GENOMICS 35, 346–352 (1996)
ARTICLE NO. 0366

Mouse Autosomal Homolog of *DAZ,* a Candidate Male Sterility Gene in Humans, Is Expressed in Male Germ Cells before and after Puberty

RENEE REIJO,* JUDITH SELIGMAN,* MARY BETH DINULOS,† TOM JAFFE,* LAURA G. BROWN,* CHRISTINE M. DISTECHE,† AND DAVID C. PAGE*,[1]

*Howard Hughes Research Laboratories at Whitehead Institute, and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02142; and †Department of Pathology, University of Washington, Seattle, Washington 98195

Received April 15, 1996; accepted April 23, 1996

Deletion of the *Azoospermia Factor* (*AZF*) region of the human Y chromosome results in spermatogenic failure. While the identity of the critical missing gene has yet to be established, a strong candidate is the putative RNA-binding protein DAZ (Deleted in Azoospermia). Here we describe the mouse homolog of DAZ. Unlike human *DAZ,* which is Y-linked, in mouse the *Dazh* (*DAZ homolog*) gene maps to chromosome 17. Nonetheless, the predicted amino acid sequences of the gene products are quite similar, especially in their RNP/RRM (putative RNA-binding) domains, and both genes are transcribed predominantly in testes; the mouse gene is transcribed at a lower level in ovaries. *Dazh* transcripts were not detected in testes of mice that lack germ cells. In testes of wildtype mice, *Dazh* transcription is detectable 1 day after birth (when the only germ cells are prospermatogonia), increases steadily as spermatogonial stem cells appear, plateaus as the first wave of spermatogenic cells enters meiosis (10 days after birth), and is sustained at this level thereafter. This unique pattern of expression suggests that *Dazh* participates in differentiation, proliferation, or maintenance of germ cell founder populations before, during, and after the pubertal onset of spermatogenesis. Such functions could readily account for the diverse spermatogenic defects observed in human males with *AZF* deletions.

INTRODUCTION

One-sixth of human couples are infertile but otherwise healthy (Hull *et al.,* 1985). In most cases of human infertility, the underlying cause cannot be identified with certainty. In theory, some cases could be due to genetic defects that disrupt germ cell development while sparing the soma. Such "pure sterile" genes have been identified in *Drosophila* and *Caenorhabditis elegans,* for example, and their characterization is now leading to an understanding of molecular processes unique to germ cells in invertebrates (Castrillon *et al.,* 1993; Ellis and Kimble, 1994). In mammals, by contrast, no pure sterile factor has been unequivocally, biochemically defined, though in some cases the responsible genetic loci have been mapped.

Perhaps the best characterized pure sterile locus in humans is the *Azoospermia Factor* (*AZF*), on the long arm of the Y chromosome. A role for the human Y chromosome in spermatogenesis was first suggested in 1976 by Tiepolo and Zuffardi, who reported the occurrence of grossly deleted Y chromosomes in six men with azoospermia (no sperm in semen). Based on these findings, they hypothesized that Yq harbors an *Azoospermia Factor* gene or gene complex required for spermatogenesis. In recent years, strong evidence in support of Tiepolo and Zuffardi's model has been obtained, and *AZF* has been precisely localized. We and our colleagues have demonstrated that a particular portion of Yq—the "*AZF* region"—was deleted in 13% of azoospermic men (Reijo *et al.,* 1995). The fathers of these azoospermic men had intact Y chromosomes, confirming that the *AZF* deletions in their sons were the cause of the sons' infertility.

Testis biopsies from azoospermic men with *AZF* deletions revealed a surprisingly wide range of histologic findings. Germ cells were found to be completely absent in some individuals ("Sertoli cell only syndrome"), while early spermatogenic cells were present in others ("testicular maturation arrest"). In two of the latter cases, spermatogenesis sometimes progressed beyond meiosis, to the stage of condensed spermatids. As previously suggested, this wide array of testicular phenotypes could be explained by a defect (with variable expressivity) in spermatogonia, the stem cells responsible for constantly replenishing the spermatogenic lineages

* Sequence data from this article have been deposited with the GenBank/EMBL Data Libraries under Accession No. U46694.

[1] To whom correspondence should be addressed. Telephone: (617) 258-5203. Fax: (617) 258-5578.

0888-7543/96 $18.00

in the testes, or in the differentiation of primordial germ cells to spermatogonia (Reijo *et al.*, 1995).

The molecular identity of *AZF* has not been established definitively, but a strong candidate is *DAZ* (*Deleted in Azoospermia*), the only transcription unit so far identified in the *AZF* region (Reijo *et al.*, 1995). (*YRRM1* and *YRRM2*, corresponding to the cDNAs MK5 and MK29, respectively, had also been proposed as *AZF* candidates (Ma *et al.*, 1993) but subsequent studies revealed that these transcription units mapped outside the *AZF* region (Reijo *et al.*, 1995)). *DAZ* encodes a putative RNA-binding protein and, as judged by Northern blotting of human tissue RNAs, is expressed predominantly in testes (Reijo *et al.*, 1995).

Given the strong possibility that *DAZ* might be *AZF*, a pure male sterile factor, we wished to learn more about its pattern of expression. Is *DAZ* expressed in germ cells or in somatic cells of the testis? Is *DAZ* expressed early or late in the pathway of spermatogenesis? Would the pattern of *DAZ* expression readily account for the testicular pathology observed in *AZF*-deleted men? These questions may be difficult to pursue in humans given the limited availability of suitable tissue specimens. To pursue these questions in an experimentally tractable species and to explore the degree to which the *DAZ* gene and its product have been conserved during mammalian evolution, we characterized the mouse homolog of human *DAZ*.

MATERIALS AND METHODS

Mice. Wildtype (BALB/c) mice were from the laboratory of R. Jaenisch; mice with W^v or Sl^d mutations (in the *White spotted* and *Steel* genes, respectively) were obtained from Jackson Laboratories (Bar Harbor, ME).

Isolation of DNA and RNA. Preparation of mouse DNA was as previously described (Simpson and Page, 1991). RNA was prepared using Trizol reagent (Gibco BRL, Grand Island, NY). Essentially, cells or tissue were resuspended in approximately 10 vol Trizol, and 0.2 vol of chloroform was added to each sample. After centrifugation to remove cell debris, RNA in the supernatant was precipitated with isopropanol, rinsed with ethanol, and resuspended in deionized water.

Hybridizations and sequencing. Northern blotting, Southern blotting, and library screening techniques were as previously described (Simpson and Page, 1991; Page *et al.*, 1987) with hybridizations as follows: 20 h at the appropriate temperature (42°C for all Southerns and Northerns and 37°C for screening the cDNA library) in 50% formamide, 5× SSC, 1× Denhardt's, 20 m*M* Na phosphate, pH 6.6, 0.005% denatured salmon sperm DNA, 1% sodium dodecyl sulfate, 10% dextran sulfate. The blots were washed three times for 15 min each at 65°C in 0.1× SSC, 0.1% sodium dodecyl sulfate. The gene encoding the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) was used as a reference probe to control for the loading of different quantities of RNA (Tso *et al.*, 1985). Nucleotide sequencing of *DAZ* cDNA clones was performed as previously described (Fisher *et al.*, 1990).

The mouse *Dazh* gene was mapped by genetic linkage analysis using an interspecific backcross: (C57BL/6 × *Mus spretus*) F1 × *M. spretus* (Jackson Laboratories). Genomic DNAs from backcross progeny were digested with *Taq*I, Southern blotted (2.5 μg per lane), and hybridized with *Dazh* cDNA pDP1580, which was found to detect *M. spretus Taq*I fragments of approximately 7.6, 2.3, and 0.5 kb as distinct from C57BL/6 fragments of 4.5, 2.5, 2.3, 0.6, and 0.5 kb. Ninety-four backcross progeny were genotyped for this *Taq*I polymorphism, and comparison with genotype data from other markers (Jackson Laboratory collaborative data) allowed *Dazh* to be positioned.

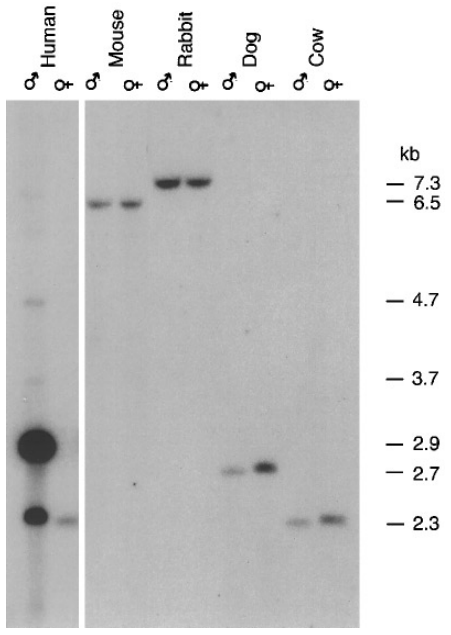

FIG. 1. *DAZ* homologs in mammals. Autoradiogram produced by hybridizing *DAZ* exon probe 325.7 to Southern blot of *Eco*RI-digested genomic DNAs. Sizes (in kilobases) of hybridizing fragments are indicated to the right.

Fluorescence in situ hybridization. Dazh genomic clone pDP1642 was isolated from a mouse library (strain 129; Wu *et al.*, 1994) by hybridization of plaque lifts with *Dazh* cDNA pDP1580 at 42°C. Sequencing of the ends of three *Eco*RV fragments subcloned into the Bluescript KS(+) vector (Stratagene, Inc., La Jolla, CA) revealed that pDP1642 contained sequence colinear with *Dazh* cDNA. The 10-kb genomic insert was labeled with biotin-11–dATP by nick-translation (Gibco BRL, Gaithersburg, MD). Metaphase chromosomes were prepared from male C57BL/6J and *M. spretus* lymphocytes using 0.075 *M* KCl as hypotonic buffer and methanol:acetic acid (3:1, v/v) as fixative. The hybridization was carried out as previously described (Edelhoff *et al.*, 1993). After incubation with goat anti-biotin antibody, slides were rinsed in 2× SSC, 0.1% Tween 20, 0.15% bovine serum albumin. A second incubation with fluorescein-labeled anti-goat IgG and a rinse in modified 2× SSC followed. The chromosomes were banded using Hoechst 33258-actinomycin D staining and propidium iodide counterstaining. Chromosomes and hybridization signals were visualized by fluorescence microscopy using a dual-band pass filter (Omega, Brattleboro, VT) and a Vector detection system (Vector, Burlingham, CA).

RESULTS

DAZ Homologs Are Autosomal in Mice and Other Mammals

We previously demonstrated that, in apes, homologs of the human *DAZ* gene are located on the Y chromosome, where they may exist in multiple copies (Reijo *et al.*, 1995). To identify homologs in more distantly related mammals, we hybridized a human *DAZ* exon (325.7; Reijo *et al.*, 1995) to a "Noah's ark" Southern blot of genomic DNAs from male and female mice, rabbits, dogs, and cattle (Fig. 1). In each of the four species,

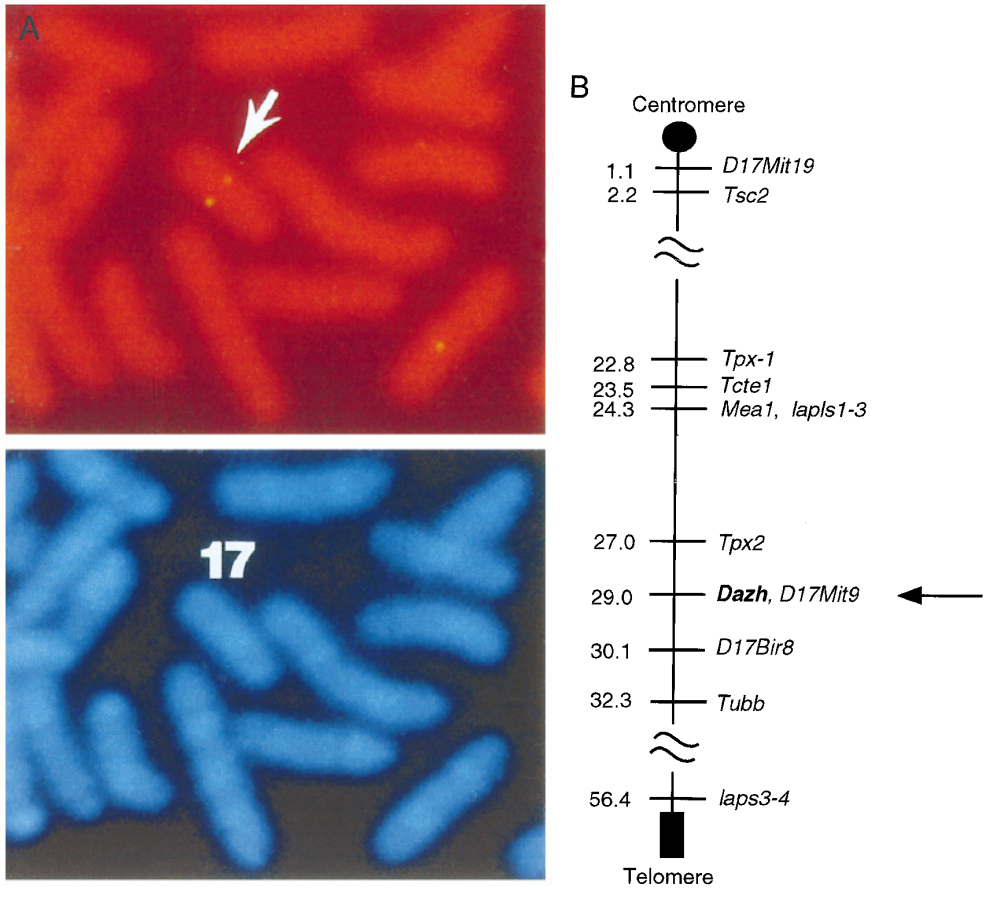

FIG. 2. Mapping of *Dazh* to mouse chromosome 17. (A, Top) Fluorescence *in situ* hybridization of mouse *Dazh* genomic clone pDP1642 to lymphocyte metaphase chromosomes from a male C57BL/6J mouse. (Bottom) Chromosomes stained with Hoechst 33258-actinomycin D and counterstained with propidium iodide. (B) Genetic map location determined by following segregation of a *Dazh Taq*I restriction fragment length polymorphism in a (C57BL/6 × *M. spretus*)F1 × *M. spretus* backcross.

hybridization to a single fragment was observed, and in each species the single fragment was present in both males and females. Thus, these more distantly related mammalian species all possess a *DAZ* homolog, but the homologs are apparently not located on the Y chromosome.

To resolve whether these non-Y homologs were autosomal or X-linked, we mapped the mouse homolog, which we will refer to as *Dazh* (*DAZ homolog*), by two independent methods. First, *Dazh* was mapped to mouse chromosome 17 by fluorescence *in situ* hybridization (FISH) to metaphase chromosomes. Similar results were obtained using metaphase spreads prepared from either C57BL/6J, an inbred lab strain, or the species *M. spretus* (Figs. 2A and 2B). Of 51 C57BL/6J cells examined, 15 (29%) showed signals on both chromatids of chromosome 17 at regions C–D. Of 73 *M. spretus* cells examined, 28 (38%) showed signals on both chromatids of chromosome 17 at regions C–D. This chromosomal assignment was independently confirmed and refined by genetic linkage mapping. Following the segregation of a *Taq*I restriction fragment length polymorphism in a C57BL/6 × *M. spretus* backcross (Jackson Laboratory Panel BSS), we observed that *Dazh* did not recombine with the marker *D17Mit9* and mapped between the markers *Tpx2* and *D17Bir8*. Although we have not rigorously mapped the rabbit, dog, and cow homologs, normalization of the Southern autoradiogram shown in Fig. 1 (by hybridizing known autosomal probes to the same blot; not shown) suggests that the homologs in these species are also autosomal rather than X-linked.

Mouse Dazh Encodes a Putative RNA-Binding Protein

To determine whether the mouse homolog is transcribed, we hybridized human *DAZ* cDNA clone pDP1576 to a Northern blot of RNAs prepared from various adult mouse tissues. We observed a 3.5-kb transcript in the testis, much as we had previously seen (Reijo *et al.*, 1995) in human tissues using the same hybridization probe (results not shown; see similar experiment using mouse cDNA probe below).

To characterize mouse *Dazh* transcription further, we screened an adult testis cDNA library (Mardon and Page, 1989) by hybridization using as probe human *DAZ* exon 325.7. The inserts of three cDNA clones identified by this screen were sequenced in their entirety

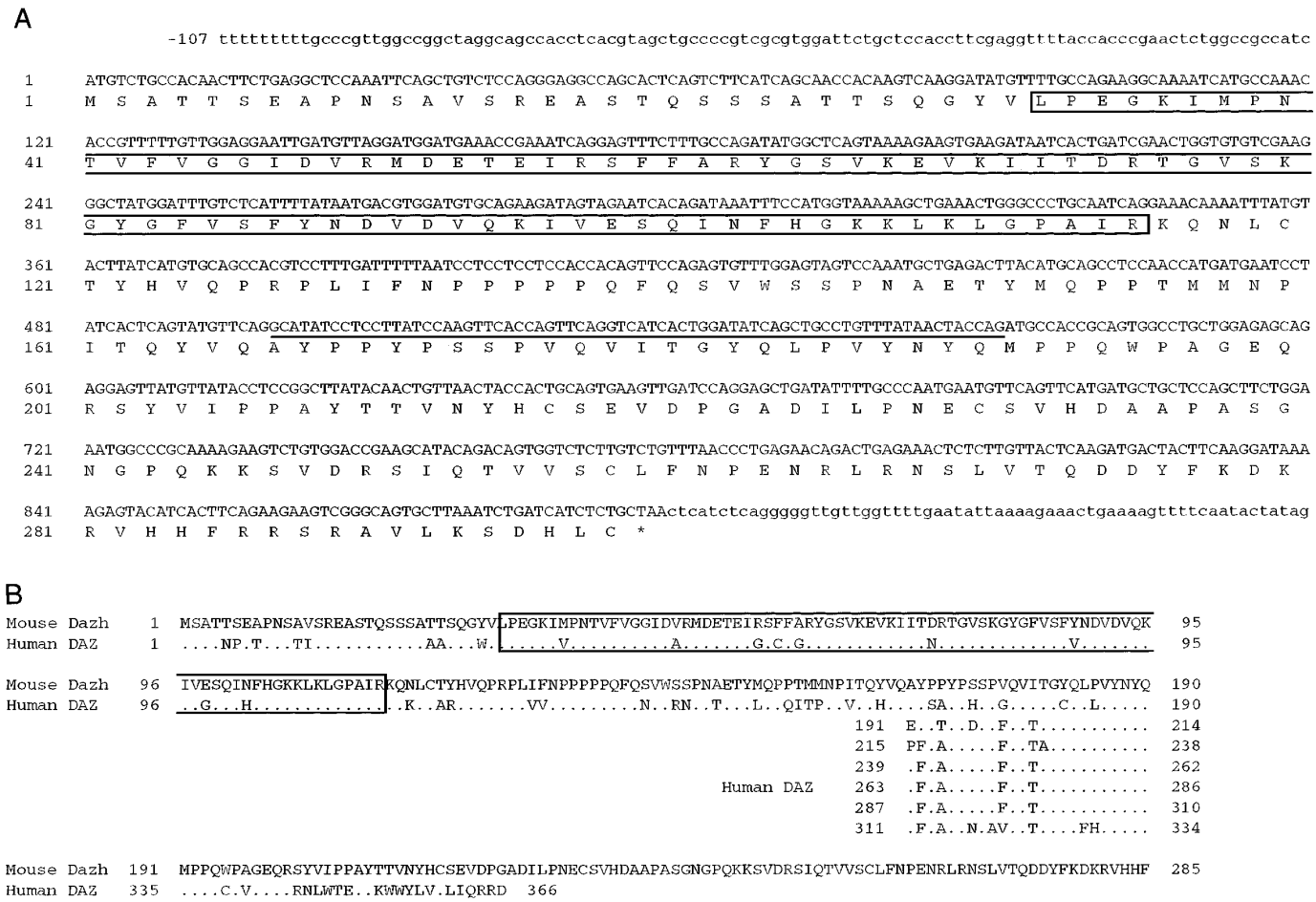

FIG. 3. (A) Nucleotide sequence of mouse *Dazh* cDNA clone pDP1580 and, immediately beneath, predicted amino acid sequence of the encoded protein. The RNP/RRM domain is boxed. A 72-nucleotide unit tandemly repeated seven times in human *DAZ* (5) but present only once here in mouse *Dazh* is underlined. The GenBank accession No. is U46694. (B) Comparison of mouse Dazh and human DAZ (5) proteins. Dots represent identity to mouse Dazh. Note that human DAZ contains seven tandem repeats of a 24-amino-acid unit present only once in mouse Dazh. The RNP/RRM domain is boxed.

and found to be completely colinear. The longest of these three cDNA clones, pDP1580, represents a 1069-nucleotide portion of the *Dazh* transcript and contains a single long open reading frame in its entirety (Fig. 3). The first AUG in this frame (position 1 in Fig. 3) occurs in a favorable context for translation initiation (Kozak, 1986). Beginning at this initiation codon, the open reading frame would encode a protein 298 amino acids in length, with a predicted molecular weight of 33,314.

Like the human DAZ protein (Reijo *et al.*, 1995), the predicted mouse Dazh protein contains an RNP/RRM motif of the sort found in many proteins that bind RNA (or in some cases single-stranded DNA) (Fig. 3B). In this 85-amino-acid domain, the human and mouse proteins differ at only 9 residues. Outside the putative RNA-binding domain, the mouse and human proteins exhibit less conservation, though both are rich in proline, glutamine, and tyrosine residues, as are many RNP/RRM proteins (Burd and Dreyfuss, 1994; Kenan *et al.*, 1991).

The greatest differences between the mouse and the human proteins are found in their carboxy-terminal portions. Among the most striking features of human DAZ are seven tandem repeats of a 24-amino-acid unit; this tandem array constitutes most of the carboxy-terminal half of the predicted protein. The mouse Dazh protein contains only one copy of this 24-residue unit. Near their carboxy termini, there is little sequence similarity between the proteins, with mouse Dazh extending an additional 76 residues beyond the carboxy terminus of human DAZ.

Mouse Dazh Is Expressed in Male Germ Cells before and after the Pubertal Onset of Spermatogenesis

As described above, Northern analysis employing a human *DAZ* probe suggested that mouse *Dazh* is expressed predominantly in the testis. We confirmed and extended these findings using mouse *Dazh* cDNA clone pDP1580 as probe. By Northern analysis, we searched for *Dazh* transcripts in the following adult tissues from both female and male mice: brain, gonad, heart, kidney, liver, lung, and spleen. As before, we detected expres-

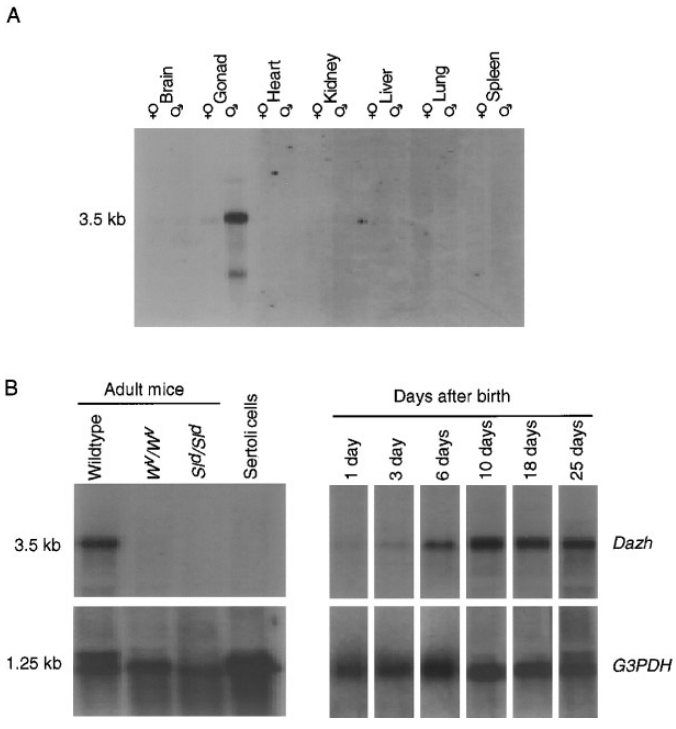

FIG. 4. Northern blotting studies of *Dazh* transcription in the mouse. (A) Survey of adult tissues. Autoradiogram produced by hybridizing *Dazh* cDNA pDP1580 to total RNAs (20 μg/lane) from mouse tissues. Hybridization at 47°C; otherwise as under Materials and Methods. (B) Germ cell dependence and temporal pattern of testicular expression. (Left) Hybridization of pDP1580 to RNAs from adult testes of wildtype BALB/c or W^v or Sl^d homozygous mutants or from Sertoli cell line TM4. (Right) Hybridization of pDP1580 (or of *G3PDH* cDNA (Tso *et al.*, 1985), as control for loading) to testes RNAs from wildtype BALB/c mice of ages indicated.

sion in the adult testis, with a 3.5-kb transcript predominating (Fig. 4A). We also detected a transcript of the same size, but much lower abundance, in the adult ovary. We did not detect transcripts outside the male and female gonads. We concluded that, in the adult mouse, *Dazh* is transcribed at relatively high levels in the testis, at a much lower level in the ovary, and little if at all in the nongonadal tissues tested.

We next addressed the critical question of whether the *Dazh* gene is expressed in the somatic cells or in the germ cells of the testis. The testis has a rich architecture composed of spermatogenic (germ) cells at many stages of development together with somatic cells of several types, including the Sertoli cells, which support the spermatogenic lineage, and the Leydig cells, which produce testosterone. Mutations in either of two mouse genes, *W* (*White spotted*) or *Sl* (*Steel*), result in greatly diminished if not obliterated germ cell populations while sparing the somatic components of the testis (Fleischman, 1993). We detected no *Dazh* transcripts in RNAs prepared from testes of animals homozygous for either the W^v or the Sl^d mutations, nor did we detect *Dazh* transcripts in RNA from TM4, a Sertoli cell line (Fig. 4B). These results demonstrate that *Dazh* transcription depends on the presence of germ cells. While it is formally possible that *Dazh* might be expressed in Sertoli or other somatic testicular cells—and be induced only in the presence of germ cells—no gene has, to our knowledge, been shown to exhibit such behavior in the testis. It is far more likely, we concluded, that *Dazh* is expressed in the germ cells of the testis.

We next set out to determine the stage or stages of male germ cell development in which *Dazh* is expressed. During the first postnatal week, spermatogonial stem cells are formed from prospermatogonia present at birth (McCarrey, 1993). During the next several weeks of life, the first cohort of fully differentiating germ cells proceed through spermatogenesis in a relatively synchronized fashion. As a result, simple Northern blotting of RNAs from testes of various ages can provide much information as to the developmental stages and cell types in which a germ-cell-specific gene is expressed. As shown in Fig. 4B, a 3.5-kb *Dazh* transcript (the same size as seen in the adult testis) was faintly detectable in the testes of 1- and 3-day-old mice, when the only germ cells present are prospermatogonia. The abundance of the *Dazh* transcript increased dramatically by Day 6, when the first spermatogonia appear; reached a plateau at about Day 10, when the first wave of spermatogenic cells entered meiosis; and remained relatively constant thereafter. Thus, the temporal and quantitative pattern of *Dazh* expression correlates with the presence of prospermatogonia and with the appearance and expansion of the spermatogonial population to which the prospermatogonia give rise.

DISCUSSION

Autosomal Homologs of DAZ in Mice and Other Mammals

We had previously shown that, in apes, the Y chromosome carries one or more homologs of human *DAZ* (Reijo *et al.*, 1995). By contrast, we found no evidence of a Y-linked *DAZ* homolog in any of the four nonprimate mammals (mice, rabbits, dogs, and cattle) studied here. As judged by Southern blotting of male and female DNAs—and more definitive mapping studies in the mouse—these mammals appear to carry a single, autosomal homolog of *DAZ* (Figs. 1 and 2). These observations substantiate the emerging view that the genetic content of the Y chromosome has been in rapid flux during mammalian evolution (e.g., Graves, 1995). This conclusion is all the more telling when one appreciates that the Y's meiotic partner, the X, has been the most stable chromosome during placental mammalian evolution (Rugarli *et al.*, 1995; Palmer *et al.*, 1995).

Nucleotide similarity between Y-linked human *DAZ* and autosomal mouse *Dazh* (85% identity in the coding region) suggests that they derived from a common ancestral gene during mammalian evolution. It is not obvious whether this ancestral gene was autosomal or Y-

linked, but a male–female common band observed on Southern blots of human and ape DNAs (Fig. 1; Fig. 5. in Reijo *et al.*, 1995) may be evidence of an autosomal homolog in these species as well.

In the mouse, *Dazh* maps to 17, a chromosome whose *t* complex is well known to students of mammalian spermatogenesis because of its male transmission distortion and recessive male-sterile loci (Silver, 1993). As *Dazh* maps about 9 cM distal to the *t* complex, it cannot be responsible for *t* complex phenomena as conventionally defined. However, chromosome 17 has also been repeatedly implicated in hybrid sterility, in which the male progeny of certain interspecies crosses suffer spermatogenic failure (Pilder *et al.*, 1993; Forejt and Ivanyi, 1974). Indeed, four of six published mammalian hybrid sterility loci map to mouse chromosome 17 (Pilder *et al.*, 1993; a fifth, unpublished locus also maps to this chromosome; S. Pilder, pers. comm. February 1996, Philadelphia, PA). The loci studied to date map within the *t* complex, and the possible existence of hybrid sterility loci elsewhere on chromosome 17 has not yet been explored. Given the expression pattern of mouse *Dazh* and that its human Y-linked homolog may be a male-sterile factor, this potential link to hybrid sterility warrants experimental study.

Dazh Expression in Prospermatogonia and Spermatogonia

Having found that mouse *Dazh* is transcribed in the adult testis, we explored the developmental timing of its expression and whether this takes place in the soma or in germ cells. We addressed these issues by Northern analysis, taking advantage of (1) the availability of mutants (W^v, Sl^d) that lack germ cells but retain the somatic components of the testis and (2) the relatively synchronized progression of male germ cells during the first month after birth, when spermatogonial stem cells are formed and the first wave of fully differentiating descendants proceed through spermatogenesis. Our studies revealed that mouse *Dazh* is expressed (1) primarily if not exclusively in the testis, (2) in germ cells but not in somatic cells, and (3) at all postnatal timepoints, including those before and after the pubertal onset of spermatogenesis. The level of *Dazh* expression increased steadily until about 10 days after birth, long before the first wave of spermatogenesis was completed, and remained relatively constant thereafter.

Taken together, these results strongly suggest that *Dazh* is expressed in prospermatogonia (the only germ cells present in newborn males) and in the expanding population of spermatogonial stem cells to which they give rise during the first postnatal week. *Dazh* continues to be expressed after puberty, most likely in the long-lived spermatogonial populations that constantly replenish the spermatogenic lineage. (At present we cannot exclude that *Dazh* might also be expressed in early spermatogenic derivatives (spermatocytes), a possibility to be explored by cell fractionation or *in situ* hybridization.) While a few other germ-cell-specific genes have been found to be expressed in prospermatogonia or spermatogonia (Starborg *et al.*, 1992; Tanaka *et al.*, 1994), the temporal expanse of *Dazh*'s expression is unprecedented (Hecht, 1993; Meistrich and van Beek, 1993) and suggests that *Dazh* participates in differentiation, proliferation, or maintenance of germ cell founder populations well before, during, and after the pubertal onset of spermatogenesis.

Dazh is also transcribed, though at a much lower level, in the adult ovary. It will be interesting to determine whether this reflects *Dazh* activity in oocytes or in the somatic components of the ovary.

Similarity of Mouse Dazh and Human DAZ

Two observations suggest that, *in vivo*, the human DAZ and mouse Dazh proteins perform similar functions on identical or near-identical RNA (or possibly single-stranded DNA) substrates. First, the amino acid sequences of the human and mouse proteins are similar throughout, and especially in the RNP/RRM domain, that is their most striking feature (Fig. 3). Second, the human and mouse genes are both expressed predominantly in the testis. Indeed, *in situ* hybridization studies have revealed that, in adult human testes, *DAZ* is transcribed in spermatogonia (D. Menke, G. Mutter, and D.C.P., unpublished results), suggesting that the human and mouse genes may be expressed in male germ cells at similar or identical developmental stages. Taken together, these data suggest that the human and other mammalian *DAZ* genes may function in spermatogonia (and their immediate precursors, prospermatogonia) throughout postnatal life.

Is DAZ the Azoospermia Factor in Humans?

If human *DAZ* plays a critical role in spermatogonia or their precursors, then this is consistent with our previous predictions for *AZF*. Men with deletions of the *AZF* region of the Y chromosome exhibit spermatogenic defects of varying severity. Severe cases are characterized by the complete absence of germ cells, including spermatogonial stem cells (Sertoli cell only syndrome; Reijo *et al.*, 1995), while in mild cases spermatogenesis proceeds to completion at reduced output, resulting in severe oligospermia (Reijo *et al.*, 1996). As we have previously suggested (Reijo *et al.*, 1995), this range of phenotypes could be readily explained by a variably expressed ("leaky") defect in spermatogonia or prospermatogonia. Although there is no definitive evidence equating *DAZ* with *AZF*, our findings provide an important circumstantial link: both *DAZ* and *AZF* may function in spermatogonia or their precursors.

ACKNOWLEDGMENTS

We thank Paul Bain for a Northern blot; Roberto Polakiewicz for a *G3PDH* clone; Rudolf Jaenisch for BALB/c mice; and Paul Bain, Ken Campbell, Mary Goodheart, Doug Menke, Steve Pilder, Richa

Saxena, and Charles Tilford for comments on the manuscript. This work was supported by the National Institutes of Health and the Howard Hughes Medical Institute. R.R. was the recipient of a Damon-Runyon/Walter Winchell fellowship.

Note added in proof. Dazh is identical to the *Dazla* gene as described by Cooke *et al.* (*Hum. Molec. Genet.* 5: 513–516, 1996).

REFERENCES

Burd, C. G., and Dreyfuss, G. (1994). Conserved structures and diversity of functions of RNA-binding proteins. *Science* 265: 615–621.

Castrillon, D. H., Gonczy, P., Alexander, S., Rawson, R., Eberhart, C. G., Viswanathan, S., Di Nardo, S., and Wasserman, S. A. (1993). Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster:* Characterization of male-sterile mutants generated by single P element mutagenesis. *Genetics* 135: 489–505.

Edelhoff, S. E., Grubin, C. E., Karlsen, A. E., Adler, D. A., Foster, D., Disteche, C. M., and Lernmark, A. (1993). Mapping of glutamic acid decarboxylase (GAD) genes. *Genomics* 17: 93–97.

Ellis, R. E., and Kimble, J. (1994). Control of germ cell differentiation in *Caenorhabditis elegans. Ciba Found. Symp.* 182: 179–188.

Fisher, E. M. C., Beer-Romero, P., Brown, L. G., Ridley, A., McNeil, J. A., Lawrence, J. B., Willard, H. F., Bieber, F. R., and Page, D. C. (1990). Homologous ribosomal protein genes on the human X and Y chromosomes: Escape from X inactivation and possible implications for Turner syndrome. *Cell* 63: 1205–1218.

Fleischman, R. A. (1993). From white spots to stem cells: The role of the Kit receptor in mammalian development. *Trends Genet.* 9: 285–290.

Forejt, J., and Ivanyi, P. (1974). Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.). *Genet. Res.* 24: 189–206.

Graves, J. A. M. (1995). The origin and function of the mammalian Y chromosome and Y-borne genes—An evolving understanding. *BioEssays* 17: 311–320.

Hecht, N. B. (1993). Gene expression during male germ cell development. *In* "Cell and Molecular Biology of the Testis" (C. Desjardins and L. L. Ewing, Eds.), pp. 400–432, Oxford Univ. Press, New York.

Hull, M. G. R., Glazener, C. M. A., Kelly, N. J., Conway, D. I., Foster, P. A., Hinton, R. A., Coulson, C., Lambert, P. A., Watt, E. M., *et al.* (1985). Population study of causes, treatment, and outcome of infertility. *Br. Med. J.* 291: 1693–1697.

Kenan, D. J., Query, C. C., and Keene, J. D. (1991). RNA recognition: Towards identifying determinants of specificity. *Trends Biochem. Sci.* 16: 214–220.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* 44: 283–292.

Ma, K., Inglis, J. D., Sharkey, A., Bickmore, W. A., Hill, R. E., Prosser, E. J., Speed, R. M., Thomson, E. J., Jobling, M., *et al.* (1993). A Y chromosome gene family with RNA-binding protein homology: Candidates for the azoospermia factor *AZF* controlling human spermatogenesis. *Cell* 75: 1287–1295.

Mardon, G., and Page, D. C. (1989). The sex-determining region of the mouse Y chromosome encodes a protein with a highly acidic domain and 13 zinc fingers. *Cell* 56: 765–770.

McCarrey, J. R. (1993). Development of the germ cell. *In* "Cell and Molecular Biology of the Testis" (C. Desjardins and L. L. Ewing, Eds.), pp. 58–89, Oxford Univ. Press, New York.

Meistrich, M. L., and van Beek, M. E. A. B. (1993). Spermatogonial stem cells. *In* "Cell and Molecular Biology of the Testis" (C. Desjardins and L. L. Ewing, Eds.), pp. 266–295, Oxford Univ. Press, New York.

Page, D. C., Mosher, R., Simpson, E. M., Fisher, E. M. C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., and Brown, L. G. (1987). The sex-determining region of the human Y chromosome encodes a finger protein. *Cell* 51: 1091–1104.

Palmer, S., Perry, J., and Ashworth, A. (1995). A contravention of Ohno's law in mice. *Nature Genet.* 10: 472–476.

Pilder, S. H., Olds-Clarke, P., Phillips, D. M., and Silver, L. M. (1993). Hybrid sterility-6: A mouse *t* complex locus controlling sperm flagellar assembly and movement. *Dev. Biol.* 159: 631–642.

Reijo, R., Lee, T. Y., Salo, P., Alagappan, R., Brown, L. G., Rosenberg, M., Rozen, S., Jaffe, T., Strauss, D., *et al.* (1995). Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet.* 10: 383–393.

Reijo, R., Alagappan, R. K., Patrizio, P., and Page, D. C. (1996). Severe oligospermia resulting from deletions of the *Azoospermia Factor* gene on the Y chromosome. *Lancet* 347: 1290–1293.

Rugarli, E. I., Adler, D. A., Borsani, G., Tsuchiya, K., Franco, B., Hauge, X., Disteche, C., Chapman, V., and Ballabio, A. (1995). Different chromosomal localization of the *Clcn4* gene in *Mus spretus* and C57BL/6J. *Nature Genet.* 10: 466–471.

Silver, L. M. (1993). The peculiar journey of a selfish chromosome: Mouse *t* haplotypes and meiotic drive. *Trends Genet.* 9: 250–254.

Simpson, E. M., and Page, D. C. (1991). An interstitial deletion in mouse Y chromosomal DNA created a transcribed *Zfy* fusion gene. *Genomics* 11: 601–608.

Starborg, M., Brundell, E., and Hoog, C. (1992). Analysis of the expression of a large number of novel genes isolated from mouse prepubertal testis. *Mol. Reprod. Dev.* 33: 243–251.

Tanaka, H., Yoshimura, Y., Nishina, Y., Nozaki, M., Nojima, H., and Nishimune, Y. (1994). Isolation and characterization of cDNA clones specifically expressed in testicular germ cells. *FEBS Lett.* 355: 4–10.

Tiepolo, L., and Zuffardi, O. (1976). Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum. Genet.* 34: 119–124.

Tso, J. Y., Sun, X. H., Kao, T. H., Reece, K. S., and Wu, R. (1985). Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. *Nucleic Acids Res.* 13: 2485–2502.

Wu, H., Liu, X., and Jaenisch, R. (1994). Double replacement: Strategy for efficient introduction of subtle mutations into the murine *Col1a-1* gene by homologous recombination in embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 91: 2819–2823.