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A Splicing Factor, Prp8: Preferential Localization in the Testis and Ovary in Adult Mice[1]

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Prp8 is a splicing factor of 220 kDa originally identified in yeast and is a component of the U5 small nuclear ribonucleoprotein particle. Mouse Prp8 cDNA was cloned and shown to share 62.6 and 68.2% sequence identity with the yeast homologue at the amino acid and nucleotide level, respectively, while it differs by only 3 amino acid residues from the human homologue. During mouse embryogenesis, Prp8 is expressed intensely at day 9.5 of gestation, and its expression decreases progressively during embryogenesis. In adult mice, Prp8 is expressed strongly in the testis and moderately in the ovary. *In situ* hybridization analysis revealed that Prp8 is preferentially expressed in the outer cell layer in the testis, probably in the spermatogonia and primary spermatocytes, and in granulosa cells in the ovary. In *Caenorhabditis elegans*, microinjection of a double stranded RNA corresponding to a portion of the Prp8 sequence results in the arrest of embryogenesis at the late-gastrulation stage. These results suggest that Prp8 plays an important role in reproduction and development. Prp8 was shown to bind to midkine (MK), a heparin-binding growth factor. Since Prp8 expression partially overlaps with the sites of action of MK, it is possible that binding to Prp8 is involved in part of MK signaling.

Key words: *Caenorhabditis elegans*, granulosa cells, midkine, Prp8, splicing factor, testis.

The splicing of nuclear pre-mRNA is a dynamic process catalyzed by the spliceosome, which assembles on the pre-mRNA by integration of several small nuclear ribonucleoprotein particles including U4, U5, and U6 (*1, 2*). Yeast genetics in combination with biochemical analysis has proven to be a powerful means for dissecting the protein components of the splicing apparatus. The identification of splicing factors has been facilitated by the isolation of *prp* (*p*re-m*R*NA *p*rocessing) mutants of *Saccharomyces cerevisiae* that have conditional lethal phenotypes due to defects in pre-mRNA splicing. At least 30 Prp genes have been identified to date (*3, 4*). A *Prp8* allele has been isolated as a genetic suppressor of the DEAD-box protein splicing factor, Prp28, a putative ATP-dependent RNA helicase (*5, 6*). The yeast Prp8 protein is a 220 kDa molecule that is a stable component of the U5 small nuclear ribonucleoprotein particle (*7–9*). During splicing, Prp8 interacts directly with the substrate RNA at the 5′ splice site region and the branch point-3′ splice site region (*10–14*). The human homologue of Prp8 (*15*), also designated as the U5-220 kDa protein, has been cloned and shown to share 63% sequence identity with the yeast homologue. This human protein has been shown to form a stable RNA-free complex with several U5-specific proteins, and also to make contact with both the pre-mRNA and the U5 small nuclear RNA (*15, 16*). Therefore, the Prp8 protein appears to play a central role in the assembly of the U5 small nuclear ribonucleoprotein particle also in vertebrates as well. However, the detailed mode of action and tissue distribution of the Prp8 protein have not been reported in vertebrates. As a step toward understanding the role of Prp8 *in vivo* in mammals, we cloned the mouse Prp8 cDNA and studied its mode of expression in adult organs and during embryogenesis.

We became interested in Prp8 because it was detected as a protein that binds to midkine (MK). MK is a heparin-binding growth factor (*17*) that promotes neurite outgrowth of embryonic neurons (*18*), the migration of neurons (*19*) and inflammatory leukocytes (*20*), prevents apoptosis (*21*) and enhances the fibrinolytic activity of endothelial cells (*22*). Furthermore, it enhances the recovery of preimplantation embryos developed *in vitro* (*23*) and prevents retinal

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Abbreviations: CMV, cytomegalovirus; DMEM, Dulbecco's modified Eagle's medium; d.p.c., embryonic day post coitus; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HA, hemagglutinin; MK, midkine; PB, phosphate buffer; PBS, Dulbecco's phosphate buffered saline; PI, propidium iodide; PMSF, phenylmethylsulfonyl fluoride; Prp, pre-mRNA processing; SSC, 16.6 mM sodium chloride/16.6 mM sodium citrate buffer, pH 7.0.

degeneration caused by exposure to constant light (*24*). The expression pattern of Prp8 was examined to evaluate whether the MK-Prp8 binding observed *in vitro* has functional implications. Finally, to test for a role of Prp8 in embryogenesis, we microinjected a Prp8 double strand RNA into the gonads of *Caenorhabditis elegans*.

MATERIALS AND METHODS

Isolation of mPrp8 cDNA—A mouse cDNA in the EST database (GenBank accession number AB047391), which shows homology to human Prp8, was obtained by PCR; the sense primer was 5′-CAGGGCTACAACATGCTCAA-3′ (nucleotide numbers 1536–1555) and the antisense primer was 5′-TTCGTTACTGTCTTTGCCAC-3′ (nucleotide numbers 2043–2062), and the template was cDNA made from mouse day 13.5 whole embryo mRNA. PCR was performed for 35 cycles of denaturation (94°C for 1 min), re-annealing (50°C for 1 min) and extension (72°C for 1 min). This cDNA fragment was labeled with [α-^{32}P]dCTP, and used as a probe. Approximately 1 × 10^6 plaques of a mouse day 11.5 whole embryo cDNA library in λgt11 (Clontech, CA, USA) were screened using the radioactive probe. Filters were hybridized overnight at 42°C in hybridization solution containing 50% formamide, 5 × SSC (16.6 mM sodium chloride/16.6 mM sodium citrate buffer, pH 7.0), 50 mM sodium phosphate buffer, 5 × Denhardt's solution, 1% SDS, and 50 μg/ml heat-denatured salmon sperm DNA. The filters were washed at 65°C in 0.2 × SSC with 0.1% SDS. Phage DNA from positive clones was digested with *Eco*RI, and the cDNA inserts were subcloned into the *Eco*RI site of the plasmid vector pBluescript SK(+). Nucleotide sequences of the fragments were determined by the dideoxy chain-termination method using an automated DNA sequencer (Licor model 4000). This procedure revealed 5.3 kb of mouse Prp8 sequences (nucleotide numbers 1–5297). To obtain a cDNA with more 3′-terminally-located sequences, a cDNA fragment (nucleotide numbers 4428–5297) was used as a probe to screen the 11.5 day mouse embryo library by plaque hybridization; this resulted in the isolation of a cDNA (nucleotide numbers 2940–6210). A further 3′ region was obtained by PCR with a sense primer based on the determined sequences of mouse Prp8 and an antisense primer designed based on human Prp8 sequences; the sense primer was 5′-CAGACTCGAACTGTCAACAA-3′ (nucleotide numbers 6168–6187) and the antisense primer was 5′-TCAACAACACAAGCACAGA-3′ (nucleotide numbers 7146–7166). PCR was carried out for 35 cycles of denaturation (94°C for 1 min), re-annealing (63°C for 1 min), and extension (72°C for 1 min). The PCR products were subcloned into the plasmid vector pGEM-T (Promega, WI, USA), and the nucleotide sequences were determined as described above.

Northern Blotting Analysis—Total RNA (5 μg) was prepared from ICR mouse tissues and whole embryos by the acid guanidinium isothiocyanate–phenol chloroform method (*25*). The probe for mouse Prp8 was prepared from a cDNA fragment of mouse Prp8 (nucleotide numbers 122–1075). RNAs were separated by agarose gel electorophoresis and transferred onto nylon membranes (*26*). The membranes were hybridized with the probe labeled with [α-^{32}P]-dCTP. The blots were washed at 65°C in 0.2 × SSC with 0.1% SDS. The membranes were exposed to a BAS-imaging plate and the radioactivity on the membrane was determined with a BAS 2000 Radioimage Analyzer (Fuji Film, Tokyo). As a control, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe (*27*) was used.

In Situ Hybridization—ICR mice, 8 weeks old, were anesthetized with Nembutal, and then perfused with 5 ml of Dulbecco's phosphate buffered saline (PBS) followed by 10 ml of 4% paraformaldehyde in PBS. Their testes and ovaries were removed, fixed in 4% paraformaldehyde in PBS overnight at 4°C, and embedded in paraffin. Sections 5 μm thick were cut, placed on silane-coated slide glasses, and then subjected to *in situ* hybridization as described previously (*28*). Sense and antisense RNA probes were prepared by *in vitro* transcription with a DIG RNA labeling kit (Boehringer Mannheim, Germany), using a fragment of Prp8 cDNA (nucleotide numbers 122–1075).

Expression of the Mouse Prp8-HA Fusion Protein in COS7 Cells—A cDNA with the whole Prp8 protein-coding sequence (nucleotide numbers 42–7049) and the hemagglutinin protein (HA) epitope (Try-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala) tagged at the C-terminal end was produced by PCR and ligation. This cDNA was subcloned in frame into the cytomegalovirus (CMV) expression vector, pcDNA3 (Invitrogen, CA, USA). COS7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. For DNA transfection, 2 × 10^4 cells were plated in the center of a 22 × 22 mm glass coverslide. Plasmid DNA was transiently transfected into COS7 cells using LipofectAMINE (Gibco BRL, Rockville, MD, USA), and the cells were cultured for 36 h at 37°C according to the instructions of the manufacturer. Cells were sequentially incubated as follows to detect the HA epitope: (a) fixed with ethanol and acetic acid (3:1) for 5 min at room temperature; (b) with PBS containing 0.1% goat serum to remove possible background for 20 min at room temperature; (c) with rat anti-HA antibodies (Boehringer Mannheim, Germany) in PBS for 1 h at 4°C; (d) with the second antibody (fluorescence isothiocyanate–conjugated anti-rat IgG; Capppel, ICN Pharmaceuticals, USA) for 1 h at 4°C. Washing was performed three times with PBS after each incubation. Nuclear staining was performed with a propidium iodide (PI) nucleic acid staining kit (Molecular Probes, Eugene, OR), and then observed under a confocal laser scanning microscope (MRC 1024; Nippon Bio-Rad, Tokyo).

Identification of Prp8 in MK Binding Proteins from Day 13 Mouse Embryos—MK binding proteins were isolated from the membrane fraction of day 13 mouse embryos by MK-Sepharose affinity chromatography as described previously (*29*), except that *Ricinus communis* agglutinin Sephrose affinity chromatography was not employed. After SDS-PAGE, the protein bands were excised, digested with trypsin in the gel, and the resulting peptides were identified by peptide sequencing after separation by high performance liquid chromatography as described previously (*29*).

Midkine Binding Properties of HA-Tagged mPrp8—pcDNA3 carrying HA-tagged mouse Prp8 cDNA was transfected into COS7 cells (2 × 10^6) using LipofectAMINE, after which the cells were cultured for 24 h in DMEM supplemented with 10% fetal calf serum at 37°C and then lysed with 1 ml of buffer A [20 mM Tris-HCl pH 7.5, 0.15 M NaCl, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. The lysate was centrifuged at 13,000 ×*g* for 10 min, and the supernatant was mixed with 0.2 ml of

MK-Sepharose, prepared as described previously (*29*), and rotated at 4°C overnight. The resin was then packed into a column, and washed with 20 column volumes of 20 mM phosphate buffer (PB), pH 7.0, and 0.15 M NaCl, and the

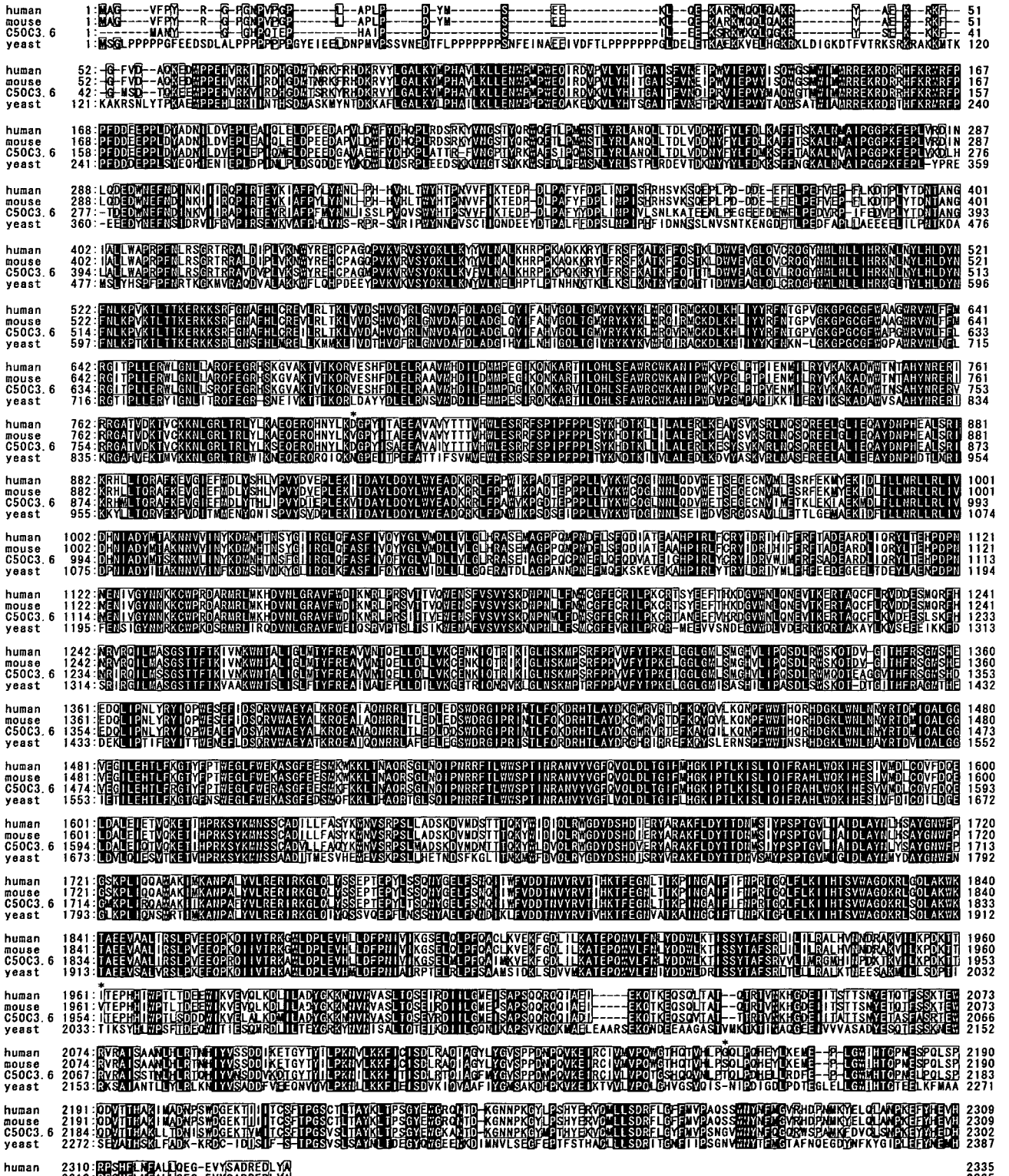

Fig. 1. **Comparison of the deduced proteins sequences of Prp8 from different species.** The amino acid sequences of mouse Prp8, human Prp8, *C. elegans* C50C3.6, and yeast Prp8 were compared. Amino acids conserved in all species are shown by white letters on a black background, while those conserved in three species are shaded. Stars indicate amino acids that differ between human and mouse Prp8.

bound proteins were eluted successively with 0.3 M NaCl, 0.5 M NaCl, 0.75 M NaCl, 1.0 M NaCl, 1.5 M NaCl, and 5 mM EDTA with 0.3 M NaCl in 20 mM PB, pH 7.0. The last 1ml wash and each eluate were precipitated with 10% trichloroacetic acid, and subjected to SDS-PAGE on a 7% gel. Then, the proteins were transferred onto a nitrocellulose membrane, which was incubated with 4% skim milk in PBS and then with rat anti-HA antibody (*30*) (Boehringer Mannheim, Germany, 100 ng/ml in PBS) for 2 h at 4°C. After washing with PBS containing 0.1% Tween 20, the membrane was incubated with affinity-purified anti–rat IgG conjugated with horseradish peroxidase, and the bound antibodies were visualized by enhanced chemiluminescence (Amersham Japan, Tokyo).

Poly(U) or Poly(I) Poly(C) Binding Properties of HA-Tagged Prp8—COS7 cells transfected with pcDNA3 carrying HA-tagged Prp8 were cultured in DMEM supplemented with 10% fetal calf serum for 24 h at 37°C and then lysed with 1 ml of buffer A. The lysates were centrifuged at 13,000 ×*g* for 10 min, and the supernatant was loaded onto a 0.2 ml of poly(U) or poly(I) poly(C) (Amersham Pharmacia) column equilibrated with 0.2 M NaCl in Buffer B consisting of 10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.5 mM dithiothreitol, and 10 mM PMSF. Proteins were eluted successively with 2 ml of 0.5 M NaCl, 1.0 M NaCl, 1.5 M NaCl, and 2.5 M NaCl in Buffer B. Proteins in each fraction were loaded onto a 7% gel for SDS-PAGE and electrophoretically transferred onto a nitrocellulose membrane (*31*). The membrane was analyzed by Western blotting as described above.

RNA Interference (RNAi) of the C. elegans Prp8 Gene—A segment of *C. elegans* DNA (C50C3.6, GenBank accession number L14433, Nucleotide numbers 1-831) was isolated by PCR using 5′-ATGGCCAACTACGGCGGTCA and 3′-AGTATGTAAATCTTTTACCA end nucleotides as primers and *C. elegans* DNA as the template. The conditions for PCR were the same as for the isolation of mouse Prp8 cDNA. The DNA segment was subcloned into Bluescript SK(+). The inserted DNAs were amplified by PCR with primers CMo24 and CMo422 and used as templates for RNA synthesis (Craig Mello, personal communication). The sense and antisense RNAs were synthesized simultaneously with T7 RNA polymerase (Boeringer Mannheim, Germany) in a single tube, and purified with an RNeasy kit (Qiagen). The RNAs (1 mg/ml in distilled water) were injected into the gonads of adult wild type *C. elegans* (N2) hermaphrodites (*32*). F1 progeny laid between 24 h and 48 h after the injection were examined under a dissection microscope. Some embryos were observed under a microscope (Axioplan, Zeiss) equipped with Nomarski optics..

RESULTS

Molecular Cloning of Mouse Prp8—Mouse Prp8 cDNA was cloned based on the sequence of human Prp8 cDNA. The protein and nucleotide sequences of mouse Prp8 have been submitted to the GenBank and DDBJ databases under the accession number AB047391. The mouse cDNA shares 90% sequence identity with the human homologue. The deduced protein sequence of mouse Prp8 comprises of 2,335 amino acids and differs from the human homologue in only 3 amino acids (Fig. 1). Its level of homology to the yeast homologue is 62.2% (Fig. 1). The remarkable homology be-tween human and mouse Prp8 as compared to that be-tween mouse and yeast suggests that some interaction with other proteins has been acquired during evolution. Using the tBLASTn program (*33*), a genomic sequence corresponding to the cDNA sequence of mouse Prp8 was found on mouse chromosome 19 (accession number AC002121).

Expression of Prp8 in Mouse Organs and during Embryogenesis—Northern blotting analysis revealed that the Prp8 mRNA is expressed most strongly at 9.5 days of gestation among the periods of mouse embryogenesis so far examined (Fig. 2A). Among adult mouse organs, Prp8 mRNA is strongly expressed in the testis, with moderate expression observed in the ovary. Other organs showed either weak or no expression (Fig. 2B); for example, the ratio of Prp8 mRNA/GAPDH mRNA (Bio-Rad Gel Doc 1000) in the kidney was 1/35 of the value in the testis. *In situ* hybridization revealed that cells in the outer layers of the testis strongly express Prp8 mRNA (Fig 3B). Cells with particularly strong expression might be primary spermatocytes and spermatogonia. In the ovary, Prp8 mRNA is detected preferentially in granulosa cells (follicle type 5b and 7, Ref. *34*) (Fig. 3, E and H).

Poly(U) Binding Capability of Prp 8—Prp8 with the HA tag was located in both the nucleus and cytoplasm of COS7 cells transfected with the expression vector (Fig. 4). The expressed protein bound to a poly(U) agarose column, and was eluted by 0.5 M NaCl (Fig. 5A). The protein showed little binding to a poly(I) poly(C) column (Fig. 5B). These results are consistent with the view that Prp8 is an RNA binding protein with nucleotide sequence specificity.

MK Binding Capability of Prp8—The initial discovery of Prp8 as an MK binding protein was brought about by extensive protein sequence analysis of arrays of proteins in the MK-binding fraction (Fig. 6). MK binding proteins were isolated by affinity chromatography of CHAPS-solubilized membrane proteins on MK-agarose. After separation on SDS-PAGE, each protein band in the gel was digested with trypsin, and the resulting peptides were sequenced after HPLC separation. The 220 kDa band yielded a peptide with the sequence of TEDPDLPAIYYDPLINPL, which is identical to a sequence in human Prp8. Since arrays of proteins bind to MK, we carried out further studies to reveal the specificity of the binding. First, we observed that Prp8 with the HA tag also bound to the MK agarose column at 0.15 M NaCl and eluted at 0.3 M NaCl (Fig. 5C), confirm-

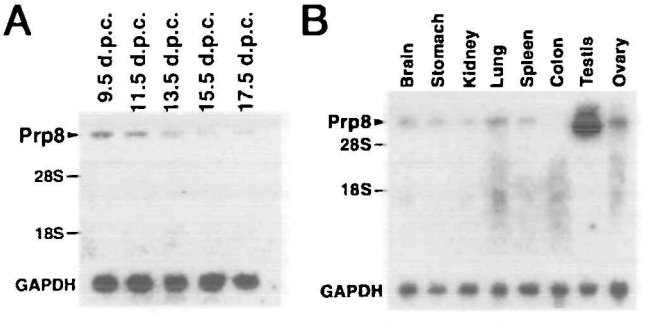

Fig. 2. **Expression of Prp8 mRNA as determined by Northern blotting analysis.** (A) Whole mouse embryos at different developmental stages. (B) Organs of adult mice. Prp8, 28S, and 18S indicate the positions of the respective RNAs. d.p.c., embryonic day post coitus; GAPDH, RNA of GAPDH.

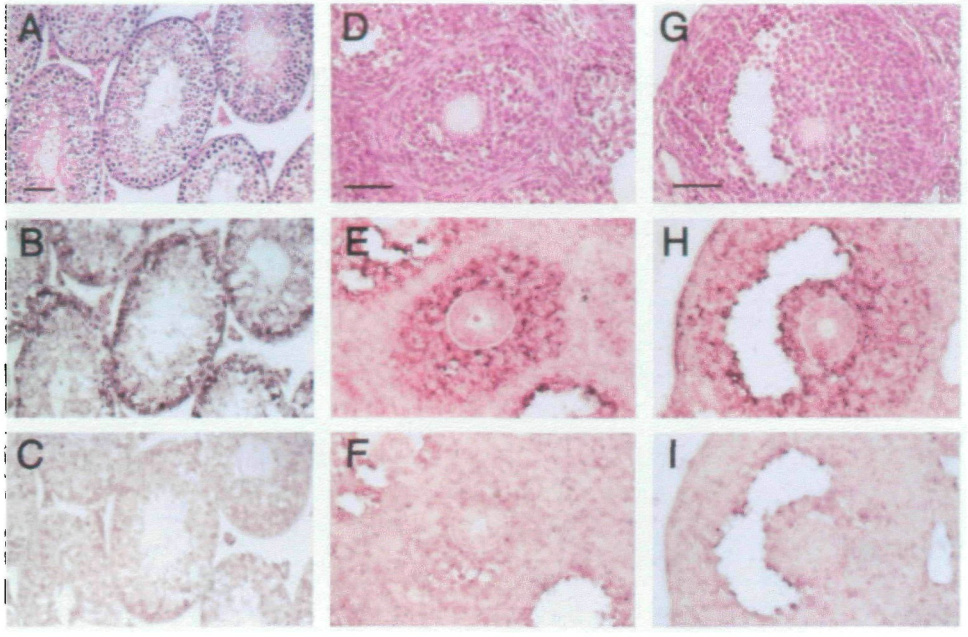

Fig. 3. **Localization of Prp8 mRNA in the testis and ovary of adult mice.** (A, B, C) Testis. (D, E, F) Ovary (follicle type 5b). (G, H, I) Ovary (follicle type 7). (A, D, G) Hematoxylin-eosin staining. (B, E, H) Staining with the anti-sense Prp8 probe. (C, F, I) Staining with the sense Prp8 probe. Bar, 50 μm.

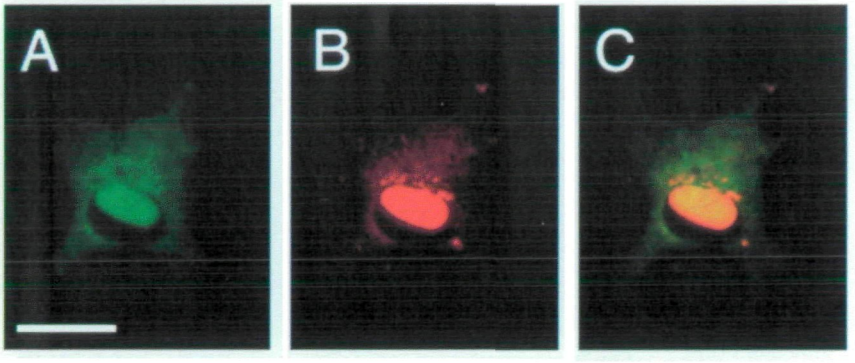

Fig. 4. **Localization of a Prp8-HA fusion protein in COS7 cells.** COS7 cells transfected with Prp8-HA expression vector were examined by confocal fluorescence microscopy for the localization of a Prp8-HA fusion protein (A) and by PI staining for the localization of nuclei (B). Two images are merged (C). Bar, 50 μm.

ing the binding capability of Prp8 to MK. In the next experiment, Prp8 with an HA tag was purified by absorption to a poly(U) column, and after ribonuclease treatment applied to an MK-agarose column. Prp8 was again absorbed to MK column, and was eluted by 0.3 M NaCl, but not by 0.2 M NaCl (Fig. 5D). Prp8 did not bind at all to a Sepharose 4B column used as a control. These results indicate that the interaction of Prp8 with MK is relatively specific.

Inhibition of Embryogenesis in C. elegans by Prp8 Double-Stranded RNA—We also examined the role of Prp8 directly in embryogenesis. For this, we employed double-stranded RNA injection into the gonads of *C. elegans* (*35*), since a protein homologous to Prp8 has been found in this organism. The progeny of animals injected with double-stranded Prp8 RNA exhibited severe embryonic arrest. Most of the progeny (349/350) did not hatch, while the one animal that did hatch was arrested at the first larval stage (L1). We chose several (n=82) embryos that failed to hatch at random, and found that all were arrested at late-gastrulation (Fig. 7). No apparent morphological abnormalities were found in the arrested embryos; they appeared identical to normal embryos at the late-gastrulation stage. In a control experiment, in which T2IB6.3 RNA was similarly injected, 98% of embryos hatched normally. This stage-specific arrest and the absence of any cytotoxic effect suggest that the double-stranded RNA specifically inhibited the function of Prp8.

DISCUSSION

One of the most interesting findings of the present investigation was the very strong expression of Prp8 in adult testis. *In situ* hybridization analysis revealed that the message is preferentially located in the outer cell layer, which corresponds to the spermatogonia and primary spermatocytes. A moderate level of expression was found in the ovary, and granulosa cells showed strong expression of the message. Furthermore, during mouse embryogenesis, the intensity of Prp8 mRNA expression progressively decreased. These results suggest that Prp8 plays an important role in reproduction and development. This appears to contradict the general suggestion that Prp8 is a fundamental component of the U5 small nuclear ribonucleoprotein particle (*13*). However, the observations that mouse Prp8 is at least partially present in the nucleus and that it binds to a poly(U) column are consistent with a role as a splicing

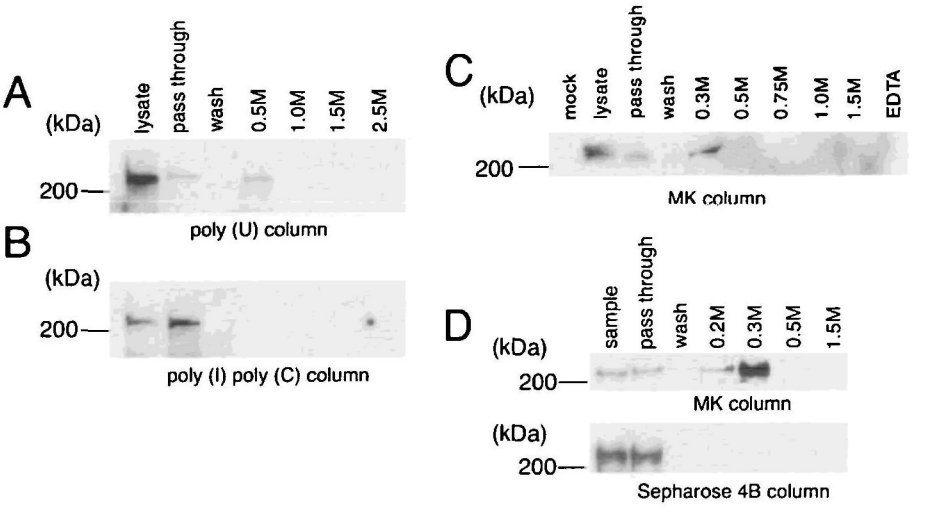

Fig. 5. **Binding of HA-tagged Prp8 to Poly(U), Poly(I) Poly(C), or MK.** HA-tagged Prp8 was subjected to affinity chromatography on the respective ligands, and eluted by increasing NaCl concentration. HA-tagged Prp8 was detected by Western blotting after SDS-PAGE of the eluate. (A) Eluate from a poly(U) column. (B) Eluate from a poly(I) poly(C) column. (C) Eluate from an MK column. (D) Eluate from an MK column after prior purification of the Prp8 by poly(U) column chromatography. The eluate from a poly(U) column was digested with 10 μg/ml ribonuclease A (Sigma, MO, USA) at 37°C for 30 min, and subjected to MK-Sepharose column chromatography as described in "MATERIALS AND METHODS," except that elution with 0.2 M NaCl in 20 mM PB was added. As a control, a sample treated in the same way was analyzed on a Sephrose 4B column.

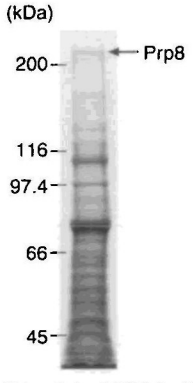

Fig. 6. **Identification of Prp8 in MK binding proteins from the membrane fraction of day 13 mouse embryos.** MK binding proteins were subjected to SDS-PAGE on a 7% running gel, and the proteins were stained with Coomassie Brilliant Blue. The positions where standard proteins migrated are shown by the molecular mass values (kDa) of the proteins. The arrow indicates Prp8.

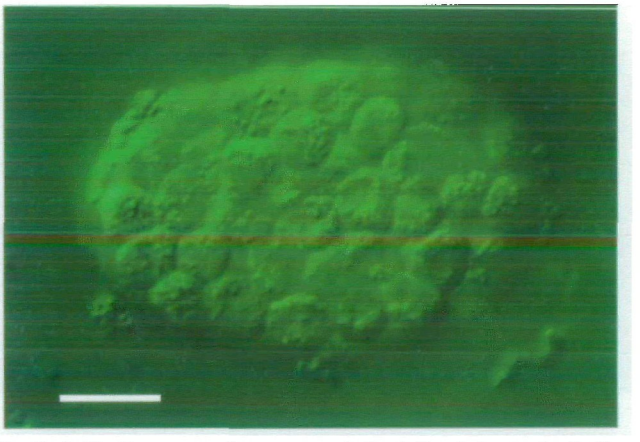

Fig. 7. **A *C. elegans* embryo arrested at the late-gastrulation stage by the injection of Prp8 double stranded RNA.** Nomarski optics were used for microscopic examination. Bar, 10 μm.

factor. Prp8 in vertebrates may have acquired some specific role in addition to that shared with the yeast counterpart.

A protein homologous to Prp8 has also been identified in *C. elegans*. Although the protein is called C50C3.6, it shows 86.6, 71.0, and 60.7% identity to human, mouse and yeast Prp8, respectively, at the protein level. Due to the presence of conserved amino acid residues among these proteins (Fig. 1), we conclude that C50C3.6 is the *C. elegans* counterpart of Prp8. Injection of a double-stranded RNA corresponding to a sequence of *C. elegans* Prp8 into the gonads of *C. elegans* resulted in the arrest of embryogenesis at the late-gastrulation stage. *In situ* hybridization using the antisense RNA corresponding to the injected double-stranded RNA revealed preferential expression of Prp8 RNA also in the *C. elegans* gonad (unpublished results). These results strongly suggest that Prp8 indeed plays a role in development, and that a specific function of Prp8 in reproduction might be conserved in multicellular organisms.

We also found that Prp8 binds to MK, a heparin-binding growth/differentiation factor with anti-apoptotic and migration-promoting activities. Although we did not confirm the physiological significance of this binding, the strong expression of *Prp8* in day 9.5–11.5 embryos suggests that MK, which is active in midgestation and is also located intracellularly during this period (*36*), may influence the splicing activity of the U5 small nuclear ribonucleoprotein particle. MK is also known to enhance the survival of preimplantation embryos cultured *in vitro*. This activity probably reflects its protective action *in vivo* (*23*). The direct target cells of MK are granulosa cells surrounding the embryos (*23*). The strong expression of Prp8 mRNA in granulosa cells determined in the present study is interesting in this respect, and there is also a possibility of a functional interaction between MK and Prp8 in these cells. An obvious question is how an extracellular protein, MK, can interact with Prp8, which functions in the nucleus as a splicing factor. The nuclear localization of MK has been demonstrated in mouse hemangioma cells (*37*). Furthermore, MK binds

to nucleolin, which is a shuttle protein located in the cytoplasm, nucleus and on the cell surface (*37*). In addition, it has been found that the binding of MK to the laminin binding protein results in nuclear transport of the molecular complex (R. Salama *et al.*, manuscript in preparation). Therefore, it is possible that MK partly functions in the nucleus after nuclear transportation and that a target of nuclear MK is Prp8.

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