Review

Special Focus – Neuronal Cell Biology

Postsynaptic ProSAP/Shank scaffolds in the cross-hair of synaptopathies

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Intact synaptic homeostasis is a fundamental prerequisite for a healthy brain. Thus, it is not surprising that altered synaptic morphology and function are involved in the molecular pathogenesis of so-called synaptopathies including autism, schizophrenia (SCZ) and Alzheimer's disease (AD). Intriguingly, various recent studies revealed a crucial role of postsynaptic ProSAP/Shank scaffold proteins in all of the aforementioned disorders. Considering these findings, we follow the hypothesis that ProSAP/Shank proteins are key regulators of synaptic development and plasticity with clear-cut isoform-specific roles. We thus propose a model where ProSAP/Shank proteins are in the center of a postsynaptic signaling pathway that is disrupted in several neuropsychiatric disorders.

ProSAP/Shank proteins and synaptopathies

The establishment and maintenance of synaptic contacts and synaptic plasticity are crucial factors for normal brain function and homeostasis, and the functional properties of a synapse are largely dependent on the molecular setup of synaptic proteins. These proteins can be grouped into molecules that modulate synaptic assembly, stability, plasticity, and/or maturation. ProSAP/Shank (alternative names: CortBP, Spank, SSTRIP, Synamon) proteins function as major scaffolding molecules at postsynaptic densities (PSDs) within the Central Nervous System (CNS). Predominantly localized beneath the postsynaptic membrane and harboring multiple protein–protein interaction domains, they cross-link receptor complexes and cytoskeletal elements, thereby forming an indispensable framework for the assembly of the PSD (Box 1; Figure 1) [1–5].

The ProSAP/Shank family was identified in 1998/1999 [6–9] and consists of three members: Shank1, ProSAP1/Shank2 and ProSAP2/Shank3. Within the brain, all three family members show a broad expression pattern, including areas of higher cognitive function like cortex and hippocampus. Through local dynamic regulation, such as activity-dependent changes in turnover and dendritic mRNA transcription, ProSAPs/Shanks essentially contribute to synaptic plasticity, which is thought to be the basic molecular prerequisite for learning and memory formation [10].

An emerging role of ProSAPs/Shanks in human neurological disease was first proposed when the loss of one ProSAP2/Shank3 copy was identified as the main genetic cause for a neurodevelopmental disorder called the Phelan-McDermid Syndrome (PMS) [11]. Moreover, within the last four years, mutations in ProSAP1/Shank2 and/or ProSAP2/Shank3 proteins were identified in patients diagnosed with autism and/or intellectual disability (ID) [12–16] or SCZ [17]. Interestingly, a dysregulation of the ProSAP/Shank platform was found in AD models and patients [18–20]. These intriguing disease-related findings led us to speculate about the specific role of ProSAP/Shank family members in synaptic homeostasis and their contribution to the molecular pathogenesis of brain disorders directly linked to the malfunction of synaptic networks, so-called synaptopathies. A disruption of ProSAP/Shank-associated pathways can lead to the loss of synaptic stability, immature synapse morphology and an imbalance between excitatory and inhibitory contacts. Given that the precise molecular pathologies underlying this disruption are only beginning to be deciphered, it is indispensable for future research to highlight recent findings converging into a model, which might help to draw conclusions for the development of targeted therapeutic strategies (Box 3).

Thus, this review briefly summarizes the role of ProSAP/Shank family members in synaptopathies and discusses a model that places synaptic assembly, synapse maturation and intact glutamatergic signaling at the center of various neuropsychiatric disorders, including non-syndromic intellectual disabilities, autism spectrum disorders (ASDs), SCZ and AD, highlighting the importance of this protein family in the molecular pathology behind the aforementioned processes.

ProSAP2/Shank3 and PMS

Haploinsufficiency of the ProSAP2/Shank3 gene as the cause of PMS; Deletion 22q13.3 Syndrome, Deletion 22q13 Syndrome [11,21,22] appears to provide the most direct link between a disrupted ProSAP/Shank protein family member and a disorder whose major features include neuropsychiatric symptoms. PMS was initially described in the medical literature in 1994 [23]. Most cases are due to a spontaneous (*de novo*) break in the long arm of chromosome 22 (q), thereby causing a microdeletion in which a portion of the distal part is lost [24]. In addition

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Box 1. ProSAP/Shank domain composition and main interactions at the PSD

Multiple protein–protein interaction domains show a high degree of conservation among all three ProSAP/Shank family members and are crucial for their molecular interplay within the PSD. Although domain composition can be differentially regulated via alternative splicing events, most ProSAP/Shank isoforms found at the PSD of excitatory synapses contain an SH3 (Src homology 3), a PDZ (PSD-95/DLG/ZO-1), a SAM (sterile alpha motif) domain and a region of proline-rich clusters. Moreover, all three family members can harbor N-terminal Ankyrin repeats [3,96]. Localization to and assembly of ProSAP1/Shank2 and ProSAP2/Shank3 at the PSD crucially depend on an intact C-terminus, as it exhibits so-called synaptic targeting signals (localization to the PSD [97]) and the SAM domain (assembly at the PSD via Zn^{2+} ions [38,59,60]). Interestingly, some of the mutations identified in the ProSAP1/Shank2 and ProSAP2/Shank3 genes of individuals diagnosed with autism, ID and/or SCZ lead to premature stops and thus to modifications of the C-terminal sequence and putative truncated proteins (Figure I; for more detailed information and references, see Table 1). Attachment of ionotropic glutamate receptor complexes happens mainly by indirect or direct interaction via the PDZ domain, a master protein–protein interaction module of PSD assembly [98] (NMDAR: PDZ domain–GKAP–PSD-95; AMPAR: either PDZ domain–GKAP–PSD-95–Stargazin or directly via the PDZ domain); attachment of metabotropic receptor complexes occurs via the Homer binding site ([Hbs]; Homer binding site–Homer1a–mGluR1a/5). Proper association with the postsynaptic cytoskeleton is mediated mainly by the Ankyrin repeats (α-fodrin) and/or the cortactin binding site (Cortactin, Abp1) and other motifs (proline-rich [Pr] clusters: Abi-1, IRSp53; PDZ domain: βPIX, Fezzins) [4].

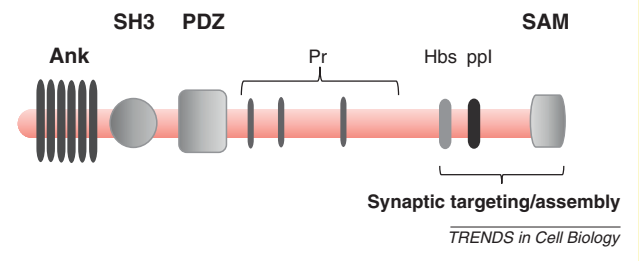

Figure I. Overview of the ProSAP/Shank domain structure.

to physical abnormalities, most affected individuals exhibit speech delay and an 'autistic-like' phenotype with tactile defensiveness, anxiety in social situations, avoidance of eye contact, and self-stimulatory behavior [25]. Given that a balanced translocation between chromosomes 12 and 22 with a breakpoint in the ProSAP2/Shank3 gene [11] and another recurrent breakpoint within intron 8 of the ProSAP2/Shank3 gene [26] were identified in unrelated PMS patients, current understanding indicates that the loss of one ProSAP2/Shank3 copy in the 22q13 region is indeed responsible for most of the disorder's neurological symptoms, including autistic features, developmental delay, hypotonia and absent or severely delayed speech.

ProSAP/Shank in ASDs and ID

ASDs like autism or Asperger Syndrome, also termed pervasive developmental disorders (PDDs), are neurodevelopmental disorders that usually manifest within the first years of life. In addition, there is a strong correlation between ASDs and ID and most ASD individuals exhibit an intelligence quotient (IQ) less than 70 [27].

Many of the genes implicated in the development of ASDs encode proteins that are crucial components of excitatory glutamatergic synapses, including ProSAP1/Shank2, ProSAP2/Shank3, Neuroligins, Neurexins, SAPAP2 (DLGAP2) from the GKAP/SAPAP family of ProSAP/Shank interacting proteins, as well as Cadherins, Contactin,

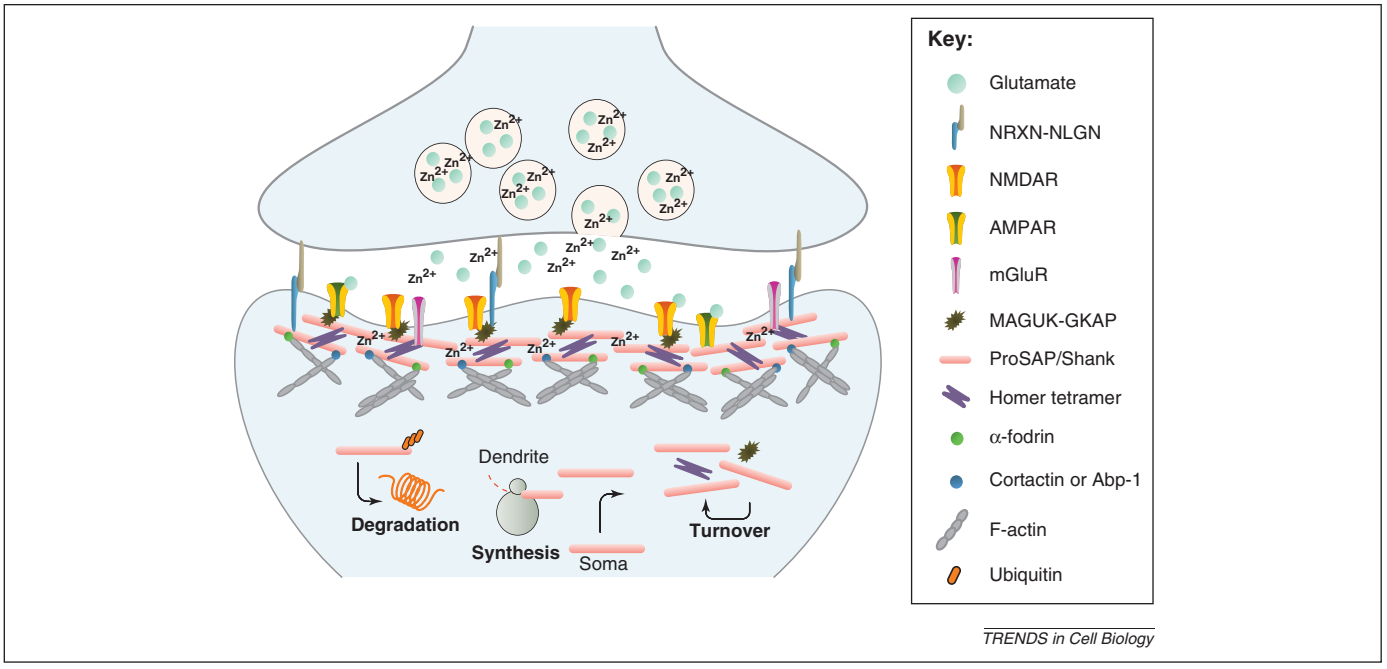

Figure 1. Functional overview of ProSAP/Shank's mode of action at CNS synapses. During development, homeostatic changes in synapse strength occur and are associated with ProSAP/Shank concentrations at PSDs. At the PSD, ProSAP/Shank proteins multimerize and build platforms providing multiple protein-protein interaction sites (e.g. linking MAGUK-GKAP-NMDAR complexes, as well as Homer-mGluR complexes and AMPA receptors, to the cytoskeleton via α-fodrin, Cortactin and/or Abp-1). ProSAP/Shank levels at the PSD, in turn, circulate via protein turnover between a PSD-bound and a soluble pool within the dendritic spine. The equilibrium between both pools is regulated by degradation and local synthesis of ProSAP/Shank, and can be shifted to a PSD-bound state by an increase in Zn^{2+} ions released from intracellular Zn^{2+} stores or from presynaptic vesicles into the synaptic cleft, where they translocate into the postsynaptic compartment.

Contactin-associated protein and other cell-adhesion molecules (CAMs), calcium channels, and neurotransmitter receptors [28–30]. ProSAP2/Shank3 was the first ProSAP/Shank family member directly related to ASD/ID-associated genomic mutations exhibiting deletions or duplications of larger areas but also point mutations (Box 1; Table 1). A guanine insertion, for example, creates a frameshift at nucleotide 3680 (InsG3680), resulting in a premature stop and thus a modification of the C-terminal sequence [12–14,16]. In these studies, many more inherited than *de novo* mutations have been identified, a phenomenon whose importance is unclear. In a large cohort study of rather high-functioning autistic individuals, no ProSAP2/Shank3 mutation was found, leading the authors to conclude that deletions of ProSAP2/Shank3 might be linked to lower functioning ASD cases [31,32].

In 2010, ProSAP1/Shank2 was added to the list of autism-associated genes with several affected individuals found to be carrying mutations in the ProSAP1/Shank2 gene; among these, two deletions were identified as disrupting the highly conserved ProSAP1/Shank2 PDZ domain. One of these patients carried a *de novo* nonsense mutation (C1384T), whose gene product, similar to the ProSAP2/Shank3 InsG3680 variant might have lost its synaptic localization ability due to disruption of the protein's C-terminus (Box 1; Table 1) [15]. A further study identified two novel *de novo* deletions in ProSAP1/Shank2 in two unrelated cases [30]. Intriguingly, so far, mutations associated with ASDs have been found in ProSAP1/Shank2 and ProSAP2/Shank3, but not in Shank1. In line with these observations, mice lacking Shank1 do not show autism-related behavioral patterns despite reduced levels of ultrasonic vocalization and scent marking behavior in social contexts [33–35].

ProSAP2/Shank3 and SCZ

SCZ is a chronic neuropsychiatric disorder characterized mainly by psychotic symptoms leading to a disruption in cognition and behavior, and is likely triggered during adolescence by poorly understood genetic and environmental factors. Interestingly, mutations in ProSAP2/Shank3 have also been associated with this disorder (Table 1) [17]. In a study screening a cohort of SCZ patients, two unrelated individuals were identified that were heterozygous for *de novo* mutations of the ProSAP2/Shank3 gene, C3349T

Table 1. *De novo* mutations in ProSAPs/Shanks associated with neurodevelopmental and neuropsychiatric disorders

Reference	Gene	Families/ individuals (n) with			*De novo* hemizygous mutations (mutations/n)	Mutations/Deletions	Clinical Phenotype[a]
		ASD	ID	SCZ			
[12]	PROSAP2/ SHANK3	227			3/227	(1) del22qter[b] with breakpoint in intron 8 (2) del22qter with complete loss of one ProSAP2/Shank3 copy, (3) sibling of (2) with partial trisomy 22qter, (4) InsG3680 (frameshift and premature stop at aa 1234)	(1) autism, absent language, mild ID, (2) autism, severe LI[c], moderate ID, (3) Asperger syndrome, (4) two brothers with: autism, severe LI severe ID, epilepsy/seizures
[13]	PROSAP2/ SHANK3	400			3/400	(1) & (2) del22qter with complete loss of one ProSAP2/Shank3 copy, (3) sibling of (2) with partial trisomy 22qter, (4) A962G (aa Q321R)	(1) autism, severe ID, (2) autism, LI, (3) ADHD, mild ID, (4) autism, severe LI
[14]	PROSAP2/ SHANK3	427			1/427	DelG in splice donor site of intron 19 (frameshift and premature stop at aa 755)	autism
[17]	PROSAP2/ SHANK3			185	2/185	(1) nonsense mutation C3349T (aa R1117X), (2) C1606T (aa R536W)	(1) three brothers: 1 with schizoaffective disorder, borderline ID, 1 with SCZ, ADHD in childhood, mild ID[d], one seizure event, 1 with SCZ, moderate ID, (2) schizoaffective disorder, LI, borderline ID
[15]	PROSAP1/ SHANK2	396	184		2/396 (1) & (2), 1/184 (3)	CNV del of exon 6/7 (premature stop in PDZ domain), (2) nonsense mutation C1384T (aa R462X), (3) CNV del of exon 7 (premature stop in PDZ domain)	(1) autism, mild ID, LI, (2) autism, (3) moderate ID, autism
[30]	PROSAP1/ SHANK2	996[e]			2/996	(1) 66 kb exonic deletion, (2) 68 kb exonic deletion	(1) autism, ID, LI, (2) PDD-NOS[f], mild ID, LI
[16]	PROSAP2/ SHANK3		95[g]		1/95	mutation in splice acceptor site of intron 5 (frameshift and premature stop at aa 211)	mild ID, severe LI
Total Score		9/1450 (0.62%)	2/279 (0.72%)	2/185 (1.1%)			

[a]according to available information; [b]del22qter = terminal deletion 22q; [c]LI = language impairment; [d]no IQ test available; [e]excluded from Total Score, since only larger deletions were detected; [f]pervasive developmental disorder – not otherwise specified (belongs to ASDs); [g]nonsyndromic ID.

and C1606T. Similar to ASD-associated InsG3680, C3349T, a nonsense mutation, results in a truncated ProSAP2/Shank3 protein lacking parts of its C-terminus, possibly disrupting synaptic localization and spine induction. The exact synaptopathic impact of the C1606T mutation, however, is unknown. Interestingly, both patients identified in this study exhibited impaired intellectual ability, as is often the case in ASD individuals (see above). Recently, a mouse model was created that lacks the C-terminus of ProSAP2/Shank3. These mice exhibit behavioral patterns associated with autism and SCZ [36]. It might be intriguing to further investigate shared etiological factors of mental retardation, SCZ and ASD that may have contributed to a patient's neuropsychiatric phenotype, because mutations in all three disorder entities have separately been associated with the ProSAP2/Shank3 gene (see Table 1 for a compendious overview of disorder-associated mutations in ProSAP2/Shank3 and ProSAP1/Shank2). Only *de novo* mutations with an assumed high penetrance are listed.

ProSAP/Shank and AD

Recent studies show that ProSAP/Shank platform disassembly is linked to the molecular pathology of AD [18,19]. Moreover, Amyloid-beta (Aβ) oligomers progressively accumulate in the forebrains of patients with AD as well as of APP transgenic mice, accompanied by a reduction in the levels of synaptic scaffold proteins, among them Shank1 and ProSAP2/Shank3 [20]. In AD, ProSAP/Shank platform disassembly caused by Aβ-induced disruption of the Homer1b and Shank1 scaffold might be linked to the molecular pathology of the disease [18–20]. Furthermore, rat frontocortical neurons in culture treated with soluble $A\beta_{1\text{-}40}$ showed a significant thinning of the PSD, decreased synaptic levels of Homer1b and Shank1, and reduced synaptic mGluR1 levels [18]. In a screen to identify candidate proteins involved in the pathology of AD, ProSAP/Shank proteins were dramatically altered in affected individuals. The protein level of ProSAP1/Shank2 in frontocortical synaptosomes of AD patients was increased, whereas that of ProSAP2/Shank3 was decreased. Additionally, ProSAP2/Shank3 was strongly ubiquitinated, indicating abnormal activity of the ubiquitin–proteasome system [19].

Taken together, research on various neuropsychiatric diseases (autism, mental retardation, SCZ, and AD) has shown that, at the molecular level, alterations of ProSAP/Shank family members occur, thereby underlining their crucial role in the molecular integrity of the synapse. Thus, understanding the functional properties of ProSAP/Shank proteins in more detail is an important goal in decoding the molecular mechanisms underlying these synaptopathies.

Contribution of ProSAP/Shank family members to synaptopathies: a functional overview

Because ProSAP/Shank family members were identified as core components of excitatory CNS synapses [7,8], malformation of their platforms at the PSD subsequently leads to a disruption of the postsynaptic protein scaffold, thereby vigorously perturbing synaptic homeostasis. This might result in the mislocalization, de-clustering and/or functional impairment of several other crucial synaptic proteins such as cytoskeletal regulators and/or neurotransmitter receptors.

A trans-synaptic pathway containing ProSAP/Shank, GKAP/SAPAP, Neuroligin–Neurexin and mGluR5

Overexpression of Shank1 leads to an enlargement of spine heads and promotes spine maturation, whereas its knockdown reduces spine density [37]. Other studies showed that overexpression of ProSAP1/Shank2 and ProSAP2/Shank3 increases the initial formation of excitatory synapses [38,39]. Overexpression of another single PSD protein, PSD-95, similarly increases the formation of excitatory synapses [40] via the recruitment of Neuroligins and the subsequent clustering of several other synaptic proteins [41]. Interestingly, *in vitro,* ProSAP/Shank proteins either interact directly with Neuroligins [42], which, along with presynaptic Neurexins bridge the synaptic cleft at glutamatergic synapses, or indirectly via interaction with GKAPs/SAPAPs and PSD-95 [9,41]. Neuroligins (NLGNs) and Neurexins (NRXNs) are synaptic CAMs (SynCAMs) forming *trans*-synaptic signaling complexes at excitatory synapses. The synaptic concentration of Neuroligins directly regulates synapse density and, via isoform-specific assembly, the ratio of excitatory/inhibitory synapses as well as morphological features and signaling strength [41,43–45]. Mutations associated with ASDs and ID have been reported in Neuroligin3, Neuroligin4, Neuroligin4Y, Neurexin1 and SAPAP2 [28,30,46–48], whereas one mutation in Neuroligin3 (R451C) was found in two independent ASD cases [46,49]. A model was thus proposed where ASD-related proteins such as ProSAPs/Shanks, Neuroligins and Neurexins are all functionally linked in a trans-synaptic signaling pathway, although there is further need to strengthen this hypothesis [50]. Another hint for a common role of these pathway-associated synaptic proteins in the pathogenesis of ASDs is the dysregulation of several microRNAs that, among others, were found to target the mRNAs of ProSAP2/Shank3 and Nrxn1 in a cohort of individuals diagnosed with autism [51].

The aforementioned proposed synaptic pathway(s) might not only be specific for ASDs, but could also contribute to the pathogenesis of other synaptopathic phenotypes, since mutations in ProSAP2/Shank3 and Nrxn1/2 have not only been associated with ASDs, but also with SCZ and ID [16,17].

Intriguingly, recent studies on the most common known genetic cause of autism, the Fragile X Syndrome (FXS), similarly proposed that ASDs might be related to a disruption of synaptic activity [52]. FXS is caused by a mutation in the FMR1 gene, thereby leading to a dysregulation of synaptic protein synthesis [53]. FMR1 encodes the Fragile X mental retardation protein (FMRP), a postsynaptically enriched mRNA binding protein found at glutamatergic synapses. Nonfunctional FMRP leads to dysregulated mGluR activity at the synapse [54] and morphological studies in FXS brains indicate the presence of abnormally long, thin dendritic spines [55]. Intriguingly, the protein levels of Shank1 and ProSAP2/Shank3, whose mRNAs are both localized to the dendritic compartment [10], are

reduced in neocortical PSDs from FMRP knockout mice [56]. ProSAP2/Shank3 is linked to mGluR5 receptors directly or indirectly via Homer [57], thereby providing another interesting link between ProSAP2/Shank3 and autism. Intriguingly, knockdown of ProSAP2/Shank3 in cultured hippocampal neurons leads to reduced expression of mGluR5, impaired mGluR5-dependent signaling and disrupted synaptic transmission. Moreover, autism-associated mutations in ProSAP2/Shank3 affect mGluR5 signaling *in vitro* [58].

Pathological zinc distribution disintegrates synaptic ProSAP/Shank clusters

The data on ProSAP/Shank and glutamatergic neurotransmission point towards a role for ProSAP/Shank proteins in synaptic pathways, where the amount of ProSAP/Shank at the PSD directly influences synaptic function. One regulator of ProSAP/Shank clustering at the synapse is Zn^{2+} [38,59,60] and ProSAP/Shank protein levels at the PSD depend on local Zn^{2+} concentration and influx [38]. Decreased Zn^{2+} concentrations at the synapse, therefore, lead to a decrease in Zn^{2+}-binding ProSAP/Shank family members at the PSD. Intriguingly, high concentrations of Zn^{2+} are observed in neuritic plaques and cerebrovascular amyloid deposits from both AD patients and AD-prone transgenic mice [61,62]. Aβ is a metal-binding protein with high affinity for copper and zinc [63] and Zn^{2+} ions promote Aβ oligomerization [64,65]. Thus, it has been speculated that one pathological role of Zn^{2+} in AD might be the promotion of Aβ oligomer aggregation. One proposed mechanism for Aβ pathology is that cognitive impairment is caused by trapping synaptic Zn^{2+} rather than through direct toxicity [66]. Thus, *trans*-synaptic movement of Zn^{2+} may be severely compromised in AD due to sequestration in Aβ oligomer aggregates, leading to enhanced plaque formation. Given that local Zn^{2+} concentrations contribute, at least in part, to ProSAP1/Shank2 and ProSAP2/Shank3 cluster stability at the PSD, trapping of Zn^{2+} within the synaptic cleft might be one pathomechanism causing ProSAP/Shank scaffold disruption and, therefore, contributing to postsynaptic instability. This conclusion is further consistent with findings showing that sequestration of Zn^{2+} by oligomeric Aβ leads to reduced availability of Zn^{2+} at the synapse, ultimately causing synapse loss accompanied by cognitive deficits [67].

Another role for synaptic Zn^{2+} dysregulation has been suggested in the pathophysiology of convulsive seizures. Mouse models for epilepsy exhibit significant decreases in Zn^{2+} concentration within the hippocampal dentate gyrus [68]. Intriguingly, epilepsy is also a major phenotype in PMS [22] and is present in a large percentage of ASD patients. Given that ProSAP1/Shank2 and ProSAP2/Shank3 are both target proteins for Zn^{2+}, the disruption of their clusters at the PSD might resemble synaptic Zn^{2+} deficiency. The role of Zn^{2+} dyshomeostasis and its impact on PSDs in epilepsy is not yet fully understood, however, and awaits evaluation by future studies.

Taken together, these data hint that ProSAP/Shank proteins not only functionally organize the molecular scaffold within the PSD, but are also core elements within synaptic pathways whose disruption in synaptopathies ultimately has a severe impact on synaptic homeostasis and, therefore, glutamatergic signaling.

ProSAP/Shank regulating synaptic homeostasis at glutamatergic synapses: a model

ProSAP/Shank proteins are strongly involved in glutamatergic synaptic signaling, including ionotropic and metabotropic glutamate receptor-mediated transmission. Via interactions with Homer and the GKAP/PSD-95 protein complex, ProSAPs/Shanks interconnect metabotropic group-I (mGluR1a/5) and ionotropic *N*-methyl-D-aspartic acid-type glutamate receptors (NMDA receptors) [57,69], whereas the GluA1 (GluR1) subunit of ionotropic amino-3-hydroxy-5-methylisoxazole-4-propionate-type glutamate receptors (AMPA receptors) directly binds to the ProSAP/Shank PDZ domain [70]. Interestingly, ProSAP2/Shank3 heterozygous knockout mice exhibit reduced AMPA-mediated basal synaptic transmission in the hippocampus [71], and one of the ProSAP2/Shank3 homozygous knockouts published thus far exhibits reduced hippocampal LTP and impaired activity-dependent trafficking of GluA1 subunits in primary hippocampal culture [72]. Moreover, a second ProSAP2/Shank3 homozygous knockout shows a dysregulation of glutamate–receptor complex-associated proteins in the striatum [73]. Furthermore, in a mouse model resembling the heterozygous state of human ProSAP2/Shank3 ASD mutations by truncation of the protein's C-terminus, disruption of the postsynaptic Homer–ProSAP2/Shank3 complex leads to increased ubiquitination and proteasomal degradation of the remaining wild type ProSAP2/Shank3 and the NMDAR subunit NR1, as well as to enhanced mGluR-dependent, long-term depression (LTD; see Box 2 for a more detailed summary of recent data on ProSAP2/Shank3 mouse models) [36].

Based on these findings, we conclude that the amount of ProSAP/Shank proteins clustering at postsynaptic compartments of excitatory synapses has an impact on proper synapse formation, maturation and transmission by directly and indirectly organizing interaction partners within the postsynaptic specialization. Thus, we propose a model where synaptic ProSAP/Shank levels have: (i) downstream effects, such as regulating the number of interaction partners within the PSD (e.g. neurotransmitter receptors, cytoskeletal-associated proteins); or (ii) upstream effects towards the presynaptic site by modulating interaction partners linked to *trans*-synaptic signaling pathways (e.g. SynCAMs such as Neuroligins and/or Neurexins) (Figures 1 and 2).

Postsynaptic scaffolds are essential for the synaptic clustering of neurotransmitter receptors and their perturbation possibly results in an imbalance between excitation and inhibition. It is well known that such an imbalance might underlie several neurological diseases like autism [27], Tourette's syndrome [74] or SCZ [75]. In healthy brains, the balance of excitation and inhibition is crucial for unimpaired cognitive performance, sleep, motor control and the processing of sensory information [76]. Transgenic mice with the ASD-associated R451C substitution in Neuroligin3 show increased inhibitory synaptic transmission. There are discrepancies, however, in studies concerning

Box 2. Mouse models harboring targeted disruption of the ProSAP2/Shank3 gene

In the CA1 hippocampus of a PMS mouse model with haploinsufficiency of the ProSAP2/Shank3 gene, a reduction of AMPA-mediated basal synaptic transmission was found alongside impaired synaptic plasticity. Behaviorally, these mice exhibit less social sniffing and reduced ultrasonic vocalization (USV) [71].

The creation of a knockout with the N-terminal Ankyrin repeats targeted for deletion resulted in an autism mouse model lacking full-length ProSAP2/Shank3, leaving shorter isoforms expressed. Besides profound behavioral abnormalities resembling an autistic-like phenotype, these animals exhibit less mature spines in the CA1 hippocampus predominantly during development and reduced levels of Homer, GKAP and the AMPAR subunit GluA1 at the synapse. This mouse model exhibits reduced synaptic plasticity (CA3-CA1 synapses) and impaired activity-dependent GluA1 redistribution in primary hippocampal cultures. Another study using a ProSAP2/Shank3 knockout model with the PDZ domain as target for deletion, showed a loss of almost all ProSAP2/Shank3 isoforms [73]. The focus of this study was on the striatum and corticostriatal circuits, which are assumed to be disturbed in ASDs, thus possibly promoting repetitive behavior. The authors identified altered molecular composition of striatal PSDs, suggesting impairment of the glutamatergic system. Medium spiny neurons showed increased neuronal complexity, while spine morphology did not seem to be dramatically altered. Consistent with the observation of a correlation between brain hypertrophy and autism [50], the authors found the striatum to be hypertrophied. During electrophysiological testing of cortico-striatal (but not hippocampal) circuits, these knockout mice exhibited impaired postsynaptic functions while presynaptic functions stayed intact. Moreover, the animals display increased anxiety-like behavior, excessive grooming and skin lesions [73]. A mouse model that is similar to specific human ProSAP2/Shank3 mutations (e.g. InsG3680 [12]) harbors a targeted disruption of the C-terminal sequence leading to the expression of a truncated ProSAP2/Shank3 isoform lacking the Homer binding site, the ppI motif and the SAM domain [36]. Analysis of synaptic protein levels in heterozygous mutants (*Shank3(+/ΔC)* revealed increased polyubiquitination of wild type ProSAP2/Shank3 and the NMDAR subunit NR1. These alterations lead to reduced NMDAR-dependent synaptic responses in the cortex and synaptic plasticity in the hippocampus (reduced LTP and LTD, respectively) without any change in synapse density or morphology. Intriguingly, *Shank3(+/ΔC)* mice do not show profound defects in cognitive function, but exhibit an ASD-like and, interestingly, a SCZ-like behavioral phenotype, especially apparent when expressing exaggerated aggressiveness to novel conspecifics, in addition to deficits in USV and social approach behavior [36].

the behavioral evaluation of this mouse model; one study shows an autism-related phenotype, while another reports wild type and knockout mice to be behaviorally indistinguishable [77,78].

The amount and distribution of excitatory and inhibitory synapses along the dendritic tree of single neurons has a significant impact on the integration of synaptic input and output [79] and E/I (excitation/inhibition) circuit dynamics can influence the normal timing of critical periods in brain development [80]. Integrated signals thereby modulate synaptic plasticity; for instance, via the alteration of long-term potentiation (LTP) [81].

The E/I balance hypothesis just described is strengthened by the fact that epilepsy, an E/I imbalance disorder, is

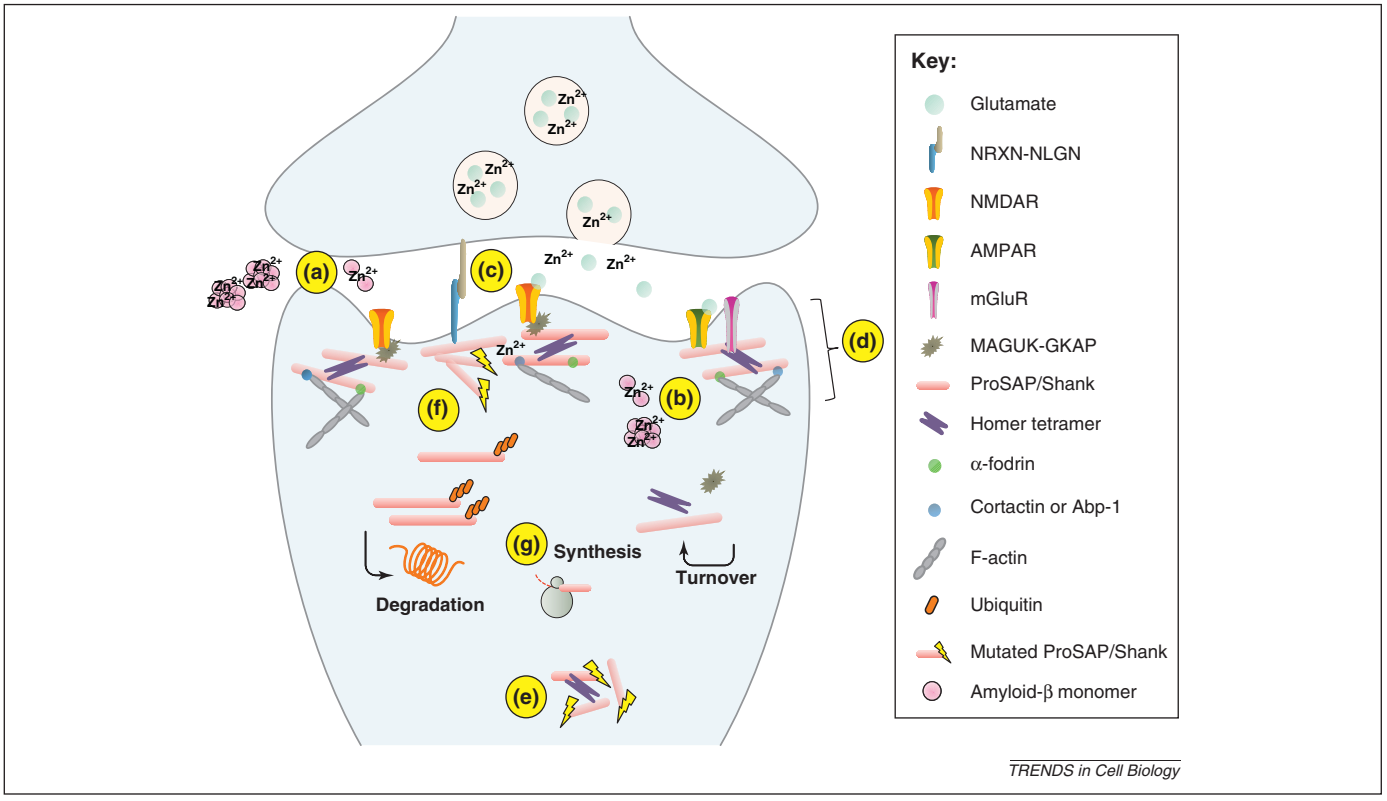

Figure 2. A model for ProSAP/Shank's mode of action in synaptopathies. Sequestration of extracellular **(a)** and/or intracellular Zn^{2+} **(b)** by Aβ might lead to a shift of ProSAP/Shank towards non-synaptic aggregates and, thus, decrease synaptic ProSAP/Shank concentration. In addition to their function in PSD scaffold assembly, however, ProSAP/Shank proteins are also part of a postsynaptic signaling pathway, and mutations in ProSAP2/Shank3 associated with ASD might impair proper synapse development by precluding coordinated changes of pre- and postsynapses via trans-synaptic signaling similar to mutations in Nlgns and Nrxns **(c)**. This might result in thinner PSDs lacking an elaborate protein meshwork **(d)**, which in turn might lead to improper connectivity, a weaker signal and an imbalance of excitatory/inhibitory synapses (E/I imbalance). ProSAP/Shank mutations might further lead to truncated proteins either promoting non-synaptic clusters thereby trapping synaptic molecules while being transported to the PSD **(e)** and/or disrupting the postsynaptic scaffold via impeding protein-protein interactions and ultimately resulting in enhanced proteasomal degradation of ProSAP/Shank and other proteins **(f)**. In addition, local translation processes might be altered **(g)** possibly followed by reduced synthesis and/or turnover of ProSAP/Shank.

a common phenotype in ASDs as seen, for example, in patients with the ProSAP2/Shank3 InsG3680 mutation [12,50]. Moreover, a downregulation of ProSAP/Shank, as found in PMS or AD patients, might lead to a loss of sufficient scaffold molecules and, therefore, affect the overall stability of the PSD. This could result in immature dendritic spines, thus leading to unstable and malfunctioning synaptic contacts.

Very recently, it was shown *in vitro* that two ASD-associated *de novo* mutations in ProSAP2/Shank3 (A962G, InsG3680) affect the development of dendritic spines by modifying ProSAP2/Shank3's functional impact on shaping spine morphology via an actin-dependent mechanism. The pathomechanistic impact on spine maturation was reflected by decreased spontaneous neuronal activity. Further, two inherited mutations were shown to have intermediate effects on spine formation and synaptic transmission [82].

Synapse maturation requires coordinated changes in synaptic organization and homeostatic changes in synapse strength likely depend upon ProSAP/Shank concentrations at PSDs in immature synapses. ASD-related mutations in ProSAP1/Shank2 and ProSAP2/Shank3 might impair proper synapse development by precluding coordinated changes of pre- and postsynapses via *trans*-synaptic signaling pathways, thereby resembling the effects of ASD-related mutations in Neuroligins and/or Neurexins. This results in thinner PSDs lacking an elaborate protein meshwork of protein–protein interaction partners like Shank1, GKAP/PSD-95 complexes, AMPA receptors and/or Homer/mGluR5 complexes [33,71].

Furthermore, evidence is provided that each ProSAP/Shank family member contributes to the maturation and molecular setup of the synapse in its own specific way. As an example, two of the three ProSAP/Shank family members (ProSAP1/Shank2 and ProSAP2/Shank3) are able to effectively bind Zn^{2+} via their SAM domains and require Zn^{2+}-binding for postsynaptic targeting. Shank1, however, seems to stabilize synapses via a Zn^{2+}-insensitive mechanism [38]. ProSAP1/Shank2 and ProSAP2/Shank3 levels at the PSD depend upon local Zn^{2+} concentration and sufficient amounts of Zn^{2+} might be necessary to stabilize developing synapses by promoting maturational processes.

Taken together, intact ProSAP/Shank function is indispensable for the glutamatergic system in mammalian brains and, thus, malfunctions in the assembly of ProSAP/Shank platforms result in disturbed excitatory transmission within neuronal networks.

Concluding remarks

ProSAP/Shank family members have been linked to a variety of diseases like PMS, autism, ID, SCZ and AD, pointing towards a key regulatory role in proper synapse function. Although the precise molecular mechanisms of ProSAP/Shank dysregulation that ultimately result in neuropsychiatric symptoms still remain unclear, an emerging model is that mutations/alterations in ProSAP/Shank family members impair neuronal circuitry by disrupting the formation, plasticity and maturation of excitatory glutamatergic transmission, thus, interfering with cognitive function and behavior.

ProSAP/Shank malfunction contributes to the development of diverse synaptopathies and this protein family might provide a highly potent target for drug development, assuming that various neurodevelopmental and neurodegenerative disorders share common pathways, in which ProSAPs/Shanks may play a key role. Given that each ProSAP/Shank family member is embedded in its own complex network of signaling and interaction partners, it is reasonable that a variety of small molecules might have effects downstream of ProSAP/Shank proteins. In addition, direct interference with the regulation of ProSAP/Shank scaffold protein levels and functions might harbor a great chance of success as a potential target for drug development.

To date, ProSAP/Shank signaling has not been investigated in detail for the purpose of therapeutic intervention. Some aspects of ProSAP/Shank signaling that could be useful therapeutically include the modulation of ProSAP/Shank clustering, degradation, activity-dependent regulation and local translation at dendritic spines.

In previous studies, it was demonstrated that the concerted action of ProSAP/Shank and Zn^{2+} ions is essential for the structural integrity of PSDs [38]. Two of the three ProSAP/Shank family members are in fact able to effectively bind Zn^{2+} via their SAM domains and do require Zn^{2+} binding for postsynaptic targeting. Thus, targeted exogenous addition of Zn^{2+} might lead to additional assembly and clustering of ProSAP1/Shank2 and ProSAP2/Shank3 at the PSD, which implies one possible mechanism to alter local ProSAP/Shank protein levels. Moreover, studies indicate that ProSAP2/Shank3 tissue-specific expression is strongly regulated by DNA methylation [83]. This might also be a potential future target for treatment. Demethylating substances like 5-aza-2′-deoxycytidine (5-AdC) could be used in neuropsychiatric disorders caused by heterozygous ProSAP2/Shank3 deletion (such as PMS) to enhance expression of the remaining gene. In contrast, methylating agents like methionine might be helpful in cases of ProSAP2/Shank3 trisomy [84]. Striatal gene expression profiles have been analyzed using microarray in rats after methylphenidate (MPH) exposure during adolescence. Homer1, MAGUK MPP3, and ProSAP1/Shank2 [85,86] were among the upregulated genes detected. Because the authors conclude that the potentiation of synaptic plasticity after MPH treatment is restricted to adolescent rats [84], some therapeutic interventions might be effective only during the time of initial synaptogenesis or early in development.

Assuming that ProSAP/Shank disruption in synaptopathic disease leads to hypoglutamatergic conditions, upregulation of the glutamatergic system could be a powerful target for treatment of synaptopathies [87]. This might include NMDA–receptor complex stimulation, positive modulation of AMPA receptors and antagonism of the serotonergic system. Pharmacological agents activating synaptic currents mediated by AMPA-type glutamate receptors (AMPAkines) might serve as a potential group of drugs to treat several disorders associated with impaired glutamatergic signaling like SCZ, autism, ID and even Huntington's disease [16,88,89]. AMPAkines facilitate the induction of LTP and the release of neurotrophic

Box 3. Outstanding questions

- What is the neurobiological output of the proposed NRXN-NLGN-GKAP/SAPAP-ProSAP/SHANK-mGluR pathway?
- Is it possible that this pathway serves to cross-link further disease-related signaling cascades (mTOR/PI3K, etc.)?
- Do mutations in pathway-associated proteins contribute to an E/I imbalance on a circuit level *in vivo*?
- What are the molecular mechanisms behind the fact that similar mutations can cause so many different phenotypes on a behavioral level?
- Do we need to apply a two-hit model, for example, to explain why some ProSAP/Shank mutations are found in patients as well as in healthy individuals?

factors, whose dysbalance is believed to play a pathogenic role in various neurodegenerative and neuropsychiatric disorders. This group of drugs has already shown positive effects in the treatment of SCZ and was used to enhance memory retention [90,91]. The effects of AMPAkines can be explained, at least partially, by upregulation of the excitatory input regulated by brain-derived neurotrophic factor (BDNF), which in turn increases local mRNA translation and is involved in synaptic plasticity and memory consolidation [92,93]. Intriguingly, AMPAkines like CX546, known to improve some sociability deficit parameters in the inbred mouse strain BTBR T+tf/J (BTBR) mice, another mouse model for autism [94], and CX-516 (Ampalex), which is suspected to be a potential therapy for AD, both reverse the defects in synaptic function and plasticity seen in ProSAP2/Shank3 heterozygous mice. Other compounds, which promote the maturation and/or functioning of glutamatergic synapses such as BDNF and (1-3)IGF-1, do as well [95]. However, since three copies of ProSAP2/Shank3 in a human patient coincides with Asperger Syndrome [12], it might be noteworthy that the exact gene dosage of ProSAP/Shank proteins is tremendously important for cognitive functions like overall intelligence, sociability, language and attention. This is underlined by the finding that an extra copy of ProSAP2/Shank3 has been associated with attention deficit hyperactivity disorder (ADHD) and mild ID in another study [13].

Taken together, the molecular mechanisms of synapse formation, plasticity and maturation are key elements for the establishment and maintenance of neuronal circuits and information processing. We hypothesize that these features are controlled by a set of molecules coordinating the assembly of multi-protein complexes that either regulate plasticity or homeostatic mechanisms that maintain synapses over time. However, future research (Box 3) is needed to reveal a putative common pathway in ASD, ID, SCZ, AD and/or other neurodevelopmental/neurodegenerative disorders and to identify the exact role of ProSAP/Shank family members within this pathway.

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