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Characterization of cDNA encoding mouse homolog of fission yeast *dhp1*$^+$ gene: structural and functional conservation

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ABSTRACT

The *dhp1*$^+$ gene of *Schizosaccharomyces pombe* is a homolog of *Saccharomyces cerevisiae HKE1/RAT1/TAP1* gene that is involved in RNA metabolism such as RNA trafficking and RNA synthesis. *dhp1*$^+$ is also related to *S.cerevisiae DST2* (*SEP1*) that encodes a DNA strand exchange protein required for sporulation and homologous recombination in *S.cerevisiae*. We isolated several clones of *Dhm1*, a mouse homolog of *dhp1*$^+$, from mouse spermatocyte cDNA library and determined its nucleotide sequence. The *Dhm1* gene consists of an open reading frame predicting a protein with 947 amino acids and molecular weight of 107 955. Northern blot analysis revealed that *Dhm1* is transcribed at high level in testis, liver and kidney. The predicted product of *Dhm1* (Dhm1p) has a significant homology with Dhp1p, Hke1p/Rat1p/Tap1p and Dst2p. In particular, Dhm1p, Dhp1p and Hke1p/Rat1p/Tap1p share strong similarity at the two regions of their N- and C-terminal parts. The *Dhm1* gene on a multicopy plasmid rescued the temperature-sensitivity of *dhp1*^{ts} and lethality of *dhp1* null mutation, suggesting that *Dhm1* is a mouse homolog of *S.pombe dhp1*$^+$ and functions similarly in mouse as *dhp1*$^+$.

INTRODUCTION

Homologous recombination that involves interactions between homologous DNA tracts is a universal cellular process observed in both prokaryotes and eukaryotes. Especially in eukaryotes, recombination is an essential step in gametogenesis. It is thought that the process of DNA strand exchange is a central reaction in homologous recombination and thus the protein that catalyzes DNA strand exchange is indispensable in homologous recombination (1–3).

Saccharomyces cerevisiae DST2 is a gene that encodes a DNA strand exchange protein (4–8). Dst2p is shown to be a multifunctional enzyme which also has exodeoxyribonuclease, G4 tetraplex DNA-dependent nuclease and exoribonuclease activities (8–10). In *dst2* mutants, frequency of homologous recombination decreased several-fold and sporulation is temperature sensitive, indicating that *DST2* has an important role on homologous recombination and sporulation (6,7). Furthermore, *DST2* is known to affect RNA splicing, nuclear fusion and plasmid DNA replication (10–12) besides homologous recombination and sporulation.

We have cloned the *dhp1*$^+$ gene from *Schizosaccharomyces pombe* by cross hybridization using the *DST2* gene as a probe and shown that the *dhp1*$^+$ gene bears significant structural similarity to the *DST2* gene and that *dhp1*$^+$ functionally substitutes for *DST2* in *S.cerevisiae* (13). Furthermore, we have shown that *S.pombe dhp1*$^+$ is similar to *S.cerevisiae HKE1* structurally and functionally (13,14). *HKE1* which is identical to *RAT1* and *TAP1* was isolated as an essential gene whose product has significant homology to Dst2p (14) and is shown to play an important role in RNA metabolism such as mRNA trafficking, rRNA processing and tRNA and rRNA synthesis (15–17). Although Hke1p has a 5′→3′ exoribonuclease activity, the relationship between the RNA metabolism and 5′→3′ exoribonuclease activity has not been elucidated (14).

S.pombe dhp1$^+$ and *S.cerevisiae HKE1* are *DST2*-related genes, but *dhp1*$^+$/*HKE1* is basically different from *DST2* in mainly three aspects: (i) the size of polypeptide which is predicted from *dhp1*$^+$/*HKE1* is much smaller than that of Dst2p; (ii) *dhp1*$^+$/*HKE1* is essential for mitotic growth, while *DST2* is not; (iii) on a multicopy plasmid, *dhp1*$^+$ rescued sporulation defect and growth delay of *dst2* mutation in *S.cerevisiae*. On the other hand, *DST2* on a multicopy plasmid could not complement the temperature-sensitivity of *hke1* mutant (13).

The fact that both budding yeast and fission yeast have two types of *DST2*-related genes although they are evolutionary far apart (18) suggests that the two genes exist in all eukaryotes. It is of interest to study whether the two genes are involved in recombination process in gametogenesis of mammalian cells. We have therefore searched the two types of *DST2*-related genes in mammalian cells. In this report, we describe the cloning and characterization of a mouse homolog of *dhp1*$^+$/*HKE1*, *Dhm1*, and demonstrate that *Dhm1* is homologous to *dhp1*$^+$ structurally and functionally.

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MATERIALS AND METHODS

Strains and media

Escherichia coli Y1090 *hsdR* (19) and JM109 (20) were used to propagate bacteriophage λ and plasmid DNA, respectively. They were grown on Luria broth (21).

Schizosaccharomyces pombe MP102 (*h*$^+$ *ade6-M216 leu1 ura4-D18 dhp1-1*^{ts}<<*ura4*$^+$) (see below) and SSP1 (*h*$^+$/*h*$^-$ *ade6-M210/ade6-M216 leu1/leu1 ura4-D18/ura4-D18 dhp1*$^+$/*dhp1*::*ura4*$^+$) (13) were used for complementation studies. Mitotic growth and sporulation media were as described by Alfa *et al.* (22).

Cloning of mouse *Dhm1* cDNA and sequencing

Mouse spermatocyte cDNA library in λgt11 (kindly provided by A. Kikuchi and T. Noce, Mitsubishi Kasei Institute of Life Sciences) was used as a template for polymerase chain reaction (PCR), primed with oligonucleotides designed to identify *DST2*/*dhp1*$^+$ homologs on the basis of amino acid sequence comparisons between the *DST2* and the *dhp1*$^+$ gene products (6,13). Primers were: 5′ primer, 5′-GC(A/C/G/T)AA(A/G)ATGAA(C/T)CA(A/G)CA(A/G)(A/C)G-3′ and 3′ primer, 5′-CAT(A/G/T)AT(C/T)TT(A/G)TG(C/T)TC(A/C/G/T)CC(C/T)TC-3′. PCR was carried out with 50 pmol of each primer and ~1 × 10^7 p.f.u. of cDNA library as followed by 30 cycles of 94°C for 30 s, 55°C for 120 s and 72°C for 60 s. The nucleotide sequence of the resulting 303 bp DNA fragment was determined after cloning into *Hin*cII site of pUC118 (Takara). *Dhm1* cDNA clones were then isolated from the mouse spermatocyte cDNA library by plaque hybridization with the 303 bp fragment at high stringency (21). The nucleotide sequence was determined by dideoxy method (23) using A.L.F. sequencer (Pharmacia). Gene Works program (Intelligenetics) was used to analyze nucleotide and peptide sequences.

Expression of mouse *Dhm1* in fission yeast

A complete mouse *Dhm1* cDNA was generated by combining overlapping region from three clones. The *Dhm1* cDNA was inserted downstream of the *nmt1* promoter (24) in pREP1 (pREP-Dhm1) and transformed into the haploid strain MP102 or the diploid strain SSP1 by the lithium acetate method (22).

MP102, which was isolated in our laboratory, cannot grow above 33°C on solid media (unpublished observation). MP102 carrying pREP-Dhm1 was streaked at permissive (25°C) or non-permissive temperature (36°C).

SSP1 carrying pREP-Dhm1 was incubated in sporulation medium for 1 day, treated with glusulase and 30% ethanol and the resulting spores were plated on minimal plates lacking leucine to obtain Leu$^+$ haploid cells. Then Leu$^+$ haploid was transferred on plates lacking uracil. Ura$^+$ Leu$^+$ haploid lacking the *dhp1*$^+$ gene can grow only when the *Dhm1* cDNA rescued the *dhp1*$^+$ gene function.

Northern blotting

Northern blotting was performed with Mouse Multiple Tissue Northern (MTN) Blot (CLONTECH) as described in its protocol. A 0.9 kb *Eco*T14I1347–*Pst*I2275 fragment of the *Dhm1* cDNA was used as a probe. The probe was labeled by the random primer method with [α-^{32}P]-dATP. Hybridization was performed at 65°C for 16 h in 5 × SSPE (0.75 M NaCl, 0.05 M $NaH_2PO_4 \cdot H_2O$, 0.005 M Na_2EDTA), 2% sodium dodecyl sulfate (SDS), 10 × Denhardt's solution (0.2% Ficoll, 0.2% Polyvinylpyrrolidone, 0.2% BSA), 100 μg/ml heat denatured salmon sperm DNA and heat denatured probe (1 × 10^6 c.p.m./ml). The signals were detected by image analyzer BAS2000 (Fujix).

RESULTS

Cloning of mouse *Dhm1* cDNA

To isolate a *dhp1*$^+$ homolog in mouse, we made use of the ability of PCR to amplify specific sequences using degenerate oligonucleotides as primers. We made two primers corresponding to regions of the Dhp1p that are highly homologous to the Dst2p. These primers were used for PCR on a cDNA library derived from the mouse spermatocyte. A PCR product of the expected size (303 bp) was seen. This DNA fragment was subsequently cloned and sequenced. This PCR product was found to be the fragment of the *dhp1*$^+$ homolog based on the sequence homology (58%). Thus, it was used to clone the full-length gene from the mouse spermatocyte cDNA library in phage λgt 11. We obtained several cDNA clones and determined their nucleotide sequences. We found an open reading frame (ORF) that encodes a protein of 947 amino acid residues with a calculated molecular weight of 107 955 (Fig. 1) and designated it *Dhm1* (*dhp1*$^+$/*HKE1* homolog in mouse 1). The initiation codon of the *Dhm1* gene was tentatively located at the similar site for the start site of the *HKE1*/*dhp1*$^+$ gene. The sequence around the putative ATG initiation codon, GTCATGGGAGTC, is a favorable one proposed by Kozac (25) in which a purine and a guanosine are located at positions –3 and +4, respectively.

Structure of the *Dhm1* gene product and its homology with Dhp1p and Hke1p

The amino acid sequence predicted from the open reading frame of the *Dhm1* gene was compared with those of *S.pombe dhp1*$^+$ and *S.cerevisiae HKE1*. The *Dhm1* gene product shows homology to two regions of Dhp1p and Hke1p (Fig. 2). Although the overall homology between the amino acid sequence of the Dhm1p and that of Dhp1p was 40%, N-terminal homologous segment (positions 1–405) of the Dhm1p and C-terminal portion (positions 509–782) showed 56 and 44% homology with the respective regions of the Dhp1p. The middle part of the Dhm1p (positions 406–508) and the C-terminal 165 residues of the Dhm1p were 20 and 17% homologous with the corresponding part of the Dhp1p. The *Dhm1* gene product also had significant homology with the *S.cerevisiae* Dst2p (Fig. 2). However, the C-terminal region of the Dhm1p had little homology with Dst2p and the molecular weight of the Dhm1p (108k) was closer to that of the Dhp1p (112k) and the Hke1p (116k) than of Dst2p (175k). These data suggest that *Dhm1* is a true homolog of *dhp1*$^+$/*HKE1*.

Neither the consensus sequence for nucleotide triphosphate-binding nor DNA binding motif was found in the Dhm1p.

Expression of *Dhm1* in mouse tissues

To examine the expression of the *Dhm1* gene in different mouse tissues, Northern blot hybridization was performed using poly(A)$^+$ RNA isolated from various mouse tissues. As shown in Figure 3, about 3.5 kb RNA was hybridized with *Dhm1* cDNA. This size matches reasonably to the 2.8 kb ORF. Although 3.5 kb

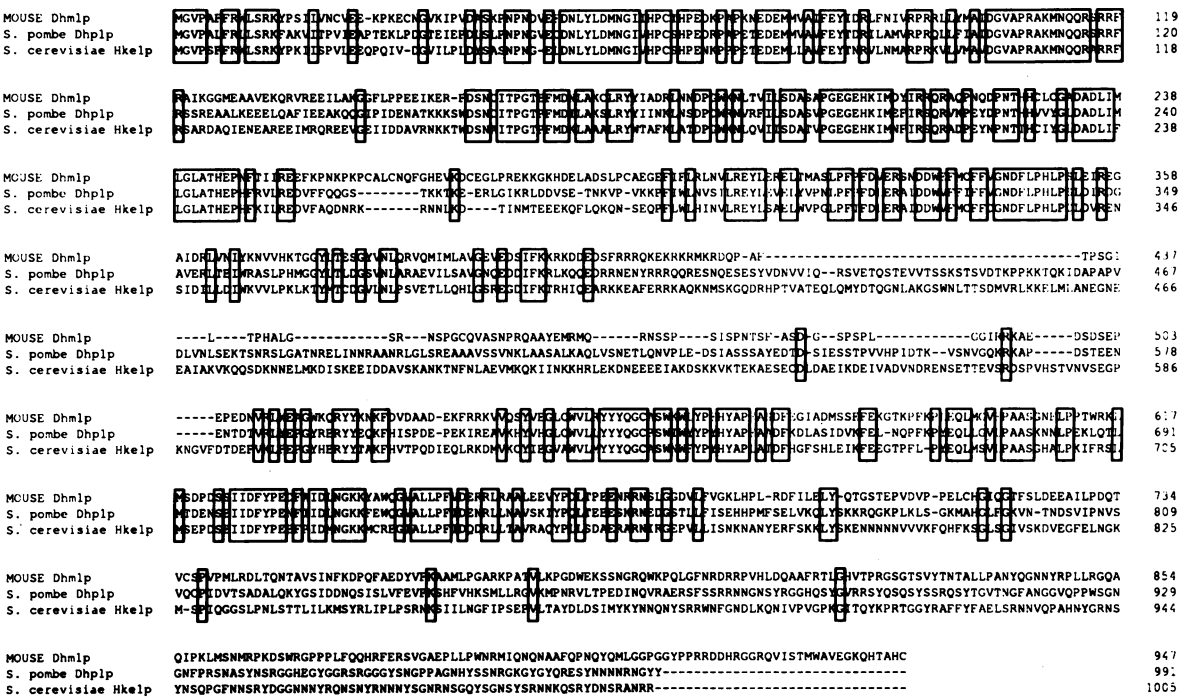

Figure 1. Amino acid sequeces (single letter amino acid code) of Dhm1, Dhp1 and Hke1 proteins are shown with alignment. Residue numbers are shown at the right side of the alignment. Gaps were introduced to maximize homology. Boxes indicate identical residues among three amino acid sequences.

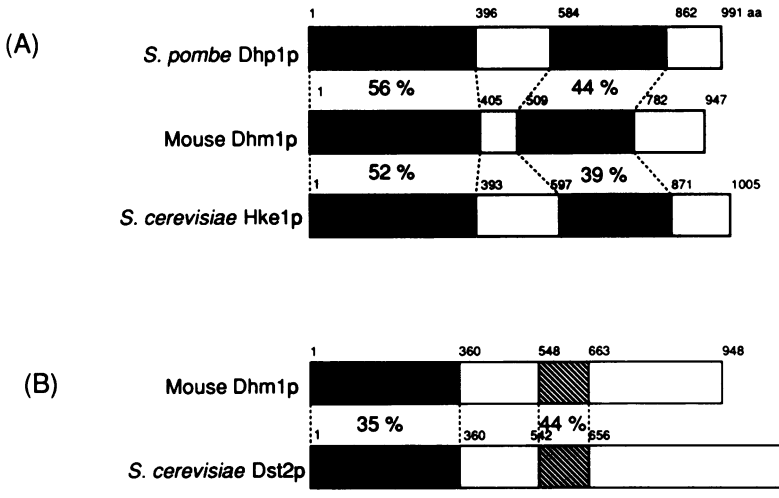

Figure 2. Structure of the mouse Dhm1 protein compared with *S.pombe* Dhp1p and *S.cerevisiae* Hke1p (**A**) and *S.cerevisiae* Dst2p (**B**). Shadowed bars indicate conserved regions.

RNA was detected in all tissues examined, this transcript was more abundant in testis, liver and kidney than in other tissues.

Complementation of *dhp1* mutation by *Dhm1* cDNA

The high degree of homology between the amino acid sequence predicted from *Dhm1* and that of *dhp1*$^+$ strongly suggested that the gene products are functionally similar. To examine this possibility, we tested whether *Dhm1* cDNA complements the temperature-sensitive *dhp1* mutation and the lethality of the *dhp1* null mutation of *S.pombe*. We constructed the plasmid pREP-Dhm1 from a multicopy shuttle vector pREP1 (24) which is able to express *Dhm1* cDNA under the control of the strong *nmt1* promoter (Fig. 4).

The fission yeast haploid strain MP102, containing a temperature-sensitive mutation in the *dhp1*$^+$ gene, was transformed with either pREP-Dhm1 or the control vector pREP1. The resulting Leu$^+$ transformants were isolated and their growth was monitored at the permissive (25°C) and non-permissive (36°C) temperatures, respectively. MP102 carrying pREP-Dhm1 could grow both at 25 and 36°C, while MP102 carrying pREP1 could grow

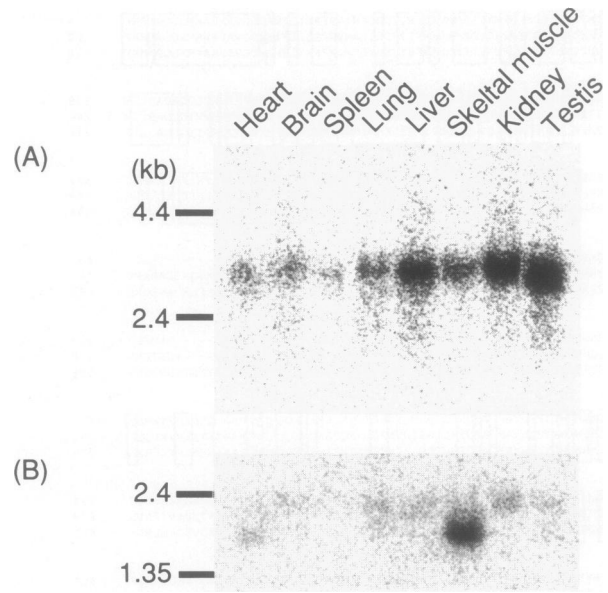

Figure 3. Northern blot analysis of the mouse *Dhm1* RNA in different mouse tissues. Each lane contains 2 μg of poly(A)$^+$ RNAs of adult mice. **(A)** The membrane was analyzed by autoradiograghy. **(B)** The same membrane was stripped and re-probed with ß-actin DNA. RNA size markers are indicated at the left.

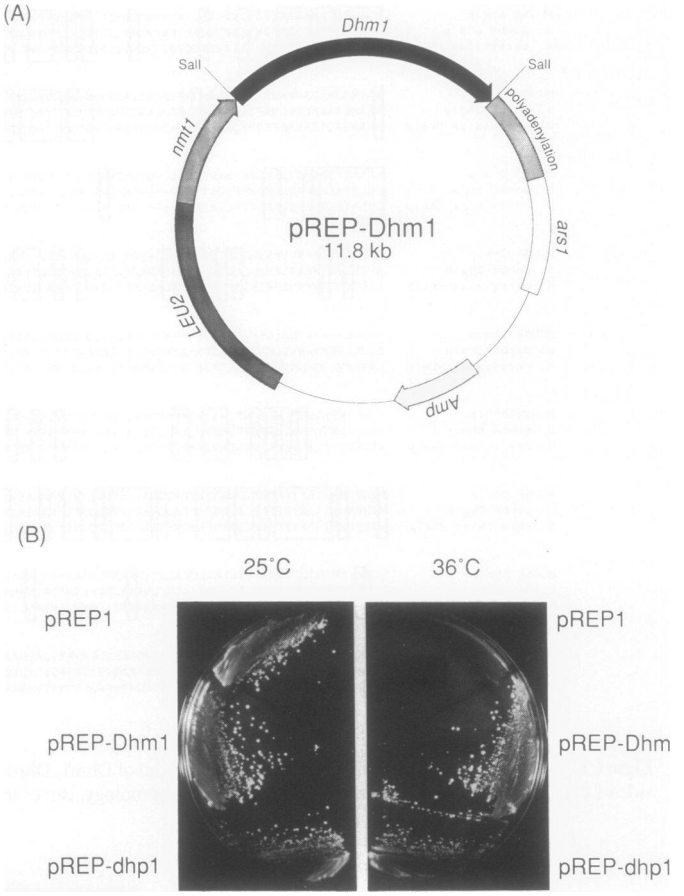

Figure 4. **(A)** Structure of the yeast expression plasmid carrying the mouse *Dhm1* gene. A 2.9 kb fragment containing the mouse *Dhm1* cDNA was ligated at the *Sal*I site of a shuttle vector pREP1 which contains the budding yeast *LEU2* gene as a selectable marker. In this plasmid the mouse *Dhm1* cDNA is expressed under the control of yeast *nmt1* promoter. *Amp*, ampicillin-resistance gene; *ars1*, portion of fission yeast autonomous replicating region. **(B)** Complementation of *S.pombe* temperature-sensitive *dhp1* mutation by the mouse *Dhm1* cDNA. Yeast strain MP102 was transformed with either the expression vector alone (pREP1) or carrying *Dhm1* (pREP-Dhm1) or *dhp1*$^+$ (pREP-dhp1) at 25°C. Transformants were streaked on MM media and incubated at 25 and 36°C. The plates were photographed on the 4th day of incubation.

at 25°C but not at 36°C (Fig. 4). Thus the *Dhm1* gene of mouse can complement the temperature-sensitive *dhp1* mutation.

The heterozygous diploid strain SSP1, in which one of two *dhp1*$^+$ genes was disrupted with the *ura4*$^+$ gene (13), was transformed with pREP-Dhm1 or pREP1. The resulting Leu$^+$ Ura$^+$ diploids harboring pREP-Dhm1 or pREP1 were sporulated, resulting in Leu$^+$ haploids. Then, Leu$^+$ haploids were transferred onto plates lacking uracil. The Leu$^+$ colonies correspond to haploid cells harboring plasmids, while Ura$^+$ Leu$^+$ correspond to *dhp1* null mutant cells harboring plasmids. If the latter cells were viable, many Leu$^+$ Ura$^+$ colonies should be obtained. Ura$^+$ Leu$^+$ haploids lacking the *dhp1*$^+$ gene can form colonies only when they carried pREP-Dhm1, while the vector pREP1 could not rescue the lethality (data not shown). This result demonstrated that *dhp1* null mutation is fully complemented by plasmid carrying *Dhm1*.

These data show that the fission yeast temperature-sensitive and null mutation of *dhp1*$^+$ can be rescued by the functional expression of mouse *Dhm1*.

DISCUSSION

We have determined the nucleotide sequence of mouse cDNA encoding a protein homologous to the *S.pombe* Dhp1p. The putative Dhm1 protein includes two highly homologous regions to the Dhp1 protein. These two regions correspond to the regions where the Dhp1p protein is highly homologous to the *S.cerevisiae* Hke1p. Since the *Dhm1* gene can compensate for the temperature-sensitivity of the *dhp1-1* mutation and the lethality of the *dhp1* null mutation, *Dhm1* functions in *S.pombe* as *dhp1*$^+$ does (see below).

It is implicated that these two highly homologous regions may be indispensable for the function of *dhp1*$^+$/*HKE1*/*Dhm1* which is essential for cell growth. In *S.pombe*, we have shown that both the N-terminal 90 amino acid residues and the C-terminal 205 amino acid residues, which are highly conserved among the *dhp1*$^+$, *HKE1* and *Dhm1* genes, are indispensable for the function, while C-terminal 125 amino acid residues, where *dhp1*$^+$ shows little homology to *HKE1* or *Dhm1*, are dispensable for mitotic growth (unpublished observation). These results are consistent with the notion that these two regions are necessary for the essential function of the *dhp1*$^+$/*HKE1*/*Dhm1* gene.

It should be noted that the high degree of conservation of the amino acid sequences among Hke1p, Dhp1p and Dhm1p reflected their functional similarities. On a multicopy plasmid and under the control of the *nmt1* promoter, the *Dhm1* gene can compensate for both the temperature-sensitivity of the *dhp1-1* mutation and the lethality of the *dhp1* null mutation. *S.cerevisiae HKE1*/*RAT1*/*TAP1* is shown to be involved in RNA trafficking and RNA synthesis (15,16) and encodes a protein of 116 kDa which has 5′→3′ exoribonuclease activity (14), suggesting its role in RNA metabolism. Dhm1p may also perform the essential

function in RNA metabolism. The significant homology between Dst2p and Dhm1p further implies that Dhm1p may have DNA strand exchange activity and participate in recombination process. The human HPP-1 that catalyzes DNA strand exchange has been purified and characterized (26,27). Although the molecular weight of HPP-1 is close to that of Dhm1p, it is not yet clear whether the human counterpart of *Dhm1* encodes HPP-1.

Northern blot analysis indicated that *Dhm1* transcript was present in all mouse tissues examined, although 3.5 kb mRNA was highly expressed in testis, kidney and liver. The high level of expression of *Dhm1* in testis suggests that Dhm1p may play a role in spermatogenesis, a process that involves mitotic proliferation, meiotic recombination, followed by reductional division and subsequent differentiation to sperm cells. We are now studying the spatial and temporal expression pattern of the *Dhm1* gene to obtain clues to its function.

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