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·*Original Article*·

Mutations of t-complex testis expressed gene 5 transcripts in the testis of sterile *t-haplotype* mutant mouse

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Abstract

Aim: To determine the possible roles of the t-complex testis expressed gene 5 (*Tctex5*) on sperm functions, the full-length sequence of mRNA was studied and compared in the testis between the normal wild-type and the sterile *t-haplotype* mutant mice. **Methods:** We applied rapid amplification of cDNA ends, Northern blot and reverse transcription polymerase chain reaction to analyze the full length of *Tctex5* mRNAs isolated from testes of the wild-type and the *t-haplotype* mice. Reverse transcription polymerase chain reaction was used to semi-quantitatively compare expression of *Tctex5* transcripts in the 16 tissues and 9.5 day stage embryos in the wild-type mice. E-translation was applied to estimate the amino acid sequences. **Results:** One long and one short transcript of *Tctex5* mRNA were discovered in mouse testis of wild-type ($Tctex5^{long\text{-}+}$ and $Tctex5^{short\text{-}+}$) and *t-haplotype* ($Tctex5^{long\text{-}t}$ and $Tctex5^{short\text{-}t}$) mice, respectively. Being enhanced only in the testis, $Tctex5^{long\text{-}t}$ had 17 point mutations and one 15-bp-deletion in the exon 1 region, comparing with the $Tctex5^{long\text{-}+}$, whereas the $Tctex5^{short\text{-}t}$ was similar to the $Tctex5^{short\text{-}+}$. The short isoforms of *Tctex5* mRNAs in the two models encoded exactly the same peptides, but the long isoforms did not. The estimated peptide encoded by $Tctex5^{long\text{-}t}$ had significant mutations on putative sites of phosphorylation and PP1 binding. **Conclusion:** We established that mutations that occur in the *Tctex5* long transcript of the *t-haplotype* mice are important for normal sperm function, whereas the short transcript of *Tctex5* might have a conserved function among different tissues. *(Asian J Androl 2008 Mar; 10: 219–226)*

Keywords: t-complex testis expressed gene 5; transcripts; testis; mice

1 Introduction

Protein phosphorylation and dephosphorylation take part in spermatogenesis, especially for the G2/M transition evidenced by the fact that pachytene spermatocytes entering metaphase within 4–6 h under stimulation *in vitro* with the protein phosphatase 1 inhibitor okadaic acid [1]. In contrast to protein kinases that add a phosphate group to the hydroxyl oxygen of serine, threonine or tyrosine residues, protein phosphatases (PP) remove the phosphate group. There are mainly four groups of PP according to their catalytic subunits: PP1, PP2A, PP2B and

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PP2C. Until now, PP1 (including subtypes PP1α, PP1β, PP1γ1 and PP1γ2), PP2A and PP2B have been found to be expressed in the testis [2]. The regulators of PP, such as inhibitor 2 (Inh2), inhibitor 3 (Inh3), sds22, 14-3-3 and hsp90, are also associated with spermatozoa both in the testis and in the epididymis [3–6].

Tctex5 is defined as t-complex testis expressed 5 [7]. The human homologue of *Tctex5* was discovered in 1996, and has been confirmed to be a protein phosphatase 1 regulator 11 (*ppp1r11*), and termed inhibitor-3 (Inh3) [8, 9]. Inh3 in humans is co-localized to the nucleoli and centrosomes with PP1γ1 and PP1α, respectively [10]. In a sterile male mice model (*t-haplotype* mutant mice), *Tctex5* and its function on sperm have drawn our attention as a candidate for the "*Curlicue*" and "*Stop*" (sperm oolemma penetration of *t-haplotype* mice) phenotype [11]. *T-haplotypes* are mutations in the proximal one-third of mouse chromosome 17. The t true homozygous embryos will die at very early developmental stages in the uterus, whereas the secondary "*t* homozygotes", which are homozygous for two non-complimentary t haplotypes (like t^{w32} and t^{w5} in the present study) originated from different genetic background (B6 and 129 mice, respectively), can survive. However, the male "t-homozygotes" (t^{w32}/t^{w5}) are absolutely sterile because of the expression of mutation-bound factors that perturb numerous and distinct sperm functions, such as motility, zona-pellucida binding and oolemma penetration [12, 13]. "*Curlicue*" and "*Stop*" are phenotypes shown by individual spermatozoa isolated from *t*-homozygotes male mice epididymis *in vitro*. The *t* sperm show extra curvicues ("*Curlicue*") when exposed to increased levels of Ca^{2+} in the medium, and they can not penetrate the oolemma ("*Stop*") when exposed to the zona-free oocytes. The genetic factors leading to these two phenotypes have been mapped to "*Ccu"* (include "*Ccua"* and *"Ccub"*) and "*Stop"* (include "*Stop1p"* and "*Stop1d*") regions in chromosome 17 [14]. Being located to both the "*Ccub"* and "*Stop1d"* locus, *Tctex5* might be involved in the regulation pathways for sperm function, including motility control, sperm flagella structure development and sperm-oolemma penetration. In our previous studies, *Tctex5* has shown a specific expression pattern in the testis undergoing spermatogenesis and a similar distribution of PP1γ2 in the spermatozoa [4]. In addition to the binding of *Tctex5* to PP1γ2 *in vitro* [15], our previous research indicates the important role of *Tctex5* in normal sperm function through PP1γ2 or other isoforms of PP1. To reveal the role(s) of *Tctex5* during spermatogenesis, in the present study, we have investigated the *Tctex5* expression pattern in the testis and compared the sequences of each transcript between the wild-type and the *t-haplotype* mice.

2 Materials and methods

2.1 Production and genotyping of mice

Wild-type male mice were produced by crossing 129-+/+ and C57BL/6- +/+ mice. All t^{w32}/t^{w5} mice (hereafter we called *t-haplotype* mice) were obtained by breeding of t^{w5}/+ and t^{w32}/+ mice and were congenic to the +/+ mice. The proximal portion of mouse chromosome 17 was genotyped by restriction fragment length polymorphism analysis of genomic DNA extracted from tail-tip biopsies. Briefly, digested genomic DNAs were separated by electrophoresis in agarose gels and blotted to nylon membrane. DNAs were bound to the membrane by ultraviolet light and hybridized with radio-labeled probes that were informative for the chromosome 17 genotype. The presence of complete t haplotypes was detected using the marker DNA clones *Tu48, Tu89. Pim1A* was used to distinguish between t^{w32} and t^{w5} haplotypes.

2.2 mRNA preparation and rapid amplification of cDNA ends

mRNA from the testes of the wild-type and the *t-haplotype* (t^{w32}/t^{w5}) mice was isolated using the Oligotex Direct mRNA Mini Kit (Qiagen, Valencia, CA, USA). Full-length 5' and 3' ends of *Tctex5* were obtained using the GeneRacer kit (Invitrogen, Carlsbad, CA, USA) based on RNA ligase-mediated and oligo-capping rapid amplification of cDNA ends (RACE). Referring to genomic *Tctex5* sequences in the GeneBank (No. AK005379), *Tctex5* specific 5' and 3' primers and nested primers were designed (Table 1). Sequencing of full length cDNA was carried out by Macrogen (Seoul, Korea). Experiments were carried out twice.

2.3 Reverse transcription polymerase chain reaction (RT-PCR), semi-quantitative analysis of Tctex5 transcripts in tissues and northern blot

After the long and short transcripts of *Tctex5* mRNA were found by RACE in the wild-type mice and the short transcript in the *t-haplotype* mice, we designed two primer sets that discriminate the long and short *Tctex5* transcript: the *Tctex5* complete and *Tctex5* lower,

Table 1. Primers used in this study. RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription polymerase chain reaction; *Tctex5*, t-complex testis expressed gene 5. F, forward; R, reverse.

Primer	Sequence
Primers used for RACE	
A(F): GeneRacer 5' primer	5'-CGACTGGAGCACGAGGACACTGA-3'
B(F): GeneRacer 5' nested Primer	5'-GGACACTGACATGGACTGAAGGAGTA-3'
C(F): GeneRacer 3' primer	5'-GCTGTCAACGATACGCTACGTAACG-3'
D(F): GeneRacer 3' nested primer	5'-CGCTACGTAACGGCATGACAGTG-3'
A(R): *Tctex5* 5' race reverse primer	5'-TAAATACAGCAGCATTTCGACGAG-3'
B(R): *Tctex5* 5' race nested reverse primer	5'-CATATGCTCATTGTCCACAGTGTC-3'
C(R): *Tctex5* 3' race forward primer	5'-ACCGAGAGTGATGAGGATGAAGA-3'
D(R): *Tctex5* 3' race nested forward primer	
Primers used for RT-PCR	
E(F): *Tctex5* complete forward primer	5'-CTCGAGGTCTCATCCCGCTTTCTC-3'
E(R): *Tctex5* complete reverse primer	5'-CTCGAGATGAATGGTGAGAGGGAT-3'
F(F): *Tctex5* lower forward primer	5'-AGGTGGAGTGGTCCAGTGAC-3'
F(R): *Tctex5* lower reverse primer	5'-GGGCTTAGAGGGATCAGGAG-3'
Beta actin forward primer	5'-AGCCATGTACGTAGCCATCC-3'
Beta actin reverse primer	5'-CTCTCAGCTGTGGTGGTGAA-3'

respectively (Table 1 and Figure 1A). RT-PCR was performed using mRNA isolated from the wild-type and *t-haplotype* testis as template to confirm the existence of the transcripts. A new long transcript in the *t-haplotype* mice testis was discovered. Expression of each transcript of *Tctex5* mRNA was screened using the mouse rapid scan gene expression panels (Origene MSCB-101, Rockville, MD, USA) in three dilutions (100 ×, 10 × and 1 ×) with the lowest dilution concentration (1 ×) with approximately 1 pg cDNA. Using the panels as templates, we carried out RT-PCR using the following cycle conditions: 95ºC for 3 min; 95ºC for 30 s, 55ºC for 30 s and 72ºC for 90 s for 35 cycles; 72ºC for 10 min, and 4ºC thereafter. Beta-actin was amplified as an internal control. Experiments were carried out twice.

Five μg of total RNA isolated from wild-type and *t-haplotype* testis and RNA markers were blotted onto membrane (Hybond-XL; Amersham, Piscataway, NJ, USA). Northern blot was then used for hybridizations. The probe was synthesized by RT-PCR using *Tctex5*-complete primer sets. Probe recovered from 1.5% low melting gel was labeled using α-^{32}P dCTP (Perkin Elmer, Waltham, MA, USA) and purified with ProbeQuant G-50 micro columns (Amersham, Piscataway, NJ, USA). The blot membrane was hybridized overnight at 65ºC. After washing, the blot was exposed to Kodak BioMax MS film (Kodak, Rochester, NY, USA).

2.4 Computer-assisted sequence analysis

Sequence analysis and *Tctex5* intron/exon analysis used in the present study were obtained from http://www.ensemble.org. E-translation of known mRNA and protein structure prediction were carried out using the following websites: http://ca.expasy.org/tools/dna.html and http://www.predictprotein.org, respectively.

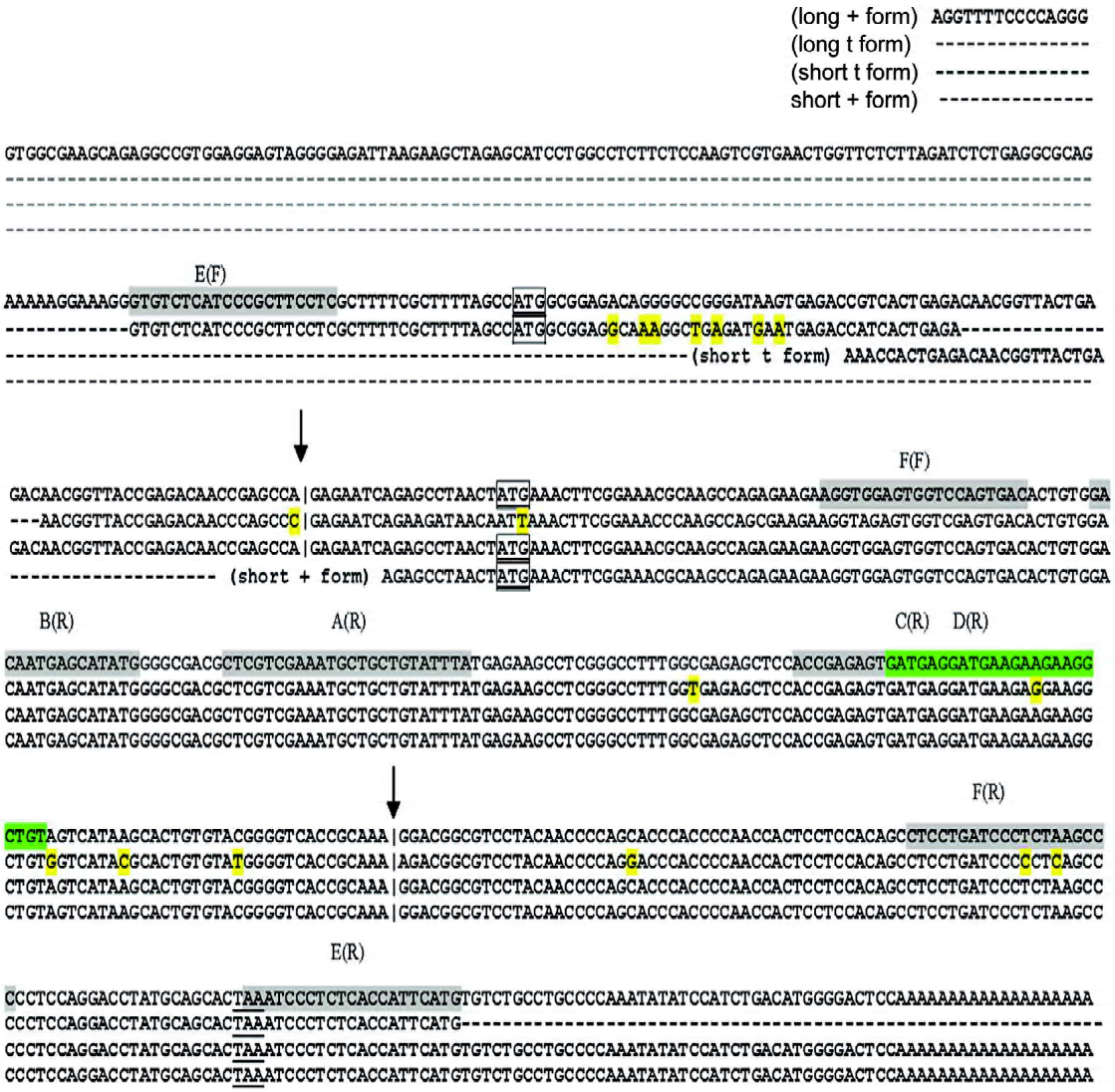
3 Results

3.1 Isoforms transcribed in the testis

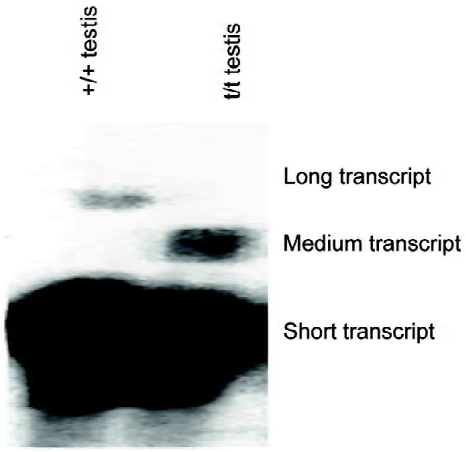
There were two isoforms of *Tctex5* mRNA transcribed in the testis. Significant mutations occurred in the long transcript of *Tctex5* between the t-haplotype and the wild-type mice. The long isoform mRNA was enhanced in the testis whereas the short isoform was more ubiquitous.

Using RACE and RT-PCR, we identified a long and a short isoform of *Tctex5* (*Tctex5*$^{long-+}$ and *Tctex5*$^{short-+}$ in wild-type, *Tctex5*$^{long-t}$ and *Tctex5*$^{short-t}$ in *t-haplotype* mutant) transcribed in the testis. The cDNA sequences of the *Tctex5*$^{long-+}$, *Tctex5*^{short} (*Tctex5*$^{short-t}$ had exactly the same

A



B



(to be continued)

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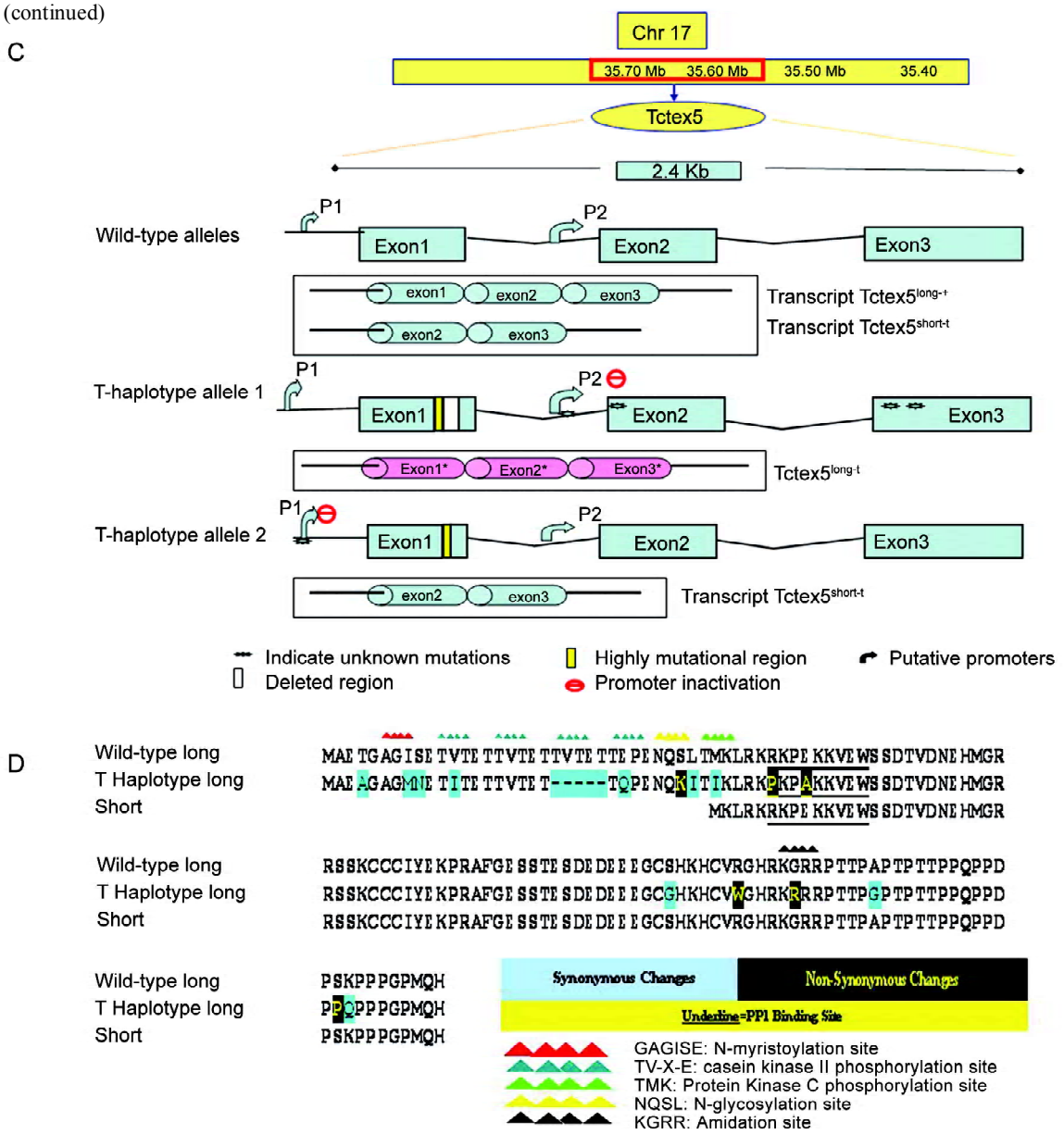

Figure 1. Transcripts of t-complex testis expressed gene 5 (*Tctex5*) expressed in the testis of the wild-type and the *t-haplotype* mice. (A): A long and a short transcript of mRNA were discovered in each type of mice using GeneRacer and reverse transcription polymerase chain reaction. Sequence alignments of the four cDNA isoforms (the long-+; the long-t; the short-+ and the short-t) are shown. In the alleles, absent or unknown sequences are represented by dashed lines. ATG were boxed and the ending code TAA is underlined. Mutations occurring in the long t isoform are highlighted yellow. Sequences in accord with primers described in Table 1 are highlighted in the long-+ isoform only with their names labeled accordingly. Exons are separated by vertical lines and indicated by arrows. (B): Northern analysis of *Tctex5* transcribed in the testis of the wild-type and the *t-haplotype* mice, respectively. The transcripts show three sizes, which were indicated as long, medium and short transcript, respectively. (C): Estimation of the expected promoters of *Tctex5* in the mouse chromosome 17. The sizes of each exon and intron are not in the appropriate ratio, as shown in the diagram. Estimated promoters 1 and 2 are indicated. The wild-type alleles of *Tctex5* are identical and are represented by green bars. The *t-haplotype* alleles are different and are denoted as 1 and 2. Mutations are represented by star symbols. (D): Estimated mutations of *Tctex5* peptide in the wild-type and the *t-haplotype* mice. The estimated peptide was E-translated from the three *Tctex5* mRNA transcripts. Mutations are highlighted and compared with the wide-type long peptide. Synonymous and non-synonymous mutations are distinguished by lighter (green) and darker (black) highlighting. Estimated influenced secondary modification sites are marked above and the PP1 binding site is underlined.

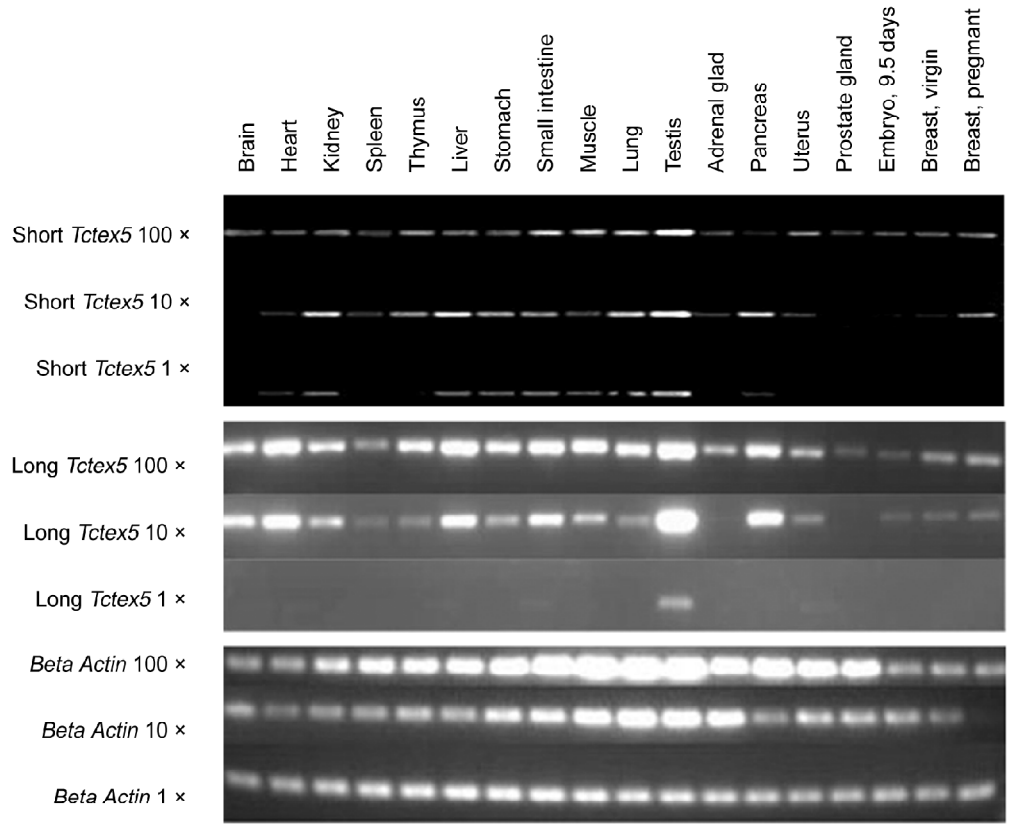

Figure 2. Transcription of t-complex testis expressed gene 5 (*Tctex5*) long and short transcripts in different tissues in wild-type mice. Reverse transcription polymerase chain reaction was performed using primer sets (*Tctex5* complete and lower primer sets, respectively) specific for the long and short transcripts of *Tctex5*. In the 18 tissues analyzed, expression of the long transcript of *Tctex5* was enhanced only in the testis, whereas expression of the short transcript of *Tctex5* was enhanced in heart, kidney, liver, stomach, small intestine, muscle, lung and testis.

reading frames as the $Tctex5^{short-+}$; therefore, we called them $Tctex5^{short}$) and $Tctex5^{long-t}$ had been sent to GenBank and been given the IDs EF528576, EF528579 and EF528577, respectively. The full lengths of $Tctex5^{long-+}$, $Tctex5^{short-+}$ and $Tctex5^{short-t}$ were 650 bp, 435 bp and 495 bp, respectively. As $Tctex5^{long-t}$ was discovered by RT-PCR, its full length was not known. The full length transcript ($Tctex5^{long-+}$) of *Tctex5* contained three exons. In the $Tctex5^{long-+}$ mRNA, exon 1 spanned from 169 bp upstream of the first initiating ATG to 91 bp downstream of ATG; exon 2 spanned 92–310 bp downstream of the first initiating ATG; exon 3 spanned 311–481 downstream of the first initiating ATG (Figure 1A). Seventeen point mutations and one 15-bp-deletion in exon 1 (from 44 to 58 bp downstream of the first initiating ATG) occurred in the $Tctex5^{long-t}$ mRNA. The most intensive point mutation (hereafter called the highly mutational region) occurred in an area 7–23 bp downstream of the first initiating ATG, which was just 11 bp upstream of the deletion region. $Tctex5^{short-t}$ and $Tctex5^{short-+}$ shared a second initiating codon ATG in exon 2 (103 bp downstream of the first initiating ATG in $Tctex5^{long-+}$ and $Tctex5^{long-t}$) without the amino acid reading frame shift comparing with their long isoform counterparts. They were similar in sequences except in the 5' UTR region.

Northern blot analysis of the *Tctex5* transcripts transcribed in the testis of the wild-type and the *t-haplotype* mice showed that the sizes of $Tctex5^{long-+}$, $Tctex5^{short-+}$, $Tctex5^{long-t}$ and $Tctex5^{short-t}$ were approximately 1.2 Kb, 0.6 Kb, 1.0 Kb and 0.6 Kb, respectively. The short isoforms were much more abundant than the long isoforms in both models (Figure 1B). Rapid-scan gene expression panel showed that, in wild-type mice, only the long transcript of *Tctex5* was enhanced in the testis. In contrast, the short transcript of *Tctex5* was not just enhanced in the testis, but also in other tissues, such as heart, kidney, liver, stomach, small intestine, muscle and lung (Figure 2).

3.2 Estimated mutations of Tctex5 peptide

The short *Tctex5* mRNAs encoded for a shorter truncated peptide compared with the long mRNAs with no reading shift. Estimated peptide sequences from the two short *Tctex5* mRNA transcripts were exactly the same in the wild-type and the *t-haplotype* mice. In contrast, estimated peptide sequences from the two long *Tctex5* mRNA transcripts were different. Two out of three sites for casein kinase II phosphorylation, one N-myristoylation site, one ASN-glycosylation site, one protein kinase C phosphorylation site, one Amidation site and the estimated PP1 binding site were mutated in the $Tctex5^{long\text{-}t}$ compared with its $Tctex5^{long\text{-}+}$ counterpart (Figure 1D).

4 Discussion

Among the two verified *Tctex5* mRNA transcripts in GenBank, only 29 bps of the AK077455 (1170 bp in length) isolated from day 8 mouse embryo whole body shared 100% identity with the genomic sequences of *Tctex5* in GenBank; whereas the BC027737 (652 bp in length) isolated from mouse mammary tumor was very similar to the long isoform mRNA sequence discovered in the present study (with only 21 bp discrepancies in the most 5' end). The long and short transcripts of *Tctex5* in the wild-type mice discovered here might be either consequences of alternative splicing in the exon1/exon2, or products of different promoters. The long transcript covered all three exons, whereas the short transcript covered only partial exon 2 and complete exon 3 in the wild-type mice (the $Tctex5^{short\text{-}t}$ covers partial exon 1 and complete exon 2 and 3 in the *t-haplotype*). In the *t-haplotype* mice, there were also two transcripts of *Tctex5*. There were several important mutations in the $Tctex5^{long\text{-}t}$ compared with $Tctex5^{long\text{-}+}$. Unlike its wild-type counterpart, $Tctex5^{short\text{-}t}$ did not have mutations, but had a different 5' UTR. The two transcripts in both mouse models were not equal. Therefore, we assumed that: (i) the two transcripts in the wild-type and *t-haplotype* mice might not originate from alternative splicing of mRNAs but from different promoters and (ii) there were two different *Tctex5* alleles (t^{w32} and t^{w5}, respectively) in the *t-haplotype* mouse testis that might be under the control of different promoters (Figure 1C).

We have searched the transcription factor binding sites using bioinformatics tools available at http://www.cbrc.jp/research/db/TFSEARCH.html. There were putative trans-acting binding sites covering both the transcription start site of the long and the short transcripts in the wild-type mice. We estimated that promoter 1 and promoter 2 located just upstream of exon 1 and exon 2, respectively, produced the long and short isoform of *Tctex5* in the wild-type mouse. Using northern blot analysis, the $Tctex5^{long\text{-}t}$ mRNA was found to be significantly shorter than the $Tctex5^{long\text{-}+}$ mRNA. Although the RNA ladder on the northern blot shifted the difference in size (approximately 0.2 Kb) between the $Tctex5^{long\text{-}+}$ and $Tctex5^{long\text{-}t}$ was even longer than the whole upstream sequences of the initiating ATG in the $Tctex5^{long\text{-}+}$ (169 bp). Therefore, we assumed that the $Tctex5^{long\text{-}t}$, which was obtained from RT-PCR, had both shorter 5' and 3' ends than those of $Tctex5^{long\text{-}+}$. We anticipated that in the *t-haplotype* allele 1, promoter 2 might be inactivated by mutations; therefore, only promoter 1 worked and $Tctex5^{long\text{-}t}$ was produced. The $Tctex5^{long\text{-}t}$ might be either much less abundant than the $Tctex5^{long\text{-}+}$, or be a splice variant of the longer transcript or hold a modified 5'-Cap structure because we could not detect it using RACE. Instead, the $Tctex5^{long\text{-}t}$ was obtained by RT-PCR using mRNAs isolated from the *t-haplotype* testis. In the *t-haplotype* allele 2, promoter 1 might be inactivated by some unknown mutations; therefore, only promoter 2 worked and the $Tctex5^{short}$ was produced (Figure 1C).

On average, there were 3.1 human putative alternative promoters (PAP) per gene and the richest tissue sources of the tissue-specific PAP were testis and brain [16]. *Tctex5* was expressed on both the germ cells and somatic cells isolated from testicular seminiferous epithelial cells (Han *et al.*, unpublished data). Functions of the two transcripts of *Tctex5* in the testis might be different and we anticipated that the long transcript might be a relatively testis-specific isoform. Enhanced expression of the long isoform in primary breast tumor cell lines and metastasic colon tumor cell lines cultured *in vitro* [17] might indicate a relation of *Tctex5* expression and cell division. The long transcript of *Tctex5* might have an important role in sperm function. As a candidate gene for "*Curlicue*" and "*Stop*", $Tctex5^{long\text{-}t}$ showed a variety of mutations when compared with $Tctex5^{long\text{-}+}$. The normal $Tctex5^{short}$ could not compensate the function loss caused by the $Tctex5^{long\text{-}t}$. Therefore, we predicted that the secondary structure of the *Tctex5* long peptide might be important for this function. The phosphorylation state of $Tctex5^{long}$ might be important and had an on/off function for the signal-

ing pathway(s). Although which pathways *Tctex5* might be involved in during spermatogenesis in the testis it is so for unknown, PP1 or other PP pathways were expected to be candidates, implied by the importance of PP1-biding site mutation in the *Tctex5*$^{long\text{-}t}$. *Tctex5* was very much like a signaling factor itself connecting PP1 or other PP to the signaling pathway(s) important for pachytene-to-metaphase transition in spermatogenesis.

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References

1 Wiltshire T, Park C, Caldwell KA, Handel MA. Induced premature G2/M-phase transition in pachytene spermatocytes includes events unique to meiosis. Dev Biol 1995; 169: 557–67.
2 Tash JS, Bracho GE. Regulation of sperm motility: emerging evidence for a major role for protein phosphatase. J Androl 1994; 15: 505–9.
3 Smith GD, Wolf DP, Trautman KC, da Cruz e Silva EF, Greengard P, Vijayaraghavan S. Primate sperm contain protein phosphatase 1, a biochemical mediator of motility. Biol Reprod 1996; 54: 719–27.
4 Han Y, Feng H, Cheung CK, Lam PM, Wang CC, Haines CJ. Expression of a novel T-complex testis expressed 5 (*Tctex5*) in mouse testis, epididymis and spermatozoa. Mol Reprod and Dev 2006; 74: 1132–40.
5 Huang Z, Khatra B, Bollen M, Carr DW, Vijayaraghavan S. Sperm PP1γ2 is regulated by a homologue of the yeast protein phosphatase binding protein sds22. Biol Reprod 2002; 67: 1936–42.
6 Huang Z, Vijayaraghavan S. Increased phosphorylation of a distinct subcellular pool of protein phosphatase, PP1γ2, during epididymal sperm maturation. Biol Reprod 2004; 70:439–47.
7 Ha H, Howard CA, Yeom YI, Abe K, Uehara H, Artzt K, *et al*. Several testis-expressed genes in the mouse t-complex have expression differences between wild-type and t-mutant mice. Dev Genet 1991; 12: 318–32.
8 Giffon T, Lepourcelet M, Pichon L, Jezequel P, Bouric P, Carn G, *et al*. Cloning of a human homologue of the mouse *Tctex-5* gene within the MHC class I region. Immunogenetics 1996; 44: 331–9.
9 Zhang J, Zhang L, Zhao S, Lee EY. Identification and characterization of the human HCG V gene product as a novel inhibitor of protein phosphatase-1. Biochemistry 1998; 37: 16728–34.
10 Huang HS, Pozarowski P, Gao Y, Darzynkiewicz Z, Lee EY. Protein phosphatase-1 inhibitor-3 is co-localized to the nucleoli and centrosomes with PP1γ1 and PP1α, respectively. Arch Biochem Biophys 2005; 443: 33–44.
11 Han Y, Lam EP, Cheung C, Haines CJ, Pilder SH. *Tctex5*, a candidate gene for the sperm t-complex-oolemma penetration ("stop") male sterility phenotype. Fertil Steril 2005; 84 (Suppl 1): S136.
12 Lyon MF. Male sterility of the mouse t-complex is due to homozygosity of the distorter genes. Cell 1986; 44: 357–63.
13 Redkar AA, Si Y, Twine SN, Pilder SH, Olds-Clarke P. Genes in the first and fourth inversions of the mouse t complex synergistically mediate sperm capacitation and interactions with the oocyte. Dev Biol 2000; 226: 267–80.
14 Redkar AA, Olds-Clarke P, Dugan LM, Pilder SH. High-resolution mapping of sperm function defects in the t complex fourth inversion. Mamm Genome 1998; 9: 825–30.
15 Cheng L, Myers K, Han Y, Pilder SH, Vijayaraghavan S. T complex protein *tctex5* is a candidate for the protein phosphatase 1 gamma 2 inhibitor in spermatozoa. The 38th Annual Meeting of Society for the Studies of Reproduction. Quebec City, QC, Canada; Oct 2005.
16 Kimura K, Eakamatsu A, Suzuki Y, Ota T, Nishikawa T, Yamashita R, *et al*. Diversification of transcriptional modulation: large-scale identification and characterization of putative alternative promoters of human genes. Genome Res 2006; 16: 55–65.
17 Barrett T, Suzek TO, Troup DB, Wilhite SE, Ngau WC, Ledoux P, *et al*. NCBI GEO: mining millions of expression profiles – database and tools. Nucleic Acids Res 2004; 33: 562–6.