The Dazh Gene Is Expressed in Male and Female Embryonic Gonads before Germ Cell Sex Differentiation

Judith Seligman*¹ and David C. Page‡

*Developmental Biology Laboratory, Department of Obstetrics/Gynecology, Rambam Medical Center, P.O.B. 9602 Haifa 31096, Israel; and ‡Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02142

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As gauged by DAZ/DAZH protein sequences and expression patterns, the newly emergent Y gene clusters retained key functional characteristics of its autosomal ancestor [4]. Northern-blotting [3] and In-situ hybridization [10] demonstrate that both, the human DAZ/DAZH and the mouse homolog of DAZ (Dazh, also known as Dazla) are expressed in spermatogonia cells of the adult testis. The specificity of DAZ expression in germ cells in early stages of spermatogenesis strongly suggests that DAZ and its homologous genes function at the first phases of spermatogenic differentiation or earlier, in the maintenance of the spermatogonial stem cell populations [3, 10]. Although no human DAZ point mutants have been reported, recent genetic findings in Drosophila and mouse provide strong support for the hypothesis that DAZ is AZF. In the Drosophila and the mouse, mutants that have lost DAZ homologous gene function (boule and Dazh, respectively) were found to be azoospermic, just as with human AZF [9, 12].

High similarity in nucleotide sequences and expression between the human and mouse autosomal genes is found. Within the 82-residue RNA-binding domain, the products of human DAZH and mouse Dazh differ by only one amino acid substitution, while both differ from human Y-encoded DAZ at nine residues. Moreover, some evidence, as demonstrated by reverse-tran-scriptase PCR (RT-PCR), suggests that the human and mouse autosomal genes are expressed in low levels in the ovaries too [4, 8]. These preliminary evidence on
ovarian expression are very interesting and have never been checked thoroughly.

The purpose of our work was to study the ovarian transcription sizes and abundance in the ovaries. We wanted to know whether Dazh expresses in the germ-cells compound of the ovary and how early Dazh is expressed during gonadal development? We found that Dazh expresses in the developing male and female embryonic gonads well before the onset of meiosis. This expression pattern suggests that these genes act at the first phase of male and female gametogenesis.

MATERIAL AND METHODS

Animals and tissue dissections. C57BL/6j and C57BL/6W/V+ mice were obtained from Jackson laboratories (Bar Harbor, ME). For all embryonic dissections it was assumed that mating took place midway through the dark period; therefore midday on the day of appearance of the vaginal plug is approximately 0.5 day post-coitum (dpc). Embryos homozygous for W/V allele were recognized by their pale appearance relative to heterozygous and wild-type littersmates. Male and female embryonic gonads were sexed under dissecting microscope and kept frozen for RNA extraction.

RNA extraction and Northern-blot hybridization. RNA samples were prepared using Trizol reagent (Gibco BRL, Grand Island, NY). Gonads were suspended in 1ml 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. Following electrophoresis, gels were stained in Ethidium bromide and photographed to assess loading differences (not shown). Northern blots were hybridized with Dazh cDNA pDP1580 [3] or with DAZH cDNA pDP1580 [4]. The gene encoding Alpha-tubulin was used as a reference probe for mouse samples [13] and the gene encoding RPS4X was used as a reference probe for human samples [14] to control the loading of different quantities of RNA. The alpha-tubulin and RPS4X autoradiograms were performed for 20 h at 42°C in 50% formamide, 5XSSC, 20% dextran sulfate. The blots were washed three times for 15 min. each at 57°C in 0.1XSSC, 0.1% sodium dodecyl sulfate.

Comparison of Dazh/DAZH expression levels by densitometry. Densitometric profiles of the Dazh/DAZH and alpha-tubulin/RPS4X were performed on the autoradiograms using the NIH Image 1.61. In order to compare the changes in Dazh/DAZH expression, the densitometry intensity (total area of the peaks) of the alpha-tubulin/RPS4X of one sample was adjusted to the corresponding other samples on the same gel.

RESULTS

Germ cell line is one of the best characterized lineage in mammals. Several different stages, time points, and/or cell types within this lineage have been described. As a result, simple Northern-blotting of RNAs from developing gonads can provide much information as to the developmental stages and cell types in which a germ cell specific gene is expressed. By using this Northern approach, we previously showed that Dazh is expressed in testicular germ cells even before puberty (in prospermatogonia and spermatogonia). To explore further Dazh expression during gonadal development, we sampled male and female embryonic gonads from 11.5 dpc to 18.5 dpc and used them for Northern blot analysis. As shown in Fig. 1A, a 3.5 kb Dazh transcript and higher less abundant transcript of about 4.5 kb were detected in embryonic gonads at 12.5 dpc. Expression of Dazh in male and female gonads was detected even earlier, at 11.5 dpc by RT-PCR (not shown), when only germ cells present in the gonad are primordial germ cells (PGCs). The Dazh transcription levels increase and reach similar levels in male and female gonads at 14.5 dpc, when first oogonia enter meiosis. In the female gonad, Dazh transcription level decreases to a half from 14.5 dpc to 16.5 dpc (as calculated by optical density of the 3.5kb bands; Fig. 1A) and remains on that level while oogonia proceed meiosis and arrest in prophase I, shortly before birth. In the male gonad, steady levels of transcription were detected through embryonic development, with a prominent decrease (half level) at 16.5 days (Fig. 1A).

We next asked the question, Is Dazh transcription
during embryonic development restricted to germ cells? To distinguish between germ cell and somatic cell expression, we used the Wv (White spotted) mutants which are deficient in germ cells as a result of impairment in proliferation and/or migration of PGCs to the gonads; the somatic element of the gonad differentiate appropriately [15]. We showed previously that Dazh does not transcribe in testis isolated from Wv mutants lacking germ cells [3], indicating that Dazh expression is restricted to germ cells. Consistent with our previous results, we did not detect Dazh transcripts in embryonic gonads isolated from male and female Wv homozygous embryos (Fig. 1B). Our results suggest that Dazh is transcribed in germ cells; in PGCs before sexual differentiation, and after it, in prospermatogonia of the testis, and in oogonia and primary oocytes of the ovary.

Atresia destroys many of the oocytes long before they are fully grown. The decrease in actual number of oocytes is very rapid during the period from birth to puberty [16]. The Dazh transcription levels are correlated with the depletion of oocytes numbers in the ovary. As shown in Fig. 2A, the levels of the two transcripts (4.5 kb and 3.5 kb) were decreased significantly from day one to day 6 after birth and during adulthood. A dramatic decrease of the levels of transcription between 17 to 70 days after birth is demonstrated in Fig. 2B. The Dazh transcription is hardly detected in ovaries depleted oocytes as a result from mutation in ZFX gene [17] (Fig 2B). These results support the observation that Dazh is expressed in the germ cell compound of the ovary, probably in primary oocytes.

In all mammals, male germ cells replicate consistently and maintain their numbers, while female germ cells do not replicate and their numbers are depleted. In the mouse, the number of spermatogonia per testis increased significantly from $0.5 \times 10^5$ at birth to about $6 \times 10^5$ cells per testis after day 25 [18], while the number of oocytes per ovary decreased significantly from about $0.1 \times 10^5$ at birth to about 4500 when females reach puberty [16]. The Dazh transcription levels in the testis and ovary reflect these differences in germ cell numbers in the gonads. The Dazh transcriptions are hardly seen (over-night exposure) in the ovary by puberty, while in the testis a highly expressed 3.5 kb with 4.5 kb and some smaller, less abundant Dazh transcripts were observed (Fig 3B). As expected, a comparison between male and female Dazh densitometry intensities demonstrated that the Dazh transcription levels in the testis are about 100 fold higher than in the ovary (Fig. 3A).

Does human DAZH show a similar expression pattern? Taking into account the fact that expression is limited to primary oocytes, we chose for this study a young human ovary (from a 23 year-old woman) with a significant number of oocytes. As shown in Fig. 3B, a transcript of about 4.5kb was detected in the human ovaries, while 3.5kb transcript is most abundant in the human testis. The ratio between the ovarian and testicular transcription levels is similar to the ratio observed in the mouse (demonstrated by densitometry, Fig 3B). These results, in addition to the observation that DAZH does not transcribe in other human tissues [4] suggest that human DAZH, like the mouse Dazh, is expressed in male and female germ-cells at a similar, if not identical manner.

DISCUSSION

We have previously showed that Dazh is expressed in testicular germ cells long before puberty. In this work, we show that Dazh is expressed in male and female embryonic gonads earlier during development, well before sex-differentiation. We detected Dazh transcripts in the male and female embryonic gonad at 11.5-12.5 days when PGCs are the only germ cells in the gonad.

The PGCs are first recognized in the extraembryonic mesoderm at 7.5 dpc and subsequently migrate to the developing genital ridges at 11.5 dpc. From 8.5 to 13.5 dpc PGCs replicate by mitosis at a uniform rate, with
and female embryonic gonads are also restricted to germ cells (most likely PGCs, oogonia, primary oocytes and prospermatogonia). The Dazh gene could belong to a group of markers that have been instrumentally used in tracking the early period of germ cells development in the mouse such as cell surface alkaline phosphates activity [21, 22] and other surface markers which may not been specific to germ-cells [20]. The Dazh expression pattern is very similar to nuclear antigen (GCNA1) which was shown to be expressed exclusively in germ cells at similar developmental stages [23].

It is most likely that human DAZH and the mouse Dazh proteins perform similar functions. The human and the mouse genes are both expressed predominantly in the testis and in lower levels in ovaries, but not in other tissues [3, 4]. Our results demonstrate similar ratio of transcription levels in testes and ovaries of human and mice and suggest that transcription is limited to germ cells at similar or identical developmental stages. However, Northern-blotting also reveals that human and mouse express different size of transcripts in the ovaries. In the human ovary a 4.5 kb DAZH transcript was observed, while a 3.5kb transcript is observed in the mouse ovary. Such 4.5 kb transcript was observed in low levels in human (not-shown) and mouse testes (see Fig. 2) and in higher levels during early embryonic development (Fig. 1A). Since the DAZ genes are organized as cluster genes [4], it is possible that the two transcripts are expressed from different copies of the genes. Another possibility is that the two different transcripts are from the same origin; RNA processing such as reported previously in DAZH mRNAs [4] may also account for differences in transcript size. The biological significant and function of the 3.5 kb and 4.5 kb transcript units remain to be studied. These transcription units may possess different functions, such as the Ddc cluster of in Drosophila which express different transcripts, some of which are involved in female fertility and some which do not restrict ovary development [24].

The decision as to whether it is oogenesis or spermatogenesis on which PGCs embark seems to depend on their environment, and not on their own chromosomes [25]. However, the ability of germ cells to enter meiosis seems to be intrinsic to germ-cells [26]. Thus, factors in the germ cells that are involved in sex differentiation and entry into meiosis would be expected to be expressed in germ cells of both sexes (XX and XY gonads) such as the Dazh gene. Our expression results are supported by the recent evidence in the mouse suggests that Dazh is essential for the differentiation of male and female germ cells [12]. As expected, disruption of the Dazh gene leads to loss of germ cells and complete absence of male and female gametes.

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