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Identification of a novel male germ cell-specific gene TESF-1 in mice

Jun Fan [a], Matthew Graham [a], Hiroto Akabane [a], Laura L. Richardson [b], Guo-Zhang Zhu [a,*]

[a] Department of Biological Sciences, Marshall University, Huntington, WV, USA
[b] Department of Anatomy and Pathology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, USA

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Abstract

Mammalian spermatogenesis is precisely regulated by many germ cell-specific factors. In search for such a germ cell-specific factor, we have identified a novel mouse gene testis-specific factor 1 (TESF-1). Messenger RNA of TESF-1 was found only in the testis and its expression appeared to be regulated in a developmental manner. Further analysis demonstrated that the expression of TESF-1 was specifically in male germ cells, supported by the observation that we were not able to detect the TESF-1 mRNA from *at*/*at* homozygous mutant testes, which lack germ cells. The deduced amino acid sequence of TESF-1 contains a leucine-zipper motif, a potential nuclear localization signal, and two cAMP- and cGMP-dependent protein kinase phosphorylation sites. The green fluorescent protein (GFP)-tagged TESF-1 fusion protein was expressed in COS-7 cells and localized primarily in the nucleus. Taken together, these results indicate that TESF-1 is a novel male germ cell-specific gene, and its protein product may function as a nuclear factor involved in the regulation of spermatogenesis.

Keywords: Spermatogenesis; Germ cell-specific; Novel gene; TESF-1

Mammalian spermatogenesis is a highly ordered, precisely orchestrated developmental process in which germ cells sequentially undergo mitotic, meiotic, and post-meiotic phases to execute specialized proliferation and differentiation programs [1–5]. Throughout this process, an undifferentiated diploid spermatogonium is developed into four highly differentiated haploid sperm, with enormous morphogenetic transformation taking place in post-meiotic spermiogenesis. Type A spermatogonia are stem cells, and thus can renew themselves through proliferation to maintain spermatogenesis over a man's reproductive life. Sperm are functional male gametes, which possess unique cellular structures, acrosome, flagellum, compacted chromatin, among others, to qualify them as carriers of the paternal genome in sexual reproduction.

The process of spermatogenesis occurs in the seminiferous tubules of testes. It begins at puberty under the sophisticated control of endocrine hormones and paracrine factors [6,7]. Besides this extrinsic control, recent studies indicate that spermatogenic cells also manage a number of intrinsic, unique regulatory programs executed by many germ-cell-specific molecules, e.g., protamine-1, an indispensable molecule for spermatid chromatin compaction [8,9]; cAMP-responsive element modulator (CREM), an essential transcription factor for spermatid development [10,11]. Thus, to further understand how regulatory programs govern the course of spermatogenesis in detail, it is now important to establish the identity of the full complement of molecules active in this process. In this report, we describe the identification and characterization of a novel, germ-cell-specific gene testis-specific factor-1 (TESF-1).

Materials and methods

Chemicals. All chemicals were purchased from Sigma unless stated elsewhere.

Isolation of the full-length TESF-1 cDNA. In the search for a testis-specific cyclin, we coincidentally obtained a clone with a 1.4 kb,

* Corresponding author. Fax: +1 304 696 7136.
E-mail address: zhu4@marshall.edu (G.-Z. Zhu).

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poly(A)-containing cDNA insert from the mouse testis marathon-ready cDNA (BD Clontech). The sequence of this cDNA was not found in the National Center for Biotechnology Information (NCBI) GenBank, based on the BLAST analysis. To obtain the full-length cDNA sequence, we performed 5′RACE (rapid amplification of cDNA ends) using the mouse testis marathon-ready cDNA (BD Clontech) as PCR template. The primers used were the forward primer, adaptor primer 1 (AP1) (5′-CCATCCT AATACGACTCACTATAGGGC-3′) and the reverse primer, gene-specific primer 1 (GSP1) (5′-CTGAGAGGTCCCAGTCTTCTGCC TTTGGCA-3′). PCR parameters were 94 °C for 2 min; 94 °C for 30 s, 72 °C for 4 min, 5 cycles; 94 °C for 30 s, 70 °C for 4 min, 5 cycles; 94 °C for 30 s, 68 °C for 4 min, 25 cycles; 72 °C for 6 min. The PCR product was cloned into the pT-Adv vector (BD Clontech) and sequenced from both strands. The sequence data from both the initial clone and the 5′ RACE product were used to compile the full-length cDNA.

Northern blot. To determine the tissue transcription profile of TESF-1, we performed the Northern blot assay on the Multiple Tissue Northern blot (MTN) membrane (BD Clontech). The digoxigenin-labeled DNA probe was prepared by using a Dig Labeling DNA Kit (Roche). The DNA template was the gel-purified PCR product generated using the forward primer GSP2 (5′-GCCAGCCTGGAGGTCCTCAA-3′) and the reverse primer GSP3 (5′-TGTCTTATCTTCAATTCTTGCT-3′). DNA hybridization and detection were performed according to the manufacturer's protocol (Roche).

Germ cell isolation and testis collection. Male ICR mice were obtained from Hilltop Lab Animals (Scottdale, PA). Enriched germ cell fractions were separated by velocity sedimentation at unit gravity [12]. Spermatogonia were obtained from 8-day-old mice. Leptotene/zygotene spermatocytes were isolated from 17-day-old mice. Adult mice at least 8 weeks old were used to obtain pachytene spermatocytes, round spermatids, and residual bodies. Testes were obtained from atrichosis (*at*) mutant mice and their normal littermates (The Jackson Laboratory, Bar Harbor, ME). The testes of the homozygous mutant mice are germ cell free [13]. Testes from 8- to 100-day-old ICR mice were also collected. The cells and testes were frozen in liquid nitrogen and stored at −80 °C until used for RNA isolation.

RT-PCR. RNA was extracted from germ cells and testes using the method of Chomczynski and Sacchi [14]. One microgram total RNA was used as template for reverse transcription by the random primer method. The primers used in the PCR were GSP2 and GSP3 (see above). For the control β-actin, the primers were the forward primer Actin-5′ (5′-GTG GGC CGC TCT AGG CAC CAA-3′) and the reverse primer Actin-3′ (5′-CTC TTT GAT GTC ACG CAC GAT TTC- 3′).

Expression of TESF-1-GFP (green fluorescent protein) in COS-7 cells. The whole open-reading frame of TESF-1 was amplified by PCR using the forward primer GFP-5′ (5′-ACTG ctgcag ATG GGT TCC CAA AGC CTG AA-3′, the *Pst*I site in lowercase letters) and the reverse primer GFP-3′ (5′- ACTG ggatcc GC TCC TCC TCT CTT CAA TAA-3′, the *Bam*HI site in lowercase letters), sequenced to rule out any mutations, and cloned into the *Pst*I/*Bam*HI sites of the vector pEGFP-N1 (BD Clontech). This construct pTESF-1-EGFP was transiently transfected into COS-7 cells using the Polyfect Transfection Reagent (Qiagene). Twenty-four hours

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                    5′    TATTGATTTCAGGGTAGGACTTAGGAGGGTAAAGCCTCCAAGGCAAGAGGAC
ATGGGTTCCCAAAGCCTGAATTCCACCTTTCTAAAAGTCAGGAGACTTTCATTAATTTCCAGTAATGAAAGATTA
 M  G  S  Q  S  L  N  S  T  F  L  K  V  R  R  L  S  L  I  S  S  N  E  R  L
CTTGGACAAACCTCTGGATTGGCCACTGGGTCGAGGTTGGTAACCCAGGAGGTTGATGACCTCAGGGTCATCCCA
 L  G  Q  T  S  G  L  A  T  G  S  R  L  V  T  Q  E  V  D  D  L  R  V  I  P
GGAAGTAGACCGGATCTGGATGATCATCAGCCTCAGTGTAGCCTGGCGGAGCTGCCTAGCACTGCTCATGGCAAG
 G  S  R  P  D  L  D  D  H  Q  P  Q  C  S  L  A  E  L  P  S  T  A  H  G  K
AGGAAGCCAGGCCATCTTCCACGCCTTAGAAGCAGTGCTGTCAAGGGCCATGCCCCTGACCCTAATCCAAGCTTG
 R  K  P  G  H  L  P  R  L  R  S  S  A  V  K  G  H  A  P  D  P  N  P  S  L
TCTATAGTCTCCAAAAGAATCTTTAAGGGCGAATCTGTGATCAAAGGGCCCGAGGACAGGCAGACATTTGTTGGG
 S  I  V  S  K  R  I  F  K  G  E  S  V  I  K  G  P  E  D  R  Q  T  F  V  G
CCCAGTGGCTTGCCAAAGATTAGCCCCAAAGCAACAGCAGGGGAGGCCCAAGGGAAGAAGAGGACAATGGAGTTG
 P  S  G  L  P  K  I  S  P  K  A  T  A  G  E  A  Q  G  K  K  R  T  M  E  L
CTTAACAAGGCTCGAAAGCAAGAAGAAAAAGTCTCAAACCTACTAGATATCAGACAGCTCCCGAAGCAAGAGGTG
 L  N  K  A  R  K  Q  E  E  K  V  S  N  L  L  D  I  R  Q  L  P  K  Q  E  V
TTTATTAACAACACACACCCATGCAAGAAACATCTGAAACAGCAACCAATGAGCCTTGAGGAATGGAGGAGGGGC
 F  I  N  N  T  H  P  C  K  K  H  L  K  Q  Q  P  M  S  L  E  E  W  R  R  G
CATCTAGGGGGAGACAACACAGGACTCATATCACAAGAACCTTTCAGGTGCTGCAAACGTCTTGGAAAGAAGGCA
 H  L  G  G  D  N  T  G  L  I  S  Q  E  P  F  R  C  C  K  R  L  G  K  K  A
CAATGTCAACTGTTGGAAGTAACTTCCCTAGAGGCAGAAGCCAGCCTGGAGGTCCTCAAGAGGAGGAGGAGGATG
 Q  C  Q  L  L  E  V  T  S  L  E  A  E  A  S  L  E  V  L  K  R  R  R  R  M
CAGGCTATGGAGATGTCCAAAAAACCTCAGGACAGAGGACTCGGTCAAGAAAAAGCAGTCTTCCTAAGCAGAGAG
 Q  A  M  E  M  S  K  K  P  Q  D  R  G  L  G  Q  E  K  A  V  F  L  S  R  E
AAAGTAAAGCCATCTTCTCATGACATGCATCTGAGCACTGCTGAAAGAAGCTTTAAACCTAAATCAATGCCAAAG
 K  V  K  P  S  S  H  D  M  H  L  S  T  A  E  R  S  F  K  P  K  S  M  P  K
GCAGAAGACTGGGACCTCTCAGTCCAGGGAACTCCTGTAGTGCTTACAGTTCGGGACCACAGCAATGTCTCACAG
 A  E  D  W  D  L  S  V  Q  G  T  P  V  V  L  T  V  R  D  H  S  N  V  S  Q
GCTCAGAAACATTTGGGCTGTGCTGAAATATTTCATTCCAGAGATGGGCGATGTACTTTATTGAAGAGAGGAGGA
 A  Q  K  H  L  G  C  A  E  I  F  H  S  R  D  G  R  C  T  L  L  K  R  G  G
GCCTAGAGAGAGTAGACACTTTCTCTGCATCCTGCTCGAGCACAATACAGGAACGAGGACCAGATTACCTTCACC
 A  .
AGCGACTGTGACTTCAATGGGCCAGGTGACTGAGGCACCCATTGTTCCACATGGGAATCAGACAGAATTCCAAGA
GTCCGTCACCATCCCGTGTATTCCATGTGAAAGCAAGAATTGAAGATAAGACAACTTTAGGAAACATCAATGAGG
AGAACAAAATAGAGTCTTGTCAAGTGTGACGAGATTATCAGTCAATTGGATGGAAATCGTACTGCACTGTAGGAT
TAGCAGCATCAGACAATGGGGGACTAGACTATTCAGTGGTTGGTGACATATGAGGCAGAATACCTGGGGCCCTGG
GCTTCTCTCTTGGGGGAGCTCCCCTATGGTGGTGCTTTAGGACCCACTAAGGAACAAATTGCTGAAGGAAATATA
AGTAGAGATATAGTATTTTTCCGGAGAAATATTTTTGTTGGACCTCAATCCACCAAGTTCCAGGCAGTTGAGCTG
CTACAGTAATTTCCTGGACACTCCAGATCTTTGGATATTGACCACCTTTACAGGGAAAAAAAAAGAATGATGTGT
TCTGTAAACACCGAGCAAGGCATAGAAAATGTAATGTCTTAAATAAAACTGTTTCAGATCTGCAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAA      3′
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Fig. 1. The cDNA and deduced amino acid sequence of TESF-1. The first methionine represents the potential starting codon of the longest open-reading frame. The conserved leucine-zipper motif is shown in bold. The underlined sequence indicates a potential nuclear localization signal. The strike through sequences represent two cAMP- and cGMP-dependent protein kinase phosphorylation sites.