

# Cloning and characterization of a novel isoform of calpastatin in human adult testis<sup>1</sup>

ZHU Hu, ZHOU Zuo-Min, LI Jian-Min, ZHU Hui, CHENG Li-Jun, SHAN Yu-Xi, YIN Lan-Lan, SHA Jia-Hao<sup>2</sup>  
(Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing 210029, China)

**KEY WORDS** testis; amino acid sequence; calpain; molecular cloning; comparative study; human; messenger RNA; nucleic acid sequence homology

## ABSTRACT

**AIM:** To clone a new gene related to human spermatogenesis. **METHODS:** cDNA probes of embryo and adult testis were used to hybridize the cDNA microarray of adult testis, and the clones of differential hybridization were sequenced and analyzed. **RESULTS:** A novel isoform of calpastatin exclusively and highly expressed in human adult testis was found. **CONCLUSION:** A novel isoform of calpastatin expresses in human testis and it is related to spermatogenesis.

## INTRODUCTION

Spermatogenesis in testis consists essentially of three phases: mitotic, meiotic, and postmeiotic phases. Genes expressed during this process encode proteins necessary for processes specific to different phases of germ cell development. The tissue, cell-type, and developmental stage specific genes expression is firmly controlled to produce spermatozoa<sup>[1,2]</sup>. Embryo testis is unable to produce sperms, because the related genes are shut down. In other words, in developing embryo testis, these genes begin to express and initiate the maturation of testis<sup>[3]</sup>.

Calpastatin is an endogenous inhibitory protein specifically acting on calpain. Calpastatin, as calpain, is known to be ubiquitous among various eukaryotic cells. Upon increase of intracellular  $Ca^{2+}$  concentration, calpain seems to segregate from its inhibitor and translocate to

membrane, and activate the downstream enzymes (such as protein kinase C)<sup>[4]</sup>. Calpastatin have five domains: a unique domain (domain I); four internal repeating structures of about 140 amino acid residues each, which are functional units of the inhibitor and exhibit inhibitory activities independently. The inhibitory activity of four domains is different: domain I > IV > III > II. Well-conserved sequence, TIPPXYR, are found in the center of the four repeating structure. Deletion of this conserved sequence will lead to a loss of inhibitory activity<sup>[5]</sup>.

Testis calpastatin (tCAST) (GenBank accession number: AF327443) and somatic calpastatin (sCAST) (GenBank accession number: D16217) are different in the sequences of mRNA and protein. Present study identified a novel testis-specific isoform of sCAST and tCAST.

## MATERIALS AND METHODS

**Human testis cDNA microarray** Twelve thousand positive phage clones were picked up randomly from Human Testis Large Insert  $\lambda$  phage cDNA Library (Clontech, HL5503U) and then converted into plasmid clones (according to Clontech's Manual PT3003-1). The inserts were amplified by PCR, forward primer 5' CCATTGTGTGGTACCCGGGAATTCG 3' and reverse primer 5' ATAAGCTTGCTCGAGTCTAGAGTCGAC 3', then 9216 PCR products were selected to make human testis cDNA microarray.

Automatic arrayer (BioRobotics, Cambridge, England) was used to spot the PCR products on an 8 cm  $\times$  12 cm Hybond-N nylon membranes (Amersham Pharmacia Biotech UK Ltd, Little Chalfont, Buckinghamshire, England), each spot is about 100 nL in volume and 0.4 mm in diameter. DNA was cross-linked to the Nylon membrane by UV light. Every DNA fragment was placed in two different spots (double-off set), so there are 18 432 spots of the 9216 PCR products. Plasmid pTriplEx2 vector without inserted cDNA fragment and

<sup>1</sup> Project supported by National 973 Foundation of China, NoG1999055901.

<sup>2</sup> Correspondence to Prof SHA Jia-Hao.

Phn 86-25-666-2908. E-mail shajh@njmu.edu.cn

Received 2001-02-23

Accepted 2002-01-22

pUC18 vector were spotted as negative controls. Eight housekeeping genes (ribosomal protein S9, beta actin, glyceraldehyde-3-phosphate dehydrogenase, ubiquitin, phospholipase A2, and ubiquitin thiolesterase) were evenly distributed, each in 12 places, on 8 cm × 12 cm array as intra-membrane controls.

**Probe labeling and hybridization** Total RNA of normal adult testis and embryo testis (6 months) was isolated using Trizol reagent according to manufacturer's instructions (Gibco BRL, Grand island, USA) and quantified by UV spectrometer and electrophoresis. The Poly (A)<sup>+</sup> mRNA was purified using a poly (dT) column (Qiagen, Hilden, Germany). Approximately 1–2 µL of mRNA was labeled by M-MLV reverse transcriptase according to manufacturer's instructions (Promega, Madison, WI, USA) in the presence of 200 microcurie [ $\alpha$ -<sup>32</sup>P]-deoxyadenosine 5' triphosphate (PerkinElmer, Life Science, Boston, MA, USA).

Human testis cDNA microarray were prehybridized with 20 mL prehybridization solution (6 × SSC, 0.5 % SDS, 5 × Denhardt, denatured salmon sperm DNA 100 mg/L) at 68 °C for 3 h. Then the membranes were hybridized overnight with <sup>32</sup>P-labeled adult testis and embryo testis cDNA probes in 6 mL hybridization solution (6 × SSC, 0.5 % SDS, salmon sperm DNA 100 mg/L) and followed by stringent washing (0.1 × SSC, 0.5 % SDS), 65 °C for 1 h.

**Array scanning and data analysis** Membranes were exposed to phosphor screen overnight and scanned using a FLA-3000A Plate/Fluorescent image Analyzer (Fuji Photo Film, Tokyo, Japan). Radioactive intensity of each spot was linearly scanned to 65 536 gray-grade in a pixel of 50 microns in an Image Reader and read out using the array gauge software (Fuji Photo Film, Tokyo, Japan). After subtracting the background chosen from area where no PCR product was spotted, clones with an intensity density over 10 were considered as positive signals. Signals thus obtained were further normalized of the sum of signals on the standard array. If the difference of signal intensity between the same house-keeping genes exceeded 1.5 fold, hybridization data would be considered invalid.

#### Clones of interest sequencing and analyzing

Clones of interest were selected and extracted by DNA extraction system (Qiagen, Hilden, Germany) and sequenced by the ABI PRISM<sup>®</sup> DNA Sequencer in HuaDa Gene Center in China. All sequences were blasted in GenBank, and nucleic acids and putative proteins were analyzed.

**Northern blot of tCAST** The calpastatin templet for labeling was amplified by PCR, the upstream primer is 5'-AGATTAAAACCCAGCCACG-3' and downstream primer is 5'-AGACAAAGCATCCAGAAGG-3'. It is the common region of tCAST and sCAST, which spans 531 bp of tCAST (from 960 bp to 1490 bp). [ $\alpha$ -<sup>32</sup>P] dCTP and Ready-To-Go<sup>™</sup> DNA Labeling Beads (-dCTP) from the Amersham Pharmacia Biotech were used to label the probes. The labeling process was following the instruction of manual.

Multiple Tissue Northern (MTN<sup>®</sup>) blots (Clontech Co) were hybridized with the probes overnight at 68 °C with continuous shaking. The blot was rinsed in wash solution 1 (2 × SSC, 0.05 % SDS) several times at room temperature, and washed twice for 30 min with continuous agitation. Finally the blots were washed twice in wash solution 2 (0.1 × SSC, 0.1 % SDS) with continuous shaking for 30 min at 50 °C.

The blot was placed on the storage phosphor screen (Packard company) and exposed for 3 h in the dark. The signal was detected at the Cyclone storage phosphor system (Packard company).

## RESULTS

A positive signal was found highly expressed in adult testis. The signal intensity hybridized with adult testis probe was 137.51, and that with embryo testis probe was 38.02. The level of adult was about 3.62 fold stronger than that of embryo (Fig 1). After comparing the sequences with GenBank data, two fragments (612 – 2267 bp, 99 % and 110 – 644 bp, 100 %) were found having higher homology with human somatic calpastatin. The difference between tCAST and sCAST might be due to alternative splicing. The nucleic acid length of tCAST was 2290 bp, and the putative protein had 590 amino acids with molecular mass of 63 673 and isoelectric point of 4.56 (Fig 2).

The cDNA sequences of tCAST and sCAST were different. Fragment of 109 bp at 5' terminus of tCAST had no homology with 290 bp in 5' terminus of sCAST. The region from 796 to 834 bp (about 39 bp) of sCAST was lost in the tCAST, and there were two single nucleotide mutations in the tCAST, C1385 and G1937 of sCAST changed to G1165 and A1717 of tCAST, respectively. The last 7 nucleotides of their 3' terminus were also different. Compared with sCAST, the putative protein of tCAST lost 105 amino acids at its N terminus

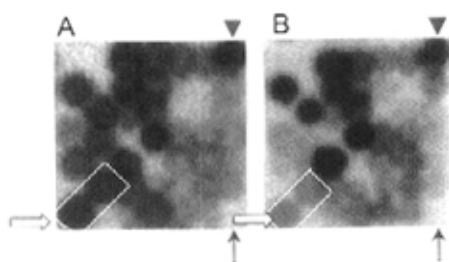


Fig 1. Hybridization results of adult (A) and embryo (B) testis cDNA probe. (↑) is the clone of interest. (▼) is the positive control. (◊) is the blank control.

and 13 amino acids in the center. And there were two single amino acid mutations, S408 and G592 of sCAST changed to C388 and E572 of tCAST, which corresponded to nucleotide mutation.

We used the common region of DNA of tCAST and sCAST as a probe to examine the distributions of two isoforms of calpastatin in various human tissues by Northern blot analysis (Fig 3). The results showed the presence of three distinct mRNA species at approximately 2.3, 2.5, and 4.6 kb. The hybridizing bands of approximate 2.5 kb and approximate 4.6 kb were ubiquitously distributed, and the level of these two transcripts appeared to be the highest in the testis. The band of approximate 2.5 kb was from sCAST. The band of 4.6 kb might be a novel isoform of calpastatin which has not been identified. The transcript of 2.3 kb was from tCAST, and it was exclusively and highly expressed in the testis.

After submitting the nucleotide acid sequences of tCAST and sCAST to GenBank, the query results showed that, in human genome draft, they all located in a DNA clone CTD2358N4, and tCAST was encoded within the sCAST. The first exon of tCAST (109 bp in 5' terminus) was a new exon between the fourth and fifth exon of sCAST. The 39 bp deleted in tCAST happened to be the 11th exon of sCAST. Furthermore after submitting the amino acid sequences of tCAST to GenBank and searching for the homologous domain, we found that tCAST had four repeating calpain inhibitory domains, but lacked the domain L of sCAST.

The prediction of hydrophobicity, phosphorylation site, and secondary structure of tCAST protein was performed by OMIGA software. The results suggested that the first sixteen amino acids had strong hydrophobicity and might be a signal peptide; 13 amino acids lost in the center of tCAST had a protein kinase C

phosphorylation site (SSK) which had a  $\beta$ -turn in the secondary structure, linking  $\alpha$ -helixes in two sides.

## DISCUSSION

Calpain and calpastatin are involved in numerous membrane fusing events. Such as neural vesicle exocytosis, platelet and red-cell aggregation<sup>[6,7]</sup>. Calpastatin modulation of m-calpain is necessary for myoblast fusion; a decrease of calpastatin accelerates fusion<sup>[8]</sup>. Yudin<sup>[9]</sup> found that calpain and calpastatin were colocalized in the region between the plasma membrane and the outer acrosomal membrane of cynomolgus macaque sperm. So calpastatin may interact with calpain and inhibit its activity. Only before the acrosome reaction, when a large scale and sudden influx of  $\text{Ca}^{2+}$  is taking place, calpastatin is detached from calpain, and calpain is activated, acrosome reaction is thus triggered.

The analysis of tCAST nucleic acid and protein suggests that tCAST may be a testis-specific form of sCAST via alternative splicing. The putative protein contains the four inhibitory domains. When compared with sCAST, tCAST protein has two single amino acid changes and large scale deletions; domain L and 13 amino acids between inhibitory domain I and II. Similar results have been reported in other papers; calpastatin cDNA of human liver has two long deletions in domain L and I<sup>[10]</sup>; calpastatin cloned from rat liver cDNA library has two deletions in domain L<sup>[11]</sup>; and there is no domain L and I in the human erythrocyte<sup>[12]</sup>. All these calpastatins have long deletion in domain L and I, but they still have inhibitory activities. The changes of tCAST might affect its three-dimension structure, inhibitory activity, and subcellular location, but more evidence is needed to confirm it.

There are two types of cells in human body, somatic cell and germ cell. From ours and others data, we know that many gene expression in germ cells are different from that in somatic cells, so expressed proteins have two isoforms; somatic type and testis-specific type. Switching of promoters from somatic sites to testis-specific sites is known in some genes, it may be caused by testis-specific promoters and its corresponding transcription factors<sup>[13-15]</sup>. In this case, the testis-specific calpastatin promoters may change the normal transcription initial site that took place in somatic cells to testis-specific transcription initial site, and transcribe a novel testis-specific isoform of calpastatin.

1	AGAACATAGGGTAC1CGAG1GAAGAATTCAGCAGCTAAGTGAAGTGTCTCTATTC		
61	ACTTTAAATACTTTTATCTACTGAATCGCAAGAAAATGGGTTTCTGGTGTCTGTGG		
121	TTTCATGAGAAAAATCCCAAGAGGAAGCCAAAAGAACACACAGAGCCAAAAGCCTAC		
181	CCAAGCAGGCATCAGATACAGGAAGTAACGATGCTCACAATAAAAAAGCAGTTCCAGAT		
241	CAGCTGAACAGAGCCATCAGAGAAATCAACAGAACCAAGACTAAACCACAGACATGA		
	<b>M</b>		
301	TTTCTGCTGGTGGAGAGAGTGTGTGCTGATCACTGCAATATCTGGCAAGCCGGGTGACA	1	
	<b>I S A G G E S V A G I T A I S G K P G D</b>	21	
361	AGAAAAAGAAAAAATCATTAAACCCAGCTGTGCCAGTTGAATCTAAACCGGATAAAC		
	<b>K K K E K K S L T P A V P V E S K P D K</b>	41	
421	CATCGGGAAAGTCAGGCATGGATGCTGCTTGGATGACTTAATAGATACTTTAGGAGGAC		
	<b>P S G K S G M D A A L D D L I D T L G G</b>	61	
481	CTGAAGAACTGAAGAAGAAAATACAAGTATACTGGACCAGAAGTTTCAGATCCAATGA		
	<b>P E E T E E E N T T Y T G P E V S D P M</b>	81	
541	GTTCCACTACATAGAGGAATTGGGTAAGAGAAAGTCACAATTCTCCAAAATATAGGG		
	<b>S S T Y I E E L G K R E V T I P P K Y R</b>	101	
601	AACTATTGGCTAAACCCATAGGCGCAGATGATGCTATAGACCGCTTGTCATCTGACTTCA		
	<b>E L L A W P I G P D D A I D A L S S D F</b>	121	
661	CCTGTGGGTGCGCTACAGCTGCTGGAAGAAAAGTGAAGAGAGGAATCTACAGAAATTT		
	<b>T C G S P T A A G K K T E K E E S T E V</b>	141	
721	TAAAGCTCAGTCAGCAGGAGCAGTCAGAAGTGTGCTCCACCCCAAGAGAAGAAAAGAA		
	<b>L K A Q S A G T V R S A A P P Q E K K R</b>	161	
781	AGGTGGAGAAGGATACAATGAGTGATCAAGCACTCGAGGCTGTGCGGCTTCACTGGGCA		
	<b>K V E K D T M S D Q A L E A L S A S L G</b>	181	
841	CCCGGCAAGCAGAACTGAGCTCGACCTCGGCTCAATTAAAGGAAGTCGATGAGGCAAAAG		
	<b>T R Q A E P E L D L R S I K E V D E A K</b>	201	
901	CTAAGAGAAAACTAGAGAAAGTGGTGAGGATGATGAACAATCCCATCTGAGTACA		
	<b>A K E E K L E K C G E D D E T I P S E Y</b>	221	
961	GATTAAACCAAGCAAGGATAAAGATGGAAGCACTATTGCCAGAGCCTGAAGAAAAAC		
	<b>R L K P A T D K D G J K P L L P E P E E K</b>	241	
1021	CCAAGCCTCGGAGTGAATCAGAACTCATGTAACCTTCAGAAAGATTTGACCGTCTG		
	<b>P K R S E S E L I D E L S E D F D R S</b>	261	
1081	AATGTAAAGAGAAACCATCTAAGCCAACTGAAAGACAGAAAGATCTAAGGCCGCTGCTC		
	<b>E C K E K P S K P T E K T E E S K A A A</b>	281	
1141	CAGCTCTCTGTGTGGAGGCTGTGTGTGGAACCTCCATGTGTAGTATACAGTCAGCACCCC		
	<b>P A P V S E A V C R T S M C S I Q S A P</b>	301	
1201	CTGAGCCGGCTACCTTGAAGGGCAGATGCCAGATGATGCTGTAGAAGCCTTGGCTGATA		
	<b>P E P A T L K G T V P D D A V E A L A D</b>	321	
1261	GCCTGGGAAAAAGGAACAGATCCAGAAGATGGAAGCACTGTGATGGATAAAGTCAAGG		
	<b>S L G K K E A D P E D G K P V M D K V K</b>	341	
1321	AGAAGGCCAAAGAAAGAACCGTGAAGAGCTTGGTGAAAAAGAAACAATTCCTCTG		
	<b>E K A K E E D R E K L G E K E E T I P P</b>	361	
1381	ATTATAGATTAGAAGAGGTCAAGGATAAAGATGGAAGGCCACTCTGCCAAAAGAGTCTA		
	<b>D Y R L E E V K D K D G K P L L P K E S</b>	381	
1441	AGGAACAGCTTCCACCATGAGTGAAGACTTCTTCTGGATGCTTGTCTGAGGACTTCT		
	<b>K E J Q L P P M S E D F L L D A L S E D F</b>	401	
1501	CTGGTCCACAAAATGCTTCATCTCTTAAATTGAAGATGCTAAACTTGCTGCTGCCATCT		
	<b>S G P Q N A S S L K F E D A K L A A A I</b>	421	
1561	CTGAAGTGGTTTCCCAACCCAGCTTCAACGACCCAGCTGGAGCCCAACCCCGTGATA		
	<b>S E V V S Q T P A S T T Q A G A P P R D</b>	441	
1621	CCTGCGCAGAGTGACAAAGCCTGATGATGCTTGGATAAACTCTCTGACAGTCTAGGAC		
	<b>T S Q S D K D L D D A L D K L S D S L G</b>	461	
1681	AAAGGCAGCCTGACCCAGATGAGAACAACCAATGGAAGATAAAGTAAAGCAAAAAGCTA		
	<b>Q R Q P D P D E N K P M E D K V K E K A</b>	481	
1741	AAGCTGAACATAGAGACAAGCTTGGAGAAAGAGATGACACTATCCCACTGAATACAGAC		
	<b>K A E H R D K L G E R D D T I P P E Y R</b>	501	
1801	ATCTCTGGATGATAATGGACAGGACAAACAGTGAAGCCACCTACAAGAAATCAGAGG		
	<b>H L L D D N G Q D K P V K P P T T K K S E</b>	521	
1861	ATTCAAAGAACTGCAGATGACCAAGACCCCATGATGCTCTCTCAGGAGATCTGGACA		
	<b>D S K K P A D D Q D P I D A L S G D L D</b>	541	
1921	GCTGTCCCTCCACTACAGAAACCTCACAGAACACAGCAAGGATAAGTGCAAGAAGGCTG		
	<b>S C P S T T E T S Q N T A K D K C K K A</b>	561	
1981	CTTCCAGCTCCAAAGCCTAAGAATGGAGGTAAGCGAAGGATTACGCAAGACAAACAG		
	<b>A S S S K A P K N G G K A K D S A K T T</b>	581	
2041	AGGAAACTTCAAGGCAAAAGATGACTAAAGAAATACAAGTTAAGGTATCTGGATCTGC		
	<b>E E T S K P K D D</b>	590	
2101	ATGTAAATCTTCAAGCTGGTGGATGGTGACTTTTGAAGAACAAAAGGCTTGGCAACAGA		
2161	AAACAATGTCTGGGTGATTTCTAGAATGGTTTGTGTGAGTCTCTGAACATCTTAAAT		
2221	ATTGGTTGTATTCTTTCCAGAAAGAAAATGAATTTGACTGGTTCACCTGCCAAAAAA		
2281	AAAAAAA		

Inhibitory domain I

Inhibitory domain II

Inhibitory domain III

Inhibitory domain IV

Fig 2. Nucleic acid sequences and putative amino acid sequences of tCAST. Amino acid sequences in four gray box are four calpain inhibitory domains, which are the queried results from GenBank homologous domain blast.

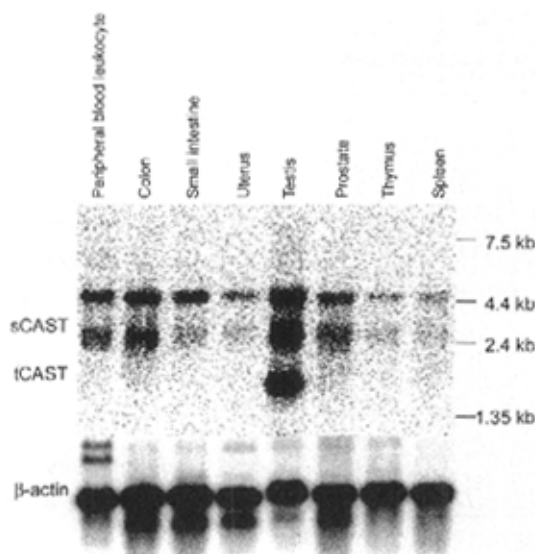


Fig 3. Northern blot of calpastatin in different tissues. sCAST: somatic calpastatin; tCAST: testis calpastatin.

In conclusion, a novel isoform of calpastatin specifically and highly expressed in human adult testis was found and it was related to spermatogenesis.

## REFERENCES

- Nayernia K, Adham I, Kremling H, Reim K, Schlicker M, Schluter G, *et al.* Stage and developmental specific gene expression during mammalian spermatogenesis. *Int J Dev Biol* 1996; 40: 379-83.
- Eddy EM. Regulation of gene expression during spermatogenesis. *Semin Cell Dev Biol* 1998; 9: 451-7.
- McCarrey JR. Spermatogenesis as a model system for developmental analysis of regulatory mechanisms associated with tissue-specific gene expression. *Semin Cell Dev Biol* 1998; 9: 459-66.
- Lee WJ, Ma H, Takano E, Yang HQ, Hatanaka M, Maki M, *et al.* Molecular diversity in amino-terminal domains of human calpastatin by exon skipping. *J Biol Chem* 1992; 267: 8437-42.
- Kawasaki H, Emori Y, Imajoh-Ohmi S, Minami Y, Suzuki K. Identification and characterization of inhibitory sequences in four repeating domains of the endogenous inhibitor for calcium-dependent protease. *J Biol Chem* 1989; 264: 274-81.
- Kosower NS, Glaster T. The calpain-calpastatin system and membrane fusion. In: Mellgren RL, Murachi R, editors. *Intracellular calcium-dependent proteolysis*. Boca Raton (FL): CRC Press; 1990. p163-79.
- Kosower NS, Bamoy S. Calpastatin (the endogenous calpain inhibitor) and membrane protein degradation in cell fusion.

*Methods Mol Biol* 2000; 144: 181-94.

- Bamoy S, Glaster T, Kosower NS. The calpain-calpastatin system and protein degradation in fusing myoblasts. *Biochim Biophys Acta* 1998; 1402: 52-60.
- Yudin AI, Goldberg E, Robertson KR, Overstreet JW. Calpain and calpastatin are located between the plasma membrane and outer acrosomal membrane of cynomolgus macaque spermatozoa. *J Androl* 2000; 21: 721-9.
- Asada K, Ishino Y, Shimada M, Shimojo T, Endo M, Kimizuka F, *et al.* cDNA cloning of human calpastatin; sequence homology among human, pig, and rabbit calpastatins. *J Enzyme Inhib* 1989; 3: 49-56.
- Ishida S, Emori Y, Suzuki K. Rat calpastatin has diverged primary sequence from other mammalian calpastatins but retains functionally important sequences. *Biochim Biophys Acta* 1991; 1088: 436-8.
- Takano E, Nosaka T, Lee WJ, Nakamura K, Takahashi T, Funaki M, *et al.* Molecular diversity of calpastatin in human erythroid cells. *Arch Biochem Biophys* 1993; 303: 349-54.
- Ito E, Toki T, Ishihara H, Ohtani H, Gu L, Yokoyama M, *et al.* Erythroid transcription factor GATA-1 is abundantly transcribed in mouse testis. *Nature* 1993; 362: 166-8.
- Goto M, Tamura T, Mikoshiba K, Masamune Y, Nakanishi Y. Transcription inhibition of the somatic-type phosphoglycerate kinase 1 gene *in vitro* by a testis-specific factor that recognizes a sequence similar to the binding site for Ets oncoproteins. *Nucleic Acids Res* 1991; 19: 3959-63.
- McCarrey JR, Berg WM, Paragioudakis SJ, Zhang PL, Dilworth DD, Arnold BL, *et al.* Differential transcription of Pkg genes during spermatogenesis in the mouse. *Dev Biol* 1992; 154: 160-8.

成人睾丸中一种新的 calpastatin 同源蛋白的克隆和分析<sup>1</sup>

R96 A

朱虎, 周作民, 李建民, 祝辉, 成丽俊, 单玉喜, 尹岚岚, 沙家豪<sup>2</sup> (南京医科大学江苏省生殖医学重点实验室, 南京 210029, 中国)

**关键词** 睾丸; 氨基酸序列; 卡配因; 分子克隆; 对比研究; 人类; 信使 RNA; 核酸序列同源性

**目的:** 克隆人精子发生相关的基因. **方法:** 使用人胚胎和成人睾丸 cDNA 探针与本院制作的人睾丸 cDNA 微阵列杂交, 差异杂交的克隆进行序列测定和分析. **结果:** 发现 calpastatin 在睾丸中表达的一种新的同源蛋白, 在睾丸中高度表达. **结论:** calpastatin 在睾丸中是以一种新的同源蛋白形式表达, 可能与精子发生相关.

(责任编辑 朱倩蓉)