Synapsins: Mosaics of Shared and Individual Domains in a Family of Synaptic Vesicle Phosphoproteins

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Synapsins are neuronal phosphoproteins that coat synaptic vesicles, bind to the cytoskeleton, and are believed to function in the regulation of neurotransmitter release. Molecular cloning reveals that the synapsins comprise a family of four homologous proteins whose messenger RNA's are generated by differential splicing of transcripts from two genes. Each synapsin is a mosaic composed of homologous amino-terminal domains common to all synapsins and different combinations of distinct carboxyl-terminal domains. Immunocytochemical studies demonstrate that all four synapsins are widely distributed in nerve terminals, but that their relative amounts vary among different kinds of synapses. The structural diversity and differential distribution of the four synapsins suggest common and different roles of each in the integration of distinct signal transduction pathways that modulate neurotransmitter release in various types of neurons.

THE RELEASE OF NEUROTRANSMITTERS IS THE FINAL COMMON pathway in all neuronal function. This release involves the Ca^{2+}-dependent exocytosis of synaptic vesicles at the nerve terminal (*1*). Under various physiological conditions, membrane depolarization and neurotransmitter release at the nerve terminal are correlated with the phosphorylation of the synapsins (*2*). Synapsins are a group of four synaptic vesicle membrane proteins: synapsins Ia and Ib (collectively referred to as synapsin I) and synapsins IIa and IIb [originally named proteins IIIa and IIIb (*2*) and together referred to as synapsin II].

It has been suggested that synapsin I links synaptic vesicles to cytoskeletal elements in the presynaptic nerve terminal (*3, 4*) and regulates neurotransmitter release (*5*). Less is known about the properties and functional role of synapsin II. All four synapsins are localized to the presynaptic nerve ending, where they constitute a significant portion of the total synaptic vesicle protein (*2, 3, 6*). Synapsin I binds to synaptic vesicles in vitro with high affinity (K_d = 10 n*M*), and phosphorylation decreases this binding affinity by a factor of five (*7*). All four synapsins are phosphorylated upon excitation of the nerve terminal (*2, 8*). In vivo and in vitro synapsin I serves as a substrate for two different Ca^{2+},calmodulin-dependent protein kinases and for cyclic adenosine monophosphate (cyclic AMP)–dependent protein kinase (*9*), and the sequences surrounding these phosphorylation sites have been determined (*10*). Synapsin II is also a substrate for cyclic AMP–dependent protein kinase and for Ca^{2+},calmodulin–dependent protein kinase I (*9*). In PC12 cells, nerve growth factor (NGF) stimulates the phosphorylation of synapsin I at another, distinct site (*11*). Through these multiple phosphorylations, synapsin I may constitute a point of convergence for several different signal transduction pathways.

Synapsin I contains at least two distinct actin-binding sites that allow synapsin I to bundle actin in vitro (*12, 13*). This formation of actin bundles is regulated by phosphorylation. In addition, synapsin I binds to tubulin, spectrin, and calmodulin in vitro (*14*). Electron microscopy data suggest that synapsin I forms the fibrillar connections that link synaptic vesicles to each other and to the cytoskeleton in the presynaptic nerve terminal in vivo (*4*). Finally, microinjection of purified dephospho-synapsin I into the presynaptic terminal of the squid giant synapse dramatically decreased the efficiency of neurotransmitter release at that synapse, whereas the injection of denatured or phosphorylated synapsin I had no effect (*5*). Conversely, injection of Ca^{2+},calmodulin-dependent protein kinase II, which phosphorylates synapsin I and decreases its affinity for synaptic vesicles in vitro, enhanced neurotransmitter release (*5*).

Little is known of the structural relations and relative distributions of synapsins Ia, Ib, IIa, and IIb. Immunoreactivity to specific antibodies has indicated that synapsin II is related to synapsin I; however, the two types of synapsins differ in several biochemical properties (*2*). In order to gain insight into the potential roles of the synapsins in the regulation of neurotransmitter release, we determined their primary sequences from cloned DNA. We have found that the four synapsins form a distinct family of homologous proteins whose structures suggest common and divergent functions. Immunocytochemical experiments with antibodies directed against

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peptides specific for each of the four synapsins show that they are differentially distributed in the central nervous system.

Primary structures of the synapsins. Synapsins I and II were purified from rat and bovine brain, and internal amino acid sequences were obtained after peptide cleavage (*15*). Oligonucleotide probes synthesized on the basis of these sequences or antibodies to synapsin I were used as probes to isolate full-length cDNA's encoding bovine synapsin Ib and rat synapsins IIa and IIb (*16, 17*). In addition, partial cDNA's for bovine synapsin Ia and rat synapsins Ia and Ib were obtained. To determine the complete structure of the 5′ end of the rat synapsin Ia and Ib mRNA's, we utilized the polymerase chain reaction (PCR) with oligonucleotides derived from the bovine cDNA to amplify the first exon on the rat synapsin I gene (*18*). The sequences of the amplified genomic and cloned cDNA's were determined by enzymatic methods on both strands, and the complete amino acid sequences for rat and bovine synapsins Ia and Ib and for rat synapsins IIa and IIb were deduced from the nucleotide sequences (Fig. 1). All amino acid sequences determined directly from purified rat and bovine synapsin I and from rat synapsin II were present in the deduced sequences (underlined in Fig. 1).

Synapsins Ia and Ib are encoded by mRNA's that are identical in nucleotide sequence except for a small stretch of sequence in the 3′ end of the coding region. Here the synapsin Ia mRNA contains a 38-nucleotide (nt) insertion that is not present in the synapsin Ib mRNA. This insertion leads to a frameshift in the remaining common nucleotide sequence encoding synapsins Ia and Ib and results in different carboxyl-terminal amino acid sequences for the two proteins. The mRNA's for synapsins Ia and Ib encode proteins of 704 and 668 amino acids in the rat, and of 706 and 670 amino acids in the cow, respectively (Fig. 1). In-frame termination codons in the 5′ untranslated regions of the mRNA's provided further evidence that the structures presented are full length.

With the exception of the 38-nt insertion, the two synapsin I mRNA's are identical, suggesting that they are generated by alternative splicing of the same primary transcript. Blotting experiments with rat genomic DNA (*19*) indicated that synapsins Ia and Ib are encoded by a single-copy gene and thus provided confirmatory evidence for differential splicing of the synapsin I transcript. The mRNA sizes for rat and bovine synapsin Ia and Ib were determined by RNA blotting (*20*). A common mechanism for an alternative splicing event resulting in the presence or absence of part of an internal sequence is the inclusion or exclusion of an individual exon (*21*). However, the sequence at the 3′ end of the insertion in the synapsin Ia mRNA resembles a consensus intron acceptor site and suggests that the differential splicing may involve the alternative use of two different splice acceptor sites. To investigate this hypothesis, we used PCR to amplify the region of the rat synapsin I gene that encodes the 3′ end of the mRNA (*22*). A single amplification product was observed. DNA sequencing revealed that the last intron

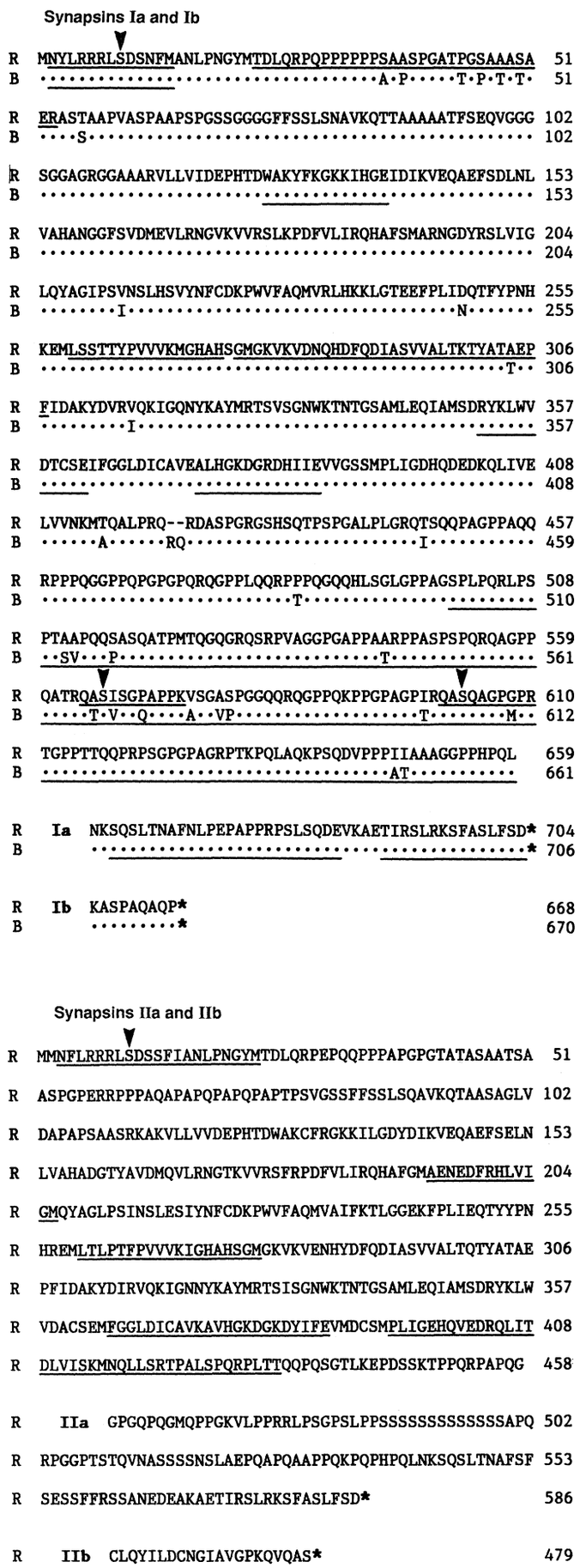

Fig. 1. The primary structures of the synapsins. The deduced amino acid sequences of rat (R) and bovine (B) synapsins Ia and Ib are shown on top and of rat synapsins IIa and IIb on the bottom. The alternatively spliced carboxyl termini of the sequences are shown at the end of each sequence preceded by the synapsin subtype symbol Ia, Ib, IIa, or IIb. Amino acid sequences determined by peptide sequencing of the purified proteins are underlined (*15*). Phosphorylation sites are indicated by arrowheads above the respective serine residues. Asterisks indicate stop codons. In the synapsin Ia and Ib sequences, residues in the bovine sequences that are identical with those in the rat sequences are indicated by periods, and only substituted residues are shown. Abbreviations for the amino acid residues are: A, Ala; C. Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

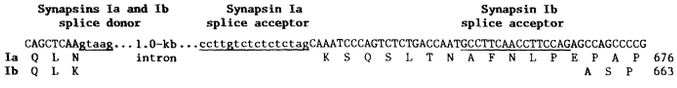

Fig. 2. The structure of the exon junctions of the last intron of the rat synapsin I gene. The last intron is followed by two splice acceptor sites that either include or exclude the 38-nt sequence specific for synapsin Ia. Splice donor and acceptor sites are underlined. Sequences were obtained from genomic DNA amplified by the PCR (*22*).

of the synapsin I gene has two splice acceptor sites (Fig. 2). The use of one or the other of these acceptor sites results in the inclusion or exclusion of the 38-nt insertion. Although the use of alternative splice acceptor sites is a common mechanism in generating multiple mRNA's, the translational reading frame is usually maintained (*21*). Splicing to generate different reading frames that results in protein diversity is a mechanism not often used.

The primary structures of both rat and bovine synapsins Ia and Ib were determined (Fig. 1). An extraordinary degree of conservation (96 percent identity) between the two species was observed. The small number of amino acid substitutions that occur are often clustered as, for example, in residues 36 to 56, which contain seven substitutions. The differentially spliced carboxyl termini and most of the amino-terminal regions are identical in the two species, with a stretch of 358 amino acids (residues 57 to 414) showing 99 percent identity. Conservative substitutions account for almost all of the differences between the amino acid sequences of rat and bovine synapsin I (*23*).

Synapsins IIa and IIb are encoded by mRNA's that also contain identical 5′ regional sequences and diverge in their 3′ coding regions (Fig. 1). In contrast to the similarities in the nucleotide sequences of synapsins Ia and Ib in their 3′ untranslated regions, the nucleotide sequences of both the 3′ coding region and the 3′ untranslated regions of the synapsin IIa and IIb mRNA's are different. RNA blotting demonstrated the presence of a different single-sized message for each protein in rat and bovine RNA (*20*). Genomic DNA blotting, however, indicated that synapsins IIa and IIb are encoded by a single gene (*19*). These results suggest that the messages for synapsins IIa and IIb are generated from the same primary transcript by a differential splicing mechanism that utilizes different 3′ exons. The two messages encode for proteins of 586 and 479 residues, respectively.

None of the four synapsins shows significant homology with any sequence in the data bases we examined (*24*). In particular, no statistically significant homology has been observed with villin, profilin, gelsolin, protein 4.1, or other actin-binding proteins that were previously proposed (*13, 23, 25*) to be related to synapsin I. Although there are limited biochemical similarities between these proteins and the synapsins, they bear no primary structural relation to each other. Accordingly, the synapsins form a novel class of actin-binding proteins. The synapsin protein family may extend beyond the four members described here. Genomic DNA blotting experiments at low stringency with probes derived from regions that are well conserved in the four synapsins suggest the presence of additional homologous sequences in the rat genome (*19*).

Common and individual domains in the four synapsins. Comparisons of the nucleotide and deduced amino acid sequences of synapsins Ia and Ib with those of synapsins IIa and IIb demonstrate a high degree of identity covering more than half of each protein. Alignment of the amino-terminal regions of synapsin I and synapsin II reveals an overall sequence identity of 70 percent covering 420 residues (Fig. 3A). Stretches of up to 27 consecutive identical residues occur, flanked by conservative substitutions. In addition to the extensive sequence homology of the four synapsins in their amino-terminal regions, the differentially spliced carboxyl-terminal sequence of synapsin Ia shares identity with the carboxyl-terminal sequence of synapsin IIa (Fig. 3B), whereas there is no homology between synapsins Ib and IIb in this region.

The four synapsins contain different sets of domain. Comparison of the primary structures of the four synapsins reveals a pattern of identity and dissimilarity that suggests a domain model for the

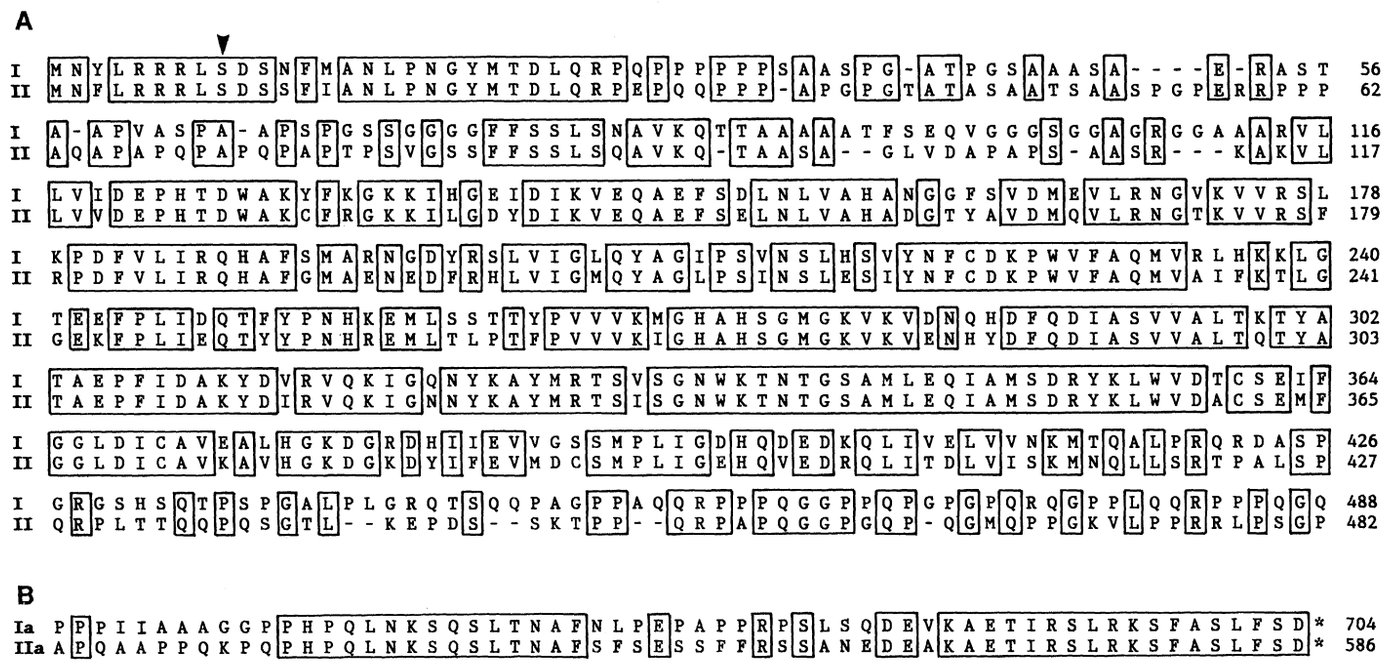

Fig. 3. Homology between rat synapsins Ia and Ib (top line) and synapsins IIa and IIb (bottom line). (**A**) The amino-terminal region common to all four synapsins (residues 1 to 488 for synapsins Ia and Ib, residues 2 to 482 for synapsins IIa, and residues 2 to 458 for synapsin IIb). (**B**) The carboxyl-terminal domain of synapsin Ia (residues 644 to 704) and synapsin IIa (residues 526 to 586). Sequences are aligned for maximal identity. Identical amino acids at a position are boxed; dashes indicate gaps.

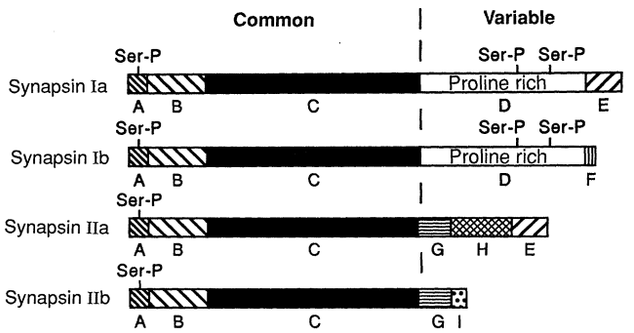

Fig. 4. Diagram of shared and individual domains in the four synapsins (*33*). Each domain is given a letter symbol and represented in scale. Numbers to the left indicate the synapsin subspecies. The large central homologous domain (domain C) is highly conserved (Fig. 3A), and domain E is found at the carboxyl terminus of both synapsins Ia and IIa (Fig. 3B). The amino acid sequence boundaries for the individual domains shown are as follows (synapsin subtype; rat sequence residue numbers): Domain A (Ia and Ib, 1 to 28; IIa and IIb, 1 to 29); domain B (Ia and Ib, 29 to 112; IIa and IIb, 30 to 112); domain C (Ia, Ib, IIa, and IIb, 113 to 420); domain D (Ia and Ib, 421 to 654); domain E (Ia, 655 to 704; IIa, 537 to 586); domain F (Ib, 655 to 668); domain G (IIa and IIb, 421 to 458); domain H (IIa, 459 to 536); and domain I (IIb, 459 to 479).

protein family (Fig. 4). The large homologous region common to all four synapsins (Fig. 3) can be subdivided into three domains. At the amino terminus, the previously characterized sequence surrounding the single phosphorylation site in synapsin I that serves as an acceptor for cyclic AMP–dependent protein kinase and for Ca^{2+}, calmodulin-dependent protein kinase I (*10*) is also found in synapsin II (domain A). Since synapsins IIa and IIb also are substrates for these kinases, all four synapsins are presumably phosphorylated by the two kinases at identical sites in domain A.

Domain A is followed by a sequence that shows a weaker resemblance between synapsins I and II and is less conserved evolutionarily in synapsin I (domain B). Domain B is rich in small side-chain amino acids such as alanine and serine. These characteristics of domain B indicate that it may serve as a link between domain A and the third domain, the central homologous region (domain C).

Domain C is characterized by a high degree of homology between synapsins I and II (78 percent identity) and a high degree of interspecies conservation in synapsin I (98 percent identity between bovine and rat). Fragments of synapsin I prepared by cysteine-specific cleavage have been used to investigate the binding sites of synapsin I to actin and to synaptic vesicle membrane proteins (*12, 13, 26*). Using our deduced primary structures, we aligned the positions of these cleavage fragments with the parent molecules (Fig. 1). On the basis of this alignment, the central homologous region of synapsin I (domain C) appears to contain independent binding sites for actin and for synaptic vesicles. The strong homology between synapsins I and II in domain C (Fig. 3) suggests that synapsin II may also bind microfilaments and synaptic vesicles with characteristics that are similar to those of synapsin I.

Domain C is both hydrophobic [39 percent M, A, I, L, V, F, and W residues (see legend to Fig. 1 for single-letter amino acid code symbols)] and highly charged (27 percent D, E, K, and R residues). Secondary structure predictions (*27*) suggest that domain C also contains multiple sequence stretches with the potential to form amphipathic α helices and β sheets. There are several clusters of hydrophobic amino acids flanked by charged residues, such as RVLLVIDE (residues 114 to 121). Studies of the association of synapsin I with phospholipid vesicles, in which hydrophobic photoaffinity labels were used, demonstrated that a region of synapsin I located within domain C partially inserts into the hydrophobic phase of the phospholipid bilayers (*26*). Again, synapsin II might be expected to behave similarly.

On the carboxyl-terminal side of domain C, the structures of the four synapsins diverge. Here, synapsins Ia and Ib contain a common, long, proline-rich, collagenase-sensitive sequence (Fig. 4, domain D) that is followed by the variably spliced regions (domains E and F), while synapsins IIa and IIb have only a short common sequence (domain G) that is followed by their respective alternatively spliced carboxyl termini (Fig. 5, domains H, I, and E).

Domain D of synapsins Ia and Ib includes two phosphorylation sites for Ca^{2+},calmodulin-dependent protein kinase II [sites 2 and 3 (*10*)] and has a very unusual composition. It consists of 27 percent proline and 17 percent glutamine residues, whereas asparagine is totally excluded. In contrast, the ratio of glutamine to asparagine is approximately 1:1 in all other domains of synapsin I. Domain D includes 10 percent basic amino acids but has only two acidic residues, conferring upon the synapsin I proteins their strongly basic isoelectric point ($pI > 11$). Seven amino acids (A, G, P, Q, R, S, and T) account for about 90% of the residues in domain D.

In synapsins IIa and IIb, domain C is also followed by a proline-rich region (domain G) which, however, is smaller than that of synapsins Ia and Ib and is not phosphorylated. On the carboxyl-terminal side of the proline-rich regions, the four synapsins contain differentially spliced carboxyl-terminal sequences (domains E, F, H, and I). A strong homology is observed in the final 50 amino acids between synapsin Ia and synapsin IIa (domain E). In this region, 76 percent of the residues are identical (Fig. 3B). Strikingly, the last 18 residues of synapsins Ia and IIa are identical, in spite of their being the products of two different genes derived by two distinct differential splicing mechanisms. No other homology exists at the carboxyl terminus between any two synapsins. This results in the presence of three different domains (E, F, and I) at the carboxyl termini of the four synapsins.

Variations in synapsins among synapses. Domain-specific polyclonal antibodies were raised to synthetic peptides corresponding either to specific sequences at the carboxyl-terminal region of each of the four synapsins or to sequences shared by two or by all four synapsins (*28*). The antibodies reacted with the synapsins on immunoblots of rat brain homogenate with the specificity predicted by the deduced amino acid sequences (Fig. 5). The results confirm the sequence assignment of the primary structures of the four synapsins and support the proposed molecular basis for generating their diversity.

Immunocytochemical experiments were performed by light and

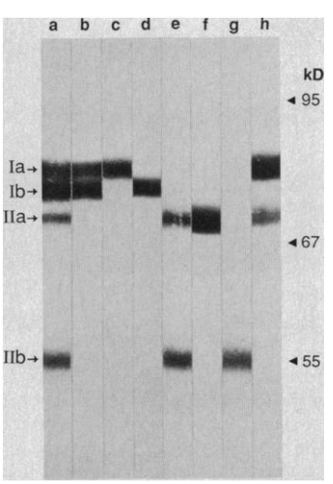

Fig. 5. Recognition of shared and individual epitopes of the synapsins by peptide antibodies. Rat brain homogenate was immunoblotted with antibodies (*28*) specific for: (Lane a) All four synapsins (residues 3 to 13 of rat and bovine synapsins I). (Lane b) Synapsins Ia and Ib (residues 587 to 609 of bovine synapsin I). (Lane c) Synapsin Ia (residues 659 to 674 of rat synapsin Ia). (Lane d) Synapsin Ib (residues 660 to 668 of rat synapsin Ib). (Lane e) Synapsins IIa and IIb (residues 414 to 432 of rat synapsin II). (Lane f) Synapsin IIa (residues 470 to 484 of rat synapsin IIa). (Lane g) Synapsin IIb (residues 462 to 476 of rat synapsin IIb). (Lane h) Synapsins Ia and IIa (residues 680 to 704 of rat synapsin Ia). Immunoreactive bands were visualized with horseradish peroxidase–coupled secondary antibodies.

electron microscopy in order to determine the distribution of the four synapsins in the nervous system (Figs. 6 and 7). For the light microscopy studies, sections immunostained for each of the four synapsins were counterstained with mouse monoclonal antibodies to synaptophysin, a well-established synaptic vesicle marker that is not related to the synapsins and that served as an internal control for nerve terminal immunostaining (*29*).

The overall immunostaining pattern was similar for all four synapsins and represented a pattern specific to nerve terminals (*30*). Many nerve terminals appeared to contain all four proteins, but the staining intensity of distinct nerve terminal populations was different for the four antisera. In general, synapsin Ib appeared to have the most widespread distribution, which appeared to be almost identical to that of synaptophysin. The staining of the other three synapsins varied in intensity between synapses and in some nerve terminals at least one of the synapsins was undetectable.

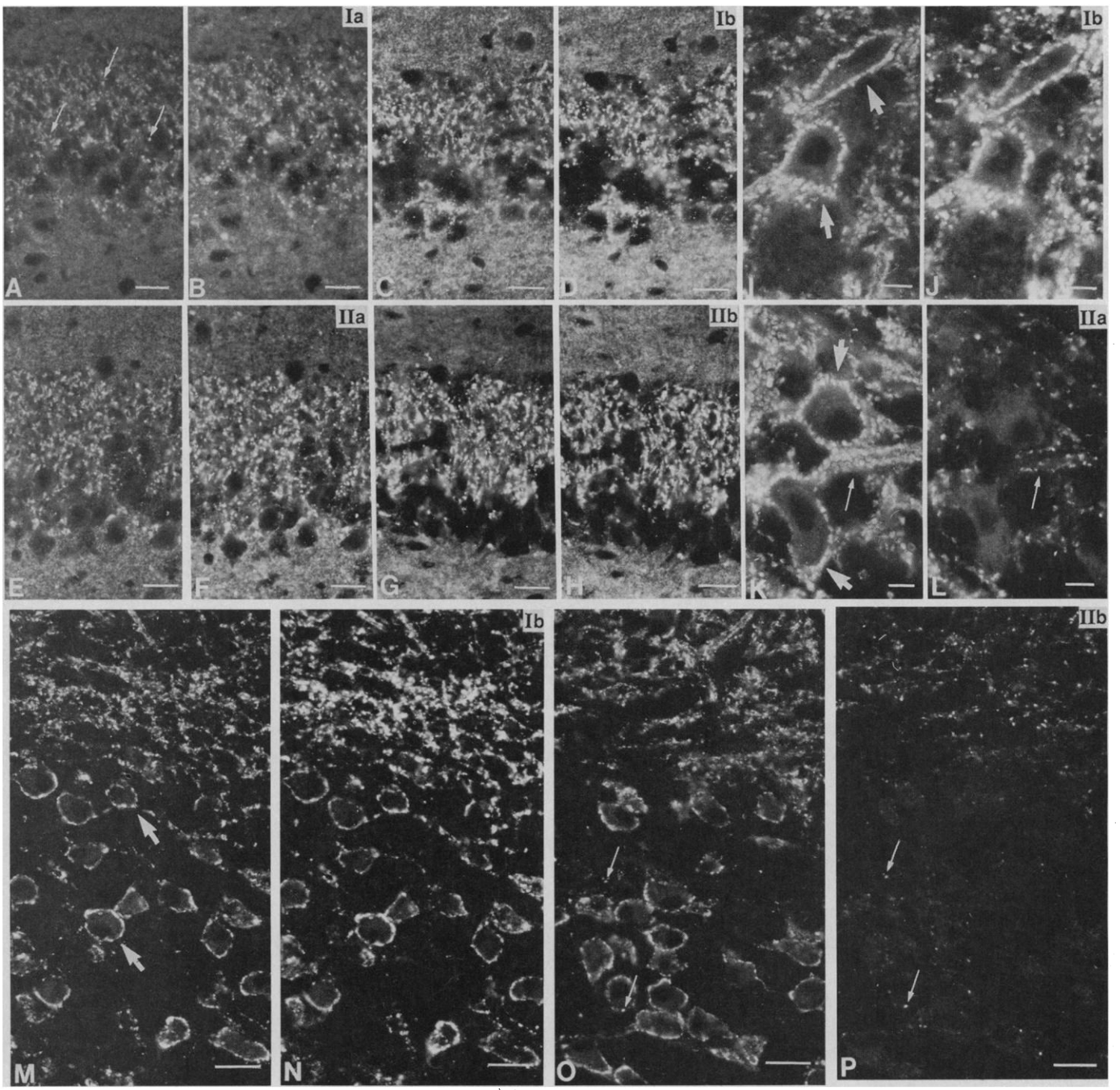

Fig. 6. Immunofluorescence localization of the four synapsins in rat brain frozen sections (*34*). Sections were double-labeled for synaptophysin and one of the four synapsins. The pairs of micrographs show the same field stained for synaptophysin on the left and for one of the four synapsins on the right. The synapsin subtype is indicated in the upper right corner. Antibodies used are those of lanes c, d, f, and g in Fig. 5. Panels show sections of the CA3 region of rat hippocampus (**A** to **H**), deep cerebellar nuclei (**I** to **L**), and the nucleus of the trapezoid body (**M** to **P**). The small arrows in (A) point to nerve terminals of mossy fibers that are equally reactive with all four synapsin antibodies (A to H). The thick arrows in (I) and (K) point to synapses on neuronal perikarya that contain synapsin Ib (J) but not synapsin IIa (L), while other synapses in the same field contain both synapsins Ib and IIa [small arrows in (K) and (L)]. Thick arrows in (M) point to synapses on neuronal perikarya that contain synapsin Ib (N) but lack synapsin IIb (P), whereas other synapses in the same field contain both [thin arrows in (O) and (P)]. Calibration: (A to H), 50 μm; (I to L), 10 μm; (M to P), 20 μm.

Sections of the CA3 region of the rat brain hippocampus were double-immunostained for each of the synapsins and for synaptophysin (Fig. 6, A to H). The large puncta observed with all five antibodies represent the mossy fiber nerve terminals that form synapses on the proximal dendrites of pyramidal neurons. The precise correspondence between the synapsin immunostain and the synaptophysin immunostain in each pair of micrographs indicates that each mossy fiber contains all four synapsins.

A very different distribution of the synapsins was observed in the deep cerebellar nuclei. Synapsin Ib was present in all nerve terminals (Fig. 6, I and J) whereas synapsin IIa was not detectable in the γ-aminobutyric acid (GABA)–containing nerve terminals that innervate the large neurons visible in the field (Fig. 6, K and L). These synapsin IIa–negative nerve terminals correspond to the Purkinje cell axon terminals (*31*). The same nerve terminals contained low, but detectable, levels of synapsin Ia and IIb immunoreactivities. Another striking example of the heterogeneous distribution of the synapsins was observed in the nucleus of the trapezoid body of the brain stem. In this nucleus, synapsin Ib was equally present in all nerve terminals (Fig. 6, M and N). In contrast, synapsin IIb was present in only a few synapses of the field (Fig. 6, O and P).

Immunoelectron microscopy was performed to determine whether the immunofluorescence pattern obtained with the peptide antibodies corresponded to a nerve terminal immunostain. Lysed nerve terminals embedded in agarose were labeled by immunogold (Fig. 7). These experiments confirmed the association of immunoreactivity specific for each of the four synapsins with synaptic vesicles in the nerve endings.

Our data, together with the results of earlier studies (*2–12*), provide a picture of synaptic vesicles as being coated on their cytoplasmic face by the four synapsins, a group of homologous and abundant extrinsic membrane proteins. This class of actin-binding proteins may connect synaptic vesicles to each other or to the cytoskeleton or to both, and these interactions are probably regulated by phosphorylation. Accordingly, the four synapsins are expected to play a role in positioning synaptic vesicles in the nerve terminal.

The four synapsins comprise common and individual domains. No two synapsins are identical, but each pair shares a different set of sequences. The four synapsins are differentially distributed among synapses, and it is possible that this differential distribution reflects differences in the functional properties of the synapses.

On the basis of the analysis of the homology and sequence characteristics of the four synapsins, a domain model was formulated for the synapsin protein family (Fig. 4). The central homologous region (domain C) that is shared by all four synapsins clearly forms a major functional domain. Domain C appears to bind synapsin I to actin and to synaptic vesicles. The presence of domain C in synapsins IIa and IIb suggests a similar function in these molecules. Depending on gene transcription, alternative splicing, and protein phosphorylation, domain C can be present in at least 12 different environments in the four synapsins (*32*). The regulation of the binding properties of domain C in the synapsins could function in positioning synaptic vesicles in the nerve terminal, thereby regulating neurotransmitter release. Our data, by elucidating the primary structures of the four synapsins and revealing the selective presence of individual members of the synapsin family in different types of nerve terminals, provide a framework for understanding how these molecules may function in organizing the presynaptic intracellular space.

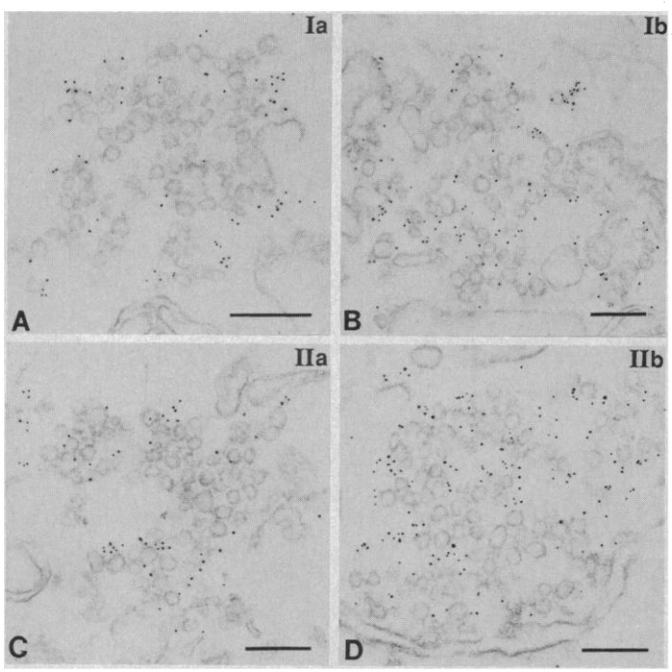

Fig. 7. Immunoelectron microscopic localization of the four synapsins in bovine brain. Bovine brain homogenates embedded in an agarose matrix were labeled with immunogold (*35*). The four fields show labeling obtained with antibodies to peptides specific for synapsin Ia (**A**), synapsin Ib (**B**), synapsin IIa (**C**), and synapsin IIb (**D**). Gold particles are concentrated in proximity to synaptic vesicles. Calibration bars, 200 nm.

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15. Synapsins I and II were purified as described (*3, 6, 10*). Rat and bovine synapsin I peptides were obtained after chemical or proteolytic cleavage then purified and sequenced. Rat synapsin II peptides were generated by cyanogen bromide cleavage and sequenced after purification. The sequences surrounding the phosphorylation sites of synapsin I were reported previously (*10*).
16. Four rat brain and two bovine brain cDNA libraries were screened with oligonucleotide probes or with antibodies (*36*). Positive clones were purified and identified by sequencing. Sequences of the largest clones were obtained on both strands by the dideoxy chain termination method (*37*).
17. The nucleotide sequences will be published in full elsewhere (T. C. Südhof *et al.*, in preparation) and are deposited in the GenBank database (bovine synapsin 1A, M27810; bovine synapsin 1B, M27811; rat synapsin 1A, M27812; rat synapsin 1B, M27924; rat synapsin 2A, M27925; rat synapsin 2B, M27926). Nucleotide sequences can be obtained on request before their publication from T. C. Südhof.
18. Studies on the human synapsin I gene (T. C. Südhof *et al.*, in preparation) indicate that the first exon contains more than 500 nt. Oligonucleotides corresponding to nt 21 to 44 and 125 to 153 and to nt 461 to 488 on the antisense strand of the bovine synapsin Ib cDNA were used to amplify the first exon of the rat synapsin I gene by the PCR [R. K. Saiki *et al.*, *Science* **230**, 1350 (1985)]. Genomic rat liver

DNA and a Perkin-Elmer thermocycler with denaturing temperatures of 95°C for 1 min and extension temperatures of 68°C for 5 min were used. The amplification product was purified, digested with restriction enzymes, and cloned into M13 vectors. Several independent isolates of the amplification products were sequenced (*37*) on both strands.

19. Genomic DNA prepared from rat liver was analyzed by DNA blotting [M. A. Lehrman *et al.*, *Science* **227**, 140 (1985)] with single-stranded, uniformly labeled DNA probes synthesized by M13 templates. Whereas hybridizations at high stringency revealed the presence of single-copy sequences, low-stringency hybridizations demonstrated the presence of several related sequences in the rat genome.
20. Purified polyadenylate-enriched RNA from bovine and rat tissues was separated by electrophoresis and blotted onto nylon membranes (*38*). Hybridizations were performed either with uniformly labeled single-stranded DNA probes (*38*) or with ^{32}P-labeled oligonucleotide probes. For the latter purpose, hybridizations were performed as for DNA-DNA hybridizations with oligonucleotides (*36*) except that the washing temperature was raised to 65°C. Messenger RNA sizes observed were as follows: in adult rat brain, two synapsin I messages of 3.3 kb and 4.3 kb were observed in a ratio of approximately 4:1, whereas in bovine brain only the 3.3-kb message was present. Both rat synapsin I messages hybridized with probes specific for synapsin Ia or Ib, suggesting alternative polyadenylation sites as the reason for the two rat mRNA sizes. For both rat and bovine synapsin IIa and IIb, single mRNA sizes were observed of 3.0 kb and 4.2 kb, respectively.
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22. Amplification reactions (*18*) were done with oligonucleotides corresponding to nt 2012 to 2040 and 2155 to 2182 (antisense) of the bovine cDNA with rat liver genomic DNA as template.
23. A partial sequence for synapsin Ia was published [C. A. McCaffery and L. J. DeGennaro, *EMBO J.* **5**, 3167 (1986)] and thought to represent synapsin Ib. The nucleotide and primary amino acid sequences we obtained indicate sequencing errors resulting in multiple frameshifts in that study. Approximately 20 percent of the amino acid sequence reported is probably from other reading frames. In addition, the sequence does not contain the three phosphorylation sites determined by direct amino acid sequencing (*10*).
24. GenBank release 59 and NBRF release 19 databanks were searched with standard programs [W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. U.S.A.* **85**, 2444 (1988)]. Several regions in the sequences of the synapsins are highly enriched in particular amino acids (such as proline and glutamine in domain D), and these regions are weakly homologous to many other proteins that are also enriched in these amino acids. However, the homologies were not judged to be significant because they were based on composition only and were independent of the residue sequences [R. F. Doolittle, *Of URFS and ORFS: A Primer on How to Analyze Derived Amino Acid Sequences* (University Science Books, Mill Valley, CA, 1986)].
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28. Peptides were synthesized by standard solid-phase methods, purified by Sephadex G-15 chromatography or reversed-phase high-performance liquid chromatography, and conjugated to bovine thyroglobulin with the use of glutaraldehyde. Rabbit polyclonal antibodies (*38*) to these peptides were affinity-purified on peptide–CH-Sepharose 4B columns. Rat cerebral cortex was homogenized [1:20 (w/v)] in hot (95°C) 2% SDS and 1% β-mercaptoethanol. Protein (500 μg) was loaded into a single large well, separated by electrophoresis on 6.5% polyacrylamide gels, and electrophoretically transferred to nitrocellulose. Immunoreactive bands on strips cut from the blot were visualized (*38*) with peroxidase-labeled secondary antibodies.
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30. In addition to yielding an intense nerve terminal staining pattern, all peptide antibodies also showed low-level staining of the perikaryal dendritic region of certain neurons (Fig. 6L).
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32. Synapsins Ia and Ib can exist in at least four phosphorylation states and synapsins IIa and IIb can exist in two phosphorylation states, excluding the state of the NGF-dependent phosphorylation site (*11*). By permutation, there are at least 12 contextual phosphorylation states in the four proteins.
33. The domain boundaries were determined on the basis of (i) amino acid sequence characteristics, (ii) degree of homology between the synapsins, (iii) functional characteristics, and (iv) alternative splicing. The exact positions of the boundaries may be slightly arbitrary. For example, the carboxyl-terminal 50 amino acids of synapsins Ia and IIa are 76 percent identical; the first five amino acids of this region are also present in synapsin Ib, but the rest of synapsin Ib is different. Accordingly, the beginning of domain E could be placed before or after these five amino acids.
34. For double immunofluorescence staining, frozen sections were first labeled with the different affinity-purified rabbit antibodies to synapsin (*28*) and then with monoclonal antibodies to synaptophysin (*29*). Each incubation was followed by a second incubation with rhodamine- or fluorescein-coupled secondary antibodies. Controls for specificity were performed as described [P. De Camilli, R. Cameron, P. Greengard, *J. Cell Biol.* **96**, 1337 (1983)].
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39. We thank the Rockefeller University Protein Sequencing Facility for peptide sequencing and synthesis, the Yale University Protein and Nucleic Acid Chemistry Facility and the Howard Hughes Medical Institute Protein Chemistry Facility at Dallas for peptide synthesis; D. Atherton, J. Elliott, and W. Roberts for their contributions; C. Carlson, I. Slizys, G. Kuzmycz, I. Leznicki, P. Barjon, N. Broyles, and R. Aparisi for technical assistance; M. S. Brown, D. W. Russell, and J. L. Goldstein for invaluable discussions. Partly supported by the Muscular Dystrophy Association (P.D.C.), by PHS grants MH 39327 and AA 06944 (P.G.), and by a cooperative agreement with the U.S. Environmental Protection Agency, CR 813826 (P.G.).

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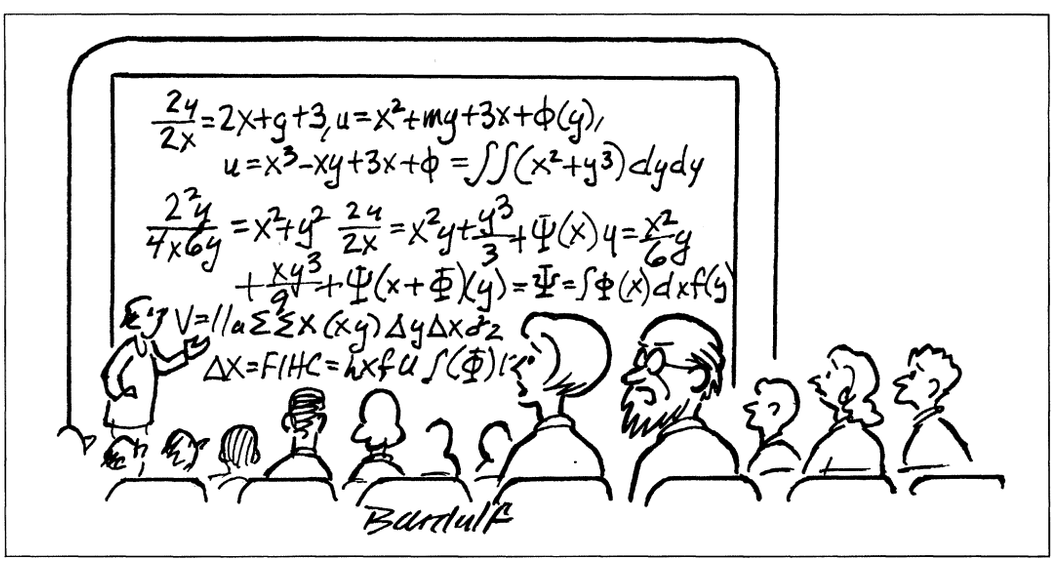

"I don't recall any speaker displaying such contempt for the intelligence of an audience."