HHS Public Access

Author manuscript

Sci Signal. Author manuscript; available in PMC 2018 June 15.

Published in final edited form as:

Sci Signal. ; 7(349): re10. doi:10.1126/scisignal.2005832.

Reciprocal Signaling Between Translational Control Pathways and Synaptic Proteins in Autism Spectrum Disorders

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Abstract

Autism spectrum disorder (ASD) is a heterogeneous group of heritable neurodevelopmental disorders whose symptoms, which include deficits in social interaction skills, impaired communication ability, and ritualistic-like repetitive behaviors, appear in early childhood and continue throughout life. Genetic studies have revealed at least two clusters of genes frequently associated with ASD and intellectual disability: genes encoding for proteins involved in translational control and proteins involved in synaptic function. We hypothesize that mutations occurring in these two clusters of genes interfere with interconnected downstream signaling pathways to cause ASD symptomatology. In this review, we focus on the monogenic forms of ASD caused by mutations in genes encoding for proteins that regulate translation and synaptic proteins. Specifically, we describe the function of these proteins, the intracellular signaling regulated by them, and the current mouse models utilized to characterize synaptic and behavioral features of these mutations. Finally, we summarize recent studies that have established a connection between these two signaling pathways in models of ASD and propose that dysregulation of one pathway has a detrimental impact of the other.

Autism spectrum disorder (ASD) is a complex group of heterogeneous neurodevelopmental disorders categorized by three key behavioral abnormalities: restricted interests accompanied by repetitive behavior, deficits in language and communication skills, and inability to engage in reciprocal social interactions (1–3). These core symptoms often are co-morbid with intellectual disability, epilepsy, motor impairment, anxiety, sleep disorder, attention-deficit hyperactivity disorder, and tics (4–6). The behavioral symptomatology of ASD encompasses a spectrum of wide ranging phenotypes, which span from mild behavioral and personality traits to severe and debilitating impairments (7).

The remarkable clinical heterogeneity that characterizes ASD is paralleled by an equally multifaceted etiological heterogeneity. ASD has been recognized to have a genetic component (twin concordance rate 73–95%) and is extraordinary heritable (>90%) (8, 9). Recently, linkage and association studies have identified numerous susceptibility genes located on multiple chromosomes, especially 2q, 7q, 15q, and the X chromosome. Thus, ASD is considered a polygenic disorder generated by the additive effect of multiple common genetic variants in combination with as of yet unidentified environmental factors (10). These forms of ASD are referred to as non-syndromic ASD (11).

In contrast to non-syndromic ASD, in approximately 10% of the cases, ASD appears as part of a syndrome with a known genetic cause (12–14). These monogenic forms of ASD can

result from genomic DNA mutations, *de novo* copy number variants (CNVs) and chromosomal rearrangements (e.g. deletion and translocation) (11). Even when ASD associated is genetically defined, the clinical symptoms are highly heterogeneous, likely due to differences in genetic background and epigenetic regulation (11). Nevertheless, monogenic forms of ASD have been paramount for understanding key neurobiological processes and complex physiological pathways that when perturbed increase the risk for ASD.

Recent studies of monogenic forms of ASD have focused on at least two different clusters of genes frequently associated with ASD and intellectual disability: genes encoding for structural synaptic proteins and genes involved in the regulation of protein synthesis (reviewed in 3, 11, 15). These studies also suggest that mutations in the same clusters of genes may be pathogenic in non-syndromic forms of ASD. We hypothesize that mutations in these two clusters of genes interfere with interconnected downstream signaling pathways resulting in ASD symptomatology.

Here, we will first review the monogenic forms of ASD caused by mutations in these two clusters of genes, the molecular function of their protein products, and the current mouse models utilized to characterize the neurobiological features of these mutations. Next, we summarize key findings that have established a connection between the protein products of these two clusters of genes in models of ASD.

Genes encoding for proteins that regulate translation

Fragile X mental retardation protein

Nearly all individuals with fragile X syndrome (FXS) present with a trinucleotide (CGG) repeat expansion adjacent to the *fragile X mental retardation 1 (FMR1)* gene promoter that leads to the transcriptional silencing and subsequent loss of its protein product (16, 17). Recently, it was discovered that the silencing of the *FMR1* gene is mediated by the formation of a DNA-mRNA duplex between the gene promoter and the trinucleotide repeat region of the mRNA (18). Epidemiological studies show that FXS is the most common known disorder associated with inherited intellectual disability and ASD (19, 20). It occurs in approximately 1:5000 males and roughly half as many females. Affected males with FXS usually have other neurological and psychiatric conditions in addition to ASD and intellectual disability, including motor abnormalities, speech delay, hyperactivity, and anxiety. Postmortem neuropathological studies have revealed an increase in spine-like protrusions on apical and basal dendrites in the cerebral cortex of individuals with FXS (21, 22).

The fragile X mental retardation protein (FMRP), the product of the *FMR1* gene, is an RNA-binding protein that is involved in many aspects of the posttranscriptional regulation of mRNA such as stability, dendritic transport, and translational control. In particular, its function as repressor of protein synthesis has been intensively studied, but the molecular mechanism responsible for this repression remains controversial. Experimental evidence indicate a role for FMRP in the initiation (23–28) and elongation (29–33) steps of protein synthesis. In the initiation model, FMRP inhibits translation initiation by interacting with

CYFIP1, a eIF4E-binding protein (4E-BP)-like protein, which is associated with eIF4E, the cap-binding translation factor for mRNAs (27). On the other hand, there is experimental evidence indicating that the function of FMRP as a translation repressor is at the level of the elongation. (29–33). Moreover, ribosomal run-off of these mRNAs demonstrated that FMRP is associated with mRNAs bound to stalled ribosomes (34) and ribosome transit assays indicate that elongation is enhanced in mice that lack FMRP (35). It is possible that FMRP acts by inhibiting both initiation and elongation steps of translation that depends mRNA identity and/or neuronal stimuli.

The most studied animal model for FXS is a mouse line in which the *Fmr1* gene has been deleted. *Fmr1* null mice display a range of phenotypes that mimic many of the symptoms observed in individuals with FXS. For example, these mice display hyperactivity, altered sensorimotor gating, deficits in learning and memory, increased susceptibility to audiogenic seizures, increased body growth rate, and macroorchidism (36). *Fmr1* null mice also exhibit increased density of dendritic spines and numerous filopodia-like spines in the cortex, recapitulating another pathological feature observed in FXS patients (21, 37–39). *Fmr1* null mice exhibit high levels of basal brain protein synthesis (40) and using HITS-CLIP, at least 842 FMRP target mRNAs have been identified (34). These mRNAs encode for both pre- and postsynaptic proteins. Importantly, the postsynaptic proteins include SHANK1–3, SAPAP1–4, SynGAP1, and neuroligins, whereas the presynaptic proteins include the neurexins, among others. These findings suggest that synaptic proteins and regulators of protein synthesis may interact to generate the FXS phenotype.

In addition to directly repressing translation, FMRP impacts protein synthesis by acting indirectly on signaling pathways involved in translational control. Elevated mammalian target of rapamycin complex 1 (mTORC1) signaling has been reported in *Fmr1* null mice (41), which likely is induced by the elevated expression of PIKE, a GTPase that connects the activation of mGluR5 to the PI3K-mTORC1 signaling pathways in the hippocampus (41, 42). Moreover, several mRNAs targets of FMRP encode for repressors of the mTORC1 signaling pathway, including tuberin (TSC2) and phosphatase and tensin homolog (PTEN) (34). Thus, it is possible that FMRP silencing may have an indirect, secondary effect on protein synthesis by repressing translation of components of the mTORC1 signaling pathway.

Eukaryotic initiation factor 4E

Several studies suggest an association between mutations in the eukaryotic initiation factor 4E (*EIF4E*) gene and ASD. Genetic variants in chromosome 4q, which contains the *EIF4E* locus, have been described in patients with ASD (43, 44). Notably, in ASD subjects several of these common genetic variants in the *EIF4E* gene are associated with a clinical phenotype characterized by repetitive and stereotyped behaviors, but not intellectual disability (45). A *de novo* chromosomal translocation involving the promoter region of the *EIF4E* gene in a boy with classic non-syndromic ASD has been described (46). In addition, a nucleotide insertion in the promoter region of the *EIF4E* gene that increases promoter activity was discovered in two unrelated families with autistic siblings. These genetic studies link

mutations in *EIF4E* to ASD; however further investigations are needed to clearly establish a causal connection.

eIF4E binds to the cap structure at the 5' terminus of mRNA and regulates the initiation step of cap-dependent translation (47, 48). The main role of eIF4E in translation initiation is in the formation of the eIF4F initiation complex, which brings mRNAs to the ribosome for correct translation initiation. The critical step in the formation of eIF4F is the direct association of eIF4E with eIF4G (49), an mRNA-ribosome bridging factor, and the indirect association with the RNA helicase eIF4A (50). The interaction of eIF4E with eIF4G is regulated by eIF4E-binding proteins (4E-BPs), which repress translation by blocking the interaction of eIF4E with eIF4G (51). Upon stimulation, 4E-BP is phosphorylated and inactivated by mTORC1, thereby allowing eIF4E to associate with eIF4G to form eIF4F (52). eIF4E also is regulated by the extracellular signal-regulated kinase (ERK) signaling pathway via phosphorylation by Mnk1/2, which is a substrate for ERK. In some experimental conditions, eIF4E phosphorylation is correlated with the rate of protein synthesis (53). Thus, eIF4E and cap-dependent protein synthesis can be regulated by both mTORC1 and ERK signaling (54).

The relationship between eIF4E, cap-dependent translation, and ASD has been recently studied by genetically increasing the levels of eIF4E in a transgenic mouse (55). eIF4E transgenic mice showed increased brain protein synthesis and aberrant behaviors reminiscent of ASD, including impairments in social interactions and repetitive/perseverative behaviors. The ASD-like behaviors were corrected by blocking the interaction of eIF4E with eIF4G with the cap-dependent translation inhibitor, 4EGI-1. Notably, mice with a genetic reduction in 4E-BP2, the predominant 4E-BP isoform in the brain, exhibit ASD-like behaviors that mimic those displayed by eIF4E transgenic mice (56). Thus, mice with elevated eIF4E-dependent translation display ASD-like behaviors, strongly suggesting a link between exaggerated protein synthesis and ASD.

TSC1 and TSC2

Tuberous sclerosis complex (TSC) is a multi-system disorder characterized by the presence of benign tumor-like lesions (hamartomas) in many organs, such as brain, skin, eye, kidneys and heart (57). TSC is an autosomal dominant inherited disorder caused by loss-of-function mutations in either *TSC1* (encoding hamartin, also referred to as TSC1) or *TSC2* (encoding tuberin, also referred to as TSC2) genes. These mutations comprise a mix of missense, nonsense, insertions, and deletions involving nearly all exons present in the *TSC1* and *TSC2* genes (4, 5, 58). The impact of the different mutations on clinical phenotypes is extremely variable with respect to symptoms and disease severity, and in part is dependent on which *TSC* gene is affected (59). Seizures are the most common neurological symptom occurring in up to 90% of the patients, whereas intellectual disability and ASD occur in approximately 50% of the patients (57).

TSC1 and TSC2 form a heterodimeric complex that can regulate protein synthesis by controlling mTORC1 activity. TSC1/TSC2 are phosphorylated by many kinases and factors, including Akt, ERK, glycogen synthase kinase-3β, AMP-activated kinase, and cyclin-dependent kinase 1 (60–63). The active TSC1/TSC2 complex inhibits mTORC1 through

activation of the small GTPase Ras homolog enriched in the brain (Rheb). Rheb activates mTORC1 when it is bound to GTP. The TSC1-TSC2 complex has GTPase-activity localized in the GAP domain of TSC2. When phosphorylated by Akt, the GAP activity of TSC1/TSC2 is increased, which in turn hydrolyzes GTP bound to Rheb, thereby inhibiting mTORC1 (60–63). Therefore, in the absence of either TSC1 or TSC2, high levels of Rheb-GTP lead to constitutive activation of mTORC1 signaling, thereby resulting in dysregulated protein synthesis and cell growth (63). Several mouse models of TSC have been employed to understand the etiology of this disorder. For example, heterozygous genetic deletion of either *Tsc1* or *Tsc2* results in cognitive and synaptic impairments consistent with ASD (64–66). *Tsc1* and *Tsc2* mutant mice display ASD-like phenotypes in absence of neuropathological brain tumors and epilepsy, suggesting that the cognitive dysfunction in TSC arise independently of brain tumors and/or epilepsy. However, it should be noted that specific genetic ablation of *Tsc1* in either astrocytes (67) or neurons (65, 68) result in epilepsy and lethality. Recently, either genetic reduction or complete depletion of *Tsc1* in cerebellar Purkinje cells (PC) (69) was demonstrated to result in ASD-like behaviors, including impaired social interaction, altered ultrasonic vocalizations, and increased repetitive behaviors that are correlated with decreased PC excitability and changes in the number and morphology of PCs.

Importantly, in the aforementioned mouse models, postnatal and post-development treatment with rapamycin, which inhibits mTORC1 activity, ameliorates multiple behavioral and synaptic phenotypes (65, 68–71). Thus, inhibition of mTORC1 activity in adulthood is sufficient to correct ASD-like phenotypes in TSC model mice, which suggests that these behaviors are caused by ongoing, elevated mTORC1 signaling rather than irreversible pathophysiological changes that occur during brain development.

Phosphatase and tensin homolog

Phosphate and tensin homolog (*PTEN*), a gene located on chromosome 10q23, is a candidate risk gene for ASD and macrocephaly (72–75). Different studies have suggested a causal role for *PTEN* mutations in a subset of individuals with ASD. Recently, a novel frameshift variant of *PTEN* was identified in a patient with extreme macrocephaly, ASD, intellectual disability, and epilepsy, confirming that mutations in this gene are involved in the etiology of ASD and macrocephaly (76). In general, *PTEN* mutation are more frequent (10–20%) in ASD children that develop macrocephaly (77, 78).

PTEN is a phosphatase with activity directed against 3’ phosphate of the phosphatidylinositol-3,4,5-triphosphate (79). PTEN is a negative regulator of the Akt-mTORC1 signaling pathway. Thus, activation of this phosphatase leads to inhibition of PI3K signaling, thereby inactivating Akt and mTORC1. In contrast, deletion of *PTEN* results in a constitutively active Akt-mTORC1 signaling pathway. Given the importance of PI3K/AkT/mTORC1 signaling in controlling cell growth, survival and proliferation, it is not surprising that PTEN inactivation leads to human cancers and neurological disorders (80).

Mouse models with *Pten* deletions have been studied mostly to clarify the role of PTEN in neuronal hypertrophy and number, since the most obvious phenotype in human patients is macrocephaly. Overall, the effect of genetic deletion of *Pten* during development is dramatic

resulting in brain enlargement and gross anatomical abnormalities that are often accompanied by the development of seizures and premature death (81, 82). Several studies have directly addressed the role of *PTEN* mutations in ASD. These studies bypassed the severe developmental phenotype by deleting *Pten* in mice either in a specific cell population or at a certain time after development using conditional genetic technology. For example, in one of the mouse models, *Pten* was ablated in a subset of postmitotic cortical and hippocampal neurons (83). These mice develop macrocephaly and display ASD-like behaviors, including impairments in social interactions, seizures, anxiety, and cognitive deficits (83). Treatment with the mTORC1 inhibitor rapamycin reverses the neuronal hypertrophy and leads to the amelioration of the impairments in social interactions and the seizures (84). Moreover, mice with germline *Pten* haploinsufficiency ($Pten^{+/-}$), exhibit an increase in total brain mass and behavioral impairments such as abnormal social behavior and sensorimotor gating (85), increased repetitive behaviors, and depressive-like behaviors (86). These ASD-like behaviors were exacerbated when $Pten^{+/-}$ mice were crossed with serotonin transporter heterozygote mice $Slc6a4^{+/-}$, which also is considered an ASD susceptibility gene (85). These findings demonstrate that deletion of two ASD risk genes, *Pten* and *Slc6a4*, can cooperate to give rise to ASD-like behavioral phenotypes.

Genes encoding for proteins involved in synaptic function

SHANK

Phelan-McDermid Syndrome (PMS) is a genetic disorder characterized by ASD and intellectual disability. Affected patients exhibit impairments in communication skills often accompanied by reduced socialization and stereotypical movements. In addition, patients with PMS also display aggressive behaviors and seizures (87–89). Genetic studies have identified deletions of variable length in the terminal region of the long arm of chromosome 22 as being responsible for the disorder. *SHANK3*, which is located in this region of chromosome 22, is one of the candidate genes for PMS (89–91). In addition, duplications, copy number variations (CNVs), microdeletions, and mutations in *SHANK3* have been described in patients with ASD and intellectual disability (92–97).

The SH3 and multiple ankyrin repeat domains (SHANK) protein family, also known as proline-rich synapse-associated proteins (ProSAPs), is encoded by three genes (*SHANK1–3*) that share a high degree of homology. The SHANK proteins are expressed abundantly in the central nervous system, are enriched in the postsynaptic density (PSD) of excitatory synapses (98–100), and interact with cytoskeleton and scaffolding proteins, which in turn bind to receptors to create a matrix for the stabilization and organization of the PSD. Indeed, SHANK proteins bind to PSD-95-binding proteins (SAPAP), which interact with PSD-95 proteins associated with glutamate receptors (101). Moreover, SHANK proteins bind to the Homer family of scaffolding proteins, which are associated with metabotropic glutamate receptors (102). Finally, SHANK proteins also are involved in the regulation of cytoskeleton by binding cortactin (103), inositol 1, 4, 5-triphosphate (IP3) receptors, and F-actin (104, 105).

Recently, multiple mouse models with genetic deletions of the *Shank* genes have been intensively studied. In particular, four different lines of *Shank3* mice have been studied, each

with a specific deletion of exons encoding for the functional interaction domains of the protein. Overall, the *Shank3* mutant mice exhibit behavioral deficits consistent with ASD, including social deficits, communication alterations, repetitive and stereotyped behaviors, and abnormal learning and memory that are accompanied generally by changes in synaptic function and molecular composition of the PSD (106), (107, 108). Notably, *Shank3B* mutant mice, carrying an ablation of the PDZ domain of the protein, exhibit a particularly severe phenotype. Consistent with the marked expression of *Shank3* in the striatum, the *Shank3B* null-mice groomed so excessively that they exhibited self-inflicted skin lesions and displayed anxiety-like behaviors and impaired social interactions. Genetic deletion of *Shank1* results in abnormal grooming behavior and impairments in ultrasonic vocalization, but normal social interactions (109, 110) as well as contextual fear memory and long-term spatial memory (111). Overall, these PMS mouse models suggest that molecular changes perturbing synaptic and structural functions at the PSD of excitatory synapses are likely to generate ASD-like phenotypes.

Neuroligins and Neurexins

Several mutations and deletions in genes encoding for neuroligin3 (*NLGN3*), neuroligin4 (*NLGN4*) and neurexin1 (*NRXN1*) have been associated with ASD and intellectual disability, including frameshift, substitution, and missense mutations, as well as CNVs and gene deletions (112–115) (116–119) (43, 116, 120–123). In addition, a *de novo* mutation resulting in a base pair substitution A335G in the promoter region of *NLGN4* gene has been reported in a boy with autism and intellectual disability. Importantly, this base pair substitution (A335G) results in an increased activity of promoter and subsequently, an increase in mRNA expression (124), suggesting that overexpression of neuroligin4 is detrimental to neuronal functions and results in ASD-like phenotypes similar to deletion or loss-of-function mutations affecting the *NLGN4* gene.

Neuroligins and neurexins are synaptic cell adhesion molecules that are critical for synaptic efficacy and plasticity (125–128) (129, 130). Neurexins are type 1-membrane proteins encoded by three genes (*NRXN1, 2, 3*), which generate larger α - neurexins and shorter β - neurexins from independent promoters (131). Furthermore, each gene undergoes extensive alternative splicing that is capable of generating thousands of neurexin isoforms (132). Neuroligins are endogenous ligands for neurexins (125) and are encoded by four different genes (*NLGN1, 2, 3, 4*) located on the X-chromosome (133). Neuroligins are type 1-membrane proteins like neurexins, but have a simpler domain structure and less diversity. All neuroligins are enriched in PSD, but neuroligin1 and neuroligin2 are exclusively localized to excitatory and inhibitory synapses, respectively, whereas neuroligin3 may be present in both (134–136) (128).

Mouse models recapitulating the genetic mutations and/or deletions of *Nrxn*s and *Nlgns* described in ASD patients have been important for understanding the association of human genetic aberrations to the clinical manifestation of the disorder. For example, mice with either a genetic deletion of *Nlgn3* (137) or carryng a knockin allele with an R451C substitution (138) in *Nlgn3* displayed ASD-like behaviors that were mostly restricted to social and communication domains, such us impairments in ultrasonic vocalization, social

interaction and memory. Similarly, mice with a deletion of the *Nlgn4* ortholog exhibited impaired social interactions and ultrasonic vocalization (139). This indicates neuroligins are important in the generation of normal social skills and vocalization.

The studies performed on mice with genetic ablation of the genes that encode the neurexins are more difficult to interpret, given the high degree of genetic redundancy. Mice with a genetic deletion that results in a lack of all the neurexin α-isoforms die prenatally, whereas mice with ablation a single genedeletion live, but they are severely compromised and still die postnatally (126). It will be interesting to study the role of neurexins in a specific neuron-type (or postdevelopmental time window) to avoid the lethal phenotype and establish a link with ASD.

SAPAP

DLGAP2, which encodes for SAPAP2, has been identified as a candidate ASD risk gene in a large study aimed to identified genome-wide rare CNVs occurring in ASD patients (140). Moreover, rare genetic variants in *DLGAP2,* also have been identified in ASD patients (141), suggesting that it could act concomitantly with other genetic mutations and/or environmental factors to contribute to ASD phenotypes in patients. Although not clearly established, a possible involvement of the proteins of the SAPAP family in ASD is intriguing given their demonstrated interaction with the proteins of the SHANK family, which have been more clearly described in ASD (see section, SHANK proteins and signaling) (93–95). However, the involvement of the *SAPAP3* gene in obsessive-compulsive spectrum disorders (OCD), trichotillomania, and Tourette syndrome is fairly well established. Indeed, genetic studies have found increased frequency in *SAPAP3* gene variants in patients with these disorders (142–145).

The members of the SAP90/PSD-95-associated proteins (SAPAP) family, also referred to as guanylate kinase-associated proteins (GKAPs), are postsynaptic scaffold proteins that are localized in the PSD and are uniquely expressed at the excitatory synapses (146). SAPAP proteins are encoded by a family of four genes that are widely, but differentially, expressed throughout the nervous system. The SAPAP proteins have been proposed to provide a link between the PSD-95 family proteins and the actin cytoskeleton via interactions with the SHANK proteins, which in turn bind the actin-binding protein cortactin. Therefore, in the current model of PSD organization, PSD-95/SAPAP/SHANK interactions play an important role in the constitution of the large postsynaptic signaling complex at glutamatergic synapses (147).

The member of the SAPAP family that has been studied in great detail is SAPAP3, which is highly expressed in the striatum (146, 148). Genetic ablation of *Sapap3* caused behavioral abnormalities consisting of extremely high levels of self-grooming accompanied by self-inflicted snout lesions and anxiety-like behaviors. Consistently, *Sapap3* null mice display synaptic, morphological, and molecular defects at striatal glutamatergic synapses. The behavioral and synaptic phenotypes of the *Sapap3* null mice is similar to those generated by genetic ablation of *Shank3* indicating that genetic changes perturbing these synaptic proteins in the striatum results in specific phenotypes that are consistent with ASD.

Based on the genetic link between *DLGAP2* and ASD, the role of SAPAP2 in ASD was investigated recently (149). Genetic ablation of *DLGAP2* in mice results in elevated aggressive behavior and impairments in social interactions in mice. Moreover, the *DLGAP2* null mice exhibit reduced dendritic spines, changes in receptor composition and decrease in PSD length and thickness (149). Overall, these results suggest that deletion of SAPAP2 leads to reduction in synaptic and postsynaptic responses.

SYNGAP1

Genetic studies of ASD patients with intellectual disability have indicated *SYNGAP1* as candidate risk gene. In one of these studies, a *de novo* deletion and a premature stop-codon was discovered in *SYNGAP1*. In this study, the genetic alterations in *SYNGAP1* were described in one child with ASD and intellectual disability and two children with intellectual disability without ASD (out of 30 children observed) (150). Another study identified an extended deletion that included *SYNGAP1* together with several other genes in a patient with ASD (140).

The *SYNGAP1* gene encodes for a RasGTPase-activating protein (RasGAP) termed synaptic GTPase-activating protein (SynGAP). The *SYNGAP1* gene has several alternative start sites and the transcripts can be spliced extensively to generate multiple SynGAP1 isoforms (151, 152). SynGAP1 is a brain-specific protein highly enriched at excitatory synapses that co-localizes and interacts with NMDA receptors and the PDZ domains of PSD-95 via its C-terminal amino acids and (151, 152). It works as a negative regulator of the signaling pathways that control NMDA receptor-mediated synaptic plasticity and AMPA receptor membrane insertion (153–155). It was shown that SynGAP1 links Ca^{2+} influx to activation of ERK pathway downstream of NMDA receptors (156). Given the multiple isoforms and the possible high degree of redundancy, the impact of deleting *SynGAP1* in neurons is not clear. In fact, deletion of *SynGAP1* in hippocampal neurons in culture has been reported to both enhance (153, 155) and suppress (154) dendritic spine formation. Mice with a homozygous genetic deletion of *Syngap1* die postnatally, but heterozygous mice survive (157). Behavioral analysis of these mice revealed hyperactivity, diminished sensorimotor gating, and enhanced startle response. Moreover, they display a reduction in social memory and tendency toward social isolation. Moreover, *Syngap1* mutant mice have enhanced ERK activation and impairment in hippocampal synaptic plasticity (156). Recently, it was demonstrated that mice with a heterozygote deletion of *Syngap1* (157) exhibited glutamatergic synapses that mature at an accelerated rate during development with a consequent disruption in the excitation/inhibition balance in hippocampal neurons (158). This study indicates that changes in synapses maturation during the development results in enduring behavioral abnormalities.

Reciprocal Signaling Links Two Clusters of ASD Genes

The studies summarized above are consistent with at least two defined clusters of genes that are involved in ASD and intellectual disability. One cluster encodes for proteins that regulate protein synthesis, a fundamental process for long-lasting changes in synaptic strength and dendritic spine plasticity underlying cognition. The second cluster of genes produces

proteins involved in the regulation of synaptic transmission and structure, which are important in the establishment and remodeling of neuronal networks. Currently, there is limited experimental evidence suggesting a direct interaction between the protein products of these two gene clusters. However, their critical and central biological functions strongly suggest that an anomaly in one of these pathways would almost necessarily perturb the other (Figures 1 and 2).

Activity-dependent changes in PSD composition and/or structure represent molecular mechanisms that drive complex brain functions, including learning and memory. These long-term synaptic and structural changes are critically dependent on dendritic protein synthesis (Figure 1). Indeed, it recently was shown that aberrant protein synthesis driven by overexpression of the cap-binding translation factor eIF4E causes synaptic impairments and ASD-like behaviors (55), indicating that exaggerated translation directly influences synaptic and structural plasticity. Consistent with this idea, a related study revealed that overexpression of neuroligins is likely responsible for the generation of certain ASD-like phenotypes in these mice (56). Moreover, the synaptic, structural, and behavioral abnormalities in mice with exaggerated eIF4E-dependent translation we corrected by reducing protein synthesis and/or diminishing the expression of neuroligins with short interfering RNAs (siRNA) (55, 56).

The examination of FMRP-regulated target mRNAs also supports this idea and demonstrates that both pre- and post-synaptic proteins are part of the transcripts dysregulated in FXS (34), which include, SHANK3, SynGAP1, neuroligin3 and neurexin1 (34), SHANK1, and SAPAPs 1 and 3 (159). This suggests that changes in synaptic and PSD proteins driven by dysregulated protein synthesis may contribute to enduring changes in synaptic plasticity, dendritic morphology, and ASD-like behavioral abnormalities. Another set of mRNA targets of FMRP are proteins directly involved in the regulation of translation, such as TSC2 and PTEN (34, 160), suggesting that synaptic proteins and regulators of mTORC1 activity may interact to give rise to the FXS phenotype.

Conversely, it is possible that the ASD associated mutations that result in changes in the level and function of synaptic and PSD proteins alter protein synthesis and contribute to the generation of ASD (Figure 2). Unfortunately, to our knowledge, there is limited information regarding the activity of translational control pathways in human patients and mouse models of ASD caused by mutations in genes encoding for synaptic proteins (reviewed above). However, a recent study investigating mGluR signaling in mice with a genetic deletion of *Fmr1* reveals a fundamental role of the PSD scaffolding protein Homer1a (161, 162). Altered mGluR5-Homer interactions contribute to abnormal mGluR signaling, altered protein synthesis, and other ASD-like phenotypes in FXS. Importantly, genetic deletion of Homer1a restores the normal mGluR5-Homer association and corrects several phenotypes in *Fmr1* null mice, including enhanced global protein synthesis (162). Although the effect of mGluR5-Homer interactions on protein synthesis is secondary to the direct role of FMRP in translation, this study indicates the possibility that alteration in synaptic proteins results in aberrant translational control. It is tempting to speculate that ASD linked to *SHANK* mutations is also associated with alterations in protein synthesis because SHANK directly interacts with Homer (102). Therefore, defects in synaptic protein function could result in

aberrant protein synthesis, resulting in abnormal synaptic plasticity and ASD-like behaviors. Future studies are necessary to conclusively address this hypothesis.

Several lines of evidence indicate that loss-of-function mutations, deletions, and overexpression of synaptic and PSD proteins are detrimental and result in ASD-like behavioral phenotypes. A good example of this bidirectional effect is illustrated in patients where deletions and a *de novo* mutation of neuroligin, which increase the activity of the promoter of *NLGN4* gene, result in ASD and intellectual disability (112, 113, 124). Similarly, animal models with genetic deletion (139, 137) or exaggerated expression (56) of *NLGNs* display behavioral and synaptic phenotypes consistent with ASD. This is in agreement with our hypothesis that increased expression of synaptic proteins generated by alteration in protein synthesis could trigger synaptic abnormalities and behaviors associated with ASD. In contrast, investigations concerning the proteins regulating translation seem to point toward a connection between exaggerated protein synthesis and ASD phenotypes in humans and animal models (55, 56, 163). However, inhibition of *de novo* protein synthesis impairs long-lasting plasticity and cognition (reviewed in (47, 164)) and likely contributes to cognitive deficits in TSC model mice (165). An intriguing possibility is that excessive translation contributes to aberrant behaviors associated with ASD whereas insufficient translation contributes to impaired cognition associated with intellectual disability, which often accompanies ASD.

To conclude, we have reviewed recent data supporting the hypothesis that proteins involved in the regulation of translation and synaptic function may be interconnected and act in concert to give rise to synaptic and behavioral aberrations associated with ASD. Future genetic studies are necessary to reveal the molecular players that link these two pathways and to understand whether it is possible to intervene therapeutically at the level of these molecular crossroads.

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Gloss

Autism spectrum disorder (ASD) is a heterogeneous group of heritable neurodevelopmental disorders characterized by repetitive behavior, deficits in communication skills and impaired social interactions. Human genetic studies have uncovered at least two clusters of genes associated with ASD and intellectual disability: genes encoding for proteins involved in the regulation of protein synthesis and proteins involved in synaptic function. We hypothesize that mutations in these risk genes impact interconnected intracellular signaling pathways to disrupt synaptic function and behavior. In this review, we describe the protein products of these two clusters of genes and the findings that connect their signaling pathways. Knowledge of these molecular and synaptic abnormalities should generate novel therapeutic targets for the treatment of ASD.

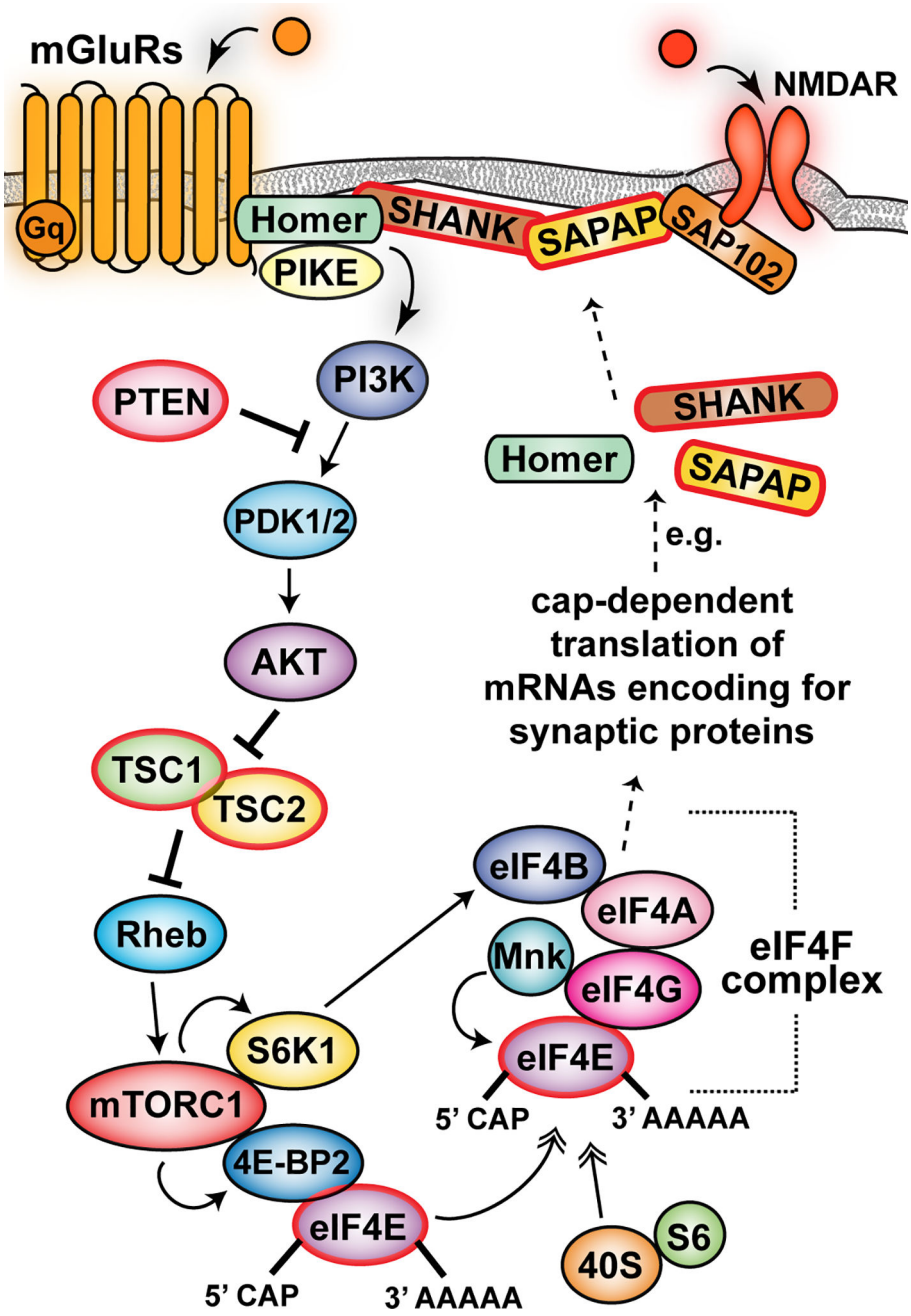

Figure 1. Schematic of the hypothetical connection between protein synthesis and synaptic proteins

Activation of group I mGluR receptors results in the activation of mTORC1 signaling, which increases protein synthesis. mTORC1 phosphorylates S6K1 and 4E-BP2.; phosphorylation of 4E-BP2 release eIF4E and results in the association of eIF4E with eIF4G to form of the active eIF4F (eIF4E-eIF4G-eIF4A) complex. eIF4F promotes the binding of mRNAs to ribosomes and recruits Mnk, which phosphorylates eIF4E, and eIF4B, which is phosphorylated by S6K1. The eIF4F complex and the poly(A) tail act synergistically together with MnK-dependent phosphorylation of eIF4E to stimulate cap-dependent translation initiation. Cap-dependent protein synthesis translates some mRNAs that encode

for synaptic proteins located in the PSD. It is possible that mutation in genes encoding for proteins involved in the regulation of the mTORC1 pathway results in aberrant synthesis synaptic proteins such as neuroligins, SHANK, SAPAP, etc. The altered synthesis of these proteins would generate changes in molecular, structural, and synaptic plasticity, ultimately leading to ASD pathophysiology. The protein products of genes associated with ASD are circled in red.

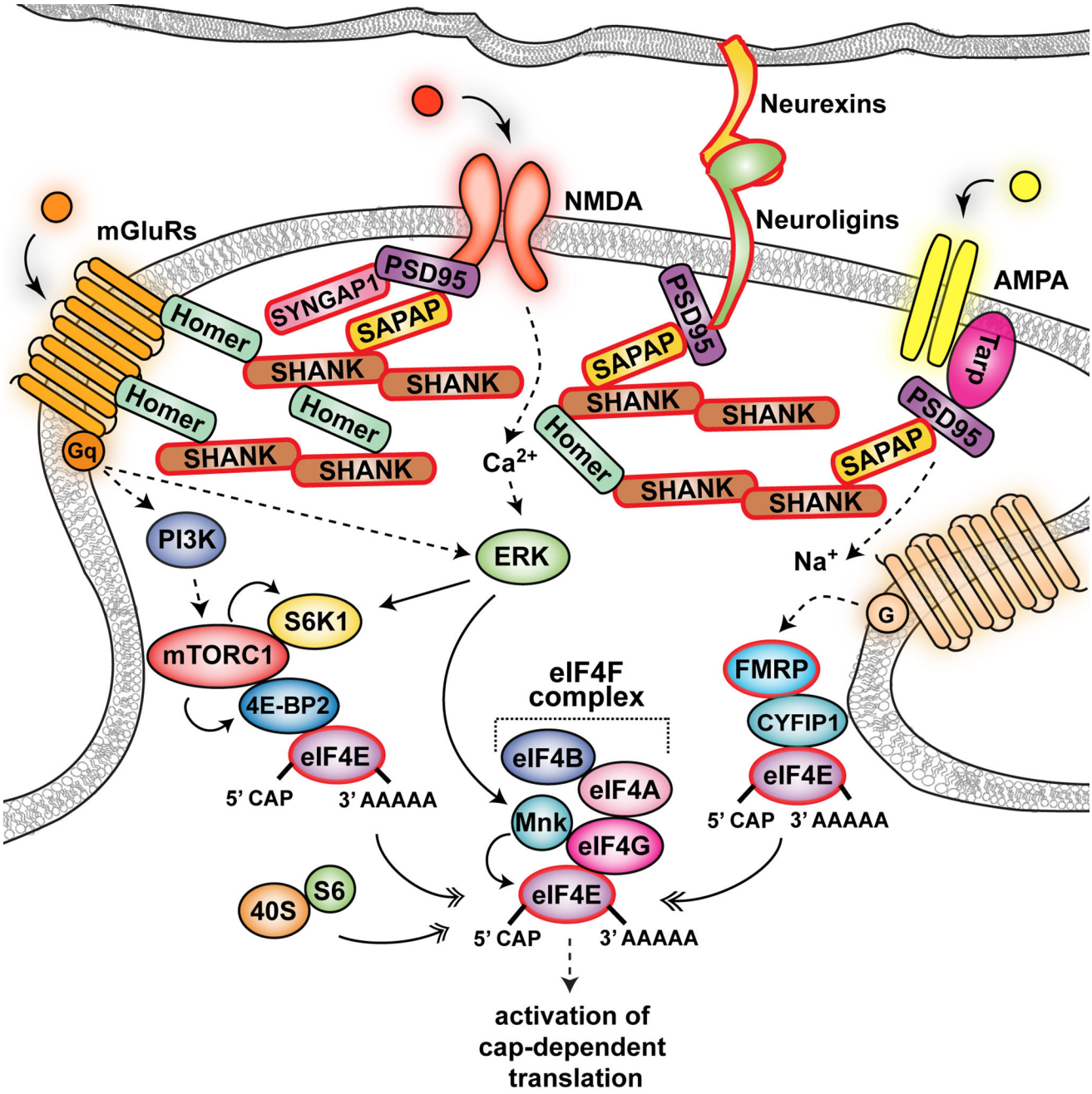

Figure 2. Schematic of the hypothetical connection between synaptic proteins and protein synthesis

Intracellular signal transduction is initiated by the activation of neurotransmitter receptors that are organized with scaffolding proteins and adhesion molecules in the PSD. Receptor stimulation triggers the activation of intracellular signaling cascades including the mTORC1 and ERK pathways, which results in increased translation (see also Fig1). Given the importance of synaptic proteins in this type of signal transduction, mutations affecting genes encoding for these proteins could result in abnormal signaling that ultimately results in

aberrant protein synthesis. The protein products of genes associated with ASD are circled in red.