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Review

Spatiotemporally resolved protein synthesis as a molecular framework for memory consolidation

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***De novo* protein synthesis is required for long-term memory consolidation. Dynamic regulation of protein synthesis occurs via a complex interplay of translation factors and modulators. Many components of the protein synthesis machinery have been targeted either pharmacologically or genetically to establish its requirement for memory. The combination of ligand/light-gating and genetic strategies, that is, chemogenetics and optogenetics, has begun to reveal the spatiotemporal resolution of protein synthesis in specific cell types during memory consolidation. This review summarizes current knowledge of the macroscopic and microscopic neural substrates for protein synthesis in memory consolidation. In addition, we highlight future directions for determining the localization and timing of *de novo* protein synthesis for memory consolidation with tools that permit unprecedented spatiotemporal precision.**

Highlights

Protein synthesis is dynamically regulated by coordinated action of numerous translational control molecules.

Pharmacological compounds have been used extensively to probe the requirement of *de novo* protein synthesis for memory consolidation; despite temporal precision these compounds lack cell type resolution.

Using pharmacology, long-term memories, including associative emotional memories and nonassociative procedural memories, have been shown to depend on *de novo* protein synthesis in discrete brain regions.

Genetic targeting of translation factors and other effectors has further established the effect of sustained protein synthesis disruption in memory consolidation.

New chemogenetic strategies combine the superior temporal resolution of pharmacology with the cell-type specificity afforded by genetic targeting and are beginning to reveal the cell type-specific requirements for *de novo* protein synthesis in memory consolidation.

Role of protein synthesis in memory consolidation

Memory is operationally defined as the capacity of an organism to encode, store, and retrieve information [1,2]. Understanding the biological basis of long-term memories is fundamental for deciphering animal cognition. A memory is molded out of an experience by integrating information about convergent multisensory inputs that represent the environment. Salience of an experience is internally represented as heightened sensory and emotional arousal [3] at the time of encoding, which can lead to transformation of the memory from a labile state into a stable long-term form in a process known as consolidation [4]. Even for salient experiences, the memory initially stays in a labile state sensitive to disruption if key intracellular signaling pathways and new protein synthesis (PS) are blocked [5–7]. In a laboratory setting, long-term memories are studied using a variety of paradigms such as classical and instrumental conditioning. Originally described by Ivan Pavlov [8], classical conditioning involves presenting a conditioned stimulus (CS) to the animal that is initially emotionally neutral that is explicitly paired with a motivationally salient unconditioned stimulus (US) to cause a conditioned response (CR) [9]. The CS can be in any of the sensory modalities including audition, olfaction, vision, and gustation, whereas the US is either a negative reinforcer inflicting pain or malaise, or a positive reinforcer that typically fulfils a homeostatic drive. Long-term aversive memories are formed in a single trial with pairing of a neutral sensory CS with an innately aversive US [10]. Subsequent presentation of the CS alone elicits a CR that, depending on the experimental context, can consist of a Pavlovian defensive response such as freezing or an instrumental defensive response such as active avoidance [11,12] or aversion [13]. Long-term memories can also be formed in non-Pavlovian instrumental conditioning such as inhibitory avoidance, where animals learn motor actions to obtain a positive outcome or avoid a negative reinforcer under uncued free-operant conditions [14].

A vast body of literature has shown that memory consolidation requires *de novo* PS in the brain in analogous structures across different species [2,7,15–18] (Figure 1). We are now gradually learning that coherent cell types defined by molecular identity and/or cellular activity are recruited during

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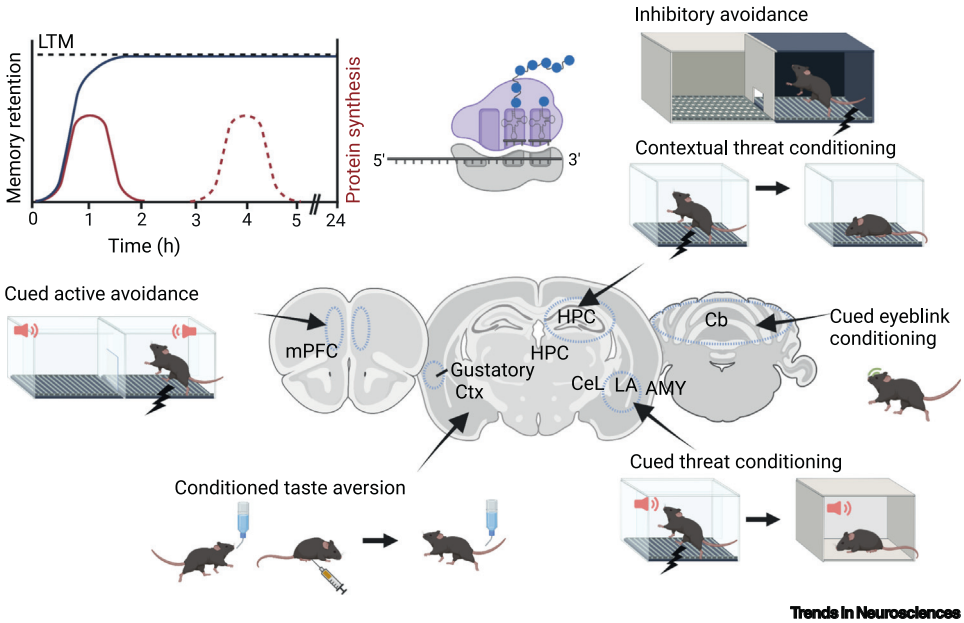

Figure 1. Memory paradigms sensitive to protein synthesis inhibition. Associative learning involves explicit pairing of a neutral sensory cue such as a specific tone, light stimulus, olfactory cue, or tastant (conditioned stimulus, or CS) with an unconditioned stimulus (US) with inherent negative valence such as footshock, lithium chloride (LiCl), or air puff for aversive US and food, water, or drug for appetitive US (not shown). After sufficient pairings, associative learning can lead to long-term memories (LTMs) that require one or multiple waves of protein synthesis depending on salience and frequency of training. Behavioral responses measured during LTM include freezing, inhibitory avoidance, active avoidance, escape, and threat discrimination for aversive memories. Focal protein synthesis inhibition in specific brain regions such as medial prefrontal cortex (mPFC), gustatory cortex (Ctx), hippocampus (HPC), lateral amygdala (LA), central amygdala (CeA), and cerebellum (Cb) have shown the requirement of protein synthesis in these brain regions for consolidation of various memory modules. Abbreviations: CeL, centrolateral amygdala; eIF, eukaryotic initiation factor.

memory formation, and that these specific cell types can store associative information crucial for naturalistic recall of memory. Burgeoning development of neurochemical sensors [19,20], light, and designer molecule-gated neural activity modulators [21–23] have advanced our knowledge of these cell types, as well as critical circuit components of various memory modules. However, we now are at the cross-roads of unraveling the causal relevance of *de novo* PS in specific cell types and circuit components with the recent development of genetically encoded and drug-inducible PS inhibitors [24,25] as well as methods to label and profile time-defined *de novo* proteomes [26,27].

Dynamic regulation of PS by endogenous molecules

The molecular machinery for PS involves dynamic ribosomes [28,29] consisting of ribosomal RNAs and small and large ribosomal subunits, whose function is to decode the nucleotide sequence of the mRNA and translate it into an amino acid primary structure by the catalysis of peptide bonds [30]. A multitude of protein factors transiently associate with ribosomes to coordinate the dynamics of PS [30], which takes place in three steps – initiation, elongation, and termination. The first two steps are tightly regulated (Figure 2). Translation initiation involves the anchoring of the ribosome at the initiation codon of an mRNA and is intricately regulated by over 25 proteins [31]. In particular, two key protein complexes are crucial for translation initiation – the ternary complex (TC) and the cap-binding complex also referred to as eukaryotic initiation factor (eIF)4F. The TC is formed by the interaction of the initiator methionyl-tRNA (Met-tRNA$_i^{Met}$) with eIF2 in the GTP-bound state and delivers the Met-tRNA$_i^{Met}$ to the small ribosomal subunit. After the resulting preinitiation complex binds an mRNA and scans to select a start codon for PS, eIF2·GTP is

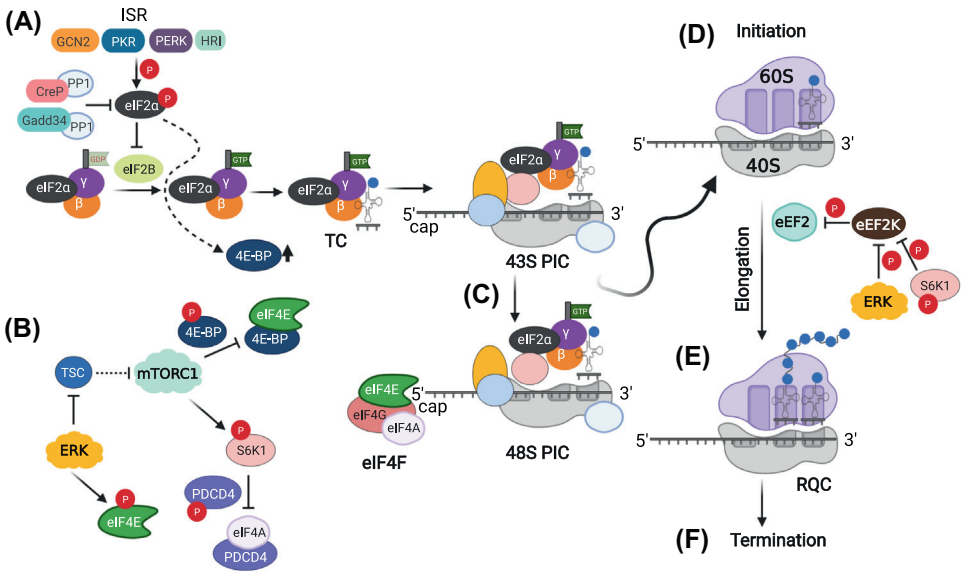

Figure 2. Protein synthesis requires three steps – initiation, elongation, and termination. (A) During the integrated stress response (ISR), eIF2α kinases (GCN2, PKR, PERK, and HRI) are activated and phosphorylate Ser51 of eIF2α, which converts it into an inhibitor of eIF2B, the guanine exchange factor (GEF) for eIF2. eIF2α is dephosphorylated by PP1 bound to either CreP or Gadd34 scaffolding proteins. The eIF2 ternary complex (TC) is formed with the binding of eIF2-GTP with the initiator methionyl-tRNA, which constitutes the 43S preinitiation complex (PIC) along with several other translation factors. (B) ERK and mammalian target of rapamycin complex I (mTORC1) are major intracellular signaling complexes closely associated with protein synthesis. ERK phosphorylates and inhibits TSC, the molecular brake on mTORC1. ERK also phosphorylates translation initiation factor eIF4E, which recognizes the cap in the 5′UTR of mRNAs. mTORC1 phosphorylates 4E-BP and promotes the formation of cap-binding complex eIF4F by releasing eIF4E from the inhibitory constraint of 4E-BP. mTORC1 also phosphorylates S6K1, which in turn phosphorylates and inhibits PDCD4, the molecule that sequesters eIF4A. (C) The assembly of 43S PIC and eIF4F into the 48S preinitiation complex (PIC) sets the stage for (D) the recruitment of the large ribosomal subunit to the mRNA marking the end of the initiation step. (E) Initiation is followed by elongation, which requires the catalytic activity of elongation factor eEF2 that is inhibited via phosphorylation by its kinase eEF2K. Both S6K1 and ERK phosphorylate and inhibit eEF2K, thus releasing the brake on eEF2. During elongation, the ribosome moves along the mRNA and peptide synthesis proceeds. (F) Termination occurs when the ribosome encounters a stop codon at which stage the peptide exits the ribosome. Abbreviations: eIF, eukaryotic initiation factor; GCN2, general control nonderepressible 2; HRI, heme-regulated inhibitor; PDCD4, programmed cell death 4; PERK, PKR-like ER kinase; PKR, protein kinase R; PP1, protein phosphatase 1; RQC, ribosome quality control; TSC, tuberous sclerosis complex.

hydrolyzed to eIF2·GDP, which is then released from the ribosome. To participate in another round of translation initiation, the GDP on eIF2 must be exchanged for GTP. During the cellular integrated stress response (ISR), the α subunit of eIF2 is phosphorylated on Ser51 by protein kinases including protein kinase R (PKR), PKR-like ER kinase (PERK), general control nonderepressible 2 (GCN2), and heme-regulated inhibitor (HRI), which are activated by specific stressors including viral infection, endoplasmic reticulum stress, amino acid deprivation, and heme depletion, respectively [32]. Recent studies have discovered new stress pathways for activating certain eIF2α kinases – for instance, GCN2 can be activated by ribosome stalling and HRI by proteosome inhibition [32,33]. Phosphorylated eIF2α is a potent inhibitor of eIF2B, a guanine exchange factor that converts inactive eIF2·GDP to active eIF2·GTP. Thus, phosphorylation of eIF2α stops the recycling of the TC and inhibits general translation, while simultaneously inducing translation of a subset of mRNAs harboring upstream open reading frames (uORFs) such as activating transcription factor 4 (ATF4). By contrast, dephosphorylation of eIF2α by protein phosphatase 1 (PP1) bound to a regulatory subunit, which can be either the constitutive repressor of eIF2α phosphorylation (CReP) or growth arrest and DNA damage-inducible protein 34 (GADD34), promotes general translation [24].

The cap-binding complex eIF4F comprises three proteins: eIF4E, the cap recognizing protein; eIF4A, an RNA helicase; and eIF4G, the scaffolding protein. Once assembled, eIF4F binds to the cap structure (m7GpppN where N is any nucleotide) in the 5′ untranslated region (UTR) of mRNAs. The cap-binding complex recruits the 43S preinitiation complex consisting of the 40S ribosomal subunit and TC and stimulates the binding of the 60S large ribosomal subunit to form a translationally active and elongation-competent 80S ribosome, thereby facilitating the initiation of cap-dependent translation, especially for mRNAs with 5′ UTRs bearing the terminal oligopyrimidine tract (TOP) and complex secondary structure. The assembly of the eIF4F complex is under positive regulatory control of mammalian target of rapamycin complex I (mTORC1) and extracellular signal-regulated kinase (ERK). Both mTORC1 and ERK are activated downstream of diverse anabolic cues in the intracellular and extracellular milieu and mediate post-translational modifications of key translation factors. mTORC1 phosphorylates eIF4E repressors known as 4E-binding proteins (4E-BPs), which when unphosphorylated inhibit eIF4E by sequestering it away from eIF4F complex, to promote translation initiation [34]. In addition to 4E-BPs, mTORC1 phosphorylates p70 S6 kinase 1 (S6K1), which targets programmed cell death 4 (PDCD4) for phosphorylation and proteasome-mediated degradation, and thus releases the inhibitory block of PDCD4 on eIF4A [35]. ERK activation, moreover, leads to phosphorylation of eIF4E on Ser209, which alters the affinity of eIF4E to mRNA cap [36]. ERK also phosphorylates tuberous sclerosis complex (TSC) and prevents the inhibitory block of TSC on mTORC1 activity, exemplifying cross-talk between the mTORC1 and ERK pathways. Cross-talk also occurs between the TC and eIF4F through the GCN2-ATF4 pathway, which mediates transcriptional induction of 4E-BPs, thereby causing an inhibition of cap-dependent translation in parallel with ternary complex depletion [37]. Inhibition of the eIF4F complex causes an increase in translation of uncapped mRNAs that have internal ribosome entry sites (IRES), such as the fragile X mental retardation protein (FMRP). FMRP is the binding partner of a noncanonical 4E-BP, known as CYFIP1, which binds eIF4E and inhibits the assembly of eIF4F [38].Thus cap-dependent translation initiation is under inhibitory control of canonical and noncanonical 4E-BPs.

At the end of initiation, the Met-tRNA$_i^{Met}$ occupies the P site of the ribosome, and another aminoacyl-tRNA bearing an amino acid corresponding to the next codon settles on the A site with the help of elongation factor 1α (eEF1A). During elongation, amino acids are added to the nascent polypeptide chain by the formation of peptide bonds and the 80S ribosome moves along the mRNA to the subsequent codon. Also crucial for elongation is elongation factor 2 (eEF2), which promotes the GTP-dependent translocation of the ribosome. eEF2 is inactivated by Ca2+/CaM-dependent eEF2 kinase (eEF2K) [39], which makes it the step most directly regulated by calcium and synaptic activity. Downstream of mTORC1, S6K1 phosphorylates eEF2K, which inhibits its kinase activity and promotes translation elongation [40]. An ERK-90 kDa ribosomal S6 kinase (p90RSK) pathway also leads to phosphorylation of eEF2K [40]; hence, mTORC1 and ERK both regulate translation elongation. Elongation not only depends on elongation factors but is subject to surveilling ribosome quality control (RQC) mechanisms. Part of RQC, ribosome stalling is induced by cellular stress, such as oxidative stress and amino acid or aminoacyl-tRNA deprivation, and is aided by proteins such as FMRP [41] and others [42]. Ribosome stalling often causes cotranslational degradation of both the aberrant mRNA and the incomplete polypeptide [43]. Overall, there are several checkpoints during the initiation and elongation steps of translation. The intricate coordination of various translation factors and modulators ensures the synthesis of cellular context-based basal and activity-dependent protein outputs of the translation machinery.

Molecular profiling of the translation landscape during memory consolidation

mRNA association with translating ribosomes is widely used as a proxy for estimating translation rates and output. Biochemical tagging of ribosomes in specific cell populations with translating-

ribosome affinity purification (TRAP) and the related Ribotag technique have been used to determine snapshots of translation profiles following memory processes including cued threat learning [44], retrieval [45], and extinction [46]. TRAP RNA-seq after Pavlovian cued threat learning revealed learning-related changes in somatic and axonal translatome of lateral amygdala projectors in the rat auditory cortex. Gene expression was upregulated for genes in a range of gene ontologies (GOs) such as postsynaptic density, myelin sheath, actin binding, neuron projection, and protein complex binding in the somatic translatome, whereas genes belonging to the GO categories – poly(A) ribosome binding, ribosome, oxidative phosphorylation were upregulated in the axonal translatome [44]. The ribosome-tagging techniques, while powerful, lack the resolution to distinguish mRNAs bound with few or high numbers of ribosomes, which is necessary to determine the translation efficiency. By comparison, ribosome profiling enables position-sensitive survey of translation on a genome-wide scale. Ribosome profiling involves purification of mRNA-ribosome complexes and nuclease treatment, leaving short ribosome-protected mRNA fragments that can be identified and quantified at single nucleotide resolution. Using ribosome profiling, multiple translation alterations have been detected in the mouse hippocampus following contextual threat conditioning [47–49].

Advances in proteomics-based methods now allow direct identification of nascent proteins at global scale or with cell-type specificity. This is achieved by labeling nascent proteins by pulse-labeling with specific chemical conjugates followed by quantification of purified proteins via mass spectrometry. Noncanonical amino acid tagging methods introduce bio-orthogonal functional groups into nascent proteins using the cell's own translation machinery that subsequently allow for identification of newly synthesized proteins *in vitro* and in intact organisms using click chemistry. New mouse strains have been developed that express mutant methionyl-tRNA synthetase, NLL-MetRS [26], or MetRS* [27], in a cell type-specific manner. NLL-MetRS mediated labeling of *de novo* proteome in hippocampal CamK2α-expressing cells has been used to profile nascent proteins synthesized following an accelerated version of active place avoidance, that is, instrumental conditioning. The learning-associated proteins included gene clusters related to mRNA splicing, vesicle-mediated transport, and others [26]. Thus, both proteomics and mRNA/ribosome association-based RNA-seq have begun to elucidate changes in the translation landscape during memory consolidation.

Querying memory consolidation with PS inhibition using pharmacology

Extensive work using pharmacology and genetic strategies has illuminated the macroscopic neural substrates for consolidation of associative memories. Before the advent of ligand-gating genetic strategies, temporal control of protein synthesis inhibition (PSI) was achieved with pharmacology where the drug inhibitor can be administered during well-defined peri-mnemonic time intervals. Using pharmacology, consolidation of a long-term memory was first postulated to require PS in 1948 by Ludwik MonnéA [50], and empirically demonstrated in 1963 by Josefa Flexner and colleagues [5]. Flexner *et al.* administered puromycin in mice to show that disrupting PS impairs consolidation of long-term discriminative avoidance memory in a Y maze.

Various drugs have been used in rodent memory research to interrogate PS at different steps (Table 1). Puromycin mimics tyrosyl-tRNA and gets attached to the growing polypeptide, which causes the truncated product to prematurely exit the ribosome, thereby inhibiting translation elongation [51]. Anisomycin, a drug that blocks peptidyl transferase activity during translation elongation, has been the most widely used pharmacological PSI for studies of the brain due to its ability to cross the blood–brain barrier, high efficiency (~90%) at blocking PS, and relatively low toxicity [1,10,52–56]; however, it can also affect catecholamine release [57] and activate stress signaling pathways [58]. Cycloheximide, a near-complete inhibitor of ribosome translocation, was

Table 1. Pharmacological approaches to mediate protein synthesis manipulation in memory processes

Drug	PS step (effect)	Mode of action	Brain region	Effects on LTM
4EGI-1	Initiation (–)	Inhibitor of cap-binding complex	Lateral amygdala	Impairs cued threat LTM consolidation [61]
Anisomycin	Elongation (–)	Inhibitor of peptidyl transferase activity	Whole-body	Impairs LTM consolidation ([1,18] and others)
			Infralimbic cortex	Impairs cued active avoidance LTM and enhances cued threat-induced freezing LTM [81]
			Lateral amygdala	Impairs cued threat LTM consolidation [6]
			Central amygdala	Impairs cued threat LTM consolidation [77]; enhances cued active avoidance [81]
			Hippocampus	Impairs contextual threat LTM consolidation [78,79]; impairs inhibitory avoidance LTM [14]
			Cerebellum	Impairs conditioned eyeblink LTM [56]
Cycloheximide	Elongation (–)	Immobilizes ribosome	Whole-body	Impairs LTM consolidation [59,60]
Emetine	Elongation (–)	Interacts with E-site of ribosomal small subunit	Whole-body	Impairs spatial memory [63]
GSK2606414	Initiation (+)	Inhibits PERK, a kinase for eIF2α	Whole-body	Enhances LTM [76]
			Gustatory cortex	Enhances conditioned taste aversion [86]
ISRIB	Initiation (+)	Activates eIF2B and renders it insensitive to p-eIF2α mediated inhibition	Whole-body	Enhances spatial memory and contextual threat LTM [73]
PKRi	Initiation (+)	Inhibits PKR, a kinase for eIF2α	Whole-body	Enhances contextual threat LTM and cued threat LTM [75]
Puromycin	Elongation (–)	Mimics tyrosyl-tRNA	Whole-brain	Impairs discriminative avoidance LTM [5]
Rapamycin	Initiation (–), elongation (–)	Inhibits mTORC1 complex	Whole-body	Impairs LTM consolidation [67–69]
			Amygdala	Impairs inhibitory avoidance LTM [69]
			Hippocampus	Impairs inhibitory avoidance LTM [69]
Sal003	Initiation (–)	Inhibits phosphatase PP1 and increases abundance of p-eIF2α	Dorsal hippocampus	Impairs contextual threat LTM consolidation [93]
U0126	Elongation (–)	Inhibits MAPK ERK1/2	Whole-body	Impairs cued threat LTM [6]
			Dorsal hippocampus	Impairs inhibitory avoidance LTM [87]

used by other groups for blocking PS to examine memory processes [59,60] but later discontinued due to toxicity. 4EGI-1, an inhibitor of eIF4E-eIF4G interactions, reduces general translation by ~40% when administered centrally in the brain and leads to impaired memory consolidation [61]. Emetine, a drug that interacts with the E-site of the ribosomal small subunit and blocks translocation, has also been used to block PS by ~50% in cultured cells [62]. Emetine has been used to show that consolidation of spatial memory requires PS [63]. Among the pathway-specific inhibitors of translation modulators, rapamycin, a drug inhibitor of mTORC1, leads to inhibition of PS by ~50% in lymphocytes and peripheral skeletal tissue [64–66]. Rapamycin has been used in several studies to show the PS dependence for memory consolidation [67–69]. U0126, an inhibitor of ERK activity, reduces PS by ~40% in cultured hippocampal neurons [70] and has been shown to inhibit memory consolidation of cued threat [71]. By contrast, a small molecule inducer of PS, integrated stress response inhibitor (ISRIB), activates eIF2B [72] and effectively uncouples the inhibitory effect of eIF2α phosphorylation on general PS leading to enhanced memory [73,74]. Similarly, drug inhibitors of the eIF2α kinases 0 (GSK2606414) and PKR (PKRi) also have memory enhancing effects when applied locally in the hippocampus [75,76].

Local delivery of anisomycin in various brain regions has been used to demonstrate the requirement of translation elongation, and by proxy PS, for different memory modules. For instance,

consolidation of associative aversive memories across most paradigms depends on translation elongation in amygdala – a crucial brain region for emotional processing [61,77]. Consolidation of aversive memories requires PS in additional brain substrates depending on the sensory modality and task complexity. For instance, multimodal context-based threat memories require PS in hippocampus [78,79], whereas consolidation of conditioned eye-blinking depends on PS in the cerebellum [80]. Cued threat-related active avoidance has been shown to depend on PS in infralimbic cortex [81]. Conditioned taste aversion memory similarly requires PS in gustatory cortex [82,83] in addition to amygdala [84]. In instrumental conditioning, consolidation of inhibitory avoidance (IA) long-term memory (LTM) has been shown to depend on PS in the hippocampus [14]. Overall, aversive memories are generally thought to require one or several waves of PS depending on the stimulus salience and training complexity [85].

Along the translation axis, the ternary complex-translation pathway is causally implicated in consolidation of aversive associative memories. Genetic and pharmacological inhibition of PERK in the hippocampus leads to enhanced memory in trace threat conditioning, and, in congruence, the same manipulations in the insular cortex cause conditioned taste aversion [76,86]. In addition, pharmacological inhibition of PS modulators ERK or mTORC1 signaling in dorsal hippocampus or basolateral amygdala both impair memory retention of IA [69,87]. Amygdalar S6K1 inhibition post-reactivation of cued threat memory also results in impaired memory persistence [88]. Nonassociative spaced learning paradigms such as spatial learning in Morris water maze (MWM) that produces long-term spatial memories depend on PS in dorsal hippocampus [63,89,90]; similarly, object recognition memory requires PS in both dorsal hippocampus and entorhinal cortex [91]. Thus, focal PSI in relevant brain areas using pharmacological inhibitors of translation factors or modulators has continued to shed light on the requirement of brain region-specific translation in memory processes (Figure 3).

Querying memory consolidation with genetic targeting of endogenous PS modulators

Key translation factors and modulators have been genetically targeted to understand their role in PS and their effect on behavioral correlates of memory and other cognitive processes in mice (Table 2). Earlier studies have found that constitutive, that is, germline, deletion of ISR mediators such as GCN2 and PKR lead to enhanced memory strength in a wide array of behavior paradigms, including spatial learning in MWM as well as contextual and auditory threat memory [75,92]. Similarly, constitutive hypo-phosphorylation of eIF2α increases translation output and results in enhanced memory strength and lowers threshold for consolidation in associative aversive and appetitive conditioning paradigms [93–95]. However, in the case of PERK, which is the most abundant eIF2α kinase in all cells, constitutive gene deletion in forebrain excitatory neurons has no effect on memory strength, but instead impairs cognitive flexibility [96]. Genetic deletion of S6K1 leads to early onset impairment in contextual threat memory and conditioned taste aversion [97]. In the case of 4E-BP2, knocking out this translation repressor leads to impaired spatial learning, motor skill memory, and associative threat memory [98,99]. Constitutive expression of kinase-defective eEF2K, that neutralizes the translation repressor function, also leads to impaired associative taste learning [100]. Genetic deletion of negative translation modulators has similarly resulted in memory impairments. Heterozygous deletion of TSC2, the catalytic component of TSC complex, leads to reduced PS [101] and impaired spatial memory [102]. Likewise, genetic deletion of FMRP causes enhanced translation [103] and yet causes impaired spatial memory in MWM [104,105] and trace threat memory [106]. These findings indicate that sustained translation aberration in either direction causes suboptimal memory retention.

Cell type-specific manipulations can unravel essential contributions of the targeted cell populations in processing and storing mnemonic information (Table 2). The availability of driver mouse

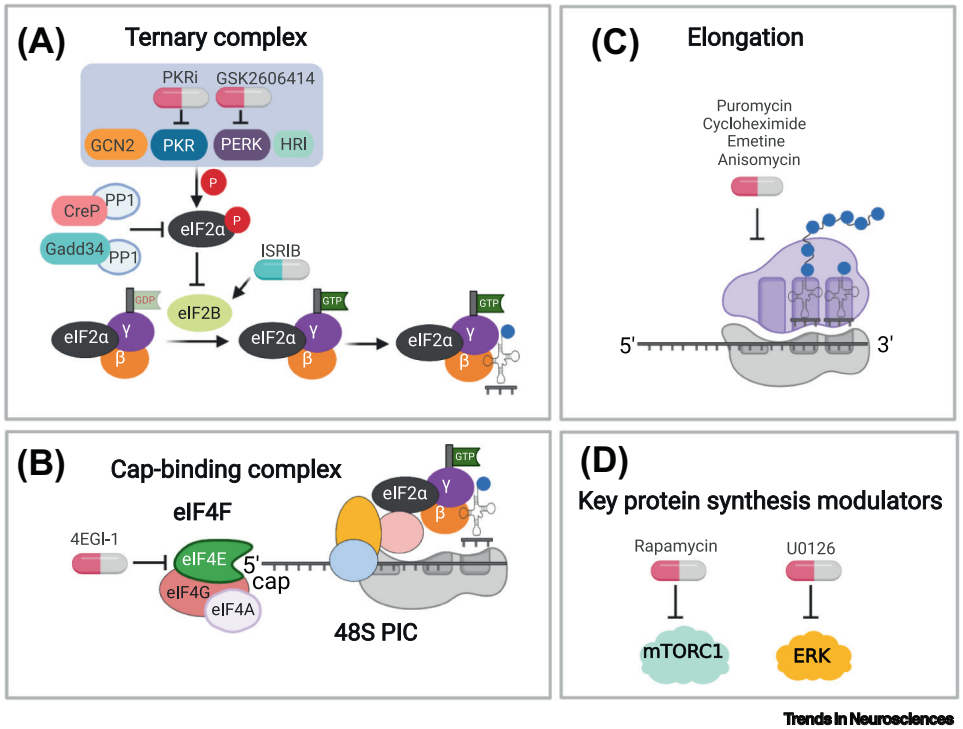

Figure 3. Pharmacological compounds targeting mammalian protein synthesis in the context of memory processes. (A) Drugs such as PKRi and GSK2606414 inhibit eIF2α kinases PKR and PERK, respectively, and thereby inhibit the integrated stress response (ISR). ISRIB promotes the GEF activity of eIF2B and uncouples the effect of phosphorylated eIF2α on general protein synthesis. (B) 4EGI-1 blocks the interaction of eIF4E and eIF4G, and thus blocks the formation of cap-binding complex eIF4F, and subsequently the 43S preinitiation complex (PIC). (C) Drugs such as puromycin, cycloheximide, emetine, and anisomycin block the elongation step of translation. (D) Key protein synthesis modulators include mTORC1 and ERK, which are inhibited by rapamycin and U0126, respectively. Abbreviations: eIF, eukaryotic initiation factor; GCN2, general control nonderepressible 2; GEF, guanine exchange factor; HRI, heme-regulated inhibitor; ISRIB, integrated stress response inhibitor; mTORC1, mammalian target of rapamycin complex 1; PERK, PKR-like ER kinase; PKR, protein kinase R.

strains and viral vehicles for Cre recombinase delivery have enabled access to specific cell populations for manipulation of translation factors or modulators. Germline manipulations of PS effectors in specific cell populations using the Cre-loxP recombinase strategy have elucidated the recruitment of PS machinery in functionally coherent cell populations for normal cell physiology and cognition. Among broad cell types, neuronal overexpression of eIF4E, which is predicted to increase cap-dependent translation, leads to enhanced contextual threat memory [107]. Microglial overexpression of eIF4E conversely does not affect memory strength, instead it results in autism-related phenotypes [107]. Conditional TSC1 deletion in astrocytes results in impaired spatial memory in MWM and contextual threat memory that are hippocampus dependent [108]. Forebrain-specific deletion of PERK in excitatory neurons resulted in impaired behavior flexibility and threat extinction [96]. Interestingly, hypo-phosphorylation of eIF2α across all forebrain or lateral amygdala excitatory neurons causes enhanced strength of cued threat memories [109,110], but the unchecked translation in the eIF2α mutant mice also causes behavior inflexibility [110]. Further bolstering the critical need for homeostatic negative feedback on translation load in cells, hippocampus-specific knockdown of ATF4 impairs both synaptic plasticity and spatial memory in MWM [111]. Conditional deletion of FMRP in cerebellar Purkinje cells attenuates eyeblink conditioning [112]. These findings indicate that genetic targeting of

Table 2. Cell type-specific protein synthesis manipulation in memory processes with genetic targeting

Gene driver	Cell type	Manipulation (effect on PS)	Temporal control	Effects on LTM
Whole body wide manipulation				
Syn1+	Neurons	eIF4E overexpression (+)	No	Enhanced contextual threat LTM [107]
Cx3cr1+	Microglia	eIF4E overexpression (+)	Postnatal	Unchanged contextual threat LTM [107]
Gapdh+	Astrocytes	Tsc1 deletion (not shown)	No	Impaired spatial memory and contextual threat LTM [108]
SOM+	Somatostatin-expressing neurons	eIF2α phospho-mutation (+)	No	Enhanced contextual threat LTM [109]
PV+	Parvalbumin-expressing neurons	eIF2α phospho-mutation (+)	No	Unchanged contextual threat LTM [109]
CamK2α+	CamK2α expressing neurons	eIF2α phospho-mutation (+)	No	Enhanced contextual threat LTM [109]
		PERK deletion (+)	No	Unchanged cued and contextual threat LTM, impaired threat extinction LTM [96]
DAT+	Dopaminergic neurons	eIF2α phospho-mutation (+)	No	Impaired contextual threat LTM, cued threat LTM, and spatial memory [113]
		PERK deletion (+)	No	Impaired contextual threat LTM, cued threat LTM, and spatial memory [113]
L7/Pcp2+	Purkinje cells in cerebellum	FMR1 deletion (ns)	No	Impaired conditioned eyeblink LTM [112]
Brain region restricted manipulation				
LA: CamK2α+	CamK2α expressing neurons in lateral amygdala (LA)	eIF4E knockdown (–)	Yes; in days	Impaired cued threat LTM [24]
		eIF2α phosphorylation (–)	Yes; in hours	Impaired cued threat LTM [24]
		eIF2α phospho-mutation (+)	Adult stage	Impaired cued threat LTM [24]
CeL:SOM+	Somatostatin-expressing neurons in centrolateral amygdala (CeL)	eIF4E knockdown (–)	Yes; in days	Impaired cued threat LTM [110]
		eIF2α phosphorylation (–)	Yes; in hours	Impaired cued threat LTM [110]
CeL:PKCδ+	Protein kinase C δ expressing neurons in centrolateral amygdala (CeL)	eIF4E knockdown (–)	Yes; in days	Impaired cued safety LTM [110]
		eIF2α phosphorylation (–)	Yes; in hours	Impaired cued safety LTM [110]
VTA:Th+	Dopaminergic neurons in ventral tegmental area (VTA)	eIF2α phospho-mutation (+)	Adult stage	Impaired contextual and cued threat LTM [113]
SN:Th+	Dopaminergic neurons in substantia nigra (SN)	eIF2α phospho-mutation (+)	Adult stage	Unchanged contextual threat LTM, cued threat LTM, and spatial memory [113]

translation effectors in broad cell types impacts different modules of memories owing to selective vulnerability in those paradigms caused by constitutive PS disruption.

Narrow categories of cell types targeted for PSI include subpopulations of excitatory and inhibitory neurons across the brain or in focal brain regions. Knocking down cap-binding protein eIF4E in centrolateral amygdala (CeL) inhibitory neuron subpopulations, SOM+ and PKCδ+ neurons, causes impairment in cued threat and cued safety memories, respectively [110]. In the latter case, impaired cued safety memory manifests as stimulus generalization. By contrast, enhancing translation in SOM+ neurons brainwide with constitutive expression of phosphomutant eIF2α results in enhanced memory in cued and contextual threat memory paradigms [109]. It is equally insightful to learn which cell types are dispensable for memory consolidation in specific paradigms. For example, PS in CeL PKCδ+ cells is dispensable for cued threat memory [110] whereas enhancing PS in brainwide PV+ inhibitory neurons does not alter the strength of aversive memories [109]. Moreover, genetic deletion of PERK in midbrain-wide or ventral tegmental area (VTA)-localized dopamine (DA) neurons causes impairment in spatial memory in MWM as well as in associative aversive memories including cued and contextual threat-conditioning paradigms [113]. The parallel approach of introducing phospho-mutant eIF2α in DA neurons resulted in consistent behavior phenotypes for associative and nonassociative long-term memories [113]. Inhibiting dephosphorylation of eIF2α using virogenetic expression of CreP in striatal cholinergic neurons also enhances performance and memory strength in spatial MWM paradigm [114]. Thus, the translation machinery is mobilized for specific behavioral tagging of long-term memories in a cell type-specific manner.

Querying memory consolidation with spatiotemporally resolved PS manipulation

Beginning from the macroscopic manipulation of PS at the level of whole brain and brain areas, the continually growing toolkit of gene-transfer, biochemical, and imaging technologies are making it possible to probe PS at the microscopic level of individual cell types and subcellular compartments with unprecedented temporal resolution (Figure 4). A new chemogenetic strategy

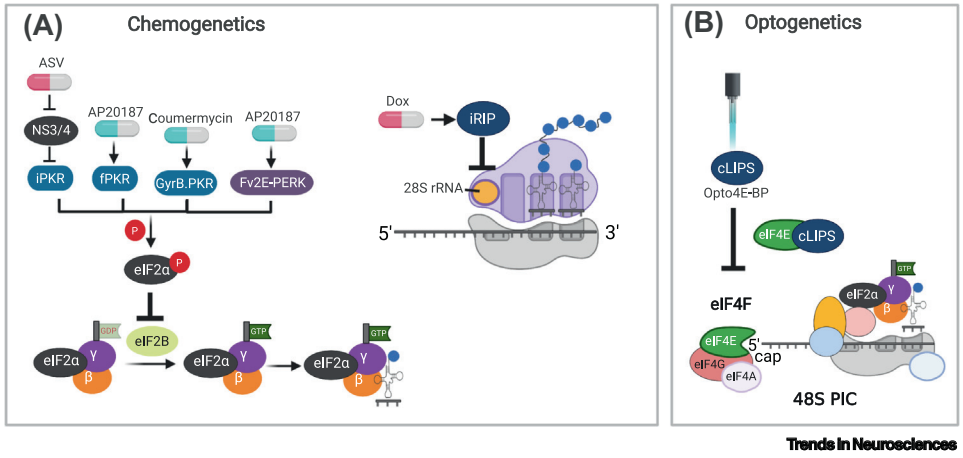

Figure 4. Spatiotemporally resolved protein synthesis manipulation with chemo- and optogenetics. (A) Chemogenetic strategies to block protein synthesis during initiation phase include targeting eIF2α kinase PKR with iPKR, fPKR, GyrB.PKR, and PERK with Fv2E-PERK. These chemogenetic protein synthesis inhibitors (ciPSIs) phosphorylate eIF2α and block eIF2B, thereby inhibiting the formation of ternary complex. Inducible RIP (iRIP) is another chemogenetic PSI that targets 28S rRNA, an essential structural component of the large subunit, and thus inhibits translation elongation. (B) Optogenetic strategies include cLIPS, a circularly permuted cLOV inducible protein synthesis inhibitor, that constitutes light-activatable 4E-BP that binds eIF4E and thus blocks cap-dependent translation. Abbreviations: ASV, asunaprevir; Dox, doxycycline, eIF, eukaryotic initiation factor; GyrB, gyrase B; PERK, PKR-like ER kinase; PIC, preinitiation complex; PKR, protein kinase R; RIP, ribosome inactivating protein.

for cell type-specific drug-inducible PSI (ciPSI) is based on Cre-conditional and drug-mediated disinhibition of engineered PKR kinase domain from the protease activity of NS3/4 [24]. With iPKR, ~50% general PSI is achieved in the brain of behaving mice. Pan-neuronal induction of iPKR demonstrated that rapid PS was required for memory consolidation [24]. The iPKR-based chemogenetic strategy for ciPSI is based on Cre-conditional and drug-mediated disinhibition of engineered PKR kinase domain from the protease activity of NS3/4. iPKR follows years of efforts to engineer eIF2α kinases to be drug-inducible such as fPKR [115], Fv2E-PERK [116], and gyrase B (GyrB)-PKR [117]. Among these efforts, fPKR combines dimerizing domain from FK506-binding protein (FKBP) with full-length PKR such that the drug AP20187 swiftly activates fPKR to act on eIF2α in Cre-expressing cells. Although fPKR induces eIF2α phosphorylation and ATF4 expression, it has no effect on general translation as assessed with S^{35} methionine labeling; hence, the amnesic effect of fPKR activation in hippocampal CA1 pyramidal neurons has been attributed to the transcriptional repression of target genes by ATF4 [118]. Similar to fPKR, Fv2E-PERK is based on FKBP and is induced by AP20187 to phosphorylate eIF2α and causes near-complete block of PS [116]. GyrB-PKR, on the other hand, is based on bacterial GyrB domain fused to PKR kinase domain, which allows the fusion protein to be dimerized and thus activated by the drug coumermycin to act on eIF2α [117]. Fv2E-PERK and GyrB-PKR have only been examined *in vitro* and their utilization to examine memory processes *in vivo* have yet to be tested.

Targeting translation elongation, another chemogenetic strategy involves the near-complete inhibition of PS with inducible activation of ricin, a plant-derived ribosome inactivating protein (RIP) [15,25], that depurinates A4324 on the sarcin-ricin loop of the 28S rRNA and blocks translation elongation. Temperature-inducible ricin was used in the fruit fly, *Drosophila melanogaster*, to demonstrate that sequential PS is required for odor-related associative memory [15]. Ricin is also the basis of genetically encodable protein synthesis inhibitor (gePSI) that uses the Tet-on system to express the α and β subunits of ricin, thus constituting the active holoenzyme for blocking translation elongation in cultured neurons [25]. The development of optogenetic inhibitors of cap-dependent PS such as cLIPS [119] further enables probing spatiotemporally resolved translation in subcellular loci in specific cell types. This is a significant advance for light-activated systems to control gene-specific translation similar to caged siRNAs [120] and antagomirs that act on miRNAs [121], and caged versions of pharmacological PSIs [122]. The reversible nature of cLIPS and its gene-blind ability to target nascent PS machinery in specific cell types with the potential for further spatial and temporal precision afforded by light-gating is an exciting prospect.

Concluding remarks and future directions

Based on the knowledge gained from artificial manipulation of neuronal activity with expression of opsins and engineered membrane-bound receptors that are gated by either light or ligand, memory formation and retrieval processes involve selective recruitment of cell populations across a distributed cellular network in the brain [123–125]. The recruitment of cells seems to be defined by both basal gene expression and activity. PS is metabolically expensive and hence acts as a filter on information processing to select only the salient or nontrivial information for long-term storage. There are several new pieces of evidence for transient recruitment of specific cell populations for storage of disparate long-term memories in mice. For instance, disruption of PS immediately after training with drug-induced release of iPKR in CeL SOM+ neurons blocks consolidation of cued threat memory in a differential threat-conditioning paradigm [110]. Complementing SOM+ neuronal function, iPKR induction in CeL PKCδ+ neurons immediately after training leads to impaired consolidation of cued safety memory and stimuli discrimination [110]. A similar strategy involving fPKR induction in hippocampal CA1 principal neurons pretraining resulted in impaired memory strength in both instrumental active avoidance as well as contextual threat-conditioning paradigms [115]. It is posited that activity-defined cells, that have robust learning-induced immediate early gene (IEG) expression, across the

Outstanding questions

Does the strength and quality of memory directly scale with the level of PS?

What is the function of different temporal waves of PS during memory consolidation? Is there a role for fast versus protracted PS during memory maturation?

Is local PS required in the processes distant from soma to fulfil local demand for new relevant proteins during memory consolidation?

Are there different PS modules, such as mTORC1 versus ERK-regulated translation, that are executed in specific cell types in response to specific environmental context and learning paradigms?

How does PS accommodate the persistence of long-term memories at remote time scale? Are there periodic waves of PS during system consolidation of memories in functionally connected brain areas that support memory persistence?

What determines the recruitment of specific cell types for forming the cellular substrate for long-term memories: baseline molecular identity or stochastic prior cellular activity?

What are the identities of PRPs newly synthesized in specific cell types during memory consolidation?

brain are preferentially recruited to the memory trace and are thus referred to as engram cells [126]. Artificial reactivation of these cells has been shown to elicit CR even when PS is inhibited post learning [127]. A possible interpretation of these findings is that cellular PS facilitates the CS to access and activate the cells that are crucial substrates for the associated memory during naturalistic memory recall, which can be bypassed by artificial tagging and direct reactivation.

Subcellular PS fulfils the local demand for new proteins with exogenous stimuli-driven synaptic plasticity, which is the basis for long-lasting long-term potentiation, a cellular correlate of memory consolidation. Although there is converging evidence for local PS in both dendritic [128] and axonal compartments [29,44] largely driven by local environmental cues and behavioral training, whether local translation is required for consolidation of long-term memories has not been resolved in mammalian systems, but with tools such as ciPSI, gePSI, and cLIPS2 this issue is on the verge of being interrogated (see Outstanding questions). The strongest evidence for the necessity of local translation in memory processes actually comes from gene-specific manipulations for CamK2α [129] and BDNF [130]; in both cases deletion of 3′ UTR from their mRNA abolished dendritic targeting and resulted in impaired synaptic plasticity and memory consolidation in mice. Combining dendrite targeting elements [131] is an attractive idea to localize ciPSI, gePSI, and cLIPS2 to subcellular compartments to establish causality for local PS in memory consolidation. In addition, it is of particular interest to profile plasticity-related proteins (PRPs) in specific cell types recruited during memory consolidation. Methods to profile cell type-specific ribosome-associated transcripts [132,133] are rapidly maturing and already have been used to profile cellular and subcellular translation in specific cell types. All in all, considering these many outstanding questions, it is an exciting time to study the molecular basis of memory consolidation with high spatiotemporal resolution with respect to both the regulation and output of translation.

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Declaration of interests

The authors declare no competing interests in relation to this work.

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