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RESEARCH ARTICLE

Cloning of a Gene Encoding Testis Specific Serine/Threonine Kinase 3 from *Mus musculus*

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ABSTRACT

Testis specific protein kinases are important because of their potential role in spermiogenesis, sperm maturation and sperm function. To continue our studies on the testis specific serine/threonine kinase (TSSK) family, we isolated a PCR fragment encoding a member of testis specific serine/threonine kinase3 (TSSK3) from *Mus musculus* testis RNA using reverse transcription polymerase chain reaction (RT-PCR) with oligonucleotides. The sequence length of the target gene was identified as 804 bp encoding a protein of 268 amino acids.

INTRODUCTION

Spermatogenesis, the production of functional sperm cells in the testis, represents a complex process involving specific interaction between the developing germ cells and their supporting Sertoli cells as well as hormonal regulation by the androgen-producing Leydig cells. During spermatogenesis, protein phosphorylation is essential for signaling pathways and regulation of protein activity. Among protein kinases catalyzing phosphorylation, the testis-specific serine/threonine kinase (TSSK) family is a particular one with exclusive or predominant expression in testis and functions in sperm differentiation, capacitation, and fertilization (Bielke *et al*., 1994, Chen *et al*., 2005, Hao *et al*., 2004, Kueng *et al*.,1997, Xu *et al*., 2007, Zuercher *et al*., 2000). Up to date, the family of TSSK comprises of five members, including TSSK1, TSSK2, TSSK3, TSSK4, and TSSK6, and all of the human homologs have been cloned. TSSK1 and TSSK2 are present in the stage of late spermatid to sperm (Hao *et al*., 2004, Kueng *et al*.,1997, Xu *et al*., 2007). TSSK3 is exclusively expressed in testis and it may function in the differentiated Leydig cells (Zuercher *et al*., 2000). TSSK6, also named small serine/threonine kinase (SSTK), is found predominantly in the elongating spermatids and is involved in postmeiotic chromatin remodeling (Hao *et al*., 2004, Spridonov *et al*., 2005).

Characteristically, TSSK3 was identified using low-stringency hybridization with a partial sequence obtained from cDNA amplification utilizing degenerated primers (Wilks 1991). The complete sequence of *hTSSK3* was published by Visconti *et al.*, 2001, shortly after it became available as a part of accessible Human Genomic Project sequences. The human *TSSK3* gene maps to chromosome 1 and is syntenic with the mouse *Tssk3* gene on chromosome 4 (Visconti *et al.*, 2001). Both the mouse and human sequence encode for a small protein of 29 kDa, consisting of a catalytic domain only that differs this protein from two previous members TSSK1 and 2 containing C-terminally extended sequence. Immunohistochemical studies in mice indicate that Tssk3 is present exclusively in testicular Leydig cells that synthesize androgens. Expression of TSSK3 was induced at puberty, persisted during adulthood and was restricted to the interstitial leytig cells of post pubertal males (Zuercher *et al*., 2000). Although exact roles of the TSSK family are still unknown, a series of studies suggests that they are involved in post meiotic germ cell differentiation. Sometimes serine threonine kinases are over expressed in tumours. To continue our studies on the TSSK family, we have now cloned TSSK3, the third member of the testis specific serine/threonine kinase family from mouse testis.

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MATERIALS AND METHODS

Sample collection

Mus musculus **(**Swiss mouse) Testis sample of 10-12 weeks old was received from the University of Madras.

RNA Isolation

Total RNA was extracted from mouse testis by trizol reagent (Biobasic Inc, Canada) according to manufactures protocol. The quality of total RNA was checked by agarose gel electrophoresis containing formaldehyde and quantitated the ratio of A260/A280.

cDNA synthesis

Aliquots (2µg) of total RNA were reverse transcribed to cDNA by using oligo dT primers and M-MuLV Reverse trasnscriptase (Fermentas, Canada).The generated cDNA was used as the template for PCR.

Polymerase chain reaction (PCR) amplification

The primers were designed based on the sequence of NM 080442 (Gen bank accession number); sense primer, 5'GCCGAATT CATGGAGGACTTTCTACTCTC3' containing an EcoR1 site; antisense primer, 5'GCCAAGCTTAGTGCTTGCTAGCCATGGGT3' containing a Hind III site (restriction sites are given in bold). The

Optimization of PCR was made and PCR was performed with 0.5µl cDNA template, 0.5µl *Taq* DNA polymerase, and 0.5µl of each primer in a 20 µl reaction mixture for 35 cycles in a thermal cycler. The PCR program consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of amplification at 94 °C for 45 s, 65 °C for 45 s and 72 °C for 60 s, followed by a final elongation step at 72 °C for 10min.

Gene cloning

After purification, the extracted PCR product was subcloned into a pTZ57R/T vector (Fermentas, Canada). Subsequently it was transformed into *E.coli*. The recombinants were determined by colony PCR and sequencing.

RESULTS

The quality of total RNA was checked by agarose gel electrophoresis containing formaldehyde and quantitated the ratio of $A_{260}/A_{280,}$ was found to be good (Fig.1). Further RNA was reverse transcribed to cDNA and the target gene was amplified from the cDNA by PCR (Fig.2). The desired fragment was extracted and cloned in pTZ57R/T vector and subsequently it was transformed into *E.coli* (Fig.3). The recombinants were determined by colony PCR (Fig.4) and sequencing (Data not shown).

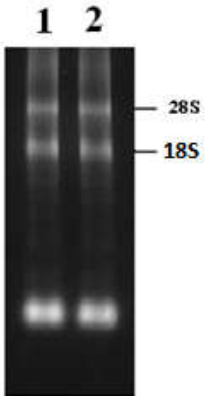

Figure 1. Total RNA extraction

Lane 1,2 - Total RNA isolated from *Mus musculus* testis.The 18S and 28S ribosomal RNA bands are clearly visible in the intact RNA sample.

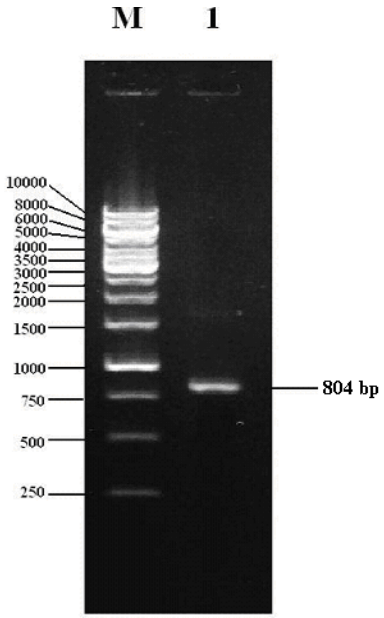

Figure 2. PCR amplification of target gene

M- Marker (1kb ladder). Lane 1 – PCR Amplified product of target gene (approximately 804bp)

Figure 3. Transformation of target gene

The cells containing recombinant TSSK3 appeared as White colonies.

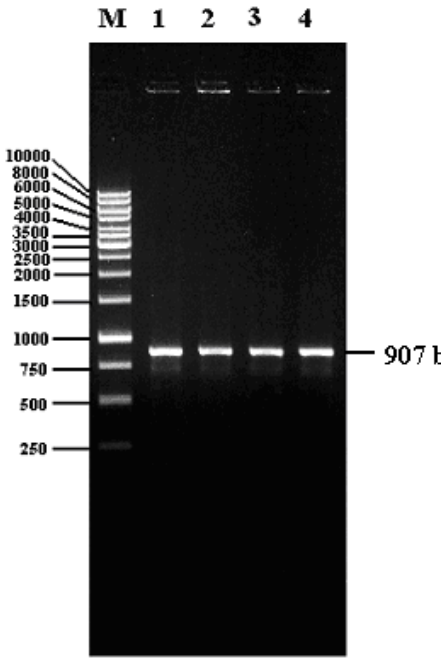

Figure 4. Colony PCR

M – Marker. Lane (1-4) are the screened product of target gene (TSSK3) (approximately 907 bp).

DISCUSSION

Protein kinases play a pivotal role in intracellular signal transduction systems involved in the regulation of cell proliferation, differentiation, metabolism, and other activities. An increasing number of genes encoding putative protein kinases have been isolated by cDNA cloning (Cance *et al*., 1993, Lindberg *et al*., 1990). The results showed that the isolated cDNA has an open reading frame of 804bp encoding a protein of 268 amino acids. Our results correlated with the work of Visconti *et al.* (2001), who isolated a member of the testis specific serine/ threonine kinase (STK) from male mouse mixed cell mRNA and he found that the isolated cDNA has an open reading frame of 804bp encoding a protein of 268 amino acids. Earlier it was performed by Zuercher *et al.* (2000) and he identified the open reading frame of TSSK3 encoding a protein of 275 amino acid, consisting essentially of a serine threonine kinase domain only. Our results were relevant with the work of Justyna *et al.* (2005), who cloned and analysed the biochemical properties, subtrate specificity and *invitro* activation.

Conclusion

The results showed that the cDNA has an open reading frame of 804bp encoding a protein of 268 amino acids. Further studies will be carried out for expression analysis of recombinant TSSK3 protein, to find its interactive proteins and its inhibitors, etc.

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