The role of selected postsynaptic scaffolding proteins at glutamatergic synapses in autism-related animal models

Veronika Meliskova[1,2], Tomas Havranek[1,3], Zuzana Bacova[1], Jan Bakos[1,2,*]

[1] Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, 845 05 Bratislava, Slovakia

[2] Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava, 811 06 Bratislava, Slovakia

[3] Institute of Anatomy, Faculty of Medicine, Comenius University in Bratislava, 811 06 Bratislava, Slovakia

*Correspondence: j.bakos@savba.sk (Jan Bakos)

Pathological changes in synapse formation, plasticity and development are caused by altered trafficking and assembly of postsynaptic scaffolding proteins at sites of glutamatergic and gamma-aminobutyric acid synapses, suggesting their involvement in the etiology of neurodevelopmental disorders, including autism. Several autism-related mouse models have been developed in recent years for studying molecular, cellular and behavioural defects to understand the etiology of autism and test potential treatment strategies. In this review, the role of alterations in selected postsynaptic scaffolding proteins in relevant transgene autism-like mouse models is explained. A summary is also provided of selected animal models by paying special attention to interactions between guanylate kinases or membrane-associated guanylate kinases, as well as other synapse protein components which form functional synaptic networks. The study of early developmental stages of autism-relevant animal models help in the understanding the origin and development of diverse autistic symptomatology.

Keywords

Scaffolding proteins; SH3 domain and ankyrin repeat containing proteins (SHANKs); HOMER1; Fragile X mental retardation protein (FMRP); Contactin-associated protein-like 2 (CNTNAP2); Autism

1. Introduction

To understand the etiology of neurodevelopmental disorders, it is necessary to systematically examine the structural consequences of synaptic remodeling. The relevant players involved in cytoskeletal remodeling and synaptic plasticity include various actin-binding proteins, microtubule-binding proteins and cell-adhesion molecules [1]. To date, a wide panel of *Shank* mutant mouse models has been developed with characteristic features that are collectively called "shankopathies" [2, 3]. For instance, abnormal patterns of cell-adhesion molecules and scaffolding proteins, in association with altered neuronal morphology, have been observed in the "SH3 domain and ankyrin repeat containing protein 3" (*Shank3*) deficient mouse model [4]. Moreover, it has recently been demonstrated that altered expression of specific genes are involved in processes of neurite elongation and synapse formation in the early developmental periods

of *MAGE* Family Member L2 (*Magel2*)-deficient mice [5], which is another model known for the presence of autism-like symptoms. Currently, it appears that crucial factors that play a role in the pathogenesis of autism are defects and abnormalities found in postsynaptic density proteins [6–8]. Alterations for a wide range of postsynaptic density molecules and scaffolding proteins are currently being investigated at the molecular and cellular level in the context of neurodevelopmental disorders, with membrane-associated guanylate kinases representing the largest group amongst them [9]. Several lines of evidence have suggested that pathological changes in synapse formation, plasticity and development are caused by altered trafficking and assembly of postsynaptic scaffolding proteins at glutamatergic and gamma-aminobutyric acid–(GABA)ergic–synapses, thereby suggesting their involvement in the etiology of neurodevelopmental disorders [10, 11].

Scaffolding proteins link several membrane proteins and ion channels with a dynamic network of cytoskeletal proteins. The specific interactions among protein complexes are associated with activation of diverse signaling pathways in subtypes of neuronal cells. In particular, there are known differences in the composition of postsynaptic sites at glutamatergic and GABAergic neurons in cortical and subcortical areas of the brain [12–14]. Structural scaffolding proteins allow for the recruitment of large, functional complexes that serve to modulate signaling pathways in both glutamatergic and GABAergic neurons. Moreover, processes such as receptor stabilization, subcellular localization and trafficking are determined by the functional interactions of scaffolding proteins. Structural and functional changes of these proteins are associated with the pathologies observed in neurodevelopmental disorders at numerous cellular and subcellular levels [1]. One group of scaffolding molecules includes SHANK proteins, which represent a large and multidomain family. *Shank* genes are important candidates for the modelling of autism spectrum disorders in mice. In this context, a variety of genetic models have been generated within the last

few years, among them *Shank1–3*-deficient mice. Additionally, Contactin-associated protein-like 2 (CNTNAP2), an organising and adhesive molecule known for its role in axons, dendrites and synaptic connections, is also widely-replicated as an autism-predisposition gene [15]. Genetic variation in *Cntnap2* is considered an important variable in synaptic phenotypes and associated with both excitatory and inhibitory synaptic abnormalities [16, 17]. It is very likely that excitatory and inhibitory synaptic changes could specifically manifest, depending on alterations in scaffolding proteins and thus result in heterogeneous autistic behavioural phenotypes.

For the purpose of this review, scaffolding proteins and their associated molecules were selected, whose variability is likely to be casually-linked with the etiology of autism spectrum disorders. The role of synaptic components in excitatory/inhibitory balance in the brain in relation to autism is also discussed. The role of major postsynaptic density proteins that are typically present in the excitatory synapses in relevant transgene autism-like mouse models are described. The animal models chosen are useful for understanding the normal and altered function of postsynaptic scaffolding proteins at excitatory synapses. The involvement of membrane-associated guanylate kinases in regulating synaptic vesicle proteins in autism-related conditions is also particularly explained. Due to the great complexity of synaptic composition, this review is limited to clarifying the links between excitatory glutamate receptors and postsynaptic density proteins, which are most relevant for the pathological processes found in autism.

2. Postsynaptic density (PSD) and scaffolding proteins

The postsynaptic density (PSD) is typically defined by the presence of numerous scaffolding proteins, receptors and signaling molecules that are in close proximity to the postsynaptic membrane [18]. Since they are important components of the PSD, scaffolding proteins play a crucial role in synaptic plasticity mediated by anchoring and clustering of glutamate receptors and interactions with cell-adhesion molecules [19, 20]. Additionally, they are involved in connections between postsynaptic receptors and downstream signaling molecules and also adjust the cytoskeletal dynamics [21]. Various groups of scaffolding proteins occur in different layers of the PSD. The spatial distribution and localization of individual proteins can be adjusted by neuronal activity, which may lead to changes in their post-translational modifications, as well as changes in their binding partners. The groups of scaffolding proteins differ from one another in their modular domain compositions, distinct interaction and binding complexes.

2.1 Membrane-associated guanylate kinases at postsynaptic density

Membrane-associated guanylate kinases (MAGUKs) comprise one of the largest groups of scaffolding proteins at the postsynaptic density. MAGUKs have a critical role

in the physiology and development of several tissues of metazoan organisms [22]. The family of MAGUKs includes a guanylate kinase-like domain, which contains members such as PSD-93, PSD-95, as well as the synapse-associated proteins SAP-97 and SAP-102. In humans, the SAP-97 and SAP-102 proteins are known to be encoded by genes, "discs large homolog" *DLG1* and *DLG3*. MAGUKs contribute to maintaining excitatory and inhibitory synaptic inputs in the brain by regulating the formation of excitatory glutamatergic synapses. Therefore, MAGUKs are involved in processes of synaptic plasticity by regulating the localization and compartmentalization of glutamate receptors [23]. In the PSD architecture of excitatory synapses, MAGUKs—mainly PSD-95, participate in the organization of ionotropic glutamate receptors and associated signaling proteins, which adjust the synaptic strength. Diverse adaptations of MAGUK proteins in glutamatergic synapses, such as altering interactions between MAGUKs and ionotropic glutamate receptor subunits, often occur in neurodevelopmental disorders [24]. For instance, a mutation in the gene for the glutamate receptor subunit *GRIN2B* has been found in a patient with autism [25]. These authors explain that impaired GluN2B subunit binding to MAGUK proteins is associated with decreased dendritic spine density. Furthermore, it has recently been widely discussed that developmental clustering and coupling of N-methyl-$_D$-Aspartate (NMDA) receptor subunits could be affected by MAGUK family proteins [9]. One recent study has suggested that a deficiency in a member of MAGUK family of proteins PSD-95 affects GABAergic inhibitory synapses. The authors of that study explain their results by a PSD-95 deficiency that enhances the inhibitory synapse activity by enhancing transport mechanisms of inhibitory cell adhesion molecules [26]. The relationship between MAGUKs and excitatory/inhibitory balance in the brain is therefore quite complex and depends upon the interactions and trafficking of cytoskeletal components from excitatory to inhibitory synapses.

2.2 SHANK proteins at postsynaptic density

Another large protein family at the postsynaptic density includes SHANK proteins. They are characterized as multidomain scaffolding proteins, which are associated with neurotransmitter receptors, signaling molecules and actin filaments. In conjunction with their binding partners, SHANK and guanylate kinase-associated proteins (GKAP) contribute to the organization of glutamate receptors in the PSD [27]. Based on their interactions with proteins, they recruit molecular machineries for G-protein-mediated signaling, leading to the control of calcium homeostasis in dendritic processes of neurons. They also participate in morphological changes which lead to the maturation of dendritic spines and synapse remodeling [28]. The proteins of the SHANK family are encoded by the three related *SHANK* genes: 1, 2 and 3. SHANK family proteins are mostly developed in excitatory glutamatergic synapses in mammalian brains [29, 30]. Although they share high, structural similarity and preva-

lently localize to the postsynaptic terminal, SHANK proteins are differentially-expressed during brain development, with SHANK2 and SHANK3 finalizing before SHANK1. SHANK2 and SHANK3 are therefore considered critical for several aspects of neuroplasticity during development [31]. Studies have demonstrated that SHANK1 protein levels increase from birth through early development, followed by a slight decrease in adulthood in the cerebellum and cortex [32]. In fact, SHANK1–3 protein components represent critical determinants of excitatory synaptic function. Therefore, whenever balanced expression of the different SHANK proteins is disturbed or diverse alterations in Shank isoforms occur, these events result in various dysfunctions of synaptic transmission (as described below in section 3.5).

SHANK full-length proteins contain ankyrin repeat domains, a SH3 domain, a single PDZ domain, a Proline-rich region (PRD) and a Sterile alpha motif domain (SAM). The PDZ SHANK domain interacts with the "SAPAP" (Synapse-associated protein (SAP) 90/Postsynaptic density (PSD)-95-associated protein) family. The PRD binds to HOMER and Cortactin and this interaction is important for cytoskeletal regulation, as well as for modification of synaptic transmission [33]. SHANK3 is highly enriched in corticostriatal glutamatergic synapses [34], which are a part of neural circuits that are thought to be dysfunctional in ASD. High *Shank1* gene expression levels occur in cortical and subcortical brain regions, including the amygdala and hippocampus, while *Shank2* gene expression is enriched in cortical areas and the thalamus [34]. In the corticostriatal projections, activity from different areas of the cerebral cortex converges into the striatum and the output is then returned to the cortical layers through thalamic pathways, serving as an aid in motor decisions and behaviour. Therefore, different synaptic errors in different parts of the circuit will ultimately affect cortical activity—this could also be a convergence mechanism for recurrent behaviour with distinct genetic causes particularly in some disorders [35]. Functional deficits of a single copy of SHANK3 are one of the major causes of ASD, Phelan-McDermid syndrome (PMS) and mental disability [6].

2.3 HOMER protein homolog at postsynaptic density

The HOMER protein homolog represents an important postsynaptic density scaffolding element, which is involved in various processes of synaptic plasticity, including changes in intracellular calcium. To date, three gene families have been characterized—*HOMER 1, 2* and *3* with both long and short-form variants. Long forms of HOMER proteins are a part of the PSD and are consecutively expressed, unlike the short forms, which are known as cytosolic molecules with low expression under basal conditions [36]. Alternative splicing of HOMER into long and short forms has been investigated in the context of the postsynaptic polymeric network structure. For instance, stoichiometric structures connecting HOMER, SHANK and MAGUK proteins have previously been suggested [37]. Indeed, one recent study found that the glutamatergic synapse protein interaction network includes interactions between HOMER and SHANK proteins [38]. The balance between long and short forms of HOMER contributes to normal synapse functioning; therefore, it is assumed that alterations in HOMER expression, folding, and binding to other scaffolding proteins could be associated with the pathological processes found in autism. Multiple controversies are associated with the functional consequences of mutations in HOMER proteins, particularly in the context of autism spectrum disorders and are currently being discussed [39]. HOMER proteins are responsible for the links between metabotropic glutamate receptors and PSD proteins [40], so disruption of the group-I metabotropic glutamate (mGlu) receptor mediated signaling can contribute to the etiology of autism [41].

2.4 Calmodulin-dependent protein kinase II-alpha at postsynaptic density

The calmodulin-dependent protein kinase II-alpha (CaMKIIα) is a well-defined and abundant protein in the PSD and participates in the control of signal modality in synaptic connections. Activity of CaMKIIα has previously been demonstrated as an important factor in the phosphorylation of synaptic Ras GTPase-activating proteins (synGAP —expressed in the postsynaptic density), distribution of synGAP from postsynaptic sites and for the activation of postsynaptic Ras-proximate-1 or Ras-related protein 1 (Rap1). SynGAP represents a neuron-specific Ras and Rap GTPase-activating protein (GAP) found in large amounts in PSD fraction [42]. Additionally, CaMKIIα plays an essential role in the regulation of memory and learning processes, as well as an activity-dependent role in spine density limitation during postnatal development [42, 43]. One recent study found that CaMKIIα is recruited to the scaffolding protein SHANK3 sub-compartment of the PSD [44]. Moreover, the binding of SHANK3 to CaMKIIα potentially modulates dendritic spine morphology [45]. This complicated concept could be useful for explanation of the role of CaMKIIα in the abnormal neuronal morphology observed in autism [1, 46]. In fact, mutation in CaMKIIα contributes to alterations in dendritic morphology as well as synaptic transmission and thus consequently leads to ASD-related behavioural phenotypes [8].

3. Autism-related mouse models

Mouse experimental models provide an option for studying autism spectrum disorder (ASD) at cellular, molecular, neuronal circuit and behavioural levels, offering possibilities in which behavioural abnormalities and their compensation by potential drug compounds can be screened before translation to humans. The main advantages of mouse models of ASD are that they are genetically flexible and practical. Additionally, various ASD models that present basic phenotypes are commercially available and ready to use in current research. However, mouse models have their own limitations, as certain behavioural phenotypes in neuropsychiatric disorders cannot be evaluated in mice. For instance, the com-

munication problems and language development delay often present in autistic children can only be poorly addressed by the study of ultrasonic calls [47, 48]. Moreover, some of them are obstructed in mouse models based on noticeable behaviours. A further risk is that the evaluation of the underlying phenotypes of ASD may be confounded with familiar comorbidities, including locomotor dysfunction, sensory dysfunction, learning loss and anxiety [49]. There are several types of genes in which deficiency plays a significant role in the pathophysiology of neurodevelopmental diseases with autism symptoms. The limitations of mouse models also include the fact that in humans most of the patients with ASD-related genetic variants are heterozygous, while in experimental conditions homozygous mice are usually investigated. Therefore, allele-specific gene expression differences in humans and experimental models should also be taken into account. The interpretation of data coming from experimental models should be carefully considered, as the concept of diverse consequences of different versions of the genes adds another component to the variability of behavioral phenotypic spectrum in ASD patients. Even more complicated, some consequences appear during specific stages in development in a brain region dependent manner.

3.1 T-box brain transcription factor 1

The T-Box Brain Transcription Factor 1 (*Tbr1*) represents a gene that encodes a transcription factor with distinct prenatal and postnatal molecular actions. This gene has a high probability of alterations in ASD [50]. Neuron-specific *Tbr1* adjusts the regional and laminar character of cerebral cortex regions in the developing brain [51]. Numerous studies have previously found that *Tbr1* regulates the identity of neocortical regions in the regional and laminar pathways of the developing brain [51–54]. The Calcium/Calmodulin Dependent Serine Protein Kinase (*Cask*) gene codes the MAGUK protein, wherein the N-terminal half consists of a calcium/calmodulin independent kinase (CaMK) domain and two LIN-2,7 (L27) domains, as well as the C-terminus half, which consists of the PDZ domain, Src homology 3 (SH3) and the guanylate kinase (GuK) domain [55]. Wang *et al.* [56] evidenced that overexpression of *Tbr1* and *Cask* increased promoter activities of some of these genes, including the NMDA receptor 2b (*Nr2b*), glycine transporter and interleukin 7 receptor (*IL7R*). It was shown that expression of *Nr2b* was consistently downregulated in *Tbr-1* deficient mice. The TBR-1/CASK protein complex is involved in downstream control of the expression of the mentioned genes, thereby modulating brain function. The expression of *Tbr1* is upregulated by neuronal activation together with the synaptic PDZ protein CASK and the nucleosome assembly protein CINAP (CASK-interacting nucleosome assembly protein), which forms a complex participating in the control of the glutamatergic receptor subunits [46]. Deriziotis *et al.* [57] and Sakai *et al.* [58] investigated direct interaction between TBR1 and Forkhead Box P2 (FOXP2), which is a transcription factor known for its role in brain develop-

ment. TBR1 was identified as a binding partner for CASK since it requires a C-terminal region. This interaction results in an increase of transcriptional activity of *Tbr1* by engagement of the nucleosome assembly protein CINAP (CASK interacting nucleosome assembly protein, also known as testis specific protein Y-encoded like 2, TSPYL2) into a promotor region containing the DNA binding motif for the T-box [59]. Numerous rodent studies have shown that the neurodevelopmental phenotypes associated with the mutation in PDZ protein CASK led to loss-of-function. Heterozygous mice display postnatal progressive microcephaly, hypoplasia of the optic nerves and cerebellum, growth retardation, as well as scoliosis [55, 60, 61]. Mutations in *Tbr1* are accompanied by synaptic, neuronal, and behavioural dysfunctions associated with ASD. $Tbr1^{+/K228E}$ mice displayed increased self-grooming, altered social interaction and increased anxiety-like behaviours [49]. *Tbr1*-deficient mice develop axonal (presynaptic) defects and other deficiencies in neuron morphology, such as immature dendritic spines and reduced synaptic density [48]. Moreover, it has been found that $Tbr1^{-/-}$ mice suffer from several other postnatal abnormalities in lamination of the cerebral cortex. In heterozygous animals, the reduction of functional amygdala interconnections has been demonstrated along with autistic-like behaviours [62]. Huang *et al.* [63] demonstrated that $Tbr1^{+/-}$ mice manifested less-developed anterior commissures and also olfactory bulbs, a reduced population of interneurons and an atypical morphology of dendrites in olfactory mitral cells. The olfactory sense was not disturbed; however, *Tbr1* haploinsufficiency affected olfactory discrimination. Characterization of cellular functions of *Tbr1* with the use of mouse models is essential for understanding abnormalities in cortical lamination and in a broader context, it contributes to the deciphering of the complicated origin of neurodevelopmental disorders, including autism.

3.2 Fmr1 gene

The fragile X mental retardation 1 (*Fmr1*) gene is located on the X chromosome and the expansion of trinucleotide CGG leads to its silencing with a consequent absence of the encoded fragile X mental retardation protein (FMRP), which is a synaptic RNA binding protein that modulates the duration of the action potential, as well as synaptic plasticity. These consequences result in a disease known as Fragile X syndrome (FXS) [64]. It has been demonstrated that neuroplasticity disorders belong to the main findings in animal models of FXS and that the lack of balance in inhibitory and excitatory neuronal circuits is the basis of many clinical manifestations of this disorder [65]. *Fmr1* gene knockout mice have shown many deformities in neuronal plasticity and learning. Recent studies have suggested that dendritic spines are reduced in juvenile *Fmr1* knock-out mice; however, the differences are not noticeable in adult mice [66, 67]. Sitzman *et al.* [64] demonstrated that *Fmr1* deficient mice display deficits in synaptic plasticity and neurite extension. Several studies have also shown metabolic

changes, namely that *Fmr1* knockout mice have an increased rate of glucose metabolism, exalted oxidative and metabolic stress and deformities in their nitric metabolism of oxygen [64, 68, 69]. Todd *et al.* [70] demonstrated that FMRP levels are rapidly up-regulated in primary cortical neurons in response to the activation of the type I metabotropic glutamate receptor (mGluR1 and mGlur5) by agonist S-3,5-dihydrophenylglycine. Further, that mRNA for PSD-95 is a scaffolding protein involved in synaptic plasticity, which contains a highly conserved canonical FMRP binding site within its 3' UTR. Furthermore, PSD-95 translates rapidly in response to the activation of glutamate receptors, since mGluR5 changes in PSD-95 expression are lost in neurons derived from FMRP deficient mice [71]. They hypothesize that FMRP is required for mGluR1 and mGluR5-dependent translation of PSD-95 and provide insight into the pathophysiology of FXS. DeMarco *et al.* [72] have suggested that FMRP regulates PSD-95 translation via activation of *miR-NAs*, thus working together to mediate the reversible inhibition of the PSD-95 expression in neurons. In addition to alterations in excitatory glutamatergic neurotransmission, recent studies have suggested that *Fmr1* knockout mice also suffer from abnormalities in inhibitory GABAergic neurotransmission [73]. Sabanov *et al.* [73] found that a significantly-reduced amplitude of evoked inhibitory postsynaptic currents decreased expression levels of GABA receptor subunits. Overall, most of the above-mentioned studies support the integrated view of the FMRP role in synaptic plasticity and postsynaptic structural remodeling. It is also clear that mutations or modifications of FMRP play an important role in developmental delays and the etiology of ASD. With the exception of PSD-95, it remains to be elucidated how FMRP might target other postsynaptic density proteins and affect synaptic remodeling.

3.3 Contactin-associated protein-like 2 (CNTNAP2)

Contactin-associated protein-like 2 (CNTNAP2) is a transmembrane cell-adhesion molecule type I which occurs in the central and peripheral nervous system. It is highly expressed throughout the spinal cord and brain, especially in the frontal and temporal lobes, dorsal thalamus, striatum and specific cortical layers [74]. It is a member of the neuronexin family and has an essential role in neural development, belonging to one of the most susceptible genes for autism spectrum disorders [17]. *Cntnap2* deficient mice are characterized by social deficits and repetitive seizures, and behaviours. Vogt *et al.* [75] assessed the CNTNAP2 function in mouse development and infection of cortical interneurons, utilizing a *Cntnap2* null mouse. Lazaro *et al.* [76] found a significant decrease in excitatory and inhibitory synaptic inputs on pyramidal neurons of the prefrontal cortex of *Cntnap2* knock-out mice. This means that reduced synaptic inputs can gain temporal coordination of neuronal firing in cortical collections. Scott *et al.* [77] report loss of CNTNAP2 in the rat causes autism-related alterations in social interactions, stereotypic behaviour and sensory processing.

$Cntnap2^{-/-}$ rats lack sociability and social behaviour. Other findings include alterations in sensory processing, increased avoidance to sounds of moderate intensity, deficit of rapid audiovisual temporal recalibration and exaggerated acoustic startle responses. Gao *et al.* [78] characterized an interaction between CNTNAP2 and the Partitioning-defective 3 protein (PAR3). These authors demonstrated that the two proteins interact through PDZ-mediated binding, since $CNTNAP2^+/PAR3^+$ complexes are largely associated with clathrin-coated endocyte vesicles in heterologous cells. Overexpression of *Par3*, however, not a knockout lacking all PDZ domains, can cluster endogenous CNTNAP2 in primary neurons. Altogether, the aforementioned studies suggest a strong association of CNTNAP2 alterations in excitatory glutamatergic neurons with autism-related behavioural phenotypes. Although the mechanisms associated with how CNTNAP2 regulates glutamatergic synapses are not fully understood, it has been suggested that CNTNAP2 plays a role in the AMPA receptor subunit's trafficking [79]. Moreover, *Cntnap2* knockdown decreased the number of functional excitatory synapses and the consequences observed in altered social behaviour were compensated by enhancement of the AMPA receptor function [80]. Taken together, CNTNAP2 is clearly a part of the molecular architecture of the postsynaptic density, which may be pathologically modified in autism and other neuropsychiatric disorders.

3.4 Magel2 gene

The MAGEL2 protein occurs at high levels in the hypothalamus and plays a crucial role in the regulation of subcellular processes that contribute to the recycling of membrane proteins from endosomes [81]. Protein truncation mutations in *MAGEL2* result in Schaaf-Yang syndrome and *MAGEL2* comes from a small array of genes deleted in Prader-Willi syndrome. Abnormal daytime sleepiness and nocturnal and/or early morning awakening occur in people with Schaaf-Yang syndrome and Prader-Willi syndrome, while mice bearing a deletion of the *Magel2* gene suffer from disrupted circadian rhythms [82]. Although Prader-Willi syndrome is a multigene neurodevelopmental disorder with various physiological and behavioural deficits, the clinical characteristics include severe hypotonia, a newborn's poor feeding due to reduced suckling reflexes, childhood-onset obesity, lower cognitive functioning and autistic symptoms [83]. *Magel2*-deficient mice manifest altered gene and protein expression of PSD-95 in early brain developmental stages [5]. *Magel2*-deficient mice have shown altered levels of oxytocin in the hypothalamus, as well as 50% neonatal mortality due to suckling problems [84]. Male mice who survive were shown to have deficits in the exploration of novel objects, social novelty and dimensional learning [84, 85].

3.5 Shank family of genes

Numerous *Shank*-deficient mouse models (with a deleted gene belonging to the *Shank* family) have been generated to provide clarification of the role of *Shank* proteins *in vivo*.

By comparing individual *Shank* members, deletion of *Shank1* proved to have milder phenotypic deficits than *Shank2* and *Shank3*. *Shank2* and *Shank3* genes are more structurally similar compared to *Shank1* [86]. Here, it needs to be emphasized that marked differences in alterations in behaviour and synaptic function of *Shank* deficient mice can be explained by remaining *Shank* isoforms [87, 88]. Moreover, endophenotypes within ASD patients with different types of mutations likely depend on isoform-specific disruption of SHANKs [2].

Shank1 mRNA and proteins are found almost exclusively in the central nervous system when compared to *Shank2* and *Shank3* in rodents, which are expressed in many tissues, such as the kidney, brain, heart, spleen and liver [34]. Overexpression of *Shank1* alters spine morphology [89]. The first mouse *Shank* model was created by Hung *et al.* [90] via disruption of the *Shank1* gene through deletion of exons 14 and 15, which leads to complete knockout of all *Shank1* isoforms. Many studies have provided evidence of an ASD-related phenotype, which is defined by deficits of social communication, modifications in repetitive behaviour and deterioration in cognitive function. Behavioural phenotypes of *Shank*-mouse models depend on specific gene mutations and modifications, resulting in intellectual disability, schizophrenia and autism symptoms [91]. *Shank1* deficient mice are characterized by lower psychomotor activation and higher levels of anxiety. Additionally, they have impaired contextual fear memory and improved acquisition [90, 92]. A study by Mao *et al.* [93] showed that *Shank1* is highly present in Parvalbumin-expressing (PV+) fast-spiking inhibitory interneurons in the hippocampus. It was shown that a lack of *Shank1* in these neurons leads to devaluation of afferent excitatory synaptic activity and inhibitory efferent synaptic activity to pyramidal neurons. The hippocampal CA1 pyramidal neurons in *Shank1* deficient mice display a change in the excitatory/inhibitory balance, which can be considered a pathophysiologic feature of ASD. Dysregulation of excitatory/inhibitory balance in the hippocampus was displayed by lower expression of gephyrin (a scaffolding component of inhibitory synapses). Mossa *et al.* [86] investigated how simultaneous deletion of *Shank1* and *Shank3* affects brain development and behaviour of mice. They showed a low survival rate and a big devaluation in the activation of intracellular signaling pathways associated with Akt, S6, ERK1/2 and eEF2 during development, as well as serious behavioural disorders. It was suggested that Shank1 and Shank3 proteins are essential for developmental adjustment of Akt activation and other intracellular pathways that are critical for mammalian postnatal brain development and synaptic plasticity. It is important to note that a full description of the complex role of the PI3K/AKT/mTOR pathways in brain development is beyond the scope of this review. Nevertheless, the diversity of binding partners is being expanded, as a recent study has discovered new components of mammalian targets of rapamycin (mTOR) signaling pathways in neurodevelopment [94]. In this context, another scaffolding protein which is present in high concentrations

in the PSD of neurons located in various brain areas is the Insulin receptor substrate p53 (IRSp53, also known as BA-IAP2) [95]. IRSp53 directly interacts with PSD95, SHANK proteins and small GTPases like "Cell division control protein 42 homolog" — Cdc42 [96]. Activation of the Rac/Cdc42 cascade regulates actin filaments polymerization in neuronal dendritic spines [97]. Therefore, IRSp53 belongs to crucial elements linked to the Akt pathway that participates in recruiting cytoskeletal elements and synaptic scaffolds, including PSD-95 and SHANK proteins [96]. Studies have shown that IRSp53 is involved in various brain disorders, including ASD [98, 99], attention deficit hyperactivity disorder [100] and schizophrenia [101]. A deletion of *Irsp53* leads to sex-dependent changes in behaviour, such as social deficit, hyperreactivity and excitatory/inhibitory imbalance [102]. A lack of IRSp53 activity leads to NMDA receptor hyperreactivity in the hippocampus [103] and prepulse inhibition in cortical excitatory neurons [102]. The suppression of the mGluR5 and NMDA receptors resulted in improvement of the social deficit in the *Irsp53* mutant mice [104].

Knockout mice for the *Shank2* gene also display ASD-like behaviour, including reduced social interaction, reduced social communication through ultrasound vocalizations, repeated jumping and also showed a significant decrease in function of the NMDA (*N*-methyl-$_D$-aspartate) glutamate receptor (NMDAR) [105]. Conditional *Shank2* deficient mice with deletion in excitatory and inhibitory neurons were used in a study by Kim *et al.* [106]. Significant changes in synaptic transmission in the hippocampus and striatum were demonstrated, which are associated with different behavioural abnormalities in the social, recurrent, locomotor and anxiety domains. Lee *et al.* [107] found that *Shank2* is expressed in GABAergic neurons, including parvalbumin (PV)-positive neurons, which is a neuronal cell type involved in brain excitation and rhythms, as well as brain dysfunctions, including ASD. A novel *Shank2* transcriptional variant in a *Shank2* deficient mouse model of ASD has shown that two lines of *Shank2* knockout mice generated by deleting different exons (exon 6-7 or exon 7) showed distinct cellular phenotypes. Lee *et al.* [7] also found expression of a novel exon (exon 4′ or e4′) between the existing exons 4 and 5 in the *Shank2* model of e6-7 *Shank2* knockout mice. The results suggested an example of genetic indemnity, leading to phenotypic heterogeneity among ASD patients with the same gene mutation. The causative contribution of rare point *SHANK2* gene mutations in schizophrenia has also been suggested [108]. Furthermore, new transcriptional variants can work as modifier genes, thereby contributing to differences between two *SHANK2* lines of mutation. Bey *et al.* [109] investigated determined preliminary behaviour in *Shank3*-knockout mice in striatal inhibitory neurons and self-grooming lesions were noticed in excitatory neurons of the forebrain. In contrast, social, communicative and instrumental learning behaviours were not significantly affected in these mice. Unique patterns of changes in biochemical and electrophysiological findings
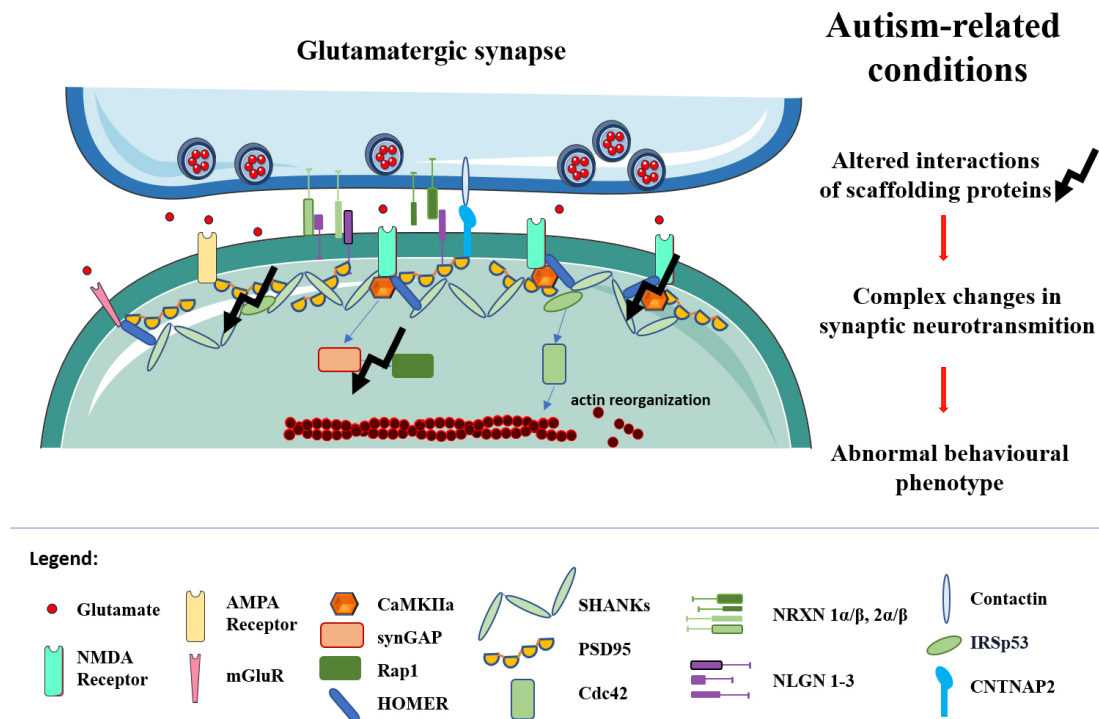
Fig. 1. Schematic representation of glutamatergic synapse, showing altered interactions (thunderbolt sign) of scaffolding proteins at the postsynaptic density observed in autism-like animal models. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor, Calmodulin-dependent protein kinase II-alpha (CaMKIIα), Cell division control protein 42 (Cdc42), contactin-associated protein-like 2 (CNTNAP2), Insulin receptor substrate p53 (IRSp53), N-methyl-$_D$-aspartate receptor (NMDA) receptor, metabotropic glutamate receptor 1 and 5 (mGluR), Postsynaptic density protein 95 (PSD-95), Neurexins (NRXNs), Neuroligins (NLGNs), synaptic Ras GTPase-activating protein (synGAP), Ras-proximate-1 or Ras-related protein 1 (Rap1), SH3 domain and ankyrin repeat containing proteins (SHANKs).

in the relevant brain regions have been discovered, which reflects the complex nature of *Shank3* transcriptional regulation. Decreased HOMER1b/c and hyper-excitability of the membrane were observed with striatal loss of SHANK3. In comparison, the *Shank3* deletion in hippocampal neurons led to an increase in NMDA receptor mediated excitatory current [109]. In individuals with autism spectrum disorders, hyper-reactivity with sensory input is a common feature. In a study by Chen *et al.* [110], both male and female *Shank3*-deficient mice were more sensitive to relatively weak tactile stimulation. Spontaneous and stimulus-induced calcium jets in pyramidal neurons were detected in population-based calcium imaging of the population; however, activity in interneurons was reduced. Preferential deletion of *Shank3* in inhibitory interneurons indicated pyramidal neuron hyperactivity. This study suggests that cortical GABAergic interneuron dysfunction plays a key role in sensory hyper-reactivity in the *Shank3* mouse model of ASD and identifies a potential cellular target for the exploration of therapeutic interventions.

4. Future perspectives

It is clear that pathological changes in synapse formation, plasticity and development are caused by altered trafficking and assembly of postsynaptic scaffolding proteins, in-cluding MAGUKs, SHANK proteins and HOMERs (Fig. 1). Although beyond the main scope of this review, it is essential to mention that alterations in presynaptic scaffolding proteins also play a role in synaptic abnormalities observed in autism-related conditions. Recent studies have investigated a role of several components of the presynaptic active zone such as liprin-α, glutamine/leucine/lysine/serine-rich protein (ELKS), Rab3a-interacting molecule (RIM), RIM-binding protein (RIM-BP), Bassoon/Piccolo and Munc13 in a neurotransmitter release [111–113]. For example, in a study by Liang *et al.* [113], it was shown that liprin-α2 determines distribution of presynaptic proteins in an active zone; therefore it is critical for presynaptic vesicular release. Defects in presynaptic protein distribution and alterations in a whole spectrum of cell-adhesion molecules, including liprins and neurexins, are associated with the etiology of neurodevelopmental disorders such as autism [3].

Postsynaptic protein complexes dynamically interact with CaMKIIα assuring synaptic plasticity, which is altered under the conditions of autism. Transcriptome analysis of *Shank3*-overexpression has revealed modification of postsynaptic membrane-related genes in mice [114]. The most recent results indicate that *Shank3* deficiency is accompanied by alterations in synaptic proteins in the hippocampus that could

be reversed by oxytocin treatment [4]. These findings thus open an avenue for further use of *Shank3*-deficient mice in examining potential treatment strategies. Indeed, other behavioural and pathological consequences in different autism-associated models could be reversed by manipulation with the oxytocin signaling [115]. ASD patients bearing *SHANK1-3* mutations may also benefit from the knowledge gathered from autism-related mouse models that provide information on how different mutations and genetic variants influence the behavioural phenotypes [2, 91]. A comparison of molecular, neuronal and behavioural abnormalities found in animal models of FXS and models of ASD represented by manipulations with *Cntnap2* and *Magel2* genes are essential in distinguishing and understanding the risk for the development of neuropsychiatric disorders.

The comprehensive analysis of neuronal subtypes via single-cell sequencing in autism-related mouse models should elucidate relevant data on molecular therapeutic targets. Chen *et al.* [116] revealed clusters of glutamatergic and GABAergic neuronal subtypes based on their differential expression; however, nothing is known of their developmental patterns in the context of autism spectrum disorders. Therefore, studying early developmental stages of autism-relevant animal models will help the understanding of the origin and development of autistic symptomatology. Specific molecular targets of postsynaptic proteins affected in ASD at different developmental stages may help development of new therapeutic approaches. Association of concrete alterations in postsynaptic scaffolding proteins, as well as their interactions with binding synaptic partners in conjunction with evaluation of behavioural symptoms, represents a valuable approach for elucidating the role of concrete autism relevant dysfunctions of synapses.

Abbreviations

3' UTR, 3' untranslated region; Akt, protein kinase B; AMPA receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ASD, autism spectrum disorder; BAIAP2, brain-specific angiogenesis inhibitor 1-associated protein 2; CA1, cornu ammonis 1; CaMK, calcium/calmodulin independent kinase; CaMKIIα, calmodulin-dependent protein kinase II-alpha; Cask, calcium/calmodulin dependent serine protein kinase; Cdc42, cell division control protein 42; CINAP, cask-interacting nucleosome assembly protein; CNTNAP2, contactin-associated protein-like 2; DLG1, discs large homolog; eEF2, eukaryotic elongation factor 2 protein; ELKS, glutamine/leucine/lysine/serine-rich protein; ERK1/2, extracellular signal-regulated kinases; Fmr1, fragile X mental retardation 1; FMRP, fragile X mental retardation protein; FOXP2, forkhead box P2; FXS, fragile x syndrome; GABA, gamma-aminobutyric acid; GAP, gtpase-activating protein; GKAP, guanylate kinase-associated proteins; GluN2B, NMDA receptor subunit 2B; GRIN2B, glutamate receptor subunit; GuK, guanylate kinase; IL7R, interleukin 7 receptor; IRSp53, insulin receptor substrate p53; LIN-2,7 (L27), protein LIN-2; MAGEL2, mage family member L2; MAGUKs, membrane-associated guanylate kinases; mGlu, metabotropic glutamate; mGluR1, type I metabotropic glutamate receptor; mGluR5, type V metabotropic glutamate receptor; miRNAs, micro ribonucleic acids; mRNA, messenger ribonucleic acid; mTOR, mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate glutamate receptor; Nr2b, NMDA receptor 2b; PAR3, partitioning-defective 3 protein; PDZ, post-synaptic density protein 95 - drosophila disc large tumor suppressor - zona occludens 1 domain; PI3K, phosphoinositide 3-kinase; PMS, phelan-mcdermid syndrome; PRD, proline-rich region; PSD, postsynaptic density; PV, parvalbumin; PV+, parvalbumin-expressing; Rac, Ras-related C3 botulinum toxin substrate protein; Rap1, ras-related protein 1; Ras, rat sarcoma virus protein; RIM, rab3a-interacting molecule; RIM-BP, rim-binding protein; RNA, ribonucleic acid; S6, ribosomal protein S6; SAM, sterile alpha motif domain; SAP, synapse-associated protein; SAP, synapse-associated proteins; SAPAP, synapse-associated protein (SAP) 90/postsynaptic density (PSD)-95-associated protein family; SHANK3, SH3 domain and ankyrin repeat containing protein 3; synGAP, ras gtpase-activating proteins; Tbr1, t-box brain transcription factor 1; TSPYL2, testis specific protein y-encoded like 2.

Author contributions

VM, JB designed the research study, VM, TH performed the research, VM, TH, ZB, JB wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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The authors declare no conflict of interest.

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