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COMMENTARY

SYNAPSIN I: A SYNAPTIC VESICLE-ASSOCIATED NEURONAL PHOSPHOPROTEIN

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The discovery of cAMP-dependent protein kinase [1] and of its ubiquitous tissue distribution [2] prompted a search in the early seventies for endogenous substrates for the kinase in individual tissues. This approach offered the hope of understanding how cAMP regulates known enzymatic processes in different cell types and of identifying previously unknown major intracellular regulatory systems.

Synapsin I was the first such substrate to be discovered in mammalian brain [3–5]. For this reason, it was referred to for several years as Protein I, until its virtually ubiquitous and specific localization at synapses was demonstrated [6–8]. Over the last decade, synapsin I has been one of the most thoroughly studied neuronal proteins. Its precise function is still unknown, but we now have several important clues as to what that function might be. In addition, studies of synapsin I have shed light on previously unknown aspects of nerve terminal function. In this short commentary, we shall summarize the major known properties of synapsin I (for a comprehensive review of such properties, see Ref. 9) and we shall discuss its possible function.

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Physico-chemical properties of synapsin I

Synapsin I is the collective name for synapsin Ia and synapsin Ib, two closely related peptides with slightly different apparent molecular weights (around 80,000 in sodium dodecyl sulfate polyacrylamide gels) which appear to be coded by distinct mRNAs [10, 11]. Synapsin I was first purified to homogeneity from bovine brain [5]. Some of its properties are illustrated in Table 1. It is an extremely basic, acid-soluble, asymmetric protein composed of two major domains, a highly elongated collagenase-sensitive (tail) region, which is rich in proline and glycine and a collagenase-insensitive (head) region [5] (Fig. 1).

Synapsin I is a physiological substrate for at least three distinct protein kinases and undergoes multisite phosphorylation on serine residues [12–16]. The head region can be phosphorylated on a specific serine residue with high affinity by either cAMP-dependent protein kinase [13, 14] or Ca^{2+}/calmodulin-dependent protein kinase I [15, 16]. The tail region can be phosphorylated on two serine residues with high affinity by Ca^{2+}/calmodulin-dependent protein kinase II [15, 17–19]. In addition, the tail region can also be phosphorylated by a Ca^{2+}/diacylglycerol-dependent protein kinase (protein kinase C) in cell-free systems [20]; however, studies with synaptosomes suggest that this phosphorylation reaction may be of limited physiological significance [21].

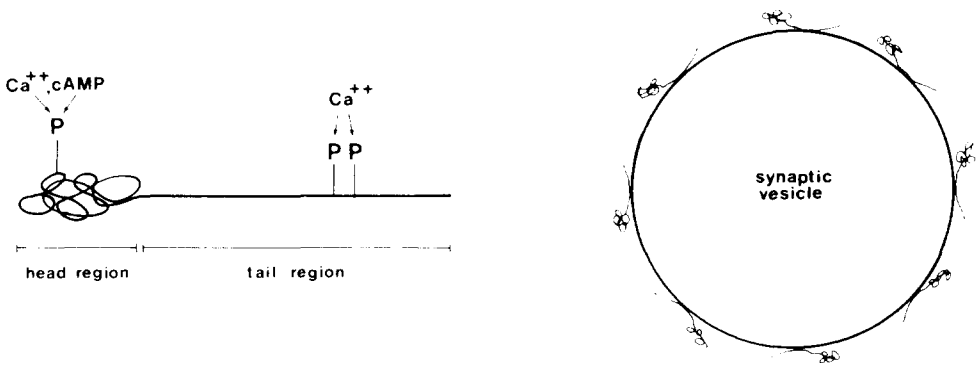

Fig. 1. Schematic illustration (left) of the structure of synapsin I showing its head and tail regions with their phosphorylation sites and (right) attachment of several synapsin I molecules, via the tail region, to the cytoplasmic surface of the synaptic vesicle membrane. The attachment to the vesicle surface is probably mediated by an intrinsic membrane protein.

Table 1. Physico-chemical properties, protein kinase specificity and distribution of synapsin I (modified from Ref. 9)

(A) Physico-chemical properties (bovine synapsin I)

	Synapsin Ia	Synapsin Ib
Molar proportion	1	2
Molecular weight (M_r)	86,000	80,000
Isoelectric point	>10.0	>10.0
Stokes radius	59 Å	59 Å
Sedimentation coefficient	2.9 S	2.9 S
Frictional ratio	2.2	2.2
Acid soluble	Yes	Yes
Amino acid composition	Rich in proline and glycine	
Other structural features	Elongated, highly asymmetric molecule with collagenase-insensitive domain (head region) and highly basic collagenase-sensitive domain (tail region)	

(B) Protein kinase specificity

Synapsin I undergoes multisite phosphorylation.

1. One serine residue in the head region of synapsin I is phosphorylated by cyclic AMP-dependent protein kinase and by Ca^{2+}/calmodulin-dependent protein kinase I.
2. Two serine residues in the tail region of synapsin I are phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II.
3. Not a physiological substrate for cyclic GMP-dependent protein kinase or for Ca^{2+}/diacylglycerol-dependent protein kinase (protein kinase C).

(C) Distribution

1. Present only in nervous system (both central and peripheral)
2. Within nervous system, present only in neurons
3. Within neurons, concentrated in nerve terminals
4. Within terminals, associated with small (40–60 nm) synaptic vesicles
5. Present at virtually all synapses
6. Appears simultaneously with synapse formation during development

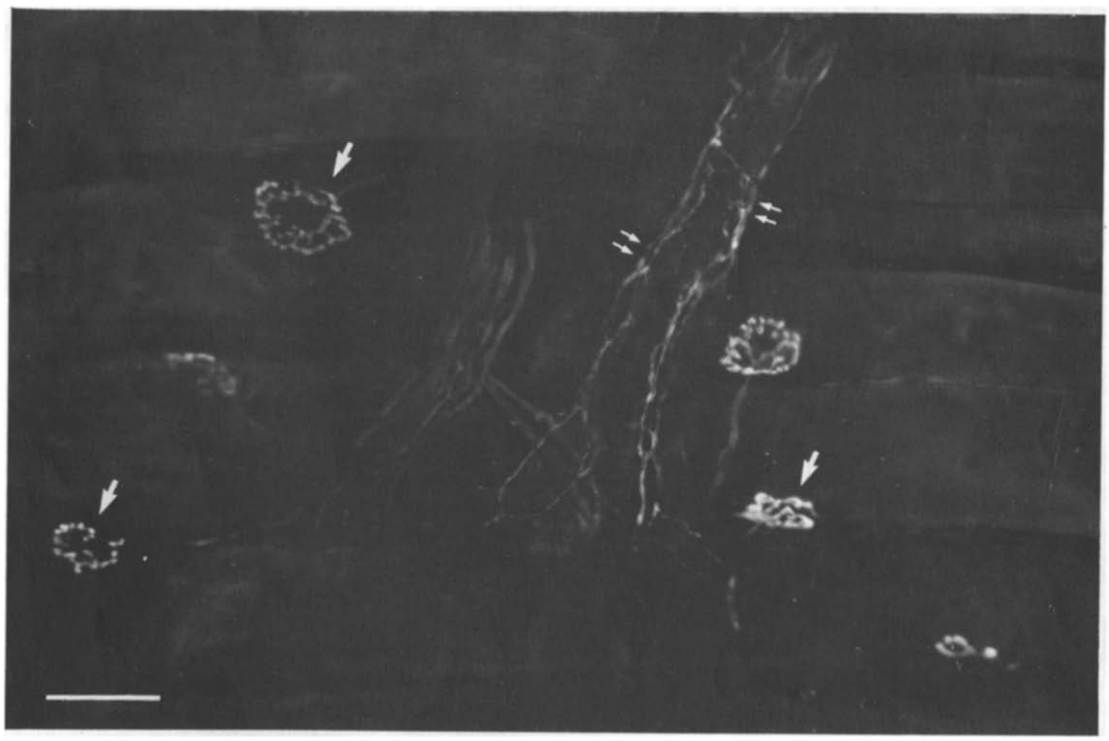

Fig. 2. Localization of synapsin I in nerve terminals of skeletal muscle demonstrated by light microscopy immunofluorescence in a frozen section. Immunoreactivity is concentrated in the cholinergic motor nerve endings (large arrow) as well as in the varicose terminals (probably catecholaminergic) of the autonomic nervous system which innervate (and outline) a blood vessel (double small arrows). (Calibration bar: 50 μm.)

Synapsin I—A synaptic vesicle-associated nerve terminal protein

Synapsin I is a neuron-specific protein [4, 6, 22] present at very high concentration in the mammalian brain [23]. It constitutes 0.3–0.4% of total brain protein [23] and must constitute an even higher percentage of total neuronal protein, considering that neurons represent only a subpopulation of brain cells. Synapsin I is present at very low concentrations in non-neuronal tissues [22], and this low amount is probably attributable to the neuronal innervation of those tissues [6, 22, 24]. Proteins exhibiting immunological similarities to synapsin I have been detected in nervous systems of diverse species of the animal kingdom [25, 26].

Results of early studies involving cell fractionation and immunocytochemistry demonstrated that synapsin I is concentrated at the synaptic regions of nerve cells [22, 27, 28]. Synapsin I was subsequently shown by light and electron microscopic (EM) immunocytochemistry to be a presynaptic protein, present in virtually all nerve endings of the central and peripheral nervous system, irrespective of their neurotransmitter content [6, 7] (Figs. 2 and 3). (One possible exception to the ubiquitous presence of synapsin I in nerve terminals appears to be the highly specialized synapse of photoreceptor cells [6]). During ontogenesis, expression of the synapsin I gene (as measured by levels of synapsin I and of synapsin I mRNA) correlates temporally [10, 29], and appearance of immunohistochemically-detectable synapsin I in the developing neurophil correlates temporally and spatially [6, 30], with synapse formation. In regions of neurons other than axon terminals, synapsin I is present at very low levels that can be detected biochemically [31] or by sensitive immunocytochemical procedures (unpublished results). Synapsin I in the cell body may represent newly synthesized synapsin I, and that in the axons synapsin I on its way to the terminals, since no cytoplasmic ribosomes (necessary for synapsin I synthesis) are present in the axon terminals [32]. Intense perikaryal staining for synapsin I is observed in some neurons at certain

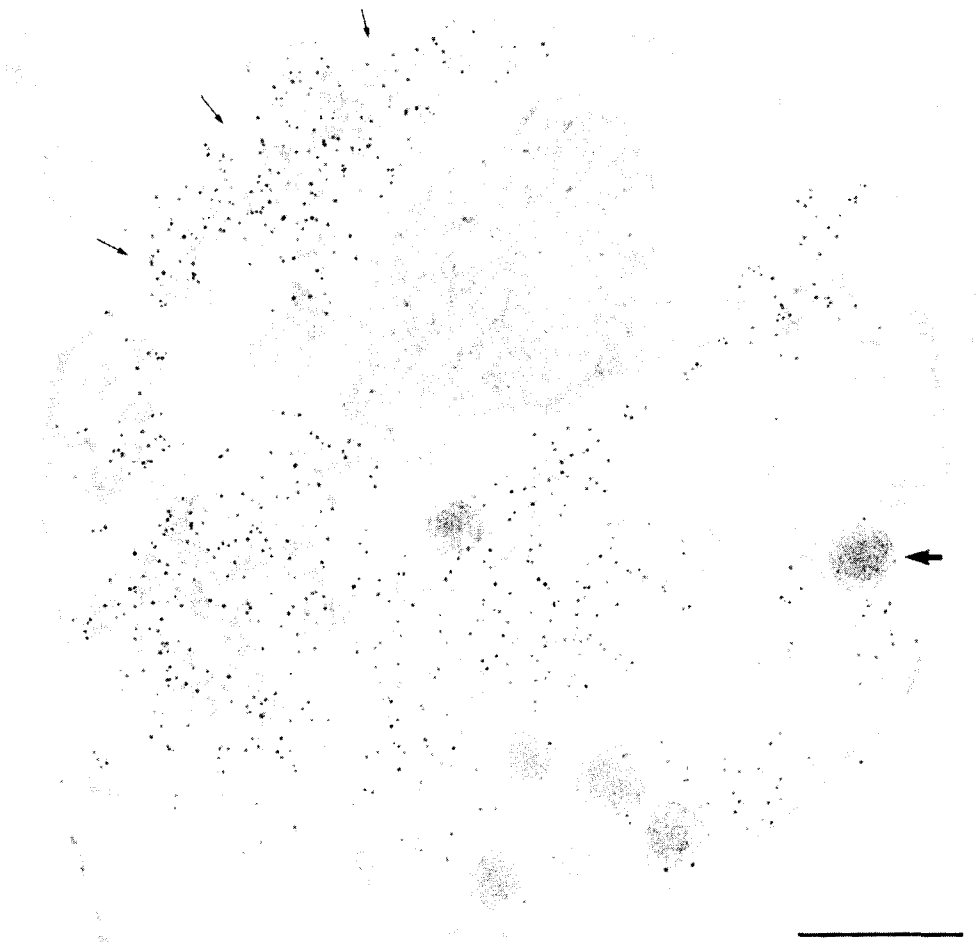

Fig. 3. Electron micrograph showing the localization of synapsin I by immunogold in an isolated lysed nerve ending of bovine hypothalamus. The figure shows the selective association of immunoreactivity with small synaptic vesicles. Large dense-core vesicles are unlabeled. Small synaptic vesicles heavily decorated by gold particles are indicated by thin arrows and a large dense-core vesicle is indicated by a thick arrow. (Calibration bar: 200 nm.) (Reprinted with permission from Navone *et al. Science* **226**, 1209–1211, Fig. 1, 7 December, 1984. Copyright 1984 by the American Association for the Advancement of Science).

ontogenetic stages. These stages probably correspond to the peaks of synaptogenetic activity at the axon terminals of these neurons [33]. Synapsin I can be readily demonstrated in axons by immunocytochemistry where a focal accumulation of axonally transported material is induced on the proximal side of a nerve ligature, while distally only little immunoreactive synapsin I is observed ([24]; A. Dahlstrom, personal communication).

At the light microscope level, synapsin I immunoreactivity is concentrated in those subregions of the nerve terminal in which synaptic vesicles are clustered [6]. Upon lysis of the terminal in low ionic strength, synapsin I remains associated with 40–60 nm-diameter synaptic vesicles (small synaptic vesicles) as can be shown by both EM immunocytochemistry and subcellular fractionation [7, 8, 27, 34] (Fig. 3). In a highly purified preparation of small synaptic vesicles from cerebral cortex, synapsin I represents 6% of the total protein [8].

Interaction of synapsin I with the surface of small synaptic vesicles

Synapsin I is associated with the vesicle surface but is not an integral membrane protein [5, 8]. It can be dissociated from the vesicle by increasing the ionic strength of the suspending medium or by markedly lowering the pH [5, 8, 35]. In agreement with these observations, it has been found to be synthesized by free ribosomes [10], which synthesize peripheral membrane proteins [36], rather than by membrane-bound ribosomes which synthesize proteins to be inserted into, or translocated across, the lipid bilayer [37]. Binding of synapsin I to the vesicle seems to involve the collagenase-sensitive domain of the molecule [8, 38]. The binding is specific, saturable, of high affinity (K_D 10 nM at 40 mM KCl, when synapsin I is in the non-phosphorylated state), and appears to be mediated by a specific protein on the vesicle surface [8, 39]. Phosphorylation of the tail region (but not of the head region) decreases the affinity of synapsin I for the vesicle surface and highly purified synaptic vesicles contain an endogenous Ca^{2+}/calmodulin-dependent protein kinase activity which is able to promote the phosphorylation of the tail region [8, 39].

Regulation of the state of phosphorylation of synapsin I

A variety of physiological and pharmacological manipulations of intact neurons or nerve endings have been shown to produce changes in the state of phosphorylation of synapsin I. A summary of the results of these studies is presented in Table 2 (see also Ref. 9). In general, manipulations known to induce release of neurotransmitter, or to potentiate release evoked by other stimuli, produce an increase in the ratio of phospho-synapsin I to dephospho-synapsin I [13, 31, 35, 40–48]. Most such manipulations are associated with increased levels of cAMP or Ca^{2+}, or both, in the nerve terminal. The sites on the synapsin I molecule that become phosphorylated depend upon the stimulus. When cAMP is involved as second messenger, the head region of synapsin I becomes phosphorylated, whereas when Ca^{2+} is involved, both the head and tail regions become phosphorylated [13, 31, 41].

These correlations between phosphorylation of synapsin I and increased rate of neurotransmitter release, together with the localization of synapsin I on synaptic vesicles, suggest that phosphorylation of synapsin I may be involved in controlling the release process. In the nerve terminal, Ca^{2+} is thought to play both an obligatory effector role as well as a modulatory role in neurotransmitter release [49–51]. In contrast, cAMP is thought to play primarily a modulatory role [9, 51, 52]. The ability of the head region to be phosphorylated at the same site by either cAMP-dependent or a Ca^{2+}-dependent protein kinase suggests that phosphorylation of this region may be involved in modulation rather than in mediation of the release process. Phosphorylation of the head region appears to provide a mechanism of convergence for modulatory effects of cAMP and Ca^{2+}. The property of the tail region to serve as a substrate only for Ca^{2+}-dependent protein kinases is compatible with either a modulatory or a mediatory role for the phosphorylation of this region.

Physiological evidence for a role of synapsin I in the regulation of neurotransmitter release based on injection experiments in the squid giant synapse

Experiments that provide evidence for a role of synapsin I in the regulation of neurotransmitter release have been carried out recently in the squid giant synapse [26]. Injection of dephospho-synapsin I into the presynaptic terminal digit greatly reduced the amount of neurotransmitter released during a depolarizing step. Conversely, injection of Ca^{2+}/calmodulin/dependent protein kinase II (which phosphorylates the tail region of synapsin I) potentiated the secretory response to a depolarizing step.

Table 2. Regulation of the state of phosphorylation of synapsin I

(1) In synaptosomes and in slices of nervous tissue, manipulations that increase the concentration of Ca^{2+} or cAMP inside the axon terminal increase the state of phosphorylation [13, 35, 40–42].
(2) In specific anatomical regions of central and peripheral nervous system, relevant neurotransmitters (serotonin, dopamine, norepinephrine, adenosine) increase the state of phosphorylation [41, 43–46, S. I. Walaas, unpublished results].
(3) In isolated superior cervical ganglion and in posterior pituitary, impulse conduction under physiological conditions increases the state of phosphorylation [31, 41, 47].
(4) In whole animals, convulsants increase and depressants decrease the state of phosphorylation in cerebrum [48].

Two distinct secretory pathways in neurons

The high and selective concentration of synapsin I on small synaptic vesicles in nerve terminals indicates that this protein plays a role in some aspect of small synaptic vesicle function. However, the lack of synapsin I in non-neuronal cells specialized for vesicular secretion, such as exocrine and peptide-secreting endocrine cells, indicates that synapsin I is not involved in some aspect of the secretory process *per se*, but rather that it plays a role which is specifically related to neuronal secretion. It is therefore highly relevant to compare the secretory pathways of neurons and non-neuronal tissues for similarities and differences.

Until recently, only a small number of neurotransmitter molecules were known [53, 54]. They were all nonpeptide molecules (now collectively referred to as classical neurotransmitters [53]) and ideas about neuronal secretion learned from studies on simple model systems, such as the cholinergic terminal of the neuromuscular junction, could be applied to terminals secreting other neurotransmitters. One such idea was that synaptic vesicles can undergo repeated exo-endocytotic cycles in nerve endings and that at each cycle they can be reloaded with neurotransmitter, since all classical neurotransmitters can be synthesized and loaded into vesicles in the nerve terminal itself [51, 55, 56]. In this respect, the secretory pathway of neurons, although regulated, appeared to differ from the typical "regulated" vesicular secretion of endocrine and exocrine cells, which involves loading and assembly of peptide-containing secretory granules in the area of the Golgi complex [57, 58]. A few neurons were known to secrete peptides (for example, in mammalian species the neurons of the hypothalamic-hypophyseal system) via secretory organelles assembled in the Golgi area as in the case of peptide-secreting non-neuronal cells. These, however, were considered a specialized family of neurons [59].

Recent discoveries that a much larger number of neurons (conceivably all neurons) can secrete peptides via a regulated pathway, and that peptides can be co-secreted together with classical neurotransmitters from the same ending [53, 60], have underlined the need for a more detailed understanding of neuronal secretion. Co-storage of peptides and classical neurotransmitters in the same peripherally-recycling vesicles is not likely since peptide molecules can only be synthesized and loaded into vesicles in the perikarya. It can therefore be presumed that, after exocytosis and reinternalization, the membranes of the peptide-containing vesicles are transported back to the perikaryon to be reloaded in the Golgi area. In fact, evidence is emerging that while typical small (40–60 nm) synaptic vesicles contain only classical neurotransmitter, peptide neurotransmitters are contained in a separate class of organelles larger and with a dense core ("large dense-core vesicle"*) [60–63]. Evidence also exists that release of neurotransmitters from small synaptic vesicles and from large dense-core vesicles may be differently regulated [60, 66, 67]. Thus, large dense-core vesicles and small synaptic vesicles in neurons appear to be secretory organelles for two distinct and independent regulated secretory pathways.

The neuronal secretory pathway involving large dense-core vesicles has important similarities with the secretory pathway of peptide-secreting endocrine cells. Peptide-containing large dense-core vesicles of neurons resemble granules of endocrine cells in content, morphology, site of assembly (the Golgi area), and mechanism of assembly [51, 57, 58, 68, 69]. An increasing number of peptide hormones and other secretory peptides (e.g. chromogranins and secretogranins) thought to be specific for certain endocrine cells have also been found in large dense-core vesicles of neurons [53, 54, 61, 64, 65, 70]. Thus, the peptidergic secretory pathway of neurons may be equivalent to the "regulated" secretory pathway of endocrine cells. In contrast, small synaptic vesicles are part of a secretory pathway specific to neurons.

Synapsin I—Its possible association with a secretory pathway specific to neurons

The exclusive presence of synapsin I in neurons could be explained if it were selectively involved in the function of small synaptic vesicles. To test this possibility, we have been investigating, by immunocytochemistry and subcellular fractionation, the localization of synapsin I in a variety of nerve terminals containing relatively high concentrations of large dense-core vesicles in addition to small synaptic vesicles. Thus far, the results support the idea of a selective association of synapsin I with small synaptic vesicles (Fig. 3) ([34]; F. Navone and P. De Camilli, unpublished observations). Little, if any, synapsin I appears to be associated with large dense-core vesicles [34]. It is possible, therefore, that the presence of synapsin I exclusively in neurons is attributable to its being involved in a secretory pathway that is unique to nerve cells. In nerve terminals synapsin I may provide one basis for the differential regulation by intracellular second messengers of the release of classical and peptide neurotransmitters.

Does synapsin I bind synaptic vesicles to the cytoskeleton?

The demonstration that synapsin I is bound selectively to small synaptic vesicles provides an important clue to understanding its role. This role is presumably to be found among those functions which are unique to small synaptic vesicles and common to all of them irrespective of their neurotransmitter content. One function common to all small synaptic vesicles, and one in which they differ from large dense-core vesicles, concerns their traffic in nerve terminals, i.e. their ability to undergo a local exo-endocytotic

* The term "dense-core vesicle" has been used to define two types of vesicles in nerve endings [63]. One type, the small dense-core vesicle, acquires an electron-opaque core under certain fixation conditions and has a diameter of 40–60 nm. The dense core is thought to represent a catecholamine-containing precipitate. We consider this type of vesicle to be part of the small synaptic vesicle population. The other type is larger and has an electron-opaque core irrespective of the fixation procedure used. These vesicles generally appear to contain secretory peptides (e.g. chromogranins, secretogranins, peptide neurotransmitters) instead of, or in addition to, a classical neurotransmitter [60–65]. The term large dense-core vesicle refers to the latter vesicle population.

recycling. The intracellular traffic of all subcellular organelles is presumably regulated by specific interactions of their membranes with components of the cytoplasmic matrix. Thus, interaction of small synaptic vesicle membranes with a specialized cytoskeletal matrix of the nerve terminal seems likely. The observation that small synaptic vesicles are not randomly interspersed with other organelles, but tend to occur in clusters which do not readily dissociate upon nerve ending lysis [7, 34], supports the idea of such an interaction. We have hypothesized that synapsin I, an extrinsic membrane protein, present at high concentration at the vesicle surface, may act as a link between a constituent of the cytoskeletal matrix and the vesicle surface [7, 8, 34]. Phosphorylation of specific domains of the molecule might regulate this linkage by changing binding affinities for constituents of the matrix and/or the vesicle. Recently it has been found that purified synapsin I binds *in vitro* to spectrin [71], to neurofilaments [72], to microtubules [71–73] and to actin (M. Bähler and P. Greengard, unpublished observations). If any one of these types of binding proves to be of physiological significance, it will provide support for the hypothesis that synapsin I is involved in linking vesicles to the cytoskeleton.

Are synapsin I and erythrocyte protein 4.1 closely related proteins?

Other proteins thought to play a role in linking the cytoplasmic side of cellular membranes to the cytoskeletal matrix are extrinsic membrane proteins. Studies carried out on the erythrocyte plasma membrane have shown that the membrane proper is lined at its cytoplasmic side by a stabilizing infrastructure that anchors some of its intrinsic membrane proteins to cytoskeletal proteins. Major components of this infrastructure are extrinsic membrane proteins [74–76]. Moreover, evidence is now accumulating that certain structural components of the erythrocyte plasma membrane may be shared by other membranes in a variety of cell types [75–78]. It is of interest that protein 4.1, which in red cells is believed to cross-link glycophorin to spectrin and actin [74–76], is immunologically related to synapsin I [71] and has a number of important physico-chemical similarities to synapsin I. These include a similar molecular weight, a highly basic isoelectric point, and elongated structure, with multiple phosphorylation sites regulated by Ca^{2+} and cAMP, and the ability to bind spectrin [74–77, 79–81]. Moreover, protein 4.1 is present in erythrocyte membranes at a level (6% of the total protein [80]) remarkably similar to that of synapsin I on vesicle membranes [8]. In addition, the association of synapsin I with vesicle membranes isolated in low ionic strength media, and the lack of evidence for other synapsin I-binding proteins in such preparations [8], are reminiscent of the property of protein 4.1 to remain associated with membranes but to lose its interaction with cytoskeletal proteins (spectrin and actin) under low ionic strength conditions [75, 76].

Although it is likely that protein 4.1 and synapsin I are related, the nature and extent of the relationship remain an open issue. First, the peptide map of synapsin I is very distinct from the peptide map of erythrocyte protein 4.1 [71]. Second, the morphological distribution of erythrocyte protein 4.1 immunoreactivity in the brain, as assessed by immunocytochemistry [82], appears to be different from the distribution of synapsin I (compare [82] and [6]; T. Petrucci and P. De Camilli, unpublished observations). Instead, the distribution of 4.1 [82] resembles the distribution of the bulk of brain spectrin [83]. Third, the cross-reactivity between synapsin I and erythrocyte protein 4.1 is weak and, fourth, the relative abilities of synapsin I and erythrocyte protein 4.1 to serve as substrates for various types of protein kinases differ substantially (M. Bahler, A. C. Nairn, R. Jahn, K. Krebs, S. R. Goodman and P. Greengard, unpublished observations).

The specific association of synapsin I with synaptic vesicle membranes in neurons constitutes compelling evidence for the idea that synapsin I plays a neuron-specific role. If protein 4.1 and protein 4.1-like proteins are general components of all plasmalemmas and plasmalemma-related membranes, as may be the case [75, 76], brain could be expected to contain more than one type of protein 4.1-related protein. Synapsin I would be only one of them, yet the most abundant because of the extremely high concentration in brain of small synaptic vesicles.

Protein III

Protein III is a protein which appears highly related to synapsin I. Protein III, so called [84] because it was the third major phosphoprotein to be discovered in brain [35], is the collective name for two peptides, Protein IIIa and Protein IIIb which, in spite of their rather different apparent molecular weights (74,000 and 55,000, respectively, in sodium dodecyl sulfate polyacrylamide gels), have many common properties [9, 84].

Similarities of Protein III with synapsin I include the following. Protein III is a major brain phosphoprotein concentrated in nerve endings where, like synapsin I, it appears to be specifically localized on small synaptic vesicles ([9, 85]; F. Navone, M. Browning, C. Ouimet, P. De Camilli and P. Greengard, unpublished observations). A number of antibodies (including some monoclonal) raised against Protein III also recognize synapsin I [9, 85]. Protein III can be phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase I and by cAMP-dependent protein kinase as can the head region of synapsin I [84, 85]. The state of phosphorylation of Protein III in intact nerve endings varies in parallel with the state of phosphorylation of the head region of synapsin I in response to physiological and pharmacological manipulations [9, 35, 41]. In contrast to synapsin I, Protein III has a roughly neutral isoelectric point [84] and lacks the tail region of synapsin I, which contains the phosphorylation sites for Ca^{2+}/calmodulin-dependent protein kinase II. Protein III is more tightly bound to the vesicle surface [e.g. it is not released by exposing vesicles to media of moderate ionic strength (M. Browning and R. Jahn, unpublished results)] and it is also present, although at low concentration, in adrenal chromaffin cells [86]. It would appear that Protein III plays a role distinct from, but presumably related to, that of synapsin I.

Possible roles of synapsin I in vesicle function

The life-cycle of small synaptic vesicles in nerve endings includes: (1) resting phase in the cytoplasm of the terminal, (2) translocation to subplasma membrane sites, (3) binding-attachment to specific sites on the plasma membrane, (4) fusion with the plasma membrane, (5) selective retrieval from the plasma membrane, and (6) selective recruitment for local recycling (while other internalized membrane components are addressed to the perikarya). The fusion event *per se* is not likely to be regulated by synapsin I because it is a process common to exocytosis of all types of secretory granules. Any of the other steps is more likely to be regulated by synapsin I.

One hypothetical model that can be proposed on the basis of the available evidence follows. In the resting nerve ending, dephospho-synapsin I may mediate the attachment of synaptic vesicles to a cytoskeletal infrastructure of the terminal. Following an excitatory stimulus, phosphorylation of synapsin I may allow dissociation of vesicles from this restraining matrix and make them available for interaction with the presynaptic membrane. This mechanism may not account for the immediate secretory response evoked by a nerve terminal depolarization, but could be a preparatory event controlling the magnitude of the response to future stimuli by altering "vesicle availability". Such a mechanism might be involved in the potentiation of neurotransmitter release produced both by electrical impulses (facilitation, augmentation, potentiation, post-tetanic potentiation [50]) and by activation of excitatory presynaptic receptors (presynaptic facilitation [9, 52]). Similarly, inhibitory presynaptic receptors might produce their effects by decreasing the ratio of phospho-synapsin I/dephospho-synapsin I. In the retrieval limb of the exo-endocytotic cycle, synapsin I dephosphorylation might be a mechanism whereby internalized vesicle membrane material reestablishes association with the cytoskeleton of the terminal. In this way vesicle membrane would be selectively recruited for local recycling as opposed to other internalized membrane material destined to reach the perikarya via retrograde axoplasmic flow. The model is undoubtedly oversimplified. Presumably the traffic of vesicles in the ending requires a coordinate sequence of biochemical interactions among which interaction with synapsin I has a key but not a unique role. Yet the model may be a useful tool to guide further studies.

Highly relevant to the testing of our model is the establishment of the precise relationship of synapsin I to synaptic vesicles during the exo-endocytotic cycle. Our model implies a dissociation of synapsin I from synaptic vesicles prior to exocytosis. This idea, however, is supported so far only by the finding that, under certain experimental conditions, Ca^{2+} acting via calmodulin-dependent phosphorylation, reduces the affinity of synapsin I for the vesicle membrane in a purified synaptic vesicle preparation [8, 39] and by the apparent lack of synapsin I on plasma membranes of nerve terminals [7]. The latter result, however, might be explained by an extremely short residence time of synaptic vesicle membranes at the cell surface.

Future trends

Several lines of investigation currently being pursued should give us additional clues concerning the function of synapsin I. These include determination of its amino acid sequence, identification and characterization of synapsin I-binding proteins, *in vitro* reconstitution experiments, characterization of the intracellular traffic of synapsin I in relation to that of intrinsic proteins of the synaptic vesicle membrane, studies involving genetic manipulations of the synapsin I gene, and more extensive experiments aimed at testing directly the effect of synapsin I phosphorylation-dephosphorylation on neurotransmitter release.

Small synaptic vesicles have traditionally been considered primarily as secretory vesicles and, consequently, analogies have been sought between small synaptic vesicles and other secretory organelles. However, much might be learned by focusing on the endocytotic nature of small synaptic vesicles and by studying their relationship to other endocytotic organelles. An endo-exocytotic recycling of membrane proteins independent of the pathway involved in regulated secretion of peptides takes place in all cells (membrane receptors for peptide hormones, for example, are internalized and recycled in this way) [87, 88]. The small synaptic vesicle system of nerve terminals might have evolved from such an exo-endocytotic pathway.

Finally, we have found recently that an intrinsic membrane protein (protein P38) originally discovered in brain synaptic vesicles [89, 90] is also present in some non-neuronal cells [peptide-secreting endocrine cells (F. Navone, R. Jahn, P. Greengard and P. De Camilli, submitted)]. In neurons, protein P38, like synapsin I, is selectively associated with small synaptic vesicles, and in endocrine cells it is localized on smooth surfaced vesicles distinct from peptide-containing secretory granules (F. Navone, R. Jahn, P. Greengard and P. De Camilli, submitted). These results support the idea that an endomembrane system related to brain synaptic vesicles may also be present in some non-neuronal cells. Synapsin I, which is present only in neurons, might be involved in the special adaptation of this endomembrane system for neuronal secretion. Identifying the adaptations of this endomembrane system that are specific to neurons will help to determine the function of synapsin I.

Concluding remarks

Synapsin I, discovered as a phosphorylated "band in a gel", has become of considerable interest in studies of synapses and synaptic vesicle function. Its specific location in neurons and in nerve endings makes synapsin I a unique marker for anatomical and biochemical studies of synapses during development and in various normal and pathological conditions [6, 30, 33, 91–94]. In fact, it is the only marker protein identified thus far that is specific for nerve terminals and common to all nerve terminals. By studying the state of phosphorylation of synapsin I, one can study the pharmacology of presynaptic receptors [45, 46]. It can be expected that a further clarification of its function will lead to an increased understanding

of the mechanisms which control neurotransmitter release and possibly to the elucidation of regulatory mechanisms of more general significance in cell biology.

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REFERENCES

1. D. A. Walsh, J. P. Perkins and E. G. Krebs, *J. biol. Chem.* **243**, 3763 (1968).
2. J. F. Kuo and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **64**, 1349 (1969).
3. E. M. Johnson, T. Ueda, H. Maeno and P. Greengard, *J. biol. Chem.* **247**, 5650 (1972).
4. T. Ueda, H. Maeno and P. Greengard, *J. biol. Chem.* **248**, 8295 (1973).
5. T. Ueda and P. Greengard, *J. biol. Chem.* **252**, 5155 (1977).
6. P. De Camilli, R. Cameron and P. Greengard, *J. Cell Biol.* **96**, 1337 (1983).
7. P. De Camilli, S. M. Harris, W. B. Huttner and P. Greengard, *J. Cell Biol.* **96**, 1355 (1983).
8. W. B. Huttner, W. Schiebler, P. Greengard and P. De Camilli, *J. Cell Biol.* **96**, 1374 (1983).
9. E. J. Nestler and P. Greengard, *Protein Phosphorylation in the Nervous System.* John Wiley, New York (1984).
10. L. J. DeGennaro, S. D. Kanazir, W. C. Wallace, R. M. Lewis and P. Greengard, *Cold Spring Harb. Symp. quant. Biol. Molec. Neurobiol.* **48**, 337 (1983).
11. M. W. Kilimann and L. J. DeGennaro, *EMBO J.* **4**, 1997 (1985).
12. W. Sieghart, J. Forn and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **76**, 2475 (1979).
13. W. B. Huttner and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5402 (1979).
14. W. B. Huttner, L. J. DeGennaro and P. Greengard, *J. biol. Chem.* **256**, 1482 (1981).
15. M. B. Kennedy and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **78**, 1293 (1981).
16. A. C. Nairn and P. Greengard, *Abstr. 13th Annual Meeting Soc. Neurosci.* 1029 (1983).
17. M. B. Kennedy, T. McGuinness and P. Greengard, *J. Neurosci.* **3**, 818 (1983).
18. M. K. Bennett, N. E. Erondu and M. B. Kennedy, *J. biol. Chem.* **258**, 12735 (1983).
19. T. L. McGuinness, Y. Lai and P. Greengard, *J. biol. Chem.* **260**, 1696 (1985).
20. K. A. Albert, W. C-S. Wu, A. C. Nairn and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **81**, 3622 (1984).
21. J. K. T. Wang, S. I. Walaas and P. Greengard, *Abstr. 15th Annual Meeting Soc. Neurosci.* 846 (1985).
22. P. De Camilli, T. Ueda, F. E. Bloom, E. Battenberg and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5977 (1979).
23. S. E. Goelz, E. J. Nestler, B. Chehrazi and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **78**, 2130 (1981).
24. G. Fried, E. J. Nestler, P. De Camilli, L. Stjärne, L. Olson, J. M. Lundberg, T. Hökfelt, C. C. Ouimet and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2717 (1982).
25. S. E. Goelz, E. J. Nestler and P. Greengard, *J. Neurochem.* **45**, 63 (1985).
26. R. Llinas, T. McGuinness, C. S. Leonard, M. Sugimori and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **82**, 3035 (1985).
27. T. Ueda, P. Greengard, K. Berzins, R. S. Cohen, F. Blomberg, D. J. Grab and P. Siekevitz, *J. Cell Biol.* **83**, 308 (1979).
28. F. E. Bloom, T. Ueda, E. Battenberg and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5982 (1979).
29. S. M. Lohmann, T. Ueda and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **75**, 4037 (1978).
30. P. Levitt, P. Rakic, P. De Camilli and P. Greengard, *J. Neurosci.* **4**, 2553 (1984).
31. E. J. Nestler and P. Greengard, *J. Neurosci.* **2**, 1011 (1982).
32. A. Peters, S. L. Palay and H. de F. Webster, *The Fine Structure of the Nervous System.* W. B. Saunders, Philadelphia (1976).
33. P. De Camilli, R. Cameron and P. Greengard, *J. Cell Biol.* **87**, 72a (1980).
34. F. Navone, P. Greengard and P. De Camilli, *Science* **226**, 1209 (1984).
35. J. Forn and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **75**, 5195 (1978).
36. E. Lazarides and R. T. Noon, *Cell* **37**, 354 (1984).
37. W. J. Wickner and H. F. Lodish, *Science* **230**, 400 (1985).
38. T. Ueda, *J. Neurochem.* **36**, 297 (1981).
39. W. Schiebler, R. Jahn, J-P. Doucet, J. Rothlein and P. Greengard, *J. biol. Chem.*, **261**, 8383 (1986).
40. B. K. Krueger, J. Forn and P. Greengard, *J. biol. Chem.* **252**, 2764 (1977).
41. K. Tsou and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6075 (1982).
42. J. Meldolesi, W. B. Huttner, R. Y. Tsien and T. Pozzan, *Proc. natn. Acad. Sci. U.S.A.* **81**, 620 (1984).
43. A. C. Dolphin and P. Greengard, *Nature, Lond.* **289**, 76 (1981).
44. E. J. Nestler and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **77**, 7479 (1980).
45. P. Mobley and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **82**, 945 (1985).
46. M. Treiman and P. Greengard, *Neuroscience* **15**, 713 (1985).
47. E. J. Nestler and P. Greengard, *Nature, Lond.* **296**, 452 (1982).
48. U. Strombom, J. Forn, A. C. Dolphin and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4687 (1979).
49. R. Llinas, in *The Neurosciences Fourth Study Program* (Eds. F. O. Schmitt and F. G. Worden), p. 555. MIT Press, Cambridge, MA (1979).
50. K. L. Magleby, in *Progress in Brain Research* (Ed. S. Tûcek), Vol. 49, p. 175. Elsevier, Amsterdam (1979).
51. L. F. Reichardt and R. B. Kelly, *A. Rev. Biochem.* **52**, 871 (1983).
52. E. R. Kandel and J. H. Schwartz, *Science* **218**, 433 (1982).
53. T. Hökfelt, O. Johansson, A. Ljundahl, J. M. Lundberg and M. Schultzberg, *Nature, Lond.* **284**, 515 (1980).
54. S. H. Snyder, *Science* **209**, 976 (1980).
55. B. Ceccarelli, W. P. Hurlbut and A. J. Mauro, *J. Cell Biol.* **57**, 499 (1973).
56. J. E. Heuser and T. S. Reese, *J. Cell Biol.* **57**, 315 (1973).
57. G. E. Palade, *Science* **189**, 347 (1975).
58. R. B. Kelly, *Science* **230**, 25 (1985).
59. E. Scharrer and B. Scharrer, *Recent Prog. Hormone Res.* **10**, 183 (1954).
60. J. M. Lundberg and T. Hökfelt, *Trends Neurosci.* **6**, 325 (1983).
61. B. Neuman, L. J. Wiedermann, R. Fischer-Colbrie, M. Schober, G. Sjerk and H. Winkler, *Neuroscience* **13**, 921 (1984).

62. G. Fried, L. Terenius, T. Hökfelt and M. Goldstein, *J. Neurosci.* **5**, 450 (1985).
63. R. L. Klein, H. Lagercrantz and H. Zimmermann (Eds.), *Neurotransmitter Vesicles*. Academic Press, New York (1982).
64. P. Somogyi, A. J. Hodgson, R. W. DePotter, R. Fischer-Colbrie, M. Schober, H. Winkler and I. W. Chubb, *Brain Res. Rev.* **8**, 193 (1984).
65. R. Rosa, A. Hille, R. W. H. Lee, A. Zanini, P. De Camilli and W. Huttner, *J. Cell Biol.* **101**, 1999 (1985).
66. P-O. Andersson, S. R. Bloom, A. V. Edwards and J. Jarhult, *J. Physiol., Lond.* **322**, 469 (1982).
67. J. M. Allen, P. M. M. Bircham, S. R. Bloom and A. V. Edwards, *J. Physiol., Lond.* **357**, 401 (1984).
68. M. G. Farquhar, in *Ramon y Cajal's Contribution to the Neurosciences* (Eds. S. Grisolia, C. Guerri, F. Samson, S. Norton and F. Reinoso-Suarez), p. 67. Elsevier, Amsterdam (1983).
69. M. J. Brownstein, J. T. Russell and H. Gainer, *Science* **207**, 373 (1980).
70. D. T. Krieger, *Science* **222**, 975 (1983).
71. A. J. Baines and V. Bennett, *Nature, Lond.* **315**, 410 (1985).
72. J. R. Goldenring, R. DeLorenzo, F. S. Lasker, M. L. Vallano and T. Ueda, *Soc. Neurosci. Abstr.* 644 (1985).
73. A. J. Baines and V. Bennett, *Nature, Lond.* **319**, 145 (1986).
74. D. Branton, C. M. Cohen and J. Tyler, *Cell* **24**, 24 (1981).
75. V. Bennett, *A. Rev. Biochem.* **54**, 273 (1985).
76. V. T. Marchesi, *A. Rev. Cell Biol.* **1**, 531 (1985).
77. B. L. Granger and E. Lazarides, *Cell* **37**, 595 (1984).
78. J. R. Glenney, P. Glenney and K. Weber, *Proc. natn. Acad. Sci. U.S.A.* **79**, 4002 (1982).
79. T. L. Leto and V. T. Marchesi, *J. biol. Chem.* **259**, 4603 (1984).
80. K. A. Shiffer and S. R. Goodman, *Proc. natn. Acad. Sci. U.S.A.* **81**, 4404 (1984).
81. E. Ling and V. Sapirstein, *Biochem. biophys. Res. Commun.* **120**, 251 (1984).
82. S. R. Goodman, L. A. Casoria, D. B. Coleman and I. G. Zagon, *Science* **224**, 1433 (1984).
83. I. S. Zagon, P. J. McLaughlin and S. R. Goodman, *J. Neurosci.* **12**, 3089 (1984).
84. C-K. Huang, M. D. Browning and P. Greengard, *J. biol. Chem.* **257**, 6524 (1982).
85. M. D. Browning and P. Greengard, *Abstr. 14th Annual Meeting Soc. Neurosci.* 196 (1984).
86. J. W. Haycock, M. D. Browning and P. Greengard, *Abstr. 14th Annual Meeting Soc. Neurosci.* 196 (1984).
87. J. L. Goldstein, M. S. Brown, R. G. W. Anderson, D. W. Russell and W. J. Schneider, *A. Rev. Cell. Biol.* **1**, 1 (1985).
88. A. Helenius, I. Mellman, D. Wall and A. Hubbard, *Trends biochem. Sci.* **8**, 245 (1983).
89. R. Jahn, W. Schiebler, C. Ouimet and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **82**, 4137 (1985).
90. B. Wiedenmann and W. Franke, *Cell* **41**, 1017 (1985).
91. J. J. M. Chun and C. J. Shatz, *Abstr. 13th Annual Meeting Soc. Neurosci.* 692 (1983).
92. C. Mason, *Neuroscience*, in press.
93. E. Perdahl, R. Adolfsson, I. Alafuzoff, K. A. Albert, E. J. Nestler, P. Greengard and B. Winblad, *J. neural Transm.* **60**, 133 (1984).
94. J. L. Bixby and L. F. Reichardt, *J. Neurosci.* **5**, 3070 (1985).