Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12258–12262, December 1994
Developmental Biology

Isolation of a DEAD-family protein gene that encodes a murine homolog of *Drosophila* vasa and its specific expression in germ cell lineage

YUKO FUJIWARA*†, TOHRU KOMIYA*, HIROMI KAWABATA*, MICHIO SATO‡, HIROKAZU FUJIMOTO‡, MITSURU FURUSAWA*§, AND TOSHIAKI NOCE‡¶

*Furusawa Morphogene Project, ERATO, Research Development Corporation of Japan (JRDC), 2-5-2 Nagata, Chiyoda, Tokyo 100, Japan; ‡Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan; and §Molecular Biology Research Laboratory, Daiichi Pharmaceutical Co., Ltd., 16-13 Kitakasai, Edogawa, Tokyo 134, Japan

Communicated by Allan C. Spradling, August 9, 1994 (received for review April 18, 1994)

ABSTRACT **In an effort to study the molecular basis of the determination processes of the mammalian germ cell lineage, we have tried to isolate a mouse gene homolog to vasa, which plays an essential role as a maternal determining factor for the formation of *Drosophila* germ cell precursors. By reverse transcriptase PCRs of mouse primordial germ cell cDNAs using family-specific primers, we obtained a gene (*Mvh*) encoding a DEAD-family protein that showed a much higher degree of similarity with the product of the *Drosophila* vasa gene (*vas*) than previously reported mouse genes. In adult tissues, *Mvh* transcripts were exclusively detected in testicular germ cells, in which Mvh protein was found to be localized in cytoplasm of spermatocytes and round spermatids including a perinuclear granule. The protein was also expressed in germ cells colonized in embryonic gonads but was not detected in pluripotential embryonic cells such as stem cells and germ cells. These results suggest the possibility that the Mvh protein may play an important role in the determination events of mouse germ cells as in the case of *Drosophila* vasa.**

In mice, germ cell precursors, termed primordial germ cells (PGCs), are generated in the epiblast. They consist of putative pluripotential cells that can be first identified as a small cluster of alkaline phosphatase-positive cells in the extraembryonic mesoderm at 7.25 days postcoitum (dpc). Then they move down to the embryonic mesoderm at the posterior end of the primitive streak (8.0 dpc), migrate through the hindgut endoderm, and colonize the developing genital ridges (10.5–11.5 dpc), in which they are destined to form functional germ cells (1, 2). Despite these detailed morphological observations, molecular mechanisms regulating this developmental pathway still remain unclear.

Unlike mammals, it has been well established that several maternal factors are involved in germ cell determination of many animal species. In particular, in *Drosophila* it has been shown that the pole plasm localized at the posterior pole of the oocyte contains determining substances for the abdomen and the germ lineage. After fertilization, only the nuclei that migrate into the pole plasm are destined to form the germ-line progenitors (pole cells). Genetic identification of genes whose function is required for pole cell formation has revealed at least eight maternally active genes, cappucino, spire, staufen, oskar, vasa, valois, mago-nashi, and tudor (3, 4). Among these genes, vasa (*vas*) is one of the best characterized. Homozygous mutant vasa females produce no eggs. In ectopic formation of pole cells induced by mislocalization of oskar mRNA to the anterior pole of the oocyte, only vasa and tudor were required for ectopic pole cell formation (5–10). The gene *vas* encodes a DEAD (Asp-Glu-Ala-Asp)-family protein of putative RNA helicases, which is found to be present as a component of both the polar granules at the posterior end of the oocyte and the nuage structure in the germ cells, and zygotic expression is also restricted to the germ lineage (6–9).

In this study, to help analyze the molecular mechanisms of mammalian germ-line determination, we have isolated a mouse DEAD protein gene (*Mvh*) by PCR cloning.‖ Amino acid sequence analyses showed that *Mvh* displayed a greater degree of similarity to *vas* than other mouse DEAD-family genes, such as *p68* (11, 12), *PL10* (13), and *eIF4A* (14). The expression profile suggested that *Mvh* was a true homolog of *vas*, and it should be a valuable molecular marker to specify a key step of the fate determination of mammalian germ cells.

MATERIALS AND METHODS

cDNA Cloning of *Mvh*. cDNAs as templates for PCR were prepared from PGCs purified from 12.5-dpc embryonic gonads of mice (ICR, Shizuoka) as described by Abe (15). Degenerate oligonucleotide mixtures for DEAD-family-specific primers were derived from the two conserved amino acid sequences (MACAQTG and VLDEADRM) in the putative ATP-binding domain of *vas*. The sequences of the 5′-sense and 3′-antisense primers were 5′-CCGGATCCATGGCNTG(T/G)GCNCA(A/G)ACNG-3′ (28-mer) containing a *Bam*HI site and 5′-CCAAGCTTAANCCCAT(A/G)TCNA(A/G)CATNC(G/TorT/C)TC-3′ (31-mer) containing a *Hin*dIII site, respectively. PCR amplification was performed in 50 mM Tris·HCl, pH 8.3/150 mM KCl/2 mM $MgCl_2$/0.2 mM dNTP/20 μM each primers/2.5 units of Amplitaq polymerase/500 ng of template cDNA. After reaction of 30 cycles (denaturing for 30 sec at 94°C, annealing for 60 sec at 55°C, and extension for 60 sec at 72°C), three DNA fragments of ≈0.4 kb were obtained and subcloned into pBluescript SK plasmids (Stratagene). For the secondary screening, ≈1 × 10^6 recombinant phages of an adult mouse testis λgt11 cDNA library (Clontech) were screened and the resulting three positive phage clones (see Fig. 1, clones E, F, and J) were subcloned into plasmids and sequenced. Moreover, most upstream cDNA clones were isolated by the 5′-RACE method (rapid amplification of cDNA ends) (Ampli-finder kit; Clontech) using an antisense primer and adult testis cDNAs as templates, according to the manufacturer's protocol.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PGC, primordial germ cell; dpc, days postcoitum; EG cell, embryonic germ cell; ES cell, embryonic stem cell.

†Present address: Roche Institute of Molecular Biology, 340 Kingsland Street, Nutley, NJ 07110.
¶To whom reprint requests should be addressed.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. D14859).

Northern Blot and *in Situ* Hybridization Analyses. The conditions of Northern blot and *in situ* hybridization were according to the methods described by Noce *et al.* (16). A 700-bp *Hin*dIII fragment (Fig. 1*A*) of *Mvh* cDNA was labeled with [^{32}P]dCTP by using a multiprime labeling kit (Amersham) and was used as a probe for Northern hybridization. Adult testes fixed in Bouin's solution were embedded in paraffin and sectioned for *in situ* hybridization analyses. Probes of sense and antisense digoxigenin-labeled RNA strands were transcribed *in vitro* from a linearized clone E plasmid (Fig. 1*A*) by using an RNA labeling kit (Boehringer Mannheim). Hybridization signals were detected by using alkaline phosphatase-conjugated anti-digoxigenin antibody and NBT as the chromogen.

Immunoblotting and Immunocytochemistry. Synthetic 30-mer oligopeptides corresponding to amino acids 113–143 (underlined in Fig. 1*B*) were used to produce specific antibodies against Mvh protein. The peptide coupled to bovine serum albumin was injected subcutaneously into a rabbit six times at 2-week intervals. The antibodies to the peptide were purified by affinity chromatography using synthetic peptide-conjugated Sepharose beads. Details of immunoblotting and immunocytochemistry have been described (16). The EG (embryonic germ) cell lines used were established by Y. Matsui from the migratory PGCs of 8.5-dpc embryos of DBA/2 and BALB/c mouse strains (17). ES (embryonic stem) cell lines used were D3 and CCE1.

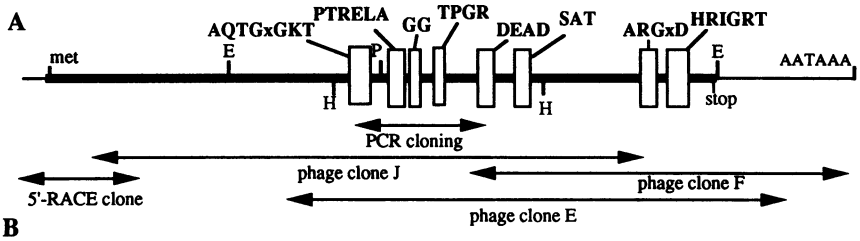
RESULTS

Isolation of Mouse *vas* Homolog cDNA. To isolate a mouse homolog of *Drosophila vas*, PCR cloning was performed with degenerated primers that were designed to amplify a part of the ATP-binding sequence conserved in DEAD-family genes. As a result, two bands of ≈400 bp were exclusively produced. Nucleotide sequences of these DNA fragments revealed three types of clones: (*i*) the larger one was a corresponding part of *PL10*, which had been previously reported as a *vas*-related gene specifically expressed in spermatogenetic cells (13); (*ii*) a minority of clones derived from the smaller band matched the mouse *p68*, which encodes a nuclear protein expressed in meiotic testicular cells and also in various other tissues (11, 12); (*iii*) the remainder, named V6, encode a DEAD-family protein. The V6 nucleotide sequence showed a higher similarity to *Drosophila vas* and *Xenopus XVLG1* (18) than the other two clones, as described below.

A transcript of ≈3.5 kb was specifically detected in adult testis by Northern blot analysis using the V6 cDNA probe (Fig. 2). Therefore, secondary screening was carried out in adult testis cDNAs. By screening of the phage library and using 5′-RACE cloning, several overlapping clones were isolated (Fig. 1*A*), and nucleotide sequence analyses (2.7 kb) revealed a full-length coding region of 735 amino acids (M_r, 79,952), which was redesignated as *Mvh* (mouse *vas* homolog). Compared with the size of the transcript, it was presumed that a 5′ nontranslational region of ≈0.7 kb was missing.

Southern blot analyses with *Mvh* showed that a single locus was detected in the mouse genome and revealed the presence of highly homologous genes in mammals such as human and caw (unpublished data).

Putative Amino Acid Sequences of Mvh. The predicted Mvh amino acid sequence contains eight absolutely conserved stretches (Fig. 2*A*), showing that Mvh is a member of the DEAD-box helicase family. Comparison with *Drosophila* vasa protein over the entire alignment indicated 38.9% identity between both (Fig. 1*B*), which is a clearly higher score than those between vasa and other mouse DEAD-family proteins: PL10 (35.9%), p68 (26.3%), and eIF-4A (27.9%). This predominance in homology to vasa is also observed when the conserved helicase domain of Mvh (amino acids 292–363, aligned in Fig. 2*A*) is compared with those domains of other DEAD-family proteins. First, the similarity of the Mvh domain to vasa (54.8% identity) is significantly higher than those to other newly isolated members of the *Drosophila* DEAD-family protein that are also found to be expressed in germ cells: 27.3% to ME31B (19) and 24.7% to Dbd73d (20). Furthermore, the identity to vasa (54.8%) and *Xenopus* XVLG1 protein (70.9%) is higher than those of any other pairs; for example, 50.7% between PL10 and vasa and 50.3%



B

```
          1                                                  50
Mvh       MGDEDWEAEILKPHVSSYVPVFEKDKYSSGANGDTFNRTSASSEMEDGPSGRDDFMRSGFPSGRSLGSRDIGESS
Drosophila | | ||  |   | |              |   |       | | |    |       |   |   | ||
vasa      MSD-DWDDE---PIVDTRGAR-------GGDWSDDEDTAKSSGERE----GDGNGGSGGEGGGYQGGNRDVFGR-
                     100                                                 150
KKENTSTTGGFGRGKGFGNRGFLNNKFEEGDSSGFWKESNNDCEDNQTRSRGFSKRGGCQDGNDSEASGPFRRGGRGSFRGCRGGF
        ||   |  |||                                  ||   |   |    || || | |||  ||
---IGGGRGGGAGGYRGGNRD-----------------------------------GGGFHGGRREGERDFR-GGEGGFRGGQGGS
                                  200
GLGRPNSESDQDQGTQCGGGFLVLGKPAASDSGNGDTYQSRSGSG-RGGYKGLNEEVVTGSGKNSWKSETEGGESSDSQGPKVT-Y
  |   |   |       |||         |   ||    |     |||  |                                  |
RGGQGGSRGGQGGFRGGEGGFR-GRLYENED---GDERRHRLDREERGGERGERGDGGFARRRRNEDDINNNNNIAEDVERKREFY
    250                                 291               644   650
IPPPPPEDEDSIFAH-YQTGINFDKYDTILVEVSGHDAPPAILTFEE -----352aa----- DTDSDNHLAQPLVKVLSDAQQD
|||  |   |   ||      || | ||  | | | | | | |     (conserved region)   |   |  ||| |    |
IPPEPSNDAIEIFSSGIASGIHFSKYNNIPVKVTGSDVPQPIQHFTS -----344aa----- HPEKDRAIAADLVKILEGSGQT
                                700                                735
VPAWLEEIAFSTYVPPSFSSSTRGGAVFASVDTRKNYQGKHTLNTAGISSSQAPNPVDDESWAGIPAWVVstop
||  |                      |  ||| |         |  |     | | |  | |
VPDFLRTCGAGGDGGYSNQN-------FGGVDVR------GRGNYVGD----ATN-VEEEQWDstop
```

FIG. 1. Deduced amino acid sequence of Mvh and alignment with vasa protein. (*A*) Schematic structure containing eight conserved motifs (boxed) and coding regions of five cDNA clones isolated are indicated. E, *Eco*RI; H, *Hin*dIII; P, *Pst* I. (*B*) Comparison of the entire sequence of Mvh with that of *Drosophila* vasa (5), except for the conserved region indicated in Fig. 2*A*. Amino acid sequence used for preparing the specific antibodies is underlined.

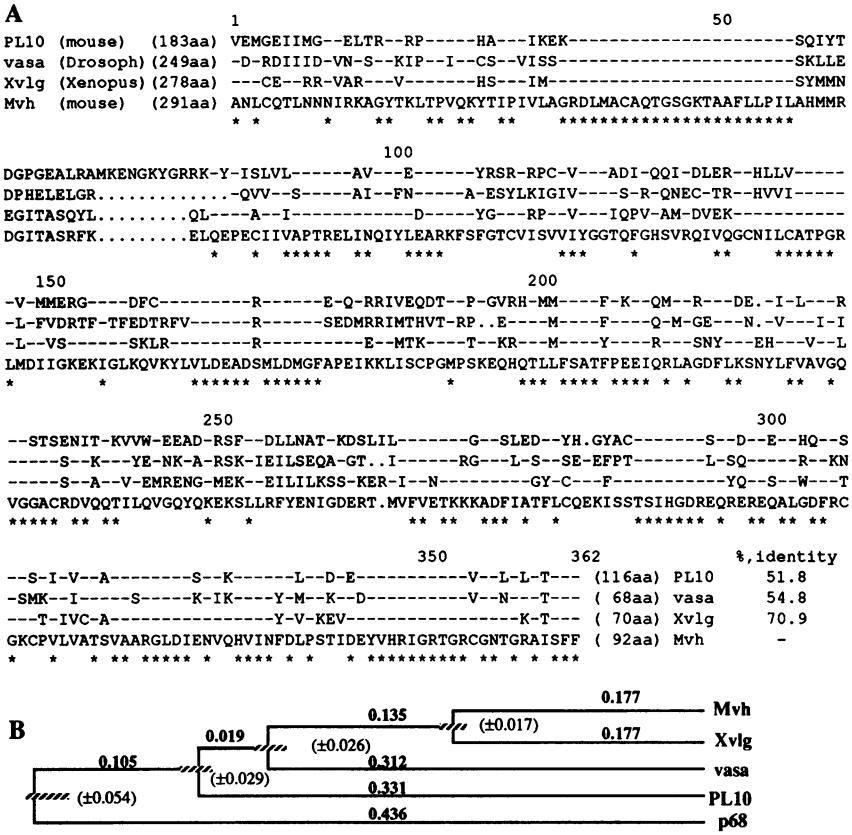


FIG. 2. Comparison of conserved regions of vasa-related gene family members. (*A*) Comparison of conserved regions of PL10 (13), vasa (5), XVLG1 (18), and Mvh proteins. Amino acids identical with Mvh protein are indicated by hyphens and amino acids conserved among vasa, XVLG1, and Mvh are indicated with asterisks in the sixth row. Percentages of identity of three other members against Mvh are shown (lower right). (*B*) UPGMA (unweighted pair group method with arithmetic mean) tree of five vasa-related proteins. Average similarities in the amino acid sequences of the conserved regions are calculated by using GENE-WORKS(2,2,1) software (IntelliGenetics). Resulting scores reflect the relationship of one sequence to another.

between PL10 and XVLG1, indicating that Mvh and XVLG1 are in the same evolutionary pathway as vertebrate vasa homologs (Fig. 2*B*).

Thus, structural similarity to *Drosophila* vasa is also notable in the nonconserved region (Fig. 1*B*). Acidic amino acid stretches close to a tryptophan (W) residue located at the N-terminal and C-terminal site are common characteristics among vasa, XVLG1, and Mvh proteins. A glycine (G)-rich region found in a number of putative RNA binding proteins is present in the N-terminal region of Mvh (22% in residues 50–250), which contains four RGG sequences showing similarity to RGGE/QGG repeats found in the corresponding region of *Drosophila* vasa (5, 8).

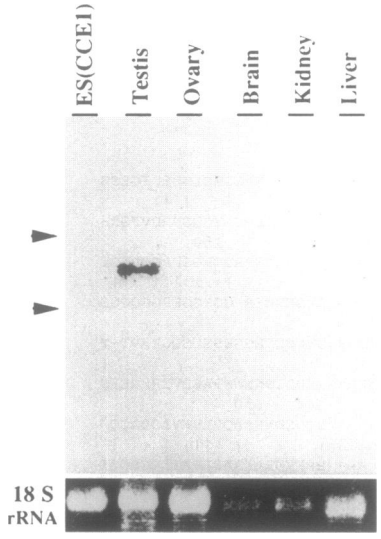


FIG. 3. Northern blot analyses of *Mvh* expression. (*Upper*) Total RNA (20 μg) from the indicated adult mouse tissues and ES (CCE1) cells was loaded for each lane. Positions of 28S and 18S rRNAs are indicated (arrows). (*Lower*) 18S rRNA bands stained with acridine orange.

Expression Pattern of Mvh. *Mvh* gene expression was initially examined by Northern blot analysis of adult tissues and ES cells. *Mvh* transcripts were exclusively detected as a single band (3.5 kb) in the testis among adult tissues examined and were not detected in ES cells (Fig. 3). To define the cellular localization of *Mvh* transcripts in the testis, *in situ* hybridization using digoxigenin-labeled RNA probes corresponding to *Mvh* cDNA was performed (Fig. 4 *A* and *B*). Hybridization signals were found to be highly concentrated within spermatocytes prior to the first meiotic division. Relatively weaker signals were detectable in early round spermatids just after the second meiotic division, and no significant signals were detected either in the subsequent stages of elongated spermatids to sperm or in testicular somatic cells such as interstitial cells and Sertoli cells, indicating that *Mvh* expression is developmentally regulated during spermatogenesis.

To investigate the temporal and spatial expression pattern of Mvh protein, specific antibodies were raised against 30-mer oligopeptides corresponding to the N-terminal unique region of Mvh (Fig. 1*B*). By immunoblot analysis using the affinity-purified antibodies, a single band of ≈85 kDa was detected in adult testis but was not detected in other tissues

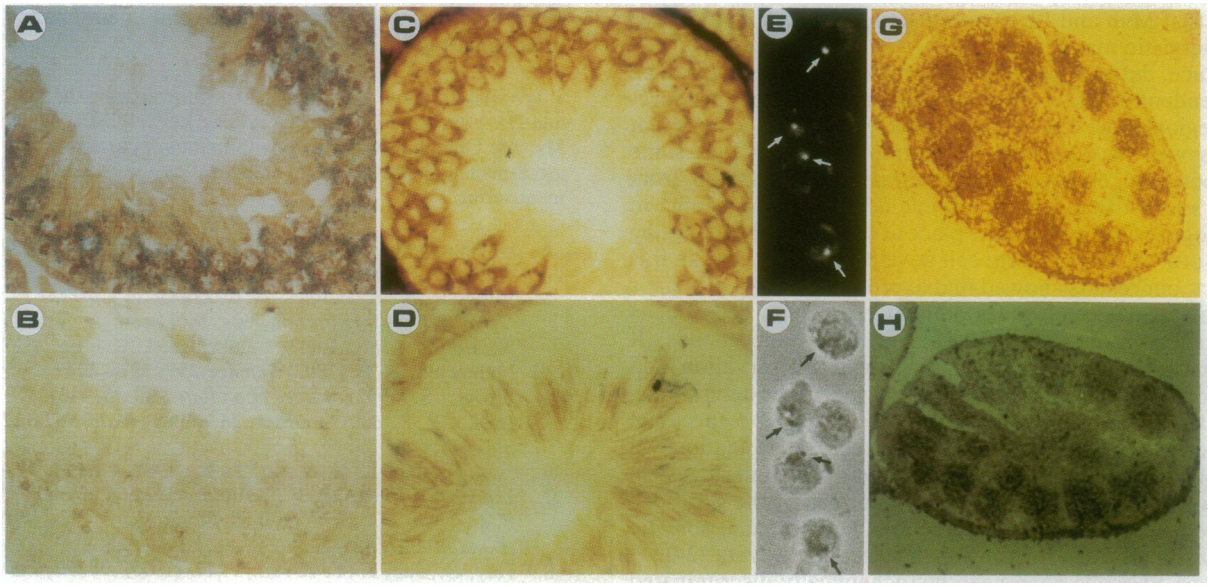


FIG. 4. Mvh expression in adult testis and embryonic gonad. *In situ* hybridization to adult testis sections with the antisense RNA strand (*A*) and the sense strand (*B*). Sections of the adult testis (*C* and *D*) and the 12.5-dpc male embryonic gonad (*G*) were immunostained with anti-Mvh antibodies (*C* and *G*) or preimmune antibodies (*D*). Signals were detected with secondary antibodies and enzymatic colorization by alkaline phosphatase-conjugated anti-digoxigenin antibody (*A* and *B*) or horseradish peroxidase-conjugated anti-rabbit IgG (*C*, *D*, and *G*). No significant staining was detected with preimmune antibodies in sections of adult testis (*D*) and embryonic gonad (data not shown). Fluorescent microscopic view of dispersed testicular cells stained with anti-Mvh antibody, followed by Texas Red-conjugated anti-rabbit IgG (*E*), and corresponding phase-contrast view (*F*). Arrows point to granular stains (*E*) and the corresponding structures (*F*) observed in the perinuclear zone of round spermatids. (*H*) To show the localization of PGCs in the embryonic gonad, endogenous activity of alkaline phosphatase was histochemically detected in a neighboring section of *G*, indicating that cells stained in *G* were gonadal germ cells.

(Fig. 5*A*). Moreover, coincidentally with the distribution of *Mvh* mRNA, immunocytochemical staining of adult testis sections revealed that the Mvh protein was localized in spermatogenic cells of stages from the spermatocyte to the round spermatid (Fig. 4 *C* and *D*). Interestingly, immunostaining of testicular cells showed that, in addition to the diffuse distribution of Mvh protein in the cytoplasm, a strong granular staining was observed in a perinuclear zone of the round spermatid (Fig. 4 *E* and *F*). The intracellular localization of stained granules appears to be coincident with that of a so-called chromatoid body, which has been well characterized as an electron-dense structure localized in the vicinity of mammalian spermatid nuclei (21, 22). Further analyses of proteins from various stages of embryonic gonads, embryos, and cultured cells showed that Mvh protein was expressed in both male and female embryonic gonads isolated from embryos after 11.5 dpc, whereas it was undetectable in 7- to 10-dpc embryos. Mvh protein also was undetectable in several pluripotential cell lines of EG and ES cells. Immunostaining of embryonic gonads revealed the restricted expression of Mvh protein in gonadal germ cells (Fig. 4 *G* and *H*), in which diffuse staining in the cytoplasm was observed. However, by staining sections of 7.5- to 10.5-dpc embryos under the same conditions as described above, no staining was detected in migrating PGCs before colonization of the genital ridges (data not shown).

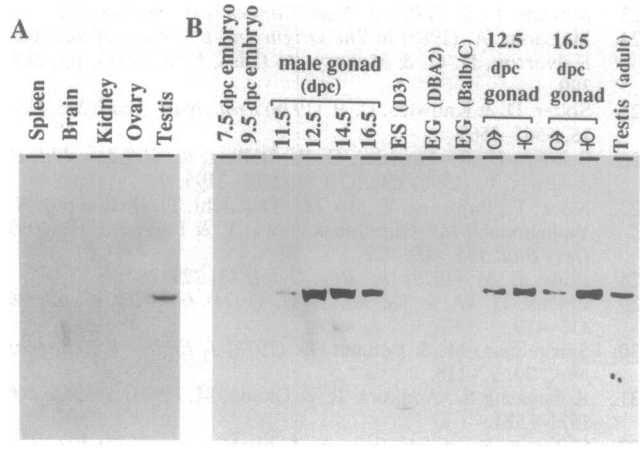


FIG. 5. Immunoblot analyses of Mvh protein. (*A*) Twenty micrograms of protein from the indicated adult tissues was used for each lane and immunostained with anti-Mvh antibodies after alkaline phosphatase conjugated anti-rabbit IgG. (*B*) Proteins (20 μg) from the indicated gonads, embryos, and cultured cell lines were analyzed as in *A*. Gonads of 11.5-dpc embryos were mixed male and female because it was impossible to distinguish the sex at this stage.

DISCUSSION

We report a member of the murine DEAD-family protein gene, *Mvh*, which appears to be a a true homolog of *Drosophila vas*, based on sequence similarity, germ cell-specific expression, and intracellular localization of the protein. Compared with PL10, Mvh showed only a moderate similarity (51.8%) even in the conserved region (Fig. 2*B*). In this region, PL10 contains a unique insertion sequence of 9 amino acids (amino acids 70–78; see Fig. 2*B*), and 21 of 162 residues identical among vasa, XVLG1, and Mvh are substituted in PL10; for example, GACXDV (amino acids 22–233) is replaced by STSXNI. Recently, Liang *et al.* (9) demonstrated that the conserved region of the *Drosophila* vasa protein actually carries activities of an ATP-dependent RNA helicase and is also involved in translocalization of vasa protein to the posterior pole by interaction with other protein(s). Therefore, these substitutions of the amino acid residues in the putative functional domain may reflect some functional differences between both proteins, such as binding specificity to RNAs and/or to other proteins. Although the intracellular localization of PL10 protein has not been reported, our result that *PL10* sequences are also detectable in PGC cDNAs may

imply that Mvh and PL10 proteins function in a cooperative or synergistic manner.

In mice, determination of the germ lineage is believed to be independent of maternal factors that are localized in a specified region of the oocyte cytoplasm. Moreover, the facts that EG as well as ES cells are pluripotential and that some multipotential embryonal carcinomas are originated from PGCs suggest that the irreversible determination leading to gametogenesis does not take place at least until the migrating phase of PGC during germ cell development (17, 23). In this respect, our results showed that expression of Mvh protein is not detected in such pluripotential embryonic cells but is detected in germ cells colonized in embryonic gonads. If structural homology reflects functional conservation and therefore Mvh could play an essential role in the determining event like *Drosophila* vasa, it seems reasonable to speculate that a crucial step of mammalian germ cell determination will take place after the entry of PGCs into the genital ridges. In this case, it is likely that fate determination of the germ cell is dependent on cell–cell interactions with gonadal somatic cells, as is the case for the sex-determination event (24).

Mouse EG cells have been histochemically discriminated by enzymatic activity of endogenous alkaline phosphatase (1) or by immunostaining with SSEA-1 antibody reactive to a particular cell-surface antigen (2, 25). The expression of *Oct3/4* and *Zfp40* transcripts is also found to be an excellent marker of the germ cell lineage (26, 27). However, since these characteristics reflecting an undifferentiated stem state of cells are commonly expressed in early embryonic cells and embryo-derived cell lines such as EG and ES cells, as well as developing PGCs, they are not available for distinguishing germ-line cells prior to the meiotic phase from pluripotential embryonic cells. Mvh protein, which is exclusively detected in developing germ cells, is therefore regarded as a molecular marker demonstrating that the germ cell, at least after colonizing the embryonic gonad, is not equivalent to the pluripotential cell but is a type of differentiated cell.

The perinuclear dense body (nuage) has been known to be a characteristic structure of various germ-line cells (28, 29). In mice, nuage-like structures are observed in PGCs but are not detected in pluripotential cells of early stage embryos (30). The chromatoid body of mammalian spermatogenic cells is comparable in structure and distribution to the polar granule of insects. Both structures consist of basic proteins and some RNA and exhibit a close interaction to mitochondrial cluster and nuclear envelope during their maturation process (28, 29). Our results showed that Mvh protein is predominantly localized in a perinuclear granule at the early spermatid stage. Taken together with localization of vasa to the polar granule (9), it is worth examining ultrastructural localization of Mvh in the germ cell cytoplasm including the nuage-like structure and the chromatoid body during germ cell differentiation. In *Drosophila*, vasa protein is assumed to interact with other maternal factors such as oskar protein, tudor protein, and mitochondria large rRNA (9, 31) and to function at the translational level including storage or transportation of some specific mRNAs (6–9). In this connection, it has been noted that most mRNAs newly transcribed at the late pachytene spermatocyte stage are under translational arrest during the round spermatid stage (32). Therefore, it is of particular interest to examine whether the Mvh protein is functionally involved in translational regulation during spermatogenesis. Further investigation of the developmental function of *Mvh* should provide an important insight into the mechanism of the determination and maintenance of the mammalian germ cell lineage.

We wish to thank Dr. Kuniya Abe for preparing the PGC cDNAs and Dr. Yasuhisa Matsui for providing EG cell lines. We also thank Ms. Jun Ohnuma for her help with immunocytochemical work. We are especially grateful to Dr. Tom Gridley for critically reading the manuscript.

1. Ginsburg, M., Snow, M. H. L. & McLaren, A. (1990) *Development (Cambridge, U.K.)* **110,** 521–528.
2. Eddy, E. M. & Hahnel, A. C. (1983) in *Current Problem in Germ Cell Differentiation*, eds. McLaren, A. & Wylie, C. C. (Cambridge Univ. Press, Cambridge, U.K.), pp. 41–70.
3. Mahowald, A. P. (1992) *Science* **255,** 1216–1217.
4. Strome, S. (1992) *Nature (London)* **358,** 368–369.
5. Lasko, P. F. & Ashburner, M. (1988) *Nature (London)* **335,** 611–617.
6. Hay, B., Jan, L. Y. & Jan, Y. N. (1988) *Cell* **55,** 577–587.
7. Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Nieilsen, P. J., Nishi, K., Schnier, J. & Slonimski, P. P. (1989) *Nature (London)* **337,** 121–122.
8. Hay, B., Jan, L. Y. & Jan, Y. N. (1990) *Development (Cambridge, U.K.)* **109,** 425–433.
9. Liang, L., Diehl-Jones, W. & Lasko, P. F. (1994) *Development (Cambridge, U.K.)* **120,** 1201–1211.
10. Ephrussi, A. & Lehmann, R. (1992) *Nature (London)* **358,** 387–392.
11. Hirling, H., Scheffer, M., Restle, T. & Stahl, H. (1989) *Nature (London)* **339,** 562–564.
12. Lemaire, L. & Heinlein, U. A. O. (1993) *Life Sci.* **52,** 917–926.
13. Leroy, P., Alzari, P., Sassoon, D., Wolgemuth, D. & Fellous, M. (1989) *Cell* **57,** 549–559.
14. Nielsen, P. J. & Trachsel, H. (1988) *EMBO J.* **7,** 2097–2105.
15. Abe, K. (1992) *Mamm. Genome* **2,** 252–257.
16. Noce, T., Fujiwara, Y., Sezaki, M., Fujimoto, H. & Higashinakagawa, T. (1992) *Dev. Biol.* **153,** 356–367.
17. Matsui, Y., Zsebo, K. & Hogan, B. L. M. (1992) *Cell* **70,** 841–847.
18. Komiya, T., Itoh, K., Ikenishi, K. & Furusawa, M. (1994) *Dev. Biol.* **162,** 354–363.
19. De Valoir, T., Tucker, M. A., Belikoff, E. J., Camp, L. A., Bolduc, C. & Buckingham, K. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2113–2117.
20. Patterson, L. F., Harrey, M. & Lasko, P. F. (1992) *Nucleic Acids Res.* **20,** 3063–3067.
21. Fawcett, D. W., Eddy, E. M. & Phillips, D. M. (1970) *Biol. Reprod.* **2,** 129–153.
22. Parvinen, M. & Parvinen, L.-M. (1979) *J. Cell Biol.* **80,** 621–628.
23. Stevens, L. C. (1967) *J. Natl. Cancer Inst.* **38,** 549–552.
24. McLaren, A. (1985) in *The Origin and Evolution of Sex*, eds. Halvorson, H. O. & Monroy, A. (Liss, New York), pp. 289–300.
25. Solter, D. & Knowles, D. P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5565–5569.
26. Schöler, H. R., Dressler, G. R., Balling, R., Rohdewohld, H. & Gruss, P. (1990) *EMBO J.* **9,** 2185–2195.
27. Noce, T., Fujiwara, Y., Ito, M., Takeuchi, T., Hashimoto, N., Yamanouchi, M., Higashinakagawa, T. & Fujimoto, H. (1993) *Dev. Biol.* **155,** 409–422.
28. Eddy, E. M. (1975) *Int. Rev. Cytol.* **43,** 229–280.
29. Beams, H. W. & Kessel, R. G. (1974) *Int. Rev. Cytol.* **39,** 413–479.
30. Spiegelman, M. & Bennett, D. (1973) *J. Embryol. Exp. Morphol.* **30,** 97–118.
31. Kobayashi, S., Amikura, R. & Okada, M. (1993) *Science* **260,** 1521–1524.
32. Geremia, R., d'Agostino, A. & Monesi, V. (1978) *Exp. Cell Res.* **111,** 23–30.