Functional roles of synapsin: lessons from invertebrates

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Functional roles of synapsin: Lessons from invertebrates

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Abstract

Data collected from the invertebrate models have allowed to establish several of the basic mechanisms of neuronal function and pioneered the studies on the molecular and cellular mechanisms involved in behavioral responses. In the 1970s, the first synaptic proteins – including synapsin – being identified, the first attempts to evaluate their synaptic function were done using available invertebrate preparations. Forty years later, it appears that deductions made from invertebrate synapsin were largely validated in vertebrates, probably reflecting the phylogenic conservation of some specific synapsin sub-domains. In this review, in light of insights got from invertebrate preparations, we discuss the role of synapsin in synaptogenesis and synaptic function, especially on short term plasticity.

Highlights

► Pioneer functional synaptic studies about synapsins were first obtained in giant invertebrate preparations. ► A prototypical invertebrate synapsin emerge from phylogenetic analysis structure/function ► The reported or potential molecular interactions of synapsins and functional observations at synapses were compared. ► We finally discuss the role of synapsins in some key steps of vesicular cycle at presynapses.

Keywords: Invertebrates; Synaptic models; Short term synaptic plasticity; Phylogenetic; Synapsin

1. Introduction

Since the 1950s, neurobiological studies on invertebrates (i.e. worms, mollusks, insects, and crustaceans) allowed to monitor elementary events underlying not only neurotransmission, but also the synaptic and neuronal correlates of behavior. The neural circuit architecture and functioning in the invertebrate central nervous system were characterized far before equivalent studies were possible in vertebrate preparations, with the exception of the vertebrate neuromuscular junction and certain autonomic ganglia (reviewed in [1], [2] and [3]). Overall, a belief shared by most of the investigators has been that, despite the rules of nervous system organization may differ between vertebrates and invertebrates, studies made with invertebrates are suitable to get insights of general value: "Fortunately, in the brains of all animals that have been studied there is apparent a uniformity of principles for neurological signaling. Therefore, with luck, examples from a lobster or a leech will have relevance for our own nervous system." [4]. For any new fundamental phenomenon described in invertebrates, a parallel has been found, sooner or later, in vertebrates [3]. The data collected from the invertebrate models have allowed to establish several of the basic mechanisms underlying neuronal membrane excitability, action potential conduction, some aspects of neurotransmission, and let emerge the molecular and cellular mechanisms involved in behavioral responses. After 60 years of success, invertebrate neurobiology started shrinking, mainly due to technical progresses allowing new investigations on vertebrate models. The advent of molecular biology and genetic manipulations has not dismissed the high value of invertebrates as biological models illustrated by the recent insights from studies using the fruit fly Drosophila melanogaster, and more recently the nematode worm, Caenorhabditis elegans.

In the 1970s, the first synaptic proteins started to be identified (Synapsin in [5]). No way to evaluate their synaptic function in vitro, except by using the few invertebrate preparations affording the possibility to micro-inject large macromolecules (purified synaptic proteins, enzymes, antibodies, or mRNA) directly into the presynaptic compartment. Works on the concepts of adaptation, habituation, and desensitization in invertebrates pioneered the notion of functional synaptic plasticity. Discovery of the prolonged heterosynaptic facilitation [6], defined as a sustained increase in the efficacy of synaptic transmission between a test interneuron and a postsynaptic neuron, inaugurated studies on the synaptic correlates of behavioral sensitization. This initiated a long series of studies on the synapses involved [7], underlying molecular mechanisms [8], [9] and [10], including macromolecular synthesis, and associated morphological changes [11] and [12], mechanisms of heterosynaptic inhibition [13] and interplay with short term plasticity [14]. Overall, investigations on mechanisms of memory in invertebrates have focused on the presynaptic aspects, whereas, after the seminal work of Bliss and Lomo [15], most of the investigations performed with mammalian preparations addressed the postsynaptic correlates of learning and memory. However, postsynaptic processes are involved in memory mechanisms in invertebrates [16] and, conversely, presynaptic events participates in learning in mammals [17], [18] and [19].

In this review, we will comment on some key experiments performed in invertebrate synaptic preparations, which first allowed to describe the fundamental role of synapsin in neurotransmitter release and short term plasticity. A discussion of the "seminal" models of synapsin function will also be presented in the manuscript.

2. Invertebrate preparations and synaptic physiology

There are several invertebrate models belonging to different phyla. These include arthropods (e.g. crayfish, lobster, cricket, cockroach, horseshoe crab), mollusks (gastropods as snails and Aplysia, cephalopods as squids, cuttlefish as sepia), annelids (as leech and earthworms), nematodes, coelenterates, etc. To stick on the scope of this paper, we will just comments on few examples dealing with the mechanisms of synaptic transmission and synaptic plasticity as addressed in mollusks.

Since the early studies in neurobiology, two groups of mollusks have been studied extensively with the aim of elucidating the basic mechanisms involved in neurotransmission: namely, the cephalopods (squid) and gastropods (Aplysia, snail) giving some major concepts on membrane excitability [20], on the nature of excitatory versus inhibitory neurotransmission [21], [22] and [23], or on the crucial role of Ca2+ ions in triggering neurotransmitter release [24], [25], [26], [27], [28] and [29]. Ultrastructure of the giant synapse in the stellate ganglion of the squid revealed presence of active zones similar as at the frog neuromuscular junction [30].

The giant presynaptic nerve terminals of the squid stellate ganglion have been widely exploited [29], [31] and [32]. Indeed, in addition to a direct access to the intra-presynaptic nerve terminal compartment, this model system affords the possibility to address the ultra-structural aspects of the presynapse. However, any material injected into the giant nerve terminals undergoes diffusion sinking into the axonal trunk. A valuable alternative model is represented by identified pairs of giant neurons in gastropods ganglia. For example the synapses made by one presynaptic cholinergic interneurone (either B4 or B5) and one postsynaptic neuron (either B3 or B6) in the buccal ganglion of Aplysia have been extensively exploited. In these preparations, macromolecules such as enzymes, neurotoxins, mRNA or neutralizing antibodies [33], [34], [35] and [36] can be injected into the presynaptic cell body from which they diffuse quickly to the nerve terminals and affect neurotransmitter release machinery. Albeit studies on the ultra-structure of the corresponding synapses is virtually impossible due to the intricacy of the ganglion tissue, the effect of the injected material can be examined at a quasi steady state, thus permitting a rather good analysis of the kinetics of changes in synaptic efficacy. At these mollusk preparations, low impedance of the neuronal membrane prevents detection of individual miniature postsynaptic events. However, insights on the quantal parameters can be obtained by analyzing the fluctuations in amplitude of post-synaptic responses, either during long lasting asynchronous quantal release (Aplysia [37]; Squid [38]) or action-potential-evoked synchronous release [39] and [40], and by analyzing their changes upon the injection of macromolecules into the presynaptic compartment. In a similar line, studies on the synaptic contacts formed between co-cultured neurons (from Aplysia, terrestrial snails or leech) made it possible to correlate changes in the cytology of the nerve terminals with those of synaptic efficacy [12], [41], [42], [43] and [44]. Of the various vertebrates, only few preparations yield the possibility to apply macromolecules into the presynaptic element from the tip of patch pipettes: lamprey axons, cultured rat sympathetic neurons and the calyx of the Held synapse in the auditory stem. Finally, a main advantage of the invertebrate preparations over the vertebrate ones is their robustness to prolonged recordings due to a quasi insensibility to anoxia ([36] quotes recordings made for >20 h on the same synapse).

3. Invertebrate synapsins: phylogenetic analysis of synapsin structure/function

3.1. Synapsins are a phylogenetically conserved family of synaptic vesicle associated proteins

Synapsins are a family of synaptic vesicle (SV)-associated phospho-proteins identified in several vertebrate and invertebrate species [45], [46] and [47]. Three synapsin genes, termed synapsin (Syn) I, II and III, have been identified in mammals, with alternative splicing creating at least ten different synapsin isoforms (SynIa, Ib, IIa, IIb, IIIa–IIIf). Synapsins have been also described in vertebrates such as frog Xenopus laevis [47], teleost fish Fugu rubripes [48] and lamprey Lampetra fluviatilis [47], a representative of our most distant vertebrate relatives.

The first invertebrate gene with homology with synapsin family has been demonstrated in the fruit fly D. melanogaster in which only one synapsin gene gives rise to 5 different isoforms by alternative splicing [49]. The hypothesis of the ancestral condition of a single synapsin gene in invertebrates has been subsequently supported also in the nematode C. elegans and in several species of mollusks (Loligo pealei, Aplysia californica and Helix pomatia) [43], [50] and [51]. The availability of an increased number of complete genomes from a variety of prokaryotes and eukaryotes has provided a better support for inference about the origin of this gene family. Recently, homologous of synapsins were found across the metazoan tree in the phylum Cnidaria, Nematoda, Annelida, Echinodermata, and Chordata [52]. Such analysis reveals that metazoan synapsin is a single copy gene except for vertebrates and provides evidence that the appearance of multiple synapsin genes has arisen from the genome duplications that occurred between the chordata and the vertebrata radiations. Finally, a pre-metazoan origin for synapsin has been also postulated on the basis of a synapsin-like sequence in protozoans [52].

3.2. Domain structure of mammalian and mollusk synapsins

Synapsins are multidomain proteins constituted by a large globular head at the N-terminus and by an elongated tail in the remainder of the molecule (Fig. 1A and B). The globular head can be subdivided into three domains termed A, B and C in common to all mammalian isoforms. The primary structure of the elongated tail presents a different domain architecture in the mammalian isoforms: a domain D in SynIa and Ib, domains G and H in Syn IIa (Fig. 1B), and domain J in the Syn IIIa (data not shown). In particular, three different synapsin genes are alternatively spliced to give rise to a-type isoforms containing a COOH-terminal region (domain E), and b-type isoforms lacking of this domain. Moreover, SynIa and Ib share an elongated, proline-rich and highly basic C-terminal region, while Syn IIa and IIb possess a short proline-rich sequence but lack the longer “tail” domain of synapsin I (Fig. 1B).

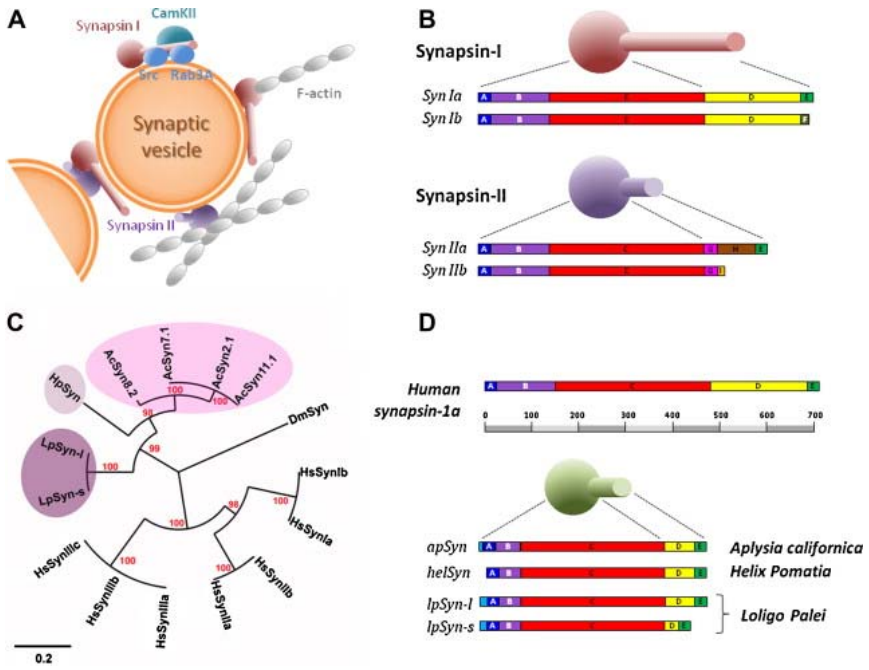

Fig. 1. (A) Scheme of major synapsin interactions (adapted from Cesca et al. [70]). (B) Structure of mammalian synapsin I and II including important sub-domains (see text for more details). (C) Circular cladogram showing the evolutionary relationships between synapsin proteins. Helix, Aplysia, Loligo, Drosophila and human synapsins sequences were aligned by Clustal W, and a phylogenetic tree was generated using the neighbor joining method and visualized using Tree view. Bootstrap confidence limits were obtained by 1000 replicates. (D) Comparison between sub-domain composition of mammalian synapsin I and invertebrate synapsins from Helix, Aplysia and squid.

Phylogenetic analysis adds the synapsin gene family to the list of gene families that show gene duplication specific to the vertebrate lineage. Single synapsin genes have been identified in several invertebrate species, whereas two and three synapsin genes are known to exist in lowest vertebrates (lamprey) and frog and mammals, respectively. In particular, our molecular phylogenetic analysis [52], shows that mollusk synapsins was situated basal to the three clades of mammals genes, indicating that the duplications generating these clades occurred in the vertebrate lineage after its separation from invertebrates (Fig. 1C).

As shown in Fig. 1 and Fig. 2, the mollusk synapsins (Aplysia synapsin, apSyn; H. pomatia synapsin, helSyn; L. pealei synapsin, lpSyn) show a similar modular structure characteristic of the mammals synapsins with a central, highly conserved ‘C’ domain (between 59 and 67% with human synapsins) and less conserved A and E domains (between 17–27% and 26–33% identity of human synapsins, respectively). Domains B, D, G, H and J show little primary sequence identity between mammals and all invertebrate synapsins analyzed to date, but these regions share conserved features with mammals synapsins, amino acid composition is characteristics of mammals synapsin, such as the proline-rich regions and the presence of consensus sites for phosphorylation by mitogen-activated protein kinase in domain B (MAPK). As expected, all mollusk synapsins exhibit a high degree of sequence similarity with each other along the entire molecule, except for the domain D that is more divergent (32% identity) (Fig. 2B). Interestingly, alternative transcripts have been identified in mollusks: for instance apSyn gene can generate four different protein isoforms named apSyn 11.1, 2.1, 7.1 and 8.2 differing only for the insertion or deletion of a short fragment in the NH2-terminus (apSyn 2.1/8.2 and apSyn7.1/11.1, respectively) or insertion of a short stretch of amino acids in the central part of domain C (apSyn 7.1 and 8.2). Moreover, apSyn 8.2 exhibits some substitutions in domain C and such region

possesses a higher sequence similarity with helSyn, lpSyn, and mammal synapsins (Fig. 2A). lpSyn can give rise to two alternative transcripts encoding for short and long isoforms (lpSyn-s and lpSyn-l) and differing only by a 37 amino acid deletion/insertion in the domain D. Such pattern of splice variants has also been demonstrated in the invertebrate chordate amphioxus Branchiostoma floridae [52]. The above-mentioned data suggest that most of the alternative splicing events are divergent between mollusks and mammals: neither isoforms in mollusk share some similarity with the alternative splice isoforms b of human synapsins. On the other hand, all mollusk splice variants contain the E-domain and resemble a-type isoforms of mammals synapsins. This conservation of mRNA splice forms over a wide phylogenetic distance implies conservation of protein function and supports the functional significance of domain E.

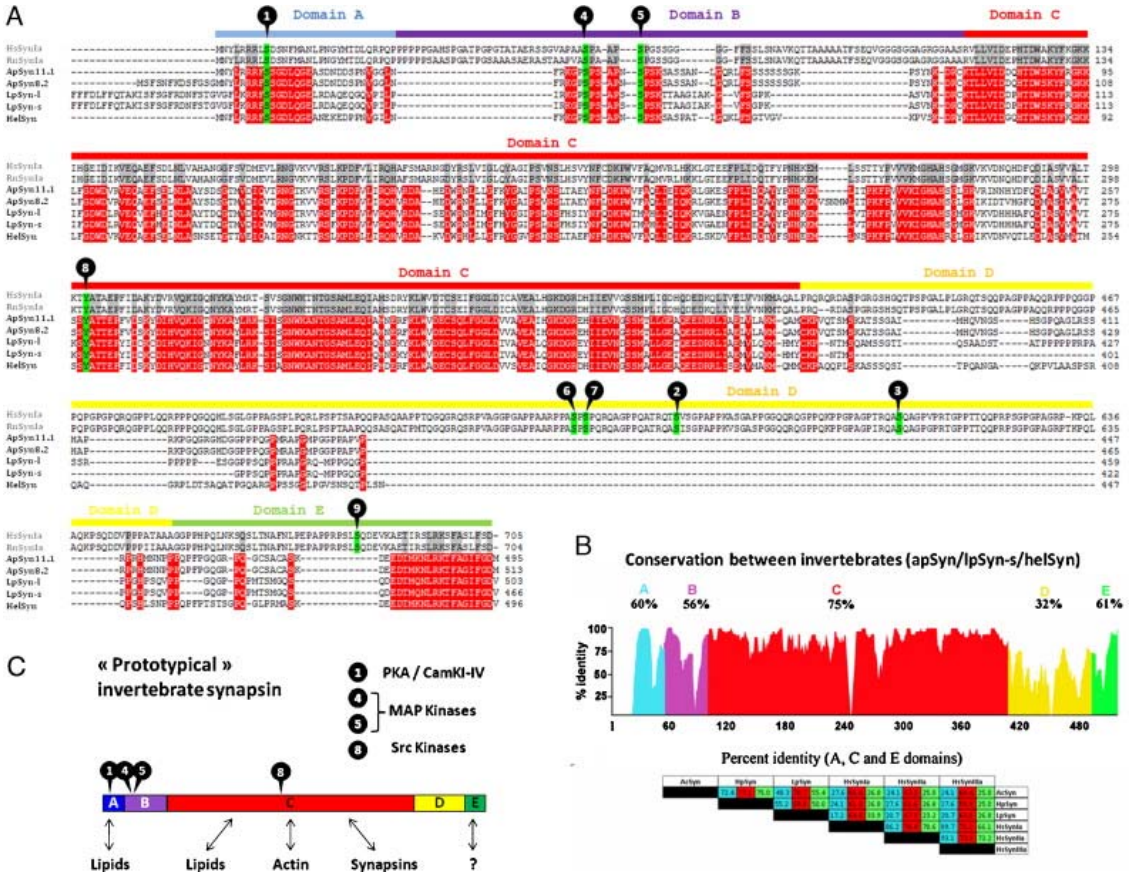

Fig. 2. (A) Sequence alignment of synapsins. Multiple sequence alignment was constructed using AlignX program of Vector NTI. Only two synapsin splice variants of Aplysia have been used because they summarize the major characteristics of all splice variants identified in this organism (see text for details). Domains A and B are indicated by azure and purple bars. Domains C, D and E are indicated by red, yellow and green bars. Phosphorylation sites corresponding to sites from 1 to 9 in human and rat synapsin I are indicated in green. Identical residues in mollusk synapsin are shown in red, and those conserved in human and rat synapsins in grey. Amino acid positions are numbered on the right. Taxa are abbreviated as follows: Ap, Aplysia californica; Lp, Loligo pealei; Hel, Helix pomatia; Hs, Homo sapiens; Rn, Rattus norvegicus. (B) (upper panel) Conservation between invertebrate synapsins. Above each domain is reported the median percent identity. (lower panel) Sequence identity matrix of A, C and E synapsin domains of human and mollusk synapsins. Comparisons of domains A, C and E were performed using GeneWorks (Clustal W method with PAM250 weighting and identical gap costs). Colored boxes indicate percent identity of domain A (azure), domain C (red) and domain E (green). In both analysis, ApSyn8.2 and LpSyn-l isoforms were used. (C) Schematic representation of prototypical invertebrate synapsin. Conserved phosphorylation sites for PKA/CaMKI–IV (site1), MAP Kinases (sites 4 and 5) and Src Kinase (site 8) are shown. The putative synapsin interactions with lipid, actin and synapsins are also indicated.

3.3. Evolutionary conservation of specific sites in mammals and mollusks

Synapsins are family of presynaptic proteins identified as major substrates for a variety of protein kinases, which all contribute to the modulation of synapsin function (see Section 4). At present, nine phosphorylation sites, embedded in a particular structural domain of synapsin, have been characterized in mammals (Fig. 2A). Domain A contains the phosphorylation site (named site 1, Ser9) for cAMP-dependent protein kinase (PKA), Ca2+/calmodulin-dependent protein kinase I (CaMKI) and Ca2+/calmodulin-dependent protein kinase IV (CaMKIV) [53]; domain B contains two phosphorylation sites (sites 4 and 5, Ser62 and Ser67) for mitogen-activated protein kinase (MAPK) [54] and [55], while domain C possesses a phosphorylation site for Src (site 8, Tyr301) [56]. Domain D presents two phosphorylation sites (sites 2 and 3, Ser566 and Ser603) for Ca2+/calmodulin-dependent protein kinase II (CaMKII) and two sites (site 6 and 7, Ser549 and Ser551) for cyclin-dependent protein kinase 5 (cdk5) [54], [55] and [57]; Ser551 may be phosphorylated also by MAPK [55]. Recently, a phosphorylation site in domain E has been also demonstrated: site 9: Ser 682 is phosphorylated by the ataxia-telangiectasia mutated (ATM) kinase [58]. Some of those phosphorylation sites are evolutionary conserved in mollusks and other invertebrates. For instance, all synapsin isoforms share a consensus sequence for phosphorylation by PKA/CaMKI–IV in domain A (site 1) (Fig. 2A and C). In mammals, phosphorylation at this site reduces the affinity of synapsin I and II for actin and lipid bilayer of synaptic vesicle (SVs) [59], [60], [61] and [62] leading to the dissociation of synapsin from synaptic vesicles. Moreover, site 1 has been also implicated in neurite outgrowth [63], SV mobilization in developing vertebrate neurons [64], in synapsin distribution [51], neurotransmitter release [65] and [66], and plasticity [43]. More details are given in Section 4.

Based on location and sequence, sites 4 and 5, consensus sites for MAPK/Erk, are present in all mollusk sequences in N-terminal B domain and have been experimentally characterized in Aplysia and Helix (Fig. 2A–C) [44] and [51], see Section 4. Finally, mollusk synapsins show a consensus phosphorylation site for Src in C-domain, that might represent the homologous phosphorylation site 8 (Tyr301) of mammalian synapsin [47] and [52]. In mammals, phosphorylation by Src was found to modulate Syn I binding to both SVs and actin [56].

In conclusion, the prototypical invertebrate synapsin is characterized by at least three conserved domains A, C and E (Fig. 2C). Amino acid sequence analyses revealed over 50% identity between mammal and invertebrate synapsin domain C, implying a crucial role for this domain in many of the conserved functions of the synapsins [47] and [66]. To date, specific residues in domain C are necessary for the binding of synapsin I to the lipid bilayer of synaptic vesicles [67], actin filaments [59] and ATP [68]. Interestingly, all those amino acids residues are well conserved in mollusks [69].

4. Synaptic functions of synapsins in invertebrates

4.1. Role of invertebrate synapsins during synapse formation and maturation

Many evidence show that synapsins have a developmental role in the processes of axon elongation and synapse formation in vertebrates (see [70]). In particular, it has been shown that synapsin I and II modulate presynaptic vesicles and synaptic functions in the period following the early neurogenesis in mouse brain [71] and regulate synaptic maturation at neuromuscular synapses of Xenopus [72], [73] and [74], while synapsin III is expressed mainly in early phases of neuronal development and is highly concentrated in growth cones [75].

As in vertebrates, synapsin expression in Helix and Aplysia correlates well with the time course of presynaptic terminals maturation and synaptogenesis. The contact and the formation of a chemical connection with an appropriate target induce a redistribution of the synapsin mainly in varicosity-like structures immunoreactive for the neurotransmitter serotonin along the neurites close to the target neuron that parallels the increase in synaptic efficacy [42]. The role of synapsins in synaptic differentiation has been addressed using co-cultures of identified neurons from Helix. When a C1 neuron makes a synaptic contact with its physiological target B2, both MAPK/Erk and PKA pathways are activated [76]. In contrast, the presence of a non physiological target (C3 neuron) results in a general inhibitory effect on the maturation of the presynaptic terminals of C1 neuron [77] involving down regulation of both MAPK/Erk and PKA pathways [76], an effect which is quickly reversed by the intra-C1 injection of bovine synapsin I [65].

The primary structure of Aplysia and Helix synapsin orthologues (apSyn and helSyn) shows the conservation of sites 1, 4 and 5 (see Section 3). The role of these sites has been studied and is detailed below. In C1–C3 co-cultures, phosphorylation of apSyn domain A by either PKA or CaMKI/IV is necessary for ultra-structural rearrangement of C1 neurons – i.e. dense inter-digitations of microtubule-packed neurite-like processes and dense core synaptic vesicle clusters – and enhancement of neurotransmitter release [78]. These changes are virtually absent in control C1–C3 or after injection of the non phosphorylatable domain A mutant [78]. However, these results are not due to mis-targeting of synapsin, because over-expression of fluorescent Helix synapsin (helSyn-GFP) shows that domain A phosphorylation is not required for the correct synaptic targeting of Helix synapsin [43]. In addition to PKA pathway, phosphorylation of synapsin by MAPK/Erk kinase of Ser36 and Ser42 in N-terminal B-domain (see Fig. 2) plays a critical role in the formation of synapses between Helix neurons in culture [44]. When non-phosphorylatable or pseudo-phosphorylated Helix synapsin mutants at the MAPK/Erk sites are overexpressed in Helix neurons, these mutants interfere with presynaptic differentiation, synapsin clustering and neurotransmission indicating that MAPK/Erk-dependent synapsin phosphorylation has indeed a role in the establishment of functional synaptic connections [44]. Overall, these data suggest that cycles of MAPK/Erk phosphorylation may play a fundamental role in regulating synapsin activity during synaptogenesis, perhaps acting on cytoskeletal assembly and vesicle clustering at synaptic terminals, as suggested by previous work in vertebrate preparations [54].

4.2. Role of invertebrate synapsins in synaptic plasticity/function

4.2.1. Role of synapsin in basal neurotransmitter release

Synapsin I – first termed protein 1 – is among the very first synaptic proteins to have been isolated and characterized. Its enrichment on synaptic vesicle membranes, identification as being a substrate for Ca2+-dependent kinases and its phosphorylation following neuronal stimulation [79] pushed toward the examination of the physiological role of synapsin in neurotransmitter release. The synaptic role of synapsin was pioneered at the stellate ganglion giant terminal of the squid by Rodolfo Llinás and collaborators [28] and [31]. Presynaptic injection of bovine dephospho-synapsin 1 has been found to inhibit neurotransmitter release, whereas injection of exogenous CaMKII induced strong increase in synaptic efficacy [31], leading to the proposal of a seminal model in which synapsin I regulates SV availability in a phosphorylation-dependent manner: at rest, dephospho-synapsin I binds to SVs thereby decreasing the fraction of SVs able to undergo fusion with plasma membrane. Upon phosphorylation by CaMKII, synapsin I is released from SVs, thereby increasing the fraction of SVs susceptible to fuse with plasma membrane [31]. The fraction of SVs trapped into the cytoskeleton meshwork by dephospho-synapsin was envisioned as the main determinant of the size of the SV

reserve pool [28]. However, cloning of squid synapsin has shown that the CaMKII/phosphorylation sites 2–3 are absent in LpSyn (Fig. 2) raising a doubt on the specificity of the CaMKII results. Such a possibility has been already addressed by Llinás et al. [28]: 'we cannot yet exclude the possibility that calmodulin kinase II produced its effects by acting on some other nerve terminal protein(s) instead of, or in addition to, synapsin I'. The physiological relevance of the inhibitory action of bovine dephospho-synapsin I reported at the squid synapse has been questioned too. Indeed, micro-injection of dephospho-synapsin in squid axoplasm stops movements of SV as well as other organelles [80]. No such inhibitory effect was reported after injection of rat- or bovine dephospho-synapsin I in Aplysia or Helix neurons (while short term plasticity was modified) [36] and [76], but overexpression of Aplysia synapsin reduces basal transmission and enhances homosynaptic depression [81]. In addition, the activation of neuromodulatory cascades known to induce synapsin phosphorylation (e.g. 5-HT and TGF) does not lead to changes in basal neurotransmission [51], [82] and [83]. Thus, a role of synapsin in regulating basal release remains an unclear issue.

4.2.2. Does synapsin play role in late steps of exocytosis?

The possibility that synapsin may play a role in the membrane events of vesicular release of neurotransmitter is supported by several data obtained from invertebrates. Following intrabeuronal injection of bovine dephosphorylated into presynaptic nerve terminals or neurons of bovine dephosphorylated synapsin I [38], peptides mimicking synapsin subdomains [50] and [84] or neutralizing antibodies directed against synapsin [36], there are alterations in the kinetics of evoked (multi-quantal) post-synaptic responses. However, injection of rat dephosphorylated synapsin I into Aplysia synapses did not lead to such effect [36]. Power spectrum analysis of synaptic noise due to spontaneous release, before and after micro-injection of bovine dephosphorylated synapsin I into squid giant nerve terminals has shown no change in the kinetics of the quantal events [38]. Thus, interfering with synapsin function can produce de-synchronization of the evoked vesicular fusion events. Hilfiker et al. [84], have discussed the possibility that the gating of the fusion pores is changed, too. To conclude, synapsin can play a role in a late stage of exocytosis. This is consistent with observations made in vertebrates: only 50% of synapsins dissociates from synaptic vesicles during neuronal activity (Frog neuromuscular junction [85]; mouse synaptosomes [61]. Thus synapsin is likely to be present on most, if not all, of the SVs tethered to the active zone. No kinetic change in post-synaptic responses has been observed in mouse lacking synapsin 1 and 2 [86]. Thus, the control of release kinetics by synapsin may be specific to invertebrate synapses, raising the possibility that during evolution this role has not been preserved in the vertebrates or assigned to another synapsin isoform or related protein.

4.2.3. Role of synapsins during low and high frequency stimulation episodes

At synapses, one of the most common consequences of increasing the spiking frequency is a decrease in the quantity of neurotransmitter released at each stimulus, referred to as synaptic depression. At Aplysia synapses, low frequency induced depression (LFD) can be induced using sustained stimulations at low frequency (1 Hz) which induce conversion of presynaptic release sites from an active to a silent status in a reversible manner [87], [88] and [89]. The molecular mechanisms involved in release site silencing during sustained stimulation remain unclear, however several evidence suggests that site silencing results from an arrest in the cascade of events leading to acquisition of a fusogenic status by the docked SVs (i.e. during the priming events) or/and that of the corresponding membrane fusion sites (discussed in [89] and [90] and see also related discussions [39], [40] and [91]. Moreover, time course of LFD at Aplysia synapses is shaped by the intermingling of different forms of synaptic plasticity: initial phase of LFD decay results from a quick silencing of a fraction of release

sites that is compensated for a while by a fast but transient increase in release probability [89]. The role of synapsin during LFD and recovery from it has been analyzed at Aplysia synapses [36] and [89]. Manipulations aimed at severing the synapsin functions (i.e. intraneuronal application of neutralizing anti-synapsin antibodies or ApSyn with a point mutation at the PKA site – viz ApSyn-Ala-) do not modify the plateau fixed level reached during LFD, but affect the kinetics of LFD and recovery from depression. Intraneuronal neutralization of ApSyn leads to a faster decay in LFD likely corresponding to suppression of the transient phase of compensatory increase in release probability [36]. When presynaptic Aplysia neurons are injected with either a recombinant apSyn bearing a Ser9-Ala mutation (apSyn Ala), which suppresses its ability to be phosphorylated by PKA and CaMKI/IV [43] and [78], or a highly specific inhibitor of PKA (peptide 6-22 amide; PKAi6-22) [92], LFD time course is faster and its plateau lower pinpointing a crucial role for PKA/synapsin pathway in induction of LFD. Release site unsilencing has a very slow time constant (>40 min) in absence of any neuronal activity [89] but faster when neurons are stimulated. This recovery is speeded up when synapsin is neutralized by anti-Syn antibodies [36] but no such effect is detected after injection of ApSyn Ala or PKA-inhibitor [89]. This clearly reveals a role for PKA-phosphorylated synapsin in the release site reactivation. Possibly application of a burst of high frequency spiking induces elevation of cAMP concentration in the vicinity of release sites and stimulates phosphorylation of synapsin present at the release site ([36], see above) thereby bypassing or reversing the molecular events underlying silencing of release sites.

4.2.4. Role of synapsins in post-tetanic potentiation

Bursts of action potentials at 20–50 Hz rates often trigger post-tetanic potentiation (PTP), a suggested neural substrates of short-term memory [93], [94] and [95], and drive behavior in invertebrates [96]. The current model for PTP propose the mobilization of the reserve pool of SVs following a rise in intra-terminal Ca2+ concentration [94], [97] and [98]. In the last years, a central role of synapsins in PTP has been uncovered in invertebrates [36], [43] and [44].

At Aplysia synapses, inactivation of synapsin by specific antibodies [36] or injection of nonphosphorylated forms of ApSyn – (ApSyn-Ala in [89]) –, completely abolished PTP, without affecting basal neurotransmission. Importantly, the same results were obtained using presynaptic injection of a PKA peptidic inhibitor [89]. Similarly, at Helix synapses, the phosphorylation of ser-9 site of helSyn, provided both by PKA and CaMKI/IV, is necessary for PTP [43] and [44]. Moreover, recent evidence pointed for a crucial role of the MAPK/Erk phosphorylation at the conserved sites 4 and 5 of synapsin in the expression of PTP at this synapse [44], suggesting that PTP is associated with the phosphorylation of several synapsin sites by distinct kinases that are concurrently activated by tetanic stimulation.

Interestingly, together with vertebrate data obtained using various synapsin mutants [99] and [100], invertebrate data consistently go against the seminal model implicating phosphorylation of sites 2 and 3 by CaMKII during PTP, leading to the mobilization of the reserve SV pool [94] and [98]. Indeed, none of invertebrate synapsin contains domain D bearing sites 2 and 3 (see Fig. 2), a likely explanation for their poor phosphorylation by CaMKII [78]. Note that PTP was nevertheless present in all these invertebrate preparation.

4.2.5. Alteration of PTP after tetanus toxin-induced dysfunction of synapsin

Tetanus toxin (TeNT) is a zinc-metalloproteases that cleave VAMP-synaptobrevin thereby blocking neurotransmitter exocytosis (reviewed in [101] and [102]). TeNT has been also established to bind and activate GTP-binding protein type h/tissue transglutaminase type II (TG2) [103] and [104]. TG2

is abundant in many nerve endings of vertebrates and invertebrates such as Aplysia [103] and [105]. It can crosslink its substrates, induce amine incorporation, deamidation, or act as an isopeptidase. TG2 substrates include RhoA GTPase, several cytoskeletal proteins [106], SNAP-25 and Synapsin I [104] and [105]. When endogenous TG2 is stimulated by TeNT, Synapsin I and SNAP-25 are covalently modified. TeNT-TG2-induced SNAP-25 modification may explain why TeNT mutants devoid of proteolytic activity against VAMP-synaptobrevin are still able to inhibit neurotransmitter release at Aplysia synapses [105]. Consistent with a key role of Synapsin in PTP, TeNT-TG2-induced synapsin modification may explain why the amplitude of PTP at Aplysia synapses declines faster than basal neurotransmitter release upon TeNT treatment [91].

5. Conclusions

An emerging picture is that the prototypical invertebrate synapsin is characterized by three conserved domains A, C and E (Fig. 2C), which play crucial roles in synaptic functions [47] and [65]. Interestingly, phylogenetic analysis on synapsin gene family has suggested that the appearance of new specific synapsin domains in animal kingdom is correlated with the increase in complexity of the nervous system [52]. Indeed, protozoans, which lack nervous system, express a proto-synapsin consisting in a single domain, the ancestor of the highly conserved C-, domain; Cnidaria, whose nervous system is very primitive, express a form of synapsin comprised of two, C- and E-related, domains. Domain A has been acquired in the protostomia group (Drosophila and mollusks), seemingly playing similar roles in invertebrates and vertebrates (i.e. phosphorylation dependent regulation of neurotransmitter release and plasticity). In the synapsin domain C, evolution has preserved all the specific residues implicated in the binding of synapsin I to the lipid bilayer of synaptic vesicles [66], actin filaments [59] and ATP [67], explaining why this domain plays similar crucial roles in vertebrates and invertebrates [47] and [65]. However, during evolution some functional properties of the synapsins seem to have not been retained or acquired in the vertebrates synapsins: for example, the role of synapsin E domain in regulating the kinetics of neurotransmitter release may be specific of the mollusks [84]. Acquisition by mammals of synapsin 1 D domain allows additional regulation by neuronal activity and CaMKII, initially envisioned in invertebrates.

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