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The ERK pathway in the autistic synapse

Troubled in translation control?

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Abstract

Autism is unequivocally a complex genetic disorder. However, its complexity converges into the activity-dependent synapse plasticity. Compelling evidence suggests that the local dendritic protein synthesis constitutes a critical molecular mechanism underlying synapse plasticity. In this regard, the extracellular signal-regulated kinase (ERK) pathway has recently attracted considerable attention in ASD pathogenesis. At synapses, ERK regulates the recruitment of components of local translation machinery and thus stimulates protein synthesis in response to activity. Mutations in multiple ERK components occur in autism and disrupt ERK signaling. *In vivo*, these mutations disturb synapse function and cognition. Dysfunction of the ERK pathway additionally lies upstream of altered translation and contributes to synapse pathology in syndromic forms of autism. Collectively, these findings suggest that activity-dependent and ERK-mediated local protein synthesis may be an important component of the molecular basis of ASD.

Contents

1 Introduction

In 1943, the psychiatrist Leo Kanner first described the autistic disorder [51] of which diagnosis nowadays relies on a triad of core behavioral deficits: impairments in social reciprocity, disturbances of verbal and nonverbal communication, and stereotyped and repetitive patterns of behavior accompanied by restrictive interests. Manifestation of clinical signs typically occurs prior to the age of three years [71]. Beyond this unifying definition, however, the phenotypes considerably vary among individual cases, ranging from deliberating behavioral impairments to mild personality traits. Additionally, intellectual disability and seizure disorder accordingly impact $\sim$ 70% and 25% of the autistic individuals while co-morbid anxiety and mood disorders often occur in autism. Thus, autism is not a single disease entity. Instead, it describes a clinically heterogeneous group of neurodevelopmental disorders, collectively termed "autism spectrum disorders" (ASDs) [109, 128]. ASDs encompass the classic autistic disorder (Kanner's autism), Asperger and Rett syndromes, pervasive developmental disorder not otherwise specified (PDD-NOS), and childhood disintegrative disorder (see Table 1 for differential diagnostic features of ASDs) [3]. They are unequivocally among the most heritable neuropsychiatric disorders (estimated heritability up to 90%) [1]. Insights into ASD genetic etiology, though, reveal substantial heterogeneity and complexity. An impressive array of genetic studies has identified rare, single-gene mutations yielding syndromes with highly-penetrant features of ASD (syndromic autism) or causing idiopathic (non-syndromic) autism, and *de novo* copy number or single nucleotide variants in multiple loci which accordingly impact gene dosage expression or function and synergistically enhance ASD susceptibility [1, 55, 89, 93, 99]. These known genetic causes account for the minority of ASD cases ($<$ 20%) and, yet, much of the ASD genetic basis remains unexplained.

Identification of molecular pathways in which rare, highly penetrant, and common genetic variations overlap is essential to gain mechanistic insights into ASD neurobiology and further devise effective therapeutic strategies. Interestingly, recent findings suggest that these known genetic causes interfere with the activity-dependent synapse development and plasticity – events that determine the structural establishment and functional refinement of neuronal connectivity in the brain as the latter learns and adapts to a changing environment (for review, see; [26]). Particularly, experience triggers the release of neurotransmitters at and stimulates specific synapses. Synaptic activity, in turn, can induce long-lasting changes in synaptic strength, namely long-term potentiation (LTP) and long-term depression (LTD). During neonatal life, the mechanisms of LTP and LTD accordingly drive the stabilization and elimination of nascent synapses. In addition, their coordinated regulation refines transmission efficacy between synaptic contacts that underlies memory storage and behavioral plasticity throughout postnatal life and adulthood [9, 49, 76]. Persistent changes in synaptic strength involve modifications of actin cytoskeletal organization in the spine and protein composition at the contact site which impact volume and amplitude of synapse [14, 68, 69]. Thus, they are highly dependent on protein synthesis and function [14, 68, 69]. In this regard, the extracellular signal-regulated kinase (ERK) pathway has recently attracted considerable attention in ASD pathogenesis. It is a highly-conserved cascade pathway that, in neurons, plays a key role in trancription and translation regulation underlying synapse plasticity and memory consolidation [56, 114, 118, 124].

Neuronal ERK signaling corresponds to a variety of extracellular stimuli and, most notably, to membrane depolarization following synaptic glutamate release and to neurotrophin stimulation (Figure 1) [114]. These last two stimuli profoundly control many aspects of synapse plasticity [14, 34, 69]. Signal transduction occurs via the sequential phosphorylation and activation of protein kinases at three distinct tiers of ERK cascade. Initially, ligand binding to the cell surface receptor stimulates the exchange of guanosine-5'-diphosphate (GDP)

	Autism	**Asperger's syndrome**	**PDD-NOS**
Age of onset	0-3 years	>3 years	Variable
Age of diagnosis	3-5 years	6-8 years	Variable
Social reciprocity	Poor; >2 DSM-IV	Poor	Variable
Communication	Delayed & deviant; might be not verbal	No early delay, though qualitative and pragmatic impairments later	Variable
Behavior	More impaired than in Asperger's sydrome or PDD-NOS (includes stereotypy)	Variable (restrictive interests)	Variable
ID	$>60\%$	Mild to none	Mild to severe
Seizures	25% over lifespan	$\sim 10\%$	$\sim 10\%$
Prevalence (per 10^3)	1-2	0.6	3.7
Sex Ratio (Male:Female)	4:1	$>11:1$	N/A (more males)
Regression	$\sim 25\%$ (social or communication)	No	Variable
Outcome	Poor to fair	Fair to good	Poor to good

Table 1: **Differential diagnostic features of autism spectrum disorders.** Table was adapted and reprinted by Levy *et al.* [71]. According to the *Diagnostic and Statistical Manual for Mental disorders* (DSM-IV, 4^{th} edition [3]), Rett syndrome and childhood disintegrative disorder (CDD) are subsets of the pervasive developmental disorders (PDDs) in autism spectrum. Rett is a rare cause for severe intellectual disability in females accompanied by seizure dirorder and autistic core traits. CDD is additionally rare neurophychiatric disorder characterized by late onset of developmental delay in communication, social reciprocity, and motor skills. The diagnostic strategies applied in Rett syndrome and CDD are different and not addressed in the current table. Abbreviations: ID – intellectual disability; N/A – not applicable; PDD-NOS – pervasive developmental disorder not otherwise specified.

for guanosine-5'-triphosphate (GTP) on and activates the small G protein protein Ras. Biochemical mechanisms for Ras activation involve the enhanced activity of guanyl nucleotide exchange factors (GEFs, which directly catalyze the exchange of GDP for GTP on Ras), the inhibition of GTPase-activating proteins (GAPs, which accelerate the slow Ras-catalyzed intrinsic hydrolysis of GDP to GTP), the altered cytoplasmic localization of these enzymes or a combination of all these processes depending on the activated type of receptors (for detailed description of the biochemical mechanisms, see [77, 114]). Thus, this GDP/GTP exchange elicits a conformation change in Ras (*i.e.,* into its active GTP-bound form) that enables Ras protein to interact with and promote the activation of the protein kinase Raf. In turn, Raf phosphorylates and activates the mitogen-activated protein kinases (MAPK) MEK1 and MEK2 (MEK1/2). MEKs lie upstream of the activation of the protein kinases ERK1 and ERK2 (also known as p44 and p42 MAPK, respectively). ERKs are serine/threonine kinases of which downstream targets include nuclear proteins and transcription factors for gene expression, cytoskeletal components, and, most importantly, other kinases for translation and transcription control (Figure 1) [118, 124].

Compelling evidence suggests that the local dendritic protein synthesis constitutes a critical molecular mechanism for synapse plasticity [12, 14, 20, 44, 50, 69]. This hypothesis originates from the discovery of polyribosome complexes selectively accumulated at the distal processes beneath the post-synaptic sites on neuron dendrites in a rosette-like structure [111]. The polyribosome complexes consist of a cluster of ribosomes, which are the translation units, bound to a strand of a messenger RNA (mRNA) and surrounded by membranous cisterns [111]. Subsequent immunohistochemical studies have identified ribosomal proteins, translation regulators, initiation and elongation factors, and endoplasmic reticulum

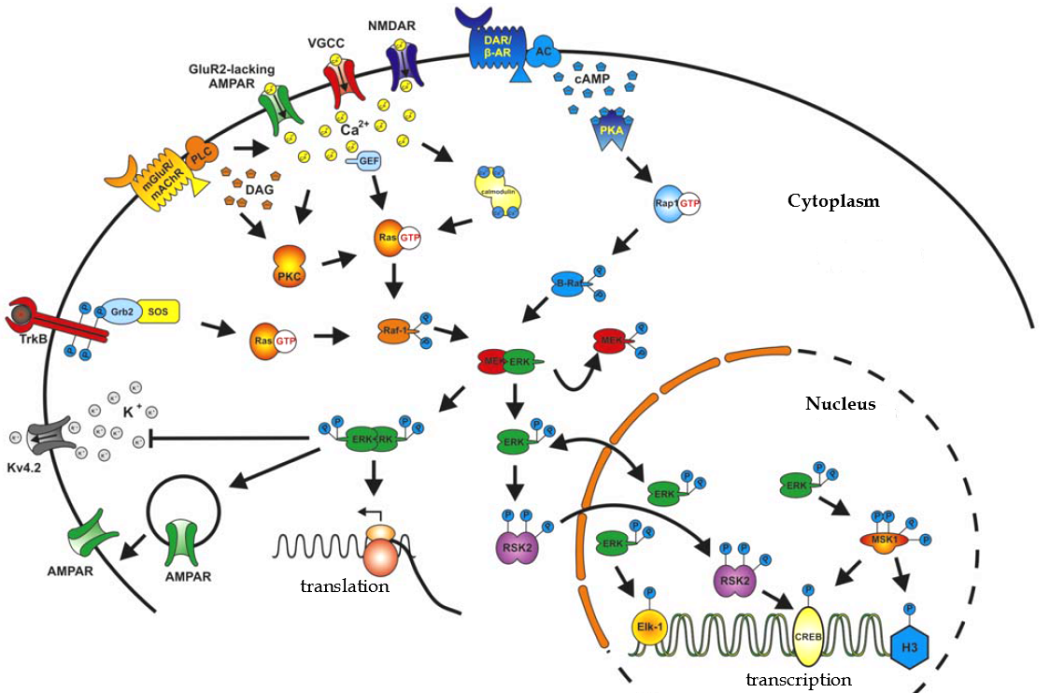

Figure 1: **ERK signal transduction and major downstream targets upon synapse activity.** Figure was adapted and reprinted by Wiegert and Bading [124]. Many extracellular stimuli, such as neurotrophic factors, norepinephrine, dopamine, acetylocholine, glutamate and calcium influx can trigger ERK activation in neurons [114]. Although the specific components of the cascade vary greatly among the different stimuli and activated type of receptors (the biochemical mechanisms have been extensively reviewed elsewhere [77, 114]), these stimuli result in an increase of the active, GTP-bound form of the small G protein Ras. In turn, Ras-GTP triggers the activation of the protein kinase Raf, which lies upstream of the activation of MEK1/2. MEKs subsequently phosphorylate and activate ERK1/2 [118]. Phosphorylated ERK1 and ERK2 dissociate from MEKs and further form a dimer with another ERK molecule or remain monomeric. ERK monomers can probably enter the nucleus where they phosphorylate local kinases (*i.e.,* MSK1) and transcription factors to regulate gene expression [124]. In the cytoplasm, ERK1/2 regulate AMPAR trafficking and neuronal excitability through phosphorylation of the receptor subunits (GluR1 and GluR2) and of the voltage-dependent K^+ channel $K_v4.2$, respectively [112, 114]. Additionally, ERKs phosphorylate and activate other kinases (*e.g.,* RSK and Mnk1/2) which, in turn, promote protein synthesis and gene transcription [118]. Abbreviations: AMPAR – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; βAR – β adrenergic receptor; DAR – dopamine receptor; ERK – extracellular signal-regulated kinase; K^+ – potassium; mAChR – muscarinic acetylcholine receptor; MEK – mitogen-activated protein kinase (MAPK); mGluR – metabotropic glutamate receptor; Mnk – MAPK-interacting protein kinase; MSK1 – mitogen- and stress-activated protein kinase 1; NMDAR – N-methyl-D-aspartate receptor; RSK – ribosomal S6 kinase; TrkB – tropomyosin receptor kinase B for neurotrophins; VGCC – voltage-gated calcium channel.

(co-translational sorting membranous organelle) in dendrites. *In situ* hybridization and RNA amplification studies have further revealed that mRNA families encoding glutamate receptor subunits, cell adhesion molecules, scaffolding proteins, compartment-specific cytoskeletal components (*e.g.,* microtubule-associated protein 2 – MAP2), other regulatory proteins for plasticity (such as the activity-regulated cytoskeleton-associated protein – Arc which regulates actin dynamics and receptor trafficking), and transcription factors concentrate in the post-synaptic vicinity of hippocampal neurons (for review of these studies, see; [113]). Markers of RNA translation have revealed that synapse activity triggers protein synthesis locally at or near the synapses [78, 113] while the peak of the translation rate occurs during

postnatal life [92]. Targeted disruption of local protein synthesis impairs the persistence of hippocampal LTP and results in long-term memory and behavioral deficits both in juvenile and young rodents [56, 80]. Collectively, these findings underscore the importance of local protein synthesis in synapse modification underlying memory formation and behavioral plasticity throughout lifetime.

The current thesis explores the relative contribution of the ERK signaling in the ASD etiology. Mutations in multiple ERK components occur in autism and disrupt pathway activity [38, 55, 66, 93, 123]. *In vivo,* these mutations defect synapse plasticity and result in behavioral deficits [37, 57, 63]. Conversely, ERK pathway plays a key regulatory role in the recruitment of the protein-synthetic-machinery components and translation initiation locally in dendrites [56]. Although the current weight of scientific evidence is not conclusive, aberrant ERK signaling and subsequent dysregulation of the ERK-dependent local protein synthesis upon activity may be an important component of the molecular basis of ASD. In support of this proposal, robust ERK1/2 activation in response to synapse stimulation lies upstream of the aberrant protein synthesis and contributes to the synapse pathology and phenotypic outcome in syndromic forms of autism [84, 85]. Thus, in the following sections, I discuss seminal studies in ERK signaling and defects of these molecules with respect to local dendritic translation and synapse modification. As mentioned above, ERK profoundly regulates gene transcription required for synapse plasticity and memory consolidation. However, this topic has been systematically reviewed in normal conditions [114, 118, 124] and recently in ASD etiology [48]; hence, it is beyond the scope of the current essay.

2 Local Protein Synthesis Regulation in Synapse Plasticity

Protein synthesis has been extensively studied in the context of memory formation and activity-dependent cortical development [39, 61]. In both contexts, the information processing and its temporary storage ($\sim 1 - 2\,\mathrm{h}$) within the circuits are independent on protein synthesis. However, its retention for longer periods, lasting hours to days, requires gene expression [39]. Like memory, synapse plasticity exhibits short- and long-lasting forms with distinct molecular requirements and temporal characteristics. Specifically, LTP and LTD occur in persistent late phases (L-LTP and L-LTD, respectively) which require gene transcription and *de novo* protein synthesis, and in transient early phases (E-LTP and E-LTD, respectively) which solely depend on post-translational modifications of preexisting proteins [18, 96].

Compelling evidence suggests that the local dendritic protein synthesis constitutes the critical mechanism underlying long-lasting synapse modification [12, 20, 44, 50, 80, 120]. Specifically, Kang and Schuman have demonstrated that the brain-derived neurotrophic factor or neurotrophin-3 application triggers long-lasting enhancement of synaptic transmission in hipocampus which characteristically exhibits an immediate requirement for protein synthesis [50]. This translation-dependent, neurotrophin-evoked LTP persists even after the dendritic layers of CA1 and CA3 pyramidal neurons have been surgically severed from their cell bodies in hippocampal slices [50]. In a similar slice preparation, isolated dendrites effectively support protein-synthesis-dependent LTD upon pharmacological or paired-pulse low-frequency stimulation of group I metabotropic glutamate receptors (Gp I mGluRs) [44]. Similarly, subsequent work has demonstrated that local translation is both sufficient and necessary for the establishment and maintenance of L-LTP in hippocampus [20, 120]. Consistent to this hypothesis, genetic and pharmacological evidence has revealed that local translation inhibition impairs L-LTP in an earlier temporal window than the gene transcription inhibition [5, 56]. The latter further suggests that local upregulation of the translation of preexisting mRNAs mediates the transition from E- to L-LTP (establishment of L-LTP) while the local translation of newly transported mRNAs from neuron soma to the activated synapse promotes the long-lasting persistence of synapse potentiation [12].

Local protein dendritic synthesis is also crucial for the persistent spine enlargement that accompanies long-lasting changes in synaptic strength upon activity [14, 52, 68]. Spines are small protrusions on the dendrites which consist of the postsynaptic excitatory (glutamatergic) machinery, postsynaptic density (PSD), actin cytoskeleton, and membranous organelles [110]. They are highly dynamic structures of which morphology is subject to rapid alteration in response to neuronal activity and glutamate receptor activation. For instance, induction of LTD causes rapid spine enlargement. Larger spines have larger and more complex (PSDs), higher surface content in glutamate receptors, better sensitivity to glutamate release and calcium influx, and subsequently better postsynaptic responsivity to LTP [110]. Activity-dependent spine growth and remodeling depend on signal transduction pathways that modulate actin dynamics through post-translational modifications of regulatory proteins, as previously reviewed [68]. However, like synapse plasticity, spine enlargement displays immediate and gradual phases. Gradual phase and persistent forms of spine remodeling are sensitive to protein synthesis inhibitors and thus dependent on mRNA translation [116]. In addition, BDNF application and Gp-I mGluR stimulation which both induce mRNA translation locally at synapses [44, 50] trigger a protein-synthesis-dependent lengthening of spines [14, 68]. Finally, many mRNAs synthesized locally encodes proteins that regulate actin polymerization and stability (*e.g.,* Arc and MAP1B) or signaling components of the spine morphogenesis pathways [14, 68].

An understanding of the molecular mechanisms by which synaptic activity regulates local protein synthesis is beginning to emerge. Local protein synthesis occurs in response to synaptic glutamate release. Current evidence implicates two types of the post-synaptic glutamate receptors in translational regulation; the calcium-permeable N-methyl-D-aspartate receptors (NMDARs) and the G_q-coupled (GpI) mGluR1 and mGluR5 [12, 20, 44, 50, 56]. NMDAR stimulation additionally triggers the release of the brain-derived neurotrophic factor (BDNF). BDNF binds to TrkB receptors and further induces local protein synthesis [50]. Stimulation of these receptors triggers downstream mammalian target of rapamycin-raptor complex (mTORC1) and ERK signaling pathways [18, 96]. mTORC1 activation occurs in a sequential signaling cascade downstream of phosphoinositide-3 kinase (PI3K) [96] or ERK [75] (Figure 3). Both pathways regulate the recruitment of components to the translation machinery and thus stimulate protein synthesis [18, 96]. Below, we point out the critical events of translation process and further discuss their regulation by neuronal ERK and mTOR signaling.

2.1 Protein synthesis mechanism

The translation of a given mRNA comprises three sequential phases – initiation, elongation, and termination. Initiation constitutes the rate-limiting event in the translation process and thus serves as the principal target for regulation. It requires a pool of separated ribosomal units and diverse eukaryotic initiation factors (eIFs). For the vast majority of mRNAs, translation initiation occurs in a cap-dependent way in which m^7G cap (also termed as 7-methylguanosine cap) present at 5' end of the mRNA and added during transcription facilitates recognition and ribosome attachment. Alternatively, initiation on few mRNAs follows a cap-independent mechanism which involves ribosome recruitment at an internal position within the mRNA 5' untranslated region (UTR), termed internal ribosome entry site (IRES) [47, 18]. However, both the relative importance of this mechanism in synaptic plasticity and its regulation remain currently unknown. Cap-dependent initiation consists of three key events – the formation of 43S ribosomal preinitation complex, recruitment of the 43S complex to the 5' end of the mRNA, and the assembly of 80S ribosomal complex [18] (Figure 2).

Initially, eIF2 binds to GTP and initiator methionyl-tRNA (Met-tRNA$_i^{Met}$) to form a ternary complex (eIF2 TC). Subsequently, eIF2 TC associates with the small 40S ribosomal subunit, which carries eIF1, eIF3, and probably eIF5, to assemble the 43S preinitiation complex. Loading of the 43S preinitiation complex onto the 5' end of a given mRNA requires the formation and cooperation of eIF4F complex. The latter consists of the cap-binding protein eIF4E, the ATP-dependent RNA helicase eIF4A which unwinds the secondary structure of the 5'UTR of the mRNA to facilitate ribosome attachment, and the eIF4G protein which interacts with eIF3 and couples 43S preinitiation complex to the mRNA. Thus, the assembly of eIF4F complex is a critical event in cap-dependent translation while its regulation lies within the phosphorylation state of eIF4E-bindings proteins (4E-BPs), as discussed below. Once attached to the 5' end, 43S ribosomal complex scans the mRNA downstream of the cap to the initiation codon (in a direction 5'$\rightarrow$ 3') to form the 48S complex. Subsequently, eIF5 along with eIF5B promotes the hydrolysis of eIF2-GTP, eIFs displacement, and the joining of 60S ribosomal subunit. The subsequent formation of 80S ribosomal complex signals the termination of the initiation phase and the initiation of the elongation phase. For elongation of the polypeptide chain, the eukaryotic elongation factor 1A (eEF1A) recruits aminoacyl-tRNA into the ribosome while the elongation factor eEF2 mediates the translocation of the ribosome along the mRNA after peptide formation. Upon stop codon recognition,

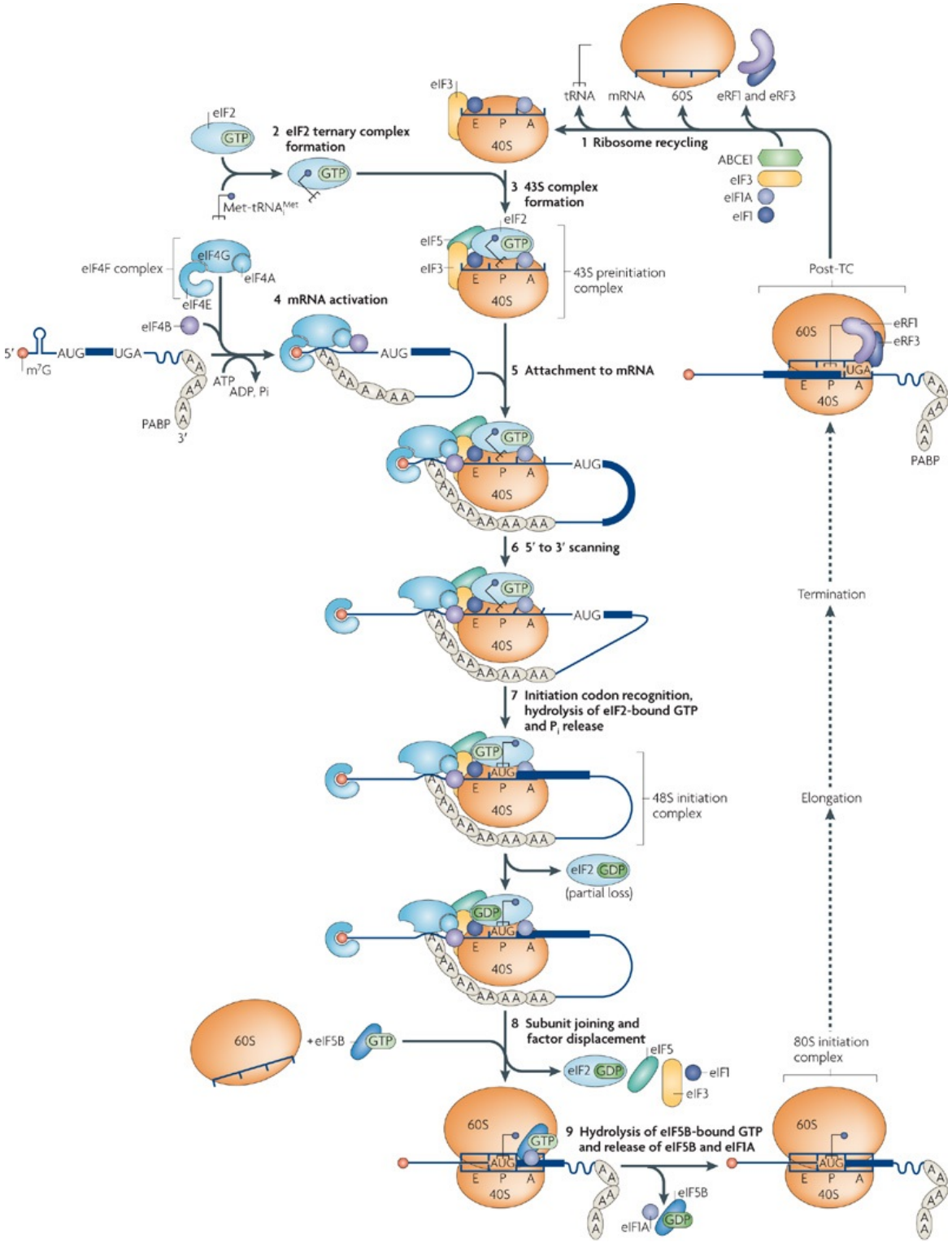

Figure 2: **Schematic representation of cap-dependent mRNA translation initiation.** Figure was adapted and reprinted by Jackson *et al.* [47]. Abbreviations: AUG – initiation codon; eIF – eukaryotic initiation factor; eRF – eukaryotic release factors; m^7G – 7-methylguanosine cap; PABP – poly-A-binding protein; UGA – stop codon;. See text for translation mechanism description and further abbreviations.

various release factors terminate the translation process, release the polypeptide chain from the mRNA and ribosome, and recycle ribosomal complexes) [18, 47, ?].

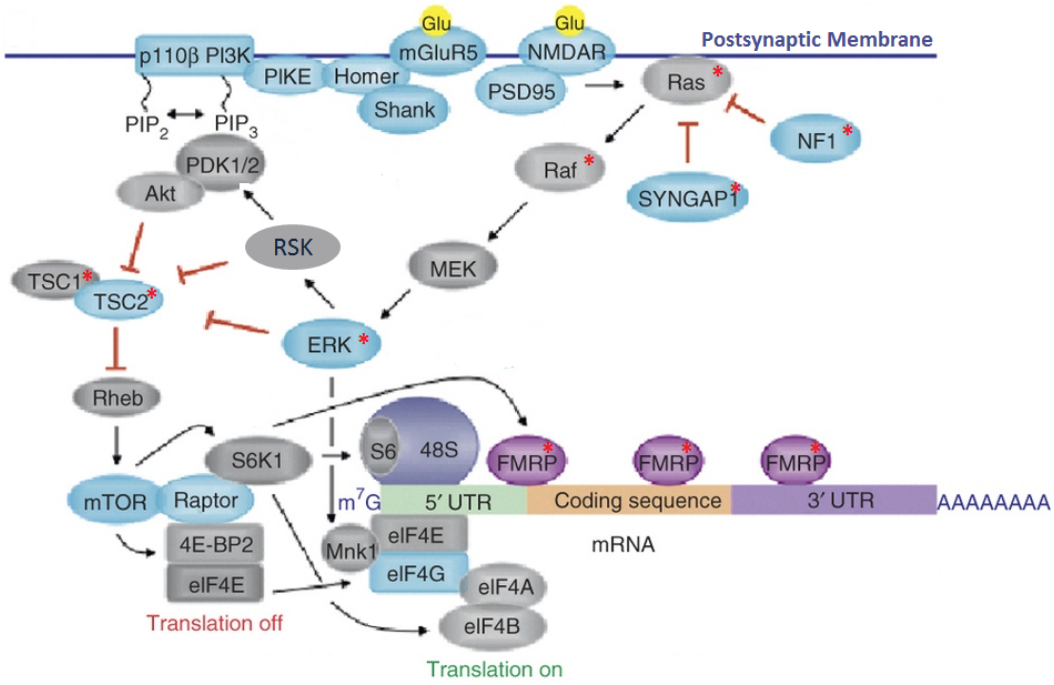

Figure 3: **Neuronal signaling pathways in regulation of translation initiation.** Figure was adapted and reprinted by Darnell and Klenn [21]. The Ras/ERK and PI3K-mTORC1 couple Gp I mGluRs, NMDARs, and TrkB receptors (not depicted in the figure) to the local translation machinery. Regulation of the availability and activity of the mRNA cap-binding factor eIF4E represents the principal effector mechanism through which ERK and mTORC1 pathways modulate local protein synthesis [56]. Nevertheless, the ERK-dependent eIF4E phosphorylation plays a pivotal role in hippocampal plasticity and memory formation [6, 56]. Mutations in multiple ERK components (such as NF1 and SynGAP1) and in the mTORC1 regulator TSC1/2 result in monogenic disorders with a high prevalence of cognitive impairments and ASD. FMRP is an mRNA-binding protein highly enriched at synapses where it represses the translation of specific mRNAs. Loss of FMRP leads to FXS – the most common form of inherited intellectual disability and autism. Red asterisks symbolize the mutations discussed in the current thesis. Abbreviations: ERK – extracellular signal-regulated kinase; FMRP – fragile X mental retardation proten; FXS – fragile X syndrome; GpI mGluR – Group I metabotropic glutamate receptor; mTORC1 – mammalian target of rapamycin-raptor complex; NMDAR – N-methyl-D-aspartate receptor; TSC – tuberous sclerosis complex. NF1 – Neurofibromatosis 1; TrkB – tropomyosin receptor kinase B for neurotrophins. See text for details and further abbreviations.

2.2 ERK and mTORC1 in local translation regulation

Regulation of the availability and activity of the mRNA cap-binding factor eIF4E represents the principal effector mechanism through which ERK and mTORC1 pathways modulate local protein synthesis. Availability of eIF4E is critical for the assembly of the eIF4F complex and thus the ribosome recruitment to the mRNA. 4E-BPs sequester eIF4E, hinder eIF4F complex formation, and suppress the cap-dependent translation *in vitro* and *in vivo*. The 4E-BPs and eukaryotic factor eIF4G share a canonical motif (YXXXXLΦ; X stands for any amino acid and Φ for a hydrodrophic residue) through which they compete for binding to and interact with the cap-binding factor eIF4E. The phosphorylation state

of 4E-BPs defines their affinity for eIF4E and thus the availability of this eukaryotic factor. Specifically, hyperphosphorylation of 4E-BPs strongly weakens their binding affinity for eIF4E which subsequently enable the latter to interact with the eIF4G protein and assemble the eIF4F complex. mTORC1 is the primary kinase that phosphorylates 4E-BPs. Pharmacological inhibition of mTORC1 activity in hippocampus attenuates 4E-BP phosphorylation and translation-dependent translation impairing long-lasting potentiation and BDNF-mediated LTP. In addition, mTORC1 signaling pathway facilitates eIF4F complex formation and translation initiation by phosphorylating and activating p70 ribosomal protein S6 kinase 1 (S6K1). In turn, S6K1 phosphorylates downstream targets, such as the ribosomal protein S6 (component of the 40S ribosomal subunit) and the eukaryotic factor eIF4B (Figure 3). Notably, EIF4B phosphorylation potentiates the RNA-helicase activity of eIF4A and stimulates eIF4F complex assembly [18].

The ERK signaling cascade can additionally modulate the phosphorylation state of 4E-BPs and eIF4B, and facilitate translation initiation [56]. Specifically, ERK phosphorylates and activates a different subfamily of S6K, namely the p90 S6K (RSK, also known as MAPK-activated protein kinase 1). In turn, RSK recruits and activates the PI3K-dependent kinase (PDK) [30] which lies upstream of the activation of the serine/threonine kinase Akt. Akt can directly phosphorylate and activate mTORC1 or indirectly through inhibiting tuberous sclerosis complex (TSC1/2) [18]. TSC2 subunit functions as GAP protein against the small GTPase Ras homolog enriched in brain (Rheb). Akt-mediated TSC2 phosphorylation suppresses its GAP activity, promotes Rheb activation, and subsequently activates mTORC1 (Figure 3) [18]. In addition, ERK or RSK can directly phosphorylate TSC2 subunit and induce mTORC1 activation (Figure 3) [75, 97]. This ERK signaling pathway to mTORC1 appears to be particularly important for translation regulation linked to hippocampal L-LTP [56]. Curiously, the ERK signaling pathway modulates phosphorylation state and, in turn, the activity of the cap-binding factor eIF4E. Particularly, MAPK-interacting kinase 1/2 (Mnk1/2) is a downstream target of ERK signaling that selectively phosphorylates eIF4E at a single serine site [18]. Phoshorylated eIF4E exhibits four-fold lower affinity for the 5' cap structure which correlates with translation suppression of bulk mRNAs [103, 129]. Nevertheless, previous studies have implicated ERK-dependent eIF4E phosphorylation in hippocampal plasticity and memory formation [6, 56]. For example, mGluR-LTD induction triggers eIF4E phosphorylation via ERK-dependent activation of Mnk1. Conversely, mGluR-LTD displays a rapid requirement for local protein synthesis. Thus, these findings suggest a regulatory mechanism in which the ERK signaling cascade may function as a cellular switch to differentially modulate translation of subset of mRNAs under certain conditions [34]. I will return to this point in a later section.

Although initiation usually constitutes the rate-limiting event in the translation process, local protein synthesis regulation can also occur in the elongation phase. Peptide chain elongation is a highly consuming process of which regulation relies on the phosphorylation of the eEF2 factor by the eEF2 kinase (eEF2K) [53]. eEF2K is a Ca^{2+}/calmodulin-dependent kinase activated in response to NMDAR and mGluR stimulation [53, 102]. It phosphorylates and inhibits the activity of the elongation factor eEF2. In turn, eEF2 phosphorylation attenuates the translation of the bulk of mRNAs. Conversely, S6K can phosphorylate and inhibit eEF2K activity to reverse translation inactivation [53]. Taken together, these findings propose an attractive model for how synapses establish translation specificity upon activity; calcium influx would repress local protein synthesis at all synapses upon stimulation. However, parallel activation mTORC1 could reverse this effect at specific synapses [18]. Paradoxically, eEF2 phosphorylation correlates to enhanced synthesis of a subset of proteins. While the underlying molecular mechanism remains elusive, current hypothesis suggests that the

inhibition of elongation releases rate-limiting initiation factors (*i.e.,* eIF4F) which, in turn, facilitates the initiation of poorly translated mRNAs [18, 35, 87, 102]. Recent findings implicate elongation inhibition in the translation regulation of specific transcripts critical for mGluR-induced LTD. Specifically, neuronal eEF2K deletion impairs LTD upon mGluR stimulation and local synthesis of Arc or MAP1B *in vivo* and *in vitro*, respectively [23, 87]. I will return to these proteins later in this thesis.

3 ERK pathway in the autistic synapse

An impressive array of human genetic studies, including high-throughput screening and exome sequencing of parent-child trios has correlated functional genetic variations in multiple components of the ERK pathway, *e.g.,* Ras-associated proteins and ERK1/2 kinase, with ASD [38, 55, 66, 93, 123]. *In vivo*, these mutations perturb cognitive functioning and adaptive behavior plasticity [37, 57, 63]. Furthermore, aberrant ERK signaling underlies the pathogenesis of neurological syndromes symptomatically overlapping with ASD [72, 104, 125]. It additionally contributes to the synaptic plasticity defects in Fragile X syndrome – a distinct syndromic form of autism – of which the causative gene product interferes with local protein synthesis [84, 85]. Below I discuss recent insights into the defects of these molecules (summarized in Figure 3) with respect to synapse modification and local translation.

3.1 Aberrant ERK signaling in syndromic autism

Fragile X Syndrome (FXS). FXS is among the most prevalent causes of inherited intellectual disability and autism in humans. Epidemiological data estimates that it impacts one per $4,000$ males and one per $8,000$ females of which $15-30\%$ entirely meet the diagnostic criteria of ASD while approximately 5% of autistic children suffer from FXS. It results in a spectrum of cognitive impairments and neuropsychiatric symptoms that include learning and memory deficits, attention deficit and hyperactivity, social anxiety, mood liability, (self-) aggressive behaviors, motor in-coordination, and seizures. Patients with FXS may additionally exhibit a physical phenotype characterized by elongated forehead, large or protruding ears, macroorchidism (in males), and hyperflexible joints. An abnormal trinucleotide (CGG) repeat expansion which triggers hypermethylation and transcriptional silencing of the *Fmr1* gene typically underlies FXS etiology. *Fmr1* gene is located on the X chromosome (one of the two sex chromosomes) and encodes the fragile X mental retardation protein (FMRP). Variations in CGG repeat size and the subsequent fluctuations in FMRP expression levels along with germline mosaicism and X inactivation in females define syndrome phenotypic severity and clinical outcome [83]. FMRP is an mRNA-binding protein highly enriched at synapses where it negatively regulates the protein synthesis of specific transcripts. Specifically, it functions to repress initiation or stall ribosome movement along the mRNA stands during elongation, though the precise mechanisms are poorly understood [7, 10].

Fmr1 knockout (KO) mice – a validated animal model for FXS [64], exhibit abnormally high levels of protein synthesis [25, 84] and exaggerated LTD upon Gp1 mGluR stimulation [43], suggesting that under normal conditions FMRP serves as 'brake' on mGluR-stimulated local mRNA translation (see also reviews; [7, 10]). In addition, FMRP loss *in vivo* manifests a decreased surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and an overabundance of long, thin, immature spines [7]. Interestingly, pharmacological and genetic inhibition of mGluR5 signaling has restored excessive basal protein synthesis, and rescued synaptic, spine, epileptogenic, and behavioral defects in *Fmr1* KO mice (for review of the studies and phenotypes corrected, see; [10]). Collectively, these findings demonstrate that excessive protein synthesis downstream of mGluR5 activation forms the pathogenic core of FXS ('mGLuR theory' [9]). Given that the FMRP regulates approximately 4% of the mRNAs in mammalian brain [7], a logical step is to identify the pathogenic proteins. Among the diverse FMRP targets, Arc and MAP1B have recently emerged as the major 'LTD' candidates [7, 10, 21]. Arc is induced as immediate early gene upon synaptic stimulation. Once encoded, its mRNA rapidly traffics to and accumulates at the activated synapses where FMRP regulates its translation. This protein functions to regulate AMPAR endocytosis through its interaction with the proteins of

the endocytic system, endophilin and dynamin [13]. mGluR5 stimulation triggers its rapid translation while the Arc-mediated AMPAR internalization represents the crucial event for mGluR-LTD [122, 87, 34]. MAP1B is another critical protein for mGluR-LTD which stabilizes the internalized AMPARs [23]. Biochemical analysis of isolated synapsoneurosomes from the *Fmr1* mice have revealed local excessive synthesis of Arc and MAP1B [73, 127], which seems to underlie the defective AMPAR trafficking. Subsequent deficient surface content in these receptors exaggerates mGluR-LTD in *Fmr1* KO hippocamus [7]. Additionally, Arc and MAP1B regulate polymerization and stability of actin cytoskeleton, suggesting that they may further contribute to spine pathology in FXS [21].

In the context of translation regulation, ERK and mTORC1 signaling cascades have been extensively studied in *Fmr1* KO hippocampus. Some reports suggest an aberrant ERK activity [41, 60] while others implicate mTOR dysregulation [42, 105] upon mGluR5 stimulation. However, none of these studies have directly correlated the aberrant ERK or mTOR signaling to the excessive local protein synthesis which represent the core pathogenic feature in FXS. Recently, Osterweil *et al.* propose that constitutive mGluR5-ERK signaling lies upstream of the excessive protein synthesis in FXS [84, 85]. Specifically, these researchers employed an *in vitro* assay to quantify protein synthesis in *Fmr1* KO hippocampus and under the same conditions that exaggerated mGluR- and translation-dependent LTD occurs *in vivo*. They have reported neither basal nor stimulated hyperactivation of ERK and mTOR pathways upon mGluR activity. Nevertheless, pharmacological inhibition of ERK1/2 normalizes protein synthesis rate to wild type (WT) value and eliminates audiogenic seizures in *Fmr1* KO model. In contrast, rapamycin treatment (mTORC1 inhibitor) fails to correct the aberrant protein synthesis and prevent epiloptogenic activity [84]. Collectively, these findings suggest that the excessive protein synthesis within *Fmr1* KO hippocampus arises from the hypersensitivity of the translation machinery to ERK signaling (not to mTORC1), basally activated by mGluR. These findings further highlight the central role of ERK pathway in translation regulation and epileptogenesis linked to FXS.

Neurofibromatosis type 1 (NF1). NF1 is a neurocutaneous single gene disorder with an estimated prevalence of one per 3500 individuals. Multiple complex cognitive impairments occur in a high incidence ($40-60\%$ of the cases) and include learning disabilities, attention deficit disorder, autistic features, executive function deficits, and motor coordination problems, as previously reviewed [106, 112]. Cutaneous symptoms involve skin pigmentation, skeletal abnormalities, optic gliomas, and an increased risk for a rare leukemia form and malignant nerve sheath tumor [29]. NF1 arises from inherited or spontaneous (*de novo*) loss-of-function mutations in *Nf1* gene which is located on the chromosome 17q and encodes the neurofibromin protein. Neurofibromin is a Ras-GAP protein that negatively regulates Ras-ERK signaling [106]. Protein loss results in constitutive activation of the Ras-ERK pathway [72] impairing LTP and learning *in vivo* [17, 108]. Particularly, mice carrying an *Nf1* heterozygous null mutation ($Nf1^{+/-}$) exhibit spatial learning and memory impairments, and deficits in contextual fear memory consolidation and attention [17, 108].

In $Nf1^{+/-}$ hippocampus, enhanced intraneuronal ERK signaling increases the activity-dependent release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Increased inhibitory currents in CA1, in turn, prevent sufficient depolarization of NMDARs, perturb the balance between excitation and inhibition within hippocampal networks, and ultimately impair LTP during learning [17, 72]. A recent DNA microarray analysis has identified disrupted expression of genes encoding proteins which regulate synaptic vesicle trafficking and recycling (*e.g.,* dynamins, Rabs and synaptostagmins), glutamate receptors (mGluR5, AMPAR4, and NMDAR1), synapse structural proteins (such as neurexin 1, integrin β6 or β7,

and NCAM1), and signaling molecules (such as αCAMKII) in *Nf1*$^{+/-}$ hippocampus [86]. For instance, NF1 deficiency *in vivo* decreases NMDAR1 expression whereas it up-regulates AMPAR4 and mGluR5 expression [86]. Defects in these molecules along with the down-regulated expression of Rab proteins involved in synaptic vesicle recycling may account for the disrupted synaptic transmission noted in the mice.

Nevertheless, excitatory synapses also express *Nf1* [45] while long-lasting synapse modifications typically require local mRNA translation [12, 18, 56]. Thus, it is interesting to consider that interfering with the mRNA levels and local translation regulation of proteins crucial for synapse plasticity and structure may cause changes synaptic connectivity and compromise network performance. This, in turn, may result in altered excitation/inhibition ratio underlying cognitive impairments in NF1. Indeed, excessive protein synthesis in FXS manifests an imbalance between excitation and inhibition in CA1 hippocampal area and cortex with hyperexcitation favored, as previously reviewed [31]. In addition, *Nf1*$^{+/-}$ hippocampus display decreased αCAMKII expression [86]. Mice in which αCAMKII mRNA restricted to the soma and proximal dendrites exhibit impaired L-LTP and memory deficits in fear conditioning and spatial tasks. This finding indicates that the dendritic localization and local translation of this signaling molecule contributes to hippocampal function and plasticity [80]. Improved understanding of the molecular defects following loss of function of *Nf1* will provide mechanistic insights into syndrome pathology. Furthermore, *Nf1* is a target of FMRP protein potentially [22] suggesting a clinical relevance between NF1 and FXS pathological pathways to autism. Thus, an unresolved question is whether disrupted mGluR5-ERK signaling and aberrant protein synthesis at excitatory synapses underlies NF1 pathogenesis.

Nooman Syndrome (NS). NS is an autosomal dominant genetically heterogeneous disorder [117] with an estimated prevalence of one per $1,000$ to $2,000$ live births [112]. Clinical features include craniofacial dysmorphias (including a webbed neck and a flat nose bridge), short stature, congenital cardiac defects (typically pulmonary valve stenosis and hypertrophic cardiomyopathy), and skeletal deformations (such as pectus excavatum and carinatum) [117]. Approximately, one third of the patients exhibits mental retardation and learning disabilities [117] while co-morbid autistic traits occur in NS [32]. This disorder primarily arises from gain-of-function germline mutations in *Ptpn11* gene which is located on the chromosome 12q24.1 and encodes the protein tyrosine phosphatase SHP2. SHP2 functions to positively regulate Ras-ERK signaling in response to growth factor receptor and Trk stimulation [104]. Genetic studies have additionally identified mutations in *Sos1* (encoding the Ras-GEF protein, SOS1), *Kras* (member of Ras family), and *Raf1* genes causal of NS. Collectively, these mutations seem to promote Ras-ERK hyperactivity impairing learning abilities [2, 104, 117], though the mechanisms remain currently unknown. Of note, mutations in transducers upstream of Ras appear to correlate less frequently with severe cognitive impairments compared to mutations in downstream components of the pathway [117].

Overexpression of the constitutively active Raf1 in primary cortical neuron cultures results in a gain of function of ERK signaling. Ras-ERK hyperactivation interferes with dendritic spine morphogenesis and maturation *in vitro*. Particularly, it dramatically decreases the total number of dendritic spines, as well as the number of the mature mushroom-type spines [125]. Although I cannot rule out the contribution of small Ras GTPases in the regulation of spine morphogenesis pathways, local protein synthesis profoundly determines spine morphology and remodeling, as previously reviewed [68, 113]. Thus, it is tempting to hypothesize that the aberrant ERK signaling and the subsequent local translation dysregulation may account for the spine defects in these neurons. In support of it, impaired

translational regulation results in immature spines in FXS [7]. Future work should address how NS-associated mutations, particularly in *Raf1*, potentially disturb local translation rate and synthesis of specific proteins crucial for spine dynamics. In addition, spine morphology defines synapse function [110]; hence, it would be of interest to examine how the spine defects noted in neurons overexpressing the constitutively active Raf1 impact synapse plasticity. Such an approach will clarify molecular mechanisms underlying syndrome's cognitive and psychiatric profile and further provide mechanistic links to autism spectrum.

3.2 More genetic hints for the involvement of the ERK pathway in autism

MAPK3. Comparative genomic hybridization studies have correlated rare copy number variants (deletion and duplication) of a $\sim 598\,\mathrm{kb}$ region on the chromosome 16p.11.2 with an increased ASD risk [66, 107, 123]. Patients with 16p.11.2 copy number variants exhibit developmental delay in language, intellectual disability, and core autistic traits [107]. Deletion of this region, though, is more penetrant for ASD than the duplication, while it further correlates with macrocephaly [40, 107]. Macrocephaly describes an abnormally large head size which results from an excessive brain growth in early infancy and occurs with high incidence in ASDs [19]. Interestingly, this locus encompasses the MAPK3 gene which encodes the ERK1 kinase [40, 107, 123]. ERK1 homozygous KO mice ($\mathrm{ERK1}^{-/-}$) manifest enhanced stratium-dependent long-term memory which correlates with LTP facilitation in nucleus accumbens [79]. At a cellular level, ERK1 ablation results in the enhancement of the ERK2 activity which, in turn, accounts for the plasticity and cognitive phenotypes in $\mathrm{ERK1}^{-/-}$ mice [79]. In normal conditions, ERK1 functions as "a built-in partial agonist" to tightly regulate ERK2 activation [48]. However, in the ERK1 absence, enhanced interaction of the ERK2 isoform with its upstream activator MEK derepresses its activity [79]. In contrast, $\mathrm{ERK2}^{-/-}$ mice were not viable; they died at embryonic state. Nevertheless, mice carrying a mutation that causes partial loss of ERK2 ($\sim 20-40\%$) were viable and grew normally. $\mathrm{ERK2}^{-/-}$ mice manifest hippocampus-dependent spatial learning impairments and associative long-term memory deficits while no compensatory increase in the ERK1 activity has been detected in their hippocampus and cerebellum [101]. Collectively, these findings indicate a prominent role of the ERK2 in synapse plasticity and cognitive function. Nonetheless, data from $\mathrm{ERK1}^{-/-}$ seems further to establish a causal link between the ERK1-dependent regulation of ERK2 and LTP changes, suggesting that the balanced activity of ERK isoforms is necessary for synapse plasticity underlying memory at least in some brain areas [79]. Consistent to these findings, loss of one copy of ERK1 due to 16p.11.2. deletion results in the ERK2 enhancement. Hence, future studies should address the downstream effects of this increased ERK2 activity and its relative link to ASD phenotypes.

HRAS. A recent high-throughput screening study has correlated single nucleotide variants in *HRAS* gene with idiopathic ASD [55]. Gain-of-function *HRAS* mutations underlie Costello syndrome (CS) which is a rare monogenic disorder characterized by delayed physical growth, cognitive impairments, facial dysmorphism, cardiac defects, and autistic-like behaviors [2, 117]. *In vivo* studies indicate that gain-of-function *HRAS* mutations may result in ERK hyperactvity which, in turn, may underlie neurobehavioral and cognitive phenotypes in CS. However, the precise molecular alterations and mechanisms remain elusive [121]. Of note, it is also currently unknown how the *HRAS* variants identified in ASD impact protein function [55]. Nevertheless, these findings together suggest that rare genetic variations in the Ras-ERK pathway may increase risk for idiopathic ASD.

SynGAP1. Human genetic screening studies in ASD and sporadic (non-syndromic) intellectual disability have recently identified *de novo* copy number variants (deletion) and a pre-

mature "stop" codon sequence in *SynGAP1* [38, 93]. These mutations truncate the encoding protein and abolish functional domains [38]. *SynGAP1* encodes the synaptic GAP protein (SynGAP) which promotes GTP hydrolysis and negatively regulates Ras enzymatic activity [63]. This protein is brain specific and highly enriched at the excitatory synapses [15, 58]. There, it binds to the PDZ domains of PSD-95 and SAP102 proteins – components of the NMDAR complex [58], and regulates signal transduction pathways underlying AMPAR trafficking during NMDAR-mediated plasticity [59, 65, 98].

SynGAP1 haploinsufficiency selectively defects hippocampal LTP [57, 63] and results in cognitive impairments and social deviant behavior [37] *in vivo*. Particularly, heterozygous KO mice of *SynGAP1* ($\text{SynGAP}^{+/-}$) display persistent hyperactivity, startle hyperresponsiveness, sensimotor gating deficits, impaired spatial learning and memory encoding, propensity to social isolation, and short-term social memory impairments [37]. In $\text{SynGAP}^{+/-}$ hippocampus, enhanced basal ERK activity occurs [63]. However, it remains controversial whether the aberrant ERK signaling underlies LTP deficits in these mice. Subsequent work implicates p_{38}MAPK signaling, in addition to ERK [98]. Better understanding of NMDAR-SynGAP downstream signaling will provide mechanistic insights into sporadic and ASD-linked intellectual disability. Intriguingly, genetic studies have shown that encoded *SynGAP1* mRNA is another FMRP target [22] while gene mutations possibly converge into the mGluR-ERK signaling which controls translation in idiopathic autism [55]. Current literature solely provides evidence on its role in NMDAR-mediated plasticity, as discussed above. Thus, an unresolved question is whether and how SynGAP truncations impact synapse depression and local protein synthesis.

4 ERK and mTORC1 may regulate the translation of separate pools of mRNAs in dendrites

Despite the identification of mutations within the ERK pathway and recent progress in our understanding of local translation regulation mechanisms, major gaps in our knowledge remain about how the dysfunction of this pathway interferes with activity-dependent protein synthesis to disturb synapse plasticity and, in turn, to result in autistic manifestations. The paradigm of FXS has been illuminating in how pathogenic excessive protein synthesis and altered synaptic signaling defect synapse pathophysiology which subsequently contributes to ASD pathogenesis. Nevertheless, disentangling ERK and mTORC1 contribution in mGluR-dependent protein synthesis has not been clear. Tuberous sclerosis (TS) is another main syndromic ASD of which pathogenic mechanism involves activity-modulated mRNA translation [26]. TS is a monogenic disorder characterized by widespread growth of benign tumors in multiple systems, high penetrance of ASD and intellectual disability. It arises from autosomal dominant loss-of-function mutations of *Tsc1* and *Tsc2* genes which encode hamartin (TSC1) and tuberin (TSC2), respectively [26, 128]. TSC1 and TSC2 form a complex that functions as signaling node to modulate mTOR activation which acts as a positive regulator of local protein synthesis in neurons [18], as discussed earlier. Deficiency of either TSC1 or TSC2 results in learning impairments and synaptic plasticity defects lying downstream of excessive mTORC1 signaling *in vivo* [4]. These findings motivate us to explore whether TSC-linked synapsopathy will provide further insights into ERK involvement in ASD.

In the CA1 area of hippocampus of mice carrying heterozygous inactivating mutations of *Tsc1* ($Tsc1^{+/-}$) or *Tsc2* ($Tsc2^{+/-}$), excessive mTORC1 activation, but not ERK, impairs basal protein synthesis resulting in deficient mGluR-LTD [4, 8]. These findings suggest that aberrant mTORC1 signaling suppresses the translation of mRNAs required for LTD. Indeed, immunoblotting along with metabolic labeling has revealed decreased Arc synthesis in $Tsc2^{+/-}$ hippocampus [4]. A possible interpretation of these biochemical and synaptic defects is that mGluR stimulation triggers activation of S6K1 activation downstream of mTORC1 signaling. Subsequently, S6K1 phosphorylates FMRP which, in turn, associates with stalled ribosomes and represses the translation of its mRNA targets [82, 7]. However, this explanation is inconsistent with the observation that genetic cross of $Tsc2^{+/-}$ and *Fmr1* KO normalizes LTD to wild type (WT) magnitude and rescues cognitive impairments *in vivo*. Furthermore, in ($Tsc2^{+/-}$) mice, allosteric augmentation of mGluR signaling restored biochemical and synaptic defects, and rescued behavioral and cognitive deficits [4]. Collectively, these findings indicate that FXS and TS show mirror symmetrical alterations in activity-dependent protein synthesis underlying synapse depression and have beneficial responses to treatments that modulate mGluR5 in opposite directions [4]. Considering the ERK involvement in FXS, but not in TS, along with the above findings, Bhakar *et al.* have hypothesized that ERK and FMRP favor the translation of a subset of mRNAs that belong to the same pool (termed as Pool I). A second pool (Pool II) regulated by mTORC1 competes with the Pool I for access to the local protein synthesis machinery (Figure 4) [10] (for the origin of this hypothesis, see also; [9]).

Consistent to this hypothesis, insights into local protein synthesis regulation (discussed on section 2) reveal that translation inhibition of bulk of mRNAs facilitates the synthesis of specific proteins, including FMRP targets crucial for synapse depression. A case in point is the translation regulation at the elongation phase. mGluR stimulation triggers eEF2K activity which phosphorylates the elongation factor eEF2 and, in turn, promotes the translation of Pool I mRNAs (including MAP1B and Arc) [87]. Conversely, S6K kinase activation downstream of mTORC1 signaling phosphorylates eEF2K to hamper its activity [18, 95]

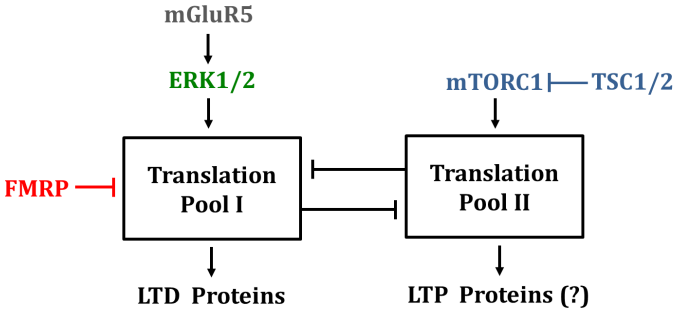

Figure 4: **"Two-pool hypothesis".** Figure has been adapted and reprinted by Bhakar *et al.* [10]. FXS and TS mirror each other in mGluR5-stimulated protein synthesis underlying synapse depression [4]. These opposing responses propose that mGlu5 stimulation triggers the translation of a subset of mRNAs (Pool I) in an ERK- and FMRP-dependent way. Pool II regulated by mTORC1 competes with Pool I for acccess in the translation machinery. Current evidence suggests that Pool I comprises mRNAs crucial for LTD establishment (LTD proteins) whereas Pool II includes mRNAs crucial for LTP establishment (LTP proteins) [10]. See text for rationale behind that model. Abbreviations: ERK – extracellular signal-regulated kinase; FMRP – fragile X mental retardation proten; FXS – fragile X syndrome; LTP – long-term potentiation; LTD – long-term depression; mGluR – metabotropic glutamate receptor; mTORC1 – mammalian target of rapamycin-raptor complex; TS – tuberous sclerosis; TSC – tuberous sclerosis complex.

which, in turn, inhibits Pool I and stimulates Pool II [10]. Furthermore, it is interesting to consider that translation regulation of the FMRP targets, MAP1B and Arc, may also occur at initiation phase in an ERK-dependent way. Specifically, an experimental model suggests that mGluR5 stimulation induces Mnk1 activation via ERK cascade. Mnk1 phosphorylates eIF4E and inhibits general translation [18, 34]. Nevertheless, this potentiates the release of a non-canonical eIF4E binding protein, namely, cytoplasmic FMRP interacting protein 1 (CYFIP1, also termed as Sra-1). In the brain, CYFIP1 forms a complex with FMRP which binds to eIF4E at 3' end and to poly-A-binding tail at 5'end of Arc and MAP1B mRNAs to repress their translation (eIF4E-CYFIP1-FMRP). Upon mGluR5 stimulation, CYFIP1 dissociation facilitates eIF4E-eIF4G interaction and thus translation of these mRNAs (Pool I) at synapses [7, 81]. However, whether and how mTORC1 can compete this potent mechanism remains elusive.

A key question arising refers to the identity of Pool II. Recently, Ehninger *et al.* reported that excessive mTOR activity results in abnormal persistence of hippocampal L-LTP disrupting cognition and behavior in *Tsc2*$^{+/-}$ mice. Interestingly, pharmacological treatment of *Tsc2*$^{+/-}$ mice with Lovastatin did not rescue synaptic and cognitive defects [27]. Conversely, the same treatment restores excessive protein synthesis and normalizes mGluR5-LTD to WT magnitude in *Fmr1* KO mice [85]. Lovastatin is an inhibitor of 3-hydroxy-3methylglutaryl-coenzyme A reductase (HMG-CoA reductase) used in treatment of hypercholesterolemia both in children and adults [24]. It can interferes with Ras-GTPase activity and inhibits downstream activation of ERK cascade [62]. Collectively, these findings along with deficient LTD in *Tsc2*$^{+/-}$ mice suggest that mTORC1-regulated Pool II may include mRNAs crucial for LTP. In contrast, Pool I potentially constitutes mRNAs crucial for LTD regulated by ERK, FMRP and mGluR signaling (Figure 4). Thus, we can hypothesize that derepression of Pool I results in exaggerated LTD in FXS while derepression leads to enhanced LTP in TS [10].

Such a simplified model could be particular useful for gaining insights into the elements and mechanisms of specific forms of plasticity. For example, proteomic comparison of

Tsc2$^{+/-}$ and *Fmr1* KO hippocampus may identify the elusive dendritic transcripts implicated in LTP and LTD. Nevertheless, gaps in our knowledge of synaptic signaling currently poses limitations in validation of this model. Particularly, many of the ERK components mutated in autism are FMRP targets and, thereby, potentially overlap into Pool I pathway. However, loss-of-function mutations in *Nf1* result in LTP impairments [17, 72]. Neurofibromin can also modulate PI(3)K - mTORC1 signaling [106]. Thus, it would be of interest to clarify whether NF1-linked synaptic defects reflect aberrant mTORC1 signaling and thereby repression of Pool II. Furthermore, a recent study has reported that pharmacological inhibition of mTORC1 stimulates translation of the mRNA encoding the $Kv4.2$ subunit of voltage-gated K^+ channel – an FMRP-regulated dendritic transcript crucial for synapse plasticity – and thus potentially derepresses Pool I. However, this occurs via dephosphorylation of FMRP in response to NMDAR stimulation [70]. Another study has implicated ERK dysfunction in hippocampal plasticity defects and epileptogenic activity of adult *Tsc2*$^{+/-}$ mice [94]. Noteworthy is that a hallmark feature of TS is the growth of benign tumors in the brain. Severity of seizure disorder, which affects up to 90% of TS cases, occurs in function of tumor growth rate and extent in cerebral cortex [67] and rather correlates to synapse dysfunction signaling underlying autistic manifestations [27]. Altogether, these findings point out the intricacy of intracellular signaling at synapses. Future studies should clarify how activity couples to mTORC1 and ERK signaling, though clarity requires consistency in type of synapses selected, animal age, and in sample preparation procedures.

5 Possible Convergence of other ASDs into the "two-pool hypothesis" and translation regulation

Notwithstanding the caveats, the model discussed previously could be particularly useful for identifying possible convergent points with other ASDs. Angelman's syndrome (AS) is a neurodevelopmental disorder characterized by intellectual disability, severe language deficits, motor incoordination, seizures, and high prevalence of autism. It primarily arises from maternal deletions of the chromosome region 15q11-13 which encompasses *Ub3A* gene. The latter encodes the ubiquitin E3 ligase protein (UB3A) which mediates protein ubiquitination and degradation [26, 128]. Arc protein is an identified substrate of UB3A [36], suggesting a convergent point among AS, FXS, and TS in ASD etiology [26, 54, 128]. Interestingly, UB3A deficiency increases Arc expression levels and decreases AMPAR cell-surface content, impairing glutamatergic transmission *in vivo* [36]. This finding suggests that aberrant ERK-mGluR signaling may contribute to ASD phenotype in AS, though current literature provides evidence solely for impairments in NMDAR-stimulated plasticity forms [100, 126]. Thus, it would be of interest to examine whether UB3A loss alters ERK activity downstream of mGluR5 stimulation and whether inhibitors of this signaling pathway can correct phenotypes in AS animal models.

The synaptic proteins neuroligin 3 (NLGN3) and neurexin 1 (NRXN1) are FMRP targets [22]. NLGNs and NRXNs are cell adhesion molecules at the post- and pre-synaptic compartment, respectively, where they mediate the trans-synaptic interaction upon neuronal activity. Multiple mutations in these molecules account for rare cases of idiopathic autism [26, 128]. Notably, recent evidence suggests that NLGNs and NRXNs regulate the balance between excitatory and inhibitory neurotransmission and thus activity-dependent synapse specificity and maturation [16, 28, 46]. Knock-in mice expressing an ASD-linked missense mutation in NGLN3 display exicitation-to-inhibition (E/I) imbalances with the inhibition favored [115]. Similarly, an ASD-linked deletion of NRXN1α selectively impairs miniature excitatory postsynaptic current (EPSC) frequency and evoked postsynaptic potential decreasing E/I balance *in vivo* [28]. Aberrant protein synthesis could similarly disrupt E/I ratio by altering net strengthening or weakening of excitatory relative to inhibitory synapses [54]. Indeed, excessive protein synthesis in FXS engenders a shift in E/I ratio towards the hyperexcitation in CA1 hippocampal area and cortex, as previously reviewed [31]. In addition, enhanced activity of the initiation factor eIF4E perturbs E/I balance in hippocampus and gives rise to the development of ASD behavioral phenotype *in vivo* [33]. Specifically, mice with the gene for 4E-BP2 protein knocked out (*Eif4ebp2* KO mice) exhibit social interaction deficits and repetitive self-grooming behavior while they bury significantly more marbles than WT littermates. Of note, marble burying test evaluates repetitive behaviors that can become compulsive in mice while pharmacological inhibition of eIF4E rescues autistic-like behavioral phenotypes noted in *Eif4ebp2* KO mice [33]. Interestingly, *Eif4ebp2* KO hippocampus displays enhanced translation rate and protein levels of NGLNs (including NGLN3) whereas mRNA levels are similar to WT, thus ruling out transcription effects. Increased E/I ratio accompanies aberrant NGLNs protein synthesis while increase mTORC1 activation seems to underlie the synaptic defects and behavioral phenotypes in *Eif4ebp2* KO mice [33]. Collectively, these findings establish a causal link between mTORC1-dependent translation regulation of NGLNs and neural network performance in ASD pathogenesis. Although the mechanisms through which mutations in NGLNs and NRXNs disturb E/I balance and result in ASD remain currently unknown, it would be of interest to address how these mutations and the subsequent perturbed synaptic transmission impact mTOC1 activity, local translation protein synthesis, and plasticity at the postsynaptic compartment.

Phelan-McDermid syndrome (PMS) is a rare, chromosome microdeletion syndrome characterized by severe neonatal hypotonia (*i.e.,* poor muscle tone), intellectual disability, global developmental delay, severely delayed or absent speech, seizures, aggressive behavior, and high prevalence of autism. It arises from a microdeletion of the chromosome 22q13 which encompasses *SHANK3* gene encoding the SH3 and ankyrin domain-containing protein (Shank3) [90, 91]. Human genetic screening studies have additionally identified protein-truncating mutations and deletions in *SHANK3* in ASD and sporadic intellectual disability (for review of these studies, see; [128]). Altogether, these findings suggest that Shank3 deficits trigger major neurobehavioral and autism-spectrum related phenotypes. Shank3 is a multi-domain protein highly enriched in the PSD of the excitatory synapses. There, it functions as a core scaffold element that regulates the organization and stability of postsynaptic signaling complexes and further links PSD to the actin cytoskeleton [88, 128]. Interestingly, Shank3 knockdown selectively impairs LTD elicited by mGluR5 stimulation and ERK1/2 activation in hippocampal neuron cultures [119]. Although it is tempting to speculate that the reduced mGluR-ERK signaling and the subsequent repression of mRNAs crucial for LTD may underlie the plasticity defects noted post suppression of Shank3 expression, allosteric augmentation of mGluR signaling reverses the deficits in LTD and ERK activation in these neurons – a finding that reminds data from $Tsc2^{+/-}$ mice (discussed on section 4, pp.15). In addition, the proline-rich domain of Shank3 interacts with Homer [128] which forms the central component in coupling mGlu5 to the downstream PI3K - mTORC1 signaling (Figure 3) [74]. Specifically, upon mGluR5 stimulation, Homer recruits and binds to the GTPase protein PI3K-enhancer (PIKE) which, in turn, activates PI3K [74]. PI3K is the principal effector of the mTORC1 signaling cascade [18, 54]. Protein levels of Homer remain unchanged in synaptosomes isolated from neurons knocked down for Shank3. This finding raises questions about the activity and potent contribution of the mTORC1 in LTD defects in these neurons. Thus, a better understanding of the downstream effects of Shank3 suppression in mGluR signaling will clarify these points. Conversely, heterozygous Shank3 KO mice ($SHANK3^{+/-}$) display impairments in basal synaptic transmission, LTP deficits, and unchanged paired-pulse low-frequency mGLuR-dependent LTD in CA1 hippocampal area [11]. Differences in sample preparation techniques (hippocampal neuron cultures *vs.* hippocampal slices), mGluR-LTD induction stimuli (pharmacological *vs.* paired-pulse low-frequency stimulation), and in Shank3 expression (suppression of Shank3 expression by $70 - 80\%$ in knockdown neurons *vs.* by 50% in $SHANK3^{+/-}$ KO mice) may account for the inconsistent findings between these two studies. Notwithstanding the caveats, these findings along with the requirement of both LTP and LTD for local protein synthesis suggest a causal link among Shank3 deficits, translation control, and synapse function in ASD etiology which requires further exploration.

6 Concluding remarks

Interest in translation has burgeoned the recent years. It is now appreciated that regulation of protein synthesis is crucial mechanism for synapse plasticity underlying learning and memory in the brain [18]. ERK pathway has emerged as key regulator of local translation while mutations in its elements give rise to synapse defects and cognitive impairments linked to autism. Furthermore, the paradigms of FXS and TS provide interesting insights into the ERK involvement in ASD etiology. Current evidence suggests that ERK signaling forms the critical pathway through which mGlu5 stimulates mRNA translation required for LTD [10, 56]. A future task is to validate this point; a promising approach is to address whether and how the mutations within ERK impact local translation generally and synthesis of specific proteins. Additionally, FXS and TS mirror each other [4] suggesting that ERK and mTORC1 regulate different subsets of mRNAs for distinct form of plasticity [9, 10]. Although future studies should address and validate this model, an important implication of it could refer to treatment strategy design. Instead of seeking for one pharmacological target, it would be more beneficial to understand first where a patient lies on the spectrum of synaptic function and signal transduction pathway, and then devise an appropriate therapy for ASD and other related psychiatric disorders.

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