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Expression of Testis Specific Gene-1 (Tesf-1) in Swiss Albino Mouse

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ABSTRACT

Spermatogenesis begins at puberty under the sophisticated control of endocrine hormones and paracrine factor. Besides this extrinsic control, spermatogenic cells also manage a number of intrinsic, unique regulatory programs executed by many germ-cell specific molecules. A novel germ cell-specific factor termed TESF-1(Gen Bank Accession No: AY552600) was already identified in mouse testis. In adult mouse testis total RNA was isolated using Trizol. RT-PCR, using M-MuLV reverse transcriptase enzyme and oligio(dt), synthesized the whole cDNA, followed by using manually designed primer for TESF-1 gene. RT-PCR results in a 1000bp fragment of TESF-1 gene, which was amplified. The desire fragment was cloned into pTZ57R/T vector and transformed in *E.coli* strain. Transformation results more white colonies. These results indicate that TESF-1 was exclusively expressed in adult testis at 77th postnatal day.

Key words: Spermatogenesis, TESF-1, Trizol, M-MuLV reverse transcriptase, Oligo(dt), pTz57R/T, *E.coli.*

1. INTRODUCTION

Spermatogenesis is a unique process of cellular differentiation in which diploid testicular stem cells differentiate into haploid spermatozoa[1,2]. 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[3,4].

The general organization of spermatogenesis is essentially the same in all mammals and can be divided into three distinct phases (i) The initial phase is the proliferative or spermatogonial phase during which spermatogonia undergo mitotic division and generate a pool of spermatocytes. (ii) The meiotic phase, which yields the haploid spermatids, (iii) Spermiogenesis whereby each round spermatid differentiates into a spermatozoon[5]. Specialized mechanisms have evolved to control the complex differentiation program of mammalian spermatogenesis. These include altered levels of general transcription factors and the expression of unique testis isoforms of transcription activators.

Transcription in germ cells during spermatogenesis follows a carefully regulated program corresponding to a series of differentiation events occurring in spermatogonial cells, spermatocytes and haploid spermatids[6]. CAMP-responsive element modulator (CREM) is an essential transcription factor for spermatid development[7, 8].

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. The present work was under taken to study the identification and characterization of a novel, germ cell specific gene testis-specific factor-1.

2. MATERIALS AND METHODS

2.1. Extraction of total RNA

Swiss albino Mouse testis sample of 11 weeks old was received from University of Madras. Total RNA was extracted from mouse testis by trizol reagent (Biobasic Inc, Canada) according to manufactures protocol. RNA quality was determined by formaldehyde agarose gel electrophoresis.

2.2. Synthesis of cDNA library

Reverse transcription was performed by M-MuLV reverse transcriptase (Fermentas, Canada) with 5µg of total RNA and oligio (dt) primers followed by DNaseI treatment and heat inactivation.

2.3. RT-PCR

Using RT-PCR, full length mouse TESF-1 was isolated. PCR was performed using *Taq* polymerase (sigma) in an eppendroff PCR system[9]. After initial denaturation of 5min at 95ºC cDNA was subjected to 35 cycles of PCR. Primer set for 1056 bp fragment mouse TESF-1 having the *BamHI* (forward) and *SacI* (reverse) restriction enzymes. The primers used were the forward primer (5'-GCCGGATCCATGGGTTCCCAAAGCCT GAA-3') and the reverse primer (5'-GCCGAGCTCGCTCCTCCTCTCTTCAAT AA-3'). PCR parameters were 94°C for 45 s (Denaturation), 65°C for 45 s (Annealing), 75°C for 60 s (Extension), 35 cycles. PCR products were separated in 1.2% agarose gel.

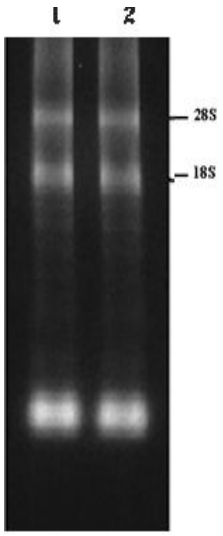
2.4. Cloning of TESF-1

The PCR product was cloned in the plasmid vector pTZ57R/T using TA cloning kit (Fermentas, Canada) and transformed into *E.coli* srain XL1 blue. For TESF-1 production bacteria were grown in LB medium containing ampicillin (100mg/ml), XGal (20mg/ml), 0.1M IPTG. The recombinants were determined by colony PCR and sequenced.

3. RESULTS

To study the expression of TESF-1 gene, we performed the RT-PCR experiment, using the total RNA samples from the testis harvested at 77th postnatal day. The total RNA of mouse testis was isolated. Formaldehyde agarose gel result showed that 18S and 28S ribosomal RNA bands were clearly visible in the intact total RNA **(Fig.1).**

Fig.1. Total RNA from 77day old mouse testis



The full length of testis specific factor was amplified by PCR based on previously known DNA sequence information obtained from a Gen Bank search. The reference DNA sequences used for RT-PCR is AY552600. To obtain a 1056bp length of fragment, RT-PCR was performed. PCR product was run on agarose gel which resulted in 1000bp of TESF-1 gene, which was amplified **(Fig.2).**

The desired fragment was extracted and cloned in pTZ57R/T vector and transformed in *E.Coli* strain. Bacteria were grown in LB medium containing ampicillin (100mg/ml), XGal (20mg/mg), 0.1M IPTG. Transformation revealed more white colonies **(Fig.3)**. Colonies were screened by colony PCR **(Fig. 4)** and sequenced (Data not shown).

Fig.2. Amplified TESF-1 in agarose gel(Approximately 1000bp) (Lane M- Marker, Lane-1-RT PCR product)

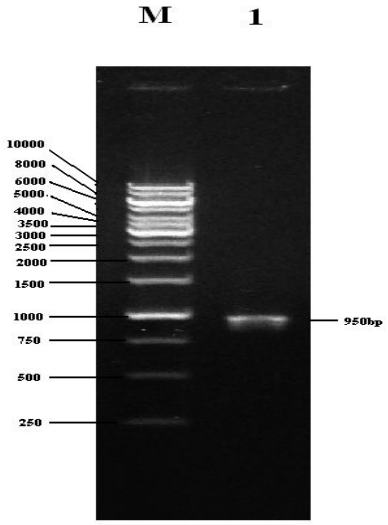
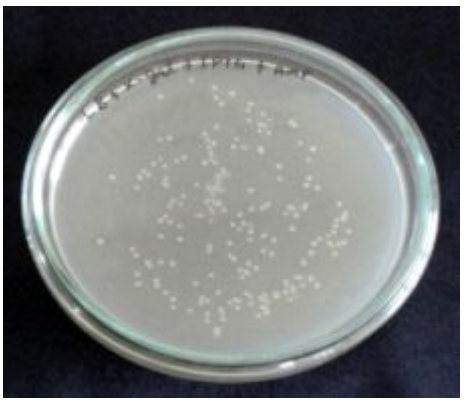


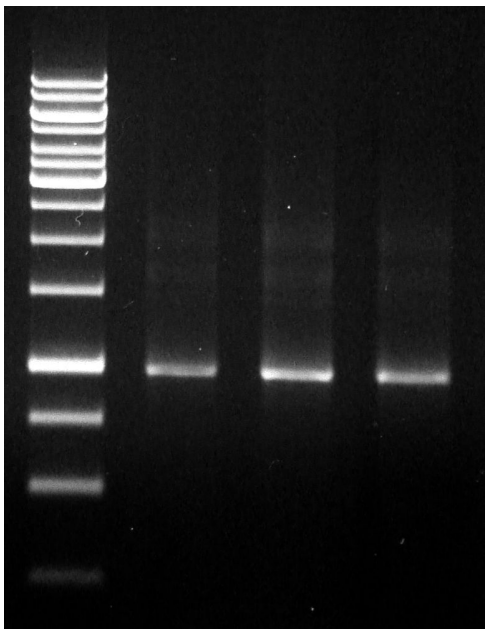
Fig.3. Blue white screening of TESF-1



TESF-1 transcript was first detected around the 20th postnatal day in the testis development. Spermatogenesis consists of several complex cellular process including the mitotic proliferation and self-renewal of spermatogonial stem cells, meiotic division of spermatocytes, and morphogenesis of haploid spermatids. These processes are highly regulated by many germ cell-specific factors[6].

The result showed approximately 1000bp which was similar to the earlier work of Jun Fan *et al.,*[10]. Transformation resulted more white colonies, which showed that TESF-1 was present in the medium containing X-gal. Colony PCR showed that the transformed colonies are expressed the desired TESF-1 gene.

Fig.4. Colony PCR of transformed white colonies of TESF-1 in agarose gel.(lane M- Marker, lane-1,2 and 3-colony PCR product)



4. CONCLUSION

In the present study it has been concluded that the expression of TESF-1 gene may occur in 77th postnatal day. Further studies are needed to identify biological function, characterization and interacting protein for TESF-1.

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