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Structural Characterization and Expression Studies of *Dby* and Its Homologs in the Mouse

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ABSTRACT: In spite of recent evidence showing the importance of *DBY* (DEAD-box RNA helicase Y) in spermatogenesis in human, the biologic role of its homolog *Dby* (also known as *Ddx3y*) in the mouse is less clear. The present study aims at characterizing the molecular structure of *Dby* and comparing its expression with its X- and autosome-linked homologs in embryonic gonads and developing germ cells in mice. Molecular cloning by rapid amplification of 3′-cDNA ends showed that the *Dby* gene in the mouse gives rise to 2 transcripts that differ only in the length of the 3′-untranslated region as a consequence of the use of alternative polyadenylation signals. Measurement by quantitative real-time polymerase chain reaction showed that both transcripts were ubiquitously expressed and were present in male germ cells and Sertoli cells. They were more abundant in type A spermatogonia compared with pachytene spermatocytes and round spermatids. Expression of *Dby* in the embryonic gonad increased from day 10.5 and reached a peak at day 17.5. The expression level of *Dby* decreased after birth and remained low in adult male gonads. Although the level of expression of *Dby* was much lower than its X chromosome homolog, *Ddx3* (also known as *Ddx3x*) in all samples examined, the pattern of expression of the 2 genes was comparable. In contrast, their autosomal homolog, *D1Pas1*(also known as *PL10*), was predominantly expressed in pachytene spermatocytes and round spermatids. This result is in accord with meiotic sex chromosome inactivation in that *Dby* and *Ddx* are replaced in pachytene spermatocytes by their autosomal retroposon. These observations indicate that unlike *DBY* in humans, the role of *Dby* in spermatogenesis is less obvious in the mouse and its biologic activity may be replaced by that of *Ddx3* and *D1Pas1*.

Key words: *Ddx3*, *D1Pas1*, 3′-UTR, gonad, spermatogonia, spermatocytes, spermatids.

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Introduction

Dby (DEAD-box RNA helicase Y; also known as *Ddx3y*) (Mazeyrat et al, 1998) is the mouse homolog of the human Y gene *DBY* (Blanco et al, 2000; Kamp et al, 2000; Sun et al, 2000). *DBY* is located in the *AZFa* (azoospermia factor a) interval on proximal Yq11 (Yq11.21), a distinct deletion interval in infertile and subfertile individuals (Vogt et al, 1996). DBY belongs to the DEAD box proteins and encodes a putative ATP-dependent RNA helicase (Abdelhaleem, 2005). This gene produces a long transcript which is ubiquitously expressed and a shorter transcript which is testis specific, suggesting that *DBY* may have both housekeeping and testis-specific functions (Foresta et al, 2000). Recent studies showed that translation of *DBY* was present only in the male germ lines while DBX protein was found in all testicular and nontesticular tissues, indicating that *DBY* is essential for human spermatogenesis (Ditton et al, 2004). The centromere to telomere gene order in the *AFZa* region (*USP9Y-DBY-UTY*) is similar in both human and mouse (Sxr^b interval) Y chromosomes indicating that they represent a conserved syntenic segment (Mazeyrat et al, 1998). In the mouse, partial deletion of the short arm of the Y chromosome (Sxr^b deletion) results in early failure of spermatogenesis and consequent sterility (Sutcliffe and Burgoyne, 1989; Simpson and Page, 1991; Wood et al, 1997). *Dby* is among the 6 genes that lie within this region (Mazeyrat et al, 1998). Even though *Dby* had been suspected to be the Y chromosome gene essential for normal spermatogonial proliferation, studies showed that *Eif2s3y* instead of *Dby* is required for spermatogenesis in the mouse (Mazeyrat et al, 2001). Thus, there appears to be species-

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specific difference between the function of *Dby* and its human homolog, *DBY*.

Similar to that in human, mouse *Dby* has a structural homolog, *Ddx3* (also known as *Ddx3x*) on the X chromosome (Mazeyrat et al, 1998). A genomic Southern blot identified further an autosomal homolog *D1Pas1*on mouse chromosome 1 (Kingsmore et al, 1989). Murine *D1Pas1*, formerly designated *PL10* (Leroy et al, 1989), was thought to be a retroposon of the X chromosome *Ddx3* (Mazeyrat et al, 1989). *D1Pas1* is probably involved in the initiation of mRNA translation, as it is able to complement the deletion of the homologous yeast gene *DED1*, which shares 53% of its amino acid composition with *D1Pas1* and has been shown to bind mRNA to the ribosome (Chuang et al, 1997). To understand the role of *Dby* in reproduction, we studied the expression pattern of *Dby* and its X and autosome homologs in male germ cells, embryonic gonads, and different somatic tissues. Results support the previous belief that the *Dby* gene may not be required for mouse spermatogenesis.

Materials and Methods

Tissue Preparation

Embryos were collected from pregnant mice at embryonic days 10.5 (E10.5), E11.5, E12.5, E13.5, E15.5, and E17.5, with the first day of vaginal plug identification defined as E0.5. Whole genital ridges (gonad and mesonephros) were dissected from the earlier embryos (E10.5, E11.5, and E12.5). In later embryos (E13.5, E15.5, and E17.5), mesonephros and gonads were separated. Sexing of the embryos was done by polymerase chain reaction (PCR) detection of *Sry* in DNA of the residual embryo (E10.5 and E11.5) or by morphology of the embryonic gonad (E12.5 to E17.5). Neonatal and adult mouse testes were collected at postnatal days 0, 3, 6, 12, 18, 24, 30, and 56. These procedures were performed at the Veterans Affairs Medical Center, University of California, San Francisco and were approved by the Institutional Animal Care and Use Committee.

Isolation of Testicular Cells

Germ cells were isolated by the STAPUT procedure (Dym et al, 1995). Six-day-old BALB/c mouse testes were used for isolation of type A spermatogonia. For the isolation of pachytene spermatocytes and round spermatids, testes from 60-day-old animals were used. Purity of germ cells was routinely higher than 95% for type A spermatogonia and higher than 90% for pachytene spermatocytes and round spermatids. Sertoli cells were isolated from neonatal (6-day-old) BALB/c mice as previously described (Dirami et al, 2001). MA-10 cells were used as Leydig cells. All germ cells and Sertoli cells were prepared at Georgetown University. The protocol was approved by the Georgetown University Animal Care and Use Committee.

RNA Preparation and cDNA Synthesis

Total RNA was isolated from mouse testicular cells and embryonic and postnatal gonads using TRIZOL reagent (Invitrogen, Gaithersburg, Md) and further purified by RNeasy Protect Mini Kit (Qiagen, Valencia, Calif) according to the manufacturer's protocol. The quality and quantity of the RNA was assessed with a 2100 Bioanalyzer (Agilent, Palo Alto, Calif). RNA of adult mouse tissues was purchased from Ambion (Austin, Tex). First strand cDNA was synthesized from RNA samples using Superscript II reverse transcriptase (Invitrogen, Gaithersburg, Md) according to the manufacturer's protocol.

Cloning of Mouse Dby Transcript Variant cDNAs

To obtain the transcript variant of *Dby*, the method of anchored PCR, rapid amplification of 3′-cDNA ends (3′-RACE), was performed using the GeneRacer Kit (Invitrogen, Gaithersburg, Md). Anchored PCR was performed using a gene-specific primer (5′-CTTACTCGTTACACTCGTCC-TACTCC-3′) and nested gene-specific primer (5′-CGTCC-TACTCCAGTGCAAAAACATGCTT-3′). After amplification, the PCR products were cloned into pGEM-T (Promega, Madison, Wis) by TA-cloning according to the manufacturer's protocol. The sequence of the cloned cDNA was determined by PCR sequencing using BigDye Primer Cycle Sequencing Kits (Applied Biosystems, Foster City, Calif). DNA sequences were analyzed using DNASIS software v2.5 (MiraiBio, Alameda, Calif). Sequences used for alignment other than those reported here were extracted from public databases from the National Center for Biotechnology Information using BLAST searches.

Quantitative Real-Time PCR

cDNA was used as a template for quantitative PCR (QPCR) of *Dby*, *Ddx3*, and *D1Pas1* mRNA levels. Gene-specific primers and Taqman probes for *Dby*, *Ddx3*, and *D1Pas1* were designed using Primer Express software (Applied Biosystems, Foster City, Calif) according to the manufacturer's instructions. The sequences of the Taqman probes and primers are shown in the Table. *Dby*-L mRNA level was determined using the *Dby*-L primers and TaqMan probe, which were specific for the nonoverlapping region of the 3′-untranslated region of *Dby*-L. Since the sequence of *Dby*-S overlapped with that of *Dby*-L, the total mRNA levels of *Dby*-L and *Dby*-S, denoted as levels of *Dby*, were obtained with *Dby* primers and a TaqMan probe designed within the coding sequence. The amount of *Dby*-S mRNA was obtained by subtracting the total *Dby* mRNA level from the *Dby*-L mRNA level. 18S ribosomal RNA (rRNA) was used as the internal control of the reaction and was quantitated using the TaqMan rRNA control reagents (Applied Biosystems, Foster City, Calif). Standard procedures for the operation of the Prism 7900 HTS Sequence Detection System (Applied Biosystems, Foster City, Calif) were followed. Thermal cycler conditions consisted of 2 minutes at 50°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. CT determinations were performed with the instrument for each reaction using

Primer sequences for Dby, Ddx3 and D1Pas1

Dby–L and *Dby*–S		
Primer	*Dby*–F	5′–GTGCTCAAACAGGGTCTGGAA–3′
	Dby–R	5′–AGCCTCTCCTGGACCATCTGTA–3′
TaqMan	*Dby*	5′–CTGCTGCATTTCTACTACCCATTCTCAGTCAGAT–3′
Dby–L		
Primer	*Dby*–L–F	5′–CTATGGCAATGACCAGACCTTATTA–3′
	Dby–L–R	5′–GAAAAACCTGACTGTGTAAAAGTAAAGC–3′
TaqMan	*Dby*–L	5′–TTGTTAATTCTGGTCTTTAATGCATGCTAGTGTTGG–3′
Dxd 3		
Primer	*Ddx3*–F	5′–GAAGACTTCATTGCTGTAGTTTGGATTA–3′
	Ddx3–R	5′–GATCCTCAGTCACAAAATTATAAATGCA–3′
TaqMan	*Ddx3*	5′–CCCTCCCGCCTACCCCTATCCC–3′
D1Pas1		
Primer	*PL10*–F	5′–GTGGAGGATTTGGTGCTAGAGA–3′
	PL10–R	5′–TTGTAAAAGCCTCCATAGCTACCT–3′
TaqMan	*PL10*	5′–CAGCAGTTCCAGCTTCAGCAGTGGC–3′

default parameters. The CT values for *Dby*, *Ddx3*, and *D1Pas1* were normalized to that of rRNA in each sample. QPCR was conducted in triplicate. The significance of the difference between expression levels was calculated using the Student's *t* test.

Results

Molecular Characterization of the Dby Transcript Variants

Dby contained 3767 bp including a 1977-bp open reading frame (ORF) identical to that published previously (GenBank NM_012008). Analysis of this sequence using the BLAST program against the mouse genome database showed that the *Dby* gene consisted of 15 coding exons (Figure 1A), 2 exons less than found for the human homolog *DBY* (Foresta et al, 2000). The ORF encoded a putative polypeptide with 88% identity to *DBY*. This is the only transcript identified to date (Mazeyrat et al, 1998). In humans 2 transcript variants of *DBY*, which have alternative polyadenylation sites, were initially identified (Foresta et al, 2000). A more recent study found at least 5 different RNA species on a Northern blot of testicular tissue (Ditton et al, 2004). To determine if any transcript variants of *Dby* existed in the mouse, a 3′-RACE experiment was performed. Two transcripts of the mouse *Dby* were identified that differed only in their 3′-untranslated region (3′-UTR) due to alternative polyadenylation signals (Figure 1B). The sequence of the longer transcript variant *Dby*-L was identical to the *Dby* published previously (GenBank NM_012008), whereas the *Dby*-S had a shorter 3′-UTR with a different polyadenylation sequence. Both *Dby* transcripts encoded the same protein containing the conserved motifs of DEAD-box RNA helicases, indicating that they likely shared a common ATP-dependent helicase function with other DEAD-box family members (Figure 1C).

Expression of Dby and Its Autosomal and X Homologs in Testicular Germ and Somatic Cells

QPCR was used to determine the expression levels of *Dby*-L and *Dby*-S in germ cells, including type A spermatogonia, pachytene spermatocytes, round spermatids, and testicular somatic cells, including Sertoli cells and Leydig cells. The results are summarized in Figure 2A. In germ cells, both *Dby*-L and *Dby*-S were expressed at a significantly higher levels ($P < .001$ for *Dby*-L comparisons and $P < .05$ for *Dby*-S comparisons) in type A spermatogonia than in pachytene spermatocytes and round spermatids. Expression levels of *Dby*-L and *Dby*-S were significantly higher ($P < .05$) in Sertoli cells than in germ cells. Both *Dby* transcripts were not found in Leydig cells.

As shown in Figure 2B, *Ddx3*, the X homolog of *Dby* was expressed in all cells examined. The expression pattern of *Ddx3* was similar to that of *Dby* except that the level of expression of *Ddx3* in type A spermatogonia and Sertoli cells was at least 3- to 4-fold higher than that in pachytene spermatocytes and round spermatids. The expression level of *Ddx3* was significantly higher ($P < .01$) than that of *Dby* in all cells examined except spermatids. Among the 3 homologous genes, *Ddx3* was the only one found to be expressed in Leydig cells. *D1Pas1* was germ-cell specific; it was expressed predominantly in meiotic pachytene spermatocytes and postmeiotic round spermatids. The expression level of *D1Pas1* in pachytene spermatocytes was more than 10-fold that of *Dby* and *Ddx3*. This relative expression pattern of the 3 genes in spermatocytes was consistent with the meiotic sex chromosome inactivation model (Handel, 2004).

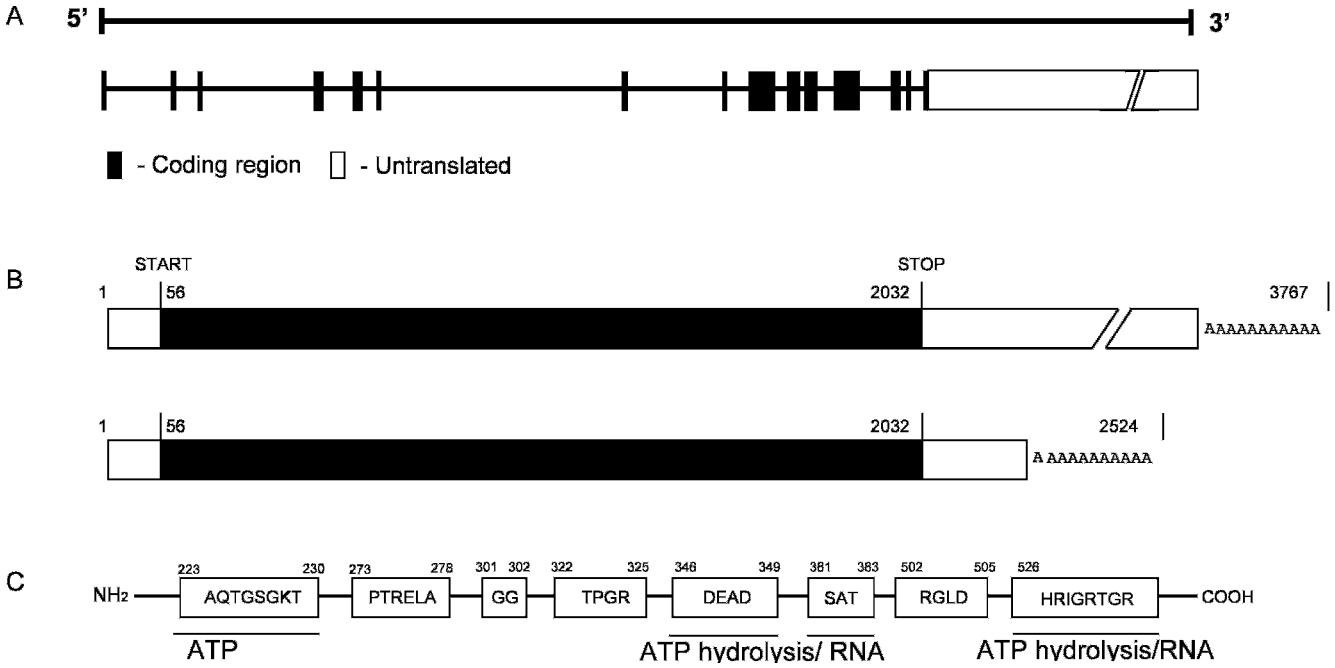

Figure 1. Mouse *Dby* gene. **(A)** Schematic representation of the intron/exon structure. Exons are represented by solid boxes; untranslated regions (UTRs) are represented by open boxes. **(B)** *Dby* transcripts. The positions of start and stop codons and poly(A) tails are indicated, with numbering of nucleotides according to the cDNA sequence in GenBank (NM_012008). The translated region is represented by solid bars; UTRs are represented by open bars. **(C)** Conserved motifs of *Dby* protein, common to the DEAD-box family. Amino acid numbering is according to GenBank NM_012008.

Expression of Dby *and Its X and Autosomal Homologs in Nontesticular Tissues*

Expression of *Dby* transcript variants was also studied in different mouse tissues including brain, heart, kidney, lung, liver, spleen, thymus, testis, ovary, and embryo (mixed male and female embryos). As shown in Figure 3A, the brain and heart were the major sites of *Dby*-L expression. *Dby*-L was found in all tissues examined with the exception of ovary, which does not have the Y chromosome and served as a negative control. Tissue distribution of *Dby*-S was similar to that of *Dby*-L, except that its expression level was significantly lower ($P < .05$) and it was absent in the thymus and ovary.

As shown in Figure 3B, *Ddx3* was expressed ubiquitously in all tissues examined, with a much higher expression level than that of *Dby*. The highest expression of *Ddx3* was observed in brain and ovary. Interestingly, thymus and testis were the organs which had significantly lower ($P < .05$) expression of *Ddx3*. On the other hand, *D1Pas1*, the autosomal homolog of *Dby* and *Ddx,* was only expressed in the testis.

Developmental Onset of the Expression of Dby *and Its X and Autosome Homologs*

The expression of *Dby*, *Ddx3*, and *D1Pas1* in the developing male gonad from E10.5 to postnatal day 56 was examined (Figure 4). The expression level of *Dby* showed a 3-fold increase from E10.5 to E17.5, and then decreased slowly after birth. The *Ddx3* expression level was 5- to 10-fold more than that of *Dby* in embryonic male gonads at all ages examined. *Ddx3* expression remained relatively stable throughout all embryonic ages and decreased after birth, although it was still significantly higher ($P < .001$) than that of *Dby* up to postnatal day 56. The expression level of *Ddx3* at postnatal day 56 was about 6-fold lower than that in the embryonic male gonads. *D1Pas1* expression was first observed at postnatal day 12 and continued to increase. It peaked at postnatal day 24 and then decreased afterwards. This pattern of expression coincides with the timing of the appearance of meiotic spermatocytes (Bellve et al, 1977).

Discussion

In humans, 2 alternative transcripts of *DBY* arising from alternative polyadenylation signals have been demonstrated. The transcript with the longer 3′-UTR was shown to be expressed ubiquitously, while the transcript with the shorter 3′-UTR is testis specific (Foresta et al, 2000). The present study demonstrated that similar to *DBY*, *Dby* also has 2 polyadenylation

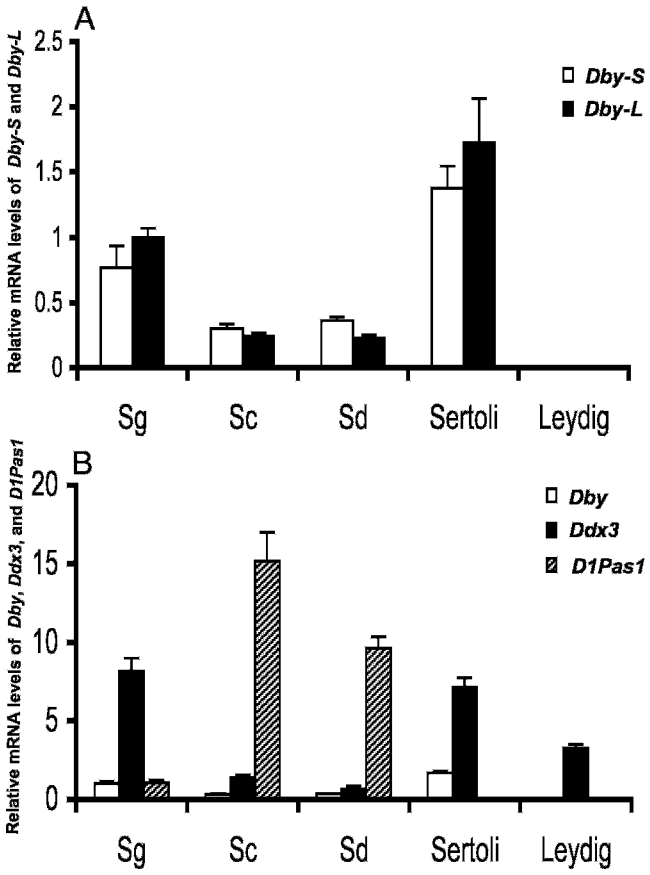

Figure 2. Expression of *Dby* variants, *Ddx3*, and *D1Pas1* in testicular germ and somatic cells of mouse. **(A)** *Dby*-L and *Dby*-S. **(B)** *Dby* (total), *Ddx3*, and *D1Pas1*. The *Dby*, *Ddx3*, and *D1Pas1* mRNA levels, measured by quantitative polymerase chain reaction, were normalized with 18S ribosomal RNA and were shown as fold induction (Y-axis) compared with that of *Dby*-L in type A spermatogonia. Sg, type A spermatogonia; Sc, pachytene spermatocytes; Sd, round spermatids; Sertoli, Sertoli cells; Leydig, Leydig cells. Values are means ± SD of triplicates.

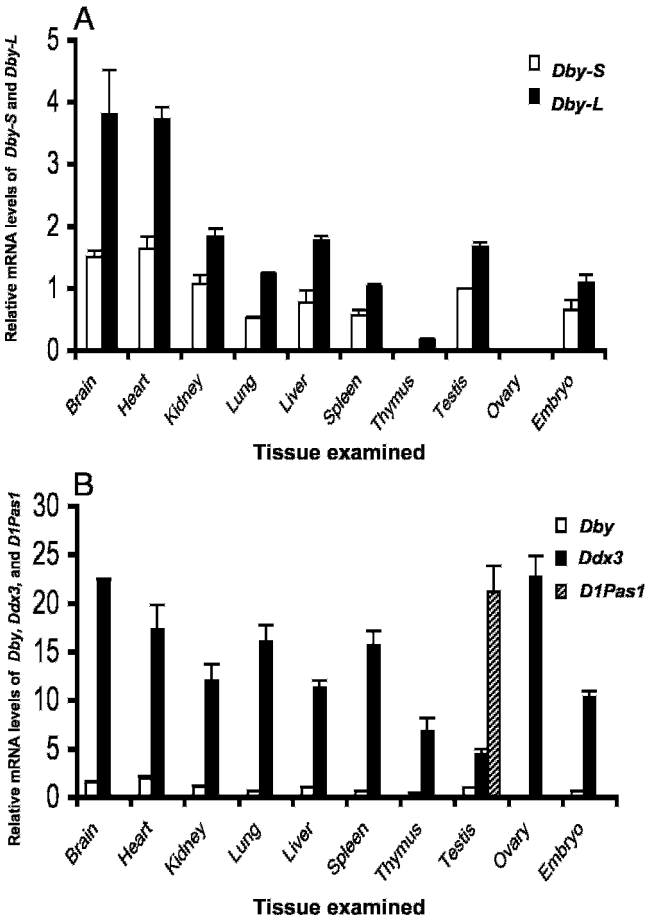

Figure 3. Expression of *Dby* variants, *Ddx3,* and *D1Pas1* in nontesticular tissues of adult mouse. **(A)** *Dby*-L and *Dby*-S. **(B)** *Dby* (total), *Ddx3*, and *D1Pas1*. The *Dby*, *Ddx3*, and *D1Pas1* mRNA levels, measured by quantitative polymerase chain reaction, were normalized with 18S ribosomal RNA and were shown as fold induction (Y-axis) compared with that of *Dby*-L in type A spermatogonia. Values are means ± SD of triplicates.

variants. However, in contrast to *DBY*, both *Dby* variants are ubiquitously expressed with similar patterns of expression in testicular germ and somatic cells and in nontesticular tissues, suggesting that their function, unlike that of the short form of *DBY* in humans, is not limited to spermatogenesis.

Sequence alignment revealed significant homology among *Dby*, *Ddx3*, and *D1Pas1*. When the coding sequences were compared, *Dby* showed 90% and 84% identity with *Ddx3* at the amino acid and nucleic acid level, respectively, while *Dby* and *D1Pas1* shared 87% and 80% homology at the amino acid and nucleic acid levels, respectively. Significant stretches of absolute identity and conserved substitutions were distributed over the entire alignment when the 3 gene products were compared (Figure 5). The proteins encoded by the 3 genes share the highly conserved RNA helicase motif of the DEAD-box family. The differences among the 3 proteins are predominantly located at the N- and C-termini. These observations suggest that these 3 proteins might all have RNA helicase function but might be involved in different pathways. The germ cell–specific expression of mouse *D1Pas1* was similar to the short form of human *DBY* (Foresta et al, 2000) and the DBY protein detected by immunostaining (Ditton et al, 2004). This implies that the spermatogenesis-specific function of *DBY* in humans is replaced by *D1Pas1*, but not *Dby*, in the mouse.

D1Pas1 maps to mouse chromosome 1 (Kingsmore et al, 1989) and is thought to be a retroposon of the X chromosome homolog *Ddx3* (Mazeyrat et al, 1998). Blasting of *D1Pas1* against the human genome revealed a sequence with 80% homology in chromosome 4. This sequence is 84% homologous to *Ddx3* and is probably the same as one of the pseudogenes reported by Kim and colleagues (2001). It is not known whether the human sequence is expressed. Interestingly, both

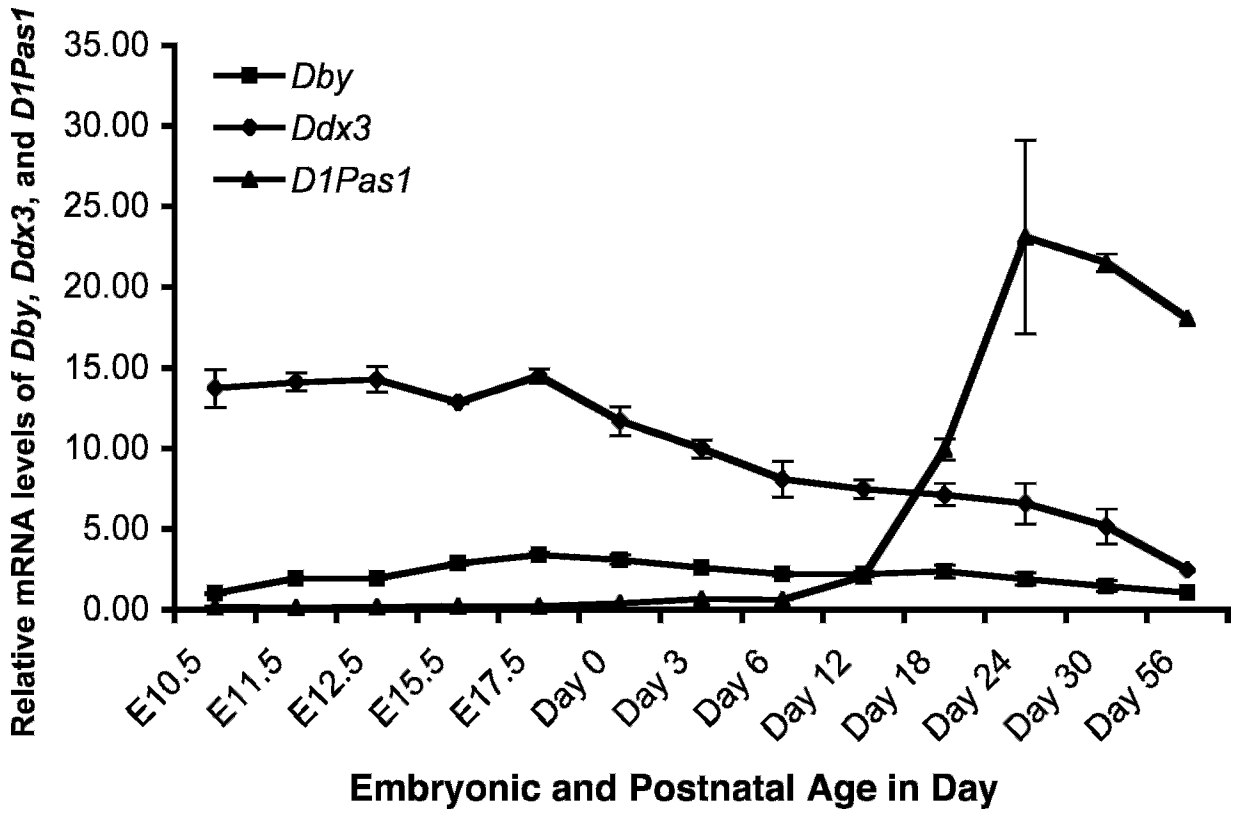

Figure 4. Expression of *Dby* (total), *Ddx3*, and *D1Pas1* in embryonic and postnatal male gonadal tissues of mouse. The *Dby*, *Ddx3*, and *D1Pas1* mRNA levels, measured by quantitative polymerase chain reaction, were normalized with 18S ribosomal RNA and were shown as fold induction (Y-axis) compared with that of *Dby* in embryo day 10.5 male gonad. Values are means ± SD of triplicates.

D1Pas1 and the human sequence are intronless (results not shown), a property common to retroposons (Sedlacek et al, 1999). It is known that the X and Y chromosomes are transcriptionally silenced during meiosis in pachytene spermatocytes because of sex chromosome meiotic inactivation (Handel, 2004). In fact, Fernandez-Capetillo and coworkers (2003) used microarrays to show that the expression of X-linked genes and 3 Y-linked genes, including *Dby,* were suppressed in histone H2AX-deficient spermatocytes which do not initiate meiotic sex chromosome inactivation. Autosomal transposons of a number of X-linked genes including *Cetn1* (Hart et al, 1999), *Cstf2t* (Dass et al, 2001), *G6pd*^{x} (Hendriksen et al, 1997), *Pdha2* (Dahl et al, 1990; Takakubo and Dahl, 1992), *Pgk2* (Boer et al, 1987; McCarrey et al, 1992), *Zfa* (Ashworth et al, 1990; Erikson et al, 1993), which are silenced during male meiotic prophase are expressed only in spermatocytes (Handel, 2004). The present study showed that *D1Pas1* is predominantly expressed in pachytene spermatocytes. This observation confirms the previous report that the D1Pas1 protein is expressed predominantly in the nuclei of germ cells undergoing meiosis (Session et al, 2001). Thus, the pattern of differential expression of *Dby* and *Ddx* and their autosomal homolog *D1Pas1* is similar to the other X and Y genes and their autosomal retrogenes and is in accord with the meiotic sex chromosome inactivation model (Handel, 2004).

Previous studies in humans showed that *DBY* is expressed mainly in spermatogonia while *DBX* is expressed mainly in spermatids. This led the investigators to conclude that *DBY* contributes mainly to the premeiotic spermatogonia phase while *DBX* functions in postmeiotic activities (Ditton et al, 2004). The fact that the expression level of *D1Pas1* in the postmeiotic spermatids is significantly higher than that of *Dbx* and *Dby* makes the *Dby*/*Ddx3*/*D1Pas1* system of the mouse distinct from the *DBY*/*DBX* system of the human and suggests that *D1Pas1* may not simply serve as the backup for *Dbx* and *Dby* during meiotic sex chromosome inactivation in the mouse.

Differential expression of *Dby*, *Ddx3*, and *D1Pas1* in gonad and germ cell development is interesting. The role of *D1Pas1* in spermatogenesis seems obvious since its expression is testis specific and peaks at the time when meiotic spermatocytes appear. On the other hand, the biologic function of *Dby* is less clear. Its expression in

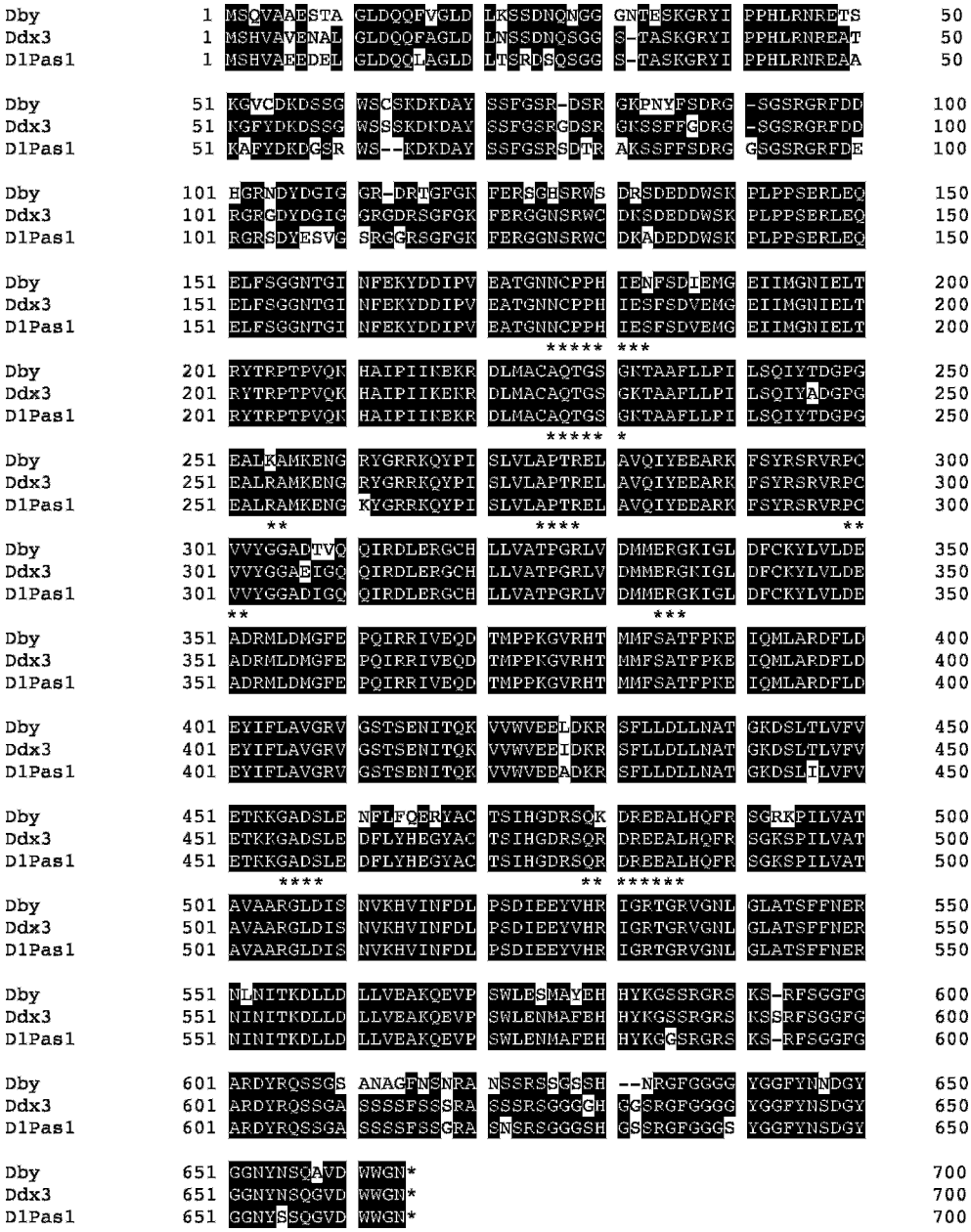

Figure 5. Amino acid alignment of mouse *Dby* with *Ddx3* and *D1Pas1*. Gaps were introduced for maximal homology. The homologous amino acid residues are shaded. The consensus motifs I–VI in proteins identified as DEAD box RNA helicases are indicated by asterisks above the sequences.

Sertoli cells is significantly higher ($P < .05$) than in germ cells. Sertoli cells are essential for supporting germ cell proliferation and differentiation (Griswold, 1998), and a number of Sertoli cell products are known to affect germ cell division, differentiation, and metabolism (Jegou, 1993; Eddy, 2002). It is tempting to speculate that *Dby* may contribute to Sertoli cell functions. However, since Sertoli cells used in the study were derived from 6-day-old animals, it is not known if *Dby* is present in more mature Sertoli cells. The expression of *Dby* in the embryonic gonads peaks at E17.5, at a time when gonocyte proliferation stops (de Rooij and Russell, 2000; Grootegoed et al, 2000). From that time on, the level of expression of *Dby* drops. Therefore, one possibility is that *Dby* plays a role in early gonocyte development. The major problem in assigning a function to *Dby* is that its expression pattern in embryonic gonads, germ cells, and somatic tissues is largely comparable to

that of *Ddx3*, but its expression levels are significantly lower, usually by several fold. This is contrary to that in humans in which the expression level of *DBY* is higher than that of *DBX* in all tissues analyzed (Ditton et al, 2004). Thus, the biologic significance of *Dby* in the mouse is not obvious. It provides further evidence that *Dby* is not required at any stage of the mouse male germ line (Mazeyrat et al, 2001).

In conclusion, this study showed that in spite of structural and organizational similarities, the significance of the *DBY*/*Dby* genes in spermatogenesis in human and mouse is different. *D1Pas1*, instead of *Dby*, plays a role in spermatogenesis in the mouse. Unlike that of humans in which *DBY* is not expressed in spermatids, *D1Pas1* expression remains high in these postmeiotic cells. There are distinct differences between the human *DBY*/*DBX* system and the mouse *Dby*/*Ddx3*/*D1Pas1* system. Further studies are required to explore the physiologic functions of the *Dby* protein and its relationship with its X and autosomal homologs. This study affirms that gene function and expression data in mice cannot always be directly translated to humans.

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