ISSN 1990-7478, Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology, 2023, Vol. 17, No. 2, pp. 69–82. © Pleiades Publishing, Ltd., 2023.
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REVIEWS

BAR Domain Proteins as Putative Regulators of the Protein Liquid Phase in Nerve Terminals in the Central Nervous System

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Received November 10, 2022; revised December 18, 2022; accepted December 20, 2022

Abstract—BAR proteins are key components of the synaptic vesicle cycle in nerve terminals. They participate in the regulation of neurotransmitter release during the fusion of synaptic vesicles with the presynaptic membrane and synaptic vesicle recycling. Localization of these proteins at the sites of liquid–liquid phase separation in nerve terminals suggests additional functions of these molecules. In the current review, we discuss the tasks of BAR proteins at different stages of the secretory cycle, including their putative role in liquid–liquid phase transitions in nerve terminals during synaptic activity. We suggest that BAR proteins, along with their established functions in exo- and endocytosis, play crucial roles in the organization of the reserve pool of synaptic vesicles and at intermediate stages of the vesicle cycle.

Keywords: synapse, synaptic vesicle cycle, exocytosis, endocytosis, BAR proteins, liquid–liquid phase transition

DOI: 10.1134/S199074782303008X

INTRODUCTION

BAR domain proteins are well-known as molecules controlling changes in the curvature of biological membranes. They play key roles in the vital activities of all cell systems. The abbreviation BAR is composed of the first letters of the names of three proteins belonging to this superfamily: **B**in1 [1] and **A**mphiphysin [2] found in mammals and **R**vs167 found in yeasts [3]. The characteristic structural feature of BAR proteins is the presence of a unique domain consisting of ~200–280 amino acids, which forms an antiparallel alpha-helix bundle. During dimerization of protein molecules, these domains form a positively charged surface, which is able to bind to the negatively charged lipids of the cell membrane and control its curvature (Figs. 1a, 1b) [4, 5]. This unique property determines the involvement of BAR proteins in many cellular processes such as the formation of intracellular vesicles, secretion, as well as the creation of membrane structures of different shapes, e.g., the T-system of skeletal muscle cells, endosomes, filopodia, mitochondria, and autophagosomes [4, 6].

In a wide range of BAR proteins, the following three groups are distinguished: N-BAR, F-BAR, and I-BAR. N-BAR proteins during dimerization form a charged crescent-shaped surface that can stabilize the maximum membrane curvature [4]. The defining feature of the BAR domain of N-BAR proteins is the N-terminal amphipathic helix, which was described for the first time for the protein amphiphysin [7]. Later it was shown that such amphipathic helix is also typical of endophilin [8], nardin (RICH1) [9], and some other proteins. The proteins stabilizing a lesser curvature of the membrane surface were named after the typical representatives of Fes/CIP4 homology-BAR proteins abbreviated as F-BAR proteins [10, 11]. The proteins with the dimers stabilizing an inverse curvature of the membrane were termed Inverse-BAR or I-BAR proteins [12–14]. The groups of BAR proteins differ both in the angle of curvature stabilization and in the length of the region interacting with the cell membrane, which determines the ability of these molecules to form membrane tubes and vesicles in vitro and in vivo with different physical properties [15–18]. The structure of BAR proteins usually also includes other domains allowing them to interact with a wide range of proteins and signaling molecules. For example, the structure of BAR proteins can include pleckstrin homology (PH) and phox-homology (PX) domains enabling the proteins to perform additional interactions with lipids, as well as GEF (guanine nucleotide exchange factor) and GAP (GTP hydrolysis-activating protein) domains regulating the activity of GTPases [7]. The most frequently occurring additional domains are Src homology 3 (SH3) domains interacting with proline-rich protein sequences,

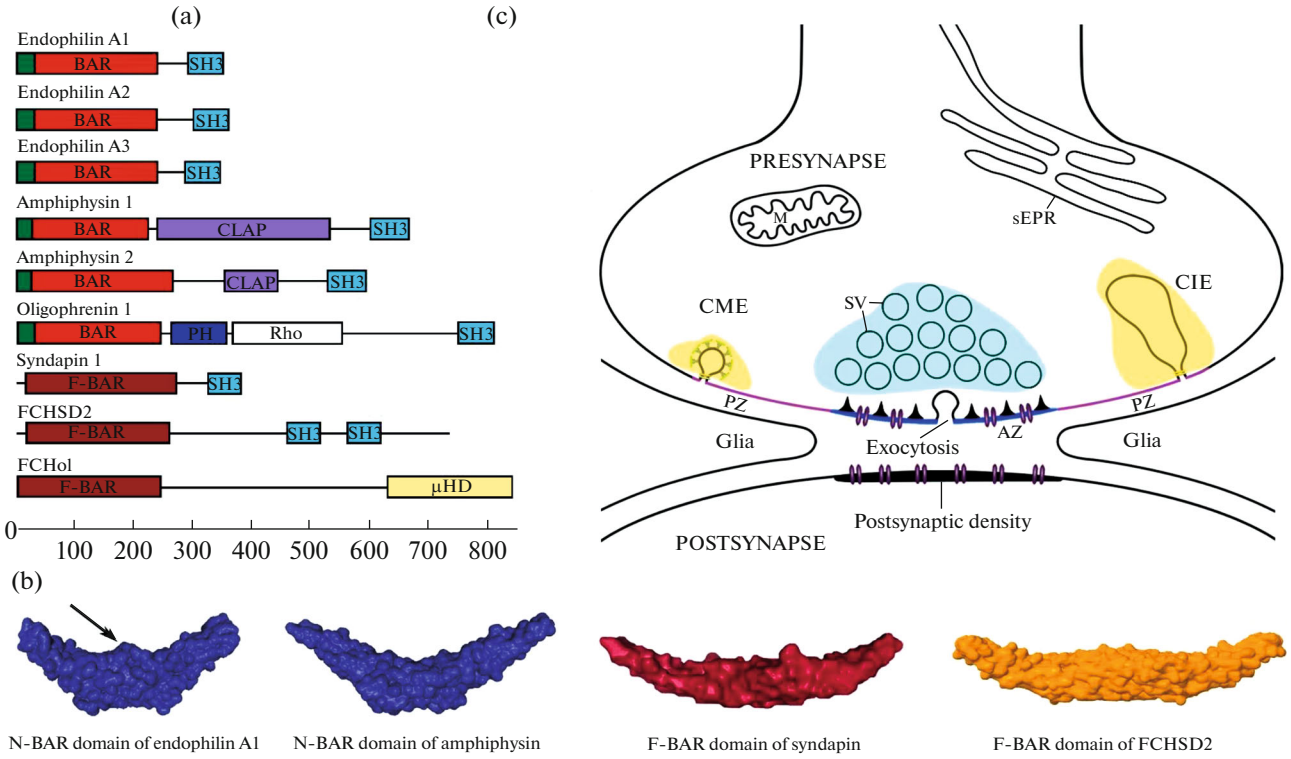

Fig. 1. BAR proteins and localization of protein liquid condensates involved in the synaptic vesicle cycle in the presynapse. (a) Schematic representation of the domain organization of BAR proteins involved in the synaptic vesicle cycle. BAR domains are shown in *red*; the additional N-terminal amphipathic helix in N-BAR proteins is *green*. CLAP, the clathrin/AP2-binding site; PH, the pleckstrin homology domain; Rho, the RhoGAP domain regulating the activity of GTPases. The scale is given for assessing the relative length of amino acid sequences of the proteins. (b) Crystal structure of the dimers of BAR domains of some proteins. The difference in the degree of curvature in N-BAR and F-BAR dimers is indicated. *Arrow* points to the central amphipathic helix (CAH) of endophilin. (c) Schematic representation of localization of liquid protein condensates involved in the synaptic vesicle cycle. The liquid protein phase organizes a cluster of synaptic vesicles in the presynapse (*light blue*) and the periactive zone (PZ), where clathrin-mediated (CME) and clathrin-independent (CIE) endocytosis takes place (*yellow*). The liquid protein phase organizing presynaptic projections in the active zone (AZ) is *blue*. SV, synaptic vesicles; M, mitochondrion; sEPR, smooth endoplasmic reticulum.

PRD [19, 20]. This interaction first and foremost plays a role in the recruitment of other proteins involved in various cellular processes. It is interesting that the SH3 domains of BAR proteins are able to occlude a proline-rich sequence inside the protein itself, thereby converting it into an inactive form [21–23].

In the present review, we discuss the functions of BAR proteins directly involved in the cycle of secretory vesicles in interneuronal synapses in the central nervous system (CNS).

SECRETORY CYCLE AND LIQUID–LIQUID PHASE SEPARATION IN CHEMICAL SYNAPSES

Interneuronal chemical synapses are specialized contacts between nerve cells, which perform the function of excitatory or inhibitory signal transduction between neurons in the CNS. Chemical synapses are structurally asymmetric and convey information from presynaptic to postsynaptic cells (Fig. 1c). The presynapse and the postsynapse are separated by a synaptic cleft of approximately 20 nm width, which is surrounded by the outgrowths of glial cells. Signal transduction is triggered by an action potential arriving at the nerve ending and implemented due to the action of the secretory cycle of synaptic vesicles, which is completed by the exocytosis of vesicles and the release of neurotransmitters into the synaptic cleft. A neurotransmitter interacts with receptors on the postsynaptic membrane, activates ion channels, and thereby performs signal transduction to another neuron. Neurotransmitters are concentrated in synaptic vesicles, or bubbles, which accumulate into a cluster on a specialized area of the presynaptic membrane, the so-called active zone (Fig. 1c). In the state of quiescence, some synaptic vesicles in the cluster are bound to the presynaptic membrane, forming a functional pool of vesicles ready for immediate fusion with the presynaptic membrane and the release of neurotransmitter into the synaptic cleft when neuronal activity occurs (readily releasable pool). In addition, there are two supple-

mentary functional pools of synaptic vesicles: the recycling pool and the reserve pool, with their vesicles being mobilized in case of moderate and intensive activity of the neuron, respectively [24].

Around the presynaptic active zone, there is the so-called peri-active zone for the recovery of synaptic vesicles after exocytosis (Fig. 1c) [25]. The basic mechanism of vesicle formation is clathrin-mediated endocytosis [26, 27]. In addition, there are other scenarios for the restoration of the pool of synaptic vesicles. These include fast and "kiss-and-run" types of endocytosis, which do not involve clathrin and occur in both peri-active and active zones [28–31]. At a high level of synaptic activity, which is accompanied by exocytosis of many vesicles within a short period of time, there may be compensatory capture and budding of large sections of the membrane by the type of "bulk" endocytosis. It results in the formation of endosomes, from which synaptic vesicles are then formed [32]. It should be noted that these mechanisms of endocytosis are auxiliary and cannot maintain the function of the synaptic cycle after the classical clathrin-mediated endocytosis has been switched off [27]. The newly formed vesicles are filled with a neurotransmitter, transported to the cluster region above the active zone, and involved again in exocytosis, thereby closing the synaptic secretory cycle [33–35].

Discoveries of liquid protein condensates and liquid–liquid phase transitions (LLPTs) in cells have changed in many respects our notions of the mechanisms of cellular processes [36, 37]. As the studies of recent years have shown, the presynaptic compartments where the cycle of synaptic vesicles occurs are also organized by the principle of liquid phase separation [38] (Fig. 1c). The ability to form an interface under certain conditions in vitro is demonstrated by: the key protein of the reserve pool, synapsin [39, 40]; presynaptic scaffolding proteins RIM, RIM-BP, and ELKS, in complex with voltage-gated calcium channels (VGCC) [38, 41–44]; and proteins initiating clathrin-mediated endocytosis (FCHo 1/2 and Eps15) [45] and clathrin-independent endocytosis (syndapin 1 and dynamin 1xA) [46]. It is noteworthy that synaptic vesicles differently interact with the liquid phase of synapsin and the liquid phase of RIM/RIM-BP/ELKS, being encapsulated in the former and distributed over the interface surface of the latter [47]. The liquid phases of synapsin and RIM/RIM-BP or RIM/ELKS also interact with each other. Irrespective of the order of addition of proteins to the mixture, the liquid phase of synapsin encapsulates protein droplets formed by RIM/RIM-BP and RIM/ELKS but does not mix with them, i.e., a kind of "a liquid phase in a liquid phase" structure is formed. The authors of the work succeeded in constructing such an organization of liquid phases in vitro. The liquid phase of RIM/RIM-BP proteins, which was formed on the membrane of a giant unilamellar vesicle with incorporated VGCC, bound synaptic vesicles on its surface and was itself encapsulated by the liquid phase of synapsin with vesicles included in the latter. These experiments confirm the hypothesis that such liquid–liquid phase separation exists also in the living synapse [47].

The biophysical basis of protein transition to the liquid phase is weak electrostatic interactions between amino acid residues of their IDR (intrinsically disordered regions), which under certain conditions become more energetically advantageous for molecules than the interaction between IDR and solvent molecules. The possibility of this transition is influenced by factors such as pH, temperature, ionic strength of the solution, and posttranslational modifications of proteins (due to the changes in charge distribution in IDR) [48]. A necessary condition for liquid phase formation is also overcoming the concentration threshold, which is promoted by the phenomenon of "molecular crowding", i.e., the presence in the medium of other high-molecular compounds competing with proteins for molecules of the solvent. In addition, threshold concentrations of proteins can be reached locally due to their interaction with other molecules. For example, experiments in vitro have shown that the liquid phase of the active zone proteins RIM/RIM-BP/ELKS is formed at lower concentrations of these proteins in the presence of VGCC, with which RIM and RIM-BP interact directly through the PDZ domain (a combination of the first letters of protein names PSD95, Dlg1, Zo-1) and the SH3 domains, respectively [42].

RIM/RIM-BP/ELKS can specifically interact with each other but not with synapsin. The liquid phases of RIM/RIM-BP/ELKS and synapsin have different densities and, accordingly, differ in the force of surface tension at the interface, which explains why they do not mix [47].

The interaction between synaptic vesicles and liquid phases is probably determined by their interactions with proteins being components of the phase. So, synapsin binds directly to the vesicular membrane through the motif in the conserved domain C and the ALPS (amphipathic lipid-packing sensor) motif, while the endocytic proteins bind through their BAR domains [7, 49]. The transmembrane proteins of synaptic vesicles, e.g., synaptophysin, play an important role in the concentration of proteins forming the liquid phase [50]. Thus, synaptic vesicles turn out to be directly inside the liquid phase of these proteins at the moment of their formation. At the same time, RIM/RIM-BP/ELKS proteins have no domains that can bind directly with membrane lipids, which reduces the probability of vesicle inclusion in the liquid phase.

During the synaptic cycle, vesicles overcome liquid phase interfaces with different organization and protein compositions depending on the level of synaptic activity (Fig. 1). A phase interface allows the diffusion of molecules between liquid protein condensates and the cytosol but is impermeable for large molecular

complexes and organelles [48, 51]. Some proteins present in such compartment act as regulatory proteins, changing the conditions of liquid phase formation [52]. The properties of BAR proteins suggest that this group of proteins is involved in the organization of liquid phases in the synapse as regulatory molecules, which, together with the known functions in exo- and endocytosis, contribute to the transition of vesicles to different presynaptic compartments during the secretory cycle.

BAR PROTEINS OF THE SYNAPTIC VESICLE CYCLE

At present, it is known that about six BAR proteins are involved in the cycle of synaptic vesicles; these are N-BAR proteins: amphiphysin, endophilin; F-BAR proteins: syndapin, FCHo, and FCHSD; as well as the BAR protein oligophrenin. Currently, there are no data on the involvement of I-BAR proteins in the synaptic cycle [14]. The studies of recent years have shown that the cycle involves specific isoforms of BAR proteins, namely: amphiphysins 1/2, endophilin A1, oligophrenin 1, syndapin 1, FCHo 1/2 and FCHSD 1/2 [53, 54]. The involvement of these isoforms is supposedly determined by the kinetic properties of the cycle per se, the major ones being the high rate and efficiency of the secretory process [53–55].

Amphiphysins 1/2. Amphiphysins 1/2 in the human genome are encoded by two different genes, *AMPH* and *BIN1* (NCBI Gene ID: 273, 274) (Fig. 1a). Amphiphysin 1 is mainly expressed in CNS [56] and is the key protein of the synaptic cycle [55]. Amphiphysin 2, also referred to as SH3P9 and BIN1 (bridging integrator-1), is expressed in other tissues too, e.g., in muscles [57, 58].

The structure of amphiphysin 1/2 includes three major domains: the N-terminal BAR domain, the clathrin/AP2-binding domain (CLAP), and the C-terminal SH3 domain [59]. The N-BAR domain s of the protein forms an arc-shaped structure during dimerization (Fig. 1b) [7]. Such a structure is able to stabilize membrane curvature by binding to the negatively charged surface of the membrane lipid layer [60]. The CLAP domain of amphiphysin 1 interacts with the key proteins of clathrin-mediated endocytosis: adaptor complex AP2 and clathrin [59]. The C-terminal SH3 domain of amphiphysin is able to recruit other proteins containing proline-rich sequences (PRD) in their structure [61]. Amphiphysins 1/2 can form homo- and heterodimers [62].

Amphiphysins have an intramolecular mechanism, which converts the protein into an inactive form as a result of locking the SH3 domain into the BAR domain [22, 23, 63]. It has been shown that the SH3 domain is able to bind with the proline-rich sequence of the H0-helix of the N-BAR domain, which leads to conformation changes in the protein; as a result, it loses the ability to participate in membrane tubulation, and the efficiency of its binding to membrane lipids decreases [63]. This property suggests the existence of mechanisms of regulation of protein activity, in particular, through phosphorylation–dephosphorylation. Studies on synaptosomes have shown that Cdk5 kinase phosphorylates amphiphysin 1 in the presence of synaptic activity [64]. Endocytosis is accompanied by dephosphorylation of amphiphysin [64–66]. It is supposed to involve the Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN) [66].

Endophilin A1. The mammalian genome contains three *SH3GL1–3* genes (NCBI Gene ID: 6455, 6456, 6457; *Homo sapiens*) encoding endophilins A1–A3 and two *SH3GLB1, 2* genes (NCBI Gene ID: 56904, 54673; *Homo sapiens*) encoding endophilins B1 and B2 (Fig. 1*a*). Endophilin A1 is specific to CNS; endophilin A2 is expressed in all tissues; endophilin A3 has been found in the brain and testicles [67–69]. Endophilins B1 and B2 are expressed in all tissues including the brain [16]. In nerve terminals, endophilins A are associated with the synaptic membrane [19]. Endophilins B have been detected mainly in association with membrane organelles such as mitochondria, autophagosomes, lysosomes, and endoplasmic reticulum (EPR) [70–72]. On the surface of some organelles, e.g., autophagosomes, endophilins A are colocalized with endophilins B [71, 72].

Determination of the crystal structure of endophilin has shown that the protein-comprises two major domains: the N-terminal BAR domain and the C-terminal SH3 domain linked through a variable region [8]. Endophilin is the only one of the N-BAR proteins that has an additional H1 insert (H1I) in the N-BAR domain structure. During dimerization, the central amphipathic helix (CAH) is formed due to the presence of this insert on the protein surface facing the membrane [8, 69] (Fig. 1b). This structural peculiarity allows endophilins not only to stabilize but also to actively generate membrane curvature; the CAH is incorporated into the lipid layer, specifying its asymmetry and, accordingly, curvature [9].

The central region of endophilin A1 can undergo phosphorylation, which entails a change in the radius of curvature of membrane structures stabilized by this protein in vitro [73]. It is supposed that BAR proteins pass into an active state during dephosphorylation by CaN. In line with this assumption, the enhanced protein kinase activity and phosphorylation of BAR proteins in the synaptic terminal led to a decrease in the efficiency of endocytosis [74].

It is known that endophilin, similar to amphiphysin, has an intramolecular locking mechanism, which is regulated by posttranslational modifications of the protein [23, 75]. The SH3 domain is also able to bind to the H0-helix of the N-BAR domain, which leads to conformational changes in the BAR domain [23].

Oligophrenin 1. Oligophrenin 1 is encoded by the *OPHN1* gene (NCBI Gene ID: 4983; *Homo sapiens*); it is a RhoGAP protein regulating the activity of Rho GTPases. Oligophrenin 1 is expressed in CNS and is present both in the nerve terminals and in the dendrites of neurons [76]. The protein structure includes the BAR domain localized at the N-terminus, the PH domain, the central RhoGAP domain, and the SH3 domains at the C-terminus of the molecule [77, 78]. The protein is involved both in the cycle of synaptic vesicles [78] and in the morphogenesis of dendritic spines [79]. The mechanism of autoinhibition has not been described for oligophrenin. Protein mutations are associated with the development of X-linked intellectual disability in humans [76].

Syndapin 1. The mammalian genome contains three genes of the F-BAR protein syndapin (*PACSIN1–3*) encoding its three isoforms: syndapins 1–3 (NCBI Gene ID: 29993, 11252, 2976; *Homo sapiens*). Syndapin 1 is expressed solely in CNS and accumulates in nerve endings. Syndapin 2 is detected in all tissues and organs. The expression of syndapin 3 is high in skeletal and cardiac muscles, but can also be detected in the lungs, the kidneys, the brain, the placenta, and the pancreas [80].

The syndapin 1 monomer comprises the F-BAR domain, the variable region, and the C-terminal SH3 domain. X-ray structure analysis has shown that the structure of the F-BAR domain of syndapin is generally similar to that in other F-BAR proteins. The F-BAR domain in the syndapin dimer has a lesser curvature of the surface compared to the N-BAR domains of endophilin and amphiphysin dimers. At the interface between the central and distal regions of the F-BAR domain of syndapin, there are also hydrophobic loops allowing syndapins 1 and 2 to actively alter the membrane curvature [81, 82].

Syndapin 1, similar to endophilin A1 and amphiphysins 1 and 2, is capable of autoinhibition, when its SH3 domains interact with the F-BAR domain, blocking some part of its membrane-binding surface. However, the interaction between the domains occurs not through the canonical SH3/PRD interaction but due to the formation of hydrogen bonds and salt bridges and, therefore, is sensitive to the pH and ionic strength of the solution [82]. Although syndapin 1 has several phosphorylation sites localized mainly at the N-terminus of the F-BAR domain and in the variable region, none of these sites can regulate the autoinhibition of syndapin. Instead, the release of syndapin from autoinhibition is regulated by the interaction between proline-rich sequences of other proteins, e.g., dynamin, and its SH3 domains; as a result, the dimer changes its conformation and again becomes capable of binding with the membrane [83].

FCHSD1/2. The vertebrate genome contains two genes of FCHSD proteins (F-BAR and double SH3 domains protein): *FCHSD1* and *FCHSD2* (NCBI Gene ID: 89848, 9873; *Homo sapiens*) [84]. The predominant isoform is *FCHSD2*, the expression of which is detected in almost all tissues, in particular, the cerebral cortex, the cerebellum, the kidneys, the liver, and the testis, while *FCHSD1* is expressed at a much lower level [85].

The FCHSD1/2 structure includes the N-terminal F-BAR domain, two SH3 domains, and the C-terminal proline-rich region. An important difference between FCHSD1/2 and other F-BAR proteins is that their membrane-binding surface has a zero curvature, i.e., FCHSD1/2 can bind to flat regions of the membrane and are incapable of its tubulation [86]. It has been shown that the FCHSD2 homolog in Drosophila, Nervous Wreck, has an intramolecular locking mechanism, which is similar to the mechanism of autoinhibition in syndapin. At the same time, the SH3 domains of the protein (the SH3b domain plays the key role here) bind to positively charged regions at the ends of the F-BAR domain due to electrostatic interactions. Interestingly, the interaction between SH3 and F-BAR domains in this protein is able not only to reduce the interaction with the membrane but also results in the formation of various oligomers on its surface in case of changes in the lipid membrane charge [21]. It still has to be elucidated how the mechanism of FCHSD2 autoinhibition works in mammals.

FCHo1/2. The mammalian genome contains two genes of FCHo proteins (from Fer/Cip4 homology domain-only): *FCHo1* and *FCHo2* (NCBI Gene ID: 23149, 115548; *Homo sapiens*), encoding the two respective protein isoforms. The expression of both isoforms is detected in numerous organs and tissues; however, *FCHo1* demonstrates a higher level of expression in the brain compared to *FCHo2* [87].

FCHo proteins comprise an F-BAR domain and a μHD domain linked through a disordered linker region [88]. The F-BAR domain in the FCHo dimer is characterized by a low curvature of membrane-binding surface and S-shape, though less marked compared to syndapin [89]. For FCHo proteins, the mechanism of autoinhibition has not been described.

It should be noted that the amino acid sequences of many BAR proteins contain IDRs that can be involved in the formation of a liquid protein phase [90]. The interaction with the vesicular membrane, the presence of IDRs and SH3 domains, and the autoinhibition ability are significant arguments in favor of the fact that these molecules can regulate the state of protein liquid phases in the nerve terminal.

FUNCTIONS OF BAR PROTEINS IN THE CYCLE OF SYNAPTIC VESICLES AND THEIR ROLE IN LIQUID PHASE REGULATION

BAR proteins in the synaptic vesicle cycle were initially identified as molecules involved in endocytosis.

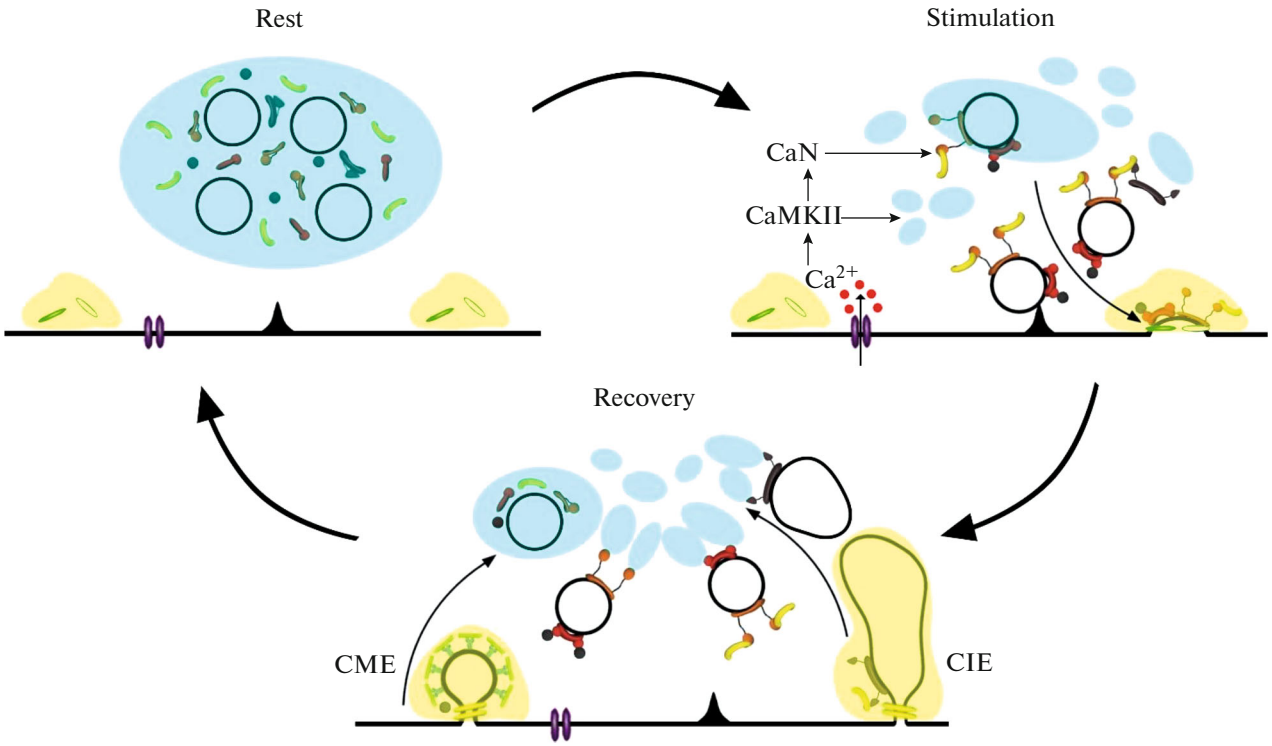

Fig. 2. Scheme illustrating the involvement of BAR proteins in the regulation of liquid protein phases formed at different stages of the synaptic cycle. The upper left-hand side of the scheme shows the organization of the reserve pool in the quiescent state (Rest). BAR proteins are in the monomeric autoinhibited state and stabilize the liquid phase of synapsin that arranges vesicles into a cluster. F-BAR proteins (FCHo, FCHSD, and partially syndapin) are localized in the periactive zone. During stimulation (the upper right-hand side of the scheme), the calcium entry through voltage gated Ca^{2+} channels results in phosphorylation of synapsin with the involvement of CaMKII and destruction of the liquid phase in the cluster. N-BAR proteins undergo dephosphorylation and dimerization, which leads to the interaction with the membrane of synaptic vesicles and other endocytic proteins, e.g., dynamin and synaptojanin, which are also localized in the cluster in the quiescent state. Dynamin here can simultaneously bind syndapin and interact with amphiphysin present on the vesicular membrane. Vesicles are shifted to the active zone and fused with the presynaptic membrane. BAR proteins involved in the regulation of these stages are in a soluble state in the axoplasm. During endocytosis (the bottom part of the scheme), BAR proteins again become components of liquid protein condensate in the periactive zone (*yellow*). Vesicle budding from the membrane with the involvement of dynamin GTPase is accompanied by post-translational modifications of the proteins and destruction of the liquid phase. BAR proteins interact with synapsin, which results in an increase in its local concentration. Dephosphorylation of synapsin, followed by intensification of its interaction with vesicles, results in the replacement of BAR proteins on the surface of vesicles and the formation of the liquid phase. Posttranslational modifications and the formation of autoinhibited monomers of BAR proteins lead to the stabilization of the liquid phase and organization of vesicles into a cluster. For details, see the text. *Large arrows* indicate the direction of the synaptic cycle. The liquid phase of synapsin is shown in *light blue*; amphiphysin, *orange*; endophilin A, *red*; syndapin 1, *gray*; dynamin, *yellow*; FCHo and FCHSD are *green*; voltage-gated calcium channels, *violet*; calcium ions, *red circles*; synaptojanin, *gray circles*. *Abbreviations*: CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CaN, calcineurin; CME, clathrin-mediated endocytosis; CIE, clathrin-independent endocytosis.

Further studies showed that BAR proteins also play an important role in synapses at other stages of the synaptic cycle, from the coupling of exo- and endocytosis to the retrieval of vesicles into the cluster in the active zone. Some BAR proteins are constantly present in the periactive zone, while other BAR proteins occur in the cluster of synaptic vesicles in the quiescent state and migrate to the periactive zone during synaptic activity [59, 91–93]. For example, endophilin, amphiphysin, and syndapin are localized in the clusters of synaptic vesicles organized by the liquid phase of synapsin, above the active zone, and are delivered to the periactive zone of the presynapse during synaptic activity after the fusion of vesicles with the membrane in the active zone [59, 91–94]. A number of endocytic proteins such as dynamin, synaptojanin, and intersectin migrate together with the BAR proteins listed above [91, 95, 96]. At the same time, the proteins undergo dephosphorylation by CaN [53, 66, 91–93] (Fig. 2).

The available data on the properties of the proteins discussed above raises the question: how do BAR proteins perform their functions in the presynaptic secretory cycle and participate in the regulation of LLPTs?

Presynaptic BAR proteins in the quiescent state. As mentioned previously, synaptic vesicles are organized into a cluster by the liquid phase of the presynaptic protein synapsin. Experiments in vitro have shown

that the liquid phase of synapsin is formed due to weak interactions between IDR of protein molecules [39]. For this purpose, its interaction with the SH3 domains of BAR proteins must be impaired. This assumption is supported by experiments demonstrating that the introduction of synapsin IDR antibodies disturbs the organization of the reserve pool of vesicles in the lamprey presynapse at rest [40]. It can be assumed that synapsin dephosphorylation and subsequent interaction with the vesicular membrane through the ALPS motif and C-domain in the liquid phase results in the displacement of BAR proteins from the membrane surface and their transition to a monomeric closed state or, like in the case of endophilin, the protein can be bound to VGLUT1 (vesicular glutamate transporter) [97]. The inactivation of SH3 interactions is supported by experiments where the blocking of the SH3 domains of amphiphysin and the SH3A domain of intersectin by antibodies were found not to lead to the destruction of the reserve pool in the quiescent state [40]. At the same time, the presence of IDRs in the structure of BAR proteins can contribute to the maintenance of the liquid phase of synapsin, as shown for other proteins [98–100]. This is also consistent with the assumption that the SH3 domains of other endocytic proteins in the formed liquid phase of synapsin are inactive [101].

Experiments have shown that synapsin, during synaptic activity, undergoes complex phosphorylation [39, 53, 102] (Fig. 2). Phosphorylation with the involvement of calcium/calmodulin-dependent protein kinase II (CaMK II) causes a transition of the protein (LLPT) from the liquid phase into the soluble form, allowing synaptic vesicles to migrate to the active zone of the presynapse [39]. Dephosphorylation of BAR proteins by Ca^{2+}/calmodulin-dependent phosphatase, CaN, in this case, makes them active, which promotes transition to the active and then periactive zone [53, 54, 66, 91].

Exocytosis. Studies in recent years suggest the involvement of BAR domain-containing proteins in different stages of exocytosis in the presynapse, including pore formation during the fusion of vesicles with the presynaptic membrane. For example, enhanced expression of endophilin increases the probability of neurotransmitter release [103]. By the example of glutamatergic neurons of the rat cerebral cortex, it has been shown that the interaction between endophilin A1 and vesicular glutamate transporter VGLUT1 decreases the efficiency of exocytosis [97]. This interaction is based on the affinity of the proline-rich sequence of VGLUT1 to the SH3 domain of endophilin A1. Thus, VGLUT1 binds endophilin A1 monomers, decreasing the amount of "active" endophilin on the membrane surface of vesicles involved in the synaptic cycle.

In addition, the interaction of the SH3 domain of endophilin may be an important factor during the positive regulation of exocytosis. The synaptic scaffold protein intersectin binds to endophilin as a result of interaction between their SH3 domains [104]. The endophilin–intersectin complex is thought to play a key role in exocytosis. This hypothesis has been supported by work in chromaffin cells of adrenal glands, which use vesicles like those in synapses for the secretion of biologically active molecules. It has been shown that endophilins A1 and A2 act in tandem with intersectin, increasing the probability of neurotransmitter release [105]. It has been hypothesized that such stimulation is implemented through the interaction between intersectin and SNARE proteins that is regulated by endophilin. Further studies are required to confirm whether this mechanism works in interneuronal synapses.

The probable role of amphiphysin 1 in exocytosis has been shown in synaptosomes isolated from mouse brain. The experiments on assessment of the efficiency of release of fluorescent dye loaded into vesicles have shown that, in the case of the amphiphysin 1 gene knockout (when reduced expression of amphiphysin 2 is also observed), the intensity of exocytosis substantially decreases compared to the wild type [55]. In addition, the involvement of amphiphysin 1 in exocytosis has been confirmed in model experiments with the fusion of vesicles with the presynaptic membrane in neuroendocrine cells. This study indicated the direct recruitment of amphiphysin 1 to the site of vesicle fusion [106].

Oligophrenin 1 is another BAR protein, the role of which is associated with the regulation of exocytosis in central synapses. Experiments in hippocampal cultures of mouse neurons have shown that this protein increases the probability of fusion of synaptic vesicles with the membrane [107]. In addition, it is thought that it plays a role in the coupling of exo- and endocytosis. On the one hand, oligophrenin 1 inactivates the RhoA/ROCK signaling pathway which, in turn, suppresses endocytosis through endophilin phosphorylation [77]. On the other hand, the PRD region of oligophrenin 1 directly interacts with the SH3 domain of endophilin A1, and the impairment of this interaction in case of mutations in the PRD domain of oligophrenin 1 slows down the recirculation of synaptic vesicles [78]. The molecular mechanism underlying this phenomenon has been recently analyzed in detail [108]. It has been proposed that oligophrenin 1 is associated with pore formation during the fusion of the vesicle with the presynaptic membrane through the GAP domain and controls the subsequent capture of the vesicular membrane using the BAR domain. Thus, the protein provides the spatial and temporal coupling of exocytosis and endocytosis.

Endocytosis. During the recovery of synaptic vesicles in the periactive zone, the protein complexes involved in various forms of endocytosis again form a liquid phase (Fig. 2). As mentioned previously, one of

the key mechanisms of the recovery of synaptic vesicles is clathrin-mediated endocytosis, which is initiated in the presynaptic membrane regions enriched in phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$). Endocytosis proceeds in several stages; as a result, a synaptic vesicle is budded from the membrane. BAR proteins are key participants in this process. A number of studies suggest that some F-BAR proteins can be constantly present in the periactive zone and recruited to the sites of endocytosis by protein complexes associated with the vesicular membrane enriched in $PI(4,5)P_2$ [46, 109]. The first F-BAR proteins recruited to the region of formation of a future vesicle are FCHo1/2 proteins, which, in turn, attract the Eps15 protein and intersectin to the site of endocytosis [110]. Studies in vitro have shown that FCHo2 homodimers form ring-shaped structures on the membrane surface, comparable to the size of the sites of clathrin-mediated endocytosis, which contributes to the clustering of $PI(4,5)P_2$ molecules [111]. In addition, the linker region of FCHo1/2 can interact with adaptor protein AP2, which also results in its clustering within the membrane and destabilization of its closed conformation. After that, $PI(4,5)P_2$, as well as clathrin and other endocytosis proteins, begin to compete with FCHo1/2 for the binding to AP2, which leads to the transition of the adaptor protein into an open conformation and triggers the subsequent stages of endocytosis [112]. It is believed that the liquid phase can be formed at the very initial stages of the assembly of a clathrin-coated pit [45].

Endophilin and amphiphysin are recruited to the zone of formation of a clathrin-coated pit already at early stages of endocytosis [113]. For example, the introduction of anti-endophilin antibodies leads to the cessation of endocytosis in the periactive zone in the giant axon of the lamprey at the initial stages of membrane invagination of the clathrin-coated pit [114], while the introduction of antibodies against the CLAP domain of amphiphysin results in disturbance of the shape of clathrin-coated vesicles, which also indicates impaired assembly of the clathrin coat at early stages [59].

The experiments with living organisms and the model experiments with liposomes in vitro have shown active participation of N-BAR proteins endophilin A1 and amphiphysins 1/2 in the stages of vesicle budding from the membrane and clathrin coat disassembly [53, 54]. For example, the impaired interaction between amphiphysins and dynamin in the giant synapse of the lamprey as a result of the introduction of the SH3 domain of amphiphysin leads to the accumulation of invaginated clathrin-coated pits in the periactive zone [20]. At the same time, under conditions of excessive expression of the SH3 domains of endophilin in the presynapse, together with the accumulation of invaginated clathrin-coated pits around the active zone, there are also numerous clathrin-coated vesicles [115]. Triple knockout of the endophilin A1–A3 genes in mice also results in the accumulation of clathrin-coated vesicles in the axoplasm [116]. Endophilin shows an affinity both to dynamin and to synaptojanin involved in clathrin coat disassembly from vesicles [19, 116]. Taken together, these data indicate a decisive role of amphiphysin at the stages of preparation for the scission of a clathrin-coated pit from the membrane surface and a role of endophilin in the recruitment of synaptojanin and the subsequent clathrin coat disassembly in the newly formed vesicles.

Amphiphysin interacts with the proline-rich region of dynamin through the SH3 domain, recruiting dynamin GTPase for polymerization around the neck of the clathrin-coated pit for the subsequent vesicle budding from the membrane [20, 53]. It is a result of the mechanochemical activity of dynamin [117]. Vesicle budding is followed by rapid disassembly of its clathrin coat. This process involves a polyinositol phosphatase synaptojanin [53, 118]. Synaptojanin is recruited by the SH3 domain of endophilin and hydrolyzes $PI(4,5)P_2$ to PI(4)P, which results in the detachment of all adaptor proteins from the vesicle surface and formation of a platform for the recruitment of auxilin and other molecules involved in clathrin coat disassembly [53, 119].

A recent study has also shown that intersectin, through an SH3–SH3 interaction, recruits the F-BAR protein FCHSD2 to the base of the clathrin-coated pit; the latter protein binds to the flat membrane regions at the pit base and triggers actin polymerization mediated by the activation of N-WASP and Arp2/3. Presumably, actin polymerization directed from the pit base to the clathrin coat of a future vesicle facilitates the final stages of clathrin-mediated endocytosis [86]. It should be noted that the homolog of this protein in the neuromuscular synapses of Drosophila, Nervous Wreck (NWK), is localized solely in the periactive zone during the synaptic cycle, which suggests analogous localization of FCHSD2 in central synapses [86]. The impaired recruitment of NWK to the sites of vesicle assembly results in vesicle size abnormality, indicating the important role of this F-BAR protein even at the early stages of endocytosis [120].

BAR proteins are also involved in other nonclassical scenarios of endocytosis. For example, it has been shown that endophilin A is involved in the recycling of synaptic vesicles through ultrafast endocytosis [28–30] and fast endophilin-mediated endocytosis (FEME) [121–123, review 124]. In addition, syndapin 1 has been shown to participate in ultrafast endocytosis and to act, as demonstrated by experiments with primary neuronal cultures and with isolated proteins, as an adapter between the presynaptic membrane and the molecules of dynamin splice isoform Dyn1xA with the formation of a protein condensate, or a liquid phase. Apparently, this is the way how preparative local accu-

mulation of dynamin occurs in the zone of endocytosis, thereby accelerating the kinetics of the process [46].

Syndapin 1 also plays an important role in bulk endocytosis. Experiments with the giant axons of the lamprey as well as with mammalian primary neuronal cultures, have demonstrated that bulk endocytosis requires not only the presence of functionally active syndapin but also its interaction with dynamin 1 [82, 94]. The condition of this interaction is the dephosphorylation of dynamin 1 by the Ca^{2+}/calmodulin-dependent phosphatase calcineurin [125]. The blocked interaction between CaN and dynamin 1 impairs the formation of synaptic vesicles on the surface of bulk endosomes and makes it impossible to restore the reserve pool [126].

Formation of the presynaptic cluster of vesicles. There are grounds to believe that, after vesicle budding from the presynaptic membrane and clathrin coat disassembly, proteins such as endophilin and amphiphysin remain on the surface of vesicles (Fig. 2). It is not improbable that syndapin falls into the cluster with vesicles of a larger diameter, which are formed during clathrin-independent endocytosis [46]. An interesting property of the SH3 domains of endophilin, amphiphysin, and syndapin is the ability to interact with synapsin [127–129]. These interactions may contribute to the increase in the local concentration of synapsin on the surface of newly formed vesicles for the subsequent transition of synapsin into the liquid phase. Currently, there is no clear evidence of the role of BAR proteins in the restoration of the cluster of vesicles during synaptic activity. Further studies are required to elucidate the detailed mechanisms of involvement of these proteins. Our hypothesis is supported by the experiments, where the knockout of endophilin genes is accompanied by the decrease in the size of clusters of synaptic vesicles in synapses [116]; however, there may be other explanations for the above observation, e.g., impaired endocytosis. The knockout of the amphiphysin 1 and syndapin 1 genes has no significant effect on the organization of the clusters of synaptic vesicles in nerve endings [55, 130]. It is not improbable that the functions of these proteins are redundant because other presynaptic molecules also work at this stage of the secretory cycle. The function of concentrating synapsin near the presynaptic active zone is performed, e.g., by the scaffold protein intersectin, which has a cassette of SH3 domains and can form multimeric synapsin-binding complexes [39, 40, 101, 131]. Biochemical experiments have shown that, in the case of intersectin, the interaction with synapsin is possible only under conditions stimulating protein phosphorylation. This fact suggests that synapsin binds to the SH3 domains in the phosphorylated state [101, 131].

CONCLUSIONS

The experiments of recent years indicate that BAR proteins are involved in the work of the synaptic secretory cycle at many of its stages. The ability of some synaptic proteins to undergo phase transition from a solution into a liquid phase with unique properties has fundamentally changed our ideas of the principles of work of the synaptic secretory cycle. Some of the hypotheses presented in this review have to be further confirmed. It should be noted that the functions of BAR proteins in nerve endings are not confined to the cycle of synaptic vesicles. For example, it has been shown that endophilins are involved in autophagy and mitochondrial membrane modifications [71, 72, 132–134]. Amphiphysins and NWK/FCHCD2 can participate in the coupling of membrane processes with cytoskeletal rearrangement [86, 109, 135, 136]. New data indicate the involvement of LLPTs in these processes too [137–139]. Further studies of the role of LLPTs in central synapses will contribute to a broader understanding of cellular mechanisms, which provide signal transduction in nerve endings, and potential sources of pathologies in the central nervous system.

FUNDING

The work was supported by the Russian Science Foundation (project no. 21-15-00227) and the Swedish Research Council (grants 2020-01731, 2020-01952), as well as by grants from St. Petersburg State University (ID 93026594) and the Swedish Brain Foundation (Hjärnfonden).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not include any experimental studies involving animals or human participants.

REFERENCES

1. Sakamuro D., Elliott K. J., Wechsler-Reya R., Prendergast G. C. 1996. BIN1 is a novel MYC-interacting protein with features of a tumour suppressor. *Nat. Genet.* **14**, 69–77.
2. Lichte B., Veh R.W., Meyer H.E., Kilimann M.W. 1992. Amphiphysin, a novel protein associated with synaptic vesicles. *EMBO J.* **11**, 2521–2530.
3. Sivadon P., Bauer F., Aigle M., Crouzet M. 1995. Actin cytoskeleton and budding pattern are altered in the yeast rvs161 mutant: The Rvs161 protein shares common domains with the brain protein amphiphysin. *Mol. Gen. Genet.* **246**, 485–495.
4. Nishimura T., Morone N., Suetsugu S. 2018. Membrane re-modelling by BAR domain superfamily proteins via molecular and non-molecular factors. *Biochem. Soc. Trans.* **46**, 379–389.
5. Itoh T., De Camilli P. 2006. BAR, F-BAR (EFC) and ENTH/ANTH domains in the regulation of membrane-cytosol interfaces and membrane curvature. *Biochim. Biophys. Acta.* **1761**, 897–912.
6. Frost A., Unger V.M., De Camilli P. 2009. The BAR domain superfamily: Membrane-molding macromolecules. *Cell.* **137**, 191–196.
7. Peter B.J., Kent H.M., Mills I.G., Vallis Y., Butler P.J., Evans P.R., McMahon H.T. 2004. BAR domains as

sensors of membrane curvature: The amphiphysin BAR structure. *Science.* **303**, 495–499.
8. Weissenhorn W. 2005. Crystal structure of the endophilin-A1 BAR domain. *J. Mol. Biol.* **351**, 653–661.
9. Gallop J.L., Jao C.C., Kent H.M., Butler P.J., Evans P.R., Langen R., McMahon H.T. 2006. Mechanism of endophilin N-BAR domain-mediated membrane curvature. *EMBO J.* **25**, 2898–2910.
10. Itoh T., Erdmann K.S., Roux A., Habermann B., Werner H., De Camilli P. 2005. Dynamin and the actin cytoskeleton cooperatively regulate plasma membrane invagination by BAR and F-BAR proteins. *Curr. Biol.* **9**, 791–804.
11. Frost A., De Camilli P., Unger V.M. 2007. F-BAR proteins join the BAR family fold. *Structure.* **15**, 751–753.
12. Ahmed S., Goh W.I., Bu W. 2010. I-BAR domains, IRSp53 and filopodium formation. *Semin. Cell. Dev. Biol.* **21**, 350–356.
13. Zhao H., Pykalainen A., Lappalainen P. 2011. I-BAR domain proteins: Linking actin and plasma membrane dynamics. *Curr. Opin. Cell Biol.* **23**, 14–21.
14. Chatzi C., Westbrook G. L. 2021. Revisiting I-BAR proteins at central synapses. *Front. Neural Circuits.* **15**, 787436.
15. Takei K., Slepnev V.I., Haucke V., De Camilli P. 1999. Functional partnership between amphiphysin and dynamin in clathrin-mediated endocytosis. *Nat. Cell. Biol.* **1**, 33–39.
16. Farsad K., Ringstad N., Takei K., Floyd S.R., Rose K., De Camilli P. 2001. Generation of high curvature membranes mediated by direct endophilin bilayer interactions. *J. Cell. Biol.* **155**, 193–200.
17. Richnau N., Fransson A., Farsad K., Aspenstrom P. 2004. RICH-1 has a BIN/Amphiphysin/Rvsp domain responsible for binding to membrane lipids and tubulation of liposomes. *Biochem. Biophys. Res. Commun.* **320**, 1034–1042.
18. Carman P.J., Dominguez R. 2018. BAR domain proteins–a linkage between cellular membranes, signaling pathways, and the actin cytoskeleton. *Biophys. Rev.* **10**, 1587–1604.
19. Ringstad N., Nemoto Y., De Camilli P. 1997. The SH3p4/Sh3p8/SH3p13 protein family: Binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain. *Proc. Natl. Acad. Sci. USA.* **94**, 8569–8574.
20. Shupliakov O., Low P., Grabs D., Gad H., Chen H., David C., Takei K., De Camilli P., Brodin L. 1997. Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. *Science.* **276**, 259–263.
21. Kelley C.F., Messelaar E.M., Eskin T.L., Wang S., Song K., Vishnia K., Becalska A.N., Shupliakov O., Hagan M.F., Danino D., Sokolova O.S., Nicastro D., Rodal A.A. 2015. Membrane charge directs the outcome of F-BAR domain lipid binding and autoregulation. *Cell Rep.* **13**, 2597–2609.
22. Kojima C., Hashimoto A., Yabuta I., Hirose M., Hashimoto S., Kanaho Y., Sumimoto H., Ikegami T., Sabe H. 2004. Regulation of Bin1 SH3 domain binding by phosphoinositides. *EMBO J.* **23**, 4413–4422.
23. Chen Z., Chang K., Capraro B.R., Zhu C., Hsu C.J., Baumgart T. 2014. Intradimer/intermolecular interactions suggest autoinhibition mechanism in endophilin A1. *J. Am. Chem. Soc.* **136**, 4557–4564.
24. Rizzoli S.O., Betz W.J. 2005. Synaptic vesicle pools. *Nat. Rev. Neurosci.* **6**, 57–69.
25. Roos J., Kelly R.B. 1999. The endocytic machinery in nerve terminals surrounds sites of exocytosis. *Curr. Biol.* **9**, 1411–1414.
26. Maycox P.R., Link E., Reetz A., Morris S.A., Jahn R. 1992. Clathrin-coated vesicles in nervous tissue are involved primarily in synaptic vesicle recycling. *J. Cell. Biol.* **118**, 1379–1388.
27. Granseth B., Odermatt B., Royle S.J., Lagnado L. 2006. Clathrin-mediated endocytosis is the dominant mechanism of vesicle retrieval at hippocampal synapses. *Neuron.* **51**, 773–786.
28. Watanabe S., Liu Q., Davis M.W., Hollopeter G., Thomas N., Jorgensen N.B., Jorgensen E.M. 2013. Ultrafast endocytosis at *Caenorhabditis* elegans neuromuscular junctions. *Elife.* **2**, e00723.
29. Watanabe S., Rost B.R., Camacho-Perez M., Davis M.W., Sohl-Kielczynski B., Rosenmund C., Jorgensen E.M. 2013. Ultrafast endocytosis at mouse hippocampal synapses. *Nature.* **504**, 242–247.
30. Watanabe S., Mamer L.E., Raychaudhuri S., Luvsanjav D., Eisen J., Trimbuch T., Sohl-Kielczynski B., Fenske P., Milosevic I., Rosenmund C., Jorgensen E.M. 2018. Synaptojanin and endophilin mediate neck formation during ultrafast endocytosis. *Neuron.* **98**, 1184–1197.e6.
31. Shin W., Wei L., Arpino G., Ge L., Guo X., Chan C.Y., Hamid E., Shupliakov O., Bleck C.K.E., Wu L.G. 2021. Preformed omega-profile closure and kiss-and-run mediate endocytosis and diverse endocytic modes in neuroendocrine chromaffin cells. *Neuron.* **109**, 3119–3134.
32. Wu W., Wu L. G. 2007. Rapid bulk endocytosis and its kinetics of fission pore closure at a central synapse. *Proc. Natl. Acad. Sci. USA.* **104**, 10234–10239.
33. Cousin M.A., Nicholls D.G. 1997. Synaptic vesicle recycling in cultured cerebellar granule cells: Role of vesicular acidification and refilling. *J. Neurochem.* **69**, 1927–1935.
34. Cousin M.A., Robinson P.J. 1999. Mechanisms of synaptic vesicle recycling illuminated by fluorescent dyes. *J. Neurochem.* **73**, 2227–2239.
35. Rizzoli S. O. 2014. Synaptic vesicle recycling: Steps and principles. *EMBO J.* **33**, 788–822.
36. Banani S.F., Lee H.O., Hyman A.A., Rosen M.K. 2017. Biomolecular condensates: Organizers of cellular biochemistry. *Nat. Rev. Mol. Cell. Biol.* **18**, 285–298.
37. Shin Y., Brangwynne C.P. 2017. Liquid phase condensation in cell physiology and disease. *Science.* **357,** eaaf4382.
38. McDonald N.A., Fetter R.D., Shen K. 2020. Assembly of synaptic active zones requires phase separation of scaffold molecules. *Nature.* **588**, 454–458.
39. Milovanovic D., Wu Y., Bian X., De Camilli P. 2018. A liquid phase of synapsin and lipid vesicles. *Science.* **361**, 604–607.
40. Pechstein A., Tomilin N., Fredrich K., Vorontsova O., Sopova E., Evergren E., Haucke V., Brodin L., Shupliakov O. 2020. Vesicle clustering in a living syn-

apse depends on a synapsin region that mediates phase separation. *Cell Rep.* **30**, 2594–2602.e3.

41. Wang S.S.H., Held R.G., Wong M.Y., Liu C., Karakhanyan A., Kaeser P.S. 2016. Fusion Competent synaptic vesicles persist upon active zone disruption and loss of vesicle docking. *Neuron*. **91**, 777–791.
42. Wu X., Cai Q., Shen Z., Chen X., Zeng M., Du S., Zhang M. 2019. RIM and RIM–BP form presynaptic active-zone-like condensates via phase separation. *Mol. Cell*. **73**, 971–984.e5.
43. Emperador-Melero J., Wong M.Y., Wang S.S.H., de Nola G., Nyitrai H., Kirchhausen T., Kaeser P.S. 2021. PKC-phosphorylation of Liprin-alpha3 triggers phase separation and controls presynaptic active zone structure. *Nat. Commun*. **12**, 3057.
44. Liang M., Jin G., Xie X., Zhang W., Li K., Niu F., Yu C., Wei Z. 2021. Oligomerized liprin-alpha promotes phase separation of ELKS for compartmentalization of presynaptic active zone proteins. *Cell Rep.* **34**, 108901.
45. Day K.J., Kago G., Wang L., Richter J.B., Hayden C.C., Lafer E.M., Stachowiak J.C. 2021. Liquid-like protein interactions catalyse assembly of endocytic vesicles. *Nat. Cell. Biol.* **23**, 366–376.
46. Imoto Y., Raychaudhuri S., Ma Y., Fenske P., Sandoval E., Itoh K., Blumrich E.M., Matsubayashi H.T., Mamer L., Zarebidaki F., Sohl-Kielczynski B., Trimbuch T., Nayak S., Iwasa J.H., Liu J., Wu B., Ha T., Inoue T., Jorgensen E.M., Cousin M.A., Rosenmund C., Watanabe S. 2022. Dynamin is primed at endocytic sites for ultrafast endocytosis. *Neuron*. **110**, 2815–2835.e13.
47. Wu X., Ganzella M., Zhou J., Zhu S., Jahn R., Zhang M. 2021. Vesicle tethering on the surface of phase-separated active zone condensates. *Mol. Cell.* **81**, 13–24.e7.
48. Alberti S. 2017. Phase separation in biology. *Curr. Biol.* **27**, R1097–R1102.
49. Krabben L., Fassio A., Bhatia V.K., 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.
50. Park D., Wu Y., Lee S.E., Kim G., Jeong S., Milovanovic D., De Camilli P., Chang S. 2021. Cooperative function of synaptophysin and synapsin in the generation of synaptic vesicle-like clusters in non-neuronal cells. *Nat. Commun.* **12**, 263.
51. Boeynaems S., Alberti S., Fawzi N.L., Mittag T., Polymenidou M., Rousseau F., Schymkowitz J., Shorter J., Wolozin B., Van Den Bosch L., Tompa P., Fuxreiter M. 2018. Protein phase separation: A new phase in cell biology. *Trends Cell. Biol.* **28**, 420–435.
52. Ghosh A., Mazarakos K., Zhou H.X. 2019. Three archetypical classes of macromolecular regulators of protein liquid-liquid phase separation. *Proc. Natl. Acad. Sci. USA*. **116**, 19474–19483.
53. Saheki Y., De Camilli P. 2012. Synaptic vesicle endocytosis. *Cold Spring Harb. Perspect. Biol.* **4**, a005645.
54. Chanaday N.L., Cousin M.A., Milosevic I., Watanabe S., Morgan J.R. 2019. The synaptic vesicle cycle revisited: New insights into the modes and mechanisms. *J. Neurosci.* **39**, 8209–8216.
55. Di Paolo G., Sankaranarayanan S., Wenk M.R., Daniell L., Perucco E., Caldarone B.J., Flavell R., Picciotto M.R., Ryan T.A., Cremona O., De Camilli P. 2002. Decreased synaptic vesicle recycling efficiency and cognitive deficits in amphiphysin 1 knockout mice. *Neuron*. **33**, 789–804.
56. Slepnev V.I., Ochoa G.C., Butler M.H., De Camilli P. 2000. Tandem arrangement of the clathrin and AP-2 binding domains in amphiphysin 1 and disruption of clathrin coat function by amphiphysin fragments comprising these sites. *J. Biol. Chem.* **275**, 17583–17589.
57. Cowling B.S., Prokic I., Tasfaout H., Rabai A., Humbert F., Rinaldi B., Nicot A. S., Kretz C., Friant S., Roux A., Laporte J. 2017. Amphiphysin (BIN1) negatively regulates dynamin 2 for normal muscle maturation. *J. Clin. Invest.* **127**, 4477–4487.
58. Prokic I., Cowling B.S., Laporte J. 2014. Amphiphysin 2 (BIN1) in physiology and diseases. *J. Mol. Med. (Berl.)*. **92**, 453–463.
59. Evergren E., Marcucci M., Tomilin N., Low P., Slepnev V., Andersson F., Gad H., Brodin L., De Camilli P., Shupliakov O. 2004. Amphiphysin is a component of clathrin coats formed during synaptic vesicle recycling at the lamprey giant synapse. *Traffic*. **5**, 514–528.
60. Arkhipov A., Yin Y., Schulten K. 2009. Membrane-bending mechanism of amphiphysin N-BAR domains. *Biophys. J.* **97**, 2727–2735.
61. Loll P.J., Swain E., Chen Y., Turner B.T., Zhang J.F. 2008. Structure of the SH3 domain of rat endophilin A2. *Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.* **64**, 243–246.
62. Wigge P., McMahon H.T. 1998. The amphiphysin family of proteins and their role in endocytosis at the synapse. *Trends Neurosci.* **21**, 339–344.
63. Farsad K., Slepnev V., Ochoa G., Daniell L., Haucke V., De Camilli P. 2003. A putative role for intramolecular regulatory mechanisms in the adaptor function of amphiphysin in endocytosis. *Neuropharmacology.* **45**, 787–796.
64. Tomizawa K., Sunada S., Lu Y.F., Oda Y., Kinuta M., Ohshima T., Saito T., Wei F. Y., Matsushita M., Li S.T., Tsutsui K., Hisanaga S., Mikoshiba K., Takei K., Matsui H. 2003. Cophosphorylation of amphiphysin I and dynamin I by Cdk5 regulates clathrin-mediated endocytosis of synaptic vesicles. *J. Cell. Biol.* **163**, 813–824.
65. Micheva K.D., Ramjaun A.R., Kay B.K., McPherson P.S. 1997. SH3 domain-dependent interactions of endophilin with amphiphysin. *FEBS Lett.* **414**, 308–312.
66. Bauerfeind R., Takei K., De Camilli P. 1997. Amphiphysin I is associated with coated endocytic intermediates and undergoes stimulation-dependent dephosphorylation in nerve terminals. *J. Biol. Chem.* **272**, 30984–30992.
67. Giachino C., Lantelme E., Lanzetti L., Saccone S., Bella Valle G., Migone N. 1997. A novel SH3-containing human gene family preferentially expressed in the central nervous system. *Genomics*. **41**, 427–434.
68. So C.W., Sham M.H., Chew S.L., Cheung N., So C.K., Chung S.K., Caldas C., Wiedemann L.M., Chan L.C. 2000. Expression and protein-binding studies of the EEN gene family, new interacting part-

ners for dynamin, synaptojanin and huntingtin proteins. *Biochem. J.* **348**, Pt 2, 447–458.

69. Kjaerulff O., Brodin L., Jung A. 2011. The structure and function of endophilin proteins. *Cell Biochem. Biophys.* **60**, 137–154.
70. Pierrat B., Simonen M., Cueto M., Mestan J., Ferrigno P., Heim J. 2001. SH3GLB, a new endophilin-related protein family featuring an SH3 domain. *Genomics.* **71**, 222–234.
71. Soukup S.F., Verstreken P. 2017. EndoA/endophilin-A creates docking stations for autophagic proteins at synapses. *Autophagy.* **13**, 971–972.
72. Hernandez-Diaz S., Ghimire S., Sanchez-Mirasierra I., Montecinos-Oliva C., Swerts J., Kuenen S., Verstreken P., Soukup S.F. 2022. Endophilin-B regulates autophagy during synapse development and neurodegeneration. *Neurobiol. Dis.* **163**, 105595.
73. Ambroso M.R., Hegde B.G., Langen R. 2014. Endophilin A1 induces different membrane shapes using a conformational switch that is regulated by phosphorylation. *Proc. Natl. Acad. Sci. USA.* **111**, 6982–6987.
74. Matta S., Van Kolen K., da Cunha R., van den Bogaart G., Mandemakers W., Miskiewicz K., De Bock P.J., Morais V.A., Vilain S., Haddad D., Delbroek L., Swerts J., Chavez-Gutierrez L., Esposito G., Daneels G., Karran E., Holt M., Gevaert K., Moechars D.W., De Strooper B., Verstreken P. 2012. LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. *Neuron.* **75**, 1008–1021.
75. Vazquez F.X., Unger V.M., Voth G.A. 2013. Autoinhibition of endophilin in solution via interdomain interactions. *Biophys. J.* **104**, 396–403.
76. Billuart P., Bienvenu T., Ronce N., des Portes V., Vinet M.C., Zemni R., Roest Crollius H., Carrie A., Fauchereau F., Cherry M., Briault S., Hamel B., Fryns J. P., Beldjord C., Kahn A., Moraine C., Chelly J. 1998. Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. *Nature.* **392**, 923–926.
77. Khelfaoui M., Pavlowsky A., Powell A.D., Valnegri P., Cheong K.W., Blandin Y., Passafaro M., Jefferys J.G., Chelly J., Billuart P. 2009. Inhibition of RhoA pathway rescues the endocytosis defects in Oligophrenin1 mouse model of mental retardation. *Hum. Mol. Genet.* **18**, 2575–2583.
78. Nakano-Kobayashi A., Kasri N.N., Newey S.E., Van Aelst L. 2009. The Rho-linked mental retardation protein OPHN1 controls synaptic vesicle endocytosis via endophilin A1. *Curr. Biol.* **19**, 1133–1139.
79. Govek E.E., Newey S.E., Akerman C.J., Cross J.R., Van der Veken L., Van Aelst L. 2004. The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nat. Neurosci.* **7**, 364–372.
80. Kessels M.M., Qualmann B. 2004. The syndapin protein family: Linking membrane trafficking with the cytoskeleton. *J. Cell. Sci.* **117**, 3077–3086.
81. Wang Q., Navarro M.V., Peng G., Molinelli E., Goh S.L., Judson B.L., Rajashankar K.R., Sondermann H. 2009. Molecular mechanism of membrane constriction and tubulation mediated by the F-BAR protein Pacsin/Syndapin. *Proc. Natl. Acad. Sci. USA.* **106**, 12700–12705.
82. Rao Y., Ma Q., Vahedi-Faridi A., Sundborger A., Pechstein A., Puchkov D., Luo L., Shupliakov O., Saenger W., Haucke V. 2010. Molecular basis for SH3 domain regulation of F-BAR-mediated membrane deformation. *Proc. Natl. Acad. Sci. USA.* **107**, 8213–8218.
83. Quan A., Robinson P.J. 2013. Syndapin–a membrane remodelling and endocytic F-BAR protein. *FEBS J.* **280**, 5198–5212.
84. Katoh M., Katoh M. 2004. Identification and characterization of human FCHO2 and mouse Fcho2 genes in silico. *Int. J. Mol. Med.* **14**, 327–331.
85. Uhlen M., Fagerberg L., Hallstrom B.M., Lindskog C., Oksvold P., Mardinoglu A., Sivertsson A., Kampf C., Sjostedt E., Asplund A., Olsson I., Edlund K., Lundberg E., Navani S., Szigyarto C. A., Odeberg J., Djureinovic D., Takanen J.O., Hober S., Alm T., Edqvist P.H., Berling H., Tegel H., Mulder J., Rockberg J., Nilsson P., Schwenk J.M., Hamsten M., von Feilitzen K., Forsberg M., Persson L., Johansson F., Zwahlen M., von Heijne G., Nielsen J., Ponten F. 2015. Proteomics. Tissue-based map of the human proteome. *Science.* **347**, 1260419. https://doi.org/10.1126/science.1260419
86. Almeida-Souza L., Frank R.A. W., Garcia-Nafria J., Colussi A., Gunawardana N., Johnson C.M., Yu M., Howard G., Andrews B., Vallis Y., McMahon H. T. 2018. A flat BAR protein promotes actin polymerization at the base of clathrin-coated pits. *Cell.* **174**, 325–337.e14.
87. Fagerberg L., Hallstrom B.M., Oksvold P., Kampf C., Djureinovic D., Odeberg J., Habuka M., Tahmasebpoor S., Danielsson A., Edlund K., Asplund A., Sjostedt E., Lundberg E., Szigyarto C.A., Skogs M., Takanen J.O., Berling H., Tegel H., Mulder J., Nilsson P., Schwenk J.M., Lindskog C., Danielsson F., Mardinoglu A., Sivertsson A., von Feilitzen K., Forsberg M., Zwahlen M., Olsson I., Navani S., Huss M., Nielsen J., Ponten F., Uhlen M. 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol. Cell. Proteomics.* **13**, 397–406.
88. Reider A., Barker S.L., Mishra S.K., Im Y.J., Maldonado-Baez L., Hurley J.H., Traub L.M., Wendland B. 2009. Syp1 is a conserved endocytic adaptor that contains domains involved in cargo selection and membrane tubulation. *EMBO J.* **28**, 3103–3116.
89. Henne W.M., Kent H.M., Ford M.G., Hegde B.G., Daumke O., Butler P.J., Mittal R., Langen R., Evans P.R., McMahon H.T. 2007. Structure and analysis of FCHo2 F-BAR domain: A dimerizing and membrane recruitment module that effects membrane curvature. *Structure.* **15**, 839–852.
90. Brodin L., Milovanovic D., Rizzoli S.O., Shupliakov O. 2022. alpha-Synuclein in the synaptic vesicle liquid phase: Active player or passive bystander? *Front. Mol. Biosci.* **9**, 891508.
91. Shupliakov O. 2009. The synaptic vesicle cluster: A source of endocytic proteins during neurotransmitter release. *Neuroscience.* **158**, 204–210.
92. Bai J., Hu Z., Dittman J.S., Pym E.C., Kaplan J.M. 2010. Endophilin functions as a membrane-bending molecule and is delivered to endocytic zones by exocytosis. *Cell.* **143**, 430–441.

93. Denker A., Krohnert K., Buckers J., Neher E., Rizzoli S.O. 2011. The reserve pool of synaptic vesicles acts as a buffer for proteins involved in synaptic vesicle recycling. *Proc. Natl. Acad. Sci. USA*. **108**, 17183–17188.
94. Andersson F., Jakobsson J., Low P., Shupliakov O., Brodin L. 2008. Perturbation of syndapin/PACSIN impairs synaptic vesicle recycling evoked by intense stimulation. *J. Neurosci.* **28**, 3925–3933.
95. Haffner C., Takei K., Chen H., Ringstad N., Hudson A., Butler M.H., Salcini A.E., Di Fiore P.P., De Camilli P. 1997. Synaptojanin 1: Localization on coated endocytic intermediates in nerve terminals and interaction of its 170 kDa isoform with Eps15. *FEBS Lett.* **419**, 175–180.
96. Evergren E., Gad H., Walther K., Sundborger A., Tomilin N., Shupliakov O. 2007. Intersectin is a negative regulator of dynamin recruitment to the synaptic endocytic zone in the central synapse. *J. Neurosci.* **27**, 379–390.
97. De Gois S., Jeanclos E., Morris M., Grewal S., Varoqui H., Erickson J.D. 2006. Identification of endophilins 1 and 3 as selective binding partners for VGLUT1 and their co-localization in neocortical glutamatergic synapses: Implications for vesicular glutamate transporter trafficking and excitatory vesicle formation. *Cell Mol. Neurobiol.* **26**, 679–693.
98. Willet A.H., Igarashi M.G., Chen J.S., Bhattacharjee R., Ren L., Cullati S.N., Elmore Z.C., Roberts-Galbraith R.H., Johnson A.E., Beckley J.R., Gould K.L. 2021. Phosphorylation in the intrinsically disordered region of F-BAR protein Imp2 regulates its contractile ring recruitment. *J. Cell. Sci.* 134.
99. Su M., Zhuang Y., Miao X., Zeng Y., Gao W., Zhao W., Wu M. 2020. Comparative study of curvature sensing mediated by F-BAR and an intrinsically disordered region of FBP17. *iScience.* **23**, 101712.
100. Mangione M.C., Snider C.E., Gould K.L. 2019. The intrinsically disordered region of the cytokinetic F-BAR protein Cdc15 performs a unique essential function in maintenance of cytokinetic ring integrity. *Mol. Biol. Cell.* **30**, 2790–2801.
101. Winther A.M., Vorontsova O., Rees K.A., Nareoja T., Sopova E., Jiao W., Shupliakov O. 2015. An endocytic scaffolding protein together with synapsin regulates synaptic vesicle clustering in the drosophila neuromuscular junction. *J. Neurosci.* **35**, 14756–14770.
102. Evergren E., Benfenati F., Shupliakov O. 2007. The synapsin cycle: A view from the synaptic endocytic zone. *J. Neurosci. Res.* **85**, 2648–2656.
103. Weston M.C., Nehring R.B., Wojcik S.M., Rosenmund C. 2011. Interplay between VGLUT isoforms and endophilin A1 regulates neurotransmitter release and short-term plasticity. *Neuron.* **69**, 1147–1159.
104. Pechstein A., Gerth F., Milosevic I., Japel M., Eichhorn-Grunig M., Vorontsova O., Bacetic J., Maritzen T., Shupliakov O., Freund C., Haucke V. 2015. Vesicle uncoating regulated by SH3-SH3 domain-mediated complex formation between endophilin and intersectin at synapses. *EMBO Rep.* **16**, 232–239.
105. Gowrisankaran S., Houy S., Del Castillo J.G.P., Steubler V., Gelker M., Kroll J., Pinheiro P.S., Schwitters D., Halbsgut N., Pechstein A., van Weering J.R.T., Maritzen T., Haucke V., Raimundo N., Sorensen J.B., Milosevic I. 2020. Endophilin-A coordinates priming and fusion of neurosecretory vesicles via intersectin. *Nat. Commun.* **11**, 1266.
106. Somasundaram A., Taraska J.W. 2018. Local protein dynamics during microvesicle exocytosis in neuroendocrine cells. *Mol. Biol. Cell.* **29**, 1891–1903.
107. Khelfaoui M., Denis C., van Galen E., de Bock F., Schmitt A., Houbron C., Morice E., Giros B., Ramakers G., Fagni L., Chelly J., Nosten-Bertrand M., Billuart P. 2007. Loss of X-linked mental retardation gene oligophrenin1 in mice impairs spatial memory and leads to ventricular enlargement and dendritic spine immaturity. *J. Neurosci.* **27**, 9439–9450.
108. Houy S., Estay-Ahumada C., Croise P., Calco V., Haeberle A.M., Bailly Y., Billuart P., Vitale N., Bader M.F., Ory S., Gasman S. 2015. Oligophrenin-1 connects exocytotic fusion to compensatory endocytosis in neuroendocrine cells. *J. Neurosci.* **35**, 11045–11055.
109. Del Signore S.J., Kelley C.F., Messelaar E.M., Lemos T., Marchan M.F., Ermanoska B., Mund M., Fai T.G., Kaksonen M., Rodal A.A. 2021. An autoinhibitory clamp of actin assembly constrains and directs synaptic endocytosis. *Elife.* **10**, e69597.
110. Henne W.M., Boucrot E., Meinecke M., Evergren E., Vallis Y., Mittal R., McMahon H.T. 2010. FCHo proteins are nucleators of clathrin-mediated endocytosis. *Science.* **328**, 1281–1284.
111. El Alaoui F., Casuso I., Sanchez-Fuentes D., Arpin-Andre C., Rathar R., Baecker V., Castro A., Lorca T., Viaud J., Vassilopoulos S., Carretero-Genevrier A., Picas L. 2022. Structural organization and dynamics of FCHo2 docking on membranes. *Elife.* **11,** e73156.
112. Zaccai N.R., Kadlecova Z., Dickson V.K., Korobchevskaya K., Kamenicky J., Kovtun O., Umasankar P.K., Wrobel A.G., Kaufman J.G.G., Gray S.R., Qu K., Evans P.R., Fritzsche M., Sroubek F., Honing S., Briggs J.A.G., Kelly B.T., Owen D.J., Traub L.M. 2022. FCHO controls AP2's initiating role in endocytosis through a PtdIns(4,5)P2-dependent switch. *Sci. Adv.* **8**, eabn2018.
113. Sundborger A., Soderblom C., Vorontsova O., Evergren E., Hinshaw J.E., Shupliakov O. 2011. An endophilin-dynamin complex promotes budding of clathrin-coated vesicles during synaptic vesicle recycling. *J. Cell. Sci.* **124**, 133–143.
114. Ringstad N., Gad H., Low P., Di Paolo G., Brodin L., Shupliakov O., De Camilli P. 1999. Endophilin/SH3p4 is required for the transition from early to late stages in clathrin-mediated synaptic vesicle endocytosis. *Neuron.* **24**, 143–154.
115. Gad H., Ringstad N., Low P., Kjaerulff O., Gustafsson J., Wenk M., Di Paolo G., Nemoto Y., Crun J., Ellisman M.H., De Camilli P., Shupliakov O., Brodin L. 2000. Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of endophilin. *Neuron.* **27**, 301–312.
116. Milosevic I., Giovedi S., Lou X., Raimondi A., Collesi C., Shen H., Paradise S., O'Toole E., Ferguson S., Cremona O., De Camilli P. 2011. Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoating after fission. *Neuron.* **72**, 587–601.

117. Antonny B., Burd C., De Camilli P., Chen E., Daumke O., Faelber K., Ford M., Frolov V.A., Frost A., Hinshaw J.E., Kirchhausen T., Kozlov M.M., Lenz M., Low H.H., McMahon H., Merrifield C., Pollard T.D., Robinson P.J., Roux A., Schmid S. 2016. Membrane fission by dynamin: What we know and what we need to know. *EMBO J.* **35**, 2270–2284.
118. Cao M., Wu Y., Ashrafi G., McCartney A.J., Wheeler H., Bushong E.A., Boassa D., Ellisman M.H., Ryan T.A., De Camilli P. 2017. Parkinson sac domain mutation in synaptojanin 1 impairs clathrin uncoating at synapses and triggers dystrophic changes in dopaminergic axons. *Neuron*. **93**, 882–896.e5.
119. Massol R.H., Boll W., Griffin A.M., Kirchhausen T. 2006. A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. *Proc. Natl. Acad. Sci. USA*. **103**, 10265–10270.
120. Shupliakov O., Akkuratova N., Korenkova O., Onochin K., Sopova E., Winther Å.M.E. 2020. Targeting of an F-BAR domain protein to the synaptic periactive zone ensures a uniform size of synaptic vesicles. *Eur. Neuropsychopharm*. **40**, 440–441.
121. Boucrot E., Ferreira A.P., Almeida-Souza L., Debard S., Vallis Y., Howard G., Bertot L., Sauvonnet N., McMahon H.T. 2015. Endophilin marks and controls a clathrin-independent endocytic pathway. *Nature*. **517**, 460–465.
122. Renard H.F., Simunovic M., Lemiere J., Boucrot E., Garcia-Castillo M.D., Arumugam S., Chambon V., Lamaze C., Wunder C., Kenworthy A.K., Schmidt A.A., McMahon H.T., Sykes C., Bassereau P., Johannes L. 2015. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. *Nature*. **517**, 493–496.
123. Ferreira A.P.A., Casamento A., Roas S.C., Panambalana J., Subramaniam S., Schützenhofer K., Halff E.F., Wah Hak L.C., McGourty K., Kittler J.T., Thalassinos K., Martinvalet D., Boucrot E. 2021. Cdk5 and GSK3β inhibit fast endophilin-mediated endocytosis. *Nat. Commun*. **12**, 2424.
124. Casamento A., Boucrot E. 2020. Molecular mechanism of Fast Endophilin-Mediated Endocytosis. *Biochem. J*. **477**, 2327–2345.
125. Clayton E.L., Anggono V., Smillie K.J., Chau N., Robinson P.J., Cousin M.A. 2009. The phospho-dependent dynamin–syndapin interaction triggers activity-dependent bulk endocytosis of synaptic vesicles. *J. Neurosci*. **29**, 7706–7717.
126. Cheung G., Cousin M.A. 2019. Synaptic vesicle generation from activity-dependent bulk endosomes requires a dephosphorylation-dependent dynamin-syndapin interaction. *J. Neurochem*. **151**, 570–583.
127. Modregger J., Schmidt A.A., Ritter B., Huttner W.B., Plomann M. 2003. Characterization of endophilin B1b, a brain-specific membrane-associated lysophosphatidic acid acyl transferase with properties distinct from endophilin A1. *J. Biol. Chem*. **278**, 4160–4167.
128. Qualmann B., Roos J., DiGregorio P.J., Kelly R.B. 1999. Syndapin I, a synaptic dynamin-binding protein that associates with the neural Wiskott–Aldrich syndrome protein. *Mol. Biol. Cell*. **10**, 501–513.
129. Onofri F., Giovedi S., Kao H.T., 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.
130. Schneider K., Seemann E., Liebmann L., Ahuja R., Koch D., Westermann M., Hubner C.A., Kessels M.M., Qualmann B. 2014. ProSAP1 and membrane nanodomain-associated syndapin I promote postsynapse formation and function. *J. Cell. Biol*. **205**, 197–215.
131. Gerth F., Japel M., Pechstein A., Kochlamazashvili G., Lehmann M., Puchkov D., Onofri F., Benfenati F., Nikonenko A. G., Fredrich K., Shupliakov O., Maritzen T., Freund C., Haucke V. 2017. Intersectin associates with synapsin and regulates its nanoscale localization and function. *Proc. Natl. Acad. Sci. USA*. **114**, 12057–12062.
132. Takahashi Y., Meyerkord C.L., Wang H.G. 2009. Bif-1/endophilin B1: A candidate for crescent driving force in autophagy. *Cell Death Differ*. **16**, 947–955.
133. Karbowski M., Jeong S.Y., Youle R.J. 2004. Endophilin B1 is required for the maintenance of mitochondrial morphology. *J. Cell. Biol*. **166**, 1027–1039.
134. Yang Y., Chen J., Guo Z., Deng S., Du X., Zhu S., Ye C., Shi Y.S., Liu J.J. 2018. Endophilin A1 promotes actin polymerization in dendritic spines required for synaptic potentiation. *Front. Mol. Neurosci*. **11**, 177.
135. Meunier B., Quaranta M., Daviet L., Hatzoglou A., Leprince C. 2009. The membrane-tubulating potential of amphiphysin 2/BIN1 is dependent on the microtubule-binding cytoplasmic linker protein 170 (CLIP-170). *Eur. J. Cell. Biol*. **88**, 91–102.
136. Chapuis J., Hansmannel F., Gistelinck M., Mounier A., Van Cauwenberghe C., Kolen K. V., Geller F., Sottejeau Y., Harold D., Dourlen P., Grenier-Boley B., Kamatani Y., Delepine B., Demiautte F., Zelenika D., Zommer N., Hamdane M., Bellenguez C., Dartigues J.F., Hauw J.J., Letronne F., Ayral A.M., Sleegers K., Schellens A., Broeck L. V., Engelborghs S., De Deyn P.P., Vandenberghe R., O'Donovan M., Owen M., Epelbaum J., Mercken M., Karran E., Bantscheff M., Drewes G., Joberty G., Campion D., Octave J.N., Berr C., Lathrop M., Callaerts P., Mann D., Williams J., Buee L., Dewachter I., Van Broeckhoven C., Amouyel P., Moechars D., Dermaut B., Lambert J.C., GERAD consortium. 2013. Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. *Mol. Psychiatry*. **18**, 1225–1234.
137. Noda N.N., Wang Z., Zhang H. 2020. Liquid-liquid phase separation in autophagy. *J. Cell. Biol*. **219,** e202004062.
138. Fujioka Y., Alam J.M., Noshiro D., Mouri K., Ando T., Okada Y., May A.I., Knorr R.L., Suzuki K., Ohsumi Y., Noda N.N. 2020. Phase separation organizes the site of autophagosome formation. *Nature*. **578**, 301–305.
139. La Cunza N., Tan L.X., Thamban T., Germer C.J., Rathnasamy G., Toops K.A., Lakkaraju A. 2021. Mitochondria-dependent phase separation of disease-relevant proteins drives pathological features of age-related macular degeneration. *JCI Insight*. **6**, e142254.

Translated by E. Makeeva