

Alternative splicing of synaptotagmins involving transmembrane exon skipping

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Received 6 August 1999; received in revised form 6 October 1999

Abstract The synaptotagmin gene family currently includes 12 members. Analysis of the three known genomic synaptotagmin sequences reveals conserved exon-intron patterns which delineate the synaptotagmin structural domains. We used expressed sequence tag, reverse transcription PCR and RNase protection assay analysis of synaptotagmin messenger RNAs to demonstrate the occurrence of alternative splicing events involving a number of exons. Exon-skipped messages where transmembrane sequences have been removed or altered were found to be abundantly expressed by synaptotagmins 1, 4, 6 and 7. Although the expression of most synaptotagmins predominates in neural tissue, we find that by contrast, synaptotagmin 6 is more abundant in thymus.

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Key words: Synaptotagmin; Exon skipping; Alternative splicing

1. Introduction

Synaptotagmin genes are expressed mainly in neural tissue and share a common domain structure, the defining elements of which are a N-terminal transmembrane sequence and two C-terminal C2 domains [1,2]. A great deal is known about the structure and function of synaptotagmin 1. Studies in vitro and in vivo such as biochemistry, microinjections and gene transfections with wild-type and mutant sequences [3–7], together with the solution of crystal and NMR structures of the C2 domain [8–10] and the production of knockout mice [11], clearly establish synaptotagmin 1 as a crucial component of synaptic vesicles, responsible for the fast component of neurotransmitter release. It binds calcium, possibly acting as a calcium sensor, as well as to a number of other proteins and lipids [12–22].

Much less is known about the other 11 members of the synaptotagmin family, but biochemical experiments with their C2 domains reveal that the different sequences have different affinities for their various binding partners [23–29]. In situ hybridisation studies show that the different genes have distinct but overlapping expression patterns [30–36], with some being rapidly induced by seizures [37,38], whereas another is induced by thyroid hormone [39]. Some synaptotagmins have been found to play a role in calcium-regulated vesicle exocytosis in peripheral tissues, for example catecholamine secretion from chromaffin cells, pancreatic insulin secretion and mast

cell lysosome exocytosis [40–43]. It seems unlikely, however, that all synaptotagmins are functionally equivalent to synaptotagmin 1, with an integral vesicle location and a role in calcium-regulated exocytosis. Isolated synaptotagmin C2 domains can bind to membranes and lipids with or without calcium [44–46] and recent work shows that in brain, synaptotagmins 3 and 6 are clustered mainly at sites on the cytoplasmic plasma membrane rather than on synaptic vesicles [47]. It is thus becoming apparent that the synaptotagmin family encompasses a functional diversity which is not restricted to the calcium regulation of synaptic vesicle exocytosis. Here, we show that tissue-specific expression and alternative splicing further add to the potential functions of some family members.

2. Materials and methods

2.1. Sequence analysis

Complementary DNA and genomic sequences were aligned using the align program [48] and the sip4 program [49]. The nucleotide sequence databases were searched using the BLAST server at NCBI [50] in order to identify novel synaptotagmin expressed sequence tag (EST) sequences.

2.2. RNA analysis

Tissues were dissected from adult or E17 embryo Sprague-Dawley rats and immediately frozen on dry-ice. Total RNA was prepared using guanidine isothiocyanate and poly(A) selected using oligo-dT cellulose. Reverse transcription PCR (RT-PCR) was performed with Pfu polymerase (Stratagene). Full-length RT-PCR products were cloned into pBSIIKS⁺ (Stratagene). RNase protection assay (RPA) probes were produced from these clones using the Maxiscript kit (Ambion) and were hybridised and digested with the Hybspeed RPA kit (Ambion). RPA products were run on 4% acrylamide sequencing gels. Results were collected on a Molecular Dynamics phosphorimager. RT-PCR from human adult brain mRNA (Clontech) employed primers corresponding either to rat synaptotagmin 7 cDNA sequence accession U20106, positions 161–180 and 524–543, or to human synaptotagmin 5 genomic sequence accession X96783, positions 392–411 and 4301–4320. RT-PCR products were digested with *Pst*I (synaptotagmin 7) or *Pst*I and *Sac*I (synaptotagmin 5) and cloned into puc18.

3. Results

We had previously determined the genomic organisation of the human synaptotagmin 5 gene and described an alternative splicing event between exons 5 and 6 of its eight exons [51]. Subsequently, RT-PCR analysis of human brain synaptotagmin 5 mRNA revealed another intriguing alternative splicing event, exon skipping. Exon 2, which encodes the transmembrane sequence, was found to be precisely skipped out of a significant proportion of messages. Several different primer pairs flanking exon 2 gave equivalent exon-skipped RT-PCR products. An example is sequence accession Y19236 (see Ta-

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Abbreviations: EST, expressed sequence tag; RT-PCR, reverse transcription PCR; RPA, RNase protection assay

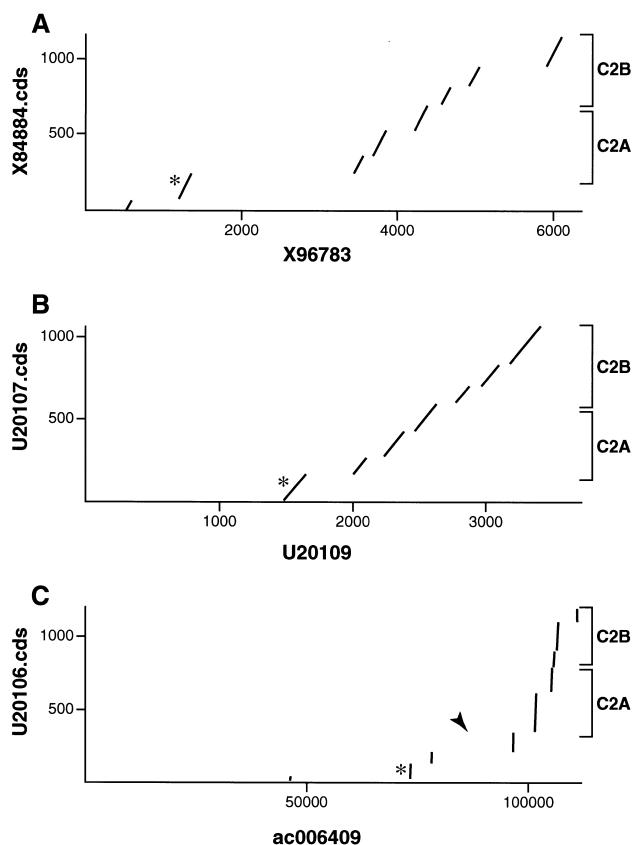


Fig. 1. cDNA versus genomic sequence plots produced by sip4 [49]. Transmembrane exons are indicated by asterisks. C2A and C2B exons are indicated. A: Comparison of rat synaptotagmin 5 coding sequence (accession X84884) and human synaptotagmin 5 genomic sequence (accession X96783). B: Comparison of mouse synaptotagmin 8 coding sequence (accession U20107) and mouse genomic synaptotagmin 8 sequence (accession U20109). C: Comparison of rat synaptotagmin 7 coding sequence (accession U20106) and human genomic synaptotagmin 7 sequence (accession AC006409). The arrowhead marks the position of novel exons (see Fig. 2 and Table 1).

ble 1), which includes exons 1, 3, 4 and 5 but excludes exon 2. The vast majority of known synaptotagmin gene sequences are derived from cDNA clones and almost all of the individual synaptotagmin family members are represented by those from rat, so we chose to investigate the rat synaptotagmin family to see whether exon skipping as observed in human synaptotagmin 5 is a unique event or a more general phenomenon.

Although there is no genomic sequence indicating the exon-intron organisation of the rat synaptotagmin genes, the complete genomic sequence is available for human synaptotagmin 7 (accession AC006409) and partial genomic sequences for human synaptotagmin 5 (accession X96783) and mouse synaptotagmin 8 (accession U20109). cDNA sequences plotted against the genomic sequences graphically reveals the intron-exon structure (Fig. 1). All three genes have a separate transmembrane exon and their C2B domain exons are similarly organised. All of the introns shared between synaptotagmins 5 and 8 have exactly the same location and phase. The C2B domain intron positions of synaptotagmin 7 also have the same phase but their location is not precisely equivalent. The arrangement of the region between the transmembrane domain and the C2B domain is different in synaptotagmin 7. The C2A domain is encoded by only two exons and is preceded by two exons which encode the relatively longer linker sequence between the transmembrane domain and the C2A domain. RT-PCR-derived sequences of human synaptotagmin 7 brain mRNA together with an examination of the synaptotagmin 7 EST sequences at NCBI [50] indicate that at least another five novel exons may be included in some synaptotagmin 7 mRNAs, as indicated by the arrowhead in Fig. 1. The RT-PCR sequences (accession numbers Y19237, Y19238, Y19239 and Y19240) and EST sequences (accession numbers AI511081, AI713274, AI704933 from rat and R63907, R63992 from human) are described in Table 1. Together, they reveal five more exons between exons 3 and 4 of the nine exons of rat synaptotagmin 7 cDNA sequence accession U20106. Fig. 2

Table 1

RT-PCR/EST* accession	Structure in terms of reference sequence	Reference sequence	Accession	Transmembrane exon (exon 2)
Y19236	Join: 537–587 (exon 1), 3424–3543 (exon 3), 3672–3839 (exon 4), 4207–4313 (exon 5)	Human synaptotagmin 5 genomic	X96783	1180–1352
Y19241	Join: 246–450 (exon 1), 534–640 (exon 3)	Rat synaptotagmin 6 cDNA	U20105	451–533
Y19242	Join: 161–191 (exon 1), 296–375 (exon 3), 376–500 (exon 4)	Rat synaptotagmin 7 cDNA	U20106	192–295
Y19242	Join: 48560–48590 (exon 1), 77915–77994 (exon 3), 96270–96394 (exon 4)	Human synaptotagmin 7 genomic	AC006409	73171–73274
Y19237	Join: 86980–87167 (exon 3d), 91124–91246 (exon 3e), 96270–96280 (exon 4)	Human synaptotagmin 7 genomic	AC006409	73171–73274
Y19238	Join: 73241–73274 (exon 2), 77915–77994 (exon 3), 96270–96280 (exon 4)	Human synaptotagmin 7 genomic	AC006409	73171–73274
Y19239	Join: 86980–87167 (exon 3d), 96270–96280 (exon 4)	Human synaptotagmin 7 genomic	AC006409	73171–73274
Y19240	Join: 73241–73274 (exon 2), 77915–77994 (exon 3), 82070–82201 (exon 3a), 96270–96280 (exon 4)	Human synaptotagmin 7 genomic	AC006409	73171–73274
AI511081*	Join: 48509–48590 (exon 1), 73171–73274 (exon 2), 77915–77994 (exon 3), 82070–82205 (exon 3a) unknown 19 nt	Human synaptotagmin 7 genomic	AC006409	73171–73274
AI713274*	Join: poly(T), unknown 38 nt, 83347–83123 (exon 3c), 82843–82719 (exon 3b), 82201–82140 (exon 3a)	Human synaptotagmin 7 genomic	AC006409	73171–73274
AI704933*	Join: poly(T), unknown 38 nt, 83347–83257 (exon 3c)	Human synaptotagmin 7 genomic	AC006409	73171–73274
R63907*	Join: 48559–48590 (exon 1), 73171–73274 (exon 2), 77915–77994 (exon 3), 82070–82186 (exon 3a)	Human synaptotagmin 7 genomic	AC006409	73171–73274
R63992*	Join: 96302–96270 (exon 4), 83347–83288 (exon 3c)	Human synaptotagmin 7 genomic	AC006409	73171–73274

<p>Exon 1</p> <p>48650 ATG TAC CGG GAC CCG GAG GCG GCC AGC CCA G 48590</p> <p>M Y R D P E A A S P</p>	<p>Exon 4</p> <p>96270 G TTG CCT GCA GGA GGG AAG GCG GTG AAC ACA GCC CCC GTG</p> <p>L P A G G K A V N T A P V</p> <p>CCA GGC CAG ACA CCC CAC GAT GAG TCC GAC CGC CGG ACC GAG</p> <p>P G Q T P H D E S D R R T E</p> <p>CCA CGT TCC TCC GTC TCA GAC CTC GTC AAC TCC CTC ACC AGC</p> <p>P R S S V S D L V N S L T S</p> <p>GAG ATG CTC ATG 96405</p> <p>E M L M</p>
<p>Exon 2</p> <p>73171 GG GCG CCC TCG CGC GAC GTC CTG CTG GTC TCT GCC ATC ATC</p> <p>G A P S R D V L L V S A I I</p> <p>ACC GTC AGC CTT AGC GTC ACT GTC GTC CTC TGC GGC CTC TGC</p> <p>T V S L S V T V V L C G L C</p> <p>CAC TGG TGT CAG CGC AAA CTG 73274</p> <p>H W C Q R K L</p>	<p>Exon 5</p> <p>101209 CTC TCC CCA GGC TCC GAG GAG GAT GAG GCC CAC GAG GGT TGC</p> <p>L S P G S E E D E A H E G C</p> <p>AGC CGA GAG AAC CTG GGC CGG ATC CAG TTC AGT GTC GGC TAC</p> <p>S R E N L G R I Q F S V G Y</p> <p>AAC TTC CAG GAG TCC ACG CTC ACC GTG AAG ATC ATG AAG GCC</p> <p>Q E L P A K D F S G T A G C A C C C</p> <p>CAG GAG CTG CCG GCC AAG GAC TTC AGC GGC ACC AGC GAC CCC</p> <p>Q E L P A K D F S G T A G C A C C C</p> <p>TTC GTC AAG ATC TAC CTG CTG CCC GAC AAG AAG CAC AAG CTG</p> <p>F V K I Y L L P D K K H K L</p> <p>GAG ACC AAG GTG AAG CGG AAG AAC CTG AAC CCC CAC TGG AAC</p> <p>E T K V K R N L N P H W N</p> <p>GAG ACC TTC CTC TTT GAA G 101479</p> <p>E T F L F E</p>
<p>Exon 3</p> <p>77915 GGC AAA CGC TAC AAG AAT TCC TTG GAG ACG GTG GGC ACG CCA</p> <p>G K R Y K N S L E T V G T P</p> <p>GAC TCA GGC CGT GGC CGC AGT GAG AAG AAG GCT ATC AA 77994</p> <p>D S G R G R S E K K A I K</p> <p>Exon 3a</p> <p>82070 TGATCTAGACAGAGACTTTTGAATAACAATGAGAGCACAGTGCAGCAGAAATG</p> <p>D L D R D F W N N N E S T V Q Q K W</p> <p>GAGCTCCTACCCCTCCCAAGGAGTTTATTCTAAACATTTCACCTACGCCCCCTTA</p> <p>S S Y P P K E F I L N I S P Y A P Y</p> <p>TGGCGACCCACGACTGTCCCTCAA 82201 GTGA 82205</p> <p>G D P R L S L K *</p>	<p>Exon 6</p> <p>104862 GT TTT CCC TAT GAG AAG GTG GTG CAG AGG ATC CTC TAC CTC</p> <p>G F P Y E K V V Q R I L Y L</p> <p>CAA GTC CTG GAC TAT GAC CGC TTC AGC CGC AAC GAC CCC ATT</p> <p>Q V L D Y D R F S R N D P I</p> <p>GGG GAG GTG TCC ATC CCC CTT AAC AAG GTG GAC CTG ACC CAG</p> <p>G E V S I P N K V D L T T Q</p> <p>ATG CAG ACC TTC TGG AAG GAT CTG AAG CCA TGC AGC GAT GGG</p> <p>M Q T F W K D L K P C S D G</p> <p>AGT 105031</p> <p>S</p>
<p>Exon 3b</p> <p>82719 CCCGCTCTTCTCCGTCGTTAACCTTCGTTGTCGTGGGATAGAGTTGGAGGTG</p> <p>R S S S V V N P S L S C G I E L E V</p> <p>GCTGCCCTCCCCCAACCCCGCCGCTGCCAGGCGGTGGGAGGGGCCCCC</p> <p>A A L P Q P P P P L P Q A V G R G P P</p> <p>CTCCGTTGTCGTG 82843</p> <p>S V V V</p> <p>Exon 3c</p> <p>83123 AGTGGCACCTCTCTGTCGGGCGCCAAAGTGGCCCGCGCGGGCTGGCGGTGGA</p> <p>S G T L L S G A K V A A A A G L A V E</p> <p>W H P P V G R Q S G R R G G A G G G</p> <p>GCGGGAAGCCCGCTGGGGGAGAAGCCGGCACCGGTGCGGCCACCCGGAGAGGAGC</p> <p>R E G R L G E K P A P V P P P P G E D</p> <p>A G R P A G G E A G T G A A T R R G R</p> <p>CCTTGAGAAGCGCGGGGCTGCCCCAGCAGCGCGGCGAGCGGTGGCAAGCGCGGG</p> <p>A L R S G G A A P S E P G S G G K A G</p> <p>L E K R R G C P Q R A G Q R W Q G G</p> <p>AGAGCGCGCTGGCGGACGGTGCAGAGCCACCTGGCCGCGAGGAAGCTCAACTGTC</p> <p>R G R W R T V Q S H L A A G K L N L S</p> <p>E R P L A D G A E P P G R R E A Q L V</p> <p>CAA 83347</p> <p>K</p> <p>Q</p>	<p>Exon 7</p> <p>105453 GGG AGC CGA GGG GAG CTG CTC TTG TCT CTC TGC TAC AAC CCC</p> <p>G S R G E L L L S L C Y N P</p> <p>TCT GCC AAC TCC ATC ATC GTG AAC ATC ATC AAA GCC CGG AAC</p> <p>S A N S I I V N I I K A R N</p> <p>CTC AAA GCC ATG GAC ATC GGG GGC ACA TCA G 105567</p> <p>L K A M D I G G T S</p>
<p>Exon 3d</p> <p>86980 CTGAGGCCACATGGCCTCGGCACCAAGGCCCAACCCCGGCCATATGGCCGGG</p> <p>L Q A H M A S A P G P N P R A Y G R G</p> <p>A G P H G L G T R P Q P P G L W P G</p> <p>CCAGGCTCGGCAGGCACCTCGGCCGCTCCAAGTACCGGGCGGAGGGGGCCGA</p> <p>Q A R Q G T S A G S K Y R A A G G R</p> <p>P G S A G H L G R L Q V P G G R G P Q</p> <p>GCCGCTCCAACCCAGGCAGCTGGGACCACTGGTGGGCGAGATTGAAACCGAGGC</p> <p>S R S N P G S W D H V V G Q I R N R G</p> <p>P L Q P R Q L G P R G G A D S K P R</p> <p>TTGGACATGAATCCTTCTCT 87167</p> <p>L D M K S F L</p> <p>L G H E I L P</p>	<p>Exon 8</p> <p>106120 AC CCC TAC GTG AAG GTA TGG CTG ATG TAC AAG GAC AAG CGG</p> <p>D P Y V K V W L M Y K D K R</p> <p>GTG GAG AAG AAG AAG ACG GTG ACG ATG AAG AGG AAC CTG AAC</p> <p>V E K K K T V T M K R N L N</p> <p>CCC ATC TTC AAT GAG TCC TTC GCC TTC GAT ATC CCC ACG GAG</p> <p>P I F N E S F A F D I P T E</p> <p>AAG CTG AGG GAG ACG ACC ATC ATC ATC ACT GTC ATG GAC AAG</p> <p>K L R E T T I I I T V M D K</p> <p>GAC AAG CTC AGC CGC AAT GAC GTC ATC GGC AAG 106319</p> <p>D K L S R N D V I G K</p>
<p>Exon 3e</p> <p>91124 GGAAGCCCGATGGTGGTCTATCCTTGGTCTTAGGGCTTTTCGGAACAGGATGACT</p> <p>E G R M V V L S L V L G L S E Q D D</p> <p>TTGCCAATATCCCTGACCTGCAAAACCCAGGAACCCAGCAGAACCAGAACGCTCAG</p> <p>F A N I P D L Q N P G T Q Q N Q N A Q</p> <p>GGGACAAGAG 91246</p> <p>G D K R</p>	<p>Exon 9</p> <p>110735 ATC TAC CTG TCC TGG AAG AGC GGG CCA GGG GAG GTG AAG CAC</p> <p>I Y L S W K S G P G E V K H</p> <p>TGG AAG GAC ATG ATT GCC CGT CCC CGG CAG CCC GTG GCC CAG</p> <p>W K D M I A R P R Q P V A Q</p> <p>TGG CAC CAG CTG AAG GCC TGA 110839</p> <p>W H Q L K A *</p>

Fig. 2. Human chromosome 11 genomic sequence accession AC006409 synaptotagmin 7 gene exon sequences. Nucleotide positions are indicated at the 5' and 3' ends of each exon. The exon phase and translation is indicated for exons 1–9. Translations of the open reading frames of exons 3a–e are indicated.

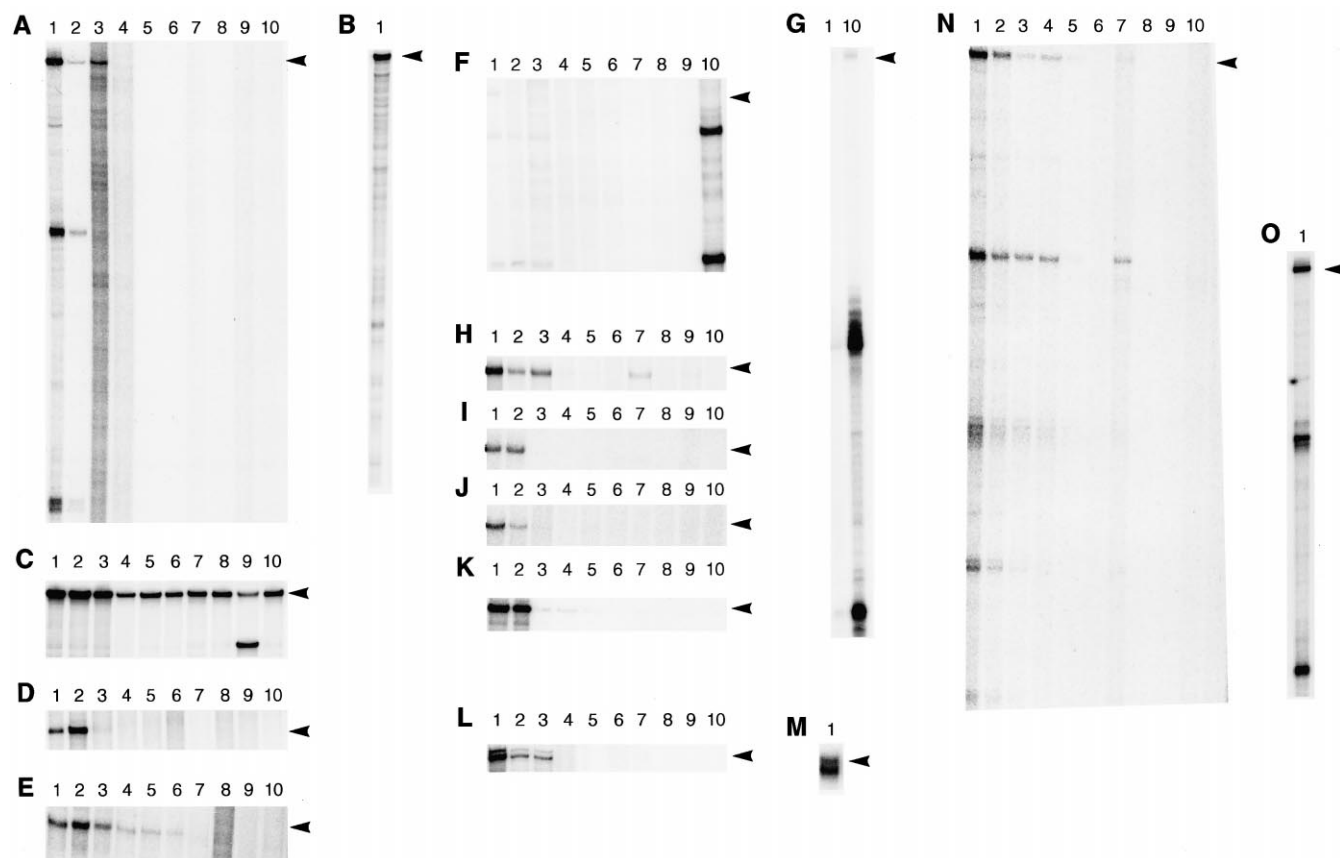


Fig. 3. RPA results. Probes A–O are detailed in Table 1. Arrowheads indicate full-length products, the sizes of which are: A 431 nt, B 430 nt, C 476 nt, D 402 nt, E 334 nt, F 357 nt, G 395 nt, H 455 nt, I 458 nt, J 330 nt, K 481 nt, L 579 nt, M 242 nt, N 520 nt, O 340 nt. Tissue mRNAs are: 1 adult brain, 2 adult spinal cord, 3 E17 embryo forebrain, 4 adult heart, 5 adult kidney, 6 adult liver, 7 adult lung, 8 adult spleen, 9 adult testis, 10 adult thymus.

shows all of these exon sequences. The known synaptotagmin 7 translation is shown beneath exons 1–9. The five novel exons, 3a–e, are shown with translations of their open reading frames. This sequence analysis indicates that it is possible that all synaptotagmin family members have similar exon-intron arrangements and that alternative splicing including transmembrane exon skipping could be widespread.

In order to examine this possibility, RT-PCR and RPA were performed on all of the N-terminally complete synaptotagmins. RT-PCR of rat brain mRNA was used to clone

portions of these synaptotagmins including the transmembrane sequence in order to generate suitable probes for RPA. Two different overlapping probes were used for each synaptotagmin gene. Where each of these probes gave a single, full-length RPA product, data are shown for only one. The probe descriptions are listed in Table 2. In every case, the RT-PCR primers comprise the first and last 20 nucleotides (nt). The vast majority of RT-PCR products of synaptotagmins 6 and 7 lacked the transmembrane region. However, full-length sequences matching the accession numbers listed were

Table 2

Panel (Fig. 3)	Gene name	Accession	Nucleotide positions	Start codon position	Transmembrane amino acid encoding region
A	Synaptotagmin 1	X52772	601–1031	526	682–762
B	Synaptotagmin 1	X52772	412–841	As for A	
C	Cyclophilin	M19533	35–510	43	N/A
D	Synaptotagmin 2	M64488	243–644	115	295–375
E	Synaptotagmin 3	D28512	217–550	103	256–333
F	Synaptotagmin 6	U20105	411–767	288	447–530 (exon 451–533)
G	Synaptotagmin 6	U20105	246–640	As for F	
H	Synaptotagmin 5	X84884	41–495	75	144–227
I	Synaptotagmin 10	U85513	63–520	133	286–363
J	Synaptotagmin srg1	U71294	111–440	221	281–340
K	Synaptotagmin 11	AF000423	239–719	243	279–362
L	Synaptotagmin 4	U14398	268–846	268	307–384
M	Synaptotagmin 4	U14398	268–509	As for L	
N	Synaptotagmin 7	U20106	161–680	161	209–286 (exon 192–295)
O	Synaptotagmin 7	U20106	161–500	As for N	

eventually obtained. The exon-skipped forms of synaptotagmin 6 (panel G, Table 2), accession Y19241, and of synaptotagmin 7 (panel O, Table 2), accession Y19242, are described in Table 1. Apart from synaptotagmins 6 and 7, all the other synaptotagmins gave RT-PCR products matching the described sequences and were easily cloned as full-length forms.

Rat mRNA from various tissues was analysed by RPA with ³²P-labelled probes of similar specific activities (Fig. 3). Cyclophilin expression (C) demonstrates that comparable mRNA levels are present in each lane. Most of the synaptotagmins are expressed in neural tissue and some are also expressed to a lesser degree elsewhere (E, N) and some in very low amounts elsewhere (H, K). A striking finding is that synaptotagmin 6 (F, G) is highly abundant, but in thymus rather than in brain. Although six of the 10 synaptotagmins examined gave single, full-length RPA products, four gave multiple products. Synaptotagmin 7 (N, O) gave several products, including full-length and transmembrane exon-skipped sequences as expected from RT-PCR, as well as some minor smaller products. Synaptotagmin 6 (F, G) gave three products: full-length (top), transmembrane exon-skipped (bottom) and a third form never obtained by RT-PCR. Full-length synaptotagmin 6 RPA products were only found in very small amounts. Synaptotagmin 1 (A, B) gave different patterns depending on the probe used. Synaptotagmin 4 (L, M) gave two RPA products, the upper of which is the full-length product expected from RT-PCR analysis. Multiple forms were not expected for synaptotagmins 1 and 4, as only full-length forms were ever produced by RT-PCR. The sizes of all the unexpected RPA products predict species of mRNA with an unknown upstream sequence spliced to a downstream region within or close to the transmembrane region. Synaptotagmins 1, 4, 6 and 7 gave identical RPA products from total RNA and mRNA (data not shown).

4. Discussion

Four members of the rat synaptotagmin gene family express alternatively spliced forms differing in their transmembrane domain regions. In contrast to human synaptotagmin 5, rat synaptotagmin 5 does not express an exon-skipped form. It is possible that both the tissue distribution and splicing patterns of rat and human synaptotagmins are different. It has been shown that the rostro-caudal expression pattern of synaptotagmin 1 is reversed in rodent and primate [31] and it seems to be the case that alternative splicing is used to increase functional diversity in higher organisms, particularly in brain [52]. In order to unravel the complexity of synaptotagmin alternative splicing, it will be necessary to obtain complete genomic sequences, together with the full repertoire of expressed mRNAs and panels of isoform-specific antibodies to track their locations and functions. Genomic sequences are likely to come soon from human and mouse genome sequencing projects. Human synaptotagmins 3 and 5 map very close to each other on chromosome 19 [51], which is currently being completely sequenced at Lawrence Livermore National Laboratory, USA. Human synaptotagmin 7 is on chromosome 11, which is being sequenced by several groups. It will be interesting to compare the exon-intron structures of all the synaptotagmins and to piece together the evolutionary history of these multidomain proteins. Apart from their C2 domains and the C-terminal neurexin binding signature [53], the other con-

served regions of the synaptotagmin family lack functional attributions. Although it has been assumed that synaptotagmins would all be integral membrane proteins due to their transmembrane domains, this work shows that some of them express forms specifically lacking this domain.

The exon-skipped forms obtained by RT-PCR all result in frame-shifted messages. It is possible that unknown upstream exons import novel N-terminal sequences to these exon-skipped forms. This is suggested by the RPA results with synaptotagmins 1, 4 and 6, where forms partially matching the RPA probes could not be obtained by RT-PCR despite using numerous primer pairs covering their known cDNA sequences. It is also possible that frame-shifted, exon-skipped forms may be initiated downstream to produce double C2 domain proteins targeted elsewhere in the cell, although synaptotagmin 4 lacks a suitable downstream methionine.

Published Western blots of synaptotagmins 1, 6 and 7 show multiple protein forms. The smaller form of synaptotagmin 1 has been assumed to be a proteolytic fragment produced by digestion at a proteolytically sensitive site immediately C-terminal to the transmembrane domain [54], but it is possible that it is indeed the product of an alternatively spliced mRNA. It is interesting to note that a p40 subsequence of synaptotagmin 1 comprising the cytoplasmic domains has been found to play a specific role in the secretion of FGF-1 [55–57]. The smaller form of synaptotagmin 6 has been speculated to be a product of alternative splicing [47]. Synaptotagmin 7 seems to appear in several more forms [24,58]. This is the first report of synaptotagmin expression in thymus. Expression in thymus is very high and may indicate a role in T-cell functions.

Acknowledgements: We thank Ross Jakes.

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