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Review

The synapsins: Multitask modulators of neuronal development

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ABSTRACT

Neurons are examples of specialized cells that evolved the extraordinary ability to transmit electrochemical information in complex networks of interconnected cells. During their development, neurons undergo precisely regulated processes that define their lineage, positioning, morphogenesis and pattern of activity. The events leading to the establishment of functional neuronal networks follow a number of key steps, including asymmetric cell division from neuronal precursors, migration, establishment of polarity, neurite outgrowth and synaptogenesis. Synapsins are a family of abundant neuronal phosphoproteins that have been extensively studied for their role in the regulation of neurotransmission in presynaptic terminals. Beside their implication in the homeostasis of adult cells, synapsins influence the development of young neurons, interacting with cytoskeletal and vesicular components and regulating their dynamics. Although the exact molecular mechanisms determining synapsin function in neuronal development are still largely unknown, in this review we summarize the most important literature on the subject, providing a conceptual framework for the progress of present and future research.

Contents

1. Introduction 378
2. Expression of the synapsins in developing neurons 379
3. Increased levels of synapsin prompt neuronal development 379
4. Synapsin knockdown and depletion models show slower neuronal differentiation 379
5. Regulation of synapsin activities through phosphorylation controls neuronal development 383
6. Modelling the molecular role(s) of synapsins during development 384
7. Conclusions 384
Acknowledgements 385
References 385

1. Introduction

Neuronal development is a finely orchestrated process that leads from a round, post-mitotic cell to a highly polarized neuron which makes thousands of connections with other neurons in a highly regulated manner, becoming phenotypically and morphologically specialized to conduct, deliver, receive and integrate electrical as well as chemical signals in a functional neuronal network.

The process of neuronal development can be subdivided into several steps, including cell migration, establishment of cell polarity, neurite outgrowth, axonal navigation and synapse formation, maturation, stabilization or elimination. This subdivision is operationally useful to describe the process; however, in the brain some of these steps can overlap, e.g. some axonal processes may have already reached their targets and established contacts with other neurons, while other processes are still navigating to reach other neuronal targets.

In vitro, the first steps in differentiation are the extension of neurites and the molecular polarization of the cell, accompanied by the establishment of distinct somatodendritic and axonal com-

Abbreviations: CaMK, Ca^{2+}/CaMK-dependent protein kinase; PKA, cAMP-dependent protein kinase; MAPK, mitogen-associated protein kinase; P-site, phosphorylation target site; KO, knockout; Syn, synapsin; SV, synaptic vesicle; WT, wild type.

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partments [1]. Axons and dendrites make contacts and establish initial synapses, which are then stabilized or eliminated in a manner strongly influenced by synaptic activity [2,3].

Synapsins (syns) are neuron-specific phosphoproteins selectively associated with synaptic vesicles (SVs) which have been implicated in neuronal development and synaptic function [4–6]. The genes encoding for the three different syn isoforms are evolutionarily highly conserved, and syn orthologues are found in lower vertebrates as wells in invertebrates (for review, see [7]). In mammals, the syns are encoded by three genes that are differentially spliced to give rise to more than ten isoforms. The genes are composed by a mosaic of conserved (A–C, E) and variable (D, F–J) domains [8,9].

Syns are targets of multiple protein kinases which phosphorylate them on either conserved or isoform-specific sites. In syn I, cAMP-dependent protein kinase (PKA) and Ca^{2+}/CaMK-dependent protein kinase (CaMK) I/IV phosphorylate one serine residue in domain A (site 1), CaMKII phosphorylates two serines in domain D (sites 2 and 3), mitogen-associated protein kinase (MAPK) phosphorylates two serines in domain B (sites 4 and 5) and one additional serine in domain D (site 6) which, is also phosphorylated by cyclin-dependent kinase 5 together with an adjacent serine (site 7). While phosphorylation by the latter kinase has not been extensively characterized, serine phosphorylation of syn I by the other kinases impairs its binding to actin and/or synaptic vesicles (SVs). On the opposite, phosphorylation of syn I by Src on a tyrosine residue in domain C (site 8) increases its binding to both SVs and actin, as well as the formation of syn dimers [10,11]. All sites located in domains A–C are conserved in the other syn isoforms, while domain D sites are exclusive of syn I.

The phosphatases that dephosphorylate the various sites in the syn molecules have been less studied. Sites 1, 2, and 3 appear to be dephosphorylated by protein phosphatase 1, whereas the MAPK sites 4, 5 and 6 are substrates for calcineurin 3, formerly known as protein phosphatase 2b [12].

It appears therefore that increases in intracellular [Ca^{2+}] activate both kinases and phosphatases acting on the syns, leading to a complex pattern in the state of syn phosphorylation during neuronal activity. A depolarizing stimulus reaching the nerve terminal leads to opening of the Ca^{2+} channels and to Ca^{2+} influx. Syn is rapidly phosphorylated on sites 1–3 and dephosphorylated on sites 4–6. At later time points, sites 4–6 are re-phosphorylated by MAPK activation. Since syn phosphorylation on sites 1–3 decreases the binding to both SVs and actin, more vesicles are released from the cytoskeleton and become available for exocytosis [13–15]. Dephosphorylation and phosphorylation of sites 4–6 adds complexity to this phenomenon, and may contribute to maintain the equilibrium among the various functional pools of SVs during various patterns of synaptic activity. Indeed, it has been shown that at high frequency of stimulation syn disperses away from the nerve terminal in a manner dependent mainly on phosphorylation of sites 4–6 by MAP kinase, whereas at low frequency of stimulation CaMK II-mediated phosphorylation of sites 2 and 3 also plays a role [16,17].

Thus, site-specific phosphorylation of the syns differentially affects the ability of the molecules to interact with various components of the neuron, leading to modulation of SV motility and exocytosis, both in mature nerve terminals and during development, and eventually influencing synapse formation and function (see below).

2. Expression of the synapsins in developing neurons

During ontogenesis, the expression of syn I and II correlates with neuropil development and synapse formation (Fig. 1). After birth, a peak in syn I and II expression is observed and in rodents the adult levels are reached at 1–2 months of age [18,4,19]. Consistently, in neurons cultured *in vitro* the expression of syn I and II gradually increases as soon as neurites develop and polarize and synaptic contacts are established.

During development syn I undergoes changes in its intracellular localization. At early stages of differentiation, the protein is distributed in the cytoplasm, albeit a preferential accumulation in the distal axon can be appreciated. As soon as synaptic contacts are formed, syn I undergoes a rapid redistribution and accumulates at presynaptic contact sites [20]. In contrast, syn III is already expressed by nestin-positive neuronal progenitors and only later becomes restricted to cells of neuronal lineage [21]. *In vitro*, it has the highest peak of expression at 7 DIV, when synaptogenesis is still ongoing, and subsequently decreases [22].

3. Increased levels of synapsin prompt neuronal development

The first clear evidence demonstrating a direct involvement of syns in the formation and maturation of presynaptic terminals comes from pioneering studies, which evaluate the effects of syn overexpression in various experimental models of neuronal synapses. Han and collaborators evaluated the effects of transfecting syn IIb in the differentiated neuroblastoma/glioma cell line NG108-15, and found that overexpression of syn IIb induced the formation of varicosities along their neurites, containing both small clear vesicles and large dense-core vesicles resembling those typically seen in neurons which were able to form synapse-like contacts [23]. Moreover, the levels of both other syn isoforms and synaptophysin were significantly increased in NG108-15 overexpressing Syn IIb, an effect which was subsequently confirmed for other presynaptic proteins, like secretogranin I [24], and for laminin, a basal membrane glycoprotein that promotes adhesion and induces neurite outgrowth and neuronal differentiation [25].

In line with these studies, early blastomere injection of either syn I or syn IIa in *Xenopus* embryonic spinal neurons co-cultured with muscle cells led to an enhancement of spontaneous synaptic currents, reflecting an increase in the number of SVs released spontaneously, and to a larger amplitude and less variable evoked synaptic currents, characteristic of more mature synapses [26,27]. This acceleration in the functional development of the neuromuscular synapse was associated with a rearrangement in the ultrastructure of the nerve terminal. Indeed, synapses overexpressing syn I were characterized by a higher number of small SVs and exhibited a precocious development of active zone-like structures [28]. Consistently, a subsequent study reported that syn I and II expression is involved in the developmentally regulated increase of the readily releasable pool of SVs at the hippocampal synapse [29], indicating a clear involvement of these isoforms in synapse maturation.

Since syns I and II share the N-terminal domains but not the C-terminal domains [8], it seems likely that the ability of both syn I and syn II to accelerate the development of synapses is mediated by their common N-terminal sequences, containing distinct target phosphorylation sites of both PKA and CaMK I/IV. Interestingly, overexpression of wild type (WT) Syn II had no detectable effect on synapse development (reference). Although Syn I and II show overlapping effects, probably due to their high degree of structural homology [7], their roles in neuronal development are distinct, as documented by studies based on loss of function approaches [30] and see below.

4. Synapsin knockdown and depletion models show slower neuronal differentiation

Studies performed in neurons where syns expression was genetically abolished or acutely reduced have confirmed the importance of the syns in neuronal development and have allowed to pinpoint

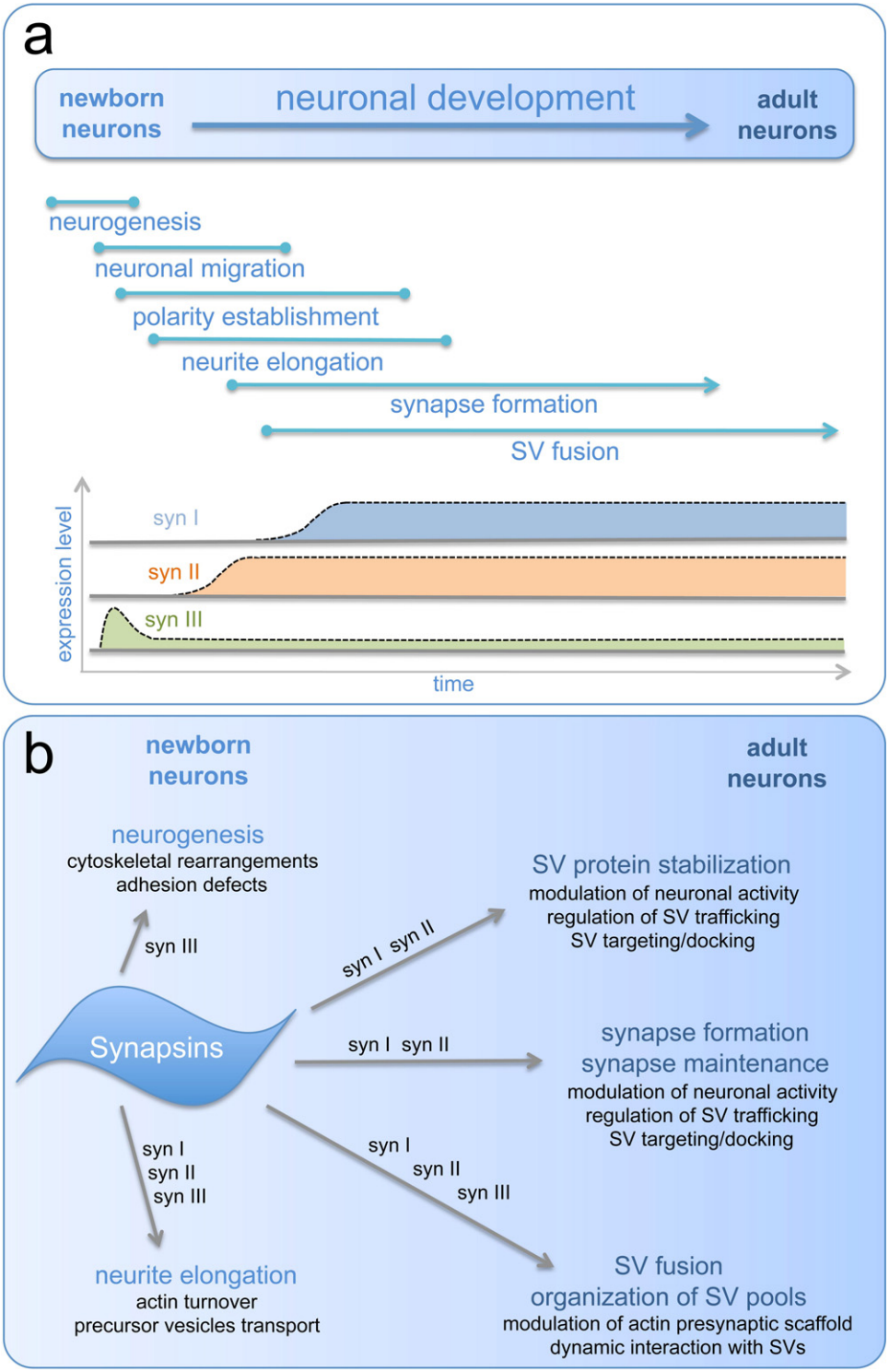


Fig. 1. Synapsins regulate several neurodevelopmental processes in the sequential stages of neuronal development. (a) Schematic representation of the temporal distribution of the key neurodevelopmental events and of the expression pattern of syn isoforms. Note that syn III is expressed from the earliest stages following neurogenesis, while the expression of syn I and II increases over time and reaches a plateau when development is completed. (b) Schematic representation of the processes regulated by syns during development, with indication of the hypothetical cellular/molecular mechanisms and the syn isoforms involved.

important differences among distinct syn isoforms (Fig. 2). In early experiments, the effects of reducing syn II levels by using antisense oligonucleotides [31] or genetic deletion of the Syn II gene [30], were evaluated in rat embryonic hippocampal neurons in culture.

The development of hippocampal neurons *in vitro* follows a well-characterized stereotyped sequence of events: once attached to the substrate, neurons form lamellipodial veils surrounding the cells (stage I), followed by their condensation into short undifferentiated neurites (stage II). Later, one of these processes begins to elongate faster than the others and differentiate into the axon (stage III) [32,33]. Hippocampal syn II-depleted neurons exhibited dramatic changes in their morphology and the timed sequence of development was markedly altered. In particular, about 80% of cultured neurons precociously treated with antisense oligonu-

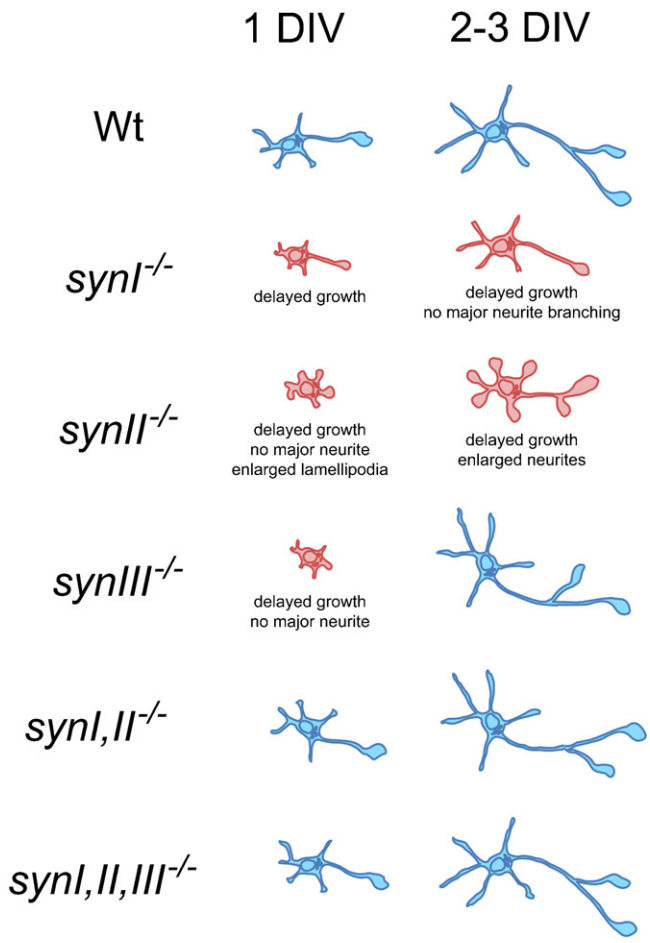


Fig. 2. Differential role of synapsin isoforms in early stages of rodent neuronal development *in vitro*. Blue colour designates normal development, while red colour indicates developmental defects. WT (WT) rodent hippocampal neurons in culture undergo a series of well-characterized developmental stages [32]. Between 1 and 3 DIV, stage III neurons show a longer neuritic process that has the highest probability to become the axon. *SynI*$^{-/-}$ mouse hippocampal neurons at 1 and 3 DIV show an overall growth delay accompanied by decreased branching of the primary neurite [35,80]. Mouse *synII*$^{-/-}$ neurons have a stronger phenotype, being devoid of a major neurite at 1 DIV and showing a slower growth pattern [30]. In addition, *synII*$^{-/-}$ neurons have enlarged lamellipodia veils. Mouse hippocampal *synIII*$^{-/-}$ neurons display a milder phenotype at 1 DIV, but they become completely normal at 2 DIV [36]. Neurons knocked out for multiple syn genes have a milder phenotype [30,37]. However, a comprehensive comparative work with all these genotypes is still missing. Reproduced with permission from [81].

cleotides against Syn II showed very short processes and failed to elongate axons, whereas about 20% of neurons did not exhibit any process. Moreover, the formed processes were morphologically altered, characterized by flattened and broad neurites surrounded by lamellipodial veil [31]. Similar results were obtained in neuronal cultures established from syn II KO mice [30].

Syn II was found to be involved not only in the consolidation of the minor processes and in axonal establishment, but also in synapse formation and maintenance. Indeed, the suppression of Syn II by using antisense oligonucleotides at a stage of development subsequent to axon formation prevented synapse formation [34]. Moreover, a more detailed analysis performed on syn II lacking cultures showed a delay in synapse formation as compared to WT cultures [30]. However, at a mature stage of development, the number of synaptic contacts was comparable between the two genotypes, indicating that syn II expression affects only the timing of development and that neurons can catch up synapse formation also in the absence of syn II. Nevertheless, syn II appears to be involved in the maintenance of synapses, as its suppression after synapse consolidation induces synapse loss [34].

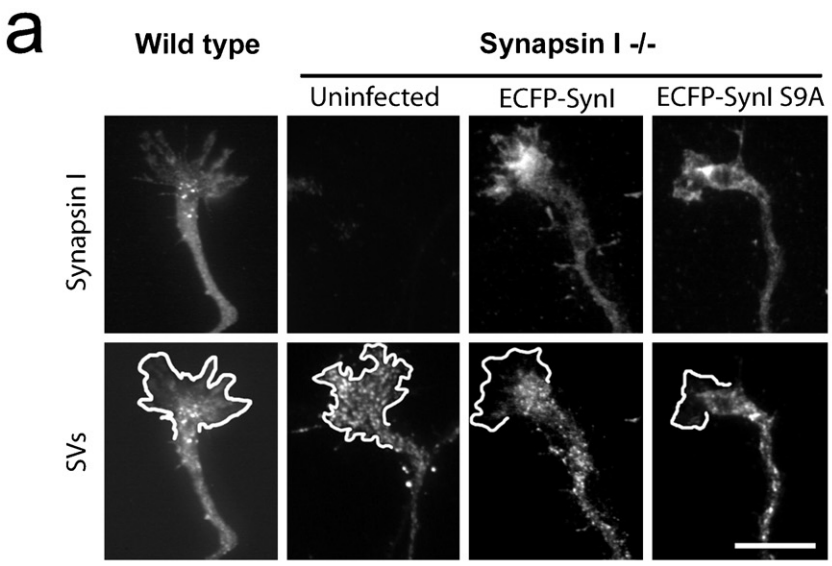
A similar but distinct role in neuronal development has been described also for the syn I isoform. Differently from the effect of syn II, the genetic ablation of syn I does not impair the timing of neurite formation or axon differentiation (stages I and II, respectively). However, this isoform affects the subsequent development of the axon. Indeed, syn I-deficient neurons exhibit a shorter axon, with slower elongation rate and less branching than their wild-type counterparts [35]. Moreover, the delay in synapse formation is greater in syn I lacking cultures than in syn II-deficient neurons, suggesting a more important effect of this isoform in synapse establishment [30]. Again, at later stage of development the number of total synapses is indistinguishable from that of WT cultures [35]. Interestingly, a recent study has shown that the acute reduction in syn I expression significantly reduces the number of synapses formed *in vitro*, indicating that syn I is also involved in synapse maintenance or formation (Perlini, Botti, Fornasiero, Benfenati and Valtorta, personal communication). All these data allow to draw a picture in which the two isoforms, syn I and II, have distinct roles during distinct stages of development: syn II acts earlier by affecting both neurite and axon formation, whereas syn I has a much prominent role during axonal elongation and synapse formation [30]. Surprisingly, the double KO mice for these two isoforms that would be expected to exhibit more severe developmental defects, are much less altered than either single KO mice. This unexpected evidence might be explained by complex compensatory mechanisms which could differentially occur in the KO mice, making the picture much more difficult to interpret.

The specific expression pattern of Syn III suggested a possible role of this isoform in neurite elongation, which was also supported by localization analysis showing that Syn III is highly enriched in the growth cones of neurons in culture [22]. Consistently, downregulation of syn III expression using antisense oligonucleotides *in vitro* specifically impaired differentiation and elongation of the axons, which was accompanied by an enlargement of the growth cones, whereas no significant effects were detected on either synapse formation or synapse maturation in older cultures. Similar results were obtained in cultures established from syn III-KO mice [36], where axonal outgrowth was delayed, thus confirming a distinct role of Syn III in the early development of axons.

Beyond these evidences obtained *in vitro*, studies performed in vivo did not show any significant alteration in the overall structure and morphology of the syns KO brain [37,30,35,36], with the exception of a decreased brain weight in syn II KO mice [30]. However, it cannot be ruled out that in the absence of syns minor differences in the wiring of the brain occur as suggested by both human and rodent phenotypes.

Concerning the molecular mechanisms underlying the effect of syns suppression on neuronal development, the evidences point toward the possible effect on cytoskeletal remodelling. Indeed, in neurites formed by syn II-deficient neurons the distribution of actin filaments appeared aberrant [30,31]. In particular, in syn II antisense treated neurons, F-actin was spread through the flattened lamellipodial veils, whereas in control neurons actin filaments were concentrated in growth cones at the tips of the axons and in minor processes [31].

Moreover, it has been postulated that reduction of Syn III expression could alter growth cone morphology leading to an enlargement of this structure through an action on actin dynamics, in view of the high structural homology of this isoform with the actin-binding domain of syn I [9]. Beside the “cytoskeleton hypothesis”, another important but unclear effect mediated by both syns’ knockdown and depletion was the reduction of synaptic proteins expression. Indeed, the reduction of Syn II expression *in vitro* was associated with the decrease in the immunoreactivity of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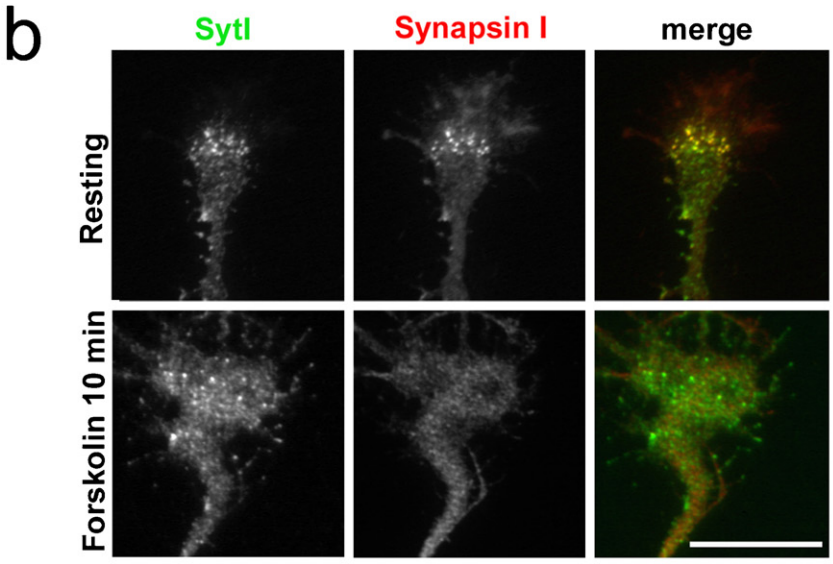
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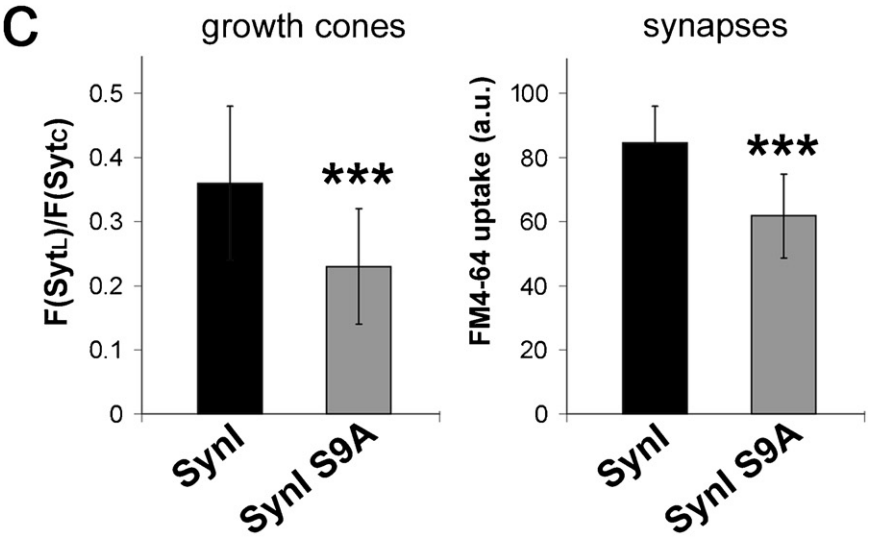
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presynaptic proteins, such as Syn I, synaptophysin and synaptotagmin [31,34]. A significant reduction of several presynaptic protein expressions was also obtained in TKO mice [37], whereas other proteins of the presynaptic terminals such as syntaxin and several post-synaptic proteins, like PSD95, NR1 and NR2B, did not change [37]. More recently, the acute reduction of syn I expression by using siRNA in cultured neurons was found to significantly reduce the expression of many pre- and postsynaptic proteins analyzed and such reduction was associated with a reduced number of synapses (Perlini, Botti, Fornasiero, Benfenati and Valtorta, personal communication). Even though the precise meaning of this phenomenon is still unknown, and also it is still unclear whether the reduction of SV proteins is a cause or a consequence of the impairment of synapse formation and maintenance, a possible speculation is that syns might be involved in the regulation of SVs stabilization and/or degradation. Due to the lack of evidence regarding this hypothesis, further studies are needed to address this point.

Interestingly, the expression of human syn I bearing either of the non-sense mutations described in epileptic and/or autistic patients in neuronal cultures led to impairment of axonal growth in the case of truncated proteins ([38]; Giannandrea, Benfenati and Valtorta, personal communication). The impairment of axonal growth associated with truncated syn I could be due to defective MAPK phosphorylation and/or c-Src or PI3K interactions, although a dominant-negative effect toward the endogenous SynII/III isoform I cannot be ruled out.

This evidence strongly supports the importance of syns in pathogenesis of various illnesses, indicating that, besides an alteration in neurotransmission, an impairment in neuronal development might underlie disorders such as epilepsy or autism.

5. Regulation of synapsin activities through phosphorylation controls neuronal development

Syn I was discovered as one of the most prominent phosphoproteins in the brain [39], and further studies have confirmed that the activity of syns can be tightly regulated through phosphorylation, integrating the inputs from several signalling cascades [40–44]. Although most of the studies on syn post-translational modifications have been focused on the regulation of neurotransmission, a number of reports indicate that syn phosphorylation can modulate key aspects of neuronal development. Thus, injection of the RNA coding for the non-phosphorylatable mutant (S9A) of syn IIa hampers neurite outgrowth in cultured embryonic neurons [45]. To further corroborate this finding the injection of phospho-specific antibodies against phosphorylation target site (P-site) 1 also decreases neurite outgrowth. Consistently, injection of pseudophosphorylated (S9E) syn IIa RNA increases neurite outgrowth, suggesting that the balance of syn IIa S9 phosphorylated conformers is important for the regulation of neurite extension. Whole-mount immunocytochemistry in *X. laevis* embryos injected with the RNA encoding for either phospho-mutant recapitulates the *in vitro* results, since S9A-expressing spinal neurons are shorter than the contralateral WT controls, while S9E-expressing spinal neurons have longer neurites.

Experiments carried out in mouse hippocampal neurons show that phosphorylation of syn I at site 1 influences the distribution and the transport of SV precursors in developing neurons. Bonanomi et al. [46] have demonstrated that syn I regulates the dynamic localization of SV precursors at the central domain of axonal growth cones in culture. In WT cultures at rest SV precursors are restricted to the central domain of axonal growth cones [47]. In contrast, in syn I −/− neurons SV precursors are dispersed throughout the actin-rich peripheral domain already at rest (Fig. 3a). The concentration of SV precursors in the central domain is restored by overexpression of syn Ia, independently of whether it is phosphorylatable or not. However, upon prolonged PKA activation syn Ia and SV precursors disperse throughout the cytoplasm (Fig. 3b). Interestingly, PKA-mediated SV dispersion is rescued by overexpression of WT syn Ia, but not by the non-phosphorylatable mutant of site 1 (S9A-syn I). From a functional point of view it is interesting to observe that neurons overexpressing the non-phosphorylatable mutant S9A show a decreased exo-endocytic rate of vesicular precursors in axonal growth cones (Fig. 3c [46]). This property is by some means conserved at later stages of development, since adult synapses overexpressing S9A syn I display a reduced SV recycling as assayed by FM4-64 uptake (Fig. 3c [48]).

Recent results indicate that the syn I phosphorylation at site 1 is relevant during the formation and/or stabilization of synaptic contacts. Overexpression of the phosphomimetic site 1 mutant (S9E-syn I) increases the number of synapses in cultured neurons at defined time points, while overexpression of the S9A non-phosphorylatable mutant decreases synapse number. Furthermore, overexpression of the phosphomimetic mutant rescues the decrease in synapse number caused by chronic treatment with tetrodotoxin. These results suggest that neuronal activity boosts synaptogenesis in a syn-dependent manner, integrating the signalling of PKA and that the correct balance between the phospho- and dephosphorylated state of syn on P-site 1, rather than the absolute expression level of WT syn I or syn II, is critical for synapse maturation, highlighting the importance of the post-translational modifications occurring during signal transduction pathways. However, due to the multilayer complexity of such biological system and because of the multiple P-sites in the syn sequence, additional mechanisms underlying syn-mediated effects on synapse development cannot be ruled out.

The role of syn phosphorylation by other kinases in neuronal development is less studied. Phosphorylation of sites 2 and 3 by CaMK II, which plays an important role in the regulation of neurotransmitter release in mature neurons, is unlikely to play a role during development, since before the establishment of synaptic contacts this kinase is active only in the somatodendritic compartment, whereas in the axon an imbalance between kinase and phosphatase activities prevents CaMK II autophosphorylation and

Fig. 3. Synapsin I determines SV precursor positioning in the central domain of the neuronal growth cone, and regulates SV dynamics in a phosphorylation-dependent manner at various stages of neuronal development. (a) Hippocampal neurons from either WT or *synI*$^{-/-}$ mice were stained with an antibody recognizing synaptic vesicles (bottom panels). WT neurons were also stained with an antibody recognizing syn I (G143), while *synI*$^{-/-}$ neurons were either uninfected, or infected with ECFP chimeric WT or S9A-syn I (the ECFP fluorescence is shown). In culture, neuronal growth cones are characterized by two defined regions: a central domain, enriched in organelles and microtubules, and a peripheral domain characterized by the absence of organelles and by the deposition of filamentous actin [47]. The white traces in the bottom panels outline the distal edges of the peripheral domain. Strikingly, SV precursors are confined to the central domain in WT neurons, but they disperse throughout the growth cone in *synI*$^{-/-}$ neurons. The dispersion phenotype is rescued by infection of either WT or nonphosphorylatable S9A-syn I. Scale bar, 5 μm. (b) Growth cones of rat hippocampal neurons incubated for 10 min at 37 °C in the presence or absence of forskolin and labelled with anti-synaptotagmin I (SytI; green in the merged images) and anti-syn I (red in the merged images) antibodies. At rest, syn I colocalizes with a fraction of synaptotagmin I-positive SVs, but after prolonged forskolin treatment both syn I and synaptic vesicles appear evenly distributed throughout the growth cone. Scale bar, 10 μm. (c) Left: quantitative analysis of the basal internalization of an antibody directed against the intraluminal domain of synaptotagmin (Syt_L) in growth cones expressing either syn I or S9A-syn I fused to ECFP. The ratio between the fluorescence intensities of Syt_L and total synaptotagmin in each growth cone is reported (mean ± SD; ***$p < 0.001$; Student's *t* test). Right: internalization of the lipophic dye FM4-64 evoked by depolarization in mature synapses (mean ± SD; ***$p < 0.001$; Student's *t* test). The exo-endocytosis of synaptic vesicle precursors and mature synaptic vesicles is reduced in both growth cones and synapses of ECFP-S9A-SynI overexpressing neurons.
Unpublished from Bonanomi and Valtorta and modified with permission from [46,48].

activation [49]. In contrast, syn I has been shown to undergo phosphorylation on the MAPK sites in axonal growth cones. Agonists acting on presynaptic glutamate [3H]-*amino*-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors elicit MAPK activation, with the subsequent phosphorylation of syn I on sites 4,5. Phosphorylation of syn I is accompanied by dispersion of the protein in the cytoplasm and increased recycling of SV precursors [50].

6. Modelling the molecular role(s) of synapsins during development

In spite of a flourishing number of observations that have linked syns to brain development, it is surprising that our knowledge of the underlying molecular mechanisms is still very speculative. This is in part due to the fact that neuronal development comprises a number of dynamic processes that are often temporally and spatially overlapping (Fig. 1a). Aside the complexity of neurodevelopmental processes, the ability of syns to contemporarily regulate numerous processes further complicates the identification of the exact cellular and molecular mechanisms modulated by these proteins. Here we provide a conceptual framework, identifying some of the most likely downstream effectors and cellular processes that need to be better studied in order to dissect the molecular role(s) of syns during brain development (Fig. 1b).

As previously reviewed, the manipulation of syn levels affects various morphological parameters of neuronal maturation, such as neurite extension and synaptic contact formation. Both processes are extensively dependent on cytoskeletal determinants that regulate the spatial coupling of adhesive and protrusive forces, ultimately determining neuronal morphogenesis. Indeed, syns bind several cytoskeletal components, including neurofilaments [51], spectrin/fodrin [52,53], microtubules [54], and both filamentous and globular actin [55–57]. Furthermore, syns contribute to actin bundling and actin polymerization in a phosphorylation-dependent manner [58–62]. These *in vitro* results suggest that the effects on neurite elongation and synaptogenesis might be primarily ascribed to the syn ability to regulate actin polymerization and cytoskeletal remodelling. Although there are evidences confirming a role of syns in cytoskeletal rearrangements in intact cells [24], it will be essential to clarify which is the role of these molecules in the stabilization of actin filaments at growing neuritic tips and at the level of presynaptic terminals. Moreover, it should be investigated whether syn can alter the coupling of actin structures with other cytoskeletal components, and if the binding of syns to microtubules, spectrin, fodrin and neurofilaments is physiologically relevant and somehow instructive for their polymerization/stability. Of particular interest is the complex pattern of phosphorylation that characterizes syns, which suggests that if specific functions are exerted in neurons, a tight regulation of these processes might have evolved.

Syns are known to interact with membranes and vesicles containing acidic phospholipids, such as phosphatidylserine and phosphatidylinositol that are present in transport vesicles and SVs [63–66]. Emerging evidences indicate that syns are relevant for the transport and localization of SV precursors and molecular constituents of newborn synapses, including piccolo-bassoon transport vesicles [46,67]. These findings constitute an intriguing clue to explain why syns are able to interact with virtually all the cytoskeletal components of neurons, and indicate that they could mediate/regulate transport either directly by interacting with vesicles and cytoskeletal components or indirectly by modulating their polymerization. Under this perspective, a new chapter of research would clarify if there are neuronal components whose traffic is directly determined by syns that in turn regulate neurite and synapse formation, e.g. adhesion molecules, scaffolding proteins, growth factors and structural components such as cytoskeletal and membrane constituents. An additional hypothesis is that syns might contribute to preserve the identity of a defined set of neuronal organelles, such as SVs, by coating them and preventing their intermixing. In agreement with this possibility, single and double synI −/− and synII −/− show decreased levels of SV proteins, whereas Rab5 levels are increased, indicating that indeed syns may contribute to preservation of packet/vesicle identity.

In spite of uncertainties concerning the putative roles exerted by syns in the regulation of vesicular and cytoskeletal constituents, it is well established that syns control neuronal activity as shown by the experiments carried out in the KO mice models [68–72,36,37,73,74]. Indeed, alterations of neuronal activity might indirectly affect the latest stages of neuronal development, including synapse formation and stabilization, as it has been recently proposed (Perlini, Botti, Fornasiero, Benfenati and Valtorta, personal communication). Up to date, it is still controversial how and to which extent neuronal electrical activity regulates synapse development [75–77], and syn KO models might be useful tools to further test this issue. Moreover, some of the effects observed in the neuropsychiatric disorders linked to syn mutations might be due to an indirect modulation of relevant neurodevelopmental processes due to a primary imbalance of neuronal activity, thus deserving more accurate investigation.

In 2008 Kao and collaborators have revealed that syn III is important for neuronal maturation. It is noteworthy that syn III is expressed prior to neural precursor cell development, and its depletion affects the proliferation, survival, and differentiation of precursors into neurons, further underlining the differences existing between syn III and the other two syns. At present, these findings are the most challenging to explain, since it is difficult to envisage a scenario in which syn III directly regulates cell-cycle exit of neuronal precursors, although we cannot exclude this possibility. It is interesting to note that syn III has a diffused staining throughout the soma of precursors, possibly indicating that its role is not confined to precise subcellular domains. The role in development of other direct interactors of syns including SH3 domain proteins and Rab 3 [78,79] also awaits clarification. Through these interactors syns might indirectly regulate yet unidentified neuronal processes at the basis of developmental phenotypes.

7. Conclusions

Although we are still far from an exact description of the cellular/molecular mechanisms at the basis of syn role(s) during neuronal development, it is clear that syns act on several processes, spanning from cytoskeletal rearrangement to the regulation of SV precursor trafficking, thus affecting key developmental phenomena such as neurite outgrowth and synapse formation and stabilization.

The syns do not appear to be essential for brain development, since in syn knocked-out animals the structure of the brain does not appear to be grossly altered, and neurons prepared from these animals form networks in culture, which are apparently normal from a morphological point of view. However, the proteins are essential for proper neuronal development and function, as demonstrated by the phenotype of knocked out animals, which are epileptic and exhibit behavioural abnormalities, and by the human pathologies (epilepsy, autistic disorders, mental retardation) which have been associated with syn mutations. From this point of view, it is important to point out that, although these pathologies can be clinically severe, from the biological point of view they can be considered to be mild, since in these patients all the basic brain functions are retained, and what is missing is the fine tuning of synaptic connectivity and plasticity.

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