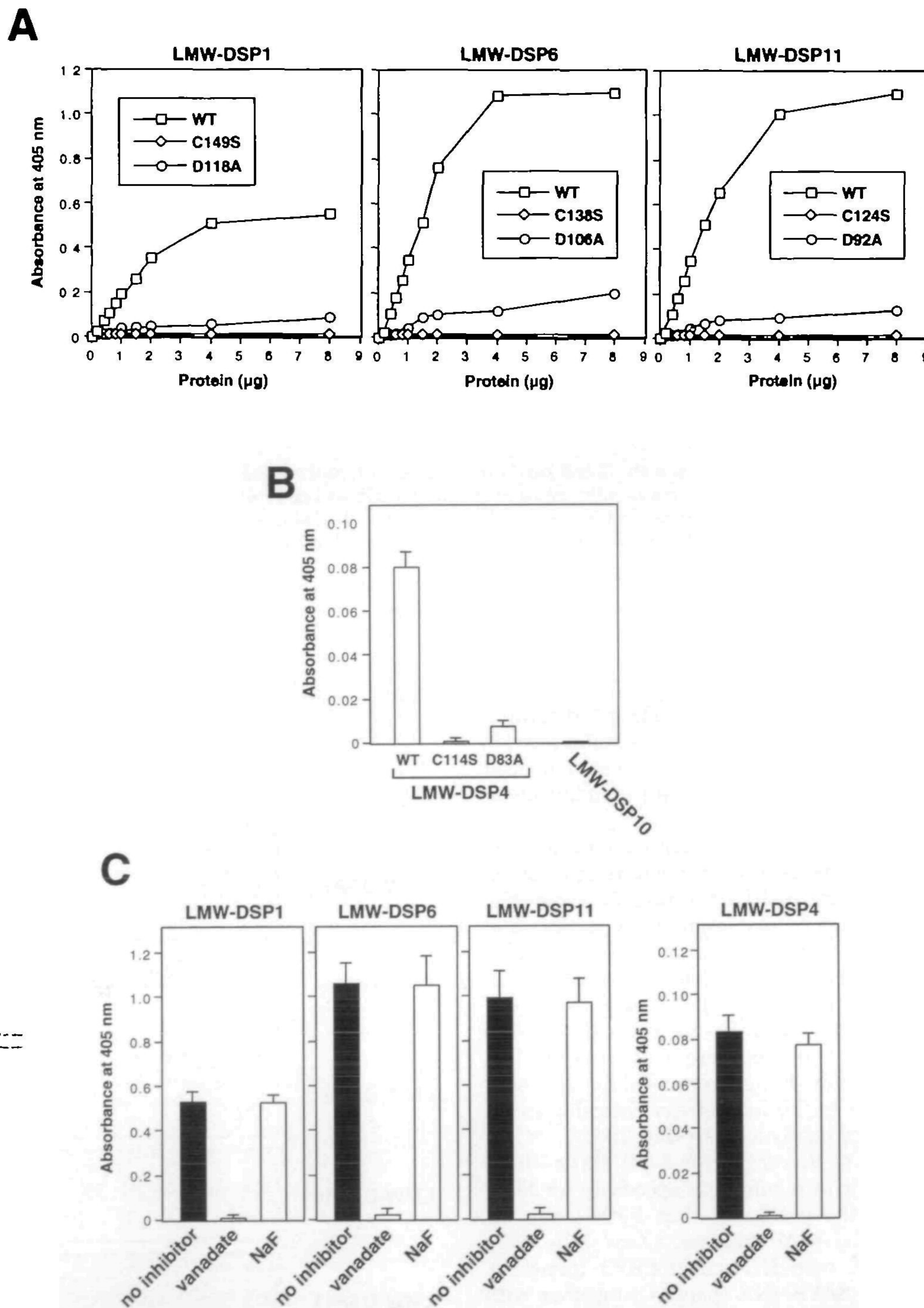

Fig. 3. **Phosphatase activity of LMW-DSPs against *para*-nitrophenyl phosphate.** A: recombinant GST-LMW-DSP1, -DSP6, and -DSP11 [wild-type (WT) and their mutants] were expressed in *E. coli* and purified on glutathione Sepharose. The indicated amounts of the proteins were subjected to assay for phosphatase activity. The reactions were terminated by the addition of 1 N NaOH and absorbance at 405 nm was determined. Data are representative of three independent experiments. B: 40 µg of GST-LMW-DSP4 (WT and their mutants) or -DSP10 was subjected to phosphatase activity assay as above. C: 4 µg (LMW-DSP1, -DSP6, and -DSP11) or 40 µg (LMW-DSP4) were incubated with *para*-nitrophenyl phosphate in the absence (no inhibitor) or presence of 1 mM sodium orthovanadate (vanadate) or 1 mM sodium fluoride (NaF) and processed as above. For panels B and C, data are expressed as means ± SEM of two or three independent experiments.

D118A, -DSP6 D106A, and -DSP11 D92A also resulted in a dramatic loss of activity, but slight activity was retained, suggesting that other Asp or amino acid residues in LMW-DSP1, -DSP6, and -DSP11 might participate in dephosphorylation as general acids. The catalytic activity of LMW-DSP1, -DSP6, and -DSP11 was strongly inhibited by a

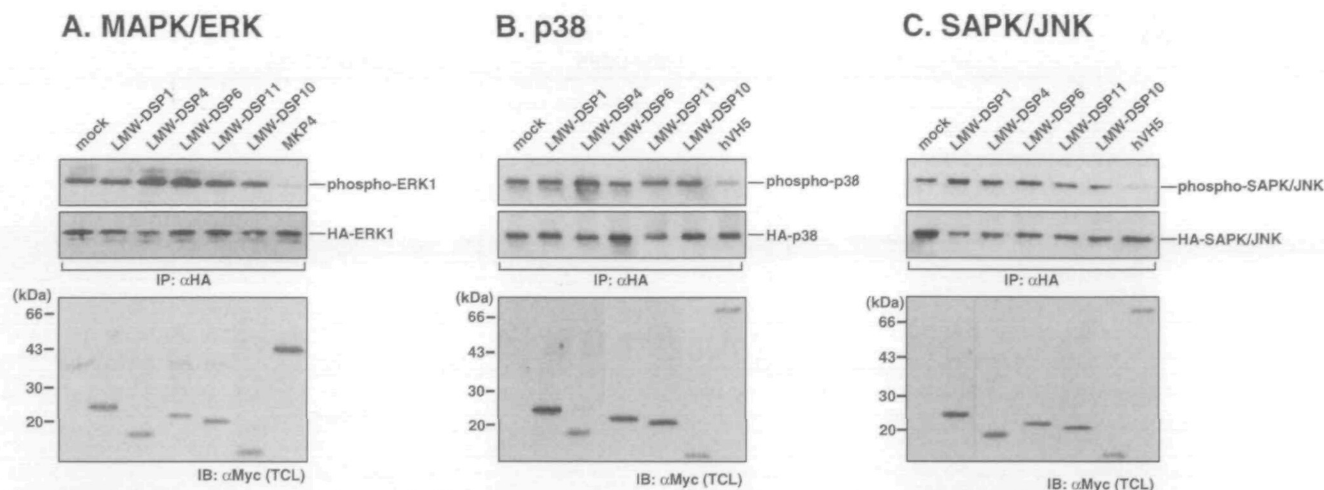

Fig. 4. **MAP kinases are not substrates for the LMW-DSPs in transfected COS7 cells.** COS7 cells were transiently transfected with HA-tagged ERK1 (panel A), p38 (panel B), or p54 SAPK/JNK (panel C) together with either an empty vector (mock), or each of the indicated Myc-LMW-DSPs. Myc-MKP4 and Myc-hVH5 were also included as positive controls for dephosphorylation of ERK1, and p38 and p54 SAPK/JNK, respectively. Cells were serum starved and stimulated with EGF (50 nM) for ERK1 activation or anisomycin (10 μg/ml) for p38 and p54 SAPK/JNK activation. Cells were lysed and the respective MAP kinases were immunoprecipitated with anti-HA antibody followed by immunoblotting with anti-phospho MAP kinase antibodies. The membranes were reprobed with anti-HA antibody. Aliquots of total cell lysate (TCL) were immunoblotted with anti-Myc antibody to confirm the expression of Myc-tagged DSPs.

potent tyrosine phosphatase inhibitor, sodium orthovanadate at a concentration of 1 mM, but not by sodium fluoride at the same concentration (Fig. 3C). Thus, it was confirmed that LMW-DSP1, -DSP6, and -DSP11 are actually members of the phosphatase family.

LMW-DSP4 exhibited much weaker but significant activity against pNPP when much higher amounts of recombinant protein (40 μg) were used for the activity assay (Fig. 3B). Substitution of the critical cysteine (Cys-114) and aspartic acid (Asp-83) resulted in a loss of activity (Fig. 3B), and the activity was strongly inhibited by sodium orthovanadate but not sodium fluoride (Fig. 3C), as in the case of LMW-DSP1, -DSP6, and -DSP11. On the other hand, GST-LMW-DSP10 exhibited no phosphatase activity, even when higher amounts of protein (40 μg) were used for the assay (Fig. 3B), possibly due to the lack of the critical cysteine residue in the conserved catalytic motif (Fig. 2D).

MAP Kinases Are Not Substrates for LMW-DSPs—Individual DSPs have their own substrate specificity for MAP kinases. To examine the possibility that LMW-DSPs dephosphorylate activated MAP kinases, COS7 cells were transiently transfected with HA-tagged ERK1 (panel A), p38 (panel B), or p54 SAPK/JNK (panel C) together with either an empty vector (mock), or each of the indicated Myc-LMW-DSPs. Myc-MKP4 and Myc-hVH5 were also included as positive controls for the dephosphorylation of ERK1, and p38 and p54 SAPK/JNK, respectively. After stimulation of the cells by appropriate agonists to activate MAP kinases (50 nM EGF for 20 min for ERK1 and 10 μg/ml anisomycin for 30 min for p38 and p54 SAPK/JNK), HA epitope-tagged MAP kinases were immunoprecipitated and subjected to immunoblot analysis using anti-phospho (activated) MAP kinases. Surprisingly, the co-expression of any LMW-DSPs had nearly no effect on the phosphorylation level of HA-ERK1, while dramatic dephosphorylation of HA-ERK1 was observed upon co-expression with MKP-4,

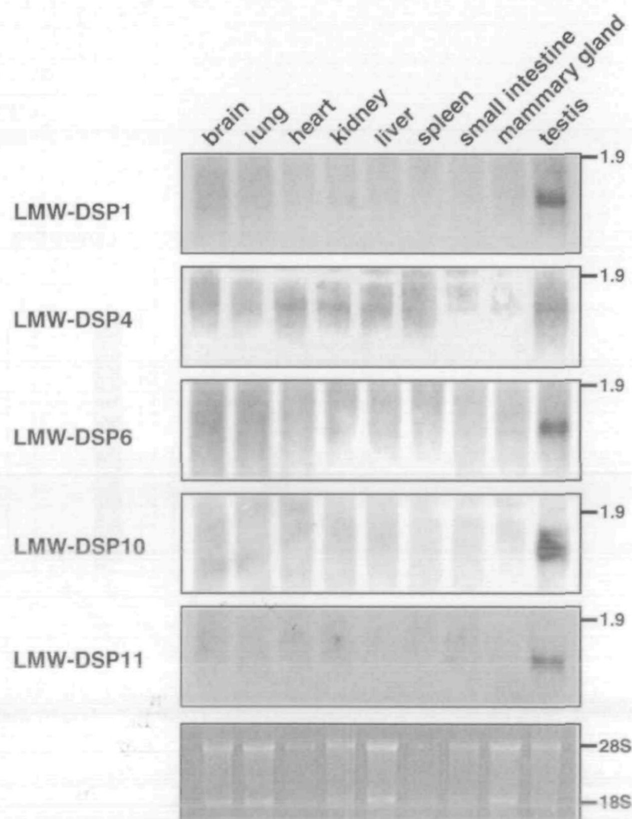

Fig. 5. **Northern blot analysis of LMW-DSPs.** Twenty micrograms of total RNAs prepared from the indicated tissues of a male mouse, except for mammary gland, which was from a female, were separated and blotted onto nylon membranes. The membranes were hybridized independently with digoxigenin-labeled RNA probes of the indicated LMW-DSPs as described under "MATERIALS AND METHODS." Ethidium bromide staining of 28S and 18S ribosomal RNAs are shown as controls.

as reported by Muda *et al.* (*21*) (Fig. 4A). Comparable amounts of LMW-DSPs and MKP4 were expressed, as assessed by immunoblotting with anti-Myc antibody. Neither H_2O_2- nor TPA-activated ERK1 was dephosphorylated by LMW-DSPs (data not shown). Moreover, the phosphorylation level of anisomycin-activated p38 and SAPK/JNK was unchanged upon co-expression with LMW-DSPs, whereas hVH5 dephosphorylated p38 and SAPK/JNK (*28*) (Fig. 4, B and C).

Northern Blot Analysis of LMW-DSPs—We examined the tissue distribution of the cloned LMW-DSPs by Northern blot analysis. As shown in Fig. 5, LMW-DSP1 and -DSP11 were detected as a single band only in testis. LMW-DSP6/TMDP was also detected specifically in testis as reported (*25*). Two transcripts for LMW-DSP10 were detected only in testis, suggesting another splice variant. In total, four LMW-DSPs were shown to be expressed specifically in testis. In contrast, LMW-DSP4 was shown to be expressed ubiquitously as a rather smeared band.

DISCUSSION

In the present study, we cloned 5 different DSPs designated LMW-DSP1, -DSP4, -DSP6, -DSP10, and -DSP11. Sequencing analysis revealed that all of them contained a single DSP catalytic domain but lacked the cdc25 homology domain, and, except for LMW-DSP4, were shown to be specifically expressed in testis. LMW-DSP6 was shown to be identical to TMDP, and, more recently, sequence information but not the biological activity of LMW-DSP1 has been reported (*29*). Based on Blast search results (April 3, 2001), LMW-DSP4, -DSP10, and -DSP11 are novel clones.

LMW-DSP4 shows the highest similarity to human YVH1 (*26*) and rat GKAP (*27*), although it lacks the C-terminal region. Since cDNA sequence alignment of LMW-DSP4 with these orthologs also showed very high similarity, LMW-DSP4 might be a splice variant of the mouse ortholog of human YVH1 and rat GKAP, which has not yet been identified. The phosphatase activity of LMW-DSP4 is much weaker than that of LMW-DSP1, -DSP6, and -DSP11 (Fig. 3), although they have well matched DSP catalytic sequences, suggesting that the activity is regulated by biological stimuli. Rat GKAP was first identified as a binding protein of glucokinase and was shown to dephosphorylate glucokinase in an *in vitro* study (*27*). While we have not determined the specific *in vivo* substrates for LMW-DSP4, glucokinase might be a candidate.

LMW-DSP10 is also an interesting example of an LMW-DSP, because it has a well matched DSP catalytic sequence, but lacks the cysteine residue that functions as a catalytic center for all other DSPs/MKPs reported. Accordingly, it exhibited no activity against pNPP (Fig. 3). Two transcripts were detected by Northern blotting, suggesting the presence of a splice variant. We are now trying to isolate other variants.

The high similarity (92%) between LMW-DSP11 and human VHR might simply imply that LMW-DSP11 is a mouse counterpart of human VHR. However, we cannot exclude the possibility that LMW-DSP11 is not a mouse ortholog, because LMW-DSP11 was shown to be expressed specifically in testis by Northern blotting (Fig. 5), whereas human VHR has been shown to be expressed ubiquitously (*30*). Furthermore, although LMW-DSP11 could not dephosphorylate EGF-activated ERK1 in transfected COS7 cells (Fig. 4A), human VHR has been shown to dephosphorylate ERK1/2 *in vivo* and *in vitro* (*30*).

Recent reports have shown that the noncatalytic domain within the cdc25-like domain of MKP3/PYST1 determines substrate specificity against ERK1/2 (*19*, *31*). When an amino-terminally truncated version of MKP3/PYST1 devoid of binding activity loses its selectivity for inactivating ERKs, it displays similar but reduced activity against all MAP kinases (*31*, *32*). Furthermore, Nishida and co-workers recently reported that a conserved docking motif in MAP kinases is essential for binding and the biological functions of their substrates, activators and regulators, and that such a docking motif also exists in substrates, activators, and regulators including DSPs/MKPs (*33*, *34*). The LMW-DSPs cloned in this study exhibited no dephosphorylation activity against MAP kinases under the conditions used, which might be explained in part by the fact that all of them lacked the cdc25 homology domain at their N-termini. However, we cannot exclude the possibility that MAP kinases are substrates for LMW-DSPs under certain physiological conditions.

Several protein phosphatases are reported to be expressed specifically in the testis. Serine/threonine protein phosphatase PP12 is abundant in rat testis and is localized in the nuclei of late spermatocytes and early spermatids (*35*). Protein tyrosine phosphatase (PTP) Typ has also been shown to be expressed specifically in testicular germ cells (*36*). We also observed that cytosolic PTP20 is abundantly expressed in testis (unpublished data) and showed that one of the splice variants of PTP36 (PTP36-B) is specifically expressed in testis (*37*). Moreover, we have obtained three partial but novel DSP clones that are also abundantly expressed in testis (unpublished data). The testis-specific or predominant expression of many phosphatases suggests their involvement in a testis-specific cellular event, especially in spermatogenesis.

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