Expression of a novel reticulon-like gene in human testis

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Identification of genes that are specifically expressed in the adult testis or the fetal testis is important for the study of genes related to the development of the testis. In this study, a human testis cDNA microarray was established. PCR products of 9216 clones from a human testis cDNA library were dotted on a nylon membrane; mRNA from adult and fetal testes were purified and probes were prepared by a reverse transcription reaction with testis mRNA as template. The microarray was hybridized with probes of adult and fetal testes, and 96.8 and 95.4% of clones were positive, respectively. In total, 731 clones were differentially expressed: 592 were highly expressed in adult testis and 139 were highly expressed in fetal testis. Among these genes, a new reticulon (Rtn)-like gene was detected and named Rtn-T. Rtn-T was highly expressed in adult human testis. The cDNA of Rtn-T contains 3491 bp and the putative protein

had 968 amino acids. This protein is homologous to the six known members of the Rtn family (KIAA0886, Rtn xL, reticulon 4a, Nogo-A, Nogo-A short form, and brain my043) but was different at the 5' end. All homologues originate from one gene, and result from both different promotor regions and different splicing. Rtn-T lacks the first exon and contains a second exon that is lacking in the other homologues. Rtn-T is shorter than KIAA0886, Rtn xL, reticulon 4a and Nogo-A, but longer than the Nogo-A short form and brain my043. Sequence analysis showed that Rtn-T protein has two hydrophobic regions that may be membrane-spanning domains. Expression profiles showed that Rtn-T is specifically and strongly expressed in testis. The results of the present study indicate that the Rtn-T gene is differentially expressed in adult and fetal testes and encodes a membrane protein that may have a function in testis development.

Introduction

The testis performs two fundamental functions: androgen secretion and spermatogenesis. Spermatogenesis is a complex and efficient process that begins with the division and differentiation of the spermatogenic stem cell within the seminiferous tubule of the testis. Because meiosis is different from mitosis, expression of genes in the testis is different from that in other organs. In addition, as spermatogenesis begins at puberty, the expression of genes differs at different stages, for example in adult and fetal testis (Eddy, 1998; Hecht, 1998; Sutton, 2000). Study of genes specifically expressed in the testis at different stages of development may reveal new genes related to testis function, particularly spermatogenesis (McCarrey, 1998). In the present study, human testis cDNA microarrays were used to identify the genes specifically expressed in adult or fetal testes and a new reticulon-like gene was found. Reticulon (Rtn) is a gene family and all Rtn proteins possess a 200 amino acid residue region of sequence similarity at the C terminus (Van de Velde *et al.*, 1994; Roebroek *et al.*, 1996, 1998; Moreira *et al.*, 1999; Morris *et al.*, 1999).

Related sequences have been recognized in human, bovine, mouse, rat, fly and worm genomes (Spillmann *et al.*, 1998; Moreira *et al.*, 1999; Schafer *et al.*, 2001). Several homologous genes have been reported, including Nogo-A, Nogo-B, Nogo-C, Rtn 1, Rtn 2, Rtn 3 and Rtn 4 (Hens *et al.*, 1998; GrandPre *et al.*, 2000). Rtn proteins are expressed in neuroendocrine cells, lung carcinoma cells, oligodendrocytes and adipocytes (Senden *et al.*, 1997; Morris *et al.*, 1999; Chen *et al.*, 2000; Prinjha *et al.*, 2000) but their function is unclear, although it might be related to axon extension and apoptosis. There have been no reports to the authors' knowledge of studies investigating the relationship between Rtn and testis function.

Materials and Methods

Construction of human testis cDNA microarrays

The Human Testis 5'-STRETCH PLUS cDNA library was ordered from Clontech, Palo Alto, CA (catalogue number HL5503U, source of insert cDNA was 25 caucasians aged 20–65 years, average length of insert cDNA = 3.4 kb). Each λ TriplEx2 clone was converted to a p λ TriplEx2 clone and excised with *Escherichia coli* BM25.8, and the complete plasmid was circularized from the recombinant phage according to the Clontech Manual PT3003-1.

The plasmids were extracted by alkaline lysis using the modified method of Sambrook *et al.* (1989) and the samples were stored in 96-well trays at -70°C .

The gene sequence was amplified from the plasmid clone by PCR to produce DNA for spotting the microarray. Primers were designed depending on the 5' and 3' sequences of λ TriplEx2 vector flanking the insert. The 5' primer was CCATTGTGTTGGTACCCGGAATTCG, interval 6 bp to insert cDNA site, and the 3' primer was ATAAGCTTGCTCGAGTCTAGAGTCGAC, interval 7 bp to insert cDNA site. In total, 9216 clones selected randomly from the cDNA library were amplified. The PCR products contained 2.0–7.0 kb. They were stored at -20°C .

A cDNA array was assembled with 9216 samples of PCR product. Each sample was dotted on an 8 cm $\times$ 12 cm nylon membrane (Amersham Pharmacia Biotech, Bucks, lot number YA1103) using an automatic arrayer (BioRobotics, Cambridge). Two dots for each sample were applied: a total of 18 432 dots representing 9216 samples. DNA was cross-linked to the nylon membrane by UV light. Eight house-keeping genes were used as positive controls: ribosomal protein S9 (RPS9), actin gamma, glyceraldehyde-3-phosphate dehydrogenase (GAPD), hypoxanthine phosphoribosyl-transferase 1 (HPRT1), *Homo sapiens* mRNA for a 23 kDa highly basic protein, ubiquitin C (UBC), phospholipase A2 and ubiquitin carboxyl-terminal esterase L1 (UCHL1). λ TriplEx2 phage DNA and PUC18 plasmid DNA were used as negative controls. Twelve spots were distributed in each membrane for each control cDNA.

Screening of differentially expressed genes in adult or fetal testis

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, NY) 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 leukaemia virus (M-MLV) reverse transcriptase. Each labelling reaction was carried out with 200 μCi [$\alpha^{33}\text{P}$]dATP according to the manufacturer's instructions (NEN Life Science, Boston, MA).

Nylon membranes spotted with cDNA fragments were prehybridized with 20 ml prehybridization solution ($6 \times$ sodium chloride–sodium citrate buffer (SSC), 0.5% SDS, $5 \times$ Denhardt compound, 100 μg denatured salmon sperm DNA ml^{-1}) at 68°C for 3 h. Overnight hybridization with the ^{33}P -labelled cDNA from testis samples was carried out in 6 ml hybridization solution ($6 \times$ SSC, 0.5% SDS, 100 μg denatured salmon sperm DNA ml^{-1}), and followed by stringent washing with 20 ml wash solution (10% SSC,

0.5% SDS) at 65°C for 1 h. Membranes were exposed to phosphor screen overnight and scanned using a FLA-3000A fluorescent image analyser (Fuji Photo Film, Tokyo). 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). 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 fetus were compared. If the difference in values of spot intensity in adult and fetus was more than threefold higher or lower, the corresponding genes were considered as differentially expressed.

Sequence identification and analysis

cDNA clones for predicted genes were proliferated. The amplified cDNA plasmids were isolated and purified in mini-preps (QIAprep Spin Miniprep Kit, Qiagen) and the inserts were sequenced using an ABI 377 automatic sequencing machine. For all clones, GenBank databases were searched for sequence homologies. The nucleic and deduced amino acid sequences were analysed using Gene Runner software.

Expression profiles of Rtn-T

After sequence identification and analysis, a reticulon (Rtn)-like gene, named Rtn-T, was identified. The expression profile of Rtn-T was determined using PCR screening. Multiple tissue cDNA panels including brain, heart, kidney, liver, lung, pancreas, placenta and skeletal muscle were purchased from Clontech (number 1420-1). Testis cDNA was amplified from normal human adult testis using RT-PCR. The Rtn-T specific primers were as follows: upstream 5' ACATTGACCCAACCGCAGT 3' (nt 40–58), and downstream 5' TTTCACGATTATTCTTCCCTA 3' (nt 517–538). The upstream primer was located in the specific region of the Rtn-T. The downstream primer is homologous with other genes of the Rtn family. The PCR product was 498 bp in size. G3PDH was used as positive control. The reagents in 30 μl PCR reaction tubes were as follows: $10 \times 3 \mu\text{l}$ PCR buffer, 2.4 μl of 25 mmol Mg^{2+} l^{-1} , 2.4 μl of 2 mmol dNTP l^{-1} , 0.2 μl of 5 iu tag DNA polymerase μl^{-1} , 5 pmol upstream primer, 5 pmol downstream primer, 3 μl cDNA sample (0.6 ng). PCR conditions were as follows: denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min. The first cycle had a denaturation period of 5 min. The last cycle had an extension period of 7 min. Thirty cycles of PCR were performed and the PCR products were analysed after electrophoresis.

Results

Differential expression of genes in adult and fetal testes

The microarrays of the human testis cDNA were hybridized with cDNA probes prepared from the mRNA of adult and fetal testes. Signals were obtained from 8925 spots (about 96.8% of the cDNA clones) in the case of adult testis, and 8795 spots (about 95.4% of the cDNA clones) in fetal testis. Most genes were expressed in both adult and fetal testes and the remainder were expressed only in adult or fetal testis. Among the cDNA clones that gave signals, 731 were differentially expressed in adult or fetal testis: 592 clones were highly expressed in adult testis, whereas 139 were expressed in fetal testis. The difference in intensity in these cases was at least threefold. The reciprocal expression characteristics of these genes indicate that different sets of genes are involved in different developmental stages.

Sequence identification and the foundation of *Rtn-T* gene

Sequence identification of all the above differentially expressed genes showed that 37 were novel genes that have not been reported and 90 have at least one homologous gene reported before. One of these novel genes was a reticulon-like gene. The hybridization intensities of this *Rtn*-like gene in adult and fetal testes were 180.64 and 50.15, respectively (Fig. 1). This gene was expressed in both adult and fetal testis, but the expression in adult testis was approximately 3.60-fold higher than that in fetal testis. The full length cDNA was 3491 bp and contained an open reading frame (nt 179–3139) that encodes a 108 kDa protein of 986 amino acids (Fig. 2). The methionine at nt 179–181 was almost certainly the site of initiation because there was an up-frame upstream stop code at nt 44–46. Blast search in the human genome database showed that this *Rtn*-like gene consisted of nine exons. Blast search of the contig map showed that all exons were located within chromosome 2p16, so this *Rtn*-like gene was mapped to chromosome 2p16. Analysis of the amino acid sequence revealed one signal sequence of 26 amino acids at the N-terminal and two long hydrophobic stretches of 37 and 35 amino acids at the C-terminal. These stretches may be transmembrane segments, although they are much longer than the typical transmembrane segments of approximately 20 amino acids. There were no other functional domains as determined by comparative sequence analysis. This *Rtn*-like gene has GenBank accession number AF333336.

Expression profiles of predicted genes

PCR and electrophoresis showed that the novel *Rtn*-like gene was particularly and strongly expressed in human testis (Fig. 3) and therefore was named *Rtn-T*. *Rtn-T* was not expressed in brain, heart, kidney, liver, lung, pancreas, placenta, or skeletal muscle.

Fig. 1. Partial cDNA hybridization images showing differential expression of *Rtn-T* in (a) 6-month-old fetal and (b) adult human testes (red circles). The hybridization intensity in fetal and adult testes was 50.15 and 180.64, respectively.

Homologous analysis between *Rtn-T* and other members of the *Rtn* family

Blast searches revealed that *Rtn-T* was highly homologous to six other genes, all of which belong to the *Rtn* family and have been identified in humans: KIAA0886 (AB020693), *Rtn-xL* (AB040462), reticulon 4a (AF148537), Nogo-A (AJ251383), Nogo-A short form (AF320999) and brain my043 (AF063601) (Fig. 4). The cDNA of *Rtn-T* is shorter than that of KIAA0886, *Rtn-xL*, reticulon 4a and Nogo-A. The first exon of *Rtn-T* at the 5' end was different from that of the above four genes. However, *Rtn-T* had a shorter ORF and coded a small protein (986 amino acids), which had a similar C terminus to all *Rtn* proteins. The cDNA of *Rtn-T* was longer than those of Nogo-A short form and brain my043. The C terminus of *Rtn-T* was also similar to that of Nogo A short form and brain my043 (Fig. 5).

Discussion

In the present study, cDNA microarray was used to identify the genes related to the development of human testis and a reticulon-like gene, named *Rtn-T*, was identified. The results indicated that the use of high-density arrays of spotted human testis cDNA clones offers a number of advantages in the identification of genes specifically expressed in adult or fetal testis. The results also showed that *Rtn-T* is differentially expressed in adult testis and encodes a membrane protein that may have a function in the development of human testis.

Different approaches have been developed to gain information on the amount of gene expression on the basis of intensity measurement related to differential expression, for example, whole-mount *in situ* hybridization (Wilkinson and Nieto, 1993), differential display technology (Liang and Pardee, 1992) and cDNA microarray (Eickhoff *et al.*, 2000). Some of these methods have been used in the identification of genes expressed in the testis. Goto *et al.* (2001) identified a testis-specific member of the olfactory receptor gene family in human primordial germ cells with differential

1	ggaggtgctgtggggagciggcagtggaagaacgtccacattgacccaaccgcagatga	1741	agtgcctgacatcttgtagttagtgaagattcctcactgattctgaaccagtgactt
61	aggagagcctccagtggtgtggtggagaaatggagagagaggttcttgatga	1801	atttagtgatgattcaactcagctgagcttcacaaaacagatgaactgtgattgtt
121	gacccttttctctctgcatcgtgagccigtgatacgtctctctcagaaaatat	1861	gaaagaagctcactgagacttcatgttcaatgatagaataatgaaaataagaaaa
181	ggacttgaagagagcagcaggttaacactatttgcgtggtcaagagatttcccatctgt	1921	actcagcttgcctcagctcagggaggaagcatttttggaactcttttaagctcagctt
241	cccttgtaaacctgctcttcttctctctctctctctctctcagcgcgtctttcaaa	1981	agatacacaaagatgacccctgttaccctgtagtgaacattgagcaaaaagagaaa
301	agaacatgaatccttgtaattgtcaacagtattaccacacgaagcaactcaaga	2041	aattccttgtagtgagagcagctcagctgagcttatttcaaaatgagacttattat
361	aatgtcagtgagcttcaagagcttcagagaagcgaagcaaaaacttactatagatg	2101	tictaagaagcagacataagagaacacgaagcttctcagattcattcctcaattgaat
421	agatttaacagagtttccagaattagaatactcagaatgggcatcctgtcagttctc	2161	tatagatgagttccctacattgacgttcttaaacctgattcttcttaattagccag
481	tcacaaagcagaatcgcgttaatagtagcaaatctctaggaaggaataatcgtgaanaa	2221	ggaatatactgacactaggaatccccacaaaagtgaaattgctaatgcccggaaggagc
541	taagacagaagaagaggttagttagtaataacatctctcataatcaacagagttacc	2281	tggtgctattggttcacagaatggccctgagcttcttgaagacatacaaccaaa
601	tacagcttacttaattgttaagagagtagtggtgtcttcagaaaaagcaaaaga	2341	agttgaagagaacaaacagtttctcagatgacgtttctaaaaatgggtctgtacatcaaa
661	cagttttaatgaaaagagagttgcagttggaagctctctatgagagaggaataatgcagactt	2401	gggtccttattgctccagatgttctgttggccactcaacagcagagatagagacat
721	caaacatttgagcagtagtggaagtgaaagatagtaaggaagatagtgatgttgc	2461	agttaaaacccaagttcttgtaagaagctgagaaaataatccttcogatacagaaaa
781	tgctggaggtaaactcagagcaacttgaaagtaaaagtggataaaaagtgttcgaga	2521	agagacagcaccatctctatatttccagcagctgagtaaaacttcagtttga
841	tagccttgagcaactaatcacgaanaagatagtgagagtagtaagtgatgatactttt	2581	ctctctgactgagagacataaagaactgagtggtgtgtgtggcgccctattctct
901	cccagtcacgcagaggtataaagatcgcttcagagcattatcacatgtgtccctt	2641	ggtgttctatgacagattacagctgtgagcgttaacacattgcttggccttggccct
961	taaccagcagcaactgagagcaatgcacaacattttcttctgttagagatcctac	2701	gctctgtagcactcattgagatatacaggggtgtagccaagctatccagaatc
1021	ticagaaaaataagacgatgaaaaaaatagaaaaggaagcccaaatgatacaga	2761	actgaagccaccctcagggcattcggatctggaattgctatctatctgaggagt
1081	gaagatactagcaacaaacatcaacccttttctgtgacgcagagattctgagac	2821	gggtacagatcacatctctcttctgtgacgtgacacgcacataaagggaactcag
1141	agattatgcacacagataatttaacaaagtgactgaggaagtggtgcaacatgcc	2881	ggcctcttcttagtgatgatttagttgattctctgagttgcagttgtagtgggt
1201	tgaagcctgactccagatttagtacagaagcagatggaagtgaattgagtgagttac	2941	attacatgtgtgtgctgtttaaagtggttcgacactgatttggctcatttc
1261	tgttacaagatttgtatgaacaaaaaaggacttggttcaaacatcagaagttatgca	3001	actcttgagttctgttattatgaagcgcacagcagcagatagattattctagg
1321	agagtcactctatcctgcagcacagcttggccatcatttgaagagtcagagctactcc	3061	acttgcaaatgaagatgttaaagatgctatggctaaatccaagcaaaaatccctggatt
1381	ttacaccagtttgctcagattgtatgaagcaccattgacttctgcagttcctagtc	3121	gaagcgcaagctgaatgaagaagcccaataatattagtaggagttcatctttaaagggg
1441	tgtgtctcctgatacagccagctcaccattagaagcttcttcagtttaattatga	3181	atattcatttgattatacggggaggttcaggaagaacgacactgacgttgagtgca
1501	aagcatalaaactgagctgaaacccccaccataagagagccattgagttgatact	3241	gtttcacagactggttagattcttatttttagccatgcactgtgtgaggaataatta
1561	aaaaaaagtatacaggaagaagaatttaagagcctgaaataattatgtagctt	3301	ccgtctgtgactgcatctgctcattcttcttaagttatgttaagctgtatgttgatt
1621	tcagaacaagaagctcttatactatctatgtagttgatttaataaagaacaaagct	3361	aaacogtaatactatttctcattcagggcagcactgggtaataaaaaaacttgaatt
1681	tctgtgaaccagctcggatttctcttgattattcagaataatggcaaaagttgaacgcc	3421	tiacttgttgtagatagttgtccgcacatcggcaggttgcaaaaatggtaggttaaa
	S A E P A P D F S D Y S E M A K A V E Q P	3481	aaaaaaaaaa

Fig. 2. Nucleic acid and deduced amino acid sequences of the cDNA for Rtn-T. Underlining shows PCR primers for the determination of expression profile. The upstream primer is located in the specific region of Rtn-T. The downstream primer is homologous with that of other genes of the Rtn family. Boxes show C-terminal hydrophobic regions.

Fig. 3. Expression profiles of (a) amplified Rtn-T and (b) amplified G3PDH as control after electrophoresis. PCR product of Rtn-T was 498 bp. Rtn-T was specifically and strongly expressed in testis and there was no expression in any other organs. G3PDH was expressed in all organs. Ma, marker; Te, testis; Pa, pancreas; He, heart; Ki, kidney; Lu, lung; Pl, placenta; Li, liver; Br, brain; Mu, muscle; BL, blank.

Fig. 4. Transcript and splicing comparison of Rtn-T with homologous genes. Seven homologues originate from one gene and consist of 10 exons (shown as boxes). Exon 2 is present only in Rtn-T and Rtn-T lacks exon 1. Nogo-A short form lacks exon 2, and brain my043 lacks exons 1 and 2. The length of exons 1 and 10 varies among the homologues (differences expressed by different colours).

A	1	-----	90
B		MEDLDQSPVSSSDSPRPQPAFKYQFVREPEDEEEEEEEEDEDEDELEEVLERKPAAGLSAAPVPTAPAAGAPLMDGNDGVFPAP	
C		MEDLDQSPVSSSDSPRPQPAFKYQFVREPEDEEEEEEEEDEDEDELEEVLER-----	
D		-----	
A	91	-----	180
B		RGPLAAPPVAPERQPSWDPSPVSSTVPAPSPLSAAVSPSKLPEDDEPPARPPPPASVSPQAEPVWTPAPAPAAPPSTPAAPKRRG	
C		-----	
D		-----	
A	181	-----MDLKEQPGNTISAGQEDFPSVLLETAASLPSLSPLSAAAFKEHYLGNLSTVLPTTEGLQENVS	270
B		SSGSVDETLFALPAASEPVIRSSAENMDLKEQPGNTISAGQEDFPSVLLETAASLPSLSPLSAAAFKEHYLGNLSTVLPTTEGLQENVS	
C		-----	
D		-----	
A	271	EASKEVSEKAKTLLIDRDLTEFSELEYSEMGSSFSVSPKAESAVIVANPREEIIIVKNKDEEEKLVSNILHNQQLPTALTKLVKEDEVV	360
B		EASKEVSEKAKTLLIDRDLTEFSELEYSEMGSSFSVSPKAESAVIVANPREEIIIVKNKDEEEKLVSNILHNQQLPTALTKLVKEDEVV	
C		-----TEFSELEYSEMGSSFSVSPKAESAVIVANPREEIIIVKNKDEEEKLVSNILHNQQLPTALTKLVKEDEVV	
D		-----	
A	361	SSEKAKDSFNEKRVAVEAPMREEYADFKPFERFVWEVKDSKEDSDMLAAGGKIESNLESKVDDKCFADSLEQTNHEKDSSESSNDTSFPST	450
B		SSEKAKDSFNEKRVAVEAPMREEYADFKPFERFVWEVKDSKEDSDMLAAGGKIESNLESKVDDKCFADSLEQTNHEKDSSESSNDTSFPST	
C		SSEKAKDSFNEKRVAVEAPMREEYADFKPFERFVWEVKDSKEDSDMLAAGGKIESNLESKVDDKCFADSLEQTNHEKDSSESSNDTSFPST	
D		-----	
A	451	PEGIKDRSGAYITCAPFNPAAATESIATNIFPLLGDPTSENKTEDEKKIEEKAQIVTEKNTSTKTSNPFLVAAQDSETDYVTTDNLTKVTE	540
B		PEGIKDRSGAYITCAPFNPAAATESIATNIFPLLGDPTSENKTEDEKKIEEKAQIVTEKNTSTKTSNPFLVAAQDSETDYVTTDNLTKVTE	
C		PEGIKDRSGAYITCAPFNPAAATESIATNIFPLLGDPTSENKTEDEKKIEEKAQIVTEKNTSTKTSNPFLVAAQDSETDYVTTDNLTKVTE	
D		-----	
A	541	EVVANMPEGLTPDLVQEACESELSEVTGTKIAYETKMDLVQTSEVMQESLYPAAQLCPSFEESEATPSVLPDIVMEAPLNSAVPSAGAS	630
B		EVVANMPEGLTPDLVQEACESELNEVTGTKIAYETKMDLVQTSEVMQESLYPAAQLCPSFEESEATPSVLPDIVMEAPLNSAVPSAGAS	
C		EVVANMPEGLTPDLVQEACESELNEVTGTKIAYETKMDLVQTSEVMQESLYPAAQLCPSFEESEATPSVLPDIVMEAPLNSAVPSAGAS	
D		-----MPEGLTPDLVQEACESELNEVTGTKIAYETKMDLVQTSEVMQESLYPAAQLCPSFEESEATPSVLPDIVMEAPLNSAVPSAGAS	
A	631	VIQPSPPLEASSVNYESIKHEPENPPPYEEAMSVSLKKVSGIKKEEIKEPENINAALQTEAPYISIACDLIKETKLSAEPAPDFSDYSE	720
B		VIQPSPPLEASSVNYESIKHEPENPPPYEEAMSVSLKKVSGIKKEEIKEPENINAALQTEAPYISIACDLIKETKLSAEPAPDFSDYSE	
C		VIQPSPPLEASSVNYESIKHEPENPPPYEEAMSVSLKKVSGIKKEEIKEPENINAALQTEAPYISIACDLIKETKLSAEPAPDFSDYSE	
D		VIQPSPPLEASSVNYESIKHEPENPPPYEEAMSVSLKKVSGIKKEEIKEPENINMQLFKKQLLIYLLHVDLIKETKLSAEPAPDFSDYSE	
A	721	MAKVEQVPVPHSELVEDSSPDSEPVDFSDDSIPDVPQKQDETVMVLKESLTETSFESMIEYENKEKLSALPPEGGKPYLESFKLSLNT	810
B		MAKVEQVPVPHSELVEDSSPDSEPVDFSDDSIPDVPQKQDETVMVLKESLTETSFESMIEYENKEKLSALPPEGGKPYLESFKLSLNT	
C		MAKVEQVPVPHSELVEDSSPDSEPVDFSDDSIPDVPQKQDETVMVLKESLTETSFESMIEYENKEKLSALPPEGGKPYLESFKLSLNT	
D		MAKVEQVPVPHSELVEDSSPDSEPVDFSDDSIPDVPQKQDETVMVLKESLTETSFESMIEYENKEKLSALPPEGGKPYLESFKLSLNT	
A	811	KDTLLPDEVSTLSKKEKIPLQMEELSTAVYSNDDLFIKSEAQIRETETFSOSSPIEIIDEFPTLISSKTDSEFKLAREYTDLEVSHKSEI	900
B		KDTLLPDEVSTLSKKEKIPLQMEELSTAVYSNDDLFIKSEAQIRETETFSOSSPIEIIDEFPTLISSKTDSEFKLAREYTDLEVSHKSEI	
C		KDTLLPDEVSTLSKKEKIPLQMEELSTAVYSNDDLFIKSEAQIRETETFSOSSPIEIIDEFPTLISSKTDSEFKLAREYTDLEVSHKSEI	
D		KDTLLPDEVSTLSKKEKIPLQMEELSTAVYSNDDLFIKSEAQIRETETFSOSSPIEIIDEFPTLISSKTDSEFKLAREYTDLEVSHKSEI	
A	910	ANAPDGAGSLPCTELPHDLSLKNIQPKVEEKISFSDDFSNGSATSQVLLLPDVSALATQAEIESIVKPKVLVKEAEKKLPDTEKEDR	990
B		ANAPDGAGSLPCTELPHDLSLKNIQPKVEEKISFSDDFSNGSATSQVLLLPDVSALATQAEIESIVKPKVLVKEAEKKLPDTEKEDR	
C		ANAPDGAGSLPCTELPHDLSLKNIQPKVEEKISFSDDFSNGSATSQVLLLPDVSALATQAEIESIVKPKVLVKEAEKKLPDTEKEDR	
D		ANAPDGAGSLPCTELPHDLSLKNIQPKVEEKISFSDDFSNGSATSQVLLLPDVSALATQAEIESIVKPKVLVKEAEKKLPDTEKEDR	
A	991	SPSAIFSAELSKTSVVDLLYWRDIKKTGVVFGASLFLLLSLTVFSIVSVTAYIALALLSVTISFRIYKGVIAIQKSDGHPFRAYLESE	1080
B		SPSAIFSAELSKTSVVDLLYWRDIKKTGVVFGASLFLLLSLTVFSIVSVTAYIALALLSVTISFRIYKGVIAIQKSDGHPFRAYLESE	
C		SPSAIFSAELSKTSVVDLLYWRDIKKTGVVFGASLFLLLSLTVFSIVSVTAYIALALLSVTISFRIYKGVIAIQKSDGHPFRAYLESE	
D		SPSAIFSAELSKTSVVDLLYWRDIKKTGVVFGASLFLLLSLTVFSIVSVTAYIALALLSVTISFRIYKGVIAIQKSDGHPFRAYLESE	
A	1081	VAISEELVQKYSNSALGHVNCITIKELRRLFLVDDLVDLSKFVLMWVFTYVGALFNGLTLLILALISLFSVPVYIERHQAQIDHYLGLAN	1170
B		VAISEELVQKYSNSALGHVNCITIKELRRLFLVDDLVDLSKFVLMWVFTYVGALFNGLTLLILALISLFSVPVYIERHQAQIDHYLGLAN	
C		VAISEELVQKYSNSALGHVNCITIKELRRLFLVDDLVDLSKFVLMWVFTYVGALFNGLTLLILALISLFSVPVYIERHQAQIDHYLGLAN	
D		VAISEELVQKYSNSALGHVNCITIKELRRLFLVDDLVDLSKFVLMWVFTYVGALFNGLTLLILALISLFSVPVYIERHQAQIDHYLGLAN	
	1		
A	1171	KNVKDAMAKIQAKIPGLKRKAE	1193
B		KNVKDAMAKIQAKIPGLKRKAE	
C		KNVKDAMAKIQAKIPGLKRKAE	
D		KNVKDAMAKIQAKIPGLKRKAE	

Fig. 5. Comparison of the deduced amino acid sequence of Rtn-T with homologues. Line A: Rtn-T; line B: KIAA0886, Rtn xL, reticulon 4a and Nogo-A; line C: Nogo-A short form; line D: brain my043. Grey highlighted areas indicate differences in amino acid sequence.

display. Raabe *et al.* (2001) also identified an SNF2 factor involved in mammalian development and cellular proliferation using this approach. Interest in and use of DNA array technology has increased markedly over the past 5 years, and there has been a surge in demand for different types of array. Although manufacturers offer a number of pre-made arrays, these are generally of utilitarian design and often cannot accommodate the specific requirements of focused research, such as a specific set of genes from a particular tissue. For this reason, some specific arrays, such as live expression array, have been established for particular studies. Rockett *et al.* (2001) produced a mouse testis expression array assembling 950 genes and used it in the study of gene expression patterns in testis. In the present study, an expression microarray was produced with 9216 clones from the human testis cDNA library. This microarray was used to find genes related to development of the testis by comparing expression in adult and fetal testes. Some genes were expressed mainly in adult testis and some mainly in fetal testis. The reciprocal expression characteristics of these genes indicates that different sets of genes are involved in different developmental stages, for example, those expressed mainly in adult testis may be related to spermatogenesis or androgen secretion. Study of these differentially expressed genes may reveal new genes related to spermatogenesis.

Sequence identification of 731 genes revealed that 37 were novel genes, whereas 90 have had at least one homologue reported before. One of these novel genes, a member of the reticulon gene family (Van de Velde *et al.*, 1994; Roebroek *et al.*, 1996, 1998; Moreira *et al.*, 1999; Morris *et al.*, 1999; Yang *et al.*, 2000), was named Rtn-T. All Rtn proteins are anchored to membranes of the endoplasmic reticulum and share a 200 amino acid residue region of sequence similarity (70% identical across the Rtn family) at the carboxyl terminus of the protein (Van de Velde *et al.*, 1994; Roebroek *et al.*, 1996, 1998; Moreira *et al.*, 1999; Morris *et al.*, 1999). The reticulon family consists of two main groups: Nogo and Rtn (also known as neuroendocrine-specific protein, NSP). DNA sequence analysis and northern blotting indicates that Nogo has three different transcripts (A, B and C) and protein products: Nogo-A (1163 amino acids), Nogo-B (360 amino acids) and Nogo-C (199 amino acids) (Chen *et al.*, 2000). Rtn also has four alternative transcripts, Rtn 1–4 (Hens *et al.*, 1998; GrandPre *et al.*, 2000). All transcripts are similar at the C-end, but different at the N-end. Reticulon-related sequences have been recognized in human, bovine, mouse, rat, fly and worm genomes (Spillmann *et al.*, 1998; Moreira *et al.*, 1999; Schafer *et al.*, 2001). Rtn proteins are expressed in neuroendocrine cells, lung carcinoma cells, oligodendrocytes and adipocytes (Senden *et al.*, 1997; Morris *et al.*, 1999; Chen *et al.*, 2000; Prinjha *et al.*, 2000). Blast searches revealed that Rtn-T was highly homologous to six genes: KIAA0886, Rtn-xL, reticulon 4a, Nogo-A, Nogo-A short form and brain my043, all of which belong to the Rtn family. Blast searches of these homologues in the human

genome revealed that all of these transcripts originate from one gene, and result from the use of alternative promoters and alternative splicing. In total, this gene has ten exons. Exon 2 occurs only in Rtn-T, which lacks exon 1. Blast search of amino acid sequences showed that the novel Rtn-T protein shared high homology with the proteins of the Rtn family at the carboxyl terminus, but the N-end of Rtn-T was shorter than that of KIAA0886, Rtn-xL, reticulon 4a and Nogo-A, and longer than that of Nogo-A short form and brain my043. The sequence upstream of the ORF in Rtn-T was also different from that of other genes. This same difference is found in other members of the Rtn family, such as KIAA0886, Rtn-xL, reticulon 4a and Nogo-A, and this may explain why the genes of this family are specifically expressed in different organs or tissues and appear to have different functions. For example, Rtn 1 is expressed exclusively in neuroendocrine cells (van de Velde *et al.*, 1994) and lung carcinoma cells with neuroendocrine phenotype (Senden *et al.*, 1997). Tissue specificity indicates that Rtn 1 is related to the function of neuroendocrine cells. Nogo was firstly purified from bovine brain (Spillmann *et al.*, 1998) and encodes at least three major protein products (Nogo-A, -B, and -C) (Chen *et al.*, 2000). Human Nogo-A was derived during the Kazusa DNA Research Institute effort to sequence random high molecular weight brain-derived cDNAs (Nagase *et al.*, 1998). Nogo-A is expressed by oligodendrocytes and contributes to the failure of axonal regeneration in the adult central nervous system (GrandPre *et al.*, 2000). Rtn 1 and Rtn XS were identified by Tagami *et al.* (2000), who reported that they were localized predominantly on the endoplasmic reticulum and could bind to Bcl-XL and Bcl-2. Rtn can also modulate anti-apoptotic activity (Tagami *et al.*, 2000). Bcl-2 and Bcl-XL serve as critical inhibitors of apoptosis triggered by a broad range of stimuli. Rtn-xL can interact with both Bcl-XL and Bcl-2, increase the localization of Bcl-XL and Bcl-2 on the endoplasmic reticulum, and reduce the anti-apoptotic activity of Bcl-XL and Bcl-2. Rtn-T is expressed in fetal and adult testis, but highly in adult testis, indicating that Rtn-T may be related to testis development and, in particular, spermatogenesis. It needs to be determined whether Rtn-T affects the process of spermatogenesis by influencing apoptosis.

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