Human Reproduction vol.13 no.2 pp.363–369, 1998

DAZ (Deleted in AZoospermia) genes encode proteins located in human late spermatids and in sperm tails

B.Habermann[1], H.-F.Mi[2], A.Edelmann[2], C.Bohring[1], I.-T.Bäckert[3], F.Kiesewetter[4], G.Aumüller[5], P.H.Vogt[2,6]

[1]Department of Andrology, Centre of Dermatology, University of Marburg, D-35033 Marburg, [2]Reproduction Genetics, Institute of Human Genetics, University of Heidelberg, D-69120 Heidelberg, [3]Department of Gynaecology and Obstetrics, University of Tübingen, D-72076 Tübingen, [4]Department of Andrology, University of Erlangen, D-91052 Erlangen, and [5]Reproduction Biology, Institute of Anatomy and Cell Biology, University of Marburg, D-35037 Marburg, Germany

[6]To whom correspondence should be addressed at: Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany

We analysed the location of proteins encoded by the *DAZ* (Deleted in AZoospermia) genes in human testis tissue and in mature spermatozoa. The *DAZ* genes are known to be expressed exclusively in the human male germ line, and are candidate genes for the expression of the azoospermia factor AZFc mapped recently to distal Yq11. They encode testis-specific RNA binding proteins, the function of which is not yet known. Immunostaining experiments with antibodies prepared for the specific peptide domain encoded by the *DAZ2* transcript (formerly *SPGY1*) revealed the presence of DAZ proteins in the innermost layer of the male germ cell epithelium and in the tails of spermatozoa. This suggests a function for DAZ proteins in the RNA metabolism of late spermatids, presumably in the storage or transport of testis-specific mRNA, the translation of which is repressed until the formation of mature spermatozoa. Deletion of *DAZ* genes is supposed not to interfere with human sperm maturation but to result in a gradual reduction of mature spermatozoa.

Key words: AZFc patients' testis pathology/*DAZ* gene family/DAZ proteins/testis-specific RNA binding proteins

Introduction

Of men seeking help at infertility clinics, ~20% suffer from severe oligozoospermia or azoospermia, the cause of which cannot readily be given (Hargreave *et al.*, 1990). It was found recently that about one in eight of them carry de-novo deletions for a specific region of the Y chromosome long arm (Yq11) encompassing the *DAZ* (Deleted in AZoospermia) gene (Reijo *et al.*, 1995). Initially, this was thought to be 'single copy', but it is now known as the *DAZ* gene family on Yq11 (Vogt *et al.*, 1997) and with an autosomal copy on the short arm of chromosome 3 (*DAZL1*, Vogt *et al.*, 1997; formerly called *DAZH*, Saxena *et al.*, 1996; *DAZLA*, Yen *et al.*, 1996, and *SPGYLA*, Shan *et al.*, 1996; Seboun *et al.*, 1997).

DAZ genes are expressed exclusively in the testis and encode proteins which contain an RNA recognition motif (RRM) suggesting a potential role in the RNA metabolism of the male germ cell. Due to their structural homology with the mouse gene *Dazla* (Cooke *et al.*, 1996) and the *Drosophila* gene *Boule* (Eberhart *et al.*, 1996), a conserved meiotic cell cycle function for *DAZ* was recently postulated (Eberhart *et al.*, 1996). However, this was inconsistent with the resulting variable phenotypes after *DAZ* deletion, and the transmission of a *DAZ* deletion from father to son (Vogt *et al.*, 1996). It suggests that the primary phenotypic effect of *DAZ* deletion is not azoospermia but subfertility, and that *DAZ* deletions are compatible with the development of motile spermatozoa, albeit in low numbers. We have now tested this hypothesis experimentally by analysing the location of DAZ proteins in the human male germ line using antibodies for DAZ2 (formerly SPGY1; Vogt *et al.*, 1996). They cross-react with testis proteins of variable molecular weight, as is expected for DAZ proteins. These proteins seemed to be present mainly in late spermatids and in sperm tails. Our results suggest a need for DAZ proteins in the storage or transport of testis-specific mRNA, the translation of which is repressed until formation of mature spermatozoa. The implications of our results for the phenotypes of men with deletions of the whole *DAZ* gene family in Yq11 (AZFc patients) will be discussed.

Materials and methods

Generation of DAZ-specific antibodies

Transcripts of the *DAZ* genes in Yq11 are homologous to the transcript of *DAZL1* on chromosome 3 but differ by a tandem repetition of a 72 bp nucleotide unit and absence of a 130 bp nucleotide unit (Shan *et al.*, 1996). The 72 bp nucleotide unit comprises the repetitive exon 7 of *DAZ* genes and the 130 bp nucleotide unit is exon 9 and part of exon 8 only in the *DAZL1* gene (Saxena *et al.*, 1996). Consequently, DAZ proteins are different from the DAZL1 protein in their *C*-terminal regions. Moreover, the sequence of the *C*-terminal peptide domain encoded by exon 8–10 of *DAZL1* contains 105 amino acids; that of each *DAZ* copy only 32 amino acids.

DAZL1, exon 8-exon 10 peptide

MPPQWPVGEQRSYVVPPAYSAVNYHCNEVDPG-
AEVVPNECSVHEATPPSGNGPQKKSVDRSIQTVVSCLFNPENR-
LRNSVVTQDDYFKDKRVHHFRRSRAMLKSV

DAZ, exon 8-exon 10 peptide:

MPPQCPVGEQRRNLWTEAYKWWYLVCLIQRRD

Antibodies specific for DAZ proteins can therefore be prepared

© European Society for Human Reproduction and Embryology

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022

from their specific *C*-terminal domain. We used this peptide part of the DAZ2 protein (formerly called SPGY1; Vogt *et al.*, 1996). *DAZ* and *SPGY1* genes were first thought to be different although homologous because *DAZ* was described as a single copy gene (Reijo *et al.*, 1995). Later it became evident that *DAZ* and *SPGY1* belong to one gene family, the members of which have an identical sequence but are heterogeneous in number and composition of a 72 bp sequence unit (DAZ repeat). Consequently, *DAZ* was renamed *DAZ1* and *SPGY1*, *DAZ2* (Vogt *et al.*, 1997).

A subfragment of the *DAZ2* gene containing the sequence for the whole *C*-terminal peptide and 11 copies of its tandem repetitive 24 amino acid domain was cloned in the glutathione-*S*-transferase fusion factor pGEX-5X-3 (Pharmacia Biotech Europe GmbH, Freiburg, Germany) and the corresponding fusion protein expressed in *Escherichia coli* after induction with 1 mM isopropyl-β-D-thiogalactoside (IPTG). This construction contained 303 amino acids of the DAZ2 protein. It was purified by affinity chromatography from the background of *E.coli* proteins and used directly for injection with complete Freund's adjuvant into a rabbit. The rabbit was boostered by additional injections complemented with incomplete Freund's adjuvant after 2, 4 and 8 weeks. Serum of the final blood sample was purified using standard protocols (Ausubel *et al.*, 1995). The resulting antiserum (anti-DAZ2) was tested for specificity by crossreaction to a single protein band in Western blots of *E.coli* protein extracts containing the glutathione-*S*-transferase–DAZ2 fusion peptide. The antiserum only stained one protein band with a molecular weight corresponding to that of the DAZ2 fusion peptide. We therefore designated it as anti-DAZ2 serum. The preimmune serum was taken from the same rabbit before the first injection.

Immunoblotting of testis proteins

Surgically removed human testis tissue (20–50 mg) homogenized in ~3 ml phosphate-buffered saline (PBS) was centrifuged at 100 000 *g* for 20 h. The pellet (nuclear and unsoluble cellular protein fraction: 'P') was resuspended in 1 ml Laemmli buffer [50 mM Tris–HCl pH 6.8, 100 mM dithiothreitol (DTT), 2% sodium dodecyl sulphate (SDS), 0.1% Bromphenol Blue, 10% glycerol]. The supernatant (soluble cellular protein fraction: 'S') was mixed with 1/3 vol. of 4× Laemmli buffer. Aliquots of both fractions were electrophoresized on 8% SDS–polyacrylamide Laemmli gels and blotted on nitrocellulose (Nytran BA 85; Schleicher and Schuell, Dassel, Germany) using standard procedures. To control protein transfer, blots were stained for 5 min with Ponceau S (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) and destained with aqua bidest (10 min).

After blocking blots for non-specific binding reactions in Tris-buffered saline (TBS) with 5% non-fat dry milk, 0.2% sodium azide at room temperature for 30 min, one blot was incubated with anti-DAZ2 (1:200 diluted in TBS), and the second blot with preimmune serum (1:200 diluted in TBS). Incubation time was 2 h at room temperature or overnight at 4°C. After a short TBS washing step, antibody reactions were visualized by incubation with goat anti-rabbit immunoglobulin (Ig)G-coupled horseradish peroxidase (Amersham Buchler GmbH and Co KG, Braunschweig, Germany) diluted 1:400 in TBS/0.5% gelatine and 0.04% H_2O_2, 0.036% 4-chloro-1-naphthol in TBS buffer.

Immunoblotting of sperm tail proteins

Spermatozoa isolated from different individuals were first purified from seminal plasma by centrifugation (5 min at 300 *g*), washed with PBS buffer, and concentrated on a Percoll step gradient (Pharmacia) according to the method of Ord *et al.* (1990). The pellets were resuspended in 0.25 M sucrose buffer [5 mM Tris–HCl pH 7.2, 1 mM EDTA, 10 mM benzamidine, 2 mM DTT, 0.2 mM phenylmethylsulphonyl fluoride (PMSF), 20 mg/l aprotinin] and sonicated on ice as described by Henkel *et al.* (1992). The sperm concentration was adjusted to 100×10^6 spermatozoa/ml.

For isolation of sperm tails sonicated sperm probes were mixed with 30 ml isotonic Percoll solution distributed in centrifuge tubes and overlayed with one fifth volume of an isotonic sodium chloride solution. After centrifugation at 3000 *g*, 4°C for 45 min, sperm tails could be collected at the interface. They were washed with sucrose buffer and resuspended in Laemmli buffer for analysis on SDS–PAGE gradient gels (7.5–20%) running for 45 min with 100 V at room temperature. After protein transfer to polyvinyldifluoride (PVDF) membranes, they were first preincubated with I-block® (Tropix, Bedford, MA, USA), then with anti-DAZ2 and preimmune antiserum (1:200 dilution in I-block® buffer) for 1 h at room temperature.

After extensive washing with 0.1% Tween 20–PBS buffer (pH 7.4) the blots were incubated with horseradish peroxidase-conjugated anti-rabbit IgG (Dako Diagnostica GmbH, Hamburg, Germany) for 1 h at room temperature. Detection of antibody binding was performed by chemical luminescence (ECL-reagent protocol; Amersham).

Immunohistochemical analysis of testis tissue sections

Testis tissue sections fixed in Bouin's solution and embedded in paraffin wax were processed for immunohistochemistry using standard protocols. Preincubation with 3% H_2O_2 in PBS buffer [including 5% bovine serum albumin (BSA)] was used to block internal peroxidases. Tissue samples were then incubated with anti-DAZ2 (1:200 dilution in PBS/5% BSA), or with sulphydryl oxidase (SOx) antiserum (1:200 diluted in PBS/5% BSA) as a positive control, or with preimmune serum (1:200 diluted in PBS/5% BSA) as a negative control, for 1 h at room temperature. After several washing steps with 0.1% Tween 20 in PBS buffer, all slides were incubated with anti-rabbit-IgG-coupled horseradish peroxidase (Dako; 1:3000 v/v). Sections were washed in 0.1% Tween 20–PBS buffer; then stained with 0.2 mg/ml diaminobenzidine (DAB) dissolved in 50 mM sodium acetate pH 6.0 and 50 µl of 30% H_2O_2 and counterstained with Mayer's haematoxylin before embedding in Corbit® Balsam (Hecht Kiel, Hasee, Germany) for microscopic analysis.

Immunofluorescence experiments with spermatozoa

In these experiments we used spermatozoa from three groups of individuals: normozoospermic men with sperm numbers of 20–100 × 10^6 spermatozoa/ml ejaculate and presence of AZFc; oligozoospermic men with sperm numbers of 5–20×10^6 spermatozoa/ml ejaculate and presence of AZFc, and oligozoospermic men with sperm numbers of 1–5×10^6 spermatozoa/ml ejaculate and absence of AZFc (AZFc patient group). Concentrations of follicle stimulating hormone (FSH) were estimated to be in the normal range or elevated (in some oligozoospermic patients, with or without AZFc deletion). Luteinizing hormone (LH) and testosterone concentrations were estimated to be in the normal range in all individuals analysed, measured according to the WHO laboratory manual (World Health Organization, 1992).

Spermatozoa were washed with PBS buffer to remove seminal plasma, briefly centrifuged (300 g, 2×5 min) and spread on poly-L-lysin coated microscope slides. After freezing sperm slides (at –70°C) for at least 24 h they were slowly thawed and first incubated in PBS buffer (pH 7.4) with 3% BSA for 30 min. Incubation with anti-DAZ2 antiserum and preimmune serum (1:50 diluted in PBS) was performed for 1 h at room temperature. After extensive washing with PBS buffer, specific binding of DAZ2 antibodies was visualized by incubation with fluorescein isothiocyanate (FITC)-marked anti-rabbit-IgG (DAKO) as the second antibody (1 h at room temperature). After several washing steps in PBS buffer (3 × 5 min) spermatozoa were embedded with Perma Fluor aqueous mounting medium (Immunotech)

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022

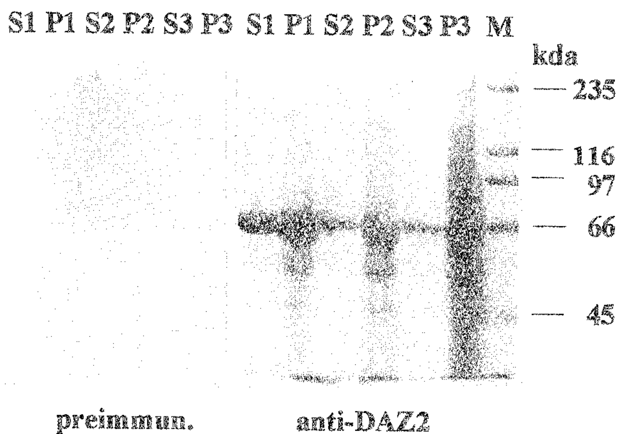

Figure 1. Western-blot of human testis proteins after incubation with the anti-DAZ2 serum respectively with the corresponding preimmune serum. The three different tissue homogenates were divided in a PBS-soluble fraction (S1–S3) and in a PBS-insoluble fraction (P1–P3) before gel loading, in order to analyse the presence of DAZ proteins in different testis tissue fractions. Molecular weights of marker proteins (M) are indicated on the right. Anti-DAZ2 bound mainly to a testis protein of 66 kDa present in both tissue fractions presumed to be the DAZ2 protein. Weaker cross-reactions to other DAZ proteins with homologous epitopes are visible in the P fractions (the high background in P3 is due to overloading). The preimmune serum was negative as expected.

and analysed by fluorescence microscopy (Zeiss fluorescence microscope, filter no. 9: exitation 490 nm, emission 520 nm).

Results

DAZ proteins are present in different testis tissue fractions

For analysis of DAZ proteins in human testis tissue the tissue homogenates were first subdivided into a cell fraction soluble in PBS buffer ('S'-fraction) and into a cell fraction insoluble in PBS buffer ('P'- fraction). The S-fraction was assumed to be rich in cytoplasmic proteins; the P-fraction was assumed to be mainly membranes and nuclear proteins. Immunostaining of protein extracts from both fractions on Western blots with anti-DAZ2 reveals a testis protein of ~66 kDa in all three testis samples (Figure 1), presumed to be the DAZ2 protein with 16 copies of the tandem repeat 24 amino acid domain resulting in a molecular weight of ~66 kDa. Weaker cross-reactions with a variable range of other testis proteins are visible in the P fractions of all testis tissue samples (in P3, the high background is due to overloading). As expected, their molecular weights were comparable with that of the other DAZ proteins with different copy numbers of the repetitive 24 amino acid domain supposed to range between 7–21 (Vogt *et al.*, 1997). Molecular weights of DAZ proteins should therefore be 41–80 kDa spaced in multiples of 2.7 kDa (the molecular weight of one 24 amino acid domain). Additional cross-reactions of anti-DAZ2 to homologous epitopes in other testis proteins present only in the P fractions can however not be excluded.

DAZ proteins are present in the cytoplasm of late spermatids

Testis tissue sections of different men with apparently normal spermatogenesis were incubated with anti-DAZ2 and anti-rabbit-IgG-coupled horseradish peroxidase in order to identify the location of DAZ proteins in the various testis cell types (germ cells, Sertoli cells, Leydig cells, interstitium) by immunohistochemical staining analysis. A significant staining pattern with anti-DAZ2 was detected only in the innermost layer of germ cells, i.e. in late spermatids (Figure 2). For analysis of reaction specificity, we analysed the same testis tissue sections with preimmune serum (negative control), and with an antiserum known to detect SOx (sulphydril oxidase) proteins (positive control). As we expected, staining with the preimmune serum was not above the experimental background (Figure 2C), and the SOx antiserum marked homologous proteins in spermatogonia and in spermatocytes (Kumari *et al.*, 1990; data not shown).

DAZ proteins are present in the tails of mature spermatozoa

Deletion of the whole *DAZ* gene family in AZFc patients is not only associated with the phenotype of azoospermia, but also with severe oligozoospermia (0.1–10 $\times$ 10^6 spermatozoa/ml ejaculate) and with subfertility (Reijo *et al.*, 1996; Vogt *et al.*, 1996). We therefore suggested that the primary mutation effect of a deletion of AZFc might be found postmeiotically and most likely in spermatozoa, and that DAZ deletions are compatible with the formation of motile spermatozoa (Vogt *et al.*, 1996). If this theory is proved correct, DAZ proteins might be present in mature spermatozoa. Their analyses were performed by immunofluorescence experiments with anti-DAZ2 and FITC-labelled secondary antibodies. For this purpose, we first obtained semen samples from men with normal sperm concentrations of 20–100 $\times$ 10^6 spermatozoa/ml and spread them on microscope slides. A strong fluorescent signal with anti-DAZ2 attached to FITC-conjugated anti-rabbit serum was observed consistently along the entire sperm tails (Figure 3A). In no case was a fluorescent sperm tail staining observed with the preimmune serum (Figure 3B). The immunofluorescence tail signal did not depend on the men's sperm count. Men with idiopathic oligozoospermia (5–20 $\times$ 10^6 spermatozoa/ml) displayed the same immunofluorescence reaction of anti-DAZ2 on their sperm tails as shown in Figure 3A for men with a normal sperm count. However, no immunofluorescence signal could be observed in oligozoospermic patients with deletion of AZFc (i.e. deletion of the whole *DAZ* gene family). An overview of the results of our immunofluorescence experiments is given in Table I. Absence of the strong immunofluorescence signal on sperm tails was consistent with deletion of the *DAZ* genes in AZFc, suggesting a link between the two events.

The presence of DAZ proteins in sperm tails was also analysed by protein extraction from a pure tail preparation and Western blotting with anti-DAZ2. For this purpose, sperm samples of two men with a normal sperm count were treated by sonication for tail separation and the sperm tails isolated in the interphase of an isotonic Percoll gradient (for details see Materials and methods section). Western blots of protein extracts prepared from both tail samples and incubated with anti-DAZ2 revealed a cross-hybridizing tail protein with a molecular weight of 61 kDa (Figure 4). This is similar to that of the main band observed on immunoblots of the total testis

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022

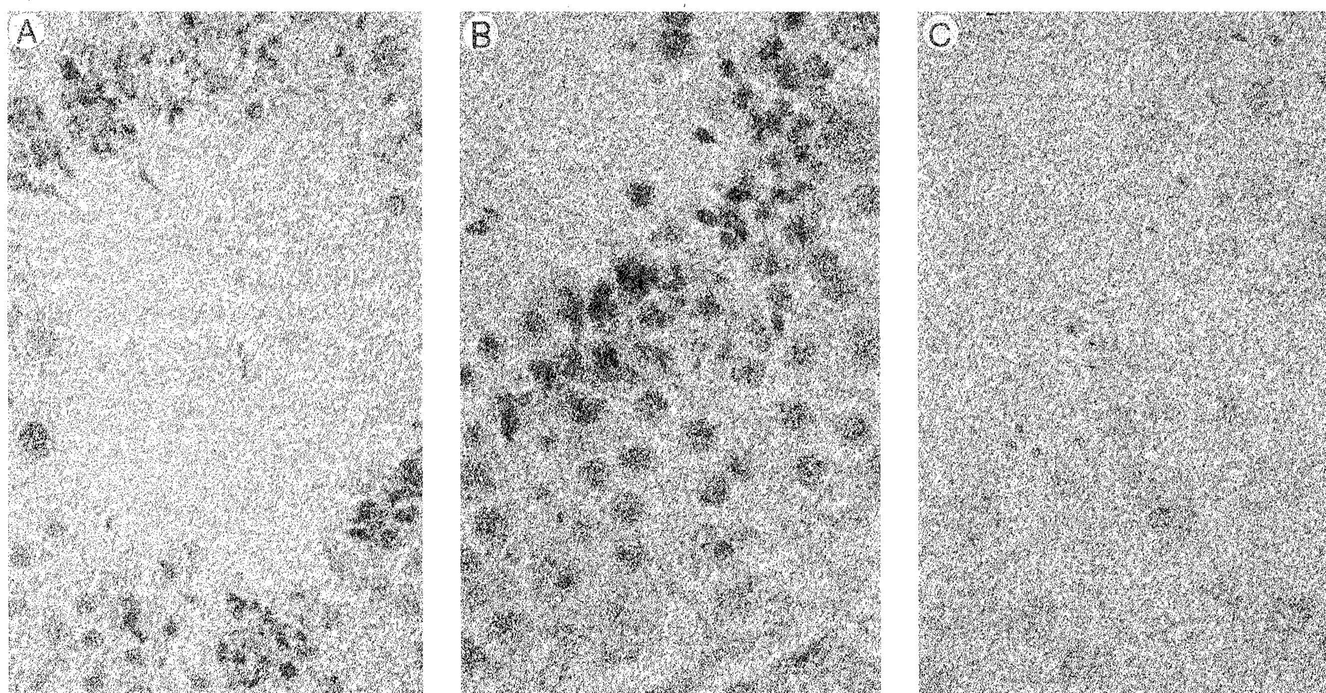

Figure 2. Immunostaining pattern of human testis tissue sections with anti-DAZ2 serum after incubation with anti-rabbit-immunoglobulin (Ig)G-coupled horseradish-peroxidase (1:3000 v/v). Sections were stained with diaminobenzidine (DAB), H_2O_2 and counterstained with Mayer's haematoxylin before embedding in Corbit® Balsam. Significant staining of spermatids located at the innermost layer of the germ cell epithelium is clearly visible in each tissue section: (**A**) original magnification ×400; (**B**) original magnification ×630; prepared from different testis tissues. (**C**) Immunostaining of testis tissue sections with the preimmune serum did not show any significant staining pattern (original magnification ×630).

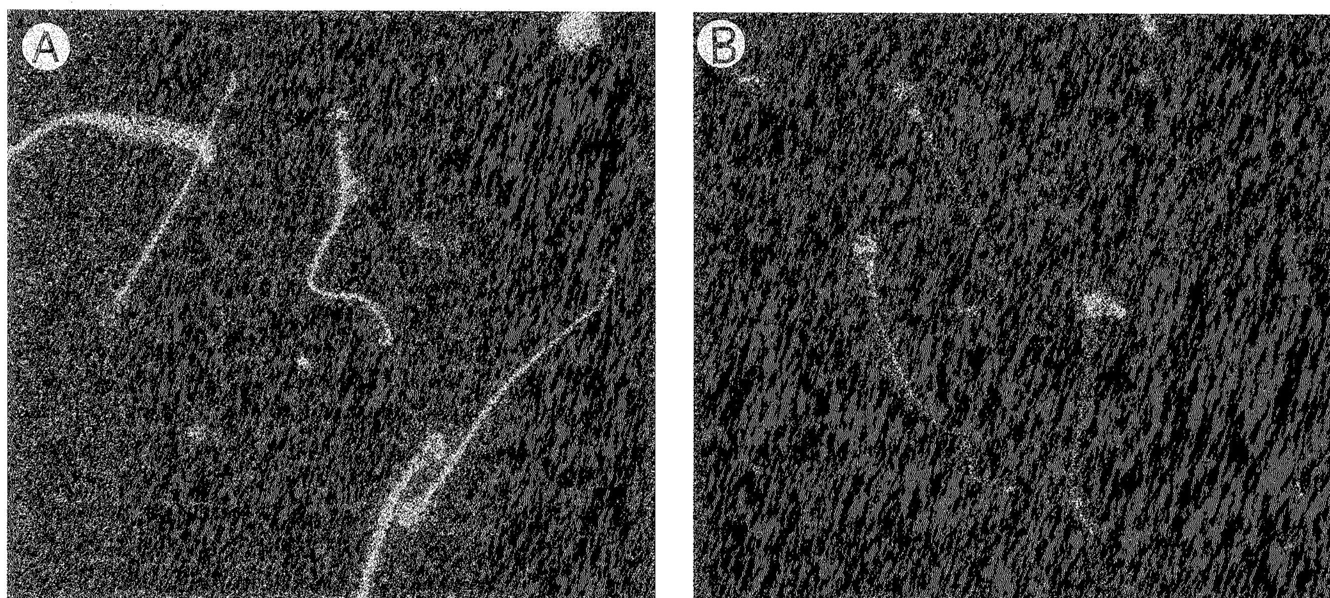

Figure 3. Immunofluorescence experiment with anti-DAZ2 antiserum (**A**) and the corresponding preimmune serum (**B**) incubated on spreads of spermatozoa from a man with a normal sperm count. After incubation with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit serum as the second antibody, a strong fluorescent signal on sperm tails is consistently observed only in (**A**). Occasionally, artificial cross-hybridization occurred to the sperm heads in both experiments.

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022

Table I. Immunofluorescence sperm analysis with anti-DAZ2

Patient groups	No. individuals	AZFc	No. fluorescent sperm tails
Normozoospermia	17	+	16
Oligozoospermia	5	+	4
AZFc patients	3	–	0

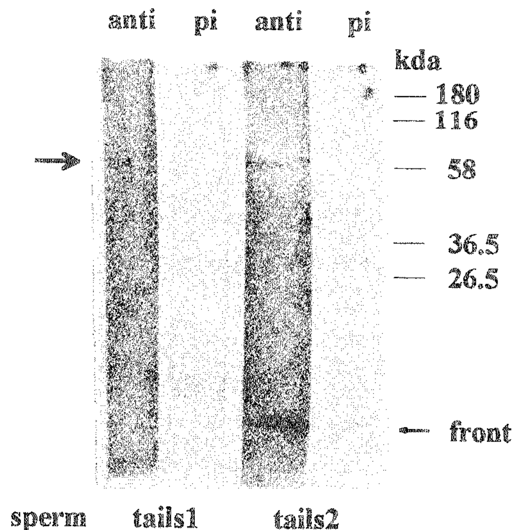

Figure 4. Western blot of sperm tail proteins after incubation with the anti-DAZ2 antiserum (anti) and with the corresponding preimmune serum (pi) respectively. We analysed the protein extracts of two different tail preparations (tails 1 and tails 2). Molecular weights of marker proteins are indicated at the right. Anti-DAZ2 cross-hybridized to a tail protein of ~61 kDa (arrow) in both sperm tail preparations. Occasionally, artificial cross-hybridization is observed to the front of the separated tail proteins (e.g. tails 2 preparation).

protein fractions in Figure 1 (66 kDa); thus confirming the presence of DAZ proteins in sperm tails.

Discussion

DAZ was first thought to be a single copy gene with high homology to the mouse gene *Dazl1* (formerly called *Dazla*; Cooke *et al.*, 1996) and the *Drosophila* gene *Boule* (Eberhart *et al.*, 1996). This suggested that the function of *DAZ* is evolutionarily highly conserved and similar in human spermatogenesis to that of *Boule* in *Drosophila* spermatogenesis and that of *Dazl1* in mouse spermatogenesis. However, this hypothesis must be re-evaluated. *DAZ* is now known to be a member of a multicopy gene family and is designated as *DAZ1* (Vogt *et al.*, 1997). This gene family has evolved only recently on the Y chromosome during primate evolution. It seems to have originated from an autosomal homologue on the short arm of chromosome 3 (*DAZL1*; Saxena *et al.*, 1996; Shan *et al.*, 1996; Yen *et al.*, 1996; Seboun *et al.*, 1997). The speculations about a different function of DAZ proteins in human spermatogenesis and Dazl1 proteins in mouse spermatogenesis were initially based only on structural sequence analysis (Shan *et al.*, 1996). They seemed to be confirmed now by analysis of the location of Dazl1 proteins in mouse testis tissue (Ruggiu *et al.*, 1997) and by analysis of the location of DAZ proteins in human testis tissue and in spermatozoa. Dazl1 proteins were found mainly in the cytoplasm of pachytene spermatocytes as well as in B spermatogonia, and in leptotene and zygotene spermatocytes, but not in post-meiotic mouse germ cells (Ruggiu *et al.*, 1997). DAZ proteins, however, were found mainly in late spermatids (Figure 2) and in the tails of mature spermatozoa (Figures 3 and 4). No protein detected with anti-DAZ2 was observed in premeiotic germ cells. This result suggests that DAZ proteins are present predominantly in post-meiotic germ cells. In this context it is interesting to note that recent hybridization experiments with a DAZ1 riboprobe on testis tissue sections had labelled mainly spermatogonia but not spermatids (Menke *et al.*, 1997). Together with our results, this suggests that DAZ transcripts in spermatogonia may not be directly translated but are complexed to a non-translatable ribonucleoprotein (RNP) fraction until the germ cells have passed meiosis. During gametogenesis, such translational regulation events are exceptionally prominent (Hecht, 1995).

On the other hand, Menke *et al.* (1997) used a riboprobe containing the *DAZ1* RRM domain. Hybridization with this probe does not distinguish transcripts of *DAZ* and transcripts of the autosomal *DAZL1* gene because their RRM domains are homologous (94% similarity). We performed similar experiments, but with a riboprobe prepared from the repetitive transcript part of *DAZ2* not present in *DAZL1*. This riboprobe marked only spermatocytes and early spermatids in testis tissue sections (Vogt, 1996). Although we cannot exclude a different transcription phase of *DAZ1* and *DAZ2* in human spermatogenesis, it is more likely that the Menke probe mainly identified transcripts of *DAZL1* in spermatogonia (which would also coincide to the Dazl1 protein results of Ruggiu *et al.*, 1997) and that *DAZ* transcripts occur later in the human male germ line.

Our imunoblotting experiments with whole testis protein extracts suggest that DAZ proteins are present in the cytoplasm but more abundantly in the nuclear and membrane protein fraction (Figure 1). However, this conclusion might be wrong and the results may simply reflect the inability of our differential extraction procedure to produce a cytoplasmic germ cell fraction from late spermatids and spermatozoa where most of the DAZ proteins are located.

DAZ proteins are involved in the RNA metabolism of late spermatids

The highly conserved *RRM* domain in DAZ proteins is most likely to be functional in RNA binding (Burd and Dreyfuss, 1994). So it is reasonable to assume that DAZ proteins are able to bind to mRNA in late spermatids. Their binding specificity might be influenced by their C-terminal domains and variable numbers of the 24 amino acid domain which are not present in the homologous autosomal DAZL1 protein.

The spermatid cell is polarized towards formation of the highly differentiated spermatozoon. The need for the transport of mRNAs within developing spermatids is therefore potentially very great. They are expected to be stored as mRNPs until their protein products are needed in a specific localization

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022

of the developing spermatozoon (Fulton *et al.*, 1980). Proper localization of mRNA to microtubules or other elements of the cytoskeleton mediated by RNA binding proteins is known in mouse spermatogenesis (Schumacher *et al.*, 1995) and in *Xenopus* and in *Drosophila* oogenesis (Yisraeli *et al.*, 1990; Pokrywka and Stephenson, 1991). All of these RNA binding proteins belong to the class of RRM proteins such as the DAZ proteins. We therefore assume that DAZ proteins also take part in such late translational control events.

DAZ proteins are involved in the structure of sperm tails

DAZ proteins seem to be not only present in late spermatids but also in the tails of mature spermatozoa. The molecular weight of the tail protein cross-reacting to anti-DAZ2 is comparable with that of the main cross-reacting protein in total testis protein extracts (Figure 1). We cannot exclude the possibility that anti-DAZ2 has cross-reacted to another sperm tail protein of 61 kDa containing *DAZ2* homologous epitopes. However, deletion of the anti-DAZ2 immunofluorescence in sperm tails seems to be related to deletion of the *DAZ* gene family in Yq11 (see AZFc patient group in Table I). Therefore, the cross-reacting 61 kDa tail protein is most likely related to the DAZ2 protein and the presence of at least the DAZ2 protein in sperm tails is expected. We concede that the number of sperm samples analysed from AZFc patients is small. However, AZFc patients with sperm numbers sufficient for immunofluorescence experiments ($>1\times10^6$ spermatozoa) are rare. Most of them are azoospermic or have only a low sperm count (Reijo *et al.*, 1996; Vogt *et al.*, 1996). It has been argued that AZFc microdeletions are compatible with the formation of normal spermatozoa (Kent-First *et al.*, 1996), but a reduction of their numbers (most likely age-dependent) is consistently observed in this patient group (Vogt *et al.*, 1996). If this holds true, deletion of all *DAZ* genes in AZFc patients and the consequent loss of all DAZ proteins in the sperm tail might not interfere with human sperm maturation but induce a degenerative effect on mature spermatozoa. This would imply a low selection pressure on the *DAZ* genes and might explain why the incidence of AZFc deletions in men with nonobstructive azoospermia is high, although most of these cases seem to have an intact Y chromosome (Vereb *et al.*, 1997).

Translational activities in sperm tails have not yet been reported, and seem to be unlikely because of the highly differentiated cell structure. The presence of DAZ proteins in sperm tails might therefore hint at a structural function for these proteins in motile spermatozoa. This may be to act as a scaffold protein, such as the Spnr RNA binding proteins for the subcellular localization of other flagellum proteins in the mouse germ line (Schumacher *et al.*, 1995), or to act as an architectural component of the tail structure itself. Further analysis of the location of DAZ proteins in the human sperm tail structure by electron microscopy will help to distinguish these possibilities in more detail.

Acknowledgements

We thank our colleagues I.Dremer, S.Pomer, W.Schmidthal and U.Kühne for sharing different samples of human testis tissue with us, isolated in their clinics for medical reasons. P.Hirschmann is thanked for technical assistance and D.Littauer for providing laboratory space and technical support in some immuno-staining experiments. R.A.Pfeiffer and U.Mau are thanked for cytogenetic analysis and counselling of AZFc patients. W.Krause is thanked for critically reading the manuscript. We are indebted to A.Hider and M.Follo for carefully improving English grammar and expression of this manuscript. A.Wiegenstein is thanked for excellent photographic assistance especially with preparing fluorescent reproductions of the original microscope slides of spermatozoa and testis tissue sections. This study was supported by a grant of the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF) to P.H.Vogt (01 KY9507/4, Göttinger Forschungsverbund).

References

Ausubel, F.M., Brent, R., Kingston, R.E. *et al.* (1995) Analysis of proteins. In *Current Protocols in Molecular Biology.* Vol. 2. John Wiley and Sons, New York, Chapter 10, pp. 1–28.

Altschul, S.F., Gish, W., Miller, W. *et al.* (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.

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

Cooke, H.J., Lee, M., Kerr, S. *et al.* (1996) A murine homologue of the human *DAZ* gene is autosomal and expressed only in male and female gonads. *Hum. Mol. Genet.*, **5**, 513–516.

Eberhart, C.G., Maines, J.Z. and Wasserman, S.A. (1996) Meiotic cell cycle requirement for a fly homologue of human deleted in azoospermia. *Nature*, **381**, 783–785.

Fulton, A.B., Wan, K.M. and Penman, S. (1980) The spatial distribution of polyribosomes in 3T3 cells and the associated assembly of proteins into the skeletal framework. *Cell*, **20**, 849–857.

Hargreave, T.B. (1990) Introduction. In Hargreave, T.B. and Soom, T.E. (eds), *Management of Male Infertility.* PG Publishing, Singapore, pp. 1–21.

Hecht, N.B. (1995) The making of a spermatozoon: a molecular perspective. *Dev. Genet.*, **16**, 95–103.

Henkel, R., Stalf, T. and Miska, W. (1992) Isolation and partial characterization of the outer dense fiber proteins from human spermatozoa. *Biol. Chem. Hoppe-Seyler*, **373**, 685–689.

Kent-First, M.G., Kol, S., Mualiem, A. *et al.* (1996) The incidence and possible relevance of Y-linked microdeletions. *Mol. Hum. Reprod.*, **2**, 943–950.

Kumari, M., Aumüller, G., Bermann M. *et al.* (1990) Stage-dependent appearance of sulfhydryl oxidase during spermatogenesis in the testis of rat and hamster. *Histochemistry*, **94**, 365–371.

Menke, D.B., Mutter, G.L. and Page, D.C. (1997) Expression of *DAZ*, an azoospermia factor candidate, in human spermatogenesis. *Am. J. Hum. Genet.*, **60**, 237–241.

Ord, T., Patrizio, P., Marello, E. *et al.* (1990) Mini-Percoll: a new method of semen preparation for IVF in severe male factor infertility. *Hum. Reprod.*, **5**, 987–989.

Pokrywka, N.J. and Stephenson, E.C. (1991) Microtubules mediate the localization of bicoid RNA during *Drosophila* oogenesis. *Development*, **113**, 55–66.

Reijo, R., Lee, T.-Y., Salo P. *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 oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*, **347**, 1290–1293.

Ruggiu, M., Speed, R., Taggart, M. *et al.* (1997) The mouse *Dazla* gene encodes a cytoplasmic protein essential for sperm and egg production. *Nature*, **389**, 73–76.

Saxena, R., Brown, L.G., Hawkins, T. *et al.* (1996) The *DAZ* gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nature Genet.*, **14**, 292–299.

Schumacher, J.M., Keesook, L., Edelhoff, S. and Braun, R.E. (1995) Spnr, a murine RNA-binding protein that is localized to cytoplasmic microtubules. *J. Cell Biol.*, **129**, 1023–1032.

Seboun, E., Barbaux, S., Bourgeron, T. *et al.* (1997) Gene sequence, localization and evolutionary conservation of *DAZLA*, a candidate male sterility gene. *Genomics*, **41**, 227–235.

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022

Shan, Z., Hirschmann, P., Seebacher, T. *et al.* (1996) 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.*, **5**, 2005–2011.

Vereb, M., Agulnik, A.I., Houston, J.T. *et al.* (1997) Absence of *DAZ* gene mutations in cases of non-obstructed azoospermia. *Mol. Hum. Reprod.*, **3**, 55–59.

Vogt, P.H., Edelmann, A., Kirsch S. *et al.* (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum. Mol. Genet.*, **5**, 933–943.

Vogt, P.H., Affara, N., Davey, P. *et al.* (1997) Report of the third international workshop on Y chromosome mapping. *Cytogenet. Cell Genet.*, **79**, in press.

World Health Organization (1992) *Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction.* 3rd edn. Cambridge University Press, Cambridge, UK, pp. 51–52.

Yen, P.H., Chai, N.N. and Salido, E.C. (1996) The human autosomal gene *DAZLA*: testis specificity and a candidate for male infertility. *Hum. Mol. Genet.*, **5**, 2013–2017.

Yisraeli, J.K., Sokol, S. and Melton, D.A. (1990) A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. *Development*, **108**, 289–298.

Received on August 27, 1997; accepted on December 2, 1997

Downloaded from https://academic.oup.com/humrep/article/13/2/363/875930 by guest on 16 August 2022