Three novel spermatogenesis-specific zinc finger genes

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Abstract **We have cloned and characterized the expression, during spermatogenesis, of three novel zinc finger genes (*Zfp94*, *Zfp95*, *Zfp96*). Analysis of the deduced protein sequences reveals that all three molecules belong to the LeR family (leucine-rich zinc fingers) and that ZFP95 contains a domain homologous to the Krüppel-associated box. All three genes were found expressed at high levels in testis among other tissues, but testis-specific transcripts were observed for *Zfp95* and *Zfp96*. Northern blot analyses of the testis-specific transcripts of *Zfp95* and *Zfp96* were performed using whole testis RNA as well as RNA isolated from enriched populations of specific spermatogenic cell types. The testis-specific transcript of *Zfp95* showed the highest expression in pachytene spermatocytes, while that of *Zfp96* was highly expressed in pachytene spermatocytes, in round spermatids and residual bodies. Northern blot analysis of RNA from the testis of mice carrying the *atrichosis* mutation further validated these expression patterns. In particular, the testis-specific transcripts of *Zfp95* and *Zfp96* were greatly reduced in heterozygous, and completely absent in homozygous testis RNA from *atrichosis* mutant mice, further defining the germ cell specificity of these transcripts.**

Key words: Transcription factor; Germ cell; Testis-specific gene expression

1. Introduction

The testis can be morphologically subdivided into two compartments, the seminiferous tubules and the interstitial space. Germ cells reside within the tubules where they progress through several well-defined stages of development in close contact with Sertoli cells [1]. The process of spermatogenesis is generally divided into three different phases: (a) a proliferative phase characterized by spermatogonia undergoing rapid mitotic divisions, (b) a meiotic phase in which spermatocytes recombine and segregate the genetic material, and (c) the differentiation or spermiogenic phase in which spermatids transform into sperm. The constant and asynchronous nature of spermatogenesis involves a series of cell–cell interactions between the different somatic cell types in the testis and the germ cells. These interactions are often cyclical and can be categorized into stages as the germ cells progress through the morphologically well defined steps of development [1,2]. Each stage (there are 14 in the rat and 12 in the mouse) is characterized by a unique complement of germ cell types at various stages of development. With the passage of time, any given stage will progress to the next stage as the germ cell complement matures [3].

The crucial interaction of germ cells with the testicular somatic cells is exemplified by the difficulty of maintaining germ cells in culture for prolonged periods of time [4]. Hofmann and colleagues have established several murine testicular cell lines, including the spermatogonial-like cell line GC-1spg, by transformation with the SV40 large T antigen [5,6]. The immortalized cell lines GC-2spd(ts) and GC-3spc(ts) were produced by co-transfection of the gene encoding SV40 large T antigen and a temperature-sensitive (ts) mutant of p53. The binding of the active form of p53 at lower (i.e. permissive) cultivation temperatures induces the cells to differentiate along the spermatogenic pathway [6]. These cell lines seemed to represent a particularly useful system to identify molecules with differential expression pattern during spermatogenesis.

2. Materials and methods

2.1. Differential display-reverse transcriptase polymerase chain reaction (DDRT-PCR)

RNA was isolated from GC-2spd(ts) cells and a cDNA library was constructed using λgt11 arms (Stratagene) and packaged using the Giga Pack Gold kit (Stratagene) according to the manufacturer's instructions. The described 3′ primers T12MA, T12MC, T12MG and T12MT [7] were used in the RT as well as in the following PCR amplification step. The following arbitrary 5′ primers were employed in the PCR reactions (all sequences 5′-3′): ARB1: GCG GAC ACA C; ARB2: CCA CCT TCG A; ARB3: GAG AAG ATC T; ARB4: GGT CAG AAG A; ARB5: AAG TCT TGG G; ARB6: TAC AAC GAG G; ARB7: TGG ATT GGT C; ARB8: CTT TCT ACC C; ARB9: TTT TGG CTC C; ARB10: GGA ACC AAT C. Primers ARB1–ARB5 were chosen arbitrarily, primers ARB6–ARB10 were derived from the sequences suggested by Bauer et al. [8]. Purified total RNA (1–0.1 μg) was reversed transcribed and subsequently amplified as described [7]. A programmable heat block (MJ Research, Watertown, MA, USA) was used with these parameters: denaturing at 96°C, 1 s, annealing at 42°C, 1 s, elongation at 72°C, 1 s for 40 cycles with an additional elongation step for 5 min at 72°C. PCR products were separated on a 6% denaturing polyacrylamide sequencing gel. Evaluation of differentially expressed fragments was done after overnight autoradiography of the dried gels. These cDNA fragments were

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excised from the dry gels and incubated at 98°C for 10 min in 100 μl TE buffer. Five microliter of the eluate was used in the subsequent reamplification of the fragments under the following conditions: 20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.4 mM $MgCl_2$, 0.1 mM of each nucleotide, 2 μM of each primer and 3.5 U Taq polymerase (Boehringer Mannheim). The cycling parameters of the PCR reaction were as for the differential display with a higher annealing temperature of 55°C after one cycle of annealing at 42°C.

2.2. Animals

Mice of the strains BALB/c and C57BL/6J were obtained from the in-house animal facility. Additionally, mice homozygous and hetero-

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   1  GAATTCCGTTGCTGTTGGAGAAGACCGGGTTCGCGTGTGTGCTGTGTCCGTAGTGGCCTTTTCTGGGCCTCGTTTTTGGAAG   82
  83  AGGGTAGCACTCCGCCTCGGTTTCAGAAAAGGTCCGTGAAGTCATTGGGCTTTGCTCTTCTAGAAAACAAGCTCGACCTGGAC  165
 166  GGTCCAGAGGCTCCA ATG GCT GCA GGC TCT GGG GTC GTC CCG CCA CCT CTC GGT GCT GGG CTT TGT  231
   1                  M   A   A   G   S   G   V   V   P   P   P   L   G   A   G   L   C   17
 232  ACG GTG AAG GTG GAG GAG GAC TCT CCG GGG AAT CAG GAG TCC TCC GGT TCC GGA GAT TGG CAG  294
  18   T   V   K   V   E   E   D   S   P   G   N   Q   E   S   S   G   S   G   D   W   Q   38
 295  AAC CCC GAA ACT TCT CGA AAA CAA TTC AGG CAG TTG CGC TAC CAG GAA GTG GCC GGC CCT GAG  357
  39   N   P   E   T   S   R   K   Q   F   R   Q   L   R   Y   Q   E   V   A   G   P   E   59
 358  GAG GCG CTG AGC CGA CTT TGG GAG CTC TGT CGG AGG TGG CTG AGG CCC GAG CTG CTC TCC AAG  420
  60   E   A   L   S   R   L   W   E   L   C   R   R   W   L   R   P   E   L   L   S   K   80
 421  GAG CAG ATC ATG GAG CTG TTG GTG CTG GAG CAA TTC CTC ACC ATC CTG CCC CAG GAG CTC CAG  483
  81   E   Q   I   M   E   L   L   V   L   E   Q   F   L   T   I   L   P   Q   E   L   Q  101
 484  GCC TAC GTG CGG GAC CAC AGC CCT GAA AGT AGG GAG GAG GCT GCG GCT TGG CGC ACT CTG CAA  546
 102   A   Y   V   R   D   H   S   P   E   S   R   E   E   A   A   A   W   R   T   L   Q  122
 547  AGA GCA CTG GAT CGG GCC TCT CCG CAG GGT TTT ATG ACC TTC AAG GAT GTG GCT GAA TCT CTG  609
 123   R   A   L   D   R   A   S   P   Q   G   F   M   T   F   K   D   V   A   E   S   L  143
 610  ACC TGG GAA GAG TGG GAA CAG CTG GCT GCA GCT CGG AAA GGT TTC TGT GAA GAG AGC ACA AAG  672
 144   T   W   E   E   W   E   Q   L   A   A   A   R   K   G   F   C   E   E   S   T   K  164
 673  GAC GCT GGG AGC ACA GTT GTG CCT GGC TTG GAA ACA CGA ACT GTG AAC ACA GAT GTG ATT CTA  735
 165   D   A   G   S   T   V   V   P   G   L   E   T   R   T   V   N   T   D   V   I   L  185
 736  AAG CAG GAA ATT TTA AAG GAG CAG AGC CAC AGG TCA TGT CTA CAA GAA GTA TCC CAG GGA ATG  798
 186   K   Q   E   I   L   K   E   Q   S   H   R   S   C   L   Q   E   V   S   Q   G   M  206
 799  GTC CCA GCA CTT ACC AAA TGT GGT GAC CCC TCT GAG GAC TGG GAA GAA AAG CTG CCA AAA GCT  861
 207   V   P   A   L   T   K   C   G   D   P   S   E   D   W   E   E   K   L   P   K   A  227
 862  GCT GTA CTG CTG CAG CTC CAG GGT TCT GAA GAG CAA GGA CGC ACT GCC ATC CCG CTT CTC ATC  924
 228   A   V   L   L   Q   L   Q   G   S   E   E   Q   G   R   T   A   I   P   L   L   I  248
 925  GGT GTG TCC AGA GAG GAA AGG GAC TCT AAA AAC AAT GAG TCT GAG AAC AGT GGC AGT TCT GTC  987
 249   G   V   S   R   E   E   R   D   S   K   N   N   E   S   E   N   S   G   S   S   V  269
 988  CTT GGT CAG CAC ATC CAG ACA GCA GAA GGA CTG GGC ACC AAC AGT CAG TGT GGG GAT GAC CAC 1050
 270   L   G   Q   H   I   Q   T   A   E   G   L   G   T   N   S   Q   C   G   D   D   H  290
1051  AAA CAA GGT TTC CAT GTG AAA TGC CAT TCA GTG AAG CCC CAC AGC TCT GTG GAC AGT GCT GTA 1113
 291   K   Q   G   F   H   V   K   C   H   S   V   K   P   H   S   S   V   D   S   A   V  311
1114  GGG CTT CTT GAG ACC CAG AGG CAG TTC CAG GAA GAC AAA CCT TAC AAA TGT GAT AGC TGT GAG 1176
 312   G   L   L   E   T   Q   R   Q   F   Q   E   D   K   P   Y   K   C   D   S   C   E  332
1177  AAG GGC TTC AGA CAG CGC TCG GAC CTC TTC AAA CAC CAG AGA ATC CAC ACA GGT GAG AAG CCC 1239
 333   K   G   F   R   Q   R   S   D   L   F   K   H   Q   R   I   H   T   G   E   K   P  353
1240  TAT CAG TGC CAA GAG TGT GGG AAA CGC TTT AGT CAG AGC GCT GCC CTC GTT AAG CAC CAG CGG 1302
 354   Y   Q   C   Q   E   C   G   K   R   F   S   Q   S   A   A   L   V   K   H   Q   R  374
1303  ACA CAC ACA GGT GAG AAG CCA TAT GCA TGC CCA GAA TGC GGG GAG TGC TTC AGG CAG AGC TCA 1365
 375   T   H   T   G   E   K   P   Y   A   C   P   E   C   G   E   C   F   R   Q   S   S  395
1366  CAT CTG AGT CGG CAT CAG AGA ACC CAT GCC AGT GAG AAG TAC TAT AAA TGT GAG GAG TGT GGG 1428
 396   H   L   S   R   H   Q   R   T   H   A   S   E   K   Y   Y   K   C   E   E   C   G  416
1429  GAG ATC GTT CAT GTT TCC AGC CTG TTC AGA CAT CAG AGA CTA CAC AGA GGG GAG AGA CCA TAC 1491
 417   E   I   V   H   V   S   S   L   F   R   H   Q   R   L   H   R   G   E   R   P   Y  437
1492  AAG TGT GGA GAC TGT GAG AAG AGC TTC AGA CAG CGC TCA GAC CTC TTC AAG CAC CAG AGA ACC 1554
 438   K   C   G   D   C   E   K   S   F   R   Q   R   S   D   L   F   K   H   Q   R   T  458
1555  CAC ACA GGA GAG AAG CCC TAT GCG TGT GTC GTC TGC GGG AGA AGA TTC AGT CAG AGC GCC ACC 1617
 459   H   T   G   E   K   P   Y   A   C   V   V   C   G   R   R   F   S   Q   S   A   T  479
1618  CTC ATT AAA CAC CAG AGA ACT CAC ACA GGA GAA AAA CCG TAT AAA TGT TTT CAA TGT GGT GAA 1680
 480   L   I   K   H   Q   R   T   H   T   G   E   K   P   Y   K   C   F   Q   C   G   E  500
1681  AGA TTT AGA CAG AGT ACA CAC CTT GTC CGA CAC CAA AGA ATC CAT CAA AAT TCA GTC TCC TAG 1743
 501   R   F   R   Q   S   T   H   L   V   R   H   Q   R   I   H   Q   N   S   V   S   *  520
1744  CTTCCATGTGTCTCTGCGTGACTCACGTGGCTTTTGTTGTTGCTACTGTTGAGAAAAGGTCTCACTGTGTAGCCCTGGCTATC 1826
1827  TGGCAGTAGCTATGTGGACCAGGCTGGCCACAAACTCAAGAGGTCATCCTGCCTCTGGCTTTACCTCCTGTATGTTGAGATTA 1909
1910  AAGGTGCCACCACCTTCCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAACAAAAAAAAAAAACCGTTGCTGTCGCGACAGCAACGG 1992
1993  AATTC                                                                              1997
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Fig. 1. The complete cDNA sequences and derived amino acid sequences (shown in one letter code) for (A) *Zfp94*, (B) *Zfp95* and (C) *Zfp96*. The following features are highlighted for *Zfp94*: bp 349–534 (aa 57–118) the LeR domain in bold face; the locations of the zinc finger domains are shown by underlined and bold Cys residues at the positions bp 1162 (aa 328), 1246 (356), 1330 (384), 1414 (412), 1495 (439), 1579 (467) and 1663 (495); the region with similarity to the B1/Alu sequence (bp 1797–1855) is underlined and the putative poly-adenylation signal at position 1906 is shown in bold face. The following features are highlighted for *Zfp95*: bp 217–438 (aa 57–118) the LeR domain in bold face; a region with homology to KRAB-A zinc finger domains (bp 718–804) is underlined and the locations of the zinc finger domains are shown by underlined and bold Cys residues at the positions bp 1087 (aa 343), 1171 (371), 1255 (399), 1339 (427), 1684 (542), 1771 (570), 1852 (598), 1936 (626), 2020 (654), 2188 (710), 2356 (766) and 2440 (794). The following features are highlighted for *Zfp96*: the poly(dG) stretch (bp 45–69) introduced by the 5′-RACE method is underlined; the LeR domain (bp 607–798, aa 59–122) is shown in bold face; the locations of the zinc finger domains are shown by underlined and bold Cys residues at the positions bp 1243 (aa 271), 1327 (299), 1411 (327), 1495 (355), 1579 (383), 1663 (411) and 1801 (457); the putative poly-adenylation signal is shown in bold at position 2233.

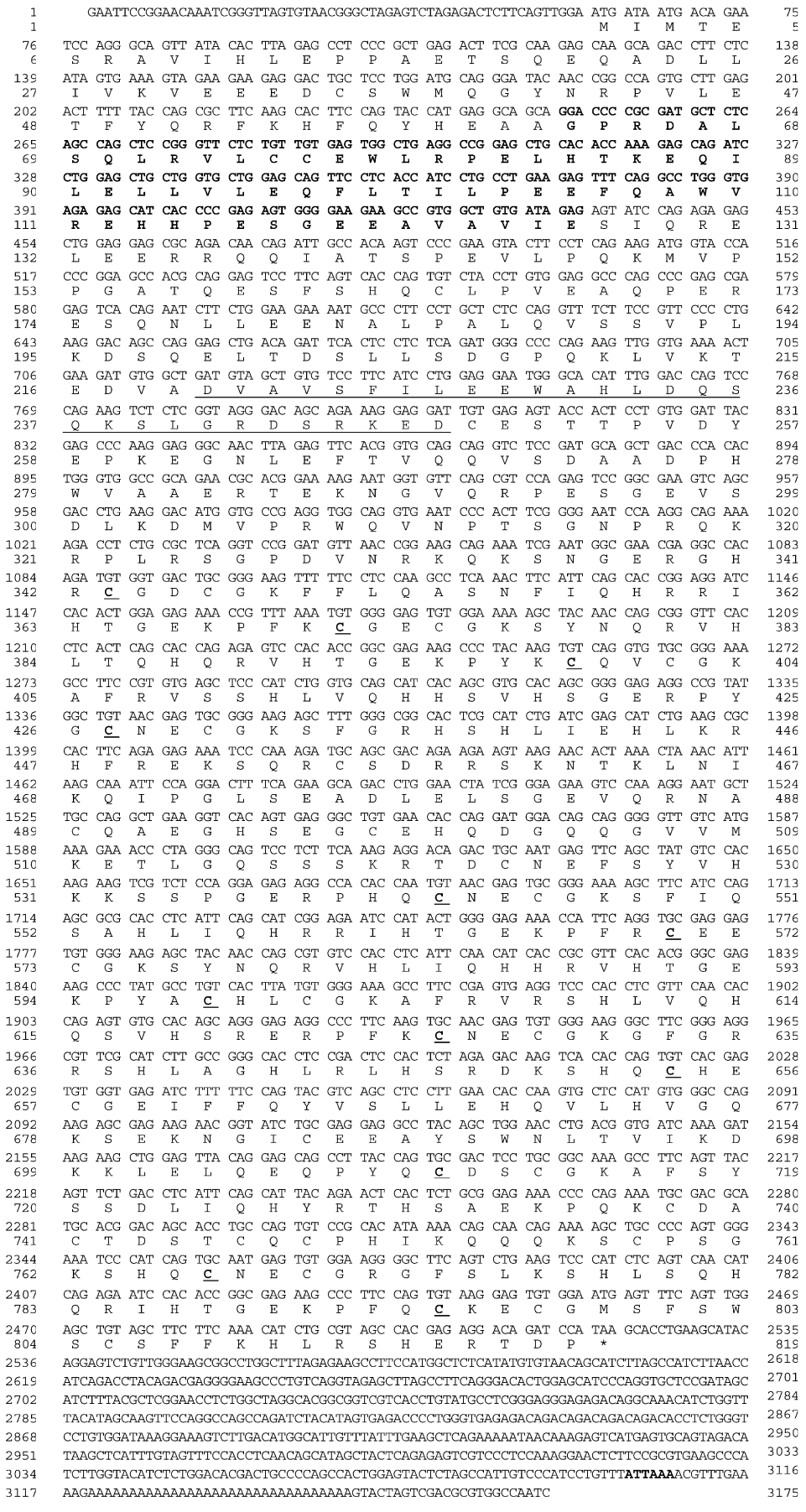

Fig. 1 (*Continued*).

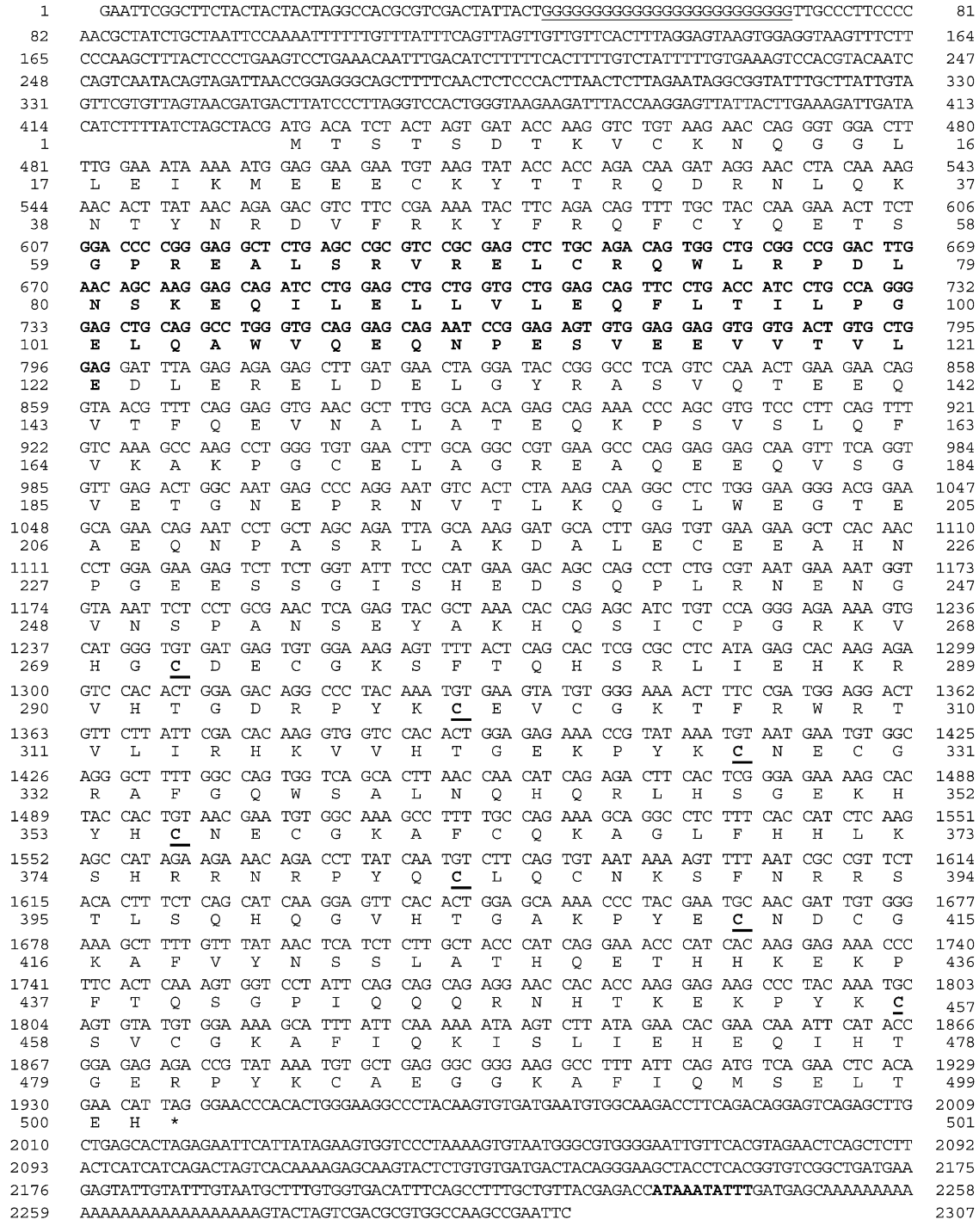

Fig. 1 (*Continued*).

zygous for the mutation *atrichosis* (*at*) [9] were purchased from the Jackson Laboratories (Bar Harbor, ME, USA).

2.3. Germ cell fractionation

Highly enriched populations of specific spermatogenic cell types were prepared by unit gravity sedimentation through a 2–4% bovine serum albumin (StaPut) gradient as described [10]. Populations of primitive type A spermatogonia (purity ≥85%) and somatic Sertoli cells (purity ≥85%) were recovered from testes of male CD-1 mice at 6 days post partum (dpp). A combined population of type A and type B spermatogonia (purity ≥85%) was recovered from CD-1 mouse testes at 8 dpp. Separate populations of pachytene spermatocytes, round spermatids, and residual cytoplasmic bodies (purity of each ≥95%) were recovered from testes of adult (>60 dpp) male CD-1 mice. Purities of each cell population were determined on the basis of cellular morphology examined under phase contrast optics.

2.4. Expression studies

Expression was studied by Northern analysis of tissues and isolated cell populations. Also, expression in the mouse testis was examined via in situ hybridization as described [11].

3. Results and discussion

We used DDRT-PCR to identify and clone cDNA ex-

pressed differentially in the spermatogenic cell lines under permissive and non-permissive conditions. We extracted RNAs from the GC-1spg cell line at 37°C and from GC-2spd(ts) after cultivation at 32°C and 37°C [6]. Out of approximately 100 differentially displayed PCR bands, 30 fragments were sequenced and the information used to search the non-redundant part of the GenBank database with the BLAST algorithm [12]. The fragment designated 2A1.32 was isolated as a differentially displayed band present in GC-2spd(ts) cells grown at 32°C but not at 37°C or in GC-1spg. This fragment was found to be homologous to many C2H2 Krüppel-like zinc finger proteins [13]. Remarkably, the four most highly homologous murine zinc finger proteins available in GenBank are expressed differentially in testis and thus this fragment was chosen for further study. Several rounds of screening of a GC-2spd(ts) λgt11 cDNA library with the fragment 2A1.32 resulted in the isolation of three homologous, but distinct, cDNAs, Zfp94 Zfp95 and Zfp96 (accession numbers: MMU 62906, 62907, 62908 respectively, see Fig. 1).

3.1. The cDNA and protein sequence of Zfp94, Zfp95 and Zfp96

The 1997 bp length of the Zfp94 cDNA is in good correlation with the approximately 2.2 kb of the Zfp94 mRNA considering that the average length of oligo(dA) tails is about 200–250 bp (Fig. 1A) [14]. The longest open reading frame, encoding 520 amino acids, extends from bp 181 to bp 1740. Zfp95 is the longest, 3175 bp, of the three novel cDNAs (Fig. 1B). The longest open reading frame was found to extend from bp 61 to bp 2520, encoding 819 amino acids. The cDNA for

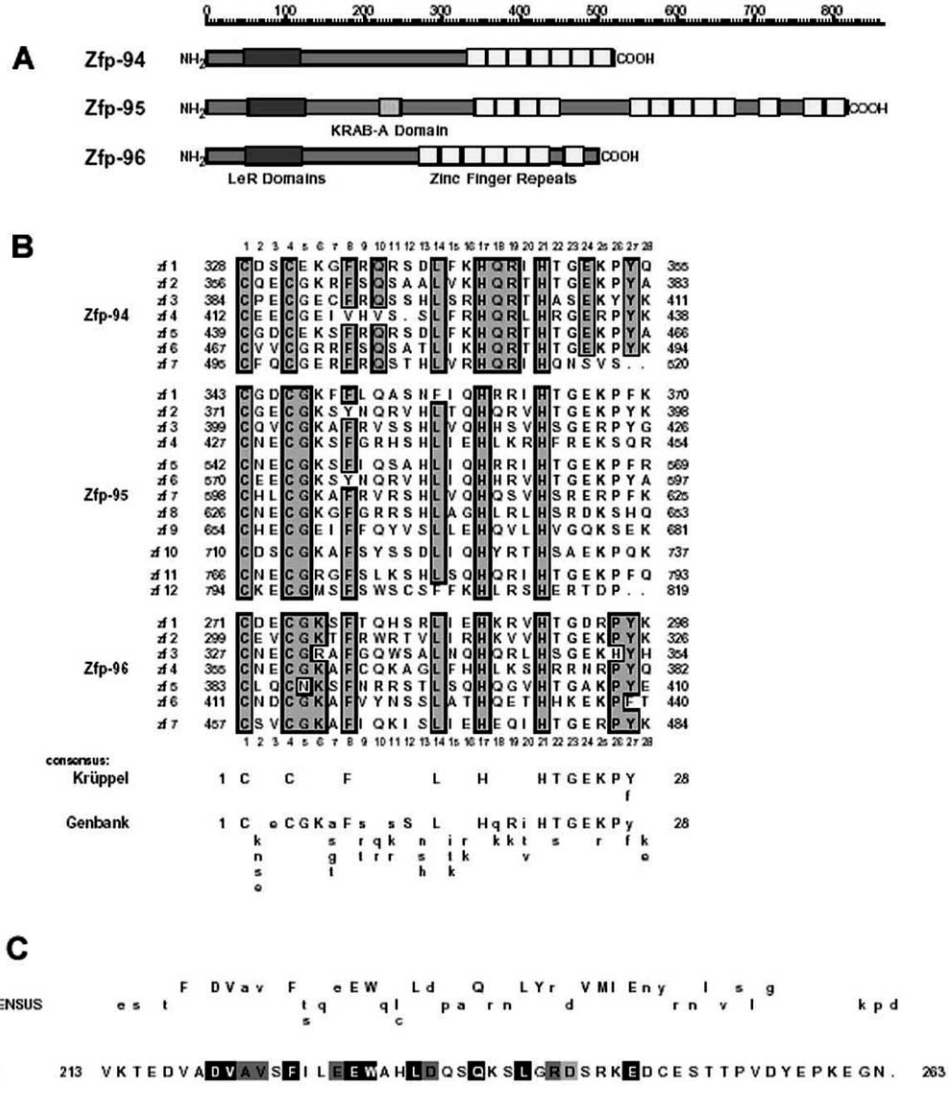

Fig. 2. A: Schematic representation of the structural features of *Zfp94*, *Zfp95* and *Zfp96*. The following domains are represented in the figure: a zinc finger consensus sequence, a LeR domain and a KRAB-A domain. B: Alignment of the zinc finger domains of *Zfp94*, *Zfp95* and *Zfp96*. Areas with at least 80% similarity are boxed and shaded. The line spacing indicates the cluster arrangement of the domains in the respective proteins. Residues matching the Krüppel consensus sequence [21] given below the alignments are shown in bold type. Residues with a frequency of more than 60% are shown in capital letters in the first row, those with a frequency of 30% or more are shown in lower case. The second and all subsequent rows list residues with frequencies above 10% in order of their respective frequencies. C: The consensus sequence for KRAB-A domains is indicated with capital letters corresponding to high conservation and lower case letters in the first row corresponding to moderate conservation in the alignment. The lower case letters in the second and third rows show the less conserved residues with the more frequent amino acids in the second row. The corresponding region of Zfp95 is colored accordingly. Those residues that differ are identified by arrowheads.

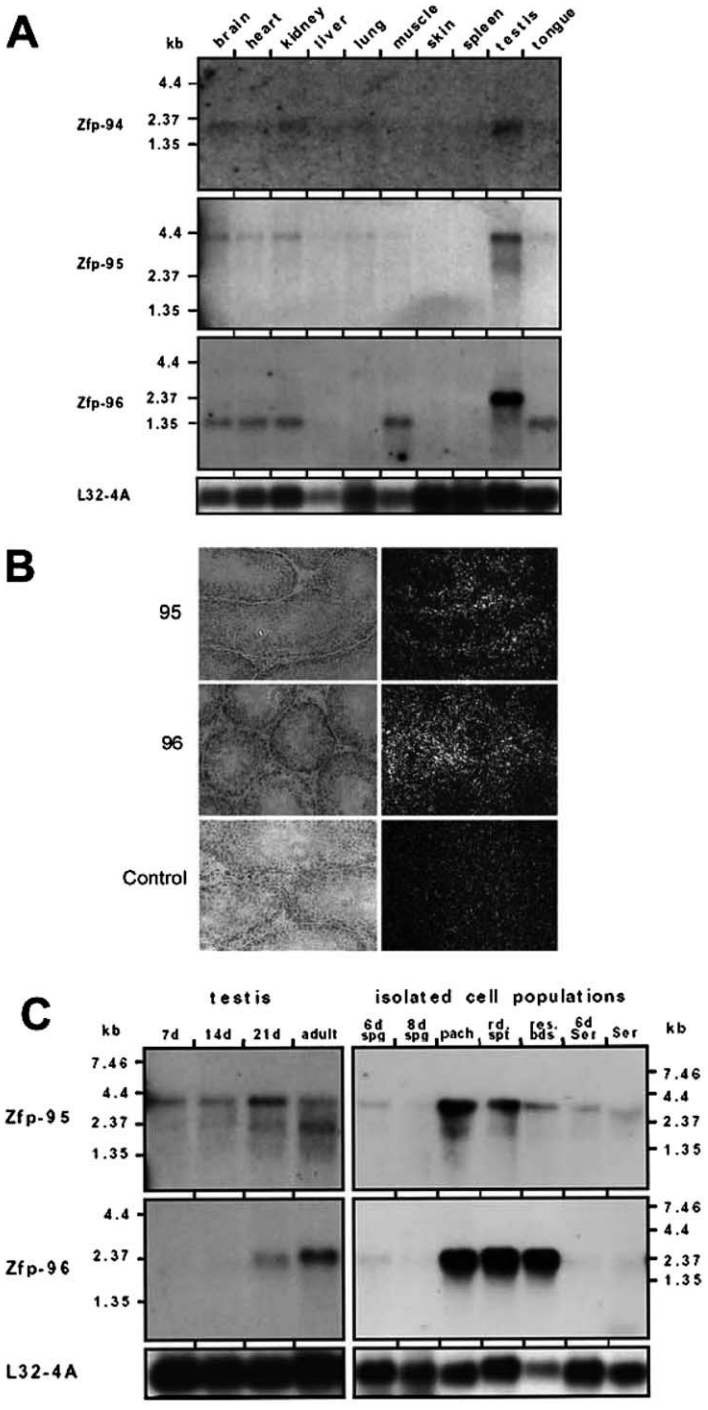

Fig. 3. Expression of *Zfp94*, *Zfp95* and *Zfp96*. A: Northern blot analysis of male tissues using 10 μg of total RNA per lane. B: In situ hybridization of *Zfp95* and *Zfp96* with sections of adult testis. Negative control using the *Zfp96* sense probe, the background for the others was equivalent to the one represented here. C: Expression of *Zfp95* and *Zfp96* in mouse testis and isolated spermatogenic cells using 10 μg of RNA per lane. Abbreviations: 6d and 8d spg, spermatogonia isolated from mice 6 and 8 days after birth respectively; pach, pachytene spermatocytes; rd. spt., round spermatids; res. bds., residual bodies; 6d Ser and Ser, Sertoli cells isolated 6 days after birth or from adult mice respectively. The mRNA of the ribosomal protein L32-4A was used as control.

Zfp96 has a total length of 2307 bp. An open reading frame of 1503 bp (bp 433–1936) was identified and encodes 501 amino acids (Fig. 1C). Mouse expressed sequence tag (EST) studies have recently determined the chromosomal location for *Zfp95* and *Zfp96*. As part of the Washington University's Mouse EST project, Marra and coworkers located *Zfp95* on chromosome 5 of the mouse at 5.5 cM. *Zfp96* was located on chromosome 13 with some ambiguity about the exact location around 61.6 cM [15]. Fig. 2A presents an overview of the protein domains identified in ZFP94, ZFP95 and ZFP96. All three molecules belong to the family of leucine-rich zinc finger proteins. The approximately 80 amino acids long leucine-rich domain (LeR) is present in a small subfamily of zinc finger proteins [16–20]. Seven zinc finger domains are found in

a contiguous cluster in ZFP94, while the 12 domains of ZFP95 are dispersed into two groups of four and five domains each, followed by a single domain and another cluster of two domains at the immediate C-terminus. The seven domains of ZFP96 are also contiguous with the exception of the last domain. Fig. 2B shows the alignments of the zinc finger domains of ZFP94, ZFP95 and ZFP96 compared with the consensus sequence of the zinc finger domains in the *Drosophila* gene Krüppel [21] and a consensus sequence calculated from 1802 different vertebrate zinc finger domains extracted from a total of 385 genes in GenBank. For this latter comparison, all sequences complying with a 'limited' C2H2 consensus (CX2CX3[FYLV]X5[LF]- X2HX3HX7, where X could be any amino acid and positions with multiple possibilities are shown in square brackets) were compiled from the vertebrate sequences of GenBank. Approximately one-third of all zinc finger proteins contain an evolutionarily conserved region of about 75 amino acids at their N-terminus, the Krüppel-associated box (KRAB). This region is split into two parts, KRAB-A and KRAB-B, both of which are found separate in different proteins, or closely associated together in the same protein [22]. Fig. 2C shows an alignment of the KRAB-A domain consensus sequence in comparison with amino acids 213–263 of Zfp95. Thirty-five percent (15 out of 43) of the amino acids in this region are identical with the conserved amino acids of the KRAB-A consensus sequence. Moreover, nine out of 13 highly conserved residues and five out of 12 moderately conserved residues are found in Zfp95. In contrast to the majority of KRAB domain containing proteins, the domain in Zfp95 is not at the immediate N-terminus of the molecule.

3.2. *Expression pattern of* Zfp94, Zfp95 *and* Zfp96

The expression of *Zfp94*, *Zfp95* and *Zfp96* was analyzed in tissues of male mice. All three genes are expressed at higher levels in testis than in any of the other tissues investigated (Fig. 3A). Expression is detectable for *Zfp94*, *Zfp95* and *Zfp96* in brain, heart, kidney, liver and tongue of male mice. *Zfp94* and *Zfp95* are also expressed in the lung, while *Zfp95* and *Zfp96* are expressed in striated muscle as well. *Zfp94* shows one transcript in the tissues mentioned as well as high expression in the testis. On the other hand, *Zfp95* and *Zfp96* show two transcripts. The 2.4 kb mRNA for *Zfp95* and *Zfp96*, detectable in testis RNA, was not present in any of the other tissues.

Since *Zfp95* and *Zfp96* show testis-specific transcripts, we decided to further analyze their expression patterns. In situ hybridization confirmed the expression of these genes in the testis (Fig. 3B). In addition, four postnatal time points, i.e. 7, 14 and 21 days old as well as adult mice, were chosen for more detailed expression analyses. The population of germ cells in the 7 day postnatal testis consists largely of type A and B spermatogonia. At 2 weeks, pachytene spermatocytes are also detectable while round spermatids become visible around 3 weeks after birth. In contrast, the somatic cell types are present throughout postnatal development [23]. *Zfp95* is expressed throughout (Fig. 3C, left panel). The expression of the 3.5 kb *Zfp95* mRNA peaks at day 21 and subsequently declines while the 2.4 kb testis-specific transcript has its highest expression in adult testis. The 2.4 kb testis-specific transcript of *Zfp96* mRNA becomes distinctly visible in RNA samples extracted from 3 week old mice and increases until adulthood.

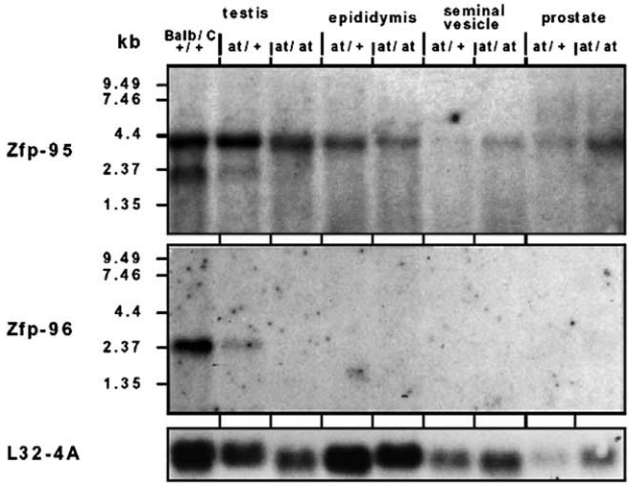

Fig. 4. Expression of *Zfp95* and *Zfp96* in RNA in reproductive tissues of mice carrying the *at* mutation. Total RNA from testis of control BALB/c, *at* heterozygous and homozygous littermates of the strain ATEB/Le as well as RNA from reproductive tissues of hetero- and homozygous mice was analyzed. The blots were hybridized consecutively to probes for *Zfp95*, *Zfp96* and L32-4A as a control. Autoradiography was carried out at −70°C for up to 4 weeks.

The mRNA levels of *Zfp95* and *Zfp96* in the isolated germ cell populations are in good correlation with the results from whole testis (Fig. 3C, right panel). The expression of the testis-specific *Zfp95* transcript is highest in pachytene spermatocytes, but it is also present in round spermatids. The strongest expression of the testis-specific *Zfp96* transcript is in residual bodies (taking into account the loading difference in this lane), suggesting that this gene is expressed in late stage spermatids.

To further substantiate the conclusions drawn from the expression pattern in whole testis at different stages of postnatal development and in isolated cell fractions, experiments were conducted employing RNA isolated from reproductive tissues (testis, epididymis, seminal vesicle and the prostate) of *at* mutant mice. The recessive *at* mutation renders affected mice almost hairless and sterile as their gonads are greatly reduced in size and contain few germ cells [9]. Expression of the testis-specific *Zfp95* transcript in *at* mutants is not detectable compared to the levels of expression in heterozygous littermates (Fig. 4). In addition, this 2.4 kb mRNA is not detectable in epididymis, seminal vesicle or prostate of mutant or heterozygous mice. Thus, *Zfp95* is expressed both in germ cells and in other as yet undefined cell types of the testis but shows differential splicing only in meiotic and post-meiotic germ cells. The expression of *Zfp96* in mice heterozygous for the *at* mutation is greatly reduced and no expression is detectable in their homozygous littermates, confirming the germ cell specificity of this expression.

Our expression data in wild type mice as well as in the *at* mutant clearly indicate a crucial role of these proteins in spermatogenesis and specifically in germ cell differentiation and maturation. It is clear that Zfp95 is differentially spliced in meiotic and post-meiotic germ cells and this expression disappears in *at* mutant mice that lack germ cells. Zfp96 shows only one transcript that also disappears in *at* mutants and seems to be specific in later stages of germ cell differentiation. The identification of novel zinc finger genes with expression enhanced in the testis and displaying splice variants that are germ cell-specific will facilitate studies of the molec-

ular mechanisms that govern germ cell-specific gene expression and spermatogenesis.

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