BIOLOGY OF REPRODUCTION **63,** 1490–1496 (2000)

DAZ Family Proteins Exist Throughout Male Germ Cell Development and Transit from Nucleus to Cytoplasm at Meiosis in Humans and Mice[1]

Renee A. Reijo,[2,3,4] David M. Dorfman,[5] Roger Slee,[7] Andrew A. Renshaw,[5] Kevin R. Loughlin,[6] Howard Cooke,[7] and David C. Page[3]

Howard Hughes Medical Institute,[3] Whitehead Institute, and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142
Department of Obstetrics, Gynecology and Reproductive Sciences,[4] and Departments of Physiology and Urology, University of California, San Francisco, California 94143-0720
Department of Pathology[5] and Department of Urology,[6] Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115
MRC Human Genetics Unit,[7] Edinburgh, Scotland EH4 2XU, United Kingdom

ABSTRACT

The human *DAZ* gene family is expressed in germ cells and consists of a cluster of nearly identical *DAZ* (*deleted in azoospermia*) genes on the Y chromosome and an autosomal homolog, *DAZL* (*DAZ*-like). Only the autosomal gene is found in mice. Y-chromosome deletions that encompass the *DAZ* genes are a common cause of spermatogenic failure in men, and autosomal homologs of *DAZ* are essential for testicular germ cell development in mice and *Drosophila*. Previous studies have reported that mouse DAZL protein is strictly cytoplasmic and that human DAZ protein is restricted to postmeiotic cells. By contrast, we report here that human DAZ and human and mouse DAZL proteins are present in both the nuclei and cytoplasm of fetal gonocytes and in spermatogonial nuclei. The proteins relocate to the cytoplasm during male meiosis. Further observations using human tissues indicate that, unlike DAZ, human DAZL protein persists in spermatids and even spermatozoa. These results, combined with findings in diverse species, suggest that DAZ family proteins function in multiple cellular compartments at multiple points in male germ cell development. They may act during meiosis and much earlier, when spermatogonial stem cell populations are established.

sperm, spermatogenesis

INTRODUCTION

Infertility affects 10–15% of couples in industrialized countries, and nearly half of all cases are traced to the male [1]. Y-chromosome deletions encompassing the *DAZ* (deleted in azoospermia) gene cluster comprise the most common molecularly defined cause of spermatogenic failure in infertile men [2–14]. In both *Drosophila* and mice, disruption of the autosomal *DAZ* homolog results in male infertility and, in mice, female infertility as well [15, 16].

The human Y-linked *DAZ* gene cluster arose during primate evolution by transposition and amplification of the still-extant autosomal gene, *DAZL* (*DAZ*-like, formerly known as *DAZLA*, *DAZH*, or *SPGYLA*), which is widely conserved among vertebrates and invertebrates [15, 17–25]. In all species examined, expression of *DAZ*, *DAZL*, and their homologs is reported only in germline cells [18, 19, 24–27]. The presence of RNA recognition motifs suggests that DAZ family proteins bind RNA [28], but little is known of their cellular functions.

Previous studies of the cellular localization of DAZ family proteins in testes of various species have reported surprisingly different results. In *Drosophila*, the DAZ-homologous protein Boule was found in the nuclei of premeiotic cells, moving to the cytoplasm at the onset of meiosis [29]. In mice, however, the DAZL protein was reported to be strictly cytoplasmic [16]. In humans, DAZ protein was stated to be restricted to postmeiotic cells [30].

Using independent antisera, we re-examined how DAZ family proteins were distributed in testicular germ cells of humans and mice. We wished to determine whether the DAZL and DAZ proteins are present in fetal as well as adult testes, whether they exist in both premeiotic and postmeiotic cells, and whether they are found in the nucleus or cytoplasm.

MATERIALS AND METHODS

Preparation of Antisera

Polyclonal antibodies were raised by injecting synthetic oligopeptides coupled to a multiple-antigen peptide resin into rabbits (Research Genetics, Inc., Huntsville, AL). Twelve to 20 wk after initial injection of peptides, serum antibodies were purified on Protein A columns according to the manufacturer's instructions (Hi-Trap; Pharmacia, Inc., Uppsala, Sweden) and preabsorbed on mouse liver acetone powder to remove nonspecific binding activities.

Western Blotting

Mouse or human testis samples or semen were lysed by heating to 95°C for 5 min in 10 volumes of 0.1 M Tris-Cl (pH 6.8), 200 mM dithiothreitol (DTT), 4% SDS, 0.2% bromophenol blue, and 20% glycerol. The samples were placed on ice and sonicated vigorously to shear DNA. Twenty micrograms of protein extract were run in each lane of a 12% polyacrylamide gel and then electrophoretically transferred to nitrocellulose filters according to the manufacturer's instructions (BioRad, Inc., Hercules, CA). The nitrocellulose filters underwent the following series of room-temperature incubations in solutions containing 5% milk powder, 0.1 M Tris-HCl (pH 7.5), 0.9% NaCl, and

[1]Supported by the National Institutes of Health.
[2]Correspondence: Renee A. Reijo, Dept. of Ob/Gyn & RS, 513 Parnassus, Box 0720, HSW1480, University of California, San Francisco, CA 94143-0720. FAX: 415 476 6145; e-mail: reijo@itsa.ucsf.edu

Received: 14 October 1999.
First decision: 10 November 1999.
Accepted: 12 July 2000.

ISSN: 0006-3363. http://www.biolreprod.org

0.2% Nonidet 40 (NP40): 1) preincubation for 1 h, 2) incubation with a 1:1000 dilution of antisera 149, 150, or 133 for 1 h, 3) three rinses of 5 min each, 4) incubation with a 1:10 000 dilution of secondary antibody, anti-rabbit IgG/peroxidase conjugate (Sigma Chemical Co., St. Louis, MO), for 1 h, and 5) two rinses of 5 min each. There were three more room-temperature rinses of 5 min each: one rinse in 5% milk powder, 0.1 M Tris-HCl (pH 7.5), 0.9% NaCl, and 0.1% Tween 20; and two rinses in 0.1 M Tris-HCl (pH 7.5), 0.9% NaCl. Peroxidase reactions were visualized using the electrochemiluminescence system according to the manufacturer's instructions (Amersham, Arlington Heights, IL).

Immunohistochemistry

Human testis sections were obtained from a 20–21-wk fetus and from a 38-yr-old man who presented with a seminoma; testes sections were obtained from the Department of Pathology, Brigham and Women's Hospital, Boston, following institutional review board approval. Mouse testis sections were obtained from embryos 18 days postcoitum and from 60-day-old adult mice; an Institutional Animal Care and Use Committee approved the use of the mice.

Formalin-fixed, paraffin-embedded human tissues were sectioned at 5 μm. After baking at 60°C for 1 h, sections were deparaffinized and rehydrated (100% xylene, four times, 3 min each; 100% ethanol, twice, 3 min each; 95% ethanol, twice, 3 min each; water, 5 min). Slides were blocked by treating with 3% hydrogen peroxide in methanol for 5 min at room temperature, then washed in water for 5 min, and microwave treated (800 W, General Electric) at 199°F for 30 min in 10 mM citrate (pH 6.0). Slides were cooled for 15 min, transferred to PBS, 0.1% Tween 20, and preincubated for 15 min at room temperature in 1.5% goat serum. Slides were incubated for 1 h at room temperature with antisera 149 (at 1:4000 dilution), antisera 150 (1:1000), or antisera 133 (1:200) in 2% goat serum. Slides were washed three times (5 min each) in PBS, 0.1% Tween 20, and then incubated for 30 min with biotinylated horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA). After three washes (5 min each) in PBS, 0.1% Tween 20, slides were incubated for 40 min at room temperature with avidin:biotinylated-peroxidase complex (Vector), followed by reaction with 3,3′-diaminobenzidine tetrachloride:hydrogen peroxide. Sections were subsequently stained with 2% Gill hematoxylin. Column-fractionated preimmune sera corresponding to fractions containing the specific antibodies were used as negative controls.

Mouse tissues were treated in a similar manner except that tissues were fixed in Bouin fixative rather than formalin, the microwave treatment was eliminated, and goat anti-rabbit horseradish peroxidase-conjugated antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) replaced the biotin:avidin system described above.

Cell types were determined by enlarging the images and examining the position, size, and morphology of cells that were immunopositive. The criteria to assign cell types were those previously reported [31–33].

RESULTS

Three peptides against which polyclonal antibodies were raised and the corresponding portions of the human DAZ (Y-encoded), human DAZL (autosomal), and mouse DAZL (autosomal) proteins are shown in Figure 1a. We anticipated that antisera 149 would recognize all three proteins, that

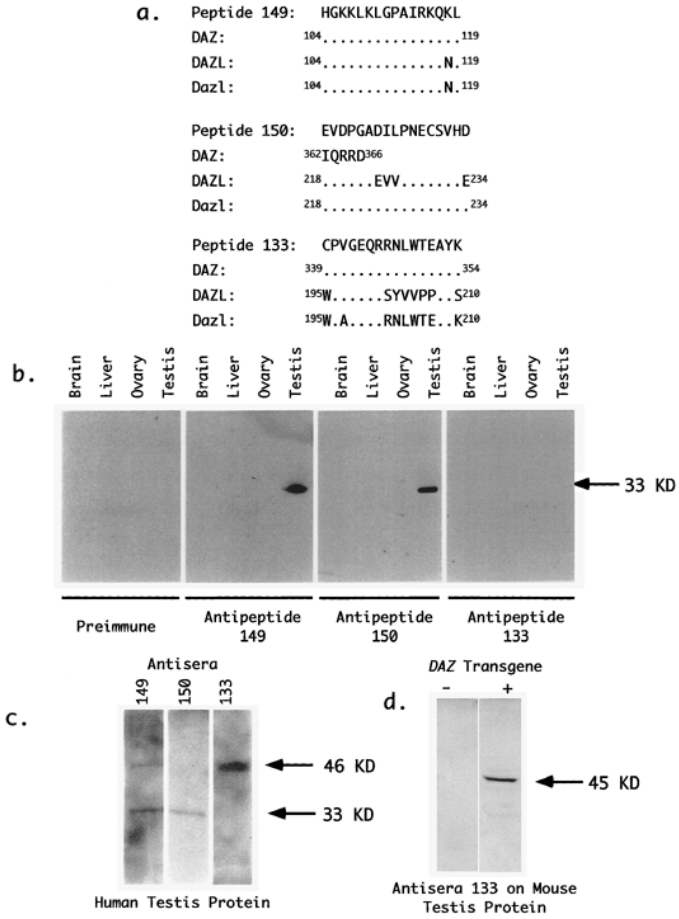

FIG. 1. Antisera to DAZL and DAZ proteins. **a**) Three peptides against which antisera were raised: peptide 149, present in both Y-encoded DAZ and autosomally encoded DAZL; peptide 150, found only in DAZL; and peptide 133, found only in DAZ. Amino acid residues in the corresponding portions of the human DAZ, human DAZL, and mouse DAZL proteins are numbered. Dots represent identity to the corresponding peptide sequence. **b**) Western blotting of adult mouse tissues. No staining was observed with preimmune serum or with antisera 133, raised against a human Y-encoded (DAZ) epitope absent in mice. **c**) Western blotting of adult human testes. Based on the apparent molecular weight of the DAZ protein in vivo (approximately 46 kDa), we estimate that it contains a tandem array of roughly nine DAZ repeats [2, 35]. **d**) Western blotting of adult human and mouse testes. No staining is observed in wild-type mouse testes without a *DAZ* transgene (−) when Western blots are probed with antisera 133 that is specific to human Y-chromosomal DAZ protein. However, mice containing a *DAZ* transgene (+) express DAZ protein in the testes. Transgene construction was as reported in Slee et al. [34]. Protein extracts of testicular tissue from mice or humans were prepared as indicated (see *Materials and Methods*).

antisera 150 would recognize the autosomally encoded (human and mouse) DAZL proteins, and that antisera 133 would recognize the human Y-encoded DAZ protein. Indeed, on Western blots of adult mouse tissues, a testis-specific protein with a molecular weight of 33 kDa, as predicted for mouse DAZL, was detected by antisera 149 and 150 (Fig. 1b). These two antisera also recognized the human DAZL protein, again with a molecular weight of 33 kDa, on Western blots of human adult testes. As anticipated, a human testis protein of higher molecular weight (46 kDa), DAZ, was detected by antisera 149 and 133 (Fig. 1c). (Competition with the immunizing peptides abolished all of these signals; not shown.) Antisera 133, raised against a peptide present in human Y-encoded DAZ but absent in the autosomal proteins, did not recognize any proteins in

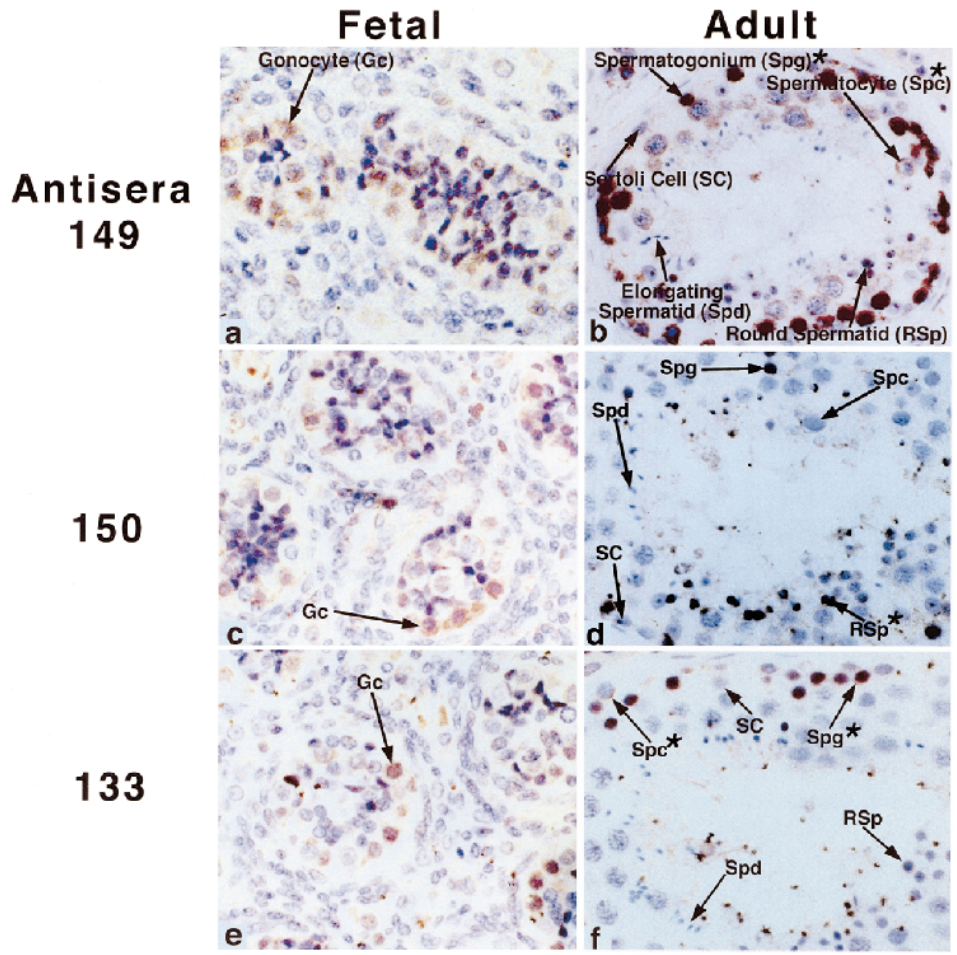

FIG. 2. Immunoperoxidase staining of human testis sections: fetal week 20–21 and adult. Antisera 149 recognizes both DAZL and DAZ. Antisera 150 recognizes DAZL. Antisera 133 recognizes DAZ. In fetal sections, arrows point to representative gonocytes. Abbreviations in adult sections: Spg, spermatogonium; Spc, spermatocyte; Spt, spermatid; SC, Sertoli cell. Note that staining with antisera 133 sometimes results in deposits of brown debris in regions of tubule lumens where no cells are found. Sections were counterstained with 2% Gill hematoxylin.

mice (Fig. 1, b and d, left-hand lane), where the Y chromosome does not carry any *DAZ* genes. However, mice carrying a *DAZ* transgene express a DAZ protein as a fusion with 14 amino acids from herpes simplex virus thymidine kinase protein that is recognized by antisera 133 (Fig. 1d, right-hand lane; transgene construction is as reported in Slee et al. [34]). This indicates that antibody 133 is specific to the DAZ protein encoded by the human Y chromosome. The size of the DAZ protein in human testes in these studies corresponds to a protein with an estimated tandem array of roughly nine DAZ repeats; no other forms of DAZ were detected. Another report, in which a single antibody was used and specificity was not confirmed with transgenic mice, suggested that the predominant form of DAZ protein expressed was 66 kDa [30]. This suggests the possibility that there may be several different DAZ proteins expressed in human testis. Although we have not observed any additional DAZ proteins on multiple blots, this may be expected because there is heterogeneity in the number of repeats in different gene copies in different men [35]. In any case, confidence that the 46-kDa protein represents authentic DAZ is based on the observations that this protein is absent from wild-type mouse testis extracts, present in human testis extracts, and present in transgenic mice expressing DAZ protein from a Y-chromosome yeast artificial chromosome transgene.

We next used these antisera to explore the distribution of DAZ and DAZL proteins in human testis sections from a 20- to 21-wk fetus and from an adult. (The adult had presented with a seminoma; the sections we studied were not adjacent to the tumor and were comprised of histologically normal seminiferous tubules with a full range of spermatogenic cell types.) We found that, in humans, the DAZ and DAZL proteins are present in male germ cells at many stages in their development, both prenatally and during spermatogenesis. Antisera 149 that recognizes both DAZ and DAZL clearly stained the gonocytes of the fetus and the spermatogonia and spermatocytes of the adult (Fig. 2); we also observed weaker staining of round and elongating spermatids. A similar pattern was obtained with antisera 150 that is specific for DAZL, although here the staining of spermatids was more pronounced (Fig. 2). Staining with antisera 133, which is specific for (Y-encoded) DAZ, was largely restricted to the less mature germ cells: gonocytes, spermatogonia, to a lesser extent, spermatocytes, and only occasionally round spermatids (Fig. 2). These results are confirmed by examination of multiple tubules in testicular biopsies (Fig. 3). Our impression that DAZL, but not DAZ, was present in spermatids throughout their differentiation was further explored by Western blotting of protein extracts from a normal semen sample. We observed the 33-kDa DAZL protein readily in these preparations, while we did

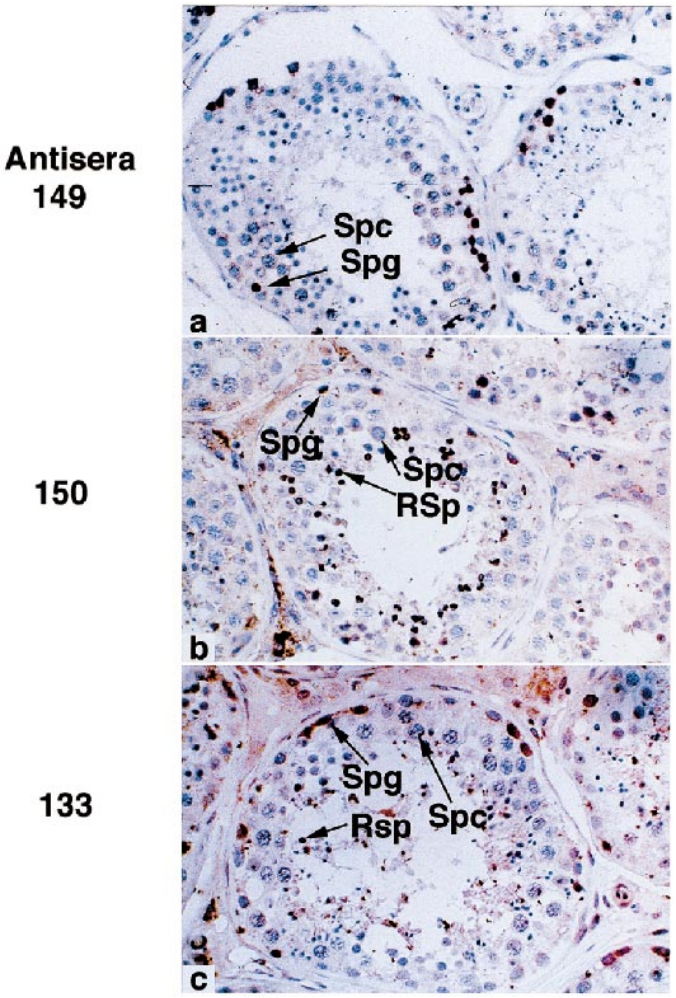

FIG. 3. Immunoperoxidase staining of human testis sections from adults indicating staining patterns in multiple tubules. Antisera 149 recognizes both DAZL and DAZ. Antisera 150 recognizes DAZL. Antisera 133 recognizes DAZ. Note that staining with antisera 133 sometimes results in deposits of brown debris in regions of tubule lumens where no cells are found. Sections were counterstained with 2% Gill hematoxylin.

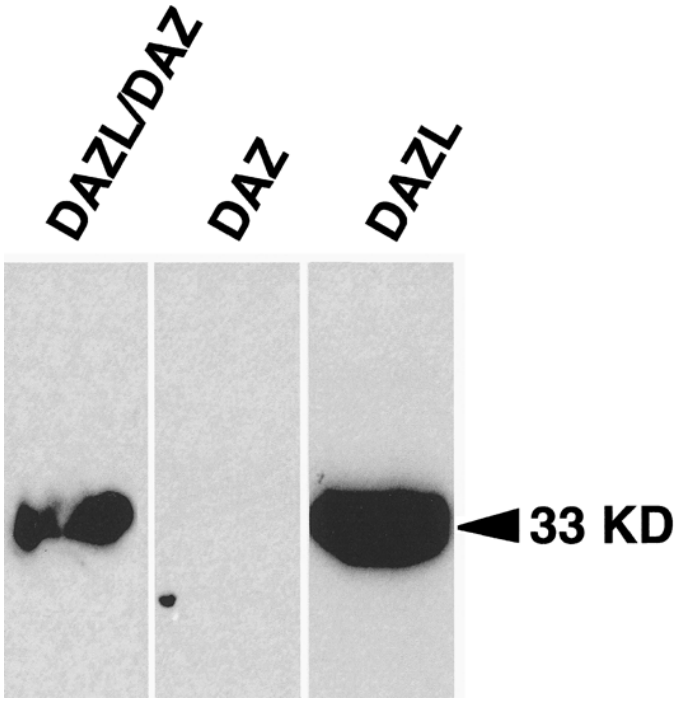

FIG. 4. Western blotting of proteins in mature human spermatozoa. Western blotting was done using antisera 133. Protein extracts of sperm from a normal semen sample were prepared as described in *Materials and Methods.*

not detect DAZ in mature spermatids (Fig. 4). We conclude that both the autosomally encoded DAZL and Y-encoded DAZ proteins are present in human male germ cells through much of their development, both prenatally and during spermatogenesis, but that only DAZL is detected in postmeiotic, mature spermatids.

By studying the immunohistochemically stained sections of human fetal and adult testes, we found that the intracellular locations of the DAZ and DAZL proteins change during the progression from fetal gonocytes to spermatogonia to spermatocytes to spermatids. In gonocytes, DAZ and DAZL were present in both the nucleus and the cytoplasm (Fig. 2). In spermatogonia, DAZ and DAZL were most abundant in the nucleus but could also be detected in the cytoplasm (Fig. 2; for example see upper right panel and cells marked with an asterisk). In spermatocytes, the proteins appear to be restricted to the cytoplasm (see especially antisera 149, adult testis in Fig. 2; cells indicated by an asterisk). Finally, as the spermatogenic cells mature into round spermatids and begin to elongate, the pattern of DAZL staining is consistent with cytoplasmic localization (see especially antisera 150, adult testis in Fig. 2; cell marked by an asterisk). Thus, our results are consistent with the hypothesis that DAZ and DAZL are predominantly nuclear in spermatogonia, but at meiosis the proteins are largely located in the cytoplasm, where DAZL persists in differentiating spermatids.

We next sought to determine whether DAZL protein is present in the germ cells of mice at the same developmental stages and in the same cellular compartments as in humans. (As mentioned earlier, there are no Y-linked *DAZ* genes in mice.) In mice, as in humans, DAZL protein is present in testicular germ cells in both fetuses and adults, as revealed by staining with antisera 149 and 150 (Fig. 5). As expected, no staining is observed with antiserum 133 that specifically recognizes human Y-chromosome-encoded DAZ protein (Fig. 5). In addition, there appears to be no staining of Sertoli cells or other somatic cells, in agreement with previous Northern blotting and in situ hybridization studies [18, 19, 27]. In mouse fetal gonocytes, DAZL appears to be present in both the nucleus and the cytoplasm (Fig. 5). In sections of adult mouse testis counterstained conventionally with hematoxylin and eosin, DAZL staining is most evident in the cytoplasm of meiotic cells (spermatocytes; Fig. 5). To ensure that no DAZL staining was obscured, we also examined adult testicular sections that were not counterstained. With no counterstaining, the localization of DAZL to spermatocyte cytoplasm was confirmed, and a second localization—to the nuclei of spermatogonia—was revealed (Fig. 6). We conclude that during spermatogenesis in mice, as in humans, DAZL shifts from a predominantly nuclear to a predominantly cytoplasmic location at meiosis.

These findings with mouse DAZL conflict with a recent report indicating that the protein is strictly cytoplasmic in murine male germ cells [16]. As in the present study, Ruggiu et al. [16] reported strong staining for DAZL in spermatocytes, where the protein is cytoplasmic. They did not observe staining in spermatogonial nuclei despite using fluorescently labeled antibodies. As the two antibodies are

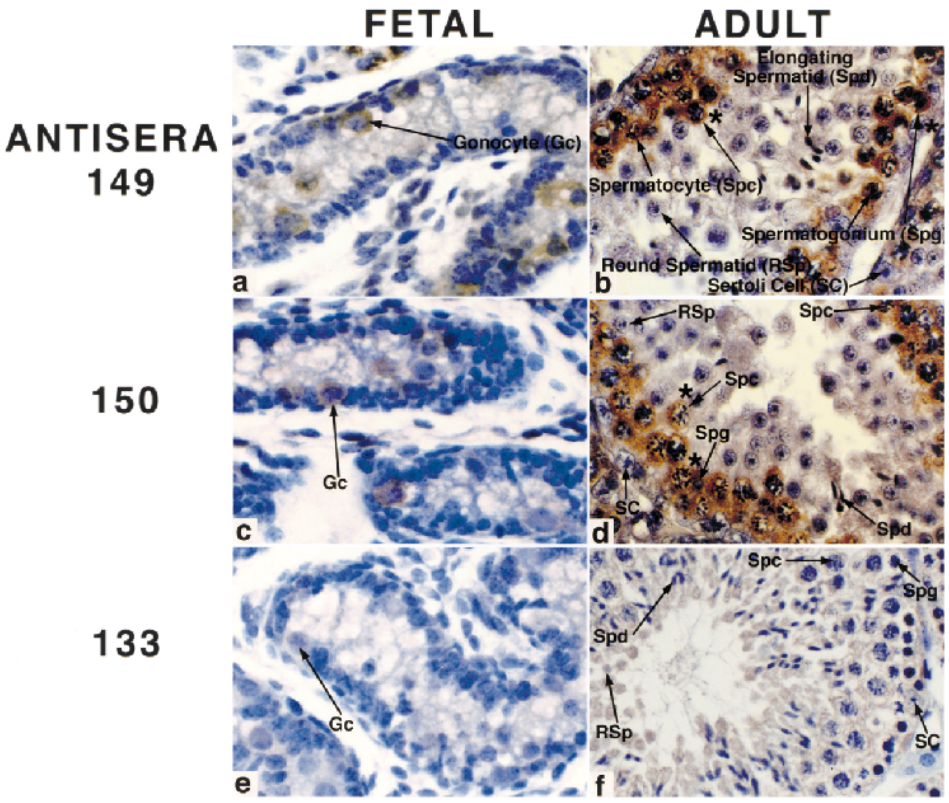

FIG. 5. Immunoperoxidase staining of mouse testis sections with antisera 149, 150, and 133: fetal (18 days postcoitum) and adult (60 days after birth). Abbreviations in adult sections: Spg, spermatogonium; Spc, spermatocyte; RS, round spermatid; ES, elongating spermatid; SC, Sertoli cell. As expected, antisera 133, specific to Y-encoded DAZ, did not stain any spermatogenic cells in mouse testis sections. In fetal sections, arrows point to representative gonocytes.

raised against different parts of the DAZL protein, it is possible that different epitopes have different availabilities in different cell types.

DISCUSSION

Our immunohistochemical studies in human and mouse, together with mutation and expression analysis of DAZ homologs in diverse organisms, provide evidence that mammalian DAZ family proteins may function at multiple points in male germ cell development. Specifically, we hypothesize that the mammalian DAZ family proteins act both during meiosis and much earlier, during the establishment of spermatogonial stem cell populations. As demonstrated here in both humans and mice, the DAZ family proteins are expressed in gonocytes of fetal testes, long before the onset of spermatogenesis and meiosis (at puberty). In mutant male mice lacking *Dazl* function, germ cells are lost between Days 15 and 19 of embryonic development [16], also long before spermatogenesis begins. Taken together, these results suggest that DAZ family proteins in both humans and mice play a critical role in early male germ cell development, when spermatogonial stem cell precursors must emerge and be maintained. The putative RNA-binding proteins of the DAZ family are largely nuclear during these early, premeiotic phases of male germ cell development, leading us to speculate that the early functions of the mammalian DAZ family proteins are directed at RNA processing or storage in the nucleus [28].

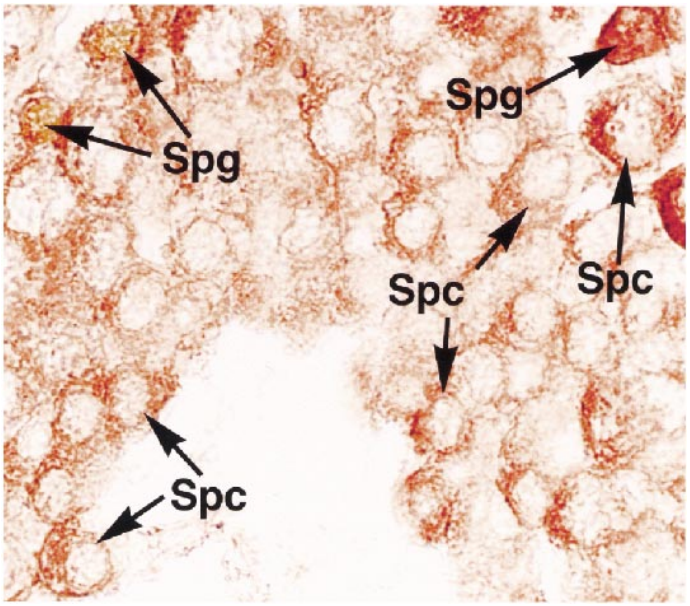

FIG. 6. Adult mouse testis stained with antisera 149 but with no hematoxylin counterstaining.

Genetic analysis of diverse species demonstrates the importance of DAZ family proteins in meiosis, in one or both sexes, and our protein localization studies hint at a similar meiotic role in male mammals. In male flies lacking *boule* function, meiotic divisions do not occur [15]. The *Xenopus DAZ* homolog *Xdazl*, when expressed in *Drosophila*, rescues the *boule* meiotic entry defect [25]. Also, loss of *Dazl* function in mutant female mice or suppression of *DAZ*-homolog function in female nematodes result in germ cell loss at meiosis [16, 24]. (In *Dazl*-mutant male mice, germ cells are lost during fetal development [16], precluding any

manifestation of the male meiotic role that we postulate here.) As we report, the mammalian DAZ family proteins appear to move from a predominantly nuclear to a predominantly cytoplasmic location at male meiosis. The protein product of the *Drosophila boule* gene, homologous to mammalian DAZ and DAZL, undergoes a similar redistribution from nucleus to cytoplasm when male meiosis is initiated [29]. The late functions of the mammalian DAZ family proteins may begin at the onset of meiosis.

Previous in situ hybridization studies had revealed that, in the human adult testis, *DAZ*/*DAZL* transcripts are most abundant in spermatogonia and primary spermatocytes [26]. For DAZ, these in situ hybridization data are in close accord with the present immunohistochemical findings. In contrast, DAZL protein appears to persist long after transcript levels have peaked. The DAZL protein observed in spermatozoa and their (postmeiotic) spermatid precursors may have been synthesized many days or even weeks earlier, during or prior to meiosis. Our findings with DAZ are at odds with the report of Habermann et al. [30] that this Y-encoded human protein is found only in late stage germ cells, spermatids, and spermatozoa. Spermatids and spermatozoa are the only male germ cells in which we do not detect the Y-chromosome-encoded DAZ protein (Figs. 2–4). Habermann et al. [30] employed a single antiserum that detected many protein species on Western blots of human testis extracts. This antiserum may not have been specific for DAZ. In the present study, three different antisera were used and results were confirmed via several different methods.

The expression patterns of the human DAZ and DAZL proteins overlap but are not identical, suggesting that the two human proteins may contribute differentially to the postulated early and late DAZ family functions. As described earlier, the human Y-encoded DAZ protein is most prominently observed in less mature germ cells, especially gonocytes and spermatogonia, while the human autosomally encoded DAZL protein is clearly present after meiosis, persisting even in mature spermatozoa. Thus, the Y-borne *DAZ* gene cluster, which arose by transposition and amplification of the autosomal *DAZL* gene during primate evolution [17], may have evolved toward specialization in early functions; the human *DAZL* gene may have evolved toward specialization in late (meiotic or postmeiotic) functions. This would fit well with previous evidence that men who are deleted for the Y-borne *DAZ* gene cluster are infertile because of a problem in the generation or maintenance of spermatogonial stem cell populations (an early function) [2, 3] and not a defect in the differentiation pathway of spermatogenesis itself (a late function). Thus, infertility in *DAZ*-deleted men may have its origins long before puberty and perhaps even during fetal development.

ACKNOWLEDGMENTS

We thank L. Brown and J. Cook-Chrysos for graphic art; and A. Bortvin, L. Brown, B. Lahn, D. Menke, R. Saxena, C. Sun, P.J. Turek, and J. Wang for comments on the manuscript.

REFERENCES

1. de Kretser DM. Male infertility. Lancet 1997; 349:787–790.
2. Reijo RA, Lee T-Y, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O, de la Chapelle A, Silber S, Page DC. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995; 10:383–393.
3. Reijo RA, Alagappan RK, Patrizio P, Page DC. Severe oligospermia resulting from deletions of the *Azoospermia Factor* gene on the Y chromosome. Lancet 1996; 347:1290–1293.
4. Vogt P, Chandley AC, Hargreave TB, Keil R, Ma K, Sharkey A. Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of *AZF*, a human spermatogenesis gene. Hum Genet 1992; 89:491–496.
5. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, Kohn FM, Schill WB, Farah S, Ramos C, Hartmann M, Hartschuh W, Meschede D, Behre HM, Castel A, Nieschlag E, Weidner W, Grone H-J, Jung A, Engel W, Haidl G. Human Y chromosome azoospermia factors (*AZF*) mapped to different subregions in Yq11. Hum Mol Genet 1996; 5:933–943.
6. Nakahori Y, Kuroki Y, Komaki R, Kondoh N, Namiki M, Iwamoto T, Toda T, Kobayashi K. The Y chromosome region essential for spermatogenesis. Horm Res 1996; 46:20–23.
7. Najmabadi H, Huang V, Yen P, Subbarao MN, Bhasin D, Banaag L, Naseeruddin S, DeKretser DM, Baker HWG, McLachlan RI, Loveland KA, Bhasin S. Substantial prevalence of microdeletions of the Y-chromosome in infertile men with ideiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. J Clin Endocrinol Metab 1996; 81:1347–1352.
8. Simoni M, Gromoll J, Dworniczak B, Rolf C, Abshagen K, Kamischke A, Carani C, Meschede D, Behre HM, Horst J, Nieschlag E. Screening for deletions of the Y chromosome involving the *DAZ* (*Deleted in AZoospermia*) gene in azoospermia and severe oligozoospermia. Fertil Steril 1997; 67:542–547.
9. Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, Roberts KP. Microdeletions in the Y chromosome of infertile men. N Engl J Med 1997; 336:534–539.
10. Kremer JAM, Tuerlings JHAM, Meuleman EJH, Schoute F, Mariman E, Smeets DFCM, Hoefsloot LH, Braat DDM, Merkus HMWM. Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. Hum Reprod 1997; 12:687–691.
11. Girardi SK, Mielnik A, Schlegel PN. Submicroscopic deletions in the Y chromosome of infertile men. Hum Reprod 1997; 12:1635–1641.
12. Vereb M, Agulnik AI, Houston JT, Lipschultz LI, Lamb DJ, Bishop CE. Absence of *DAZ* gene mutations in cases of non-obstructed azoospermia. Mol Hum Reprod 1997; 3:55–59.
13. van der Ven K, Montag M, Peschka B, Leygraaf J, Schwanitz G, Haidl G, Krebs D, van der Ven H. Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. Mol Hum Reprod 1997; 3:699–704.
14. Foresta C, Ferlin A, Garolla A, Rossato M, Barbaux S, De Bortoli A. Y-chromosome deletions in idiopathic severe testiculopathies. J Clin Endocrinol Metab 1997; 82:1075–1080.
15. Eberhart CG, Maines JZ, Wasserman SA. Meiotic cell cycle requirement for a fly homologue of human *Deleted in AZoospermia*. Nature 1996; 381:783–785.
16. Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, Dorin J, Cooke H. The mouse *Dazla* gene encodes a cytoplasmic protein essential for gametogenesis. Nature 1997; 389:73–77.
17. Saxena R, Brown LG, Hawkins T, Alagappan RK, Skaletsky H, Reeve MP, Reijo RA, Rozen S, Dinulos MB, Disteche CM, Page DC. The *DAZ* gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nat Genet 1996; 14:292–299.
18. Cooke HJ, Lee M, Kerr S, Ruggiu M. A murine homologue of the human *DAZ* gene is autosomal and expressed only in male and female gonads. Hum Mol Genet 1996; 5:513–516.
19. Reijo R, Seligman J, Dinulos MB, Jaffe T, Brown LG, Disteche CM, Page DC. Mouse autosomal homolog of *DAZ*, a candidate male sterility gene in humans, is expressed in male germ cells before and after puberty. Genomics 1996; 35:346–352.
20. Shan Z, Hirschmann P, Seebacher T, Edelmann A, Jauch A, Morell J, Urbitsch P, Vogt PH. A *SPGY* copy homologous to the mouse gene *Dazla* and the *Drosophila* gene *boule* is autosomal and expressed only in the human male gonad. Hum Mol Genet 1996; 5:2005–2011.
21. Yen PH, Chai NN, Salido EC. The human autosomal gene *DAZLA*: testis specificity and a candidate for male infertility. Hum Mol Genet 1996; 5:2013–2017.
22. Seboun E, Barbaux S, Bourgeron T, Nishi S, Algonik A, Egashira M, Nikkawa N, Bishop C, Fellow M, McElreavey K, Kasahara M. Gene sequence, localization and evolution conservation of *DAZLA*, a candidate male sterility gene. Genomics 1997; 41:227–235.
23. Carani C, Gromoll J, Brinkworth MH, Simoni M, Weinbauer GF, Nieschlag E. *cynDAZLA*: a cynomolgus monkey homologue of the human autosomal *DAZ* gene. Mol Hum Reprod 1997; 3:479–483.

24. Karashima T, Sugimoto A, Yamamoto MA. *C. elegans* homologue of *DAZ/boule* is involved in progression through meiosis during oogenesis. Worm Breeder's Gazette 1997; 15:65.
25. Houston DW, Zhang J, Maines JZ, Wasserman SA, King ML. A *Xenopus DAZ*-like gene encodes an RNA component of germ plasm and is a functional homologue of *Drosophila boule*. Development 1998; 125:171–180.
26. Menke DB, Mutter GL, Page DC. Expression of *DAZ*, an azoospermia factor candidate, in human spermatogonia [letter]. Am J Hum Genet 1997; 60:237–241.
27. Neiderberger C, Agulnik AI, Cho Y, Lamb D, Bishop CE. *In situ* hybridization shows that *Dazla* expression in mouse testis is restricted to premeiotic stages IV–VI of spermatogenesis. Mamm Genome 1997; 8:277–278.
28. Burd CG, Dreyfuss G. Conserved structures and diversity of functions of RNA-binding proteins. Science 1994; 265:615–621.
29. Cheng MH, Maines JZ, Wasserman SA. Biphasic subcellular localization of the *DAZL*-related protein *boule* in *Drosophila* spermatogenesis. Dev Biol 1998; 204:567–576.
30. Habermann B, Mi HF, Edelmann A, Bohring C, Backert IT, Kiesewetter F, Aumuller G, Vogt PH. *DAZ* (*Deleted in AZoospermia*) genes encode proteins located in human late spermatids and in sperm tails. Hum Reprod 1998; 13:363–369.
31. Orth JM. Cell biology of testicular development in the fetus and neonate. In: Desjardins C, Ewing LL (eds.), Cell and Molecular Biology of the Testis. New York: Oxford University Press; 1993: 3–42.
32. Meistrich ML, van Beek MEAB. Spermatogonial stem cells. In: Desjardins C, Ewing LL (eds.), Cell and Molecular Biology of the Testis. New York: Oxford University Press; 1993: 266–295.
33. Clermont Y, Oko R, Hermo L. Cell biology of mammalian spermiogenesis. In: Desjardins C, Ewing LL (eds.), Cell and Molecular Biology of the Testis. New York: Oxford University Press; 1993: 332–376.
34. Slee R, Grimes B, Speed R, Taggart M, Maguire SM, Ross A, McGill NI, Saunders P, Cooke HJ. A human *DAZ* transgene confers partial rescue of the mouse *Dazl1* null phenotype. Proc Natl Acad Sci U S A 1999; 96:8040–8045.
35. Yen PH, Chai NN, Salido EC. The human *DAZ* genes, a putative male infertility factor on the Y chromosome, are highly polymorphic in the DAZ repeat regions. Mamm Genome 1997; 8:756–759.