Blackwell Publishing

Asian J Androl 2005; 7 (2): 205–211
DOI: 10.1111/j.1745-7262.2005.00024.x

AJA

·*Original Article*·

Identification and characterization of *cul-3b*, a novel hominine CUL-3 transcript variant

Li Lu, Zuo-Ming Zhou, Xiao-Yan Huang, Min Xu, Lan-Lan Yin, Hui Wang, Zhi-Yang Xu, Jia-Hao Sha

Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing 210029, China

Abstract

Aim: To identify genes related to the human testis development by substrate hybridization technique. **Methods:** A human testis cDNA microarray was constructed and hybridized with probes prepared from human adult and fetal testes and spermatozoa mRNAs by reverse transcription reactions. The differentially expressed genes were sequenced. And a newly identified cullin-3 (CUL-3) transcript variant (designated *cul-3b*) was bio-informatically analyzed with an online GenBank database. Multi-tissue reverse transcription polymerase chain reaction (RT-PCR) was used to determine the tissue expression profile of *cul-3b*. **Results:** *Cul-3b*, a novel CUL-3 transcript variant, was identified. The expression level of *cul-3b* in adult testes was 3.79-fold higher than that in fetal ones. *Cul-3b* differed from *cul-3* (including NM_003590 and AY337761) in the opening reading frame and had three internal ribosomal entry sites (IRESes) in the 5'-UTR. These led to a 24 amino acid (aa) truncation at N-terminus of CUL-3b as compared with CUL-3 and a more motivated expression pattern of *cul-3b* under some strict circumstances. Additionally, *cul-3b* expressed ubiquitously in human tissues according to multi-tissue RT-PCR. **Conclusion:** *Cul-3b* is a novel transcript variant of CUL-3, which may be important not only for the development of human testis but also for that of other organs. *(Asian J Androl 2005 Jun; 7: 205–211)*

Keywords: alternative splicing; CUL-3; DNA sequence; human testis; microarray

1 Introduction

Spermatogenesis, the fundamental function of testes, occurs in successive mitotic, meiotic, and postmeiotic phases of germ cells, with the germ cells moving from the periphery to the lumen of the seminiferous tubule during this process. Spermatogenesis is controlled by a number of genes. Therefore, characterization of these genes in human fetal and adult testes and their related transcript variants are of great importance. In this study, a human testis cDNA microarray was generated to screen out differentially transcribed genes, of which a new transcript variant of CUL-3 (designated *cul-3b*) was identified and sequenced. Bioinformatical analysis revealed that CUL-3b was 24 aa shorter than CUL-3 at the N-terminus and the three internal ribosomal entry sites (IRESes) contained in the 5'-UTR of *cul-3b* (not in *cul-3*) implicated that *cul-3b* could be translated even under some strict circumstances. CUL-3 is recognized to play an important role in a ubiquitin system that selectively degrades short-lived proteins in eukaryotic cells [1, 2], which are involved in the male reproductive system [3]. Furthermore multi-tissue reverse transcription-polymerase chain reaction (RT-PCR) results showed that *cul-*

Correspondence to: Dr Jia-Hao Sha, Key Laboratory of Reproductive Medicine, Nanjing Medical University, Hanzhong Road, Nanjing 210029, China.
Tel/Fax: +86-25-8686-2908
E-mail: shajh@njmu.edu.cn
Received 2004-09-23 Accepted 2004-12-08

3b was widely transcribed in various tissues. All this implies that *cul-3b* is important not only for the development of human testes but also for that of other organs.

2 Materials and methods

2.1 Construction of human testis cDNA microarrays

The human testis 5'-STRETCH PLUS cDNA library was from Clontech (Palo Alto, CA, USA [catalogue number HL5503U, source of inserted cDNAs was 25 Caucasians aged 20–65, average length of inserted cDNAs was approximately 3.4 kb]). Each λ TriplEx2 clone was converted to a p TriplEx2 clone and excised with *Escherichia coli* BM25.8, and the complete plasmids were circularized from the recombinant phage according to the instruction manuals.

The plasmids were extracted by the conventional alkaline lysis method [4], and the samples were stored in 96 well trays at –70 ℃. The gene sequences were amplified from the plasmids clone by PCR to produce DNAs for spotting the microarray. Primers were designed from the 5' and 3' sequences of λ TriplEx2 vector flanking the insert. The 5' primer was *cca ttg tgt tgg tac ccg gga att cg*, interval 6 bp to inserted cDNA site, and the 3' primer was *ata agc ttg ctc gag tct aga gtc gac*, interval 7 bp to inserted cDNA site. In total, 9216 clones selected randomly from the cDNA library were amplified. The PCR products were 2.0–7.0 kb. They were stored at –20 ℃.

A cDNA array was assembled with 9216 samples of PCR product. PCR templates were dotted on an 8 cm × 12 cm nylon membrane (Amersham Pharmacia Biotech (England, UK), Bucks, lot number YA1103) using an automatic arrayer (BioRobotics, Cambridge, UK). Two dots for each sample were applied: a total of 18 432 dots representing 9216 samples. DNA templates were cross-linked to the nylon membrane by UV light. Eight housekeeping genes were used as positive controls: *ribosomal protein S9* (RPS9), *actin gamma*, *G3PDH*, *HPRT1*, Homo sapiens mRNA for a 23 kDa highly basic protein, *ubiquitin C*, *phospholipase A2* and *UCHL1*. TriplEx2 phage DNA and PUC18 plasmid DNA were used as negative controls. Twelve spots were distributed on each membrane for each control cDNA.

2.2 Screening of differentially expressed genes in adult or fetal testes

Testes from deceased human adults (n = 2) and spontaneously aborted 6-month-old fetuses (n = 3) were collected. Adult and fetal testes were homogenized separately. Total mRNA was extracted according to the Trizol RNA isolation protocol (Gibco BRL, Grand Island, USA) and quantified with a UV spectrometer after electrophoresis. The Poly(A)+ mRNA was purified using an affinity column filled with poly(dT) resins (Qiagen, Hilden). The probes were prepared by incorporation of [^{33} P]-dATP in a reverse transcription reaction using 2 μg purified mRNA as the template, and an oligo(dT) as the primer with Moloney murine leukemia virus (M-MLV) reverse transcriptase. Each labeling reaction was carried out with 200 μCi_[α-^{33}P] dATP according to the manufacturer instructions (NEN Life Science, Boston, USA).

Nylon membranes spotted with cDNA fragments were prehybridized with 20 mL prehybridization solution (6 × sodium chloride–sodium citrate buffer [SSC], 0.5 % SDS, 5 × Denhardt compound, 100 μg denatured salmon sperm DNA/mL) at 68 ℃ for 3 h. Overnight hybridization with the ^{33}P-labeled cDNA from testis samples was carried out in 6 mL hybridization solution (6 × SSC, 0. 5 % SDS, 100 μg denatured salmon sperm DNA/mL), and followed by stringent washing with 20 mL wash solution (10 % SSC, 0.5 % SDS) at 65 ℃ for 1 h. Membranes were exposed to phosphor screen overnight and scanned using an FLA-3000A fluorescent image analyzer (Fuji Photo Film, Tokyo, Japan). The radioactive intensity of each spot was linearly scanned with a 65 536 gray-grade and a pixel size of 50 μm, and interpreted using the array gauge software (Fuji Photo Film, Tokyo, Japan). After subtraction of the background from an area where no PCR product was spotted, clones with an intensity density >10 were considered as positive signals. Hybridization data would be considered invalid if there was a difference of >1.5-fold in the intensity of any of the 12 control spots for the same control cDNA between arrays. The hybridization intensity of corresponding dots in adult and fetuses were compared. If the difference in values of spot intensity in an adult and fetus was more than 3-fold higher or lower, the corresponding genes were considered differentially expressed.

2.3 Sequencing and gene analysis

Plasmids containing differentially expressed genes were amplified in *E. coli* bacteria and purified by using a plasmid extraction kit (QIAprep Spin Miniprep Kit, Qiagen, Hilden, Germany). Gene sequence was deter-

mined by ABI377 automatic sequencer (Applied Biosystems, CA, USA) and the related homologies were searched in GenBank with BLAST tools. The nucleotides and deduced amino acid sequences were analyzed by Omiga, GenRunner and other online softwares.

2.4 RT-PCR of cul-3b

The expression profile of a newly identified transcript variant of CUL-3, named *cul-3b*, was investigated by PCR. Multiple human tissue cDNA panels including brain, heart, kidney, liver, lung, pancreas, placenta and skeletal muscle were from Clontech (number 1420-1). G3PDH was used as a positive control. The *cul-3b* specific primers were: upstream 5'-*tac tgt gct ctg ggc tgt g*-3' and downstream 5'-*aga agg gcc caa atg ctg*-3', to amplify from nucleotide 278 to 644; the resulting product was 367 base pairs. The upstream primer was located in the specific region of *cul-3b*, and the downstream primer was homologous with *cul-3*. PCR reaction system included 2 μL cDNA template (0.4 ng), 2 μL 10 × reaction buffer, 1.5 μL $MgCl_2$ (25 mmol /L), 1.5 μL dNTP (2 mmol /L), 1μL of each primer (5 pmol/μL), 0.2 μL Taq polymerase (5 U/μL) and 10.8 μL water to a final volume of 20 μL. PCR procedure was 94℃ for 30 s, 52 ℃ for 1 min and 72 ℃ for 1 min. And the amplification cycles were forty. The PCR products were analyzed in 1.5 % agarose gel. RT-PCR of *cul-3b* in human adult and fetal testes as well as in spermatozoa (purified from collected semen) was done under the same PCR conditions described above to confirm the microarray-hybridization results. The human adult testis was taken from a deceased person (37-year-old man). The fetal testis was taken from an accidentally aborted fetus of six months. Human ejaculates were obtained from healthy volunteers of proven fertility and normal semen quality according to WHO criteria (1999). All the samples were obtained after ethics approval and consent from all participants.

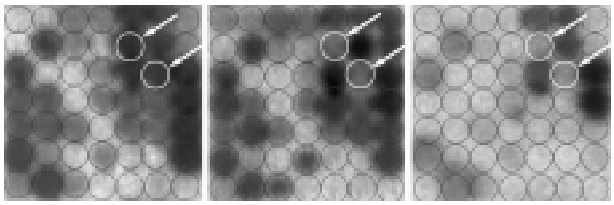
3 Results

3.1 Differential expression of genes in adult and fetal testes

The microarray containing 9216 human clones was hybridized with cDNA probes prepared from the mRNA of adult and fetal testes. There were 731 clones altogether which expressed differentially in adult or fetal testes, among which 592 clones were highly expressed in adult testes and 139 clones were highly expressed in fetal testes. The difference of expression signal was at least 3-fold. The up-regulation of 592 genes in adult testes and the down-regulation of 139 genes in fetal testes implicated that expression of these genes regulates human testis development and spermatogenesis.

3.2 Sequencing results and Bioinformatical analysis of cul-3b

Sequencing results of all the differentially expressed genes showed there were 37 novel genes and 74 novel transcript variants of known genes that had not been reported. One of the novel transcript variants was a cullin-3 gene, named *cul-3b* (GenBank accession number AY337761). This gene was expressed in both adult and fetal testes, but the expression level in adult testes was approximately 3.79-fold higher than that in fetal testes (Figure 1). *Cul-3b* was 3168 bp and contained a single ORF (nt 607-2838). The putative translation initiation site within the sequence (ACCATGG) complied with Kozak's rule [5] (Figure 2), which supported the prediction of the initiation site. The deduced peptide was 744 amino acids of 86.2 kDa. Although CUL-3b was 24 aa shorter in N-terminus than CUL-3, no difference existed between their domains when analyzed with the SMART program (http://smart.embl-heidelberg.de/). Comparisons of their exons between *cul-3* and *cul-3b* on human chromosome are presented in Figure 3. Moreover, since the 5' UTRs of *cul-3* and *cul-3b* were different (Figure 3), we searched for UTR elements in UTRHome (http://bighost.area.ba.cnr.it/BIG/UTRHome/) and found three IRESes in the 5' UTR of *cul-3b* but not in that of *cul-3*. The promoters also seemed to be different according to the online program PROSCAN version



(a) (b) (c)

Figure 1. Hybridization using human cDNA microarray revealed differential mRNA level of *cul-3b* in human adult (a) and fetal (b) testes and sperms (c) (arrowhead oriented). The hybridization intensities for *cul-3b* were 140.63, 37.13 and 55.90, respectively.

1 aggacagagttctcagggtgaaaatgatttaaatggctctggaaaagtagtatacaagag
61 atgtaggggaataatgttttctaaaagaaagctacgttatactgaggtgcatttatcata
121 gtgttattcttaaggtgcctttgttttagctcatatctgtatatttgctaagcttgaaaa
181 agctatatttctttctgacatttatttgatgactgtcgatgagcgttcaagagattctat
IRES(1)
241 cattgggcactttcctttgttgatgcatgagggatta***tactgtgctctgggctgtg***ttaa
301 atctactgacgttctcatttaatcttcatgacagccctttgagatagatgctgttattga
IRES(2)
361 acccaggacttaattttctcaaggtcacatggcttgtgagtaacaaagctgtggcacaaa
421 cctaaggctgtctgacagcaaaacccatgttctctaccagtatactaactatgtttacca
481 agataatcagtgactgtgatgttactttaccatattagcacaatggaatgtgacattagt
541 ccactgatttggactaggttttgggatatataagttctaatcccagctctatgattttgg
IRES(3)
Kozak Signal
601 atgacc**atg**gatgaaaaatatgtaaa***cagcatttgggaccttct***gaaaaatgcaattcaa
M
661 gaaatccagcgtaagaataacagtggtcttagttttgaggagctctatagaaatgcatat

Figure 2. 5'-terminus nucleotide sequence (1nt – 720 nt) of *cul-3b*. The start codon (in bold) captioned with Kozak Signal was underlined and the first amino acid (M) was shown as an indication of translation initiation. The location of primers used for PCR was presented by boxing the two italic and bold sequences. The translation-regulatory motifs of IRESes were boxed and further shadowed. The alternative splicing site is indicated by ▲.

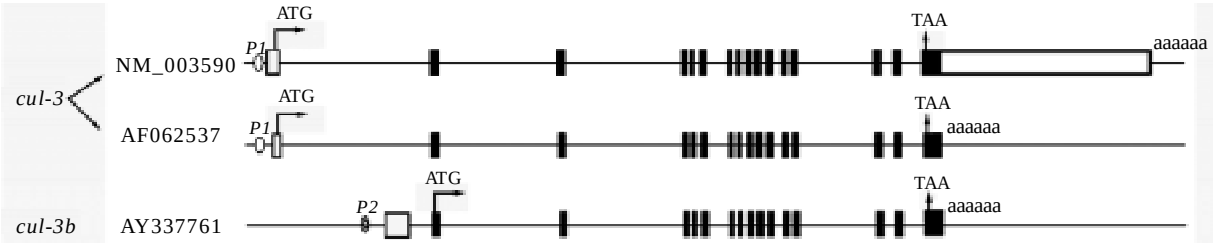


Figure 3. Transcriptional and translational patterns of cul-3b (AY337761) and cul-3 (AF062537 and NM-003590). Identical exons were shown with black rectangles and the different ones were presented with empty rectangles, in which the novel exon of AY337761 was filled with gray. The putative translation start and stop locations were arrow marked. P1(⇨) and P2 (⇨) were shown as the different promoters.

1.7 (http://bimas.dcrt.nih.gov/molbio/proscan/) when the 5000 bp upstream sequence of both *cul-3* and *cul-3b* were analyzed. The predicted promoter sequences and the significant regulatory elements within the 5000 bp upstream region are presented in Figure 4.

3.3 Multi-tissue expression

Cul-3b was ubiquitously expressed in the 16 tissues (Figure 5) and the PCR result in adult, fetal testes and sperm were consistent with that of microarray (Figure 6).

4 Discussion

In this study, a human testis cDNA microarray was used to identify genes related to the human testis development. By this method, 731 genes altogether were screened out when a significant difference was set at the

P1

CATCTCACTGCGCAGGCCGAACGTCGTCCTCCTCCTCCTCCTTCTCCTCCTCCGCCTGAG
CGAGACGAGATCCGGCCCAGAGGGTGGAGGGGGGAGGAGGGAGGGAGGAGAGCCGG
AGAGTGAGAGATGCCGGGGGAGGGAGGTGGGAGGACGGGAGGGGGAGCCCGGCGGCC
GCCGCGGGGGACGCTCAGATCTCGCGAGAAGAGGGCGAGCGCGCTGCCCCCCTGGTGG
GCGGGGCGAAGCCCGGGAGA

Sp1

P2

TCTGGGTGGCTAGGTGCATTGTCCAGTAGAAAAAATACTAAAAGGCAATCCCTTACTGA
CAAGGTACTTCCTGACGTCAGGGACAAAGCACTGATGGAACCAATCCGGAAA

CREB/ATF

AAGAGTTCTCACTGTCCAGGCCAGGCCTTTTTGAACAACCAAAAGACTGGCAGCTAGTG
TTTATCTTTTCTCTTCAAGGCTCAGGGGTTAGTAGATTTATAGATAAAGTCAGCCATGAT
CAAAAACCTGACTGCACTTGC

Figure 4. *P1*, *cul-3* promoter, confirmation score (shown by the program, which presents the probability of predicted promoter, the cutoff score is 53.00) 84.07, locates 4 bp upstream of NM_003590 transcription initiation; *P2*, *cul-3b* promoter, confirmation score 70.24, locates 2859 bp upstream of AY337761. The significant regulatory elements were boxed and further shadowed.

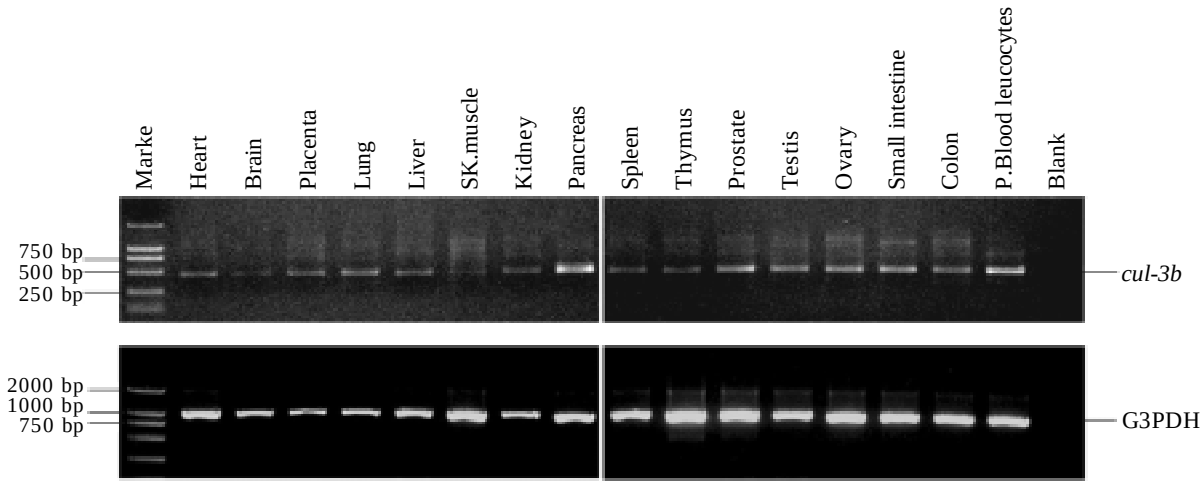


Figure 5. Tissue distribution of *cul-3b* (C*ul-3b* amplified with RT-PCR). G3PDH presents as a positive control.

level of adult/fetal or fetal/adult testis signal ratio =3-fold, of which there were 37 novel genes and 74 novel transcript variants of known genes [6]. Here, we specifically characterized a novel transcript of CUL-3, named *cul-3b*. *Cul-3b* mRNA was found to exist in human fetal testes, adult testes and spermatozoa (Figures 1, 6). The expression level of *cul-3b* in adult testes was 3.80-fold higher than that in fetal testes (Figure 1). Therefore we could reasonably postulate that *cul-3b* may play an important role in the process of human spermatogenesis.

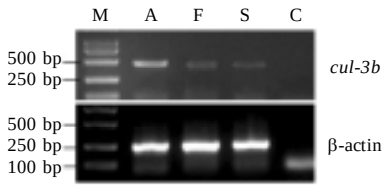


Figure 6. C*ul-3b* amplified with RT-PCR in adult (A) (one individual), fetal (F) (one individual) testes and sperm (S) (three individuals). Marker (M) on the left and negative control (C) on the right. β-actin presents as the positive control.

As the completion of the Human Genome Project facilitates gene analysis under a known background, the genomic structure of *cul-3b* could be easily deduced by a BLAST search in GenBank. Two versions of *cul-3* (AF062537, 2.8-kb and NM_003590, 4.3-kb) had been reported according to GenBank databases. *Cul-3b* was 3.2-kb, shorter than NM_003590 at 3'-terminus whereas longer than AF062537 at 5'-terminus as aligned in Figure 2. And most of all, its transcription seemed to be initiated at the location between the first and second exons of *cul-3*, thus resulting in translational initiation alteration and protein product shortening. The transcription of *cul-3b* seemed to be promoted by a predicted promoter that differed from the one that promotes *cul-3* transcription, and the two promoters could be differently controlled according to the predicted elements in them. Furthermore, the prediction of the three IRESes in 5'-UTR of *cul-3b* implicated possible IRES translational initiation, which is quite different from the normal initiation mode of Cap-dependent initiation. IRES was first identified in picornavirus for its ability to initiate mRNA translation. After that, a number of IRESes were identified in other viral and cellular mRNAs by functional experiments. Noticeably, viral IRESes were found to contain higher ordered structures whereas cellular IRESes were diverse in their structural features [7]. IRES initiation mode may efficiently initiate CUL-3b translation when some specific initiation factors (eIFs) are limited under some certain circumstances such as mitosis, apoptosis, hypoxia and viral infection [8]. However, the 5'-UTR of *cul-3* is GC-richened and should be translated by the normal cap-initiation, which is restricted by the initiation factors for the most part [9]. Therefore, *cul-3b* might have an unusual transcriptional and translational mode as compared with *cul-3*, which made it important for spermatogenesis. However, this needs to be further investigated. On the other hand, the deduced amino acid peptide of CUL-3b was 24 aa shorter than CUL-3. Bioinformatical analysis showed no difference between their domains. CUL-3 belongs to the cullin family including CUL-1, CUL-2, CUL-3, CUL-4A, CUL-4B, CUL-5, and may be more. Cullin proteins act as a scaffold in E3 ligases that selectively target substrates for ubiquitin-dependent degradation by the 26S. Subunits and substrates were previously known only for the CUL-1 and CUL-2 complexes [10, 11]. But recent researchers were directed to CUL-3 ubiquitin ligases system [12–14]. CUL-3 was reported to efficiently interact with BTB (Bric-a-brac, Tramtrack and Broad complex) domain proteins to form BCR3 (BTB-Cul3-Roc,) ubiquitin ligases [14]. A large number of BTB proteins exist in eukaryotes (refer to http://www.sanger.ac.uk/Software/Pfam) and present diverse physiological functions ranging from developmental control to oncogenesis [15]. Therefore CUL-3 could be involved in various biological events through interacting with these BTB proteins. CUL-3 ubiquitin ligases could bind directly to several BTB/Kelch proteins that are structurally similar to Kelch-like protein homolog 10 (KLHL10) [2, 14]. The latter is a testis-specific protein without which mice would be infertile [16]. This implicated that CUL-3 and CUL-3b may play important roles in spermatogenesis. Finally, it was reported that CUL-3 interacted with BTB proteins by its N-terminal domain (from Trp34 to Tyr 74) [2]. The lack of 24 residues at N-terminus of CUL-3b may not affect the interaction between CUL-3b and BTB proteins. But this needs further investigation.

Multi-tissue RT-PCR results showed that *cul-3b* was ubiquitously expressed in 16 human tissues (Figure 5). However, RT-PCR of *cul-3b* was very difficult, possibly due to the special structure of IRESes in its 5'-UTR, or its low expression level. Therefore, in order to clarify whether or not *cul-3b* expressed in the 16 tissues, forty PCR cycles were used in the amplification of *cul-3b*. *Cul-3* expression profile was previously reported in eight human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) by Northern hybridization [17]. Results showed that the 2.8-kb version of *cul-3* was ubiquitously expressed whereas the 4.3-kb version was absent in the lung and liver. We did not compare the multi-tissue result of *cul-3b* with *cul-3* in detail as they were from different experiments done in different tissues, and most of all, these data were enough to make the conclusion that the expression of *cul-3b* was greater than the 4.3-kb version of *cul-3* and at least as great as the 2.8-kb version of *cul-3*. The expression level should be analyzed with more accurate techniques in specific tissues. *Cul-3* was also amplified in adult and fetal-testes and sperm by RT-PCR and the result was similar to that of *cul-3b* (data not shown).

In conclusion, we identified *cul-3b* as a new transcript variant of CUL-3, which was different in the sequence of nucleotide and peptide. *Cul-3b* may play important roles in the processes of human testes and other organ development.

Acknowledgment

This work was supported by grants from China National 973 (No. G1999055901), National Natural Science Foundation of China (No.30425006), the Fundation of Science and Technology of Jiangsu Province, China (No. BG2003028) and the Foundation of National Department of Science and Technology, China (No. 2004CCA06800).

References

1 Singer JD, Gurian-West M, Clurman B, Roberts JM. Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. Genes Dev 1999; 13: 2375–87.
2 Furukawa M, He YJ, Borchers C, Xiong Y. Targeting of protein ubiquitination by BTB-Cullin 3-Roc1 ubiquitin ligases. Nat Cell Biol 2003; 5: 1001–7.
3 Baarends WM, Roest HP, Grootegoed JA. The ubiquitin system in gametogenesis. Mol Cell Endocrinol 1999; 151: 5–16.
4 Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, 2nd edition. New York: Cold Spring Harbor Laboratory Press; 1989.
5 Kozak M. An analysis of vertebrate mRNA sequences: intimations of translational control. J Cell Biol 1991; 115: 887-903.
6 Huang X, Lu J, Xu M, Xiao J, Yin L, Zhu H, *et al*. Novel development-related alternative splices in human testis identified by cDNA microarrays. J Androl 2005; 26: 189-96.
7 Hellen CU, Sarnow P. Internal ribosome entry sites in eukaryotic mRNA molecules. Genes Dev 2001; 15: 1593–612.
8 Vagner S, Galy B, Pyronnet S. Irresistible IRES. Attaching the translation machinery internal ribosome entry sites. EMBO Rep 2001; 2: 893–8.
9 Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002; 16: 6–21.
10 Schulman BA, Carrano AC, Jeffrey PD, Bowen Z, Kinnucan ER, Finnin MS, *et al*. Insights into SCF ubiquitin ligases from the structure of the Skp1-Skp2 complex. Nature 2000; 408: 381–6.
11 Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, *et al*. Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature 2002; 416: 703–9.
12 Kurz T, Pintard L, Willis JH, Hamill DR, Gonczy P, Peter M, *et al*. Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. Science 2002; 295: 1294–8.
13 Pintard L, Kurz T, Glaser S, Willis JH, Peter M, Bowerman B. Neddylation and deneddylation of CUL-3 is required to target MEI-1/Katanin for degradation at the meiosis-to-mitosis transition in C. elegans. Curr Biol 2003; 13: 911–21.
14 Xu L, Wei Y, Reboul J, Vaglio P, Shin TH, Vidal M, *et al*. BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature 2003; 425: 316–21.
15 Collins T, Stone JR, Williams AJ. All in the family: the BTB/POZ, KRAB, and SCAN domains. Mol Cell Biol 2001; 21: 3609–15.
16 Yan W, Ma L, Burns KH, Matzuk MM. Haploinsufficiency of kelch-like protein homolog 10 causes infertility in male mice. Proc Natl Acad Sci U S A 2004; 101: 7793-8.
17 Du M, Sansores-Garcia L, Zu Z, Wu KK. Cloning and expression analysis of a novel salicylate suppressible gene, Hs-CUL-3, a member of cullin/Cdc53 family. J Biol Chem 1998; 273: 24289–92.