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Short sequence-paper

Molecular cloning of a mammalian homologue of the yeast vesicular transport protein vps45 [1]

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Abstract

We have identified the rat homologue (rvps45) of the yeast vps45 protein, a member of the Sec1 family of proteins involved in intracellular vesicle trafficking. Sequence analysis of the full-length rvps45 cDNA obtained from a rat brain library predicts a protein of 570 amino acids which shares 36% identity with the yeast vps45 protein. The sequence shows less homology with other mammalian Sec1 family proteins. Northern blotting identified a 2.3 kb mRNA highly expressed in brain and testis. RT-PCR analysis showed that the rvps45 gene product is expressed throughout the brain. The homology of this protein with the yeast vps45 together with its high expression in brain suggests a role for rvps45 in transport from the Golgi complex to synaptic vesicles. © 1997 Elsevier Science B.V. All rights reserved.

Keywords: Sec1; Synaptic vesicle; Rat brain; Golgi apparatus; vps45; mRNA

The Sec1 family of proteins are involved in vesicular transport and synaptic transmission [1]. Four distinct Sec1 proteins have been identified in the yeast *Saccharomyces cerevisiae*. Sly1p is necessary for transport from the endoplasmic reticulum to the Golgi complex [2]. Sec1p mediates transport from the Golgi complex to the cell membrane [3], while vps45 is required for transport from the Golgi to a late endosomal, prevacuolar compartment [4,5]. Slp1p (vps33p) appears to mediate transport between the prevacuolar compartment and the lysosome-like vacuole [6,7].

Recently, homologues of some of the Sec1-family proteins have been identified in various species, including mammals, suggesting that the molecular machinery for vesicular transport is well conserved. The nematode Sec1 gene, unc-18, interacts with the synaptic vesicle protein syntaxin, and unc-18 mutations result in the accumulation of acetylcholine at the nerve terminal [8]. The mammalian Sec1p homologue, Munc-18, which was identified from rat brain (also known as n-Sec1 or rbSec1A), can also interact with syntaxin, and is involved in the regulation of transmitter release from synaptic vesicles [9–11]. In chromaffin cells it binds syntaxin 1A and is associated with chromaffin granules [12]. A number of Munc-18 isoforms have now been cloned from mammalian tissues, and each may have a specialized function in vesicle transport [13–16]. The mammalian homologue to yeast Sly1p was recently cloned

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from rat liver [17] and a similar Sly1p protein was identified from a rat brain cDNA library [18]. Given the existence of these mammalian homologues of the yeast Sec1 family, mammalian versions of the other yeast family members might exist. Here we report the molecular cloning and characterization of another member of the mammalian Sec1 family, rvps45, which is expressed at high levels in the testis and brain.

A clone (1.8 kb) was obtained during the course of screening a rat brain cDNA library (Clontech) on an unrelated project. Preliminary sequencing identified the clone as a potential homologue of the yeast vps45. Sequencing of both strands of the entire clone using the dideoxy chain-termination method (Sequenase 2.0, United States Biochemical) showed that this cDNA contained a single open reading frame of 1710 bp, corresponding to a full-length rvps45 cDNA (Fig. 1). The cDNA contained a predicted open reading frame coding for 570 amino acids. Analysis of the cloned rvps45 gene product showed 36% amino acid identity with the yeast vps45 (Fig. 2). In contrast, the identity of this sequence with the other mammalian Sec1 family members was less than 20% (Fig. 2). Northern blot analysis detected a single transcript of approx. 2.3 kb. The highest expression of the rvps45 mRNA was detected in rat brain and testis, with moderate to low levels in kidney, lung, spleen, heart and liver (Fig. 3).

For reverse-transcriptase-PCR analysis, total RNA was extracted from various tissues of adult male Sprague–Dawley rats [19]. One hundred nanograms of total RNA was reverse-transcribed using 200 units of MMLV reverse transcriptase (BRL) in a buffer containing a final concentration of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3.0 mM $MgCl_2$, 10 mM dithiothreitol, 5% dimethyl sulfoxide, 19 units RNase inhibitor (Pharmacia), 0.01% bovine serum albumin, deoxynucleotide triphosphates (dNTPs; Pharmacia) at 0.5 mM each and 0.25 μg of RT primer: 5′ AGCTACAGCTGAGCTGAGCTCAGT$_{20}$ 3′ in a final volume of 10 μl. PCR primers were used to amplify an 878 bp PCR product: forward primer: 5′ TTTCATCAGAGGCTGCAA 3′; reverse primer: 5′ CATGCAGAAAAGGCTGGT 3′. For amplification, one-twentieth of the RT reaction was used in the PCR mixture containing 10 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 2 units of Taq polymerase (BRL) and 30 pmol of each of the forward and reverse primers in a final volume of 50 μl. The mixture incubated in a thermal cycler for 32 cycles in case of rvps45 and 26 cycles in case of GAPDH. Fifteen microliters of the PCR product was run on 1% agarose gels stained with 0.2 μg/ml ethidium bromide and then visualized with UV light. A PCR product of the predicted size was obtained, subcloned into pCRII vector (version 2.1; Invitrogen) and sequenced. This confirmed the sequence information obtained from the cDNA obtained from the rat brain library. As shown in Fig. 4, RT-PCR indicated

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CAGCCAGGAAAGTGGGTGGGGAGGGTGTGGGGCACCTGTCAATTCGCCACCATGAACGTG   60
                                                  M  N  V      3
GTCTTTGCTGTGAAGCAGTATATTTCCAAAATGATAGAAGACAGCGGACCCGGCATGAAG  120
V  F  A  V  K  Q  Y  I  S  K  M  I  E  D  S  G  P  G  M  K     23
GTACTTCTCATGGATAAAGAGACGACTGGTATAGTGAGCATGGTCTACACACAGTCAGAG  180
V  L  L  M  D  K  E  T  T  G  I  V  S  M  V  Y  T  Q  S  E     43
ATTCTTCAGAAGGAAGTATACCTCTTCGAACGAATTGATTCTCAAAATCGAGAGATCATG  240
I  L  Q  K  E  V  Y  L  F  E  R  I  D  S  Q  N  R  E  I  M     63
AAACACCTAAAAGCAATTTGTTTCCTTCGACCTACAAAGGAGAATGTGGATTCTCTGATC  300
K  H  L  K  A  I  C  F  L  R  P  T  K  E  N  V  D  S  L  I     83
CAGGAGCTCCGAAGACCCAAGTATAGCATATATTTTATTTATTTCAGTAATGTGATCAGC  360
Q  E  L  R  R  P  K  Y  S  I  Y  F  I  Y  F  S  N  V  I  S    103
AAGAGTGACGTGAAGTCCTTGGCTGAAGCTGACGAGCAGGAAGTTGTGGCTGAAGTTCAG  420
K  S  D  V  K  S  L  A  E  A  D  E  Q  E  V  V  A  E  V  Q    123
GAATTTTATGGAGATTATATTGCTGTGAATCCACATTTGTTTTCCCTCAATATCTTGGGC  480
E  F  Y  G  D  Y  I  A  V  N  P  H  L  F  S  L  N  I  L  G    143
TGCTGTCAGGGTCGAAATTGGGATCCAGCCCAGCTATCCAGAACCACTCAAGGGCTGACC  540
C  C  Q  G  R  N  W  D  P  A  Q  L  S  R  T  T  Q  G  L  T    163
GCTCTCCTTTTGTCTCTGAAGAAGTGTCCCATGATTCGTTATCAGCTTTCATCAGAGGCT  600
A  L  L  S  L  K  K  C  P  M  I  R  Y  Q  L  S  S  E  A    183
GCAAAGAGACTGGGAGAGTGTGTTAAGCAAGTGATAAGTAAAGAGTATGAACTCTTTGAG  660
A  K  R  L  G  E  C  V  K  Q  V  I  S  K  E  Y  E  L  F  E    203
TTCCGGCGGACAGAGGTTCCTCCACTGCTTCTCATTCTGGATCGCTGCGATGATGCCATC  720
F  R  R  T  E  V  P  P  L  L  L  I  L  D  R  C  D  D  A  I    223
ACCCCACTGCTCAACCAGTGGACATATCAGGCCATGGTCCATGAACTACTGGGCATAAAC  780
T  P  L  L  N  Q  W  T  Y  Q  A  M  V  H  E  L  L  G  I  N    243
AACAACCGGATTGATCTTTCCAGAGTGCCAGGAATCAGCAAAGACTTAAGAGAGGTGGTC  840
N  N  R  I  D  L  S  R  V  P  G  I  S  K  D  L  R  E  V  V    263
CTGTCCGCTGAAAATGATGAATTCTATGCTAATAACATGTACCTGAACTTTGCCGAGATT  900
L  S  A  E  N  D  E  F  Y  A  N  N  M  Y  L  N  F  A  E  I    283
GGTAGCAATATAAAGAATCTCATGGAAGATTTCCAGAAGAAGAGACCGAAAGAGCAGCAA  960
G  S  N  I  K  N  L  M  E  D  F  Q  K  K  R  P  K  E  Q  Q    303
AAGCTAGAGTCCATAGCGGACATGAAGGCCTTTGTTGAAAATTATCCACAATTCAAGAAG 1020
K  L  E  S  I  A  D  M  K  A  F  V  E  N  Y  P  Q  F  K  K    323
ATGTCTGGGACTGTCTCAAAGCATGTGACAGTCGTTGGAGAACTGTCTCGGTTGGTCAGT 1080
M  S  G  T  V  S  K  H  V  T  V  V  G  E  L  S  R  L  V  S    343
GAACGAAACCTGCTGGAGGTTTCAGAGGTTGAGCAAGAACTGGCCTGTCAGAATGACCAT 1140
E  R  N  L  L  E  V  S  E  V  E  Q  E  L  A  C  Q  N  D  H    363
TCTAGTGCTCTTCAGAATGTGAAGAGACTCCTGCAGAATCCGAAAGTTACAGAATTTGAT 1200
S  S  A  L  Q  N  V  K  R  L  L  Q  N  P  K  V  T  E  F  D    383
GCAGTTCGCCTGGTGATGCTTTATGCTCTACATTATGAGCGCCACAGCAGCAACAGCCTG 1260
A  V  R  L  V  M  L  Y  A  L  H  Y  E  R  H  S  S  N  S  L    403
CCAGGGCTCATAGTGGACCTCAGGAGTAAAGGTGTCGCTGAGAAATATCGGAAGCTTGTG 1320
P  G  L  I  V  D  L  R  S  K  G  V  A  E  K  Y  R  K  L  V    423
TCTGCAGTTGTTGAATATGGTGGTAAACGGGTTAGAGGAAGTGACCTCTTCAGCCCCAAA 1380
S  A  V  V  E  Y  G  G  K  R  V  R  G  S  D  L  F  S  P  K    443
GATGCTGTGGCTATTACCAAACAGTTCCTCAAAGGCCTGAAGGGAGTGGAAAATGTGTAC 1440
D  A  V  A  I  T  K  Q  F  L  K  G  L  K  G  V  E  N  V  Y    463
ACCCAACACCAGCCTTTTCTGCATGAGACCCTGGACCATCTCATCAAAGGGAAGCTTAAG 1500
T  Q  H  Q  P  F  L  H  E  T  L  D  H  L  I  K  G  K  L  K    483
GAAAACCTCTATCCGTATTTAGGCCCCAGCACACTCAGAGACAGGCCTCAGGACATCATC 1560
E  N  L  Y  P  Y  L  G  P  S  T  L  R  D  R  P  Q  D  I  I    503
GTGTTCGTTATTGGAGGAGCCACCTATGAAGAGGCACTGACAGTCTATAACCTCAACCGT 1620
V  F  V  I  G  G  A  T  Y  E  E  A  L  T  V  Y  N  L  N  R    523
ACCACTCCTGGAGTGAGGATCGTTCTGGGAGGAACAACAATACACAACACAAAAAGTTTC 1680
T  T  P  G  V  R  I  V  L  G  G  T  T  I  H  N  T  K  S  F    543
CTAGAGGAAGTCCTGGCTTCTGGGCTGCACAGCCGCAGCAGAGAGAGCTCACAGGCCACC 1740
L  E  E  V  L  A  S  G  L  H  S  R  S  R  E  S  S  Q  A  T    563
TCAAGGTCAGCAAGCAGAAGATGAGATGGCAGTTGAGAACAGAGAGAATGTCACGTTCGA 1800
S  R  S  A  S  R  R  *                                        570
GCCGGCCG                                                      1809
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Fig. 1. Nucleotide and deduced amino acid sequence of rat vesicular transport protein vps45 (rvps45). The start and the stop codons are in bold-face.

that rvps45 is expressed in all the brain regions examined.

The Sec1 family of proteins plays an important role in the regulation of vesicle trafficking and secretory processes. Here we report the identification of the mammalian homologue of the yeast vps45, a member of the Sec1 family. Recent studies have shown that the mammalian Sly1 protein can interact with syntaxin 5 to regulate transport from the endoplasmic reticulum to the Golgi complex [17]. The various mammalian Sec1p-like proteins, the Munc-18s, are known to interact with a number of syntaxin isoforms and a role for these proteins in regulating synaptic vesicle docking or release has been suggested [9–12,14,16]. Binding of the Sec1 proteins to syntaxin appears to require the entire protein rather

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 vps45p  -------MNLFDVAD FYINKIVTSQSKLSV ANVNE-HQR--IKVL LLDKNTTPTISLCAT QSELLKHEIYLVERI ENEQREVSRHLRCLV     80
 rvps45  -------MNVVFAVK QYISKMIED------ -SGP---G---MKVL LMDKETTGIVSMVYT QSEILQKEVYLFERI DSQNREIMKHLKAIC     70
rsly1p   MVG----SKMAASIR ERQTVALKRMLNFNV PHVKNSPGEPVWKVL IYDRFGQDIISPLLS VKELRDMGITLHLLL HSD-RDPIRDVPAVY     85
munc18b  MAP----LGLKAVVG EKILSGVIR------ -SVKK-DGE--WKVL IMDHPSMRILSSCCK MSDILAEGITIVEDI NKR-REPIPSLEAIY     75
munc18   MAP----IGLKAVVG EKIMHDVIK------ -KVKK-KGE--WKVL VVDQLSMRMLSSCCK MTDIMTEGITIVEDI NKR-REPLPSLEAVY     75
munc18c  MAPPVSERGLKSVVW RKIKTAVFD------ -DCRK-EGE--WKIM LLDEFTTKLLSSCCK MTDLLEEGITVIENI YKN-REPVRQMKALY     79

 vps45p  YVKPTEETLQHLLRE LR-NP--RYGEYQIF FSNIVSKSQLERLAE SDDLE-AVTKVEEIF ---QDFFILNQDLFS FDLQP--REFLSN--    159
rbvps45  FLRPTKENVDSLIQE LR-RP--KYSIYFIY FSNVISKSDVKSLAE ADEQE-VVAEVQEFY ---GDYIAVNPHLFS LNILG---CCQGR--    148
rsly1p   FVMPTEENIDRLCQD LR-NQ--LYESYYLN FISAISRSKLEDIAN AALAANAVTQVAKVF DQYLNFITLEEDMFV LCNQNKELVSYRAIN    172
munc18b  LLSPTEKSVQALIAD FQGTPTFTYKAAHIF FTDTCPEPLFSELGR SRLAK-AVKTLKEIH ---LAFLPYEAQVFS LDAPHSTYNLYCP--    159
munc18   LITPSEKSVHSLISD FKDPPTAKYRAAHVF FTDSCPDALFNELVK SRAAK-VIKTLTEIN ---IAFLPYESQVYS LDSADSFQSFYSP--    159
munc18c  FISPTPKSVDCFLRD FGSKSEKKYKAAYIY FTDFCPDSLFNKIKA S-CSK-SIRRCKEIN ---ISFIPQESQVYT LDVPDAFYYCYSPD-    163

 vps45p  ----KLVWSEGGLTK CTNSLVSVLLSLKIK PDIR--YEGASKI-C ERLAKEVSYEIGKN- --ERTFFDFPVMDS- ---TPVLLILDRNTD    235
rbvps45  ------NWDPAQLSR TTQGLTALLLSLKKC PMIR--YQLSSEA-A KRLGECVKQVISK-- --EYELFEFRRTEVP ----PLLLILDRCDD    221
rsly1p   RPDITDTEMETVMDT IVDSLFCFFVTLGAV PIIRCSRGTAAEMVA VKLDKKLRENLRDAR NSLFTGDPLGTGQFS -FQRPLLVLVDRNID    261
munc18b  ---FRAGERGRQLDA LAQQIATLCATLQEY PSIR--YRKGPED-T AQLAHAVLAKLN--- --AFKADTPSLGEGP EKTRSQLLIMDRAAD    238
munc18   ---HKAQMKNPILER LAEQIATLCATLKEY PAVR--YRGEYKD-N ALLAQLIQDKLD--- --AYKADDPTMGEGP DKARSQLLILDRGFD    238
munc18c-  -PSNASRKEVVMEA MAEQIVTVCATLDEN PGVR--YKSKPLDNA SKLAQLVEKKLED-- --YYKIDEKGLIKG- -KTQSQLLIIDRGFD    243

 vps45p  PITPLLQPWTYQSMI NEYIGIKRNIVDLSK VPRID-------KDL --EKVTLSSKQDAFF RDTMYLNFGELGDKV KQYVTTYKDK----T    312
rbvps45  AITPLLNQWTYQAMV HELLGINNNRIDLSR VPGIS-------KDL --REVVLSAENDEFY ANNMYLNFAEIGSNI KNLMEDFQKK----R    298
rsly1p   LATPLHHTWTYQALV HDVLDFHLNRVNLEE STGVENSPTGARPKR KNKKSYDLTPVDKFW QKHKGSPFPEVAESV QQELESYRAQEDEVK    351
munc18b  PVSPLLHELTFQAMA YDLLDIEQD-TYRYE TTGLS-------ESR --EKAVLLDEDDDLW VELRHMHIADVSKKV TELLKTFCES----K    314
munc18   PSSPVLHELTFQAMS YDLLPIEND-VYKYE TSGIG-------EAR --VKEVLLDEDDDLW IALRHKHIAEVSQEV TRSLKDFSSS----K    314
munc18c  PVSTVLHELTFQAMA YDLLPIEND-TYKYK TDGK---------- --EKEAVLEEDDDLW VRVRHRHIAVVLEEI PKLMKEISST----K    315

 vps45p  QTNS---------- ---QINSIEDIKNFI EKYPEFRKLSGNVAK HMAIVGELDRQLKIK NIWEISEIEQNLSAH DANEE-----DFSDL    383
rbvps45  PKEQQ---------- ---KLESIADMKAFV ENYPQFKKMSGTVSK HVTVVGELSRLVSER NLLEVSEVEQELACQ NDHSS-----ALQNV    370
rsly1p   RLKSIMGLEGEDEGA ISMLSDNTAKLTSAV SSLPELLEKKRLIDL HTNVATAVLEHIKAR KLDVYFEYEEKIMSK TTLDK-----SLLDV    436
munc18b  RLTT---------- ---DKANIKDLSHIL KKMPQYQKELNKYST HLHLADDCMKHF-KG SVEKLCSVEQDLAMG SDAEGEKIKDAMKLI    389
munc18   RMNTG---------- ---EKTTMRDLSQML KKMPQYQKELSKYST HLHLAEDCMKHY-QG TVDKLCRVEQDLAMG TDAEGEKIKDPMRAI    390
munc18c  KATE---------- ---GKTSLSALTQLM KKMPHFRKQISKQVV HLNLAEDCMNKF-KL NIEKLCKTEQDLALG TDAEGQRVKDSMLVL    390

 vps45p  IKLLQNEAVDKYYKL KLACIYSLN-NQTSS D-KIRQLVEILSQQL P--PEDVNFFHKFKS LFSRQDK---MTQSN HDK-DDILTELARRF    465
rbvps45  KRLLQNPKVTEFDAV RLVMLYALHYERHSS N-SLPGLIVDLRSKG ----VAEKYRKLVSA VVEYGGK-----RVR GSDLFSPKDAVAITK    450
rsly1p   ISDP-DAGTPE-DKM RLFLIYYISAQQAPS EVDLEQYKKALTDAG -CNLSPLQYIKQWKA FAKMAST----PASY GNTTTKPMGLLSRVM    519
munc18b  VPVLLDASVPPYDKI RVLLLYILLRNGVSE E-NLAKLIQHANVQS -YSSLIRNLEQLGGT VTNSAGSGTSSRLER RER-MEPTYQLSRWS    476
munc18   VPILLDANVSTYDKI RIILLYIFLKNGITE E-NLNKLIQHAQIPP EDSEIITNMAHLGVP IVTDSTLRRRSKPER KERISEQTYQLSRWT    479
munc18c  LPVLLNKNHDNCDKI RAVLLYIFGINGTTE E-NLDRLIHNVKIED -DSDMIRNWSHLGVP IVPPSQQ---AKPLR KDRSAEETFQLSRWT    475

 vps45p  NSRMNSKSNTAENVY --MQHIPEISSLLTD LSKNALFRDRFKEID TQGHRVIGNQQSKDI --PQD------VILF VIGGVTYEEARLVHD    545
rbvps45  QFLKGLKG--VENVY --TQHQPFLH-ETLD HLIKGKLK-----EN LYPYLGPSTLRDR-- --PQD------IIVF VIGGATYEEALTVYN    520
rsly1p   NTGSQFVMEGVKNLV LKQQNLPVTRILDNL MEMKSNPE-----TD DYRYFDPKMLRSNDS SVPRNKSPFQEAIVF VVGGGNYIEYQNLVD    604
munc18b  PVIKDVMEDVVEDRL D-RKLWPFVSDPAPV PSSQAAVS----ARF GHWHKNKAGVEARAG --PR-------LIVY IVGGVAMSEMRAAYE    552
munc18   PIIKDIMEDTIEDKL D-TKHYPYISTRSSA SFSTTAVS----ARY GHWHKNKAPGEYRSG --PR-------LIIF ILGGVSLNEMRCAYE    555
munc18c  PFIKDIMEDAIDNRL D-SKEWPYCSRCPAV WNGSGAVS-----AR QKPRTNYLELDRKNG --SR-------LIIF VIGGITYSEMRCAYE    550

 vps45p  FNGTMNNRMRVVLGG TSILSTKEYMDSIRS -----AK-------- -----    577
rbvps45  LNRTTPGVRIVLGGT TIHNTKSFLEEVLAS GLHSRSRESSQATSR SASRR    570
rsly1p   YIKGK-QGKHILYGC SEIFNATQFIKQLSQ ----LGQK------- -----    637
munc18b  VTRATEGKWEVLIGS SHILTPTRFLDDLKT -LD-QKLE--GVALP -----    593
munc18   VTQAN-GKWEVLIGS THILTPQKLLDTLKK -LN-KTDE--EISS- -----    594
munc18c  VSQAH-KSCEVIIGS THILTPRKLLDDIKM -LN-KSKD--KVSFK DE---    592
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Fig. 2. Comparison of the amino acid sequence of rvps45 from rat brain with its yeast homologue vps45 [4,5], and the other mammalian Sec1 protein family members, rsly1p [17]; munc18b [16], munc 18 [9] and munc18c [16]. Identical amino acids in all sequences are underlined, while conserved residues are in bold-face. Initial sequence alignments were performed using the ClustalW program.

than a discrete domain [10]. Given the overall homology of the rat vps45 to the other mammalian Sec1 proteins (Fig. 2), and their similar predicted secondary structure (data not shown), it is likely that rvps45 also interacts with particular syntaxin isoforms.

The exact role and subcellular localization of the mammalian rvps45 are not known yet, but studies conducted on its yeast homologue show that vps45 is involved in the regulation of vesicle trafficking between the Golgi apparatus and the vacuole [4,5]. Null mutations of the yeast vps45 lead to the accumulation of membrane vesicles and defects in vacuolar protein sorting [4,5]. These results suggest that the site of action of the mammalian rvps45 may also be in transport from the Golgi complex into secretory vesicles. It may therefore interact with the recently identified syntaxin 6, which is localized to the Golgi apparatus [20]. The identification of the mammalian rvps45 will provide us with the tools to determine the role of this protein in mammalian secretory pathways. In particular, the high expression of this protein in the brain suggests that rvps45 could play an important role in synaptic vesicle trafficking and neurotransmission.

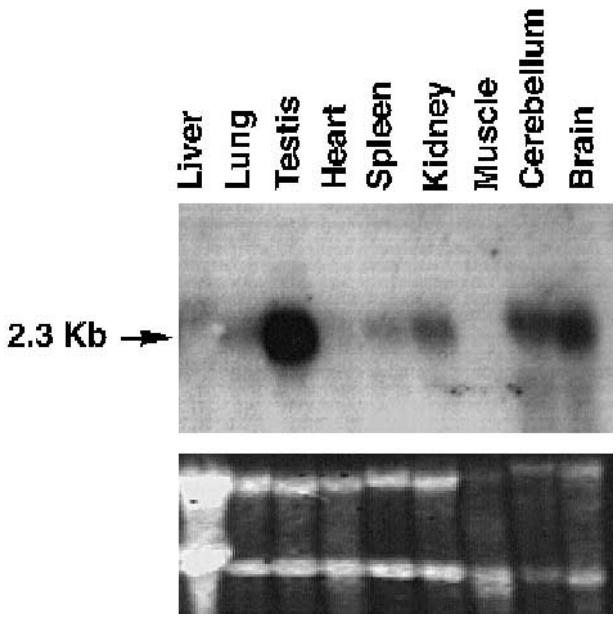

Fig. 3. Northern blot analysis of rvps45 mRNA. Twenty micrograms of total RNA from various rat tissues was electrophoresed in 1% denaturing agarose gels, transferred to a nylon membrane, and hybridized with a ^{32}P-labeled rvps45 full-length cDNA probe prepared with a random priming kit (BRL). Top panel: A 2.3 kb transcript of rvps45 is detected in all the tissues examined except skeletal muscle. Lower panel: ethidium bromide stained photograph of the blotted total RNA. Particularly high expression of rvps45 is present in brain and testis.

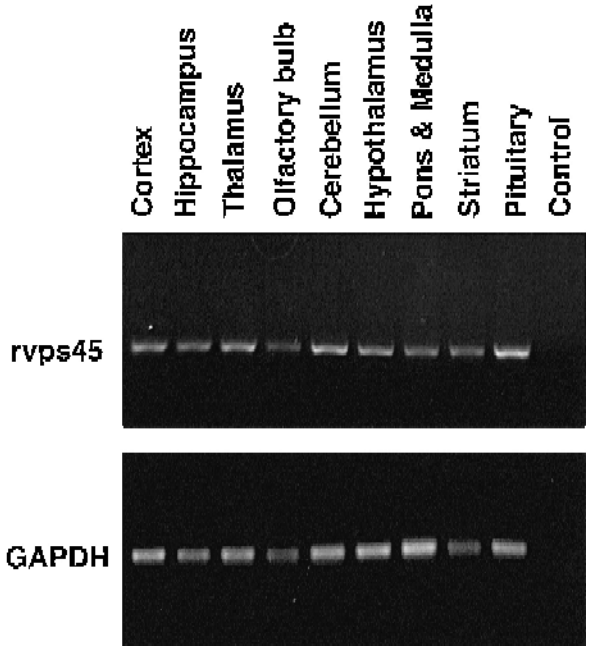

Fig. 4. RT-PCR analysis of rvps45 gene expression in various brain regions. Ethidium bromide stained gel of the rvps45 PCR product (878 bp; top panel) and GAPDH (343 bp; lower panel). Control lane contains all the RT-PCR reagents but without RNA. Expression of the rvps45 was detected in all the brain regions examined.

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