

Chapter 13

Synapsins and Synaptic Vesicle Storage

Fabrizia C. Guarnieri, Fabio Benfenati, and Flavia Valtorta

Abstract The synapsins constitute a family of evolutionarily conserved neuronal phosphoproteins associated with the cytosolic surface of synaptic vesicles. In mammals, the family comprises three members encoded by distinct genes that give rise to various splicing isoforms. In the central nervous system, the vast majority of neurons expresses at least one synapsin isoform. However, the functions of these proteins are not fully understood to date. Given their ability to bind both the vesicular membrane and actin filaments in a phosphorylation-dependent manner, the classical role attributed to synapsins is the reversible anchorage of synaptic vesicles to the cytoskeletal matrix present in the presynaptic terminal. However, recent evidences suggest the implication of synapsins in other aspects of the synaptic vesicle life cycle, such as docking, fusion and recycling. Genetic manipulation of synapsins in various in vitro and in vivo models has proved that they are dispensable for the proper development of functional neuronal networks but are essential modulators of synaptic neurotransmission and play differential roles at excitatory and inhibitory synapses. Indeed, mice lacking synapsins are viable and do not display gross brain abnormalities but exhibit generalised epileptic seizures as well as autism-related behavioural abnormalities. Consistently, several mutations have been identified in *SYN1* and *SYN2* genes in patients affected by epilepsy and/or autism spectrum disorders.

This chapter overviews the current knowledge about synapsin structure and function in the modulation of synaptic vesicle release, as well as the mechanisms leading to synaptic pathology when their properties are altered.

Keywords Synapsins • Synaptic vesicles • Synaptic transmission • Epilepsy • Autism

F.C. Guarnieri • F. Valtorta (✉)
Vita-Salute San Raffaele University and Division of Neuroscience, San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy
e-mail: valtorta.flavia@hsr.it

F. Benfenati
Department of Neuroscience and Brain Technologies, The Italian Institute of Technology and Department of Experimental Medicine, University of Genoa, Genoa, Italy

13.1 Introduction

Four decades of research have addressed the study of the synapsins in neuronal physiology, since the first description of Protein I (later renamed synapsin I) as a major target of phosphorylation by cyclic AMP-dependent kinases in synaptic membrane fractions by Paul Greengard in 1972 (Johnson et al. 1972). Though, we are still far from the complete understanding of the multiple functions that these proteins exert in neuronal development and synaptogenesis, synapse organisation, modulation of synaptic vesicle trafficking and plasticity.

Soon after their discovery, it turned out that synapsins I and II, the latter identified in 1978 and initially named Protein III (Forn and Greengard 1978), are presynaptic proteins able to reversibly associate with the cytosolic side of synaptic vesicle membrane (De Camilli et al. 1983a, b; Huttner et al. 1983) and with cytoskeletal components (Bahler and Greengard 1987; Fesce et al. 1992; Valtorta et al. 1992b) in a phosphorylation-dependent manner. Indeed, synapsins are physiological substrates of cyclic AMP-dependent and Ca^{2+} /calmodulin-dependent protein kinases that phosphorylate them upon neuronal activation (Greengard et al. 1993). Given these properties, synapsin I was postulated to be involved in the regulation of neurotransmitter release through the reversible tethering of synaptic vesicles to the actin cytoskeleton (Benfenati et al. 1991; Greengard et al. 1987, 1993; Valtorta et al. 1992a). However, recent evidences also imply synapsins in post-docking steps and vesicle recycling (Bloom et al. 2003; Fassio et al. 2006; Hilfiker et al. 1998, 2005), as well as in neurite extension and synapse formation (Fornasiero et al. 2010). More than 20 years after the identification of synapsin I, a third member of the family was described (Kao et al. 1998). In contrast to synapsins I and II, which are increasingly expressed during neuronal maturation, synapsin III is highly expressed in growth cones at early stages of development (Ferreira et al. 2000) and its specific function is still not completely elucidated. It is now clear that the three members of the family share many features but are not completely redundant. However, the precise roles and pattern of expression of each synapsin isoform remain elusive and need further clarification.

13.2 The Synapsin Family

13.2.1 *Genes and Promoter Regions*

In mammals the synapsin family is composed of three members encoded by distinct genes: *SYN1*, *SYN2* and *SYN3*. In humans, the *SYN* genes are located on chromosomes X, 3 and 22, respectively. Synapsin genes were identified in vertebrate and invertebrate organisms; however in invertebrates only a single synapsin gene is present. The intron/exon structure is conserved from *C. elegans* to humans, as well as in the three mammalian genes, suggesting that duplication events

occurred from a single ancestor, probably when vertebrates diverged from invertebrates. This interpretation is also supported by the observation that two other gene families, *raf* and *TIMP* (tissue inhibitor of metalloproteinases), are in close physical linkage to *Syn* genes. In fact, each synapsin gene lies in very close proximity to a *TIMP* and a *raf* gene family member (Kao et al. 1999).

Synapsins are widely and selectively expressed in neurons of the central and peripheral nervous systems. The proximal promoter of the *SYN1* gene is characterised by the absence of TATA and CAAT consensus motifs and is enriched in CpGs that are subjected to epigenetic regulation (Ekici et al. 2008; Paonessa et al. 2013; Sauerwald et al. 1990). Neuron-specific expression of synapsin I is driven by the presence of a binding site for the transcriptional repressor NRSF/REST (neuron-restrictive silencer factor/RE-1 silencing transcription factor), which negatively regulates *SYN1* expression in non-neuronal cells both in rodent and human promoter regions (Li et al. 1993; Schoch et al. 1996). A positive regulation is instead mediated by the binding of Sp1, a ubiquitous transcriptional activator able to bind to GC-rich regions and to recruit the transcription initiation complex to TATA-less promoters. Interestingly, it has recently been shown that Sp1 and REST binding sites lie in close proximity in the *SYN1* promoter and that the two transcription factors act in a strict functional interplay. During neuronal differentiation and development, Sp1 binding to the *SYN1* promoter is fine-tuned by REST, and the progressive decline in REST levels favours Sp1 binding and synapsin I expression (Paonessa et al. 2013). In addition, a cAMP-responsive element (CRE) is present and confers basal activation by the CRE-binding (CREB) transcription factor but does not modulate *SYN1* expression in a cAMP-sensitive manner (Hoesche et al. 1995). On the contrary, the factor *zif268/Egr-1*, which recognises specific binding sites (EBS), was shown to mediate transcriptional activation upon forskolin treatment (James et al. 2004).

The *SYN1* and *SYN2* promoters display very little sequence homology, still they share many features (absence of TATA and CAAT boxes, GC enrichment, neuronal specificity) (Chin et al. 1994). In the *SYN2* promoter, binding sites for Sp1 and *zif268/Egr-1*, but not for REST, have been identified (Petersohn et al. 1995), raising the question as to how synapsin II confinement in neuronal cells could be achieved. Two binding sites for the activating protein 2 α (AP-2 α) are also present and mediate *SYN2* activation in a cAMP/protein kinase A (PKA)-modulated manner (Skoblenick et al. 2010).

The *SYN3* promoter is completely uncharacterised. Synapsin III differs from the other two members in terms of its expression pattern: two out of the six described splicing isoforms, namely, synapsins IIIe and f, are expressed only in non-neuronal tissues (placenta and spleen), probably thanks to a second promoter that resides upstream exon 9 (Porton et al. 1999). In addition, while synapsins I and II progressively increase in their levels during development, synapsin III is predominantly expressed in the fetal brain, or during the first days in vitro when neurons are cultured, and then declines (Ferreira et al. 2000; Porton et al. 1999). In the adult brain, synapsin III is highly expressed by immature neurons in the neurogenic niches (Pieribone et al. 2002).

13.2.2 Splicing Isoforms and Protein Domain Structure

Alternative splicing events of the three synapsin genes give rise to various isoforms. Two distinct isoforms are produced for synapsin I and II (named a and b), and at least six transcripts are described for synapsin III (a–f). The resulting proteins are highly homologous at their N-termini but greatly differ in the C-terminal portion (see Fig. 13.1) (Porton et al. 1999; Südhof 1990; Südhof et al. 1989). The N-terminal region can be subdivided into three domains, namely, domains A, B and C, which, except for domain B, are highly conserved across different isoforms and species. Conversely, the C-terminal portion is composed of differentially spliced domains (domains D–J).

Domain A is a short N-terminal region that contains a conserved phosphorylation site (site 1) target of PKA and Ca²⁺/calmodulin-dependent kinases (CaMK) I and IV (Huttner et al. 1981). Domain B is the least conserved of the domains of the

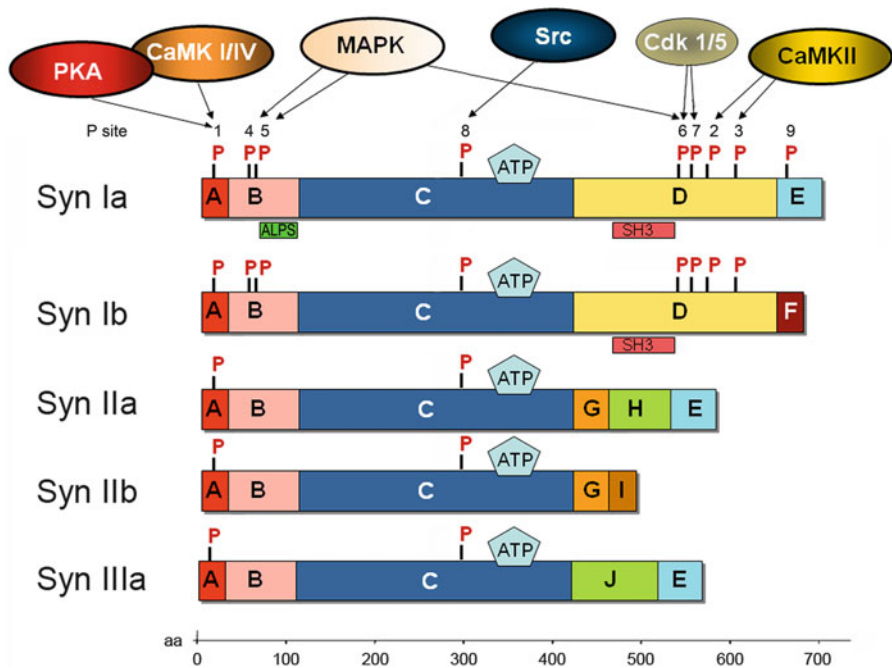


Fig. 13.1 Schematic representation of the domain structure of mammalian synapsin I, II and III proteins. Main splicing isoforms are reported. Known phosphorylation (P) sites (numbered according to the literature), as well as the ALPS motif, the ATP binding site and the proline-rich region mediating the interaction with SH3 domains, are indicated. All isoforms share a conserved N-terminal portion (A, B and C domains), while they differ in alternatively spliced C-terminal domains (D–J). PKA protein kinase A, CaMK Ca²⁺/calmodulin-dependent protein kinase, MAPK mitogen-activated protein kinase, Cdk cyclin-dependent kinase, ALPS amphipathic lipid packing sensor, ATP adenosine triphosphate, SH3 Src homology 3 domain (Adapted with authors' permission from Fornasiero et al. 2010)

N-terminal region. It is considered to be a linker region, connecting domain A to domain C. However, mutations in this domain appear to have a strong impact on the functions of this protein (Guarnieri and Valtorta, unpublished results). It is characterised by the presence of small amino acids and probably has an elongated conformation in solution (Brautigam et al. 2004). Recently, an amphipathic lipid packing sensor (ALPS) motif has been recognised in this domain. This motif is evolutionarily conserved and is deputed to the specific binding of highly curved membranes (Krabben et al. 2011). In synapsin I, domain B contains two phosphorylation sites (sites 4 and 5) for mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) (Jovanovic et al. 1996; Matsubara et al. 1996). These two sites are probably present also in synapsin II and III, although their phosphorylation has never been experimentally demonstrated (Jovanovic et al. 2001). Domain C is the core region, highly conserved among the various members of the family. It mediates the principal functions of synapsins, i.e. the interaction with actin filaments and synaptic vesicle phospholipids (Bahler and Greengard 1987; Benfenati et al. 1989b; Benfenati et al. 1989c; Cheetham et al. 2001), as well as synapsin homo- and hetero-dimerisation (Hosaka and Südhof 1999; Monaldi et al. 2010) that probably contributes to synaptic vesicle clustering. A highly conserved phosphorylation site (site 8) is present in this domain and is targeted by the tyrosine kinase Src. The phosphorylation at this site has been experimentally demonstrated for synapsins I and II (Messa et al. 2010; Onofri et al. 2007). Domain C is the only portion for which a crystal structure is available, and it appears folded as a compact structure composed of amphipathic α -helices and β -sheets. Interestingly, it has been shown that the three-dimensional structure of synapsin I strongly resembles that of an ATP-dependent glutathione synthetase and that all synapsins can bind ATP (Esser et al. 1998), even though an enzymatic activity has never been proved. After domain C, the amino acid sequence starts to diverge in the various synapsins. However, all isoforms bear an elongated proline-rich tail, namely, domain D in synapsin I, domain G in synapsin II and domain J in synapsin IIIa. In synapsin I, domain D contains phosphorylation sites 2 and 3 for CaMKII (Czernik et al. 1987). Similar consensus sites are present also in synapsin III, but their accessibility to phosphorylation remains to be determined (Kao et al. 1998). In the same region, site 6 is targeted by MAPK/ERK and cyclin-dependent kinase 5 (Cdk5), and site 7 is specifically targeted by Cdk5 (Jovanovic et al. 1996; Matsubara et al. 1996). The proline-rich domain D mediates the binding to important molecular partners, such as CaMKII (Benfenati et al. 1992) and Rab3A (Giovedi et al. 2004b), as well as the SH3 (Src homology 3 domain)-containing protein Src (Onofri et al. 1997). Downstream of the proline-rich region, domain E, common to all the 'a' isoforms, is highly conserved and it was recently shown to play an important role in modulating synapsin function. In fact, this region is required for the proper targeting of synapsins to the presynaptic terminals (Gitler et al. 2004b), as well as for synapsin I oligomerisation and synaptic vesicle clustering, and is implicated in both the pre- and post-docking steps of synaptic vesicle exocytosis (Fassio et al. 2006; Hilfiker et al. 1998; Monaldi et al. 2010). In addition, a novel phosphorylation site (site 9) targeted by the

ataxia-telangiectasia mutated (ATM) kinase has been recently identified in this domain (Li et al. 2009). The short domains F and I present in synapsin I and II 'b' isoforms, respectively, are poorly conserved and remain as yet uncharacterised.

13.3 Synapsin Expression and Biochemical Properties

13.3.1 *Spatial and Temporal Regulation of Synapsin Expression*

Virtually all neurons in the central and peripheral nervous system express at least one synapsin isoform, with the notable exception of specialised systems characterised by the presence of ribbon synapses (bipolar cells of the retina, hair cells of the inner ear, taste cells) that use distinct mechanisms of vesicle clustering and exo-endocytosis (Mandell et al. 1990). Many presynaptic terminals appear to contain all I/II isoforms, even if at differential abundance. As an example, cerebellar Purkinje cells express high levels of synapsin Ib, lower levels of synapsin Ia and IIb, while lack synapsin IIa (Südhof et al. 1989). Overall, in the cerebral cortex, synapsin I is more abundant than synapsin II, in a ratio of 2:1, and the ratios of Ia to Ib and IIa to IIb are both consistently 1:2 (De Camilli et al. 1990). In the olfactory cortex, however, synapsin II is higher than synapsin I, and the IIa to IIb ratio is 3:2 (Stone et al. 1994). Another interesting example of differential expression of synapsin isoforms is given by the distinct types of synaptic inputs coming from the retina, the visual cortex and GABAergic interneurons on relay neurons of the lateral geniculate nucleus in the thalamus. Indeed, retinogeniculate synapses do not display synapsin I or II expression, while both isoforms are present in feedback corticogeniculate synapses and only synapsin I is expressed in interneurons (Kielland et al. 2006). This characteristic pattern may reflect specific needs of each type of synaptic connection to fine-tune vesicle release in modulatory inputs or to reach powerful transmission in driver synapses. This interpretation has been strengthened by the observation that the thalamocortical input on visual cortex, which represents another driver synapse in the visual pathway, is devoid of synapsins (Owe et al. 2013). Recently, array tomography experiments confirmed that synapsin I is present in all glutamatergic and GABAergic presynaptic terminals in the mouse cortex (Micheva et al. 2010).

Synapsin III is expressed at very low levels throughout the brain, with high regional variation in the adult mouse. Its presence in presynaptic terminals suggests, at least in part, a similar function to synapsins I and II. However, this third member of the family bears the peculiarity of being expressed at high levels in the dentate gyrus and the olfactory bulb, known to be neurogenic regions of the adult brain. In addition, in these regions, synapsin III localises to the cell soma rather than to nerve terminals, indicating a completely different – but still unclear – function (Pieribone et al. 2002).

In the rat brain, synapsin I expression slightly precedes peaks of synaptogenesis, according to developmental specificity of different regions (Zurmohle et al. 1994). Thus, in the neocortex and cerebellum, synapsin I mRNA starts to increase at postnatal day 7 (P7) and peaks at P17, when synaptogenesis rate is high in these regions. Synapsin I mRNA expression is already detectable in the first postnatal week in the thalamus, piriform cortex and hippocampus (particularly in the CA3 subregion and the dentate gyrus), which mature earlier. High levels of synapsin I mRNA are maintained constant – or slightly decreased – during adulthood, especially in the CA3 region of the hippocampus, suggesting that this protein is important for synapse formation during development as well as for synapse maintenance and remodelling/plasticity during adult life (Zurmohle et al. 1994). At the protein level, synapsins I and II were shown to steadily increase during postnatal development of the mouse brain, with synapsin I being already detectable at P5. Synapsins IIa and IIb are barely detectable during the first week after birth and then increase with different kinetics (slower for IIa and much faster for IIb) (Bogen et al. 2009). These observations also indicate that the progressive increase of synapsin protein is probably due to post-transcriptional/translational mechanisms of regulation, as the rate of mRNA synthesis is about constant. Another possibility, which has been verified *in vitro*, is that synapsin proteins are progressively stabilised by their incorporation into mature synaptic vesicles produced during neuronal development, despite a constant efficiency in transcription and translation, as their half-life gradually increases (Daly and Ziff 1997).

While the levels of synapsins I and II correlate with the extent of synapse formation *in vivo* and *in vitro*, synapsin III is highly expressed in the first week of development and subsequently declines (Ferreira et al. 2000; Pieribone et al. 2002). *In vitro*, this period corresponds to active neurite elongation, and, consistently with a role in this process rather than in synapse formation, synapsin III is enriched in growth cones and at extrasynaptic sites. Indeed, depletion of synapsin III from hippocampal neurons in culture leads to a defect in axonal elongation, but not in the time course of synapse formation (Ferreira et al. 2000).

Low levels of synapsin I expression have been detected in non-neuronal cell types, such as epithelial cells, pancreatic β -cells, cell lines of neuronal and endocrine origin and, interestingly, astrocytes (see, e.g. Haycock et al. 1988; Maienschein et al. 1999; Romano et al. 1987). In astrocytes, synapsin I appears to be secreted via exosomes and to contribute to neurite outgrowth of neuronal cells (Wang et al. 2011).

13.3.2 Binding to Synaptic Vesicles

Synapsins, and in particular synapsin I, have been extensively characterised in terms of their synaptic vesicle-binding properties. The first evidences for a direct interaction of synapsin I with the cytosolic surface of synaptic vesicles came from electron microscopy immunoferritin/oxidase staining and from biochemical

purification of the organelles (De Camilli et al. 1983b; Huttner et al. 1983; Valtorta et al. 1988). These studies showed that the interaction is specific, as other membranous compartments in the terminal (such as endoplasmic reticulum cisternae, mitochondria or large dense-core vesicles) were unlabelled, and relatively stable, as it resisted to isotonic, but not to hypotonic, lysis of the nerve ending. Dephosphorylated synapsin I was proved to bind synaptic vesicles as well as artificial phospholipid membrane with high affinity ($K_d \approx 10$ nM) (Benfenati et al. 1989a, 1989b). Phosphorylation of the protein on sites 2 and 3, targets of CaMKII, causes a fivefold decrease in the binding affinity to synaptic vesicles (Schiebler et al. 1986). In intact terminals, also site 1 phosphorylation by PKA and CaMKI induces the dissociation of synapsins from the vesicle membrane (Hosaka and Südhof 1999). Recently, it was estimated that eight molecules of synapsin I are present on the surface of each vesicle (Takamori et al. 2006), confirming that synapsins represent approximately 6 % of the total vesicle proteins (Huttner et al. 1983).

The lipid-binding activity of synapsin I is predominantly mediated by the N-terminal portion of the protein, which is able to partially penetrate into the lipid bilayer after an initial electrostatic interaction with the acidic phospholipids (especially phosphatidylserine and phosphatidylinositol) of the vesicular membrane (Benfenati et al. 1989b). The specific regions involved in membrane insertion were mapped on synapsin I. Three regions, two of which contain an amphipathic α -helix, were found to be able to penetrate the vesicular membrane. They all reside in domain C and are exposed on the surface of one side of the protein that does not overlap with the dimerisation domain (Cheetham et al. 2001). These regions are highly conserved across isoforms and species and probably mediate association with synaptic vesicles also in synapsins II and III.

The C-terminal tail of synapsin I is able to bind the synaptic vesicle surface through the interaction with protein components of the vesicles rather than with phospholipids (Benfenati et al. 1989a). One of these protein binding partners is the regulatory domain of vesicle-associated CaMKII (Benfenati et al. 1992). Synapsin I is also the major binding protein of the tyrosine kinase c-Src on the synaptic vesicle membrane. The interaction between the proline-rich domain D of synapsin I and the SH3 domain of Src triggers a basal level of activation of the kinase, which in turn phosphorylates synapsin I itself on site 8 (Onofri et al. 1997). This phosphorylation confers to the surrounding sequence of synapsin I SH2 domain-binding properties and recruits the SH2 domain of Src. Thus, Src binding is reinforced by combined SH3/SH2 interactions, leading to massive activation of the kinase (Onofri et al. 2007). Through its SH3-binding regions, synapsin I is able to interact with many additional partners, such as the MAPK adaptor Grb2, the p85 subunit of PI3K, phospholipase C- γ , amphiphysins I and II, Crk, p47, α -spectrin (Onofri et al. 2000) and syndapin (Qualmann et al. 1999).

Synapsin I interacts in an SH3-independent manner with Rab3A (Giovedi et al. 2004b). Rab proteins are monomeric G proteins ubiquitously involved in the modulation of membrane trafficking. The Rab3A-C isoforms are specific for synaptic vesicles, and their GTP/GDP cycle is strictly connected to the dynamics of

synaptic vesicle exo-endocytosis (Geppert and Südhof 1998). Similarly to what was described for Src, the interaction of synapsin I and Rab3A influences the functional properties of both proteins: synapsin I stimulates Rab3A GTP binding and GTPase activity, while Rab3A inhibits synapsin I ability to bind and bundle actin and to cluster synaptic vesicles (Giovedi et al. 2004a).

Synapsin II is more tightly bound to synaptic vesicles than synapsin I, possibly because it lacks part of the C-terminal tail that contains highly charged residues, thus making hydrophobic interactions prevalent. An additional binding site for synaptic vesicles has been recognised in domain B of synapsin II, likely involving interaction with protein components (Thiel et al. 1990).

Many approaches have demonstrated that synapsins also have a stabilising activity on the vesicle membrane. Indeed, it was shown that high concentrations of synapsin I – similar to those reached at presynaptic terminals – are able to counteract the effects of destabilising stimuli on artificial liposomes (Benfenati et al. 1993; Cheetham et al. 2003) and to confer break resistance to artificial lipid bilayers (Pera et al. 2004). Given that synaptic vesicles have a very small diameter (around 40–60 nm), they are supposed to be unstable due to high curvature stress. The binding of synapsins may be important to balance the different surface pressures on the external and internal leaflet, to stabilise the small vesicles and to impede unwanted fusion events.

Interestingly, the curvature of the vesicular membrane per se may be a key determinant of the specific binding of synapsins to synaptic vesicles. Synapsin I contains an amphipathic lipid packing sensor (ALPS) motif that specifically senses highly curved membranes and facilitates synapsin binding to (and clustering of) synaptic vesicles (Krabben et al. 2011). This motif is perfectly conserved in all synapsin isoforms and likely exerts similar functions in synapsins II and III.

Thus, the association of synapsins with synaptic vesicles is mediated by multiple domains of the molecule, which interact with either phospholipids or protein components. It is conceivable that binding specificity is achieved by membrane curvature sensing, as well as by interaction with resident synaptic vesicle proteins.

13.3.3 Binding to Actin Cytoskeleton

Synapsin I was shown to interact with several cytoskeletal proteins, but the binding to actin is of particular interest given that actin is the major component of the cytoskeleton in the presynaptic terminal. The ability of synapsins to bind both the membrane of synaptic vesicles and the actin cytoskeleton has led to the hypothesis that they are involved in the cross-linking and anchoring of synaptic vesicles to the cytomatrix of the nerve terminal.

Purified dephosphorylated synapsin I is able to bind ($K_d = 1\text{--}2\ \mu\text{M}$) and bundle actin filaments *in vitro*. The bundling activity is reduced when synapsin I is phosphorylated by PKA, almost completely abolished upon CaMKII phosphorylation (Bahler and Greengard 1987), significantly reduced by MAPK (Jovanovic

et al. 1996) and not affected by Cdk5 phosphorylation (Matsubara et al. 1996). The ability to bundle actin implies the presence of multiple binding sites in each synapsin molecule and/or the ability of synapsin molecules bearing a single binding site to dimerise. At least two regions responsible for actin binding reside in the N-terminal (domain B) and middle portion of the protein (end of domain C), but the presence of the C-terminal tail is necessary for maintaining the bundling activity (Bahler et al. 1989). In addition, domain C and E, but not A, peptides interfere with the ability of synapsin I to bind and bundle actin filaments (Hilfiker et al. 2005).

Interestingly, dephospho-synapsin I also promotes monomeric actin nucleation and polymerisation, and phosphorylation by CaMKII strongly inhibits this function (Fesce et al. 1992; Valtorta et al. 1992b).

Synapsin II has a much stronger actin binding and bundling activity, which is completely abolished by PKA phosphorylation (Nielander et al. 1997).

13.3.4 *Homo- and Hetero-dimerisation of Synapsins*

Analysis of the crystal structure of synapsin I domain C showed that the protein forms tightly associated dimers and tetramers, which are stabilised by ATP binding and physiological Ca^{2+} concentrations (Brautigam et al. 2004; Esser et al. 1998). The contact area between the subunits of the oligomers is very large, indicating a stable interaction. Given that the C-domain is the most conserved region in all isoforms, synapsin hetero-dimerisation was also investigated. Yeast two-hybrid screenings and immunoprecipitation assays proved that the major synapsin binding partner is another synapsin molecule. All three synapsins are able to interact with themselves and with the other isoforms. The interaction is strong and stable, with the exception of a less intense binding between synapsins I and III, and the full-length domain C is required for the association to occur (Hosaka and Südhof 1999). Homo- and hetero-dimerisation is an important feature of synapsins, as it implies that synaptic vesicles are coated by multiple heterogeneous dimers that possibly exert very similar functions but possess different regulatory properties. Since synapsin isoforms are differentially expressed in neurons, the exact composition of the dimers in each terminal may have a functional relevance in the specific regulation of vesicle life cycle.

In addition to domain C, the highly conserved domain E has a prominent role in mediating synapsin dimerisation. Indeed, domain E peptides selectively bind to synapsins I and II and inhibit the formation of synapsin dimers (Monaldi et al. 2010). The involvement of domain E in the dimerisation of synapsins is also relevant for the specific targeting of synapsins to nerve terminals. In fact, it was shown that domain E and dimerisation are two important determinants of synaptic targeting, in particular for the correct transport of isoforms with weak targeting potential such as synapsin IIb (Gitler et al. 2004b).

13.4 Modes of Synapsin Action in the Presynaptic Terminal

13.4.1 Role in Synaptic Vesicle Pool Organisation

In the presynaptic terminal, synaptic vesicles are organised in at least three distinct and functionally relevant pools: the readily releasable pool, the recycling pool and the resting pool (Rizzoli and Betz 2005). The readily releasable pool comprises a small number of vesicles (1–10 % of the total) that are docked at the membrane, in an intermediate state of fusion and ready to be rapidly released in response to a Ca^{2+} influx. The recycling pool comprises 5–20 % of all vesicles and can be recruited after a moderate stimulation in order to substitute docked vesicles that had undergone release. The resting pool accounts for the majority of vesicles in the terminal (typically 80–90 %). This pool is quite inactive and is recruited after intense stimulation; thus it is considered the true depot for synaptic vesicles.

Given the properties described so far, i.e. the ability to bind synaptic vesicles and actin in a phosphorylation-dependent manner, synapsins were hypothesised to be primarily involved in the organisation of synaptic vesicle pools in the presynaptic terminal (Greengard et al. 1993). The first evidences supporting this idea came from microinjection studies. Delivery of dephosphorylated synapsin I into the squid giant synapse inhibited neurotransmitter release, due to a decrease in the quanta released by a constant stimulus, and this effect was abolished upon CaMKII phosphorylation. Conversely, the injection of CaMKII enhanced neurotransmitter release (Llinas et al. 1985, 1991). These observations have suggested a model in which the inhibitory effect of dephosphorylated synapsin I on neurotransmitter release is achieved through the cross-linking of synaptic vesicle to each other and to the actin cytomatrix (Benfenati et al. 1991). Direct visual proof of the anchorage of synaptic vesicles to actin filaments mediated by synapsin I came from video-enhanced microscopy of fluorescently labelled components incubated *in vitro*. The interaction of synapsin I to actin and synaptic vesicles is rapid, as after 3–20 s of incubation, synapsin I is able to bundle actin filaments or drive the polymerisation of the monomers and to tether synaptic vesicles to the formed cytoskeletal network. Again, these effects are completely abolished by CaMKII phosphorylation (Ceccaldi et al. 1995).

One mechanism by which synapsins could regulate neurotransmitter release is the reversible association/dissociation to synaptic vesicles and F-actin, according to their phosphorylation state. This was biochemically demonstrated, as depolarisation of rat synaptosomes resulted in a rapid translocation of synapsin I from the particulate to the soluble cytosolic fraction. The stoichiometry of phosphorylation of soluble synapsin I was higher with respect to that of synapsin I in the particulate fraction, and the time course of translocation paralleled that of phosphorylation, suggesting that phosphorylation promotes the dissociation of synapsin from synaptic vesicles and actin (Sihra et al. 1989). The translocation of synapsin I upon neuronal activation was also demonstrated in living hippocampal neurons in

culture through immunocytochemistry and video-imaging analysis of GFP-labelled synapsin Ia (Chi et al. 2001). It was shown that a substantial portion of synapsin molecules present in the presynaptic bouton detaches from synaptic vesicles and diffuses in the axon during electrical stimulation. This dissociation is faster than vesicle turnover itself (monitored through the uptake of the FM4-64 dye), indicating a physical separation of synapsin I from synaptic vesicles that precedes their exocytosis. Both synapsin diffusion and vesicle turnover were slowed down by transfection with non-phosphorylatable mutants on sites 1, 2 and 3 (Chi et al. 2001). These results are consistent with ultrastructural analyses performed on the frog neuromuscular junction, which indicated a partial dissociation of synapsin I from synaptic vesicles upon electrical stimulation. Indeed, upon neuronal activation, synaptophysin (a transmembrane vesicular protein) was still associated with vesicle membranes, whereas synapsin I was partially diffused in regions of the terminal devoid of synaptic vesicles (Torri-Tarelli et al. 1990, 1992).

Together these studies pointed to a model in which synapsin I (and probably synapsin II in a similar manner) is involved in the regulation of the number of synaptic vesicles available for exocytosis after a depolarisation event, through the dynamic organisation of the pools of vesicles (Fig. 13.2). In its dephosphorylated form, synapsin I may bind the majority of synaptic vesicles and link them to each other, thanks to synapsin dimerisation, and to actin filaments, both preformed or newly polymerised on vesicle membranes. In this manner, synaptic vesicles are captured in a cytoskeletal network and are unavailable for exocytosis. Upon stimulation, Ca^{2+} influx can activate protein kinases, such as CaMKII, that in turn phosphorylate synapsin I. In its phosphorylated form synapsin I loses its affinity for synaptic vesicles and the actin cytoskeleton, therefore detaching and releasing vesicles that can undergo fusion (Greengard et al. 1993). It is also important to note that not all synapsin molecules detach from synaptic vesicles upon Ca^{2+} entry; otherwise vesicle release would probably be massive rather than fine-tuned. The detachment of synapsin molecules from the resting pool appears to be a stochastic process. CaMKII phosphorylation probably proceeds from the periphery to the centre of the cluster, given that CaMKII immune-reactivity is clearly enriched around – not inside – the vesicle cluster. Thus, the number of vesicles that the kinase is able to reach would probably be dependent on the duration of the depolarising stimulus (Tao-Cheng et al. 2006).

The modulation of synapsin function by phosphorylation is rather complex. As much as nine phosphorylation sites have been extensively characterised for synapsin I up to now, but others have already been identified and wait for functional depiction (John et al. 2007; Li et al. 2009; Nuwal et al. 2011).

Phosphorylation at site 1 (Ser-9 in the rat sequence) by PKA/CaMKI induces only subtle changes in the overall conformation of the synapsin molecule (Benfenati et al. 1990) but significantly reduces the binding of synapsins to synaptic vesicles and actin in both mature (Chi et al. 2001; Menegon et al. 2006) and developing neurons (Bonanomi et al. 2005), leading to increased recycling activity.

Phosphorylation at sites 2 (Ser-566) and 3 (Ser-603) by CaMKII leads to major conformational changes (Benfenati et al. 1990) and to a drastic decrease in the

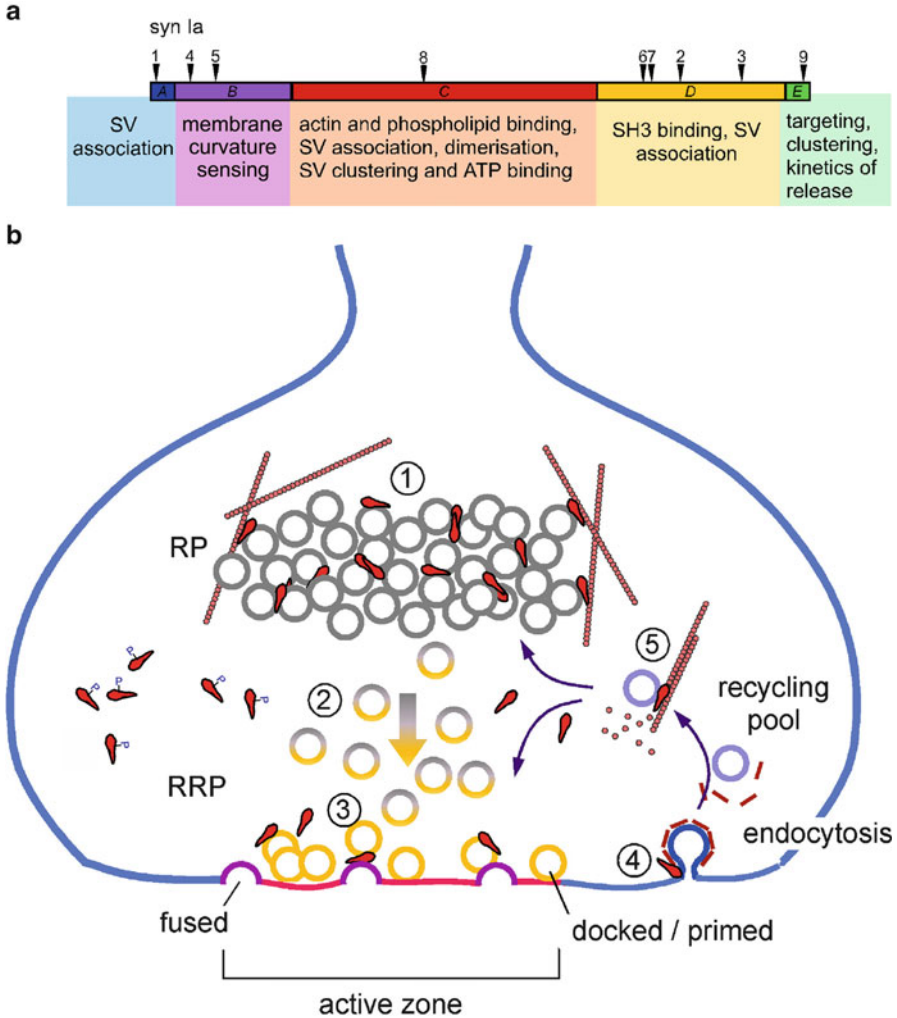


Fig. 13.2 Roles of synapsins in the presynaptic compartment. **(a)** Schematic representation of synapsin Ia, summarising the biological functions attributed to each domain. **(b)** Mechanisms of regulation of the synaptic vesicle cycle. (1) Synapsins (in red) mediate the clustering of synaptic vesicles of the resting pool (RP, in grey), distant from the active zone, by linking adjacent synaptic vesicles to each other and to the actin cytoskeleton. (2) Upon stimulation, synapsins get phosphorylated, lose affinity for synaptic vesicles and actin and relieve a subset of vesicles from physical constraint. These vesicles are now free to reach the active zone and undergo fusion. (3) Part of the synapsin molecules does not dissociate from synaptic vesicles and is found in close proximity to the active zone membrane, within the readily releasable pool (RRP, in yellow). Here, synapsins might contribute to docking, post-docking and fusion events. (4) After synaptic vesicle fusion, synapsins re-localise in endocytic regions, where they interact with proteins involved in endocytosis and probably participate in the process. (5) After the reuptake, synapsins may stimulate actin polymerisation and contribute to redirecting vesicles back to the RP or RRP. SV synaptic vesicle (Reproduced with authors' permission from Cesca et al. 2010)

ability of synapsin I to interact with actin and synaptic vesicles (Bahler and Greengard 1987; Chi et al. 2001; Schiebler et al. 1986). Interestingly, although CaMKII and synapsin I are both expressed starting from early developmental stages, it has been shown that they are functionally coupled only in mature presynaptic terminals (Menegon et al. 2002). It has been hypothesised that, before synapse specification, synapsin I is responsible for the clustering of synaptic vesicles in the growth cone and that its function is primarily modulated by PKA. As synaptogenesis begins, in addition to PKA, synapsin becomes strongly dependent on CaMKII regulation (Bonanomi et al. 2005). In mature neurons, CaMKII phosphorylation was shown to be particularly relevant for controlling synaptic vesicle mobilisation under low-frequency stimulation (Chi et al. 2003).

Similar to site 1 phosphorylation, phosphorylation at sites 4 (Ser-62), 5 (Ser-67) and 6 (Ser-549) by MAPK/ERK (or by Cdk1 at site 6) results in a significant decrease in the ability of synapsin I to bind, bundle and polymerise actin but has no marked effects on its interaction with purified synaptic vesicles *in vitro* (Jovanovic et al. 1996). Opposite to what was observed with sites 1, 2 and 3, basal levels of phosphorylation on sites 4, 5 and 6 are quite high; thus they possibly mediate a tonic control on synapsin function (Jovanovic et al. 2000). Synapsin phosphorylation by MAPK is promoted by exposure to brain-derived neurotrophic factor (BDNF) and seems to be a key step in the BDNF-driven modulation of neurotransmitter release (Jovanovic et al. 1996, 2000). On the contrary, depolarisation induced by KCl results in a fast Ca^{2+} -dependent decrease in the phosphorylation level of these sites, mediated by calcineurin (Jovanovic et al. 2001). The kinetics of site 4/5/6 phosphorylation is strictly dependent on the frequency of stimulation: at low frequencies (5 Hz), MAPK activation prevails and synapsin I is phosphorylated on these sites; at high frequencies (10–20 Hz), calcineurin is strongly activated and the net effect is synapsin I dephosphorylation. The effects of MAPK/calcineurin on sites 4, 5 and 6 are relevant in modulating synapsin activity over a wide frequency range: synapsin is phosphorylated at low stimulation frequency and dephosphorylated at high frequency, thus mobilising synaptic vesicles for release (Chi et al. 2003). MAPK phosphorylation actively drives synaptic vesicle recruitment, probably through synapsin I detachment from the actin cytoskeleton, both in growth cones and mature synapses (Schenk et al. 2005).

Phosphorylation on sites 6 and 7 by Cdk5 has been less extensively studied (Matsubara et al. 1996). Interestingly, it has been recently shown that Cdk5 has a negative effect on synaptic vesicle mobilisation from the resting pool (Kim and Ryan 2010). Recent data identify synapsin I as the central switch in mediating the synaptic effects of Cdk5. Synapsin I is constitutively phosphorylated on the Cdk5 sites at rest. Phosphorylation of synapsin I by Cdk5 increases its binding to actin, leaving the interaction with synaptic vesicles unaltered (Verstegen, Fassio and Benfenati, personal communication).

Finally, phosphorylation of synapsin I at site 8 (Tyr-301, residing in domain C) by Src results in an increase in its ability to bind actin and synaptic vesicles and to dimerise. The overexpression of a site 8 dephosphomimetic mutant in hippocampal neurons in culture leads to increased vesicle exo-endocytosis upon

electrical stimulation, suggesting that phosphorylation on this site favours the sequestration of synaptic vesicles and negatively influences their recruitment from the resting pool (Messa et al. 2010). Depolarisation induces Src phosphorylation in a Ca^{2+} -dependent manner, with a peak at 2 min upon stimulation and persistence up to 30 min, raising the possibility that tyrosine phosphorylation counteracts the effects of serine phosphorylation late after stimulation in order to reassemble synaptic vesicle pools.

The various phosphorylation sites are subjected to dephosphorylation by distinct protein phosphatases. As already mentioned, sites 4, 5 and 6 are under a dominant control of calcineurin, while sites 1, 2 and 3 are selectively dephosphorylated by protein phosphatase 2A (Jovanovic et al. 2001).

Given the complexity of the network of phosphorylating/dephosphorylating enzymes contributing to synapsin regulation, the final effect of a stimulus might be hard to predict, depending on many concomitant factors such as the intensity and duration of stimulation, the selective signal-transduction pathway activated, the presence of synaptic efficacy modulators such as BDNF and the crosstalk among the various pathways. Recent evidence showed that the blockage of exocytosis as well as of endocytosis, while not hampering phosphorylation of the protein, impairs the diffusion of synapsin I in the axonal shaft that occurs upon stimulation. These observations suggest that, in addition to phosphorylation, other unknown signals coming from the plasma membrane affect synapsin I localisation within the presynaptic terminal (Orenbuch et al. 2012b).

Further complicating the picture, synapsins are subjected to other regulatory post-translational modifications, such as the addition of O-linked N-acetylglucosamine (O-GlcNAc) on serine and threonine residues and fucosylation. O-GlcNAc sites flank phosphorylation sites in domains B and D of synapsin I and modulate the phosphorylation state and functional properties of the protein (Cole and Hart 1999; Luthi et al. 1991; Skorobogatko et al. 2014; Tallent et al. 2009). Fucosylation regulates synapsin I turnover, preventing the protein from being degraded by the Ca^{2+} -activated protease calpain (Murrey et al. 2006).

In mature synapses, many evidences suggest that synapsins are primarily involved in keeping a large proportion of synaptic vesicles unavailable for exocytosis, through reversible anchorage to each other and to the actin cytoskeleton; thereby they are thought to maintain the resting pool of synaptic vesicles (Benfenati et al. 1991). A small subset of vesicles, referred to as the readily releasable pool, is instead docked at the plasma membrane and is relatively depleted of synapsin immunoreactivity (Pieribone et al. 1995; Siksou et al. 2007). Injection of anti-synapsin I antibodies in the lamprey reticulospinal axons resulted in depletion of the distal cluster of synaptic vesicles, while the number of vesicles adjacent to the plasma membrane was unaffected (Pieribone et al. 1995). In addition, disruption of synapsin function by injection of a domain E-derived peptide dramatically decreased the number of vesicles distant from the plasma membrane, leaving docked vesicles unaltered (Hilfiker et al. 1998).

In synapsin knockout (KO) mice, the number of distal synaptic vesicles (>100 nm apart from the active zone) was greatly reduced with respect to the

wild type (WT) (Gitler et al. 2004a; Rosahl et al. 1995; Siksou et al. 2007). Analysis of the three-dimensional structure of the presynaptic terminal using advanced electron tomography showed that synaptic vesicles are linked to each other by short 30 nm-long filaments, consistent with synapsin dimension and structure. In mice deleted for all synapsin genes, the connecting filaments among synaptic vesicles were still present (Siksou et al. 2007), indicating that additional players participate in vesicle clustering (Shupliakov et al. 2011). However, in single as well as double or triple KO hippocampal neurons in culture, the reduction in the number of synaptic vesicles at presynaptic terminals was paralleled by vesicle relocalisation in adjacent axonal shafts (Fornasiero et al. 2012; Orenbuch et al. 2012a). Notably, the dispersion of synaptic vesicles could be rescued by chronic inhibition of synaptic activity, indicating that molecules other than synapsins are able to cluster synaptic vesicles at the nerve terminal but that synapsins are essential for retaining vesicles at release sites during neuronal activity (Fornasiero et al. 2012). Overexpression of synapsin IIa appears sufficient to rescue the synaptic vesicle reduction and dispersion observed in triple KO neurons (Gitler et al. 2008; Orenbuch et al. 2012a).

13.4.2 Post-docking Roles

Synapsins have been proposed to have a role also in the late stages of synaptic vesicle life cycle in the presynaptic terminal, after vesicles have docked or fused. As mentioned above, in stimulated nerve terminals, synapsins are to a great extent dissociated from the synaptic vesicle membrane. However, dissociation is not complete (Chi et al. 2001; Torri-Tarelli et al. 1990, 1992). In the lamprey giant synapses, stimulation increases the fraction of synapsin I detected in the pool of vesicles proximal to the active zone (Bloom et al. 2003).

The injection of peptides from synapsin domain E and C inhibited and slowed the kinetics of neurotransmitter release. This effect was accompanied by a reduction in the number of vesicles in the resting pool but not of the docked vesicles, suggesting that synapsins may have a second function much more related to the priming/fusion process itself (Hilfiker et al. 1998, 2005). These observations are consistent with the finding that synapsin I KO mice display glutamate release defects without changes in the number of docked vesicles (Li et al. 1995). Interestingly, in Purkinje cells, the overexpression of domain E peptides still resulted in a decrease of vesicles in the resting pool but not in the readily releasable pool, and, opposite to glutamate, it accelerated GABA release kinetics, suggesting that synapsins may exert differential modulation of vesicle fusion at glutamatergic and GABAergic synapses (Fassio et al. 2006). As previously mentioned, synapsin I, and to a lower extent synapsin II, interacts with the vesicle-associated small G protein Rab3A (Giovedi et al. 2004b). GTP-bound Rab3A is able to associate with the synaptic vesicle membrane, and it negatively influences vesicle priming and fusion. Upon GTP hydrolysis, GDP-Rab3A detaches from synaptic vesicles

and relieves the inhibition. The interaction with synapsin I stimulates the GTPase activity of Rab3A. Thus, synapsin I may stimulate priming/fusion by accelerating GTP hydrolysis and Rab3A detachment from synaptic vesicles (Giovedi et al. 2004a).

Actin filaments predominantly decorate the periphery of the synaptic vesicle cluster, while are rarely detected inside, thus providing a structural scaffold for vesicle accumulation. Upon synaptic activation, actin filaments surrounding the cluster further polymerise, probably contributing to vesicle endocytosis and recycling – even though the actual role of actin in modulating synaptic vesicle recycling is largely debated (Sankaranarayanan et al. 2003; Shupliakov et al. 2002). Interestingly, in stimulated synapses, synapsin was observed within the filamentous actin cytomatrix lateral to the active zone, where endocytosis takes place, in association with synaptic vesicles recycling back to the cluster (Bloom et al. 2003). This observation raised the possibility that synapsins participate in endocytosis of synaptic vesicles.

Synapsin was never detected on clathrin-coated intermediates, indicating that it probably dissociates before endocytosis and re-associates once synaptic vesicles have been released in the cytoplasm (Bloom et al. 2003; Torri Tarelli et al. 1992). Given these observations, the model proposed is the following: synapsin at rest is localised mainly in the resting pool and preserves its clustering; upon stimulation synapsin dissociates and accumulates in the endocytic zone, where it promotes actin polymerisation and contributes to the recycling of synaptic vesicles back to the recycling or resting pool. It has been hypothesised that this function of synapsin I could be modulated by MAPK phosphorylation: MAPK sites are phosphorylated under basal conditions and dephosphorylated by calcineurin upon stimulation (Jovanovic et al. 2001). Calcineurin is implicated in the coordinated depolarisation-induced dephosphorylation of other proteins as well, such as amphiphysin I/II, dynamin I and synaptojanin. These proteins are now classified as ‘dephosphins’; they are all involved in synaptic vesicle endocytosis, and their calcineurin-mediated dephosphorylation is necessary for triggering the process (Cousin and Robinson 2001). It is possible that in the endocytic zone synapsin interacts with some of these regulators, thus modulating synaptic vesicle recycling. Indeed, a direct interaction of amphiphysin I/II with the SH3-binding motif present in domain D of synapsin I has been demonstrated (Onofri et al. 2000). Synapsin I is also able to interact with intersectin, which in turn regulates dynamin function in vesicle fission (Evergren et al. 2006).

13.5 Role of Synapsins in Synaptic Transmission and Plasticity

A large number of studies have addressed the contribution of synapsins to synaptic transmission and plasticity, mainly taking advantage of mice deleted for one or more synapsin genes. The data generated are quite complex to interpret, and in

more than one case, they are difficult to reconcile with each other, partly because of differences in the methods used and partly because of possible compensatory mechanisms that may confound the results. Synapsin KO mice are viable and fertile, with normal life expectancy and no gross brain abnormalities (Gitler et al. 2004a; Rosahl et al. 1995), even though this family of proteins has been extensively implicated also in neuronal development and synaptogenesis (Fornasiero et al. 2010). Thus, despite their abundance in neuronal tissue, synapsins are not essential for overall brain function.

Mice deleted for synapsins, with the notable exception of synapsin III KO mice (Feng et al. 2002), experience generalised tonic-clonic seizures starting from the second month of age (Cambiaghi et al. 2013; Etholm et al. 2011; Feng et al. 2002; Ketzeff et al. 2011; Li et al. 1995; Rosahl et al. 1993, 1995). Importantly, several nonsense and missense mutations have been identified in the human *SYN1* gene in families and sporadic individuals affected by epilepsy and/or autism spectrum disorders (Fassio et al. 2011; Garcia et al. 2004; Giannandrea et al. 2013; Lignani et al. 2013). The *SYN2* gene has been recognised as a susceptibility locus for epilepsy (Cavalleri et al. 2007; Lakhan et al. 2010), and various mutations have been recently described in patients affected by autism or epilepsy (Corradi et al. 2014). This strong association with epilepsy suggests that synapsins may have a role in the control of the excitability of neuronal networks and that their absence could lead to excitation/inhibition imbalance.

13.5.1 Synapsins and Synaptic Transmission: Differential Role at Excitatory and Inhibitory Synapses

As previously mentioned, injection experiments in which either phospho- or dephosphorylated synapsin I, as well as peptides or anti-peptide antibodies, were delivered into presynaptic terminals support a model whereby dephospho-synapsins act as a physical constraint on the resting pool of synaptic vesicles, while the PKA- or CaM kinase-phosphorylated forms leave synaptic vesicles free to fuse with the plasma membrane. This action, aside from being important for the formation and maintenance of the resting pool of vesicles, implies that synapsins are involved in the fine regulation of the equilibrium between the resting and the recycling pool of vesicles, thus determining the amount of neurotransmitter released (Benfenati et al. 1991; Fassio et al. 2011 [la review di Sem. Cell. Dev. Biol.]). Indeed, the injection of dephosphorylated synapsin I into the squid giant synapse decreased the amplitude and rate of rise of postsynaptic potentials, whereas the injection of either phosphorylated or heat-inactivated protein was ineffective. Conversely, injection of CaMKII increased the amplitude and rate of rise of postsynaptic potentials (Llinas et al. 1985, 1991). The injection of antibodies directed against the domain E of synapsin I in lamprey reticulospinal presynaptic terminals caused the depletion of the resting pool of vesicles and enhanced depression following high-, but not

low-, frequency stimulation (Pieribone et al. 1995). Similar results were obtained after the injection of peptides derived from domain E and C in the squid giant synapse (Hilfiker et al. 1998, 2005).

In agreement with these observations, all synapsin KO mice, with the exception of *Syn3*^{-/-} (Feng et al. 2002), display a reduction of the resting and recycling pools and, consequently, an increased synaptic fatigue under high-frequency stimulation that is particularly pronounced in glutamatergic terminals. The study of synaptic vesicle recycling at single synaptic boutons using FM dyes showed that the number of vesicles exocytosed during brief action potential trains and the total recycling pool were reduced in *Syn1*^{-/-} neurons, while the kinetics of endocytosis was unaltered (Ryan et al. 1996). Similar results were obtained using the pH-sensitive probe synaptopHluorin in triple KO neurons, in which the recycling pool was significantly reduced, while the readily releasable pool was unaffected (Fornasiero et al. 2012). In *Syn1*^{-/-} mice glutamate release from cortical synaptosomes was impaired and the recovery of synaptic transmission after high-frequency stimulation was delayed (Li et al. 1995). A moderate increase of synaptic depression was observed also at inhibitory synapses of cultured *Syn1*^{-/-} neurons at high stimulation frequencies, accompanied by a slowdown of recovery from depression (Baldelli et al. 2007). Synaptic depression after repetitive stimulation was increased at excitatory, but not inhibitory, neurons in *Syn2*^{-/-} (Medrihan et al. 2013; Rosahl et al. 1995) and further enhanced in excitatory terminals of *Syn1/2*^{-/-} (Rosahl et al. 1995), as well as triple KO animals (Farisello et al. 2013; Gitler et al. 2004a; Vasileva et al. 2012), probably because of defective resting pool size and recruitment of vesicles to the readily releasable pool.

Overexpression of synapsin Ia in the rat calyx of Held resulted in the redistribution of vesicles in the terminal and increased depression during repetitive stimulation, possibly because the excess synapsin molecules favoured the formation of dimers not associated with the vesicle membrane, thereby interfering with synaptic vesicle clustering. Recovery from depression was accelerated, possibly through synapsin-actin interactions that speeded up vesicle endocytosis and recycling (Vasileva et al. 2013).

Conversely, mice lacking synapsin III exhibit no change in vesicle density, an increase in the size of the recycling pool and a significant reduction in synaptic depression (Feng et al. 2002), suggesting that synapsin III does not participate to the maintenance of the resting pool but is involved in limiting the number of recycling vesicles.

In addition to the pre-docking roles mainly responsible for the effects described so far, post-docking roles have also been hypothesised for synapsins, contributing to the alteration of neurotransmitter release observed upon isolated action potentials when synapsin function is perturbed. The injection of domain E and C peptides significantly reduced the amplitude and slowed the kinetics of evoked excitatory postsynaptic currents (eEPSCs), without altering the number of docked vesicles (Hilfiker et al. 1998). The lack of synapsin I induced a decrease in the amplitude of the evoked inhibitory postsynaptic currents (eIPSCs) in cultured *Syn1*^{-/-} hippocampal autaptic neurons due to a reduced number of vesicles released per

single action potential and a reduced readily releasable pool, with no change in the probability of release, quantal size or number of synaptic contacts (Baldelli et al. 2007; Chiappalone et al. 2009). Conversely, the lack of synapsin II leads to an increase in the amplitude of eIPSCs in the dentate gyrus granule neurons (Medrihan et al. 2013). A decrease of eIPSC amplitude was observed in autaptic neurons from *Syn3*^{-/-} (Feng et al. 2002).

In triple KO neurons the reduction in eIPSC amplitude was accompanied by a decreased number of docked vesicles in inhibitory terminals, as assessed by electron microscopy (Gitler et al. 2004a). Opposite to inhibitory transmission, *Syn1*^{-/-} cortical autaptic neurons displayed an increase in eEPSC amplitude attributable to an increased readily releasable pool, with no change in release probability (Chiappalone et al. 2009).

It is worth to note that spontaneous synaptic transmission is unaffected in the absence of synapsins, as the amplitude, frequency and kinetics of both miniature EPSCs and IPSCs were essentially unchanged in all synapsin KO mice (Baldelli et al. 2007; Chiappalone et al. 2009; Feliciano et al. 2013; Feng et al. 2002; Gitler et al. 2004a), indicating that the number of synaptic contacts and the postsynaptic apparatus are comparable in WT and KO neurons.

A more specific role for synapsin II in the regulation of GABA release has been recently unravelled. In granule neurons of the dentate gyrus, synapsin II deletion resulted in higher amplitude and faster kinetics of eIPSCs, due to an increase in the synchronously released fraction of the readily releasable pool. High-frequency stimulation causes, in addition to synchronous release, a delayed asynchronous release component that lasts up to seconds. The absence of synapsin II selectively and dramatically hampered asynchronous release at inhibitory, but not excitatory, synapses. Synapsin II controlled the amount of the Ca²⁺-dependent asynchronous release, through the interaction with the P/Q-type of Ca²⁺ channels. Opposite to synapsin II, synapsin I deletion increased the asynchronous release at inhibitory synapses (Medrihan et al. 2013). In this scenario, synapsins I and II appear to compete at a post-docking step on the same readily releasable pool of inhibitory vesicles, by either facilitating release synchronisation or desynchronising GABA release, respectively.

The results described so far highlight the differential effects of the synapsins in excitatory and inhibitory transmission. This difference may at least partly stem from differential expression levels of synapsin isoforms in the two neuronal subtypes. As an example, excitatory synapses positive for VGLUT1 (vesicular glutamate transporter 1) and inhibitory terminals positive for VGAT (vesicular GABA transporter) were shown to express the highest and the lowest levels of synapsin I, respectively (Micheva et al. 2010). Interestingly, other vesicular/presynaptic proteins, such as SV2B, synaptophysin, syntaxin 1A, SNAP-25 or 23 and synaptotagmins, showed a preferential expression in VGLUT1- compared to VGAT-positive synapses, suggesting that probably the release machineries in the two kinds of synaptic terminals slightly differ in order to match distinct physiological needs (Bragina et al. 2007; Gronborg et al. 2010). Synapsin ablation seems to have a stronger effect on the depression of excitatory synapses, but the milder

effect observed in inhibitory synapses could have a higher impact on the network excitability, because of the high-frequency bursting activity that characterises inhibitory neurons and makes them more sensitive to vesicle depletion. At the post-docking level, synapsin deletion mainly impairs inhibitory transmission, while leaving basal excitatory transmission unaffected or even enhanced.

The final outcome of these effects is an imbalance between excitatory and inhibitory inputs, with a hyperexcitability of the network that is probably at the basis of the epileptic phenotype observed in all synapsin KO mice, but *Syn3*^{-/-}. The absence of the epileptic phenotype in *Syn3*^{-/-} mice strengthens the hypothesis that Syn III plays a differential role with respect to the other isoforms, as suggested by electrophysiology (Feng et al. 2002) and by the observation that its expression progressively drops in the adult life.

The hyperexcitability of mature Syn-deficient neuronal networks was investigated through the use of the multi-electrode array (MEA) platform. Primary cultures of cortical neurons derived from *Syn1*^{-/-} mice displayed a diffuse spontaneous hyperactivity characterised by a general increase in firing rate, bursting rate and burst duration, as well as a higher degree of activity synchronisation, that became more evident at later stages of network maturation (Chiappalone et al. 2009). Studies in acute cortico-hippocampal brain slices of 3-week-old triple KO mice revealed that hyperexcitability of synapsin KO circuits is detectable before the incidence of manifest epileptic seizures, which arise in the animals at 2 months of age. Slices treated with the K⁺ channel blocker 4-aminopyridine were characterised by a higher incidence of fast glutamatergic inter-ictal events and a wider spread of ictal activity to the surrounding cortical areas with respect to age-matched WT slices (Boido et al. 2010). The membrane potential of CA1 hippocampal triple KO neurons was found to be more depolarised than in WT cells in presymptomatic mice and even more so in adult animals. This observation was attributed to a decrease in tonic GABA currents, due to a defective GABA release and spillover (Farisello et al. 2013).

In addition to the differential effects of synapsin deletion on the intrinsic characteristics of excitatory and inhibitory neurons, the net effect on a formed neuronal network also depends on dynamic adaptations to an altered equilibrium (homeostatic plasticity), synaptic plasticity phenomena and developmental components. The existence of compensatory adaptations was recently proposed, starting from the observation that the GABA-synthesising enzyme GAD67 was upregulated in triple KO animals (Ketzel et al. 2011). An increase in inhibitory transmission in specific developmental windows might partly contribute to suppress the seizures until 2 months of age or, vice versa, it might contribute to epileptogenesis and network hypersynchronisation. Indeed, it was recently shown that the altered inhibitory activity is subjected to developmental modulation in synapsin KO mice: sIPSC amplitude and frequency (recorded from layer 5 pyramidal neurons of the entorhinal cortex) were indeed reduced in 2/3-week-old triple KO slices with respect to WT; the differences between genotypes disappeared between 1.5 and 3.5 months of age; in mice older than 7 months of age, sIPSC amplitude and frequency were larger in KO than in WT (Ketzel and Gitler 2014). This may indicate that the failure of phasic inhibitory transmission at the beginning of KO

mice development may contribute to epileptogenesis, but then compensatory mechanisms emerge that try to counteract the established epilepsy, but ultimately aggravate the network synchronisation.

13.5.2 *Involvement of Synapsins in Synaptic Plasticity Phenomena*

A vast amount of studies have been performed, aimed at analysing the potential involvement of synapsins in short- and long-term plasticity phenomena in the brain.

Concerning short-term plasticity phenomena, the most studied paradigms are paired-pulse facilitation or depression (PPF or PPD, respectively) and post-tetanic potentiation (PTP) (Zucker and Regehr 2002). PPF was increased in excitatory cortical neurons from *Syn1*^{-/-} mice (Chiappalone et al. 2009), as well as in CA1 pyramidal neurons of *Syn1*^{-/-} hippocampal slices (Rosahl et al. 1993). Synapsin I deletion did not affect the paired-pulse ratio in inhibitory neurons (Baldelli et al. 2007; Chiappalone et al. 2009). *Syn2*^{-/-} CA1 pyramidal neurons (Rosahl et al. 1995) and *Syn3*^{-/-} cultured hippocampal neurons (Feng et al. 2002) showed no changes in PPF. PPD was not affected in *Syn2*^{-/-} at short inter-stimulus intervals but was increased at longer intervals (2–3 s) (Medrihan et al. 2013). Interestingly, in triple KO CA1 pyramidal neurons, PPF was increased in young presymptomatic animals but normalised to the WT condition in adult epileptic mice. PPD was slightly increased in young triple KO mice at short inter-stimulus intervals, while it was significantly lower in adult epileptic mice compared to WT (Farisello et al. 2013). The paired-pulse ratio at short inter-stimulus intervals has an intrinsic kinetics of milliseconds, too fast to be dependent on vesicle mobilisation from the resting pool. Thus, synapsins probably affect PPF and PPD through post-docking mechanisms.

PTP was unchanged in *Syn1*^{-/-} slices with respect to WT, while it was decreased in *Syn2*^{-/-} and even more affected in double KO preparations, indicating that both synapsins I and II participate in modulating this short-term increase in synaptic efficacy (Rosahl et al. 1995). PTP was completely abolished in triple KO slices compared to WT (Farisello et al. 2013). PTP of inhibitory transmission was not affected in *Syn1*^{-/-} neurons (Baldelli et al. 2007). PTP operates in a timescale of seconds and probably involves the Ca²⁺-dependent mobilisation of synaptic vesicles from the resting pool. Thus, synapsins are likely to affect this short-term plasticity paradigm at a pre-docking step, by facilitating vesicle recruitment upon their phosphorylation.

Recently, a new form of short-term plasticity has been described in the CA3-to-CA1 hippocampal synapses. During prolonged stimulation at 5–20 Hz, after a first rapid increase in synaptic efficacy and a subsequent decay, a second delayed response enhancement (DRE) arises in 3–4 s and lasts for approximately

75 additional stimuli. The DRE is strictly dependent on temperature, as it clearly appears at 37 °C but not at 24 °C, on the presence of an intact actin cytoskeleton and of synapsin I and/or II, as it completely disappeared in synapsin I/II double KO hippocampal slices (Bogen et al. 2009; Jensen et al. 2007). This form of plasticity may be important for maintaining synaptic activity during prolonged stimulation periods and may rely both on the synapsin-mediated recruitment of synaptic vesicles from the resting pool and on post-docking functions of these proteins.

Mice deleted for synapsin genes do not exhibit long-term potentiation (LTP) defects (Li et al. 1995; Rosahl et al. 1995; Spillane et al. 1995) and display mild memory and learning deficits (Corradi et al. 2008; Gitler et al. 2004a; Greco et al. 2013; Silva et al. 1996). However, a primary role of synapsin I in mediating LTP and learning has been recently proposed. Synapsin I and ERK were shown to be phosphorylated during contextual fear conditioning. In addition, transgenic mice overexpressing a constitutively active form of p21^{Ras}, a potent upstream synaptic activator of ERK, displayed an increased phosphorylation of both ERK and synapsin I (site 4/5) and enhanced presynaptic short-term plasticity and LTP induced by high-frequency stimulation. These functional effects were dependent on the presence of synapsin I and were probably mediated by a facilitation of neurotransmitter release due to increased recruitment of synaptic vesicles from the resting pool to the readily releasable pool, as the number of docked vesicles and the frequency of miniature EPSCs were higher in transgenic mice with respect to WT mice. The activation of the p21^{Ras}/ERK/synapsin I signalling pathway was accompanied by enhancement of hippocampus-dependent learning abilities of transgenic mice mediated by synapsin I, as this effect was abolished by crossing transgenic mice with synapsin I KO animals (Kushner et al. 2005). BDNF may be the trigger of p21^{Ras}/ERK/synapsin I signalling activation during learning (Tyler et al. 2002). A role for BDNF has already been proved for PTP, which is enhanced by BDNF-driven MAPK phosphorylation of synapsin I (Valente et al. 2012).

Synapsin I levels were found to be increased during LTP and spatial learning in the rat hippocampus (Gomez-Pinilla et al. 2001; Sato et al. 2000), as well as at the sensorimotor synapse in the invertebrate model *Aplysia* in which potentiation had been induced with serotonin treatment (Hart et al. 2011). In the latter case, the serotonin-induced increase in synapsin levels was required for the establishment of LTP, as acute administration of small interfering RNAs that blocked synapsin induction completely abolished synaptic potentiation (Hart et al. 2011).

Synapsin was also implicated in learning and behavioural paradigms in *Drosophila*. In fact, the protein is necessary for the formation of odour-sugar memory traces in the mushroom bodies and requires functional PKA phosphorylation sites to exert this effect (Michels et al. 2011). Synapsin function is specifically required in GABAergic interneurons for short-term, but not long-term, habituation of olfactory avoidance behaviour, and this effect implies CaMKII phosphorylation (Sadanandappa et al. 2013).

13.6 Concluding Remarks

Synapsins have been classically studied for their contribution to synaptic vesicle storage in the presynaptic terminal. Recent evidences described in this chapter point to the fact that, although synapsins play a crucial role in the maintenance of synaptic vesicle clusters, this phenomenon involves also other mechanisms and that, on the other hand, many other functions can be envisaged for synapsins both at the pre- and the post-docking levels. In addition, differential roles start to be depicted for each synapsin isoform. How the various isoforms distribute in the central nervous system and take part in the definition of the functional nature of each neuronal subpopulation is far from being completely understood.

Mutations in synapsins have been strongly linked to epilepsy and autism spectrum disorders (Corradi et al. 2014; Fassio et al. 2011; Garcia et al. 2004), and several polymorphisms have been positively associated with schizophrenia and other psychosis (Chen et al. 2004a, b; Lee et al. 2005; Saviouk et al. 2007). Thus, the thorough unravelling of the precise roles exerted by synapsins under physiological and pathological conditions is fundamental for envisaging new therapeutic approaches for these unresolved diseases.

References

- Bahler M, Greengard P (1987) Synapsin I bundles F-actin in a phosphorylation-dependent manner. *Nature* 326:704–707
- Bahler M, Benfenati F, Valtorta F, Czernik AJ, Greengard P (1989) Characterization of synapsin I fragments produced by cysteine-specific cleavage: a study of their interactions with F-actin. *J Cell Biol* 108:1841–1849
- Baldelli P, Fassio A, Valtorta F, Benfenati F (2007) Lack of synapsin I reduces the readily releasable pool of synaptic vesicles at central inhibitory synapses. *J Neurosci* 27:13520–13531
- Benfenati F, Bahler M, Jahn R, Greengard P (1989a) Interactions of synapsin I with small synaptic vesicles: distinct sites in synapsin I bind to vesicle phospholipids and vesicle proteins. *J Cell Biol* 108:1863–1872
- Benfenati F, Greengard P, Brunner J, Bahler M (1989b) Electrostatic and hydrophobic interactions of synapsin I and synapsin I fragments with phospholipid bilayers. *J Cell Biol* 108:1851–1862
- Benfenati F, Valtorta F, Bahler M, Greengard P (1989c) Synapsin I, a neuron-specific phosphoprotein interacting with small synaptic vesicles and F-actin. *Cell Biol Int Rep* 13:1007–1021
- Benfenati F, Neyroz P, Bahler M, Masotti L, Greengard P (1990) Time-resolved fluorescence study of the neuron-specific phosphoprotein synapsin I. Evidence for phosphorylation-dependent conformational changes. *J Biol Chem* 265:12584–12595
- Benfenati F, Valtorta F, Greengard P (1991) Computer modeling of synapsin I binding to synaptic vesicles and F-actin: implications for regulation of neurotransmitter release. *Proc Natl Acad Sci U S A* 88:575–579
- Benfenati F, Valtorta F, Rubenstein JL, Gorelick FS, Greengard P, Czernik AJ (1992) Synaptic vesicle-associated Ca^{2+} /calmodulin-dependent protein kinase II is a binding protein for synapsin I. *Nature* 359:417–420

- Benfenati F, Valtorta F, Rossi MC, Onofri F, Sihra T, Greengard P (1993) Interactions of synapsin I with phospholipids: possible role in synaptic vesicle clustering and in the maintenance of bilayer structures. *J Cell Biol* 123:1845–1855
- Bloom O, Evergren E, Tomilin N, Kjaerulf O, Low P, Brodin L, Pieribone VA, Greengard P, Shupliakov O (2003) Colocalization of synapsin and actin during synaptic vesicle recycling. *J Cell Biol* 161:737–747
- Bogen IL, Jensen V, Hvalby O, Walaas SI (2009) Synapsin-dependent development of glutamatergic synaptic vesicles and presynaptic plasticity in postnatal mouse brain. *Neuroscience* 158:231–241
- Boido D, Farisello P, Cesca F, Ferrea E, Valtorta F, Benfenati F, Baldelli P (2010) Cortico-hippocampal hyperexcitability in synapsin I/II/III knockout mice: age-dependency and response to the antiepileptic drug levetiracetam. *Neuroscience* 171:268–283
- Bonomoni D, Menegon A, Miccio A, Ferrari G, Corradi A, Kao HT, Benfenati F, Valtorta F (2005) Phosphorylation of synapsin I by cAMP-dependent protein kinase controls synaptic vesicle dynamics in developing neurons. *J Neurosci* 25:7299–7308
- Bragina L, Candiracci C, Barbaresi P, Giovedi S, Benfenati F, Conti F (2007) Heterogeneity of glutamatergic and GABAergic release machinery in cerebral cortex. *Neuroscience* 146:1829–1840
- Brautigam CA, Chelliah Y, Deisenhofer J (2004) Tetramerization and ATP binding by a protein comprising the A, B, and C domains of rat synapsin I. *J Biol Chem* 279:11948–11956
- Cambiaghi M, Cursi M, Monzani E, Benfenati F, Comi G, Minicucci F, Valtorta F, Leocani L (2013) Temporal evolution of neurophysiological and behavioral features of synapsin I/II/III triple knock-out mice. *Epilepsy Res* 103:153–160
- Cavalleri GL, Weale ME, Shianna KV, Singh R, Lynch JM, Grinton B, Szoek C, Murphy K, Kinirons P, O'Rourke D, Ge D, Depondt C, Claeys KG, Pandolfo M, Gumbs C, Walley N, McNamara J, Mulley JC, Linney KN, Sheffield LJ, Radtke RA, Tate SK, Chisoe SL, Gibson RA, Hosford D, Stanton A, Graves TD, Hanna MG, Eriksson K, Kantanen AM, Kalviainen R, O'Brien TJ, Sander JW, Duncan JS, Scheffer IE, Berkovic SF, Wood NW, Doherty CP, Delanty N, Sisodiya SM, Goldstein DB (2007) Multicentre search for genetic susceptibility loci in sporadic epilepsy syndrome and seizure types: a case-control study. *Lancet Neurol* 6:970–980
- Ceccaldi PE, Grohovaz F, Benfenati F, Chiergatti E, Greengard P, Valtorta F (1995) Dephosphorylated synapsin I anchors synaptic vesicles to actin cytoskeleton: an analysis by videomicroscopy. *J Cell Biol* 128:905–912
- Cesca F, Baldelli P, Valtorta F, Benfenati F (2010) The synapsins: key actors of synapse function and plasticity. *Prog Neurobiol* 91:313–348
- Cheetham JJ, Hilfiker S, Benfenati F, Weber T, Greengard P, Czernik AJ (2001) Identification of synapsin I peptides that insert into lipid membranes. *Biochem J* 354:57–66
- Cheetham JJ, Murray J, Ruhkalova M, Cuccia L, McAloney R, Ingold KU, Johnston LJ (2003) Interaction of synapsin I with membranes. *Biochem Biophys Res Commun* 309:823–829
- Chen Q, He G, Qin W, Chen QY, Zhao XZ, Duan SW, Liu XM, Feng GY, Xu YF, St Clair D, Li M, Wang JH, Xing YL, Shi JG, He L (2004a) Family-based association study of synapsin II and schizophrenia. *Am J Hum Genet* 75:873–877
- Chen Q, He G, Wang XY, Chen QY, Liu XM, Gu ZZ, Liu J, Li KQ, Wang SJ, Zhu SM, Feng GY, He L (2004b) Positive association between synapsin II and schizophrenia. *Biol Psychiatry* 56:177–181
- Chi P, Greengard P, Ryan TA (2001) Synapsin dispersion and recluster during synaptic activity. *Nat Neurosci* 4:1187–1193
- Chi P, Greengard P, Ryan TA (2003) Synaptic vesicle mobilization is regulated by distinct synapsin I phosphorylation pathways at different frequencies. *Neuron* 38:69–78
- Chiappalone M, Casagrande S, Tedesco M, Valtorta F, Baldelli P, Martinoia S, Benfenati F (2009) Opposite changes in glutamatergic and GABAergic transmission underlie the diffuse hyperexcitability of synapsin I-deficient cortical networks. *Cereb Cortex* 19:1422–1439

- Chin LS, Li L, Greengard P (1994) Neuron-specific expression of the synapsin II gene is directed by a specific core promoter and upstream regulatory elements. *J Biol Chem* 269:18507–18513
- Cole RN, Hart GW (1999) Glycosylation sites flank phosphorylation sites on synapsin I: O-linked N-acetylglucosamine residues are localized within domains mediating synapsin I interactions. *J Neurochem* 73:418–428
- Corradi A, Zanardi A, Giacomini C, Onofri F, Valtorta F, Zoli M, Benfenati F (2008) Synapsin-I and synapsin-II-null mice display an increased age-dependent cognitive impairment. *J Cell Sci* 121:3042–3051
- Corradi A, Fadda M, Piton A, Patry L, Marte A, Rossi P, Cadieux-Dion M, Gauthier J, Lapointe L, Mottron L, Valtorta F, Rouleau GA, Fassio A, Benfenati F, Cossette P (2014) SYN2 is an autism predisposing gene: loss-of-function mutations alter synaptic vesicle cycling and axon outgrowth. *Hum Mol Genet* 23:90–103
- Cousin MA, Robinson PJ (2001) The dephosphins: dephosphorylation by calcineurin triggers synaptic vesicle endocytosis. *Trends Neurosci* 24:659–665
- Czernik AJ, Pang DT, Greengard P (1987) Amino acid sequences surrounding the cAMP-dependent and calcium/calmodulin-dependent phosphorylation sites in rat and bovine synapsin I. *Proc Natl Acad Sci U S A* 84:7518–7522
- Daly C, Ziff EB (1997) Post-transcriptional regulation of synaptic vesicle protein expression and the developmental control of synaptic vesicle formation. *J Neurosci* 17:2365–2375
- De Camilli P, Cameron R, Greengard P (1983a) Synapsin I (protein I), a nerve terminal-specific phosphoprotein. I. Its general distribution in synapses of the central and peripheral nervous system demonstrated by immunofluorescence in frozen and plastic sections. *J Cell Biol* 96:1337–1354
- De Camilli P, Harris SM Jr, Huttner WB, Greengard P (1983b) Synapsin I (Protein I), a nerve terminal-specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes. *J Cell Biol* 96:1355–1373
- De Camilli P, Benfenati F, Valtorta F, Greengard P (1990) The synapsins. *Annu Rev Cell Biol* 6:433–460
- Ekici M, Schmitz F, Hohl M, Seigel GM, Thiel G (2008) Chromatin structure and expression of synapsin I and synaptophysin in retinal precursor cells. *Neurochem Int* 53:165–172
- Esser L, Wang CR, Hosaka M, Smagula CS, Südhof TC, Deisenhofer J (1998) Synapsin I is structurally similar to ATP-utilizing enzymes. *EMBO J* 17:977–984
- Etholm L, Linden H, Eken T, Heggelund P (2011) Electroencephalographic characterization of seizure activity in the synapsin I/II double knockout mouse. *Brain Res* 1383:270–288
- Evergren E, Zotova E, Brodin L, Shupliakov O (2006) Differential efficiency of the endocytic machinery in tonic and phasic synapses. *Neuroscience* 141:123–131
- Farisello P, Boido D, Nieus T, Medrihan L, Cesca F, Valtorta F, Baldelli P, Benfenati F (2013) Synaptic and extrasynaptic origin of the excitation/inhibition imbalance in the hippocampus of synapsin I/II/III knockout mice. *Cereb Cortex* 23:581–593
- Fassio A, Merlo D, Mapelli J, Menegon A, Corradi A, Mete M, Zappettini S, Bonanno G, Valtorta F, D'Angelo E, Benfenati F (2006) The synapsin domain E accelerates the exocytotic cycle of synaptic vesicles in cerebellar Purkinje cells. *J Cell Sci* 119:4257–4268
- Fassio A, Patry L, Congia S, Onofri F, Piton A, Gauthier J, Pozzi D, Messa M, Defranchi E, Fadda M, Corradi A, Baldelli P, Lapointe L, St-Onge J, Meloche C, Mottron L, Valtorta F, Khoa Nguyen D, Rouleau GA, Benfenati F, Cossette P (2011) SYN1 loss-of-function mutations in autism and partial epilepsy cause impaired synaptic function. *Hum Mol Genet* 20:2297–2307
- Feliciano P, Andrade R, Bykhovskaia M (2013) Synapsin II and Rab3a cooperate in the regulation of epileptic and synaptic activity in the CA1 region of the hippocampus. *J Neurosci* 33:18319–18330
- Feng J, Chi P, Blanpied TA, Xu Y, Magarinos AM, Ferreira A, Takahashi RH, Kao HT, McEwen BS, Ryan TA, Augustine GJ, Greengard P (2002) Regulation of neurotransmitter release by synapsin III. *J Neurosci* 22:4372–4380

- Ferreira A, Kao HT, Feng J, Rapoport M, Greengard P (2000) Synapsin III: developmental expression, subcellular localization, and role in axon formation. *J Neurosci* 20:3736–3744
- Fesce R, Benfenati F, Greengard P, Valtorta F (1992) Effects of the neuronal phosphoprotein synapsin I on actin polymerization. II. Analytical interpretation of kinetic curves. *J Biol Chem* 267:11289–11299
- Forn J, Greengard P (1978) Depolarizing agents and cyclic nucleotides regulate the phosphorylation of specific neuronal proteins in rat cerebral cortex slices. *Proc Natl Acad Sci U S A* 75:5195–5199
- Fornasiero EF, Bonanomi D, Benfenati F, Valtorta F (2010) The role of synapsins in neuronal development. *Cell Mol Life Sci* 67:1383–1396
- Fornasiero EF, Raimondi A, Guarnieri FC, Orlando M, Fesce R, Benfenati F, Valtorta F (2012) Synapsins contribute to the dynamic spatial organization of synaptic vesicles in an activity-dependent manner. *J Neurosci* 32:12214–12227
- Garcia CC, Blair HJ, Seager M, Coulthard A, Tennant S, Buddles M, Curtis A, Goodship JA (2004) Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. *J Med Genet* 41:183–186
- Geppert M, Südhof TC (1998) RAB3 and synaptotagmin: the yin and yang of synaptic membrane fusion. *Annu Rev Neurosci* 21:75–95
- Giannandrea M, Guarnieri FC, Gehring NH, Monzani E, Benfenati F, Kulozik AE, Valtorta F (2013) Nonsense-mediated mRNA decay and loss-of-function of the protein underlie the X-linked epilepsy associated with the W356X mutation in synapsin I. *PLoS One* 8:e67724
- Giovedì S, Darchen F, Valtorta F, Greengard P, Benfenati F (2004a) Synapsin is a novel Rab3 effector protein on small synaptic vesicles. II. Functional effects of the Rab3A-synapsin I interaction. *J Biol Chem* 279:43769–43779
- Giovedì S, Vaccaro P, Valtorta F, Darchen F, Greengard P, Cesareni G, Benfenati F (2004b) Synapsin is a novel Rab3 effector protein on small synaptic vesicles. I. Identification and characterization of the synapsin I-Rab3 interactions in vitro and in intact nerve terminals. *J Biol Chem* 279:43760–43768
- Gitler D, Takagishi Y, Feng J, Ren Y, Rodriguiz RM, Wetsel WC, Greengard P, Augustine GJ (2004a) Different presynaptic roles of synapsins at excitatory and inhibitory synapses. *J Neurosci* 24:11368–11380
- Gitler D, Xu Y, Kao HT, Lin D, Lim S, Feng J, Greengard P, Augustine GJ (2004b) Molecular determinants of synapsin targeting to presynaptic terminals. *J Neurosci* 24:3711–3720
- Gitler D, Cheng Q, Greengard P, Augustine GJ (2008) Synapsin IIa controls the reserve pool of glutamatergic synaptic vesicles. *J Neurosci* 28:10835–10843
- Gomez-Pinilla F, So V, Kesslak JP (2001) Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus. *Brain Res* 904:13–19
- Greco B, Manago F, Tucci V, Kao HT, Valtorta F, Benfenati F (2013) Autism-related behavioral abnormalities in synapsin knockout mice. *Behav Brain Res* 251:65–74
- Greengard P, Browning MD, McGuinness TL, Llinas R (1987) Synapsin I, a phosphoprotein associated with synaptic vesicles: possible role in regulation of neurotransmitter release. *Adv Exp Med Biol* 221:135–153
- Greengard P, Valtorta F, Czernik AJ, Benfenati F (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* 259:780–785
- Gronborg M, Pavlos NJ, Brunk I, Chua JJ, Munster-Wandowski A, Riedel D, Ahnert-Hilger G, Urlaub H, Jahn R (2010) Quantitative comparison of glutamatergic and GABAergic synaptic vesicles unveils selectivity for few proteins including MAL2, a novel synaptic vesicle protein. *J Neurosci* 30:2–12
- Hart AK, Fioravante D, Liu RY, Phares GA, Cleary LJ, Byrne JH (2011) Serotonin-mediated synapsin expression is necessary for long-term facilitation of the Aplysia sensorimotor synapse. *J Neurosci* 31:18401–18411
- Haycock JW, Greengard P, Browning MD (1988) Cholinergic regulation of protein III phosphorylation in bovine adrenal chromaffin cells. *J Neurosci* 8:3233–3239

- Hilfiker S, Schweizer FE, Kao HT, Czernik AJ, Greengard P, Augustine GJ (1998) Two sites of action for synapsin domain E in regulating neurotransmitter release. *Nat Neurosci* 1:29–35
- Hilfiker S, Benfenati F, Doussau F, Nairn AC, Czernik AJ, Augustine GJ, Greengard P (2005) Structural domains involved in the regulation of transmitter release by synapsins. *J Neurosci* 25:2658–2669
- Hoesche C, Bartsch P, Kilimann MW (1995) The CRE consensus sequence in the synapsin I gene promoter region confers constitutive activation but no regulation by cAMP in neuroblastoma cells. *Biochim Biophys Acta* 1261:249–256
- Hosaka M, Südhof TC (1999) Homo- and heterodimerization of synapsins. *J Biol Chem* 274:16747–16753
- Huttner WB, DeGennaro LJ, Greengard P (1981) Differential phosphorylation of multiple sites in purified protein I by cyclic AMP-dependent and calcium-dependent protein kinases. *J Biol Chem* 256:1482–1488
- Huttner WB, Schiebler W, Greengard P, De Camilli P (1983) Synapsin I (protein I), a nerve terminal-specific phosphoprotein. III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. *J Cell Biol* 96:1374–1388
- James AB, Conway AM, Thiel G, Morris BJ (2004) Egr-1 modulation of synapsin I expression: permissive effect of forskolin via cAMP. *Cell Signal* 16:1355–1362
- Jensen V, Walaas SI, Hilfiker S, Ruiz A, Hvalby O (2007) A delayed response enhancement during hippocampal presynaptic plasticity in mice. *J Physiol* 583:129–143
- John JP, Chen WQ, Pollak A, Lubec G (2007) Mass spectrometric studies on mouse hippocampal synapsins Ia, IIa, and IIb and identification of a novel phosphorylation site at serine-546. *J Proteome Res* 6:2695–2710
- Johnson EM, Ueda T, Maeno H, Greengard P (1972) Adenosine 3',5-monophosphate-dependent phosphorylation of a specific protein in synaptic membrane fractions from rat cerebrum. *J Biol Chem* 247:5650–5652
- Jovanovic JN, Benfenati F, Siow YL, Sihra TS, Sanghera JS, Pelech SL, Greengard P, Czernik AJ (1996) Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. *Proc Natl Acad Sci U S A* 93:3679–3683
- Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS (2000) Synapsins as mediators of BDNF-enhanced neurotransmitter release. *Nat Neurosci* 3:323–329
- Jovanovic JN, Sihra TS, Nairn AC, Hemmings HC Jr, Greengard P, Czernik AJ (2001) Opposing changes in phosphorylation of specific sites in synapsin I during Ca²⁺-dependent glutamate release in isolated nerve terminals. *J Neurosci* 21:7944–7953
- Kao HT, Porton B, Czernik AJ, Feng J, Yiu G, Haring M, Benfenati F, Greengard P (1998) A third member of the synapsin gene family. *Proc Natl Acad Sci U S A* 95:4667–4672
- Kao HT, Porton B, Hilfiker S, Stefani G, Pieribone VA, DeSalle R, Greengard P (1999) Molecular evolution of the synapsin gene family. *J Exp Zool* 285:360–377
- Ketzel M, Gitler D (2014) Epileptic synapsin triple knockout mice exhibit progressive long-term aberrant plasticity in the entorhinal cortex. *Cereb Cortex* 24(4):996–1008
- Ketzel M, Kahn J, Weissberg I, Becker AJ, Friedman A, Gitler D (2011) Compensatory network alterations upon onset of epilepsy in synapsin triple knock-out mice. *Neuroscience* 189:108–122
- Kielland A, Erisir A, Walaas SI, Heggelund P (2006) Synapsin utilization differs among functional classes of synapses on thalamocortical cells. *J Neurosci* 26:5786–5793
- Kim SH, Ryan TA (2010) CDK5 serves as a major control point in neurotransmitter release. *Neuron* 67:797–809
- Krabben L, Fassio A, Bhatia VK, Pechstein A, Onofri F, Fadda M, Messa M, Rao Y, Shupliakov O, Stamou D, Benfenati F, Haucke V (2011) Synapsin I senses membrane curvature by an amphipathic lipid packing sensor motif. *J Neurosci* 31:18149–18154
- Kushner SA, Elgersma Y, Murphy GG, Jaarsma D, van Woerden GM, Hojjati MR, Cui Y, LeBoutillier JC, Marrone DF, Choi ES, De Zeeuw CI, Petit TL, Pozzo-Miller L, Silva AJ (2005) Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway. *J Neurosci* 25:9721–9734

- Lakhan R, Kalita J, Misra UK, Kumari R, Mittal B (2010) Association of intronic polymorphism rs3773364 A > G in synapsin-2 gene with idiopathic epilepsy. *Synapse* 64:403–408
- Lee HJ, Song JY, Kim JW, Jin SY, Hong MS, Park JK, Chung JH, Shibata H, Fukumaki Y (2005) Association study of polymorphisms in synaptic vesicle-associated genes, SYN2 and CPLX2, with schizophrenia. *Behav Brain Funct* 1:15
- Li L, Suzuki T, Mori N, Greengard P (1993) Identification of a functional silencer element involved in neuron-specific expression of the synapsin I gene. *Proc Natl Acad Sci U S A* 90:1460–1464
- Li L, Chin LS, Shupliakov O, Brodin L, Sihra TS, Hvalby O, Jensen V, Zheng D, McNamara JO, Greengard P et al (1995) Impairment of synaptic vesicle clustering and of synaptic transmission, and increased seizure propensity, in synapsin I-deficient mice. *Proc Natl Acad Sci U S A* 92:9235–9239
- Li J, Han YR, Plummer MR, Herrup K (2009) Cytoplasmic ATM in neurons modulates synaptic function. *Curr Biol* 19:2091–2096
- Lignani G, Raimondi A, Ferrea E, Rocchi A, Paonessa F, Cesca F, Orlando M, Tkatch T, Valtorta F, Cossette P, Baldelli P, Benfenati F (2013) Epileptogenic Q555X SYN1 mutant triggers imbalances in release dynamics and short-term plasticity. *Hum Mol Genet* 22:2186–2199
- Llinas R, McGuinness TL, Leonard CS, Sugimori M, Greengard P (1985) Intraterminal injection of synapsin I or calcium/calmodulin-dependent protein kinase II alters neurotransmitter release at the squid giant synapse. *Proc Natl Acad Sci U S A* 82:3035–3039
- Llinas R, Gruner JA, Sugimori M, McGuinness TL, Greengard P (1991) Regulation by synapsin I and Ca(2+)-calmodulin-dependent protein kinase II of the transmitter release in squid giant synapse. *J Physiol* 436:257–282
- Luthi T, Haltiwanger RS, Greengard P, Bahler M (1991) Synapsins contain O-linked N-acetylglucosamine. *J Neurochem* 56:1493–1498
- Maienschein V, Marxen M, Volkandt W, Zimmermann H (1999) A plethora of presynaptic proteins associated with ATP-storing organelles in cultured astrocytes. *Glia* 26:233–244
- Mandell JW, Townes-Anderson E, Czernik AJ, Cameron R, Greengard P, De Camilli P (1990) Synapsins in the vertebrate retina: absence from ribbon synapses and heterogeneous distribution among conventional synapses. *Neuron* 5:19–33
- Matsubara M, Kusubata M, Ishiguro K, Uchida T, Titani K, Taniguchi H (1996) Site-specific phosphorylation of synapsin I by mitogen-activated protein kinase and Cdk5 and its effects on physiological functions. *J Biol Chem* 271:21108–21113
- Medrihan L, Cesca F, Raimondi A, Lignani G, Baldelli P, Benfenati F (2013) Synapsin II desynchronizes neurotransmitter release at inhibitory synapses by interacting with presynaptic calcium channels. *Nat Commun* 4:1512
- Menegon A, Verderio C, Leoni C, Benfenati F, Czernik AJ, Greengard P, Matteoli M, Valtorta F (2002) Spatial and temporal regulation of Ca2+/calmodulin-dependent protein kinase II activity in developing neurons. *J Neurosci* 22:7016–7026
- Menegon A, Bonanomi D, Albertinazzi C, Lotti F, Ferrari G, Kao HT, Benfenati F, Baldelli P, Valtorta F (2006) Protein kinase A-mediated synapsin I phosphorylation is a central modulator of Ca2+-dependent synaptic activity. *J Neurosci* 26:11670–11681
- Messa M, Congia S, Defranchi E, Valtorta F, Fassio A, Onofri F, Benfenati F (2010) Tyrosine phosphorylation of synapsin I by Src regulates synaptic-vesicle trafficking. *J Cell Sci* 123:2256–2265
- Michels B, Chen YC, Saumweber T, Mishra D, Tanimoto H, Schmid B, Engmann O, Gerber B (2011) Cellular site and molecular mode of synapsin action in associative learning. *Learn Mem* 18:332–344
- Micheva KD, Busse B, Weiler NC, O'Rourke N, Smith SJ (2010) Single-synapse analysis of a diverse synapse population: proteomic imaging methods and markers. *Neuron* 68:639–653
- Monaldi I, Vassalli M, Bachi A, Giovedi S, Millo E, Valtorta F, Raiteri R, Benfenati F, Fassio A (2010) The highly conserved synapsin domain E mediates synapsin dimerization and phospholipid vesicle clustering. *Biochem J* 426:55–64

- Murrey HE, Gama CI, Kalovidouris SA, Luo WI, Driggers EM, Porton B, Hsieh-Wilson LC (2006) Protein fucosylation regulates synapsin Ia/Ib expression and neuronal morphology in primary hippocampal neurons. *Proc Natl Acad Sci U S A* 103:21–26
- Nielander HB, Onofri F, Schaeffer E, Menegon A, Fesce R, Valtorta F, Greengard P, Benfenati F (1997) Phosphorylation-dependent effects of synapsin IIa on actin polymerization and network formation. *Eur J Neurosci* 9:2712–2722
- Nuwal T, Heo S, Lubec G, Buchner E (2011) Mass spectrometric analysis of synapsins in *Drosophila melanogaster* and identification of novel phosphorylation sites. *J Proteome Res* 10:541–550
- Onofri F, Giovedi S, Vaccaro P, Czernik AJ, Valtorta F, De Camilli P, Greengard P, Benfenati F (1997) Synapsin I interacts with c-Src and stimulates its tyrosine kinase activity. *Proc Natl Acad Sci U S A* 94:12168–12173
- Onofri F, Giovedi S, Kao HT, Valtorta F, Bongiorno Borbone L, De Camilli P, Greengard P, Benfenati F (2000) Specificity of the binding of synapsin I to Src homology 3 domains. *J Biol Chem* 275:29857–29867
- Onofri F, Messa M, Matafora V, Bonanno G, Corradi A, Bachi A, Valtorta F, Benfenati F (2007) Synapsin phosphorylation by SRC tyrosine kinase enhances SRC activity in synaptic vesicles. *J Biol Chem* 282:15754–15767
- Orenbuch A, Shalev L, Marra V, Sinai I, Lavy Y, Kahn J, Burden JJ, Staras K, Gitler D (2012a) Synapsin selectively controls the mobility of resting pool vesicles at hippocampal terminals. *J Neurosci* 32:3969–3980
- Orenbuch A, Shulman Y, Lipstein N, Bechar A, Lavy Y, Brumer E, Vasileva M, Kahn J, Barki-Harrington L, Kuner T, Gitler D (2012b) Inhibition of exocytosis or endocytosis blocks activity-dependent redistribution of synapsin. *J Neurochem* 120:248–258
- Owe SG, Erisir A, Heggelund P (2013) Terminals of the major thalamic input to visual cortex are devoid of synapsin proteins. *Neuroscience* 243:115–125
- Paonessa F, Latifi S, Scarongella H, Cesca F, Benfenati F (2013) Specificity protein 1 (Sp1)-dependent activation of the synapsin I gene (SYN1) is modulated by RE1-silencing transcription factor (REST) and 5'-cytosine-phosphoguanine (CpG) methylation. *J Biol Chem* 288:3227–3239
- Pera I, Stark R, Kappl M, Butt HJ, Benfenati F (2004) Using the atomic force microscope to study the interaction between two solid supported lipid bilayers and the influence of synapsin I. *Biophys J* 87:2446–2455
- Petersohn D, Schoch S, Brinkmann DR, Thiel G (1995) The human synapsin II gene promoter. Possible role for the transcription factor zif268/egr-1, polyoma enhancer activator 3, and AP2. *J Biol Chem* 270:24361–24369
- Pieribone VA, Shupliakov O, Brodin L, Hilfiker-Rothenfluh S, Czernik AJ, Greengard P (1995) Distinct pools of synaptic vesicles in neurotransmitter release. *Nature* 375:493–497
- Pieribone VA, Porton B, Rendon B, Feng J, Greengard P, Kao HT (2002) Expression of synapsin III in nerve terminals and neurogenic regions of the adult brain. *J Comp Neurol* 454:105–114
- Porton B, Kao HT, Greengard P (1999) Characterization of transcripts from the synapsin III gene locus. *J Neurochem* 73:2266–2271
- Qualmann B, Roos J, DiGregorio PJ, Kelly RB (1999) Syndapin I, a synaptic dynamin-binding protein that associates with the neural Wiskott-Aldrich syndrome protein. *Mol Biol Cell* 10:501–513
- Rizzoli SO, Betz WJ (2005) Synaptic vesicle pools. *Nat Rev Neurosci* 6:57–69
- Romano C, Nichols RA, Greengard P, Greene LA (1987) Synapsin I in PC12 cells. I. Characterization of the phosphoprotein and effect of chronic NGF treatment. *J Neurosci* 7:1294–1299
- Rosahl TW, Geppert M, Spillane D, Herz J, Hammer RE, Malenka RC, Südhof TC (1993) Short-term synaptic plasticity is altered in mice lacking synapsin I. *Cell* 75:661–670
- Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Südhof TC (1995) Essential functions of synapsins I and II in synaptic vesicle regulation. *Nature* 375:488–493

- Ryan TA, Li L, Chin LS, Greengard P, Smith SJ (1996) Synaptic vesicle recycling in synapsin I knock-out mice. *J Cell Biol* 134:1219–1227
- Sadanandappa MK, Blanco Redondo B, Michels B, Rodrigues V, Gerber B, VijayRaghavan K, Buchner E, Ramaswami M (2013) Synapsin function in GABA-ergic interneurons is required for short-term olfactory habituation. *J Neurosci* 33:16576–16585
- Sankaranarayanan S, Atluri PP, Ryan TA (2003) Actin has a molecular scaffolding, not propulsive, role in presynaptic function. *Nat Neurosci* 6:127–135
- Sato K, Morimoto K, Suemaru S, Sato T, Yamada N (2000) Increased synapsin I immunoreactivity during long-term potentiation in rat hippocampus. *Brain Res* 872:219–222
- Sauerwald A, Hoesche C, Oschwald R, Kilimann MW (1990) The 5'-flanking region of the synapsin I gene. A G+C-rich, TATA- and CAAT-less, phylogenetically conserved sequence with cell type-specific promoter function. *J Biol Chem* 265:14932–14937
- Saviouk V, Moreau MP, Tereshchenko IV, Brzustowicz LM (2007) Association of synapsin 2 with schizophrenia in families of Northern European ancestry. *Schizophr Res* 96:100–111
- Schenk U, Menna E, Kim T, Passafaro M, Chang S, De Camilli P, Matteoli M (2005) A novel pathway for presynaptic mitogen-activated kinase activation via AMPA receptors. *J Neurosci* 25:1654–1663
- Schiebler W, Jahn R, Doucet JP, Rothlein J, Greengard P (1986) Characterization of synapsin I binding to small synaptic vesicles. *J Biol Chem* 261:8383–8390
- Schoch S, Cibelli G, Thiel G (1996) Neuron-specific gene expression of synapsin I. Major role of a negative regulatory mechanism. *J Biol Chem* 271:3317–3323
- Shupliakov O, Bloom O, Gustafsson JS, Kjaerulff O, Low P, Tomilin N, Pieribone VA, Greengard P, Brodin L (2002) Impaired recycling of synaptic vesicles after acute perturbation of the presynaptic actin cytoskeleton. *Proc Natl Acad Sci U S A* 99:14476–14481
- Shupliakov O, Haucke V, Pechstein A (2011) How synapsin I may cluster synaptic vesicles. *Semin Cell Dev Biol* 22:393–399
- Sihra TS, Wang JK, Gorelick FS, Greengard P (1989) Translocation of synapsin I in response to depolarization of isolated nerve terminals. *Proc Natl Acad Sci U S A* 86:8108–8112
- Siksou L, Rostaing P, Lechaire JP, Boudier T, Ohtsuka T, Fejtova A, Kao HT, Greengard P, Gundelfinger ED, Triller A, Marty S (2007) Three-dimensional architecture of presynaptic terminal cytomatrix. *J Neurosci* 27:6868–6877
- Silva AJ, Rosahl TW, Chapman PF, Marowitz Z, Friedman E, Frankland PW, Cestari V, Cioffi D, Südhof TC, Bourchuladze R (1996) Impaired learning in mice with abnormal short-lived plasticity. *Curr Biol* 6:1509–1518
- Skoblenick KJ, Argintaru N, Xu Y, Dyck BA, Basu D, Tan ML, Mazurek MF, Mishra RK (2010) Role of AP-2 alpha transcription factor in the regulation of synapsin II gene expression by dopamine D1 and D2 receptors. *J Mol Neurosci* 41:267–277
- Skorobogatko Y, Landicho A, Chalkley RJ, Kossenkov AV, Gallo G, Vosseller K (2014) O-linked β -N-acetylglucosamine (O-GlcNAc) site thr-87 regulates synapsin I localization to synapses and size of the reserve pool of synaptic vesicles. *J Biol Chem* 289(6):3602–3612
- Spillane DM, Rosahl TW, Südhof TC, Malenka RC (1995) Long-term potentiation in mice lacking synapsins. *Neuropharmacology* 34:1573–1579
- Stone LM, Browning MD, Finger TE (1994) Differential distribution of the synapsins in the rat olfactory bulb. *J Neurosci* 14:301–309
- Südhof TC (1990) The structure of the human synapsin I gene and protein. *J Biol Chem* 265:7849–7852
- Südhof TC, Czernik AJ, Kao HT, Takei K, Johnston PA, Horiuchi A, Kanazir SD, Wagner MA, Perin MS, De Camilli P et al (1989) Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins. *Science* 245:1474–1480
- Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, Müller SA, Rammner B, Gräter F, Hub JS, De Groot BL, Mieskes G, Moriyama Y, Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R (2006) Molecular anatomy of a trafficking organelle. *Cell* 127:831–846

- Tallent MK, Varghis N, Skorobogatko Y, Hernandez-Cuebas L, Whelan K, Vocadlo DJ, Vosseller K (2009) In vivo modulation of O-GlcNAc levels regulates hippocampal synaptic plasticity through interplay with phosphorylation. *J Biol Chem* 284:174–181
- Tao-Cheng JH, Dosemeci A, Winters CA, Reese TS (2006) Changes in the distribution of calcium calmodulin-dependent protein kinase II at the presynaptic bouton after depolarization. *Brain Cell Biol* 35:117–124
- Thiel G, Südhof TC, Greengard P (1990) Synapsin II. Mapping of a domain in the NH₂-terminal region which binds to small synaptic vesicles. *J Biol Chem* 265:16527–16533
- Torri Tarelli F, Bossi M, Fesce R, Greengard P, Valtorta F (1992) Synapsin I partially dissociates from synaptic vesicles during exocytosis induced by electrical stimulation. *Neuron* 9:1143–1153
- Torri-Tarelli F, Villa A, Valtorta F, De Camilli P, Greengard P, Ceccarelli B (1990) Redistribution of synaptophysin and synapsin I during alpha-latrotoxin-induced release of neurotransmitter at the neuromuscular junction. *J Cell Biol* 110:449–459
- Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem* 9:224–237
- Valente P, Casagrande S, Nieuws T, Versteegen AM, Valtorta F, Benfenati F, Baldelli P (2012) Site-specific synapsin I phosphorylation participates in the expression of post-tetanic potentiation and its enhancement by BDNF. *J Neurosci* 32:5868–5879
- Valtorta F, Villa A, Jahn R, De Camilli P, Greengard P, Ceccarelli B (1988) Localization of synapsin I at the frog neuromuscular junction. *Neuroscience* 24:593–603
- Valtorta F, Benfenati F, Greengard P (1992a) Structure and function of the synapsins. *J Biol Chem* 267:7195–7198
- Valtorta F, Greengard P, Fesce R, Chierigatti E, Benfenati F (1992b) Effects of the neuronal phosphoprotein synapsin I on actin polymerization. I Evidence for a phosphorylation-dependent nucleating effect. *J Biol Chem* 267:11281–11288
- Vasileva M, Horstmann H, Geumann C, Gitler D, Kuner T (2012) Synapsin-dependent reserve pool of synaptic vesicles supports replenishment of the readily releasable pool under intense synaptic transmission. *Eur J Neurosci* 36:3005–3020
- Vasileva M, Renden R, Horstmann H, Gitler D, Kuner T (2013) Overexpression of synapsin Ia in the rat calyx of Held accelerates short-term plasticity and decreases synaptic vesicle volume and active zone area. *Front Cell Neurosci* 7:270
- Wang S, Cesca F, Loers G, Schweizer M, Buck F, Benfenati F, Schachner M, Kleene R (2011) Synapsin I is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes neurite outgrowth and neuronal survival when released via glia-derived exosomes. *J Neurosci* 31:7275–7290
- Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. *Annu Rev Physiol* 64:355–405
- Zurmohle UM, Herms J, Schlingensiepen R, Schlingensiepen KH, Brysch W (1994) Changes of synapsin I messenger RNA expression during rat brain development. *Exp Brain Res* 99:17–24