Blackwell Publishing

Asian J Androl 2005; 7 (2): 127–137
DOI: 10.1111/j.1745-7262.2005.00041.x

AJA

·Original Article·

Identification of a novel testis-specific gene and its potential roles in testis development/spermatogenesis

Lan-Lan Yin, Jian-Min Li, Zuo-Min Zhou, Jia-Hao Sha

Key Laborary of Reproductive Medicine, Nanjing Medical University, Hangzhong Road, Nanjing 210029, China

Abstract

Aim: To identify and characterize a novel gene with potential roles in testis development and spermatogenesis. **Methods:** A cDNA microarray was constructed from a human testis large insert cDNA library and hybridized with probes of human or mouse adult and fetal testes. Differentially expressed genes were isolated and sequenced. RT-PCR was used to test the tissue distribution of the genes of interest and *in situ* hybridization was performed to localize the gene expression in the mouse testis. A range of bioinformatical programs including Gene Runner, SMART, NCBI Blast and Emboss CpGPlot were used to characterize the new gene's feature. **Results:** A novel testis-specific gene, NYD-SP5, was differentially expressed in fetal and adult testes. The deduced protein structure of NYD-SP5 was found to contain an IQ motif (a short calmodulin-binding motif containing conserved Ile and Gln residues), a Carbamate kinase-like domain, a Zn-dependent exopeptidase domain and a lactate dehydrogenase (LDH) C-terminal-like domain. RT-PCR analysis revealed that NYD-SP5 was predominantly expressed in the testis but not in other 15 tissues examined. *In situ* hybridization and RT-PCR examinations revealed that the expression of NYD-SP5 was confined in the male germ cell but not present in the somatic cell in the testes. **Conclusion:** NYD-SP5 is a newly found testis-specific gene with potential roles in testis development and spermatogenesis through a calmodulin-activated enzyme. *(Asian J Androl 2005 Jun; 7: 127–137)*

Keywords: spermatogenesis; testis; calmodulin

1 Introduction

A central question in developmental genetics is how a complex organism with structurally, morphologically and functionally distinct tissues and organs can be derived from a single-cell zygote. The formation of different organs and tissues is based primarily on differential gene expression. While "housekeeping genes" that contribute to basic structural or metabolic cellular functions are expressed ubiquitously throughout the body, "tissue-specific" genes that contribute to specialized functions in differentiated cell types are expressed in a regulated fashion. Spermatogenesis, a complex process leading to the formation of male gametes, has been considered as a model system for developmental analysis of regulatory mechanisms associated with tissue-specific gene expression [1] because spermatogenesis is characterized by the expression of many genes that either are not expressed in any other cell type or are expressed in only very few

Correspondence to: Dr Jia-Hao Sha, Key Laborary of Reproductive Medicine, Nanjing Medical University, Hanzhong Road, Nanjing 210029, China
Tel/Fax: +86-25-8686-2908
E-mail: shajh@njmu.edu.cn
Received 2004-09-28 Accepted 2005-02-04

other cell types. Most of these genes also exhibit stage-specific expression during spermatogenesis, which could be considered as spermatogenic cell type-specific since the occurrence of different spermatogenic cell types is also stage-specific during spermatogenesis. Therefore, the spermatogenic cell lineage has provided a unique opportunity for developmental analysis of tissue-specific gene expression and the governing regulatory mechanisms.

In order to identify developmentally regulated genes in the testis, we have constructed cDNA microarrays from a human testis large insert cDNA library [2]. cDNA probes from human fetal and adult testes were used to hybridize the cDNA microarray. NYD-SP5 was one of the clones, which was identified as a differentially expressed gene with higher intensity in the adult testis than fetal testis. Additionally, the tissue distribution and cellular localization, together with protein structure prediction results, indicated that NYD-SP5 was a novel testis-specific gene with a potential calmodulin-binding region. Calcium plays a central role in spermatogenesis [3], spermiogenesis [4] and following fertilization [5]. Many of the calcium activating events are mediated by the intracellular calcium receptor calmodulin (CaM), which when bound to calcium can activate a variety of enzymes, including protein kinases, phosphatases and phosphodiesterases [6]. Because CaM is present in all tissues, cell-type-specific functions are determined by the complement of its downstream targets [7, 8]. The newly found CaM binding protein, NYD-SP5, in a spermatogenic cell would point to a new insight into the mechanism of spermatogenesis regulation through CaM.

2 Materials and methods

2.1 Samples

Informed consent was received from either the participants or their kin and the ethics committee of Nanjing Medical University (China) granted research approval prior to sample collection. Human adult testes (28 years old and 37 years old) were obtained from the Body Donor Center (Nanjing Medical University) and fetal testes were obtained from accidentally aborted (as a consequence of road accidents) 6-month-old fetuses (Clinical Reproductive Center, Nanjing Medical University). Testis tissue samples from five individuals with Sertoli-cell-only syndrome (SCOS) were acquired via biopsy and health volunteers with proven fertility and normal semen quality (assessed by WHO criteria, 1999) donated ejaculated sperm.

2.1 Preparation of human testis cDNA microarray

The testis cDNA microarray was constructed as described previously [2]. Briefly, this microarray contained 9216 cDNA clones that were derived from a human testis 5'-STRETCH PLUS cDNA library (Clontech, Palo Alto, CA, USA [source of insert cDNA came from 25 Caucasians aged from 20 to 65 years]). The inserts were amplified by PCR using 5'-CCATTGTGTTGGTACCCGGGAATTCG-3' (P1) as a forward primer and 5'-ATAAGCTTGC TCGAGTCTAGAGTCGAC-3' (P2) as a reverse primer. PCR products were used to make the human testis cDNA microarray. The microarray was hybridized with human testis cDNA probes from deceased adults and accidentally aborted 6-month-old fetuses. The human testis cDNA microarrays were also hybridized with probes prepared from the testes of 1- and 4-week-old mice to screen for homologous genes in testis development.

2.3 Sequence identification and analysis

For differentially expressed genes, cDNA clones were isolated for further analysis. The amplified cDNA plasmids were isolated and purified (QIAprep Spin Miniprep Kit, Qiagen, Hilden, Germany) and the inserts were sequenced using the ABI 377 automatic sequencing machine (Perkin-Elmer, Norwalk, USA). For each clone, sequence homologies were searched in the databases of GenBank. The nucleic and deduced amino acid sequences were also analyzed using a range of bioinformatical programs including Gene Runner (http://www.generunner.com),SMART (http://www.smart.embl-heidelberg.de), NCBI Blast (http://www.ncbi.nlm.nih.gov/blast),and Emboss CpGPlot (http://www.cbi.pku.edu.cn/tools/EMBOSS/cpgreport).

2.4 Analysis of NYD-SP5 gene expression in different tissues by RT-PCR

After sequence identification and analysis, a novel testis specific gene, named NYD-SP5, was found. The expression profile of NYD-SP5 was determined by using PCR screening. Multiple tissue cDNA panels, including testis, thymus, small intestine, colon, spleen, leukocyte, prostate gland, ovary, pancreas, heart, kidney, lung, placenta, liver, brain and skeletal muscle were purchased from Clontech (#1420-1). The NYD-SP5 specific primers were as follows: upstream: 5' CTCACCT TATACCTGACAAACG 3' (nt 2,627- nt 2,648) and

downstream: 5' GCCTTTCCTCAAGATCATAGC 3' (nt 2,853-nt 2,873). The PCR product was 247 bp in size. G3PDH was used as the positive control. The reagents in 50 μL PCR reaction tubes were as follows: H_2O 33.6 μL, buffer 5 μL, 10 mmol/L dNTP 1 μL, Tag DNA polymerase 0.4 μL, upstream primer 5pmol 2.5 μL, downstream primer 5 pmol 2.5 μL, and cDNA sample 5 μL. PCR conditions were as follows: denaturation at 94 °C for 1 min, annealing at 56 °C for 30 sec and extension at 72 °C for 30 sec. The first cycle had a denaturation period of 5 min; the last cycle an extension period of 7 min. Thirty-five cycles of PCR were performed. The PCR products were analyzed by 1.5 % (w/v) agarose gel electrophoresis.

2.5 Cellular localization of NYD-SP5

2.5.1 Probe preparation

Mouse homologous fragment was prepared by RT-PCR. Mouse testis total RNA was isolated using TRIzol Reagent (GIBCO BRL, Grand island, NY). Reverse transcription was performed in 15 μL of reaction mixture. First 1 μL of total RNA (about 3 μg, 1 μL random primer (0.2 μg/mL Sangon, Shanghai, China) and 7 μL DEPC water were mixed and incubated at 70°C for 5 min; then 3 μL M-MLV RT 5 × buffer, 0.75 μL dNTP (20 mmol/L), 0.35 μL RNasin(50 U/μL), 1 μL moloney murine leukemin virus (M-MLV) Reverse Transcriptase (Promega, Shanghai, China), 1 μL DEPC water were added and incubated at 37°C for 1 h, and then 95°C for 5 min. The primer sequences for amplification of mouse NYD-SP5 cDNA were: P1: 5'-CCGATATGCTGAATGTCC3'; P2: 5'-TGTCACAAAATGCTGTCC- 3'. The desired fragment was 249bp. PCR reaction mixture and conditions are the same as above except the annealing temperature was lowered to 54 °C. The PCR products were detected by staining with ethidium bromide after electrophoresis on a 1.5 % (w/v) agarose gel and purified by DNA gel extraction kit (Biorad [Hercules, CA, USA]) according to the instructions.

T7, Sp6 promoter sequences were added to the 5' end and 3' end of mouse DNA fragment, respectively also by PCR reaction. And the PCR products with T7, Sp6 promoter sequences on both sides were purified and used as the template in the *in vitro* transcription (DIG RNA labeling kit, Roche [Indianapolis, IN, USA]). The efficiency of thus obtained anti-sense and sense RNA probes were evaluated by a standard direct detection as described in The DIG System User's Guide for Filter Hybridization (Roche)(http://www.roche-applied-science.com/prodinfo_fst.htm?/PROD_INF/MANUALS/DIG_MAN/dig_toc.htm)

2.6 In situ hybridization

For preparation of paraffin-embedded sections, mouse testes at 15 days, 30 days, and 60 days were cut and fixed in 4 % (w/v) paraformaldehyde in Phosphate Buffer Saline (PBS) at 4°C overnight. The fixed testes were dehydrated with ethanol and embedded in paraffin. Paraffin-embedded sections of 5 μm thickness were cut and collected on slides pretreated with polylysine.

Sections were deparaffinized in xylene for 10 min (two changes) and rehydrated through 100 %, 70 % ethanol (two changes), DEPC H_2O and PBS (two changes). Then, the sections were treated for 10–15 min at 37°C with 2 μg/mL proteinase K in 10mmol/L Tris, pH 7.5. After that, the enzyme digestions were stopped and post-fixed by immersing the sections in 4 % paraformaldehyde in PBS for 5 min. Finally, the sections were further washed in PBS (two changes for 5min).

The sections were prehybridized and blocked in hybridization buffer (DIG Easy Hyb, Roche) without probe at 42°C for 2 h. The hybridization buffer was applied to each section with an optimal concentration of 100 ng/mL labeled RNA probes. Hybridization was carried out at 58°C for 16 h in a humidified chamber. Subsequently, the sections were washed in 4×SSC for 5 min, 2×SSC for 30 min, 1×SSC and 0.5×SSC for 10 min, and twice in 0.01 mol/L PBS for 10 min. The immunological detection of the DIG labeled signal was performed as described by the manufacturer (DIG Nucleic Acid Detection Kit, Roche).

2.7 Analysis of NYD-SP5 mRNA in normal sperm and testes of five patients with SCOS

Five male patients with SCOS were recruited in this study. Tissues from their testes were obtained via biopsy at the First Affiliated Hospital of Nanjing Medial University (Nanjing, China) for section pathologic diagnosis, and RNA was extracted using Trizol reagent. Total RNA of ejaculated sperm was also extracted with Trizol reagent. Then total RNA was reverse-transcripted to cDNA with Avian Myeloblastosis Virus (AMV) reverse transcriptase. Expression of NYD-SP5 was determined as follows: The cDNAs were amplified with the sequence specific primers (P1 and P2) as described above and PCR products were resolved by electrophoresis; the testis cDNAs were processed in a similar way to detect

the presence of β-actin mRNA.

3 Results

3.1 cDNA microarray hybridization

The hybridization of the constructed testis cDNA microarrays with adult and fetal testis probes revealed a series of clones that were highly expressed in adult but not fetal testis. Of 9216 clones analyzed, 592 had intensities at least three times higher for probes prepared from adult tissue than those from fetus tissue, whereas 139 cDNA clones had at least three times higher signals for probes prepared from the fetus testis than those from adult testis. The reciprocal expression characteristics of these genes indicated that different sets of genes were involved in different developmental stages. The hybridized signal intensities from human adult and fetal testicular probes for one of the clones, named NYD-SP5, were 26.32 and 4.87 respectively, indicating a 5-fold higher expression in the adult than that in the fetus (Figure 1A, B). Also, *in situ* hybridization with mouse testis cDNA probe the intensities from adult and fetal testes for NYD-SP5 were 12.37 and 3.0, respectively (Figure1C, D), showing the same expression time pattern with human testis.

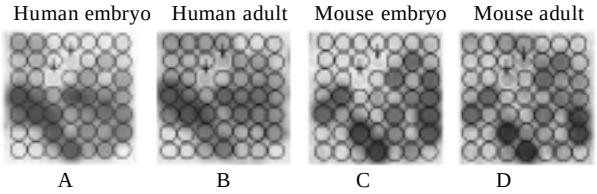

Figure 1. cDNA microarray hybridized with ^{33}P-labeled (A) human fetal testis cDNA probe, (B) human adult testis cDNA probe, (B) 1-week mouse testis cDNA probe, (C) 4-week mouse testis cDNA probe. NYD-SP5 is marked with arrow.

3.2 Structural features of the cDNA and deduced protein

NYD-SP5 (GenBank Accession No. AY014282) was found to consist of 3598 nucleotides and contain an open reading frame of 1027 amino acids (Figure 2). A nucleotide blast search against the GenBank database revealed a similar nucleotide sequence in mice (GenBank Acces-

```
1    TCCGGCTGAAGGTTTCCGTGCTTGGAAACCGCGCCTCCGCGGAGGTAGCCGTTCCCTGAC

61   CTAGCCATGGCACAGAACACTGAAAACCACGACCCTGTCGGATCCATCTTAATCCAGATC
          M  A  Q  N  T  E  N  H  D  P  V  G  S  I  L  I  Q  I
121  CATGAAGACCTTTATCAGTTAAAGGAGAAATTAACAAAATTCACACCTGAGGAAAAAGGA
     H  E  D  L  Y  Q  L  K  E  K  L  T  K  F  T  P  E  E  K  G
181  GAGACTCTAGACATTCAGAGTCTTGAAACAGCAATCAAAAGGACTGAAGTGGGGTTAAGA
     E  T  L  D  I  Q  S  L  E  T  A  I  K  R  T  E  V  G  L  R
241  ATTCACATTGAGAAGTATTTAAATGTTGTAAACCAGAATGTATTAACGACTTCTGTTAAT
     I  H  I  E  K  Y  L  N  V  V  N  Q  N  V  L  T  T  S  V  N
301  GATGAGAGCTTATATACTCCCCAGGCTTCCAAATGGTTACTTCCAACTGTAATTGATCAG
     D  E  S  L  Y  T  P  Q  A  S  K  W  L  L  P  T  V  I  D  Q
361  AAATCATTTATTTTCCCTCAGGAATCTGAGGGTACATTTTGGCAACCCCAAAGACAGCAC
     K  S  F  I  F  P  Q  E  S  E  G  T  F  W  Q  P  Q  R  Q  H
421  AGTTCATCTCTGCCTGTCTTTCCAAGAGCAAAGATAAAGGTTTCGAAGTTAATCAAAGGG
     S  S  S  L  P  V  F  P  R  A  K  I  K  V  S  K  L  I  K  G
481  TCTAACATATCCAGCCTCACGGTTCTGCCATCTTCTCATTGCACAGATCCCTATTTCACT
     S  N  I  S  S  L  T  V  L  P  S  S  H  C  T  D  P  Y  F  T
541  CCTATACCAGTCTTACAAGCAGATGCCCACAAAGGGATTTTAAGTATGATAGAACGAGGG
```

Figure 2 (continued).

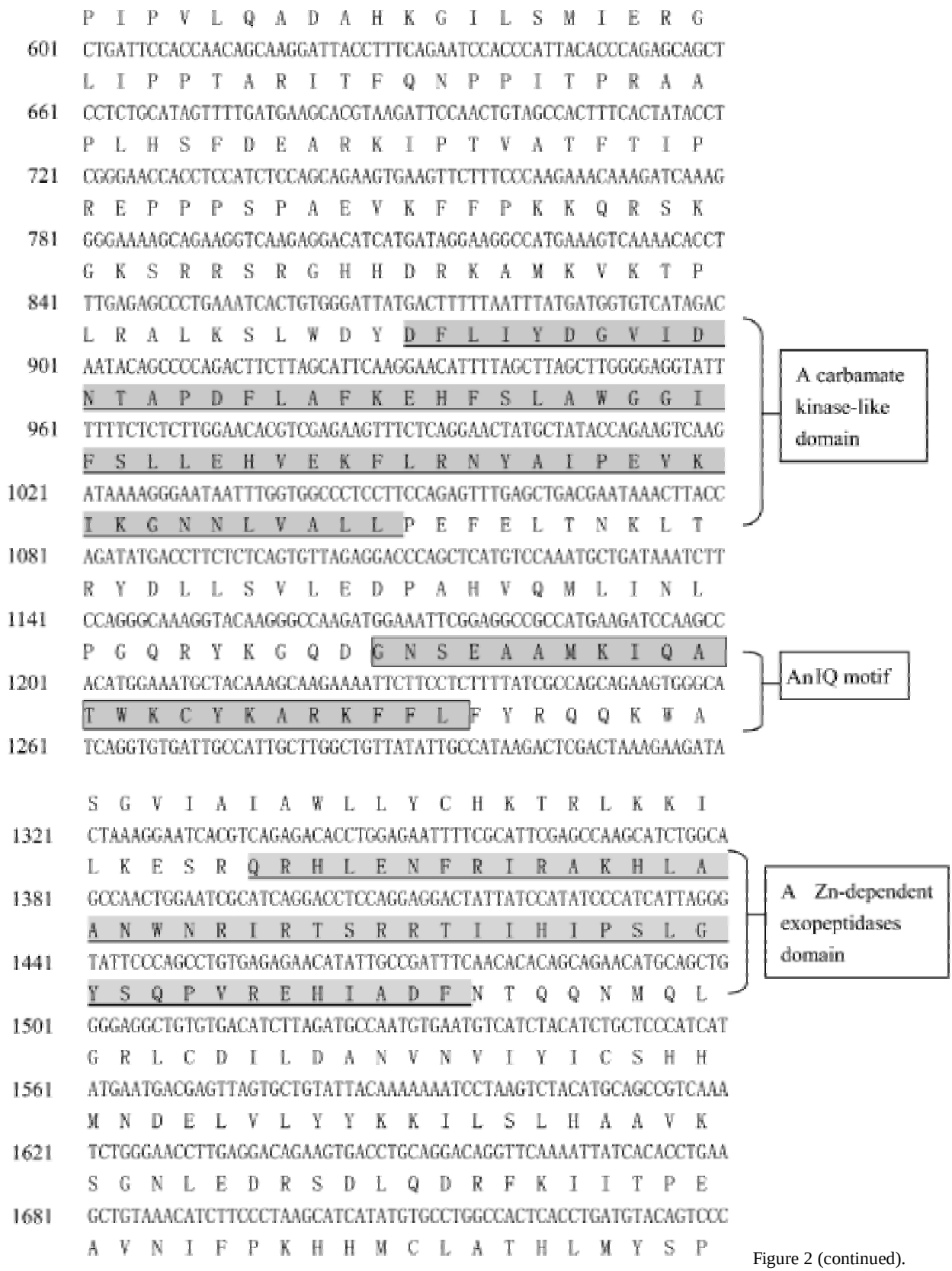

Figure 2 (continued).

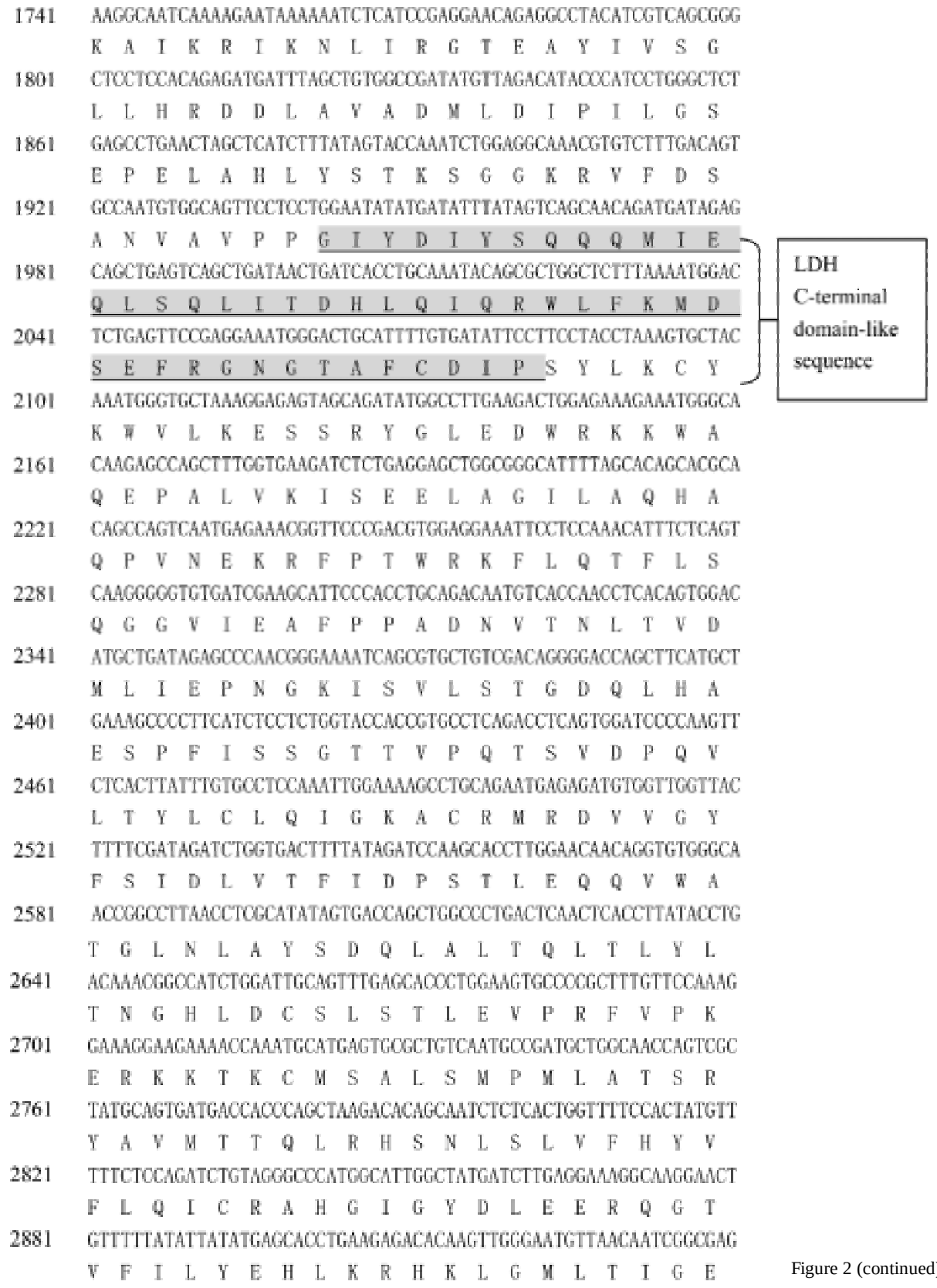

Figure 2 (continued).

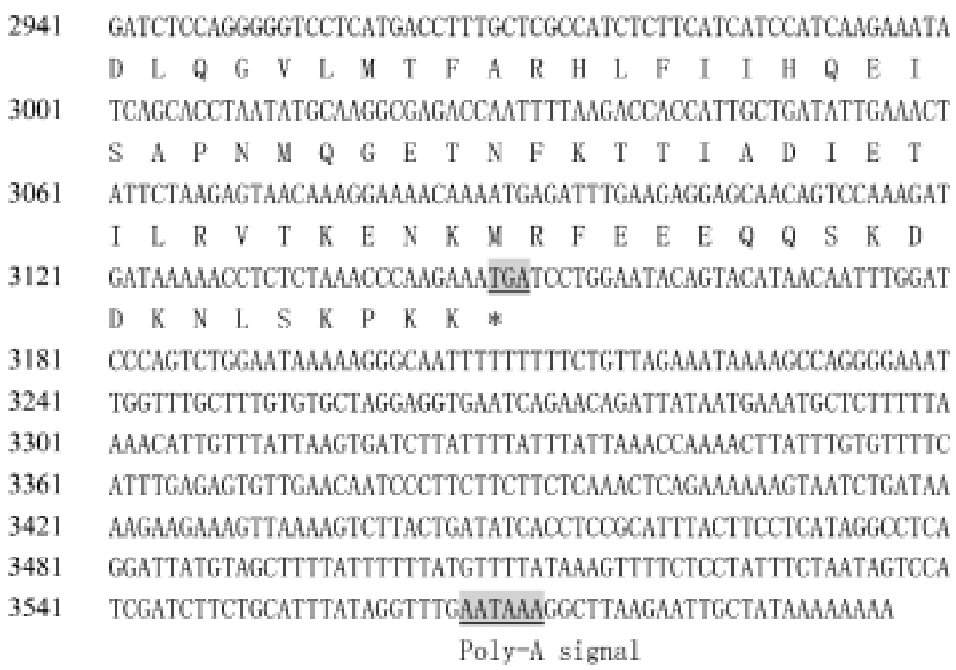

Figure 2. Nucleotide and deduced amino acid sequence of NYD-SP5. Numbering of the nucleotide sequence is shown on the right. The initiation and stop codons, as well as the polyadenylation signal, are marked by shades and underlines respectively. The nucleotide sequence appears in the GenBank databases under accession number AY014282. The predicted functional domains are underlined and shaded.

sion No. AK019535), and a similar nucleotide sequence in rats (GenBank Accession No. XM_236331).

Further, NYD-SP5 protein shares 68 % identity and 78 % positive amino acid sequence with its mouse homolog protein BAB31783 encoded by AK019535, and shares 57 % identity and 67 % positive with its rat homolog protein XP_236331 encoded by XM_236331 (Figure 3). These data indicate high levels of homology between NYD-SP5 and mice and rats at either the nucleotide or the protein level; therefore, NYD-SP5 is a human–mouse–rat homologous gene. GenBank human genome database searching mapped NYD-SP5 to chromosome 15q22.31 (NT_086827).

Simple modular architecture research tool (SMART) predicted a high possibility of an IQ motif (368–390aa) in the middle of NYD-SP5 protein sequence and three enzyme domains within NYD-SP5 sequence (Figure 2). These domains include a carbamate kinase-like domain located before the IQ motif (269–328aa), a Zn-dependent exopeptidases domain after the IQ motif (424–470aa), and a LDH C-terminal domain-like sequence that lies in the C-terminal (626–672aa) (Figure 2). In addition, CpG island revealing program, Emboss CpGPlot, reported two relatively high GC content regions occurred from –57 to –269 bp and –411 to –629 bp in the upstream of NYD-SP5 chromosome [8] (Figure 4).

3.3 Expression of NYD-SP5 in normal tissues

Tissue distribution studies using RT-PCR on a human tissue kit showed that the designed 247 bp product was expressed predominantly in the testis, but not in the other 15 tissues examined, including the ovary – the female germ-cell-producing organ (Figure 5). Therefore, NYD-SP5 was identified as a testis-specific gene.

3.4 Cellular localization of NYD-SP5

Using an *in situ* hybridization technique, we examined the localization of NYD-SP5 mRNA at mouse testes. The results showed that the expression of NYD-SP5 was confined to seminiferous tubules. Strong hybridization signals from the mouse probe was exclusively localized in the spermatocytes and spermatids, and no signal above the background level was detected outside the seminiferous tubules or in Sertoli-cell at all ages examined, namely days 15, 30 and 60 (Figure 6). It is suggested that mouse NYD-SP5 is expressed in the male germ line cell but not the somatic cell in mouse testis.

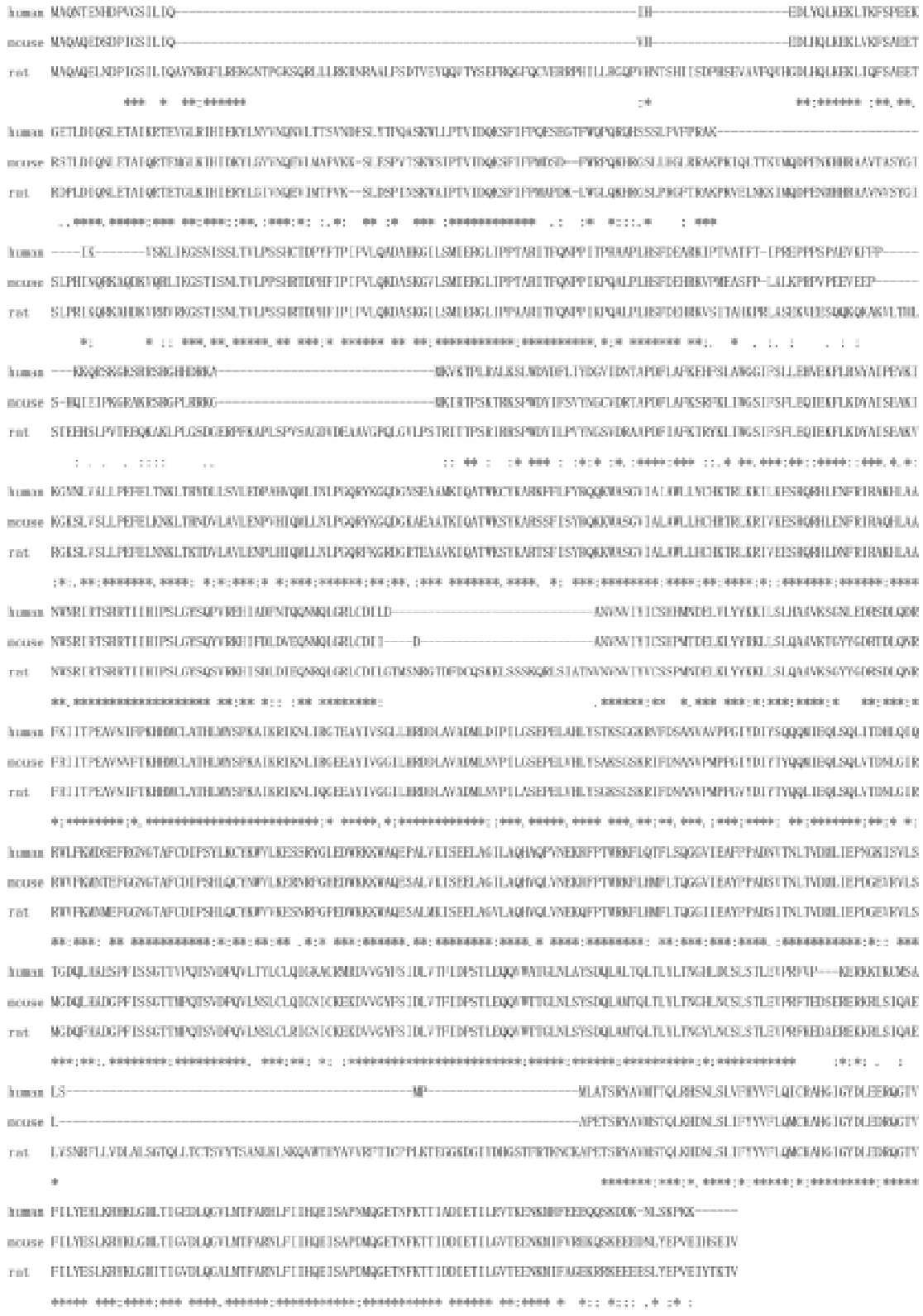

Figure 3. Sequence comparison of NYD-SP5 and its homology in mouse and rat using CLUSTER W [9].

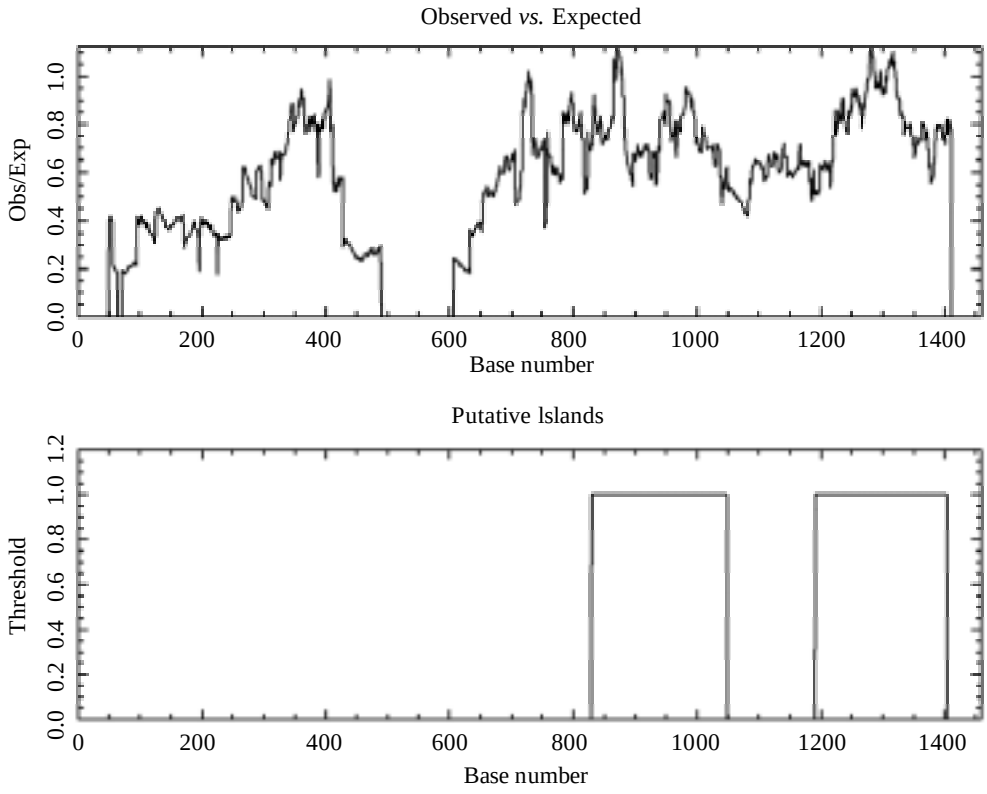

Figure 4. Report of CpG rich region prediction [10]. An island is defined as a region that satisfies the following constraints: Obs/Exp ratio >0.6; % C + % G >50 %, Length >200.

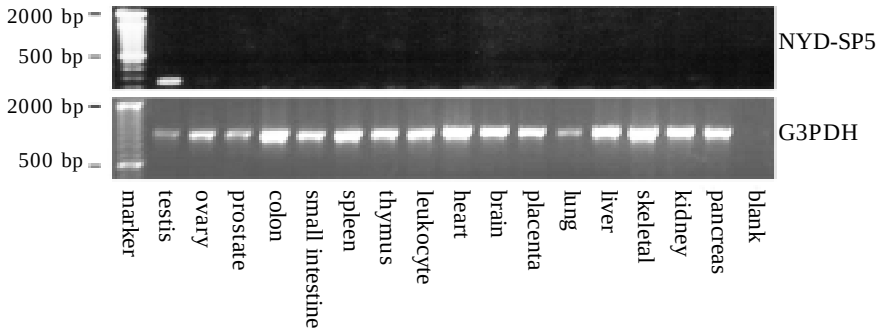

Figure 5. Electrophoresis showing expression profiles of NYD-SP5 with G3PDH as control. NYD-SP5 was specifically expressed in testis with no expression in any other organ.

3.5 Analysis of NYD-SP8 mRNA in spermatozoa and the testis of five patients with SCOS.

RT-PCR analysis showed that NYD-SP5 mRNA was also detected in spermatozoa but not in the testes of patients with SCOS that is characterized histologically by complete loss of the germinal epithelium in testicular tubules, and clinically by aspermia (Figure 7). Therefore, it has been suggested that NYD-SP5 is expressed in germ cells but not somatic cells in human testes.

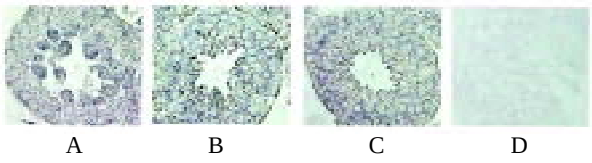

Figure 6. Cellular localization of mouse homology of NYD-SP5 mRNAs in mouse testes of different ages. Positive signal is shown in purple. (A): 15-day testis; (B): 30-day testis; (C): 60-day testis; (D): the control with sense probe.

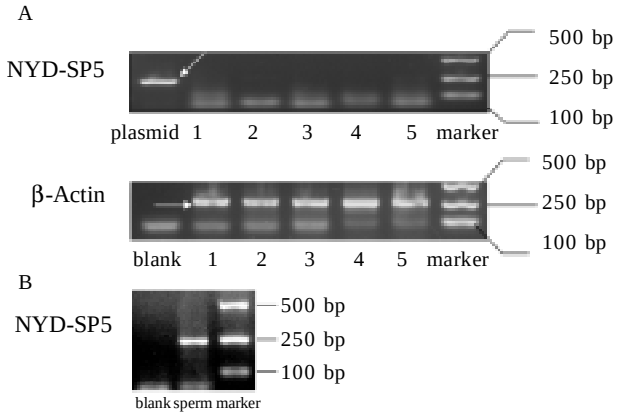

Figure 7. (A): Analysis of NYD-SP5 mRNA in the testis of patients with SCOS. As a control, the lower panel displays the expression level of β-actin in corresponding patients. Plasmid containing NYD-SP5 full length was used as the positive control. (B): Analysis of NYD-SP5 in the ejaculated spermatozoa. A designed band of 247 bp was detected in the PCR product.

4 Discussion

Using cDNA microarrays constructed from the human testis large insert cDNA library, we have identified a novel testis-specific gene, NYD-SP5, which is differentially expressed in fetal and adult testes of humans and mice. The tissue distribution and cellular localization of NYD-SP5 mRNA suggests that it is a male germ line cell specific gene with potential roles in the process of spermatogenesis. The predominant expression of NYD-SP5 mRNA in adult but not fetal testes can be confirmed by the *in situ* hybridization analysis in mouse testes, showing restricted localization of NYD-SP5 in the seminiferous tubules with signals mainly in primary spermatocytes and advanced spermatogenic cells, as well as ejaculated spermatozoa, which are absent from fetal testes.

Because of the tissue and cell-line-specific expression pattern of NYD-SP5, CpG frequency of the chromosome upstream of NYD-SP5 was examined. There is a CpG rich area in the promoter region of NYD-SP5, which might be related to the expression regulation of the gene. There is evidence indicating that methylation of the CpG island inhibits the transcription of genes [9]. The regulation of tissue-specific expression of the Pgk-2 gene, which is expressed only in spermatogenic cells in eutherian mammals, has been described to be in such a fashion that its expression is repressed in somatic cells [1]. The tissue-specific and stage-dependent expression of NYD-SP5 may also require demethylation of its CpG rich segment. Further studies along this line with NYD-SP5 may provide detailed mechanisms for developmentally regulated gene expression.

As to the function of NYD-SP5 in spermatogenesis, bioinformatical analysis provided some clues. NYD-SP5 contains three enzyme domains and one IQ motif, which is in charge of CaM binding. CaM is recognized as a major calcium sensor and orchestrator of regulatory events through its interaction with a diverse group of cellular proteins [6]. Because CaM is present in all tissues, cell-type-specific functions are determined by the complement of its downstream targets. In the testis, several calmodulin binding proteins, such as calspermin, Ca (2+)/calmodulin-dependent protein kinase IV (CaMKIV), and testis-specific calcineurin B, were isolated and demonstrated to be essential for spermatogenesis. For instance, CaMKIV is expressed in spermatids and targeted to chromatin and the nuclear matrix [12], and calspermin has been speculated to play a role in binding and sequestering CaM during the development of the germ cell [13]. NYD-SP5 might be another member of CaM targets involved in the process of spermatogenesis. The IQ motif, which NYD-SP5 contained, is one of the three recognition motifs for CaM interaction and reported as a consensus for Ca^{2+}-independent binding. Neuromodulin (GAP 43/P-57), neurogranin and Brush Border Myosin I (BBMI), all of which contain an IQ motif, interact with CaM in the absence of Ca^{2+} [14]. Neuromodulin is a major component of the motile “growth cones” that form the tips of elongating axons and plays an important role in regulation of axon growth and new connection modulation [15]. Neurogranin is the most prominent substrates of protein kinase C (PKC) in the mammalian brain [16]. BBMI is a major component of the actin assembly in the microvilli of intestinal cells, and has also been reported to have effects on membrane traffic in polarized epithelial cells [17]. All of the three IQ motif-containing mem-

bers take part in the regulatory events in the cell life. Therefore, NYD-SP5 seems to operate as a regulator in spermatogenesis. Furthermore, there are three enzyme domains occurring near the IQ motif, which advanced the possibility of regulatory function for NYD-SP5 through catalyzing some reactions in cell metabolism. In hybridization signal analysis, clones with intensities of > 10 were considered as positive signals to ensure that they were distinguished from background with statistical significance of >99.9 % [2]. Signal intensity of NYD-SP5 in adult microarray was just a little higher than the threshold value, only 26.32 and 12.37 in human and mouse adult testes, respectively, which was quite lower than skeleton protein or cell structure protein. For example, in adult microarray the signal intensity of outer dense fiber protein 2 is 581.07, and the intensity for kinesin family member 2 is 104.66. The comparatively low intensity in microarray hybridization together with predicted function domains in NYD-SP5 protein sequence indicated that NYD-SP5 might play a regulatory role in the spermatogenesis process.

In summary, NYD-SP5 is a newly found testis-specific gene with potential regulatory roles in human spermatogenesis. The tissue-specific and stage-specific expression of NYD-SP5 has suggested its importance in the fundamental understanding of spermatogenesis. Further research is required to determine the physical function of NYD-SP5 protein in spermatogenesis.

Acknowledgment

The work was supported by grants from China National 973 (No. G1999055901) and National Natural Science Foundation of China (No. 30170485). We thank Dr Min Xu for valuable discussions and Dr Monica Antenos for her critical reading of the manuscript.

References

1 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.

2 Sha J, Zhou Z, Li J, Yin L, Yang H, Hu G, *et al*. Identification of testis development and spermatogenesis-related genes in human and mouse testes using cDNA arrays. Mol Hum Reprod 2002; 8: 511–7.

3 Santi CM, Darszon A, Hernandez-Cruz A. A dihydropyridine-sensitive T-type Ca^{2+} current is the main Ca^{2+} current carrier in mouse primary spermatocytes. Am J Physiol 1996; 271: C1583–93.

4 Fernandes AP, Bao SN. Detection of calcium and calmodulin during spermiogenesis of phytophagous bugs (Hemiptera: Pentatomidae). Biocell 2001; 25: 173–7.

5 Breitbart H. Intracellular calcium regulation in sperm capacitation and acrosomal reaction. Mol Cell Endocrinol 2002; 187: 139–44.

6 Stull JT. Ca^{2+}-dependent cell signaling through calmodulin-activated protein phosphatase and protein kinases. J Biol Chem 2001; 276: 2311–2.

7 Zhang T, Brown JH. Role of Ca^{2+}/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure. Cardiovasc Res 2004; 63: 476–86.

8 Grossman SD, Futter M, Snyder GL, Allen PB, Nairn AC, Greengard P, *et al*. Spinophilin is phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II resulting in regulation of its binding to F-actin. J Neurochem 2004; 90: 317–24.

9 Corpet F. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 1988; 16: 10881–90.

10 Larsen F, Gundersen G, Lopez R, Prydz H. CpG islands as gene markers in the human genome. Genomics 1992; 13: 1095–107.

11 Bird AP. CpG-rich islands and the function of DNA methylation. Nature 1986; 321: 209–13.

12 Wu JY, Means AR. Ca^{2+}/calmodulin-dependent protein kinase IV is expressed in spermatids and targeted to chromatin and the nuclear matrix. J Biol Chem 2000; 275: 7994–9.

13 Ono T, Koide Y, Arai Y, Yamashita K. Heat-stable calmodulin-binding protein in rat testis. Inhibition of calmodulin-stimulated cyclic nucleotide phosphodiesterase activity. J Biol Chem 1984; 259: 9011–6.

14 Bahler M, Rhoads A. Calmodulin signaling via the IQ motif. FEBS Lett 2002; 513: 107–13.

15 Chen B, Wang JF, Sun X, Young LT. Regulation of GAP-43 expression by chronic desipramine treatment in rat cultured hippocampal cells. Biol Psychiatry 2003; 53: 530–7.

16 Baudier J, Deloulme JC, Van Dorsselaer A, Black D, Matthes, HW. Purification and characterization of a brain-specific protein kinase C substrate, neurogranin (p17). Identification of a consensus amino acid sequence between neurogranin and neuromodulin (GAP43) that corresponds to the protein kinase C phosphorylation site and the calmodulin-binding domain. J Biol Chem 1991; 266: 229–37.

17 Durrbach A, Raposo G, Tenza D, Louvard D, Coudrier E. Truncated brush border myosin I affects membrane traffic in polarized epithelial cells. Traffic 2000; 1: 411–24.