Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 4667–4672, April 1998
Neurobiology

A third member of the synapsin gene family

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ABSTRACT **Synapsins are a family of neuron-specific synaptic vesicle-associated phosphoproteins that have been implicated in synaptogenesis and in the modulation of neurotransmitter release. In mammals, distinct genes for synapsins I and II have been identified, each of which gives rise to two alternatively spliced isoforms. We have now cloned and characterized a third member of the synapsin gene family, synapsin III, from human DNA. Synapsin III gives rise to at least one protein isoform, designated synapsin IIIa, in several mammalian species. Synapsin IIIa is associated with synaptic vesicles, and its expression appears to be neuron-specific. The primary structure of synapsin IIIa conforms to the domain model previously described for the synapsin family, with domains A, C, and E exhibiting the highest degree of conservation. Synapsin IIIa contains a novel domain, termed domain J, located between domains C and E. The similarities among synapsins I, II, and III in domain organization, neuron-specific expression, and subcellular localization suggest a possible role for synapsin III in the regulation of neurotransmitter release and synaptogenesis. The human synapsin III gene is located on chromosome 22q12–13, which has been identified as a possible schizophrenia susceptibility locus. On the basis of this localization and the well established neurobiological roles of the synapsins, synapsin III represents a candidate gene for schizophrenia.**

Synapsins were first identified about 25 years ago as major substrates for cAMP-dependent protein kinase (1). In mammals, four members of this family have been described, synapsins Ia, Ib, IIa, and IIb (2). Molecular cloning studies revealed that these protein isoforms were derived by alternative splicing from each of two genes, synapsin I and synapsin II and that the primary sequences of the mammalian isoforms contain highly conserved regions common to all four synapsins, as well as variable regions shared by two isoforms or specific to an individual family member (2).

A large body of evidence indicates that synapsins serve multiple roles in neuronal function. Synapsins regulate neurotransmitter release and synaptic plasticity (3–9) and are essential for the proper assembly of vesicle clusters in presynaptic terminals (6, 9, 10). Synapsins also play distinct roles during the early stages of neurite outgrowth and axon elongation (11–18) and promote the formation and support the maintenance of synaptic contacts (12–17). Our prior studies suggested that additional members of the synapsin family exist (2), and we have now identified, cloned, and characterized a third member of the synapsin gene family, synapsin III, and a protein isoform, synapsin IIIa, that is expressed in several mammalian species.

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MATERIALS AND METHODS

PCR Cloning. cDNA was synthesized from human poly(A)$^+$ brain RNA (CLONTECH) by using random primers and Moloney murine reverse transcriptase (GIBCO/BRL). The cDNA served as a template for subsequent PCR using *Pfu* polymerase (Stratagene) and primers derived from putative exons of synapsin III. Primers were designed from regions of synapsin-like sequence obtained from the sequencing of human chromosome 22 (http://www.sanger.ac.uk/HGP/Chr22). PCRs were carried out in 100 μl with buffer and reaction conditions specified by Stratagene, and the reaction mixtures were incubated in a DNA thermal cycler using the following conditions: 95°C for 5 min, 55°C for 2 min, 72°C for 3 min; followed by 35 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min. PCR products were cloned into the plasmid vector pCR-blunt (Invitrogen). Plasmids were sequenced by using automated DNA sequencing technology at the Rockefeller University Protein/DNA Technology Center.

Determination of Intron–Exon Boundaries. The intron–exon boundaries of exons 1, 2, and 6–13 were obtained by direct comparison of the synapsin IIIa cDNA sequence with that of human chromosome 22. Because the sequences of exons 3–5 were not in the database, cloning approaches were used to determine their intron–exon boundaries. Analysis of exons 4 and 5 used the bacterial artificial chromosome bk766E1, provided by Ian Dunham (Sanger Research Centre, Cambridge, U.K.). Subclones of bk766E1 that contained exons 4 and 5 were sequenced to obtain their intron–exon boundaries. Inverse PCR (19) was used to clone flanking intron sequences of exon 3. Primers derived from this exon were used to amplify human genomic DNA (CLONTECH) that was previously digested with *Dpn*I and ligated. The resulting 350-bp fragment was cloned into pCR-blunt (Invitrogen) and sequenced.

Sequence Analysis. Sequence analyses were performed by using the DNA/protein analysis software packages DNA STAR (Madison, WI) and GENEWORKS (IntelliGenetics).

Northern Blot Analysis. Analysis of the tissue and brain region distribution of synapsin IIIa mRNA used human Northern blots obtained from CLONTECH. A probe derived from the 3′ untranslated region (UTR) of human synapsin IIIa mRNA (corresponding to nucleotides 1,770–2,628) was used, because this sequence was not present in the genes for synapsins I or II. The probe was synthesized by using Ready-To-Go beads (Pharmacia) and purified via Nuc-Trap columns (Stratagene). Hybridization was performed by using Express-Hyb (CLONTECH).

Production of Synapsin III-Specific Antibodies. The peptide GPPVQGRSTSQQGEESKKPAC, corresponding to residues 512–530 within domain J of synapsin IIIa, with an added N-

Abbreviations: CaM K, Ca^{2+}/calmodulin-dependent protein kinase; UTR, untranslated region.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF046873).

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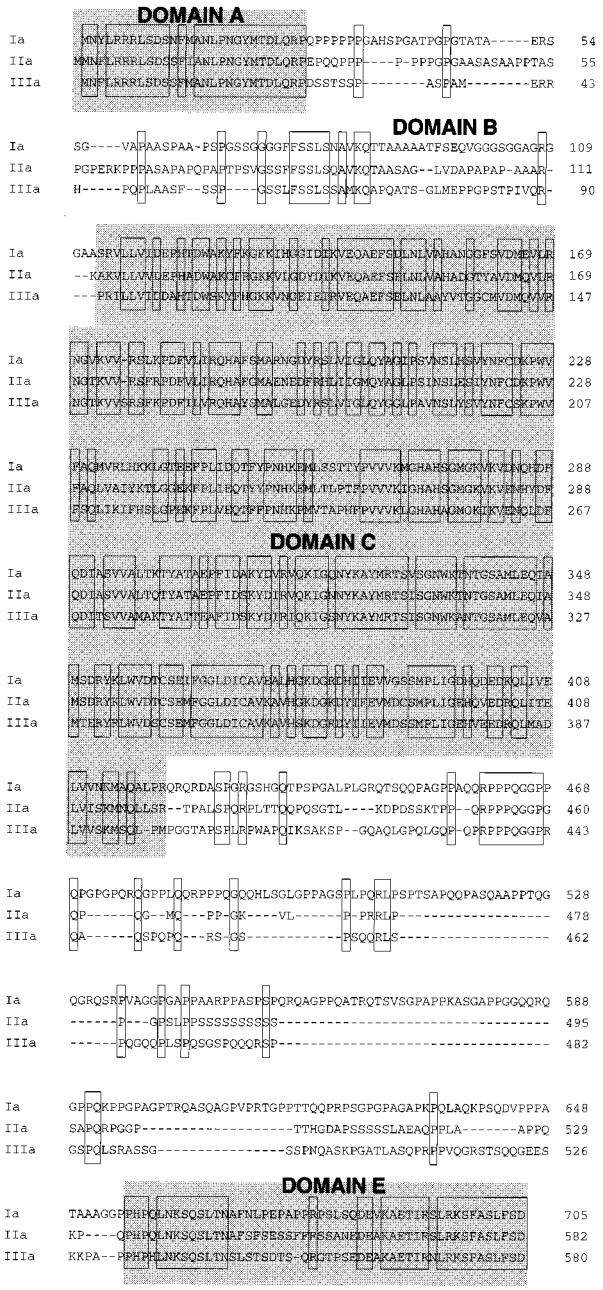

FIG. 1. Comparison of the primary sequence of human synapsins Ia, IIa, and IIIa. Sequences of human synapsins Ia, IIa, and IIIa are aligned, and identical residues present in all three isoforms are boxed. Domains A, C, and E are shaded.

terminal Gly and C-terminal Cys, was synthesized at the Keck Foundation Biotechnology Resource Laboratory of Yale University (New Haven, CT), coupled to *Limulus* hemocyanin, and injected into rabbits by Cocalico Biologicals (Reamstown, PA). Antiserum, designated RU316, displayed characteristics expected of an anti-synapsin III-specific antibody and was affinity-purified with the same antigen peptide coupled to SulfoLink resin (Pierce).

Knock-Out Mice. Mice bearing a targeted homozygous deletion in the genes for synapsin I, synapsin II, and both synapsins I and II (double knock-out) have been described (17, 18).

Immunoprecipitation and Immunoblot Analysis. Samples (500 μg) of brain homogenate were immunoprecipitated with affinity-purified antibody as described (6). Rapidly dissected tissues were homogenized in 1% SDS and incubated at 100°C for 5 min. Protein content of the extracts was quantitated with the BCA assay (Pierce). After electrophoresis using SDS/7.5% polyacrylamide gels, transfer to nitrocellulose, and blocking in nonfat dry milk, immunoblot analysis was performed as described (20). Detection of immunoreactive proteins was carried out either by incubation with alkaline phosphatase-conjugated goat anti-rabbit antibody (Vector Laboratories) followed by color development with 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indole phosphate (Boehringer-Mannheim) or by incubation with ^{125}I-labeled secondary antibodies (Fab fragments) and autoradiography. Primary antibodies used included: G304, affinity-purified anti-peptide antibody specific for domain E of synapsins (6); G95, a synaptophysin-specific polyclonal antibody; RU316 (see above); and anti-β-galactosidase (5 Prime – 3 Prime, Inc.).

Subcellular Fractionation. Subcellular fractions of rat cerebral cortex were obtained, and purification of synaptic vesicles and quantitative salt extraction of endogenous synapsins were performed as described (21).

RESULTS

Cloning and Characterization of a cDNA for Human Synapsin IIIa. A database search for additional synapsin genes revealed the presence of a homolog on human chromosome 22, residing in the expressed sequence tag H55700 (22). Because mouse and human synapsin I are localized to the X chromosome (23) and human synapsin II is localized to chromosome 3 (24, 25), the presence of homology to synapsin on another chromosome suggested the existence of another synapsin gene. A further search of chromosome 22 revealed that other synapsin-like exons were located on neighboring cosmids. On the basis of these regions of homology, primers were designed for PCR amplification by using both 3′ rapid amplification of cDNA ends (RACE) and 5′ RACE of this putative synapsin gene. The template for PCR amplification was human cDNA derived from an individual postmortem brain (CLONTECH). Several PCR clones corresponding to the full coding region of a predicted synapsin protein were obtained in this fashion. A human cDNA library was also screened with a PCR fragment and resulted in the isolation of a partial cDNA derived from this synapsin gene. Because there was expression of another synapsin mRNA, originating from the gene on chromosome 22, the gene was designated synapsin III.

Full-length synapsin III cDNA was assembled and sequenced from data obtained by PCR and cDNA cloning (Fig. 1). The coding region is 1,743 bases, and the total length of overlapping PCR clones corresponding to the 3′ UTR is a minimum of 7 kb, suggesting that the total mRNA size is very large. Long 3′ UTRs in excess of 3 kb are a feature of many brain-specific mRNAs (26). The protein encoded by the cDNA was named synapsin IIIa, because it possesses the highly conserved domain E found at the C termini of the other a isoforms, synapsins Ia and IIa. Synapsin IIIa can be divided into structural domains that are strikingly similar to those designated for synapsins I and II by homology in the primary structure (Figs. 1 and 2). Synapsin IIIa possesses a

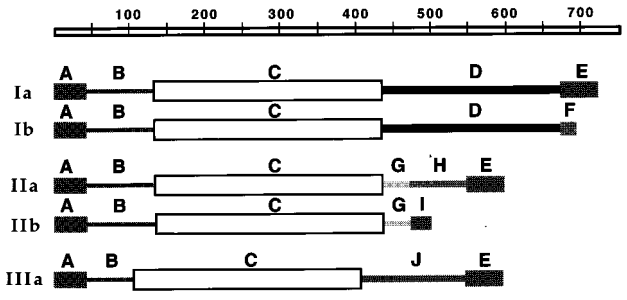

FIG. 2. Domain model of the synapsin family. Domains are schematically represented, drawn to scale, and indicated by A–J. The length of the polypeptide chains is shown at the top in number of residues.

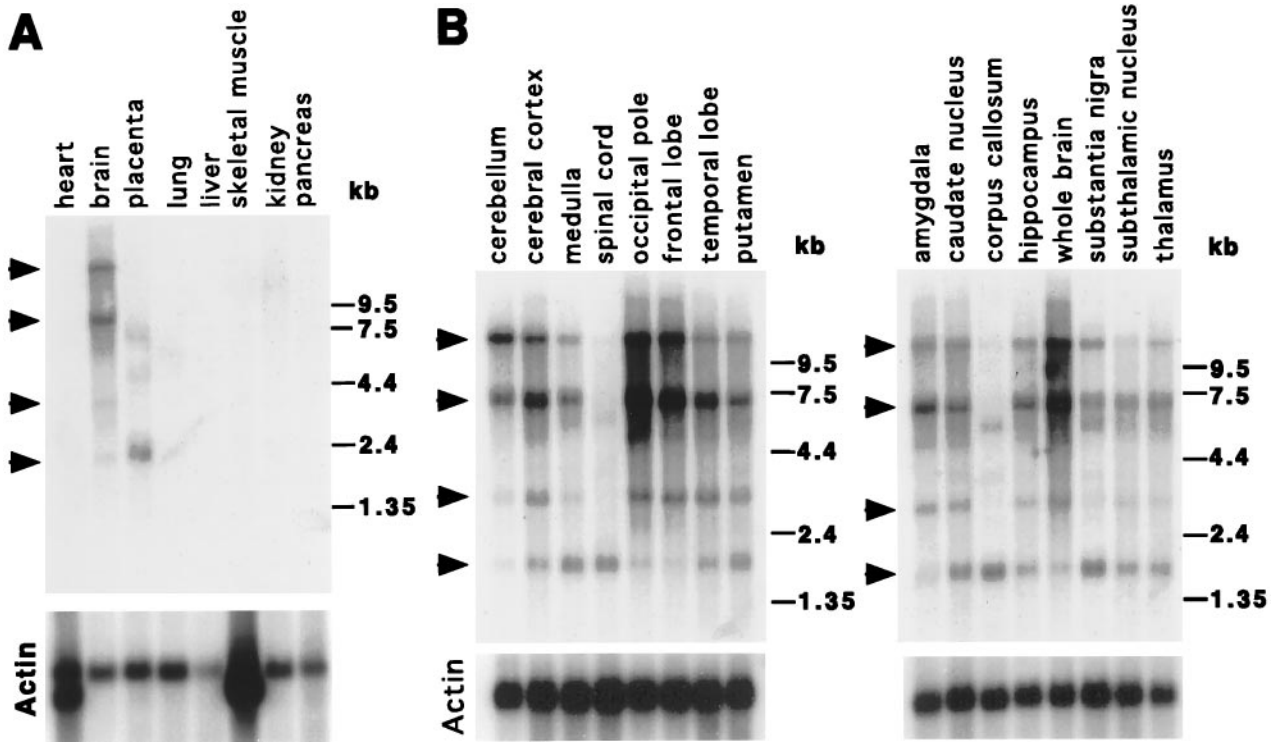

FIG. 3. Northern blot analyses using a synapsin IIIa probe. Human Northern blots (CLONTECH) were hybridized to a probe derived from the 3′ UTR of human synapsin IIIa mRNA. Results using a control actin probe are shown at the bottom of each blot. Molecular sizes are shown in kilobases (kb). Arrowheads point to the 13.0-, 7.9-, 3.5-, and 2.0-kb transcripts. (*A*) Distribution of synapsin IIIa mRNA in various human tissues. The actin probe recognizes α- and β-actin in heart and skeletal muscle but only β-actin in the other tissues. (*B*) Distribution of synapsin IIIa mRNA in human brain. The sample designated "whole brain" was derived from a different individual than the other samples on these blots.

highly conserved domain A, a less conserved linker region, domain B, and highly conserved domains C and E. When optimally aligned, domain C is 70% identical between synapsins I and III and 72% identical between synapsins II and III. A variable domain, termed domain J, is present in synapsin IIIa and is similar in amino acid composition to domains D, G, and H. Domain J is rich in proline and glutamine and is very basic (predicted pI = 12.2). A screen for cDNA or PCR clones corresponding to other isoforms was negative from the original human brain sample.

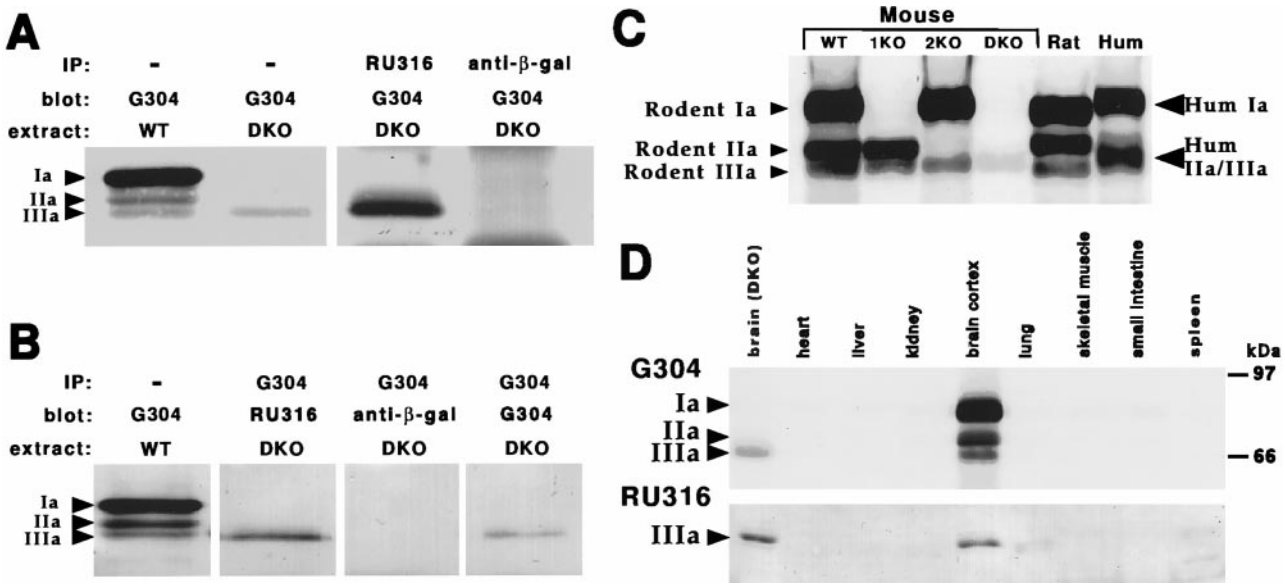

FIG. 4. Distribution of synapsin IIIa protein. Immunoblot analyses of mouse cortex (WT, wild type; 1KO, synapsin I knock-out; 2KO, synapsin II knock-out; DKO, synapsin I/II double knock-out), rat cortex (Rat), and human cortex (Hum) are shown. (*A* and *B*) Protein extracts (extract) from WT (50 μg) and DKO (200 μg) brain were loaded in the first two lanes in *A* or from WT in the first lane *B* to follow the migration of synapsins Ia, IIa, and IIIa, as indicated by the arrowheads. For immunoprecipitation, the indicated affinity-purified antibody (IP) was incubated with 500 μg of DKO brain protein extract. Antibody–antigen complexes were isolated by using protein A-Sepharose, and the samples were analyzed by immunoblot with the indicated antibody (blot). (*C*) Expression of synapsins in mouse, rat, and human brain. Protein extracts (50 μg) were loaded in each lane and blots were probed with G304. (*D*) Expression of synapsins in various mouse tissues. Protein extracts (100 μg) of various tissues were loaded in each lane, and immunoblots were probed with either G304 (*Upper*) or RU316 (*Lower*).

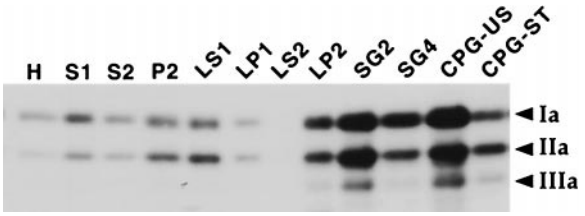

FIG. 5. Distribution of synapsins Ia, IIa, and IIIa in subcellular fractions of rat forebrain. Autoradiogram of an immunoblot of protein extract (5 μg) from the indicated subcellular fractions that was probed with G304, followed by ^{125}I-labeled anti-rabbit antibody (Fab fragments). H, homogenate; S1, postnuclear supernatant; S2, supernatant of P2; P2, crude synaptosomes; LP1, crude synaptic plasma membranes; LS1, supernatant of LP1; LP2, crude synaptic vesicles; LS2, supernatant of LP2 (synaptosol); SG2, synaptic vesicles; SG4, small synaptic membranes; CPG-US, controlled-pore glass purified, untreated synaptic vesicles; CPG-ST, controlled-pore glass purified, salt-treated synaptic vesicles.

Synapsin IIIa is likely to be a substrate for several well characterized protein kinases. There is a highly conserved consensus site for phosphorylation, Ser-9, in domain A, which is a known site for protein kinase A, Ca^{2+}/calmodulin-dependent protein kinase I (CaM KI) and CaM KIV (27) in mammalian synapsins I and II. There are three consensus sites for mitogen-activated protein (MAP) kinase present in domain B, one of which (Ser-54) is similar to site 5 (Ser-67) in synapsin I (28), and multiple consensus sites for MAP kinase are present in domain J. In addition, three consensus sites for CaM KII exist in domain J. The distribution of potential phosphorylation sites in domain J is similar to that for the known physiologically relevant sites present within domain D of synapsin I, i.e., one MAP kinase-dependent site (site 6) and two CaM KII-dependent sites (sites 2 and 3) (27, 28).

Distribution of Synapsin IIIa mRNA. To determine the tissue distribution of synapsin IIIa mRNA, Northern blot analyses were carried out with a probe derived from the 3′ UTR of synapsin IIIa. In human adult tissue, synapsin IIIa mRNA was detected predominantly in brain (Fig. 3*A*). Although cross-hybridizing bands were detected in placenta, the sizes of these RNAs differed from the RNA species detected in brain. In most human brain regions, at least four transcripts were detected, with apparent molecular sizes of 13.0 kb, 7.9 kb, 3.5 kb, and 2.0 kb (Fig. 3*B*). Several polyadenylylation signals were identified in the sequence of the 3′ UTR, suggesting that these transcripts represent four species with alternative sites of polyadenylylation, and are likely to represent synapsin IIIa transcripts with various lengths of the 3′ UTR.

Distribution of Synapsin IIIa Protein. Two affinity-purified anti-peptide synapsin antibodies were used to characterize the expression of synapsin IIIa in different tissues. On immunoblots, G304, a domain E-specific antibody (6), detected recombinant synapsin IIIa fused to oligohistidine (His-Tag-synapsin IIIa; Novagen), and RU316, directed against a synapsin III-specific sequence within domain J, reacted with His-Tag-synapsin IIIa but none of the other four isoforms (data not shown).

Table 1. Relative distribution of synapsins in subcellular fractions

	Antibody	Fold enrichment in synaptic vesicles	Depletion after salt incubation, %
Synapsin Ia	G304	13.1 ± 1.5	88 ± 5
Synapsin IIa	G304	11.6 ± 0.5	70 ± 2
Synapsin IIIa	G304	11.2 ± 1.0	78 ± 1
Synaptophysin	G95	14.8	<1

Immunoblotting of purified synaptic vesicles was carried out as described in Fig. 5 with the indicated antibodies. Enrichment values refer to the fold enrichment in synapsin immunoreactivity with respect to forebrain homogenate. Synapsins were depleted from purified synaptic vesicles by incubating the vesicles, diluted to 10 μg/ml, in the presence of 200 mM NaCl for 2 hr at 0°C. Data are the mean ± SEM (n = 3) for synapsins and a single experiment for synaptophysin.

G304 detected three clearly resolved bands, corresponding to synapsins Ia, IIa, and IIIa, in brain extracts from wild-type mice, and a single band comigrating with synapsin IIIa, in brain extracts of synapsin I/II double knock-out mice (Fig. 4*A*). In brain extracts from the double knock-out mice, RU316 immunoprecipitated a 63-kDa protein that was subsequently recognized on blots by G304 (Fig. 4*A*). An irrelevant antibody to β-galactosidase failed to immunoprecipitate a G304-reactive protein. Conversely, a 63-kDa protein immunoprecipitated by G304 was detected by RU316 (Fig. 4*B*).

Synapsins Ia, IIa, and IIIa were also clearly resolved on immunoblots of brain homogenates derived from rat, and the expected isoforms were detected in samples derived from wild-type and single and double knock-out mice (Fig. 4*C*). However, the three a isoforms from human brain were not fully resolved, presumably because the predicted size of human synapsin IIIa (581 amino acids; M_r = 63,302) is very similar to that of synapsin IIa (583 amino acids; M_r = 62,967). Immunoblots of human brain extracts probed with G304 revealed two bands (Fig. 4*C*), with the upper band representing synapsin Ia and the lower broader band the result of apparent comigration of synapsins IIa and IIIa. In addition, expression of the cloned human synapsin IIIa cDNA in COS7 cells resulted in the accumulation of a 63-kDa protein that was detected by G304 and RU316 (data not shown).

Consistent with the brain-specific expression of mRNA (Fig. 3), immunoblot analysis using either G304 or RU316 revealed that the expression of synapsin IIIa was restricted to the brain (Fig. 4*D*). Furthermore, immunocytochemistry using RU316 revealed that expression of synapsin IIIa was confined to neurons (data not shown).

In the experiments presented in Fig. 4, using G304, synapsin IIIa protein appeared to be expressed at a lower level than did synapsins Ia and IIa. Other antibodies that cross-react with all synapsin isoforms consistently detected lower reactivity for synapsin IIIa than for other synapsins on immunoblots of brain homogenates (data not shown). Synapsin IIIa levels were decreased in brain extracts of knock-out mice as compared

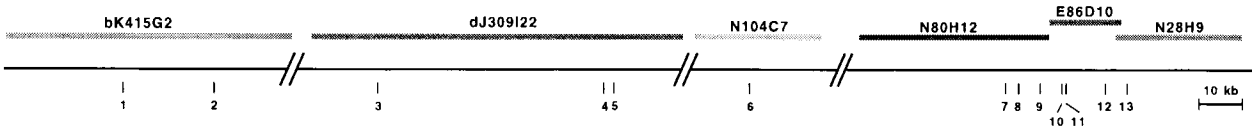

FIG. 6. Structure of the human synapsin III gene. Alignment of the exons of the human synapsin III gene with the bacterial artificial chromosomes bk415G2, the P1 artificial chromosome dJ309I22, and cosmids, N104C7, N80H12, E86D10, and N28H9, along chromosome 22. Breaks in the structure represent gaps in the nucleotide sequence. As described in the text, bk766E1 overlaps with dJ309I22. The origin of the sequences (with EMBL accession numbers in parentheses) used to align these exons are as follows: bk415G2 (Z83846) and bk766E1 originated from a previously described human library (45); dJ309I22 (Z98256) from a library constructed at the Roswell Park Cancer Institute by the group of Pieter de Jong (http://bacpac.med.buffalo.edu/); and N104C7 (Z82246), N80H12 (Z80902), E86D10 (Z82181), and N28H9 (Z71183) were derived from human chromosome 22-specific cosmid libraries constructed at the Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550. The 10-kb scale marker refers to exons in N80H12, E86D10, and N28H9 only.

Table 2. Sequence of intron–exon boundaries of human synapsin III

Exon	Sequence	
	Intron–exon	Exon–intron
1	–	ATACAGACTG**gt**aagtagga
2	ccctttac**ag**GTCGAAGTAT	AGTGGAGCAG**gt**aggtggga
3	tattcttc**ag**GCTGAATTCT	AAGTGGTGAG**gt**aagtagga
4	gctttctc**ag**CAGATCCTTC	GCCCTGGGTG**gt**atgtgaca
5	tctatcct**ag**TTCTCTCAGC	TAAGCCAATG**gt**gagtgccc
6	tctctttc**ag**GTCACAGCCC	AATGGGAAAG**gt**atgagaaa
7	tcccacac**ag**ATCAAAGTGG	AGGCTTACAT**gt**gagtctct
8	tctggtac**ag**GAGAACCTCC	TGACAGAGAG**gt**aagagaca
9	gtccctgc**ag**GTACAGGCTG	CATCATCGAG**gt**gagggatg
10	ctcccaac**ag**GTAATGGACA	CAGACCTTGG**gt**taagaccc
11	tctgctct**ag**GCTCCACAGA	CCTCCGCAAG**gt**atgaccca
12	ggcccctt**ag**GAGGCCCTCG	CGCATCTCAA**gt**aagtgctt
13	tctgtttc**ag**CAAATCTCAG	–

Intron sequences are indicated in lowercase type and exon sequences are in uppercase type. Boldface type represents intron borders.

with wild-type mice (Fig. 4*C*). This may be due in part to the dramatic decrease in the total number of synaptic vesicles in the brains of knock-out mice (8–10), which may contribute to a decrease in the metabolic stability of synapsin IIIa.

Subcellular Distribution of Synapsin IIIa. To determine the localization of synapsin IIIa protein within the cell, subcellular fractionation studies were carried out (21). As shown previously, levels of synapsins Ia and IIa were enriched in fractions containing synaptic vesicles and decreased when the vesicles were incubated in the presence of high salt (200 mM NaCl) (21). Synapsin IIIa protein appeared to localize to the same fractions as synapsins Ia and IIa (Fig. 5 and data not shown), with a comparable fold enrichment with respect to total brain homogenate (Table 1). Upon treatment with high salt, 78% of synapsin IIIa was released from the vesicles (Table 1), a value intermediate between those for synapsins Ia and IIa. Synaptophysin, an integral membrane protein of synaptic vesicles, was not affected by salt treatment. These results indicate that synapsin IIIa, like other synapsins, is a peripheral membrane protein that is localized to the cytoplasmic surface of synaptic vesicles.

Genomic Organization of the Synapsin III Gene. The human synapsin III gene was originally detected on chromosome 22 by homology to an expressed sequence tag that had been assigned to this chromosome. After the entire synapsin IIIa cDNA had been characterized, we searched this region of chromosome 22 for homologous sequences that may represent exons. Ten of 13 exons in the coding region were identified by this approach. Exons 4 and 5 were found on the bacterial artificial chromosome bk766E1, which is located between bk415G2 and N104C7 (Fig. 6). The intron–exon boundaries of exon 3 were determined by inverse PCR. The sequence of exon 3 can now be found in the database for human chromosome 22, located on the P1 artificial chromosome dJ309I22, which overlaps with bk766E1. The alignment of these exons is depicted in Fig. 6. The sequences of the intron–exon boundaries are consistent with the AG/GT rule (29) (Table 2). The intron–exon boundaries of the synapsin III gene are strikingly similar to those of synapsin I (30). The contribution of each exon to the protein sequence of synapsin IIIa is depicted in Fig. 7. Exon 1 encodes domains A, B, and a small portion of domain C. The majority of exons encode domain C. A single exon encodes domain E and an unusually long 3′ UTR that is several kilobases long.

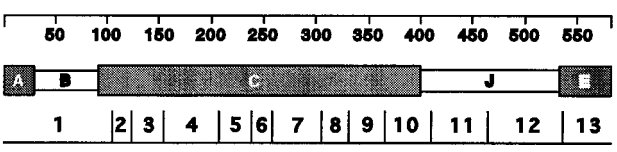

FIG. 7. Contribution of individual exons to the coding sequence of human synapsin IIIa. The predicted protein sequence encoded by each exon was aligned with the amino acid sequence for synapsin IIIa (residue numbers given at top), and mapped against the corresponding domain structure for this protein.

The synapsin III gene is located on the long arm of chromosome 22 at 22q12.3. The location of synapsin III relative to anonymous markers in the region 22q12–13 was marked by D22S280, because D22S280 is found within an intron between exons 2 and 4 (Fig. 8). This region has been previously identified as a potential schizophrenia susceptibility locus, but a susceptibility gene for schizophrenia has not been identified in this area as yet (31). D22S278, which was shown to be significantly associated with schizophrenia (31), is approximately 4.5 centimorgans away from D22S280.

DISCUSSION

In this report, we describe the cloning and characterization of a third member of the synapsin gene family that encodes a distinct synapsin isoform, synapsin IIIa. Synapsin IIIa is present in mouse, rat, and human brain and was the only synapsin III isoform found in the initial human brain sample that we analyzed. However, we have found additional spliced synapsin III transcripts in brain tissue derived from other individuals. PCR clones corresponding

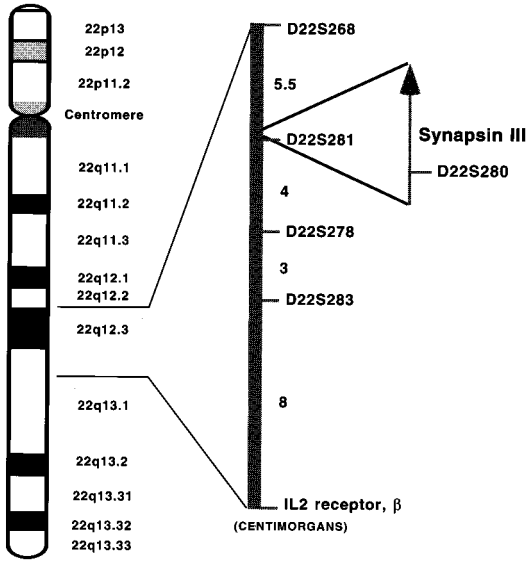

FIG. 8. Location of synapsin III on human chromosome 22. The location of synapsin III on the long arm of chromosome 22 is depicted in relationship to anonymous markers that map to 22q12.2 to 22q13.1. The distances between the markers are indicated in centimorgans.

to more than one type of spliced isoform were also isolated from mouse brain and are currently being analyzed.

The synapsin III gene is predicted to span 380–400 kb (Ian Dunham, personal communication). This size is unusually large, given that the coding region for the one known protein species derived from this gene, synapsin IIIa, is only 1,743 bases. The intron–exon organization of the synapsin III gene is very similar to that of the synapsin I gene (30), suggesting that synapsins I and III were derived from a primordial synapsin gene. Because of similarities in gene structure and sequence, it has been hypothesized that the synapsin I and II genes may have evolved through a gene duplication event (2, 30). By using protein analysis software (DNA STAR), it was found that synapsin IIIa displays virtually the same degree of similarity and divergence to synapsins Ia and IIa. This analysis suggests that, if a gene duplication event did occur, synapsins I, II, and III were generated at approximately the same time.

Studies in knock-out mice have confirmed that synapsins I and II are critical for the regulation of neurotransmitter release (7, 9, 10, 32) and synaptogenesis (17, 18). The identification of the synapsin III gene and the demonstration that at least one synapsin III isoform is expressed in the synapsin I/II double knock-out animals may account for the relatively mild phenotypic changes that were observed in those studies. To gain a more complete understanding of the role of all members of the synapsin family in the regulation of neurotransmitter release and synaptogenesis, additional studies are in progress involving targeted homozygous deletion of synapsin III and of a synapsin I/II/III triple knockout.

It is intriguing that the synapsin III gene is localized to a region of chromosome 22 previously reported to be a schizophrenia susceptibility locus. Several studies have reported potential linkage of schizophrenia to 22q12–13 (33–39), where the closest marker to date is D22S278, as confirmed by sib pair analysis (31). The synapsin III gene is located at D22S280, at a distance of 4.5 centimorgans away from D22S278. The etiology of schizophrenia is unknown, but genetic factors are a strong determinant of susceptibility (40). In addition, defects in neurotransmission have been observed in this disease (41), and abnormalities in the morphology of neurons obtained from schizophrenic postmortem brain suggest a developmental basis for this disease (42–44). Because the synapsins play a role in neurotransmitter release and synaptogenesis, the synapsin III gene is a candidate gene for schizophrenia, based not only on linkage analysis but also on the well established biological functions of the synapsins.

We thank Dr. Ian Dunham for advice regarding the sequence of human chromosome 22 and for providing the clone bk766E1. We thank Drs. Patrick B. Allen and Lars Lannfelt for critically reviewing the manuscript. This work was supported by the National Alliance for Research in Schizophrenia and Affective Disorders, the Scottish Rite Schizophrenia Research Program (B.P.), Istituto Superiore di Sanità - Sclerosi Multipla Grant 31 and Telethon Grant 753 (F.B.), and U.S. Public Health Service Grant MH39327 (P.G.). Support was received from a fund established in The New York Community Trust by DeWitt–Wallace.

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