Spermatocyte-specific expression of the gene for mouse testis-specific transcription elongation factor S-II

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Abstract Previously, we characterized a rat cDNA for testis-specific transcription elongation factor S-II (SII-T1) (Q. Xu et al., J. Biol. Chem. 269, 3100–3103 (1994)). Here, we isolated a 335-bp fragment of the cDNA for mouse SII-T1, and used it to examine the expression of the SII-T1 gene in the testis by in situ hybridization. The results indicated that the SII-T1 gene is expressed exclusively in spermatocytes, showing no appreciable expression in spermatogonia, spermatids, or Leydig cells. RT-PCR experiments using testis RNA from *W/W^v* mutant mice also suggested that SII-T1 is a specific transcription elongation factor essential for spermatogenesis.

Key words: Transcription elongation factor; S-II; SII-T1; Spermatocytes; Spermatogenesis

1. Introduction

Transcription factor S-II was originally purified as a specific stimulatory protein of RNA polymerase II from mouse ascites tumor cells, and the complete amino acid sequence of its cDNA (pSII-3) has been determined [1,2] (reviewed in [3]). Subsequently, many S-II cDNAs have been identified from a variety of organisms, including man [4], fruit fly [5] and yeast [6], and found to show high sequence homology with pSII-3. It has been demonstrated that S-II enables RNA polymerase II to read through transcription pausing sites that are present in various eukaryotic genes [7-10].

Recently, similar but distinct S-II cDNAs have been isolated from the same organisms [11-13]. In particular, our previous results clearly demonstrated that rat testis contains both pSII-3-type S-II (general S-II) and testis-specific S-II (SII-T1) [12], whose amino and carboxyl terminal amino acid sequences show marked similarity, whereas SII-T1 has a unique intervening sequence of 46 residues. To gain more insight into the biological significance of SII-T1, we performed in situ hybridization and found that the SII-T1 gene is expressed specifically in spermatocytes.

2. Materials and methods

2.1. Isolation and sequencing of a mouse SII-T1 cDNA fragment

A mouse SII-T1 cDNA fragment was isolated by reverse transcription-polymerase chain reaction (RT-PCR) using a Stratascript RT-PCR kit (Stratagene, La Jolla, CA). For this, testes from ddY male mice (9 weeks old) were homogenized, and total cellular RNA was extracted by the guanidine thiocyanate method [14]. RT was performed using testis total RNA (10 μg) as a template and oligo(dT) as a primer. The primers used for PCR were 5′-GGATGTTTCTGATGGCAAATCCAGG-3′ and 5′-TCCGAACACGGTTCTTGTACTTCAT-3′, corresponding to +317 to +341 (sense) and +627 to +651 (antisense) of rat SII-T1 cDNA, respectively. The PCR product (335 bp) was subcloned into the pGEM 3Zf vector and sequenced by the dideoxy chain termination method of Sanger et al. [15] using an ABI 373A DNA sequencer with Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems). The nucleotide sequences of both strands were determined.

2.2. Northern blotting analysis

Total cellular RNA was extracted from 9-week-old mouse brain, testis, ovary, lung, heart, thymus, spleen, liver, kidney and intestine. Samples of 20 μg of RNA from each tissue were subjected to 1.2% formaldehyde-agarose gel electrophoresis, and then the RNA was transferred to a nitrocellulose filter (Schleicher&Schuell). The filter was baked at 80°C and then hybridized with a ^{32}P-labeled probe for 12 h at 42°C. The filter was then washed twice with 2×SSC (1×SSC=150 mM sodium chloride, 15 mM sodium citrate) containing 0.1% SDS for 40 min and once with 0.1×SSC containing 0.1% SDS for 30 min at room temperature. For rehybridization, the same filter was washed in water at 80–100°C for 1 h to remove the previous probe, and then subjected to hybridization with a new probe. The probes used were a PCR product of the +26 to +145 fragment of mouse SII-T1 and the human β-actin gene (Wako).

2.3. In situ hybridization analysis

Sections (10 μm thick) from unfixed frozen testis were collected on silane-coated slides. The sections were fixed in 4% paraformaldehyde in phosphate-buffered saline and then hybridized with digoxigenin (DIG)-11-UTP-labeled single-strand sense or antisense RNA probes. The DIG-labeled RNA probes were prepared by in vitro transcription using the mouse SII-T1 subclone as a template with a DIG RNA labeling kit (Boehringer Mannheim GmbH Biochemica, Germany). After post-hybridization washes, immunocytochemical detection of DIG-labeled RNA was carried out with alkaline phosphatase-conjugated anti-DIG antibody using a DIG nucleic acid detection kit (Boehringer Mannheim) according to the manufacturer's instructions.

2.4. RT-PCR analysis of SII-T1 mRNA in W/W^v mutant mouse testis RNA

Reverse transcription was performed with 5 μg of total RNA extracted from ddY and WBB6F1-*W/W^v* mouse testis as templates and oligo(dT) as a primer. The primers for PCR were 5′-ATGCCTCAAGGACTACGGAT-3′ (sense) and 5′-GGAAGATGCACTCCTCGATC-3′ (antisense), located at +69 to +88 in the unique region and +271 to +290 in the carboxyl terminal conserved region of the mouse SII-T1 cDNA fragment, respectively. The PCR products were analyzed by agarose gel electrophoresis. As a control, primers derived from mouse cytoskeletal β-actin cDNA [16] were used.

3. Results

3.1. Isolation of a cDNA fragment of mouse SII-T1 and Northern blotting analysis

To obtain a cDNA probe for mouse SII-T1, we performed RT-PCR using mouse testis RNA with primers specific for rat SII-T1 and isolated a 335-bp fragment. This fragment encoded a peptide consisting of 111 amino acid residues that corresponded to the unique region and one-third of the conserved carboxyl terminal region of rat SII-T1 [12]. As shown in Fig. 1, the sequence identity of these regions between

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Mouse SII-T1   1: DVSDGKSRNQGRGTPLPTSSSKDASRTTDLSCKKPDPPRTPSTPRITTFPQVPITC :56
                  ******** ********************* ************** ***************
Rat SII-T1    80: DVSDGKSRDQGRGTPLPTSSSKDASGTTDLSCKKPDPPRTSSTPRITTFPQVPITC :135

Mouse SII-T1  57: DAVRNKCREMLTLALQTDHDHVAVGVNCEHLSSQIEECIFLDVGNTDMKYKNRVR  :111
                  ************************** ****************************
Rat SII-T1   136: DAVRNKCREMLTLALQTDHDHVAVGVSCEHLSSQIEECIFLDVGNTDMKYKNRVR  :190
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Fig. 1. Comparison of the amino acid sequences of mouse and rat SII-T1 [12]. The deduced amino acid sequence of the mouse SII-T1 cDNA fragment was compared with part of the rat SII-T1 sequence. Identical residues are indicated by asterisks. Numbers indicate amino acid positions in each protein.

mouse and rat was 96.4%, and only 4 residues were different, indicating that mouse testis also contains SII-T1. We confirmed this by Northern blotting analysis using this cDNA fragment as a probe. As shown in Fig. 2, SII-T1 mRNA was detected almost exclusively in the testis.

3.2. *In situ hybridization analysis of SII-T1 transcript in mouse testis*

To identify the cells expressing the SII-T1 gene in mouse testis, we performed in situ hybridization using the cDNA fragment as a probe. As is evident from Fig. 3A,B, a positive signal was detected exclusively in large round cells in the seminiferous tubules, which were thought to be spermatocytes from their morphology. This was clear in the magnified fields shown in Fig. 3C,D, but it was not certain whether Sertoli cells also expressed the SII-T1 gene. No significant signal was detected in spermatids located on the luminal side and spermatogonia located outside the layer of spermatocytes. As expected, a positive signal was detected in all the cells present in the testis when pSII-3 was used as a probe (data not shown).

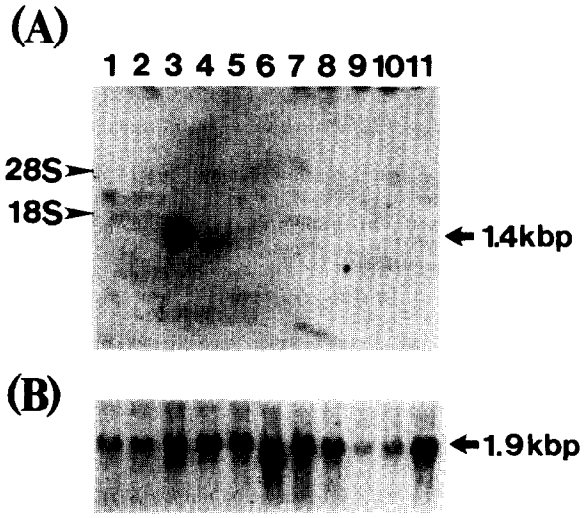

Fig. 2. Testis-specific expression of mouse SII-T1 mRNA. Northern blotting analysis was performed with 20 μg of total RNA from different mouse tissues. The probes used were +26 to +145 of the mouse SII-T1 cDNA fragment, corresponding to the unique region of rat SII-T1 (A), and the human β-actin gene (Wako) (B). The tissues used for RNA preparation were: lane 1, brain (male); lane 2, brain (female); lane 3, testis; lane 4, ovary; lane 5, lung; lane 6, heart; lane 7, thymus; lane 8, spleen; lane 9, liver; lane 10, kidney; lane 11, intestine.

3.3. *Analysis of SII-T1 mRNA in W/W^v mutant mouse testis RNA*

The W/W^v mutant mouse is known to lack the c-*kit* gene that encodes a tyrosine kinase-type receptor that is necessary for the differentiation of primordial germ cells [17-19]. Thus, the testis of this mutant mouse lacks germ cells, but contains other cells such as Leydig cells and Sertoli cells [20,21]. To confirm the spermatocyte-specific expression of the SII-T1 gene, we performed RT-PCR using testis RNA from W/W^v mutant mouse with primers specific for SII-T1. As shown in Fig. 4, no appreciable signal was detected with mutant mouse RNA compared with RNA from wild-type mouse. The molecular sizes of these RT-PCR products coincided with those expected. From these results, we concluded that SII-T1 is expressed exclusively in spermatocytes.

4. Discussion

It has become evident that transcription elongation factor SII-T1 is expressed specifically in spermatocytes, and is absent in spermatogonia and spermatids. Possibly, SII-T1 participates in the transcription of spermatocyte-specific genes [22,23] and general S-II may not compensate for the function of SII-T1 during the transcription of these genes. Other tissue-specific S-IIs may have a similar function.

We recently identified yeast S-II [6]. Gene disruption experiments revealed that the S-II null mutant is viable, but becomes sensitive to 6-azauracil due to the loss of S-II function as a transcription factor [6,24]. On the other hand, yeast S-II turned out to be the same protein as STPα, which catalyzes the transfer of a strand from a duplex linear molecule of DNA to a complementary circular single strand [25]. In fact, the meiosis-inducible homologous recombination of the S-II/STPα null mutant was decreased to 1/10 that of the wild-type strain, suggesting the participation of S-II in homologous recombination. Thus, it is possible that SII-T1 may not simply act as a transcription elongation factor, but also participate in the homologous recombination of chromosomal DNA during meiosis of spermatocytes.

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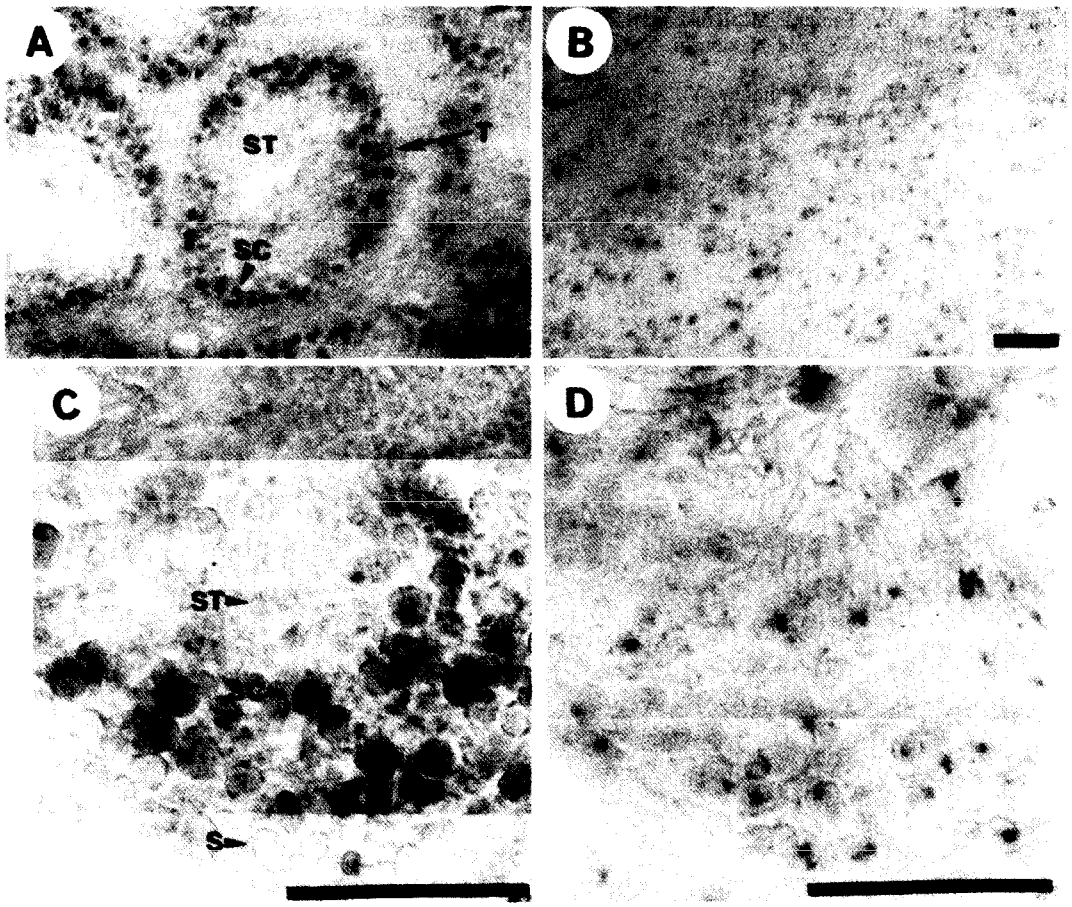

Fig. 3. Cell type-specific expression of mouse SII-T1 mRNA in the testis. (A) In situ hybridization analysis of mouse SII-T1 mRNA in the testis. Sections (10 μm thick) from frozen testis were fixed and then hybridized with a DIG-11-UTP-labeled single-strand antisense RNA probe. Probe RNA was detected with alkaline phosphatase-conjugated anti-DIG antibody. (B) Background staining with sense RNA using a section consecutive to that in A. (C) Magnified field of A. (D) Magnified field of B. SC, spermatocytes; ST, spermatids; S, Sertoli cells/spermatogonia; T, seminiferous tubule. The bar indicates 300 μm.

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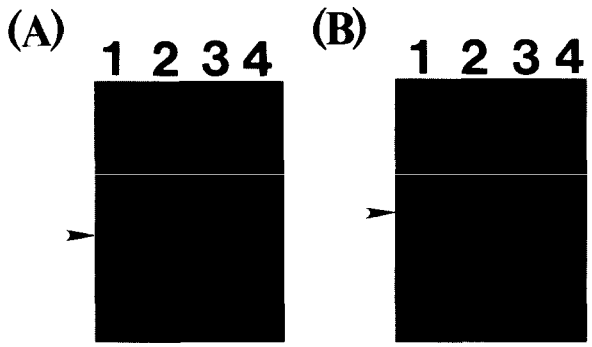

Fig. 4. Absence of SII-T1 mRNA in testis RNA from a *W/W*^{v} mutant mouse. RT was performed using 5 μg of total RNA extracted from *W/W*^{v} mutant [17-19] and wild-type mouse testes as templates, and oligo(dT) as a primer. The primers used for PCR were +69 to +88 (sense) and +271 to +290 (antisense) of the mouse SII-T1 cDNA fragment (A), and +247 to +267 (sense) and +792 to +813 (antisense) of mouse cytoskeletal β-actin cDNA [15] (B). RNA used as a template was from: lanes 1 and 2, wild-type and *W/W*^{v} mutant mouse, respectively; lanes 3 and 4, control without reverse transcription.

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