A main component of the mammalian sperm tail is a structure known as the outer dense fibers whose molecular composition as well as their function are still mostly unknown. We report here the isolation and characterization of new cDNAs (odf2) that identifies a highly variable gene locus encoding outer dense fiber proteins. Transcription of odf2 is restricted to testis and more specifically to round spermatids. Transcription starts in step 6 spermatids, which coincides with transcription of the major outer dense fiber protein gene odf1 (Burmester, S., and Hoyer-Fender, S. (1996) Mol. Reprod. Dev. 45, 10–20) and with the formation of the sperm tail. Affinity-purified anti-Odf2 antibodies identified isolated outer dense fibers immunocytochemically and detected at least three protein bands in the molecular mass range of 65,000 to 70,000 Da in total Odf protein preparations. Presence of several protein bands correlates with the presence of several transcripts and the isolation of slightly different cDNA clones, whereas Southern blot hybridization does not indicate the presence of multiple genes. Computer analyses of the structure of the encoded Odf2 protein revealed an overall α-helical structure with two regions identical to the dimerization region of the leucine zipper motif.

In mammalian sperm, the outer dense fibers consist of several proteins in the molecular mass range from about 11 kDa to about 87 kDa (4–9). Biochemical analyses have shown that in rat spermatozoa the Odf are composed of six major polypeptides in one study (7) or of at least 14 polypeptides in another (8). The major Odf protein of rat spermatozoa with a molecular mass of about 30 kDa and a high cysteine and proline content is also the main zinc-binding protein of the sperm tail (10). Recently, we have identified and characterized the gene encoding this major Odf protein (odf1) from rat, man, and mouse (1, 11–14).

To gain an understanding of the molecular composition and the function of Odf in sperm motility and the biochemical properties of the individual outer dense fiber proteins, it was our objective to isolate and characterize additional genes that encode Odf proteins or Odf-associated proteins. Here we describe the isolation and characterization of nearly identical and up to now unknown cDNA clones (odf2) that encode Odf proteins of rat spermatozoa. The cDNA clones were isolated from a rat testis cDNA library by initial screening with an antiserum directed against total Odf proteins of rat sperm and subsequent hybridization screenings with the isolated cDNA. odf2 is expressed specifically in testis, with transcript sizes of 2.2, 2.0, and 1.6 kb. By Northern blot hybridization to RNA of rat testes of different developmental stages and by in situ hybridization to rat testis sections, postmeiotic transcription was clearly demonstrated. Transcription starts in step 6 spermatids of tubular stage VI. Affinity-purified anti-Odf2 antibodies identified isolated Odf immunocytochemically and detected at least three protein bands on Western blots of total Odf proteins.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Outer dense fibers were isolated from epididymal rat spermatozoa as described previously (7). An antiserum against total Odf proteins was raised in rabbits by Eurogentec.

**Isolation of cDNA Clones and Sequence Analyses**—Screening of the Lambda ZAP II rat testis cDNA library (Stratagene) was performed by standard methods (15, 16). DNA sequences were determined (17, 18) using Sequenase 2.0 (Amersham Corp.).

**RNA Preparation and Northern Blot Hybridization**—Total RNA was prepared by guanidinium HCl lysis (19). Poly(A) RNA was isolated from total RNA with oligo(dT)12-Dynabeads (Dynal). RNA was denatured, electrophoresed on 1% agarose gels, and transferred to Hybond-N (Amersham) in TPE (60 mM Tris, 50 mM NaH2PO4, 2 mM EDTA, pH 8.0), digested with restriction endonucleases, separated on 1% (w/v) agarose gels in TPE, and blotted to Hybond-N (Amersham) (124), and hybridized in 5 × SSC, 5 × Denhardt’s solution, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA at 65 °C. Posthybridization washing was performed twice in 2 × SSC and once in 1 × SSC, 0.1% SDS and in 0.1 × SSC, 0.1% SDS at hybridization temperature. DNA probes were labeled with [32P]dATP by the random hexanucleotide primer method (25).
5′ Rapid Amplification of cDNA Ends (RACE) (26)—Isolation and cloning of the 5′ end of odf2 cDNA was performed with the 5′ RACE system, Version 2.0 (Life Technologies, Inc.) according to the instruction manual. The PCR products obtained were cloned into pGEM-T (Promega) or pCR-Script (Stratagene) and sequenced on both DNA strands.

Polymerase Chain Reaction (PCR) (27)—The conditions for polymerase chain reaction are as follows: initial denaturation for 5 min at 94 °C followed by 35 cycles of 0.5-min denaturation at 94 °C, annealing at 52 °C for 1.5 min, and elongation at 72 °C for 2.5 min, with a final step at 72 °C for 10 min.

Western Blotting—Odf proteins were separated by SDS-polyacrylamide gel electrophoresis (28) and transferred to Hybond-C (Amer sham) (29). The membrane was blocked in 5% powdered milk in TBST (10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20), and incubated with the anti-Odf antiserum, diluted 1:50 in blocking solution. Bound antibodies were detected via binding of anti-rabbit-IgG antibodies linked to horseradish peroxidase (Sigma) and chemiluminescence (ECL-Western blotting (Amersham) and Renaissance Western blot chemiluminescence reagent (Du Pont NEN)).

Expression of odf2 Fusion Protein and Affinity Purification of Antibodies—odf2 cDNA was cloned into pGEX-3X (Pharmacia Biotech Inc.). Odf2 protein, fused to glutathione S-transferase, was expressed by induction with 1 mM isopropyl-1-thio-D-galactopyranoside at 37 °C, and proteins were separated on SDS-polyacrylamide gels (28). The antiserum was first preabsorbed with immobilized total E. coli proteins including glutathione S-transferase. The preabsorbed antiserum was then incubated with total E. coli proteins containing the Odf2 protein fused to glutathione S-transferase. Elution of bound antibodies was performed as described previously (30). Binding specificity of the eluted antibodies was tested on Western blots containing total Odf proteins as described previously.

Immunocytochemistry—Rat sperm outer dense fibers were air-dried onto slides and fixed in acetone. Antibody incubation was performed with the affinity-purified anti-Odf2 antibody, diluted 1:400. Detection was performed using the Vectastain Elite ABC-Kit (Vector) and subsequent color reaction with diaminobenzidine. Controls were performed without the first antibody, but incubation with the second antibody and color reaction with diaminobenzidine.

RESULTS

Isolation of odf2 cDNA Clones—Outer dense fibers were isolated from rat spermatozoa (7). Light microscopic examination of the preparation revealed the contamination with sperm heads. The solubilized proteins were used to raise antibodies, and the antiserum was fractionated according to the molecular mass of their antigens. The antibody fraction directed against Odf proteins with molecular masses greater than 45 kDa was used to screen a rat testis cDNA library. This initial screening yielded five clones that cross-hybridize.

The DNA sequence of the largest clone consists of 1365 bp without the poly(A) tail. Nineteen bases in front of the 3′ end of the cDNA, the presumably polyadenylation signal AATAAA is present. It is obvious from Northern blot hybridizations (see Fig. 4), that the isolated clone is not a full-length cDNA clone. Screening of the rat testis cDNA library with anti-Odf antibodies and with the isolated cDNA clone itself yielded no full-length clone. Instead, several smaller clones as well as one clone of about 1.6 kb were obtained. All clones sequenced revealed that they are mostly but not completely identical. The most remarkable difference between these clones is an insertion of 69 nucleotides that does not interrupt the reading frame and therefore represents 23 additional amino acids. The missing 5′ end of the complete odf2 cDNA was obtained by reverse transcription.
transcription of rat testis mRNA and amplification of the 5' region. After a first round of PCR, two weak product bands of about 1 kb and 800 bp were present (not shown). These two PCR products were cloned and sequenced on both DNA strands. The complete nucleotide sequence of the largest odf2 cDNA as well as its derived amino acid sequence is shown in Fig. 1. This odf2 cDNA consists of 2,182 bp without the poly(A) tail. Between nucleotides 949 and 950 the 69-bp insertion was found in another cDNA clone. The largest open reading frame tail. Between nucleotides 949 and 950 the 69-bp insertion was found in another cDNA clone. The largest open reading frame translation initiation codon is marked. The amino acid sequence specific for this clone is shown in bold letters.

The 5' end of the odf2 cDNA consists of 2,182 bp without the poly(A) tail. Between nucleotides 949 and 950 the 69-bp insertion was found in another cDNA clone. The largest open reading frame of the 2,182-bp odf2 cDNA consists of 1,773 bp from base 305 to base 2,077. This open reading frame encodes a protein of 591 amino acids with a deduced molecular mass of 68,671 Da and an estimated isoelectric point of 6.04.

The sequence of the smaller 5' RACE product (Fig. 2) is in part identical to the described odf2 sequence. The 3' end of this cDNA is identical to the odf2 sequence, whereas the 5' end shows no sequence similarities. This 5' RACE product may be therefore representative for another odf2 cDNA with a different 5' end. The amino acid sequence of the putative protein may be therefore identical to the amino acid sequence of Odf2 with the exception of the first 40 amino acids and two amino acid exchanges at positions 99 and 115. The putative protein of this sequence has an estimated molecular mass of 64,486 Da.

The odf2 cDNA Sequence Is Highly Variable—It is obvious from the sequencing of a lot of cDNA clones isolated by antibody and hybridization screenings and the sequencing of the 5' RACE products that several slightly different odf2 cDNAs exist. First, between nucleotides 949 and 950 of the odf2 sequence shown in Fig. 1, a 69-bp insertion was found in another cDNA clone. Amplification of the region flanking this insertion demonstrates the presence of both sequences (with and without the insertion) in rat testis cDNA synthesized from total and poly(A) RNA (not shown). Second, the existence of nearly identical odf2 cDNAs with different 5' ends was verified by amplification of the individual 5' regions from rat testis cDNAs (Fig. 3). In Fig. 3, lanes a and b, amplification of the 5' end of odf2 was performed with a primer specific for the 5' sequence shown in Fig. 1 (oligo 86: CACCTTGTATCCATCCCC, nucleotides 2–19) and a primer specific for nucleotides 1,351–1,369 (oligo 82: CTCC-

GCATACTCTCAGTTC). In lanes c and d amplification of the 5' end of another cDNA (GenBank™ accession no. X95722, see “Discussion”) is shown, performed with a primer specific for this cDNA (oligo 87: CACGAGGAAAGGAGGAGG; see “Discussion”) and the oligo 82, and in lanes e and f amplification of the 5' end of the cDNA shown in Fig. 2 was performed with a specific primer (oligo 88: AGACTGTATGGCTTGGAG; nucleotides 6–23 in Fig. 2) and oligo 82. The results revealed that all these sequences are present in rat testis cDNA synthesized from poly(A) RNA (Fig. 3, lanes a, c, and e) as well as in rat testis cDNA synthesized from total RNA (Fig. 3, lanes b, d, and f), and are therefore not artificial. These three different 5' ends are also present in transcripts with and without the 69-bp insertion (not shown).

Three Testis-specific Transcripts Hydrbide to odf2—The odf2 gene is specifically transcribed in testis (Fig. 4). In RNA isolated from testis of adult rats, at least two hybridization signals could be obtained (Fig. 4B, lane a). The strongest signal may be composed of two RNA species at about 2 kb with slightly different lengths (see Fig. 5), whereas the weaker signal is composed of RNA smaller than 2 kb in length. In somatic rat tissues odf2 is not transcribed (Fig. 4B, lanes b–f). Hybridization of odf2 to RNA isolated from rat heart, liver, muscle, kidney, and spleen (Fig. 4B, lanes b–f), and also to rat brain and lungs (data not shown) yielded no hybridization signal. It is obvious from the gel, after staining with ethidium bromide, that the amount of total RNA from somatic tissues used for Northern blot hybridization is greater or at least the same (Fig. 4A, lanes b–f) as that of the testicular RNA (Fig. 4A, lane a).

Digestion of rat testis RNA with RNase H and subsequent Northern blot hybridization to odf2 cDNA confirmed the existence of three different odf2 transcripts (not shown). The lengths of the undigested odf2 transcripts are about 2.2, 2.0, and 1.6 kb, whereas the RNase H-digested transcripts are about 2.0, 1.8, and 1.4 kb. The poly(A) tails have been estimated to be between 120 and 180 bp in length.

Spermatid-specific Expression of odf2–odf2 transcripts could first be detected in testis RNA of 30-day-old rats (Fig. 5B, lane c), and their amount increases as spermatogenesis proceeds (Fig. 5B, lanes d and e). Increasing amounts of odf2 transcripts could be detected in RNA isolated from testis of 40-day-old rats (Fig. 5B, lane d) and adult rats that are older than 50 days (Fig. 5B, lane e). In testes of 10- and 20-day-old rats, no odf2 transcription occurs (Fig. 5B, lanes a and b). The amount of total RNA in each slot is shown after staining with ethidium bromide and demonstrates that in lanes c to e nearly the same quantities were used, whereas in lanes a and b greater amounts of total RNA were loaded onto the gel (Fig. 5A). The absence of odf2 transcripts in testes of rats younger than 30 days pointed to haploid transcription of odf2. In testis of 30-day-old rats, spermiogenesis has just started, and the germ cells have reached the stage of late round to elongating spermatids (31). In situ hybridization to testis sections confirmed the haploid transcription of odf2 (Fig. 6). In adult rat testis sections, odf2 transcripts could be clearly demonstrated.
in the cytoplasm of haploid round spermatids of tubular stages VII/VIII and in the cytoplasm of elongating spermatids of subsequent stages (Fig. 6A), whereas control hybridization to labeled odf2 sense RNA yielded no signals (Fig. 6B). Transcription starts in step 6 spermatids (not shown) of tubules of stage VI of the cycle of the seminiferous epithelium. In these tubules a slight staining could be demonstrated in the cell layer in the middle of the tubule, in which the haploid round spermatids are located. The identification of the stages of the seminiferous epithelium was performed after staining with periodic acid-Schiff (not shown) following the definitions of the stages given by Leblond and Clermont (32).

**Proteins of the Outer Dense Fibers Are Encoded by odf2 cDNA**—Antibodies specific to epitopes encoded by the largest odf2 cDNA were affinity-purified from the antiserum directed against total Odf proteins. After incubation of an immunoblot containing total Odf proteins with the affinity-purified antibodies, several proteins could be detected (Fig. 7). The two main proteins are in the molecular mass range of more than 66,000 Da (Fig. 7B). These two proteins are present in a much greater amount than that protein with a molecular mass of about 65,000 Da, which is also intensively stained with the antibodies. At least three other protein bands are also detected by the affinity-purified antibodies (Fig. 7B), whereas the major outer dense fiber protein Odf1, with a molecular mass of about 30 kDa, did not cross-react with the anti-Odf2 antibodies.

The identity of the proteins that were detected by the anti-Odf2 antibodies as outer dense fiber proteins was demonstrated immunocytologically. Incubation of isolated outer dense fibers of rat sperm with the anti-Odf2 antibodies and staining with diaminobenzidine identified the outer dense fibers (Fig. 8A), whereas no staining was obtained in identical control experiments without the anti-Odf2 antibodies. (Fig. 8B).

**odf2 at the Genomic Level**—Since all results obtained indicate the presence of several transcripts and several proteins, we performed Southern blot hybridizations to investigate the number of odf2 genes. Rat genomic DNA was digested with
Cloning of Sperm Tail odf2 cDNA

**DISCUSSION**

A remarkable feature of male germ cell differentiation is the formation of the sperm tail. The most prominent sperm tail structures are the outer dense fibers, whose composition and function are almost unknown. To characterize their protein constituents and to study their significance in sperm tail formation and function, we started to isolate cDNA clones encoding Odf proteins by an immunological approach. The isolated cDNA hybridizes to three transcripts exclusively in total RNA of rat testis, but not in RNA of somatic tissues, as was expected if it encodes a sperm tail-specific protein. The three testis-specific transcripts do not correspond to one transcript species with different poly(A) tail lengths, since no reduction in the number of RNA bands was found after digestion of poly(A) tails with RNase H. The transcripts without their poly(A) tails are about 2.0, 1.8, and 1.4 kb in length and might encode proteins of at least 70,000, 65,000, and 50,000 Da, respectively. Affinity-purified anti-Odf2 antibodies detect at least three proteins in total outer dense fiber protein preparations on Western blots. The antibodies react very strongly with two proteins in the molecular mass range of more than 66,000 Da, and with a third protein of approximately 65,000 Da. The lengths of the transcripts and the molecular masses of the proteins are calculated according to their electrophoretic migration as compared with molecular mass standards. This may explain the differences between calculated molecular masses of encoded proteins and the molecular masses of proteins reacting with the anti-Odf2 antibodies as obtained from denaturing polyacrylamide gels. But it can not be excluded that other features such as post-translational modifications may also contribute to the differences in sizes.

Although no cDNA clone encoding a full-length transcript could be isolated, the missing 5' end, and therefore the complete cDNA sequence, was obtained by 5' RACE. Sequencing of many cDNA clones and of the 5' RACE products and PCR amplification of parts of the cDNA performed with different primer pairs reveal that several nearly identical odf2 transcripts exist. These transcripts may be translated into proteins with similar amino acid sequences. The protein bands in the total Odf protein preparation, which react only weakly with the anti-Odf2 antibodies, may be proteins that share only epitopes with the Odf2 proteins, or perhaps they are those Odf2 proteins present in minor quantities or modified to different amounts. The apparent sequence variation of odf2 was found in all experiments performed with probes from different individuals and is therefore consistent across the population. Amplification of the RNA variants by reverse transcription-PCR (Fig. 3) shows that the most prominent odf2 transcript corresponds to the largest transcript (Fig. 1), whereas the lowest transcript level was found for the X95272 sequence. This is in agreement with the amount of transcripts detected on Northern blots.

**FIG. 7. odf2 cDNA encodes outer dense fiber proteins.** The largest odf2 cDNA was cloned into an expression vector and the bacterially expressed protein was used for affinity purification of anti-Odf2 antibodies from the anti-Odf antiserum. The affinity-purified antibodies were incubated with total Odf proteins of rat sperm that were separated on a denaturing SDS-gel and transferred to Hybond-C. Bound antibodies were detected by chemiluminescence. A, blot of separated Odf proteins stained with Amido Black. B, immunological detection of Odf2 proteins.

**FIG. 8. Immunocytochemistry of isolated rat sperm outer dense fiber-connecting piece complexes.** A, incubation with anti-Odf2 antibodies and subsequent color reaction with diaminobenzidine. B, incubation without the anti-Odf2 antibodies but the second antibody and color reaction. The bars represent 26 μm.

**FIG. 9. Southern blot hybridization of rat genomic DNA.** 20 μg of genomic DNA were digested with SacI (lane a), HindIII (lane b), PstI (lane c), BamHI (lane d), EcoRI (lane e), electrophoretically separated, blotted to Tropilon N (Tropix), and hybridized to odf2 cDNA.
In a data base search, no identical sequence could be found but a sequence of 2,203 bp that was deposited under accession no. X95272. This sequence is nearly fully identical to the sequence of odf2, besides three nucleotide exchanges in the 3′ region and greater differences in the 5′ region. The sequence X95272 includes the 69-bp insertion found also in one of our cDNA clones. This 69-bp insertion does not interrupt the reading frame and encodes therefore for additional 23 amino acids. The greatest difference in sequence between odf2 and X95272 is found in the 5′ region. Nevertheless, cDNAs with the 5′ region specific for X95272 could be detected in rat testis cDNAs (Fig. 3, lanes c and d). The translation initiation codon of the largest odf2 cDNA is present at position 305 and is surrounded by the sequence GCCGAATGA that resembles the consensus sequence for initiation of translation by eukaryotic ribosomes CCAGCCATGG (33) only at positions –3 and –4. The open reading frame encodes a putative protein of about 68 kDa that may represent one protein of more than 66 kDa detected in Western blots. The translation initiation codon of the putative protein encoded by the cDNA with the smaller 5′ RACE product (Fig. 2) is found at position 63 and is surrounded by the sequence GGCAATGT. This sequence resembles the consensus sequence for the initiation of translation by eukaryotic ribosomes only at positions –2 and –3. Nevertheless, the putative protein has a molecular mass of about 65 kDa and may therefore represent the 65-kDa protein detected in Western blots.

Analysis of the protein structure with the program “Protein” (DNA*, Inc.) revealed an overall α-helical structure of the complete OdF2 protein, that is in agreement with a protein component of fibrillar structures. In the C-terminal region of the derived OdF2 protein, at amino acid positions 392–413 and 530–551 (Fig. 1), two regions identical to the dimerization region of the leucine zipper motif are found. The leucine zipper motif may function in dimerization of OdF2 proteins to generate longitudinal protein complexes of the outer dense fibers.

The results obtained by Southern blot hybridization do not point to multiple odf2 genes. Although this cannot be totally excluded at the moment, these slightly different transcripts may be generated by alternative splicing. We are now working on the isolation and characterization of the gene(s).

Testis-specific and postmeiotic transcription of odf2 is clearly demonstrated, as is expected, if the gene encodes a protein important in spermatid differentiation. Transcription of odf2 starts in step 6 round spermatids with only minor transcript amounts. The start of transcription of odf2 corresponds to that of odf1, which encodes the major outer dense fiber protein (1). Even though the beginning of translation of odf2 has not been investigated, it may be similar to that of odf1, i.e. the start of translation in step 6 or 7 round spermatids. The beginning of transcription of both OdF genes corresponds to the most active period of OdF formation. Formation of OdF takes place during the acrosome phase, i.e. step 8 round spermatids to step 14 elongated spermatids, and early maturation phase, i.e. steps 15–17 elongated spermatids (34).

That odf2 indeed encodes an outer dense fiber protein was clearly demonstrated by immunocytochemical staining of isolated OdF. The isolated gene will now be used in isolation and characterization of odf2-related genes in other species to investigate the composition of OdF and to study the formation of the sperm tail. Moreover, odf2 is the second gene isolated encoding a structural component of sperm tail outer dense fibers. It should be very interesting to compare the regulation of expression of the two OdF protein genes, odf1 and odf2, and to investigate the cooperation of these proteins in formation and function of the sperm tail. Further experiments are currently under way.

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REFERENCES
1. Burmester, S., and Hoyer-Fender, S. (1996) Mol. Reprod. Dev. 45, 10–20
2. Falci, D. W. (1975) Dev. Biol. 44, 394–436
3. Baltz, J. M., Williams, P. O., and Cone, R. A. (1990) Biol. Reprod. 43, 485–491
4. Boccioni, R., Pallini, V., and Burrini, A. G. (1973) J. Submicrosc. Cytol. 5, 257–258
5. Price, M. (1973) J. Cell Biol. 59, 272a
6. Olson, G. E., and Sammons, D. W. (1980) Biol. Reprod. 22, 319–332
7. Vera, J. C., Brito, M., Zavvi, T., and Burzio, L. O. (1984) J. Biol. Chem. 259, 5970–5977
8. Oko, R. (1988) Biol. Reprod. 39, 169–182
9. Henkel, R., Stafl, T., and Miska, W. (1992) Biol. Chem. Hoppe-Seyler 373, 685–689
10. Calvin, H. I. (1979) Biol. Reprod. 21, 873–882
11. Burfeind, P., and Hoyer-Fender, S. (1991) Dev. Biol. 148, 195–204
12. Burfeind, P., Belgardt, B., Spahrer, C., and Hoyer-Fender, S. (1993) Eur. Biochem. 216, 497–505
13. Gastmann, O., Burfeind, P., Gunther, E., Hameister, H., Spahrer, C., and Hoyer-Fender, S. (1993) Mol. Reprod. Dev. 36, 407–418
14. Hoyer-Fender, S., Burfeind, P., and Hameister, H. (1995) Cytogenet. Cell Genet. 70, 200–204
15. Benton, W. D., and Davis, R. W. (1977) Science 196, 180–182
16. Young, R. A., and Davis, R. W. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1194–1198
17. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
18. Chen, E. Y., and Seeburg, P. H. (1985) DNA (N. Y.) 4, 165–170
19. Chomczynsky, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
20. McCarroll, G. K., and Carmichael, G. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4835–4838
21. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5201–5205
22. Schaefer, M., Kuhn, R., Bosse, F., and Schaefer, U. (1990) EMBO J. 9, 4319–4326
23. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
24. Southern, E. M. (1975) J. Mol. Biol. 98, 503–517
25. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
26. Frohman, M. A., Dash, M. K., and Martin, G. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8988–9002
27. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N. (1985) Science 228, 1245–1250
28. Lasse, T. (1970) Nature 227, 680–685
29. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
30. Weinberger, C., Hollenberg, S. M., Ong, E. S., Harmon, J. M., Brower, S. T., Cidlowski, J., Thompson, E. B., Rosenfeld, M. G., and Evans, R. M. (1985) Science 228, 740–742
31. Knorr, D. W., Vanha-Pertulla, T., and Lipsett, M. B. (1987) Endocrinology 116, 1298–1304
32. Leblond, C. P., and Clermont, Y. (1952) Ann. N. Y. Acad. Sci. 55, 548–573
33. Kozak, M. (1984) Nucleic Acids Res. 12, 857–872
34. Oko, R., and Clermont, Y. (1989) Anat. Rec. 225, 46–55

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Identification and Characterization of New cDNAs Encoding Outer Dense Fiber Proteins of Rat Sperm
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