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The role of CPEBs in learning and memory

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Abstract

Memory formation involves a complex interplay of molecular and cellular processes, including synaptic plasticity mechanisms such as long-term potentiation (LTP) and long-term depression (LTD). These processes rely on activity-dependent gene expression and local protein synthesis at synapses. A central unresolved question in neuroscience is how memories can be stably maintained over time, despite the transient nature of the proteins involved in their initial encoding. A key candidate addressing this "maintenance paradox" is the CPEB (cytoplasmic polyadenylation element-binding protein) family, particularly CPEB3. CPEBs are RNA-binding proteins that regulate the polyadenylation and translation of dormant mRNAs, enabling synaptic tagging and memory consolidation. CPEB3 has been shown to modulate the expression of critical synaptic proteins, including AMPA and NMDA receptor subunits, thereby influencing synaptic strength and long-term memory persistence. Structurally, CPEB3 features a disordered N-terminal domain (NTD) enriched in glutamine and proline residues, which may facilitate reversible aggregation and phase separation and an actin-binding domain, potentially supporting its localization to ribonucleoprotein granules. The highly conserved C-terminal domain (CTD) contains RNA-recognition motifs essential for mRNA binding. Together, these structural features may enable CPEB3 to function as a molecular switch, linking synaptic activity to enduring changes in protein synthesis and memory encoding. Here we review the current understanding of the function of CPEB3, highlighting current hypotheses and debates of the role(s) of protein self-assembly in memory formation.

Abbreviations

AAR: amino acid repeat; AD: Alzheimer's disease; AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaMKII: Ca^{2+}/ calmodulin-dependent protein kinase II; CPEB: cytosolic polyadenylation element binding protein; DSCAM: Down's syndrome cell adhesion molecule; FMRP: Fragile X mental retardation protein; FXS: Fragile X syndrome; IMM: intermediate mesopallium; LCD: low complexity domain; LTD: long term depression; LTP: long term potentiation; mPFC: medial prefrontal cortex; NMDA: N-methyl-D-aspartate; PPII: polyproline II; PrLD: prion-like domain; RBP: RNA-binding protein; RNP: ribonucleoprotein; RRM: RNA-recognition motif; SFPQ: splicing factor proline-glutamine rich

1. Introduction

In essence, memory is the system by which daily experiences are recorded and retained, enabling acquisition of knowledge. It operates through four stages: encoding, consolidation,

48 storage and recall [1, 2]. Consolidation of memories is predicated on synaptic modifications
49 and the strengthening of neuronal connections within specific brain regions. Neuronal
50 ensembles which are activated both during learning and retrieval processes are known as
51 engram cells, which reside, *inter alia,* in the hippocampus, amygdala and medial prefrontal
52 cortex (mPFC). However, the molecular mechanisms that connect memory acquisition to its
53 subsequent retrieval remain incompletely understood [3]. In this review we discuss knowledge
54 of the formation and retrieval of memories, alongside current knowledge of how memories
55 may be made and retrieved at a molecular level, focussing on the CPEB family of proteins
56 thought to be key players in these processes [4]. We point out key questions and current
57 hypotheses and show how by linking the fields of neuroscience and structural biology we are
58 beginning to gain a foothold into understanding this fundamental feature of human biology.
59
60 ### *The biology of long term and short-term memory*
61 The synaptic changes associated with memory acquisition and stabilisation are mediated by
62 long term potentiation (LTP) and long-term depression (LTD) [1, 2]. LTP is associated with the
63 activation of NMDA receptors and the subsequent increase of synaptic trafficking of AMPA
64 receptors (ionotropic glutamate receptors) [5], and learning tasks can promote the recruitment
65 of newly synthesised AMPA receptors to synapses [6], enhancing synaptic strength.
66 Glutamate binding to post-synaptic AMPA receptors leads to membrane depolarisation. This
67 results in the removal of the magnesium ions blocking the NMDA receptors, allowing calcium
68 influx into postsynaptic neurons which activates Ca^{2+}/ calmodulin-dependent protein kinase II
69 (CaMKII) **(Figure 1)**. This results in the phosphorylation of GluA1 subunits in AMPA receptors
70 and increases AMPA receptor trafficking to the synapse. In addition, CaMKII activity induces
71 cytoskeletal rearrangements, which also contribute to structural remodelling of synapses. In
72 late-phase LTP, which supports long-term memory persistence, the Ca^{2+} signalling activates
73 the cAMP, PKA (Protein Kinase A), MAPK (Mitogen Activated Protein Kinase) pathway. This
74 cascade leads to the phosphorylation of the transcription factor, cAMP response element-
75 binding protein (CREB), promoting the expression of genes such as *Arc* and *Fos* that are
76 critical for synaptic consolidation and memory persistence [1, 7, 8].
77
78 The key difference between short- and long-term memories is that the formation of stable
79 memories, which need to be stored and retrieved, require new gene expression. This involves
80 a dual mechanism: the transcription of new mRNAs and the translation of pre-existing,
81 quiescent mRNAs localised at synapses. Cytosolic ribonucleoprotein (RNP) granules can
82 sequester these mRNA in a translationally repressed state, preventing their translation until
83 activated by specific stimuli. The reversible assembly of RNP granules is thought to be
84 mediated by low complexity domains (LCDs) that are present in many RNA-binding proteins
85 (RBP) [9] and are known to be involved in liquid-liquid phase separation (LLPS) and
86 condensate formation in many processes spanning virus assembly, ribosome biogenesis and
87 neurodegeneration [10] and, as postulated and reviewed here, potentially also in long term
88 memory.
89
90 How then can long-term memory (LTM) be maintained and persist far longer than the lifespan
91 of the proteins initially synthesised to encode them? Francis Crick proposed that this problem
92 could be solved by the existence of a "macromolecule which is relatively immune from

93 molecular turnover", although this idea was initially considered improbable [11]. Recent
94 research has identified several plausible mechanisms that could contribute towards
95 addressing this issue. Epigenetic modifications, such as DNA methylation and histone
96 modifications, can stably alter gene expression patterns over long periods [3]. Molecular
97 scaffolds, such as the synaptic protein KIBRA, or the extracellular matrix that surrounds
98 neurones known as perineuronal nets, have also been shown to stabilise memory-related
99 synaptic changes [12]. The upsurge of interest in prion biology in the late 1990s led to the
100 suggestion of a prion-based mechanism of long-term memory storage, in which amyloid-like
101 fibrils, which inherently are highly stable and are able to self-propagate, could be involved [13].
102
103 ## 2. The CPEB family of proteins
104 ### *Discovering CPEB*
105 In 1983, Rosenthal and colleagues published a seminal paper linking mRNA adenylation and
106 translational control [14] (reviewed previously in [15]). Subsequent work in *Xenopus* identified
107 a consensus uracil (U)-rich sequence motif required for G10 mRNA polyadenylation. This motif
108 was termed the cytoplasmic polyadenylation element (CPE), and cross-linking experiments
109 identified a corresponding binding protein, named CPEB (cytoplasmic polyadenylation
110 element binding protein) [16]. Further experiments with *Xenopus* B4 mRNA identified a CPEB
111 of a different molecular weight, leading to the speculation of multiple CPEBs [17, 18]. This was
112 followed by affinity purification of a 62 kDa CPEB protein which contains two RNA-recognition
113 motifs (RRMs) and a zinc-finger motif in the C-terminal region [19-21], in addition to an N-
114 terminal PEST domain [22, 23]. Cloning of the CPEB cDNA proved to the be the gateway to
115 further functional characterisation and established CPEB as a key regulator of cytoplasmic
116 polyadenylation, activating dormant translationally inactive mRNA, especially in the context of
117 development and the cell cycle [24]. This discovery was later expanded by the identification
118 of additional CPEB family members in vertebrates named CPEB2, CPEB3 and CPEB4 [25-
119 27] (**Figure 2**) which originate from a single ancestral gene [28].
120
121 Consistent with its functional importance, CPEB is highly conserved across species, with
122 *Aplysia californica* (California sea hare, ApCPEB) having two paralogues, one in each
123 subfamily, *Drosophila* having two (Orb1 and Orb2) and vertebrates having four paralogues
124 (CPEB1-4, **Figure 2**) [29]. Furthermore, it has been suggested that CPEB1 and CPEB2-4 can
125 be separated into different sub-families [30].
126
127 The CPEB family of proteins has an unusual primary sequence composition and organisation:
128 they contain relatively few lysine and glutamate residues (compared with the average across
129 all proteins in the proteome), while serine and proline are over-represented. In addition, the
130 sequence is also asymmetrically organised, with proline and glutamine residues being
131 concentrated in the N-terminal domain (NTD) of CPEB2-4 where they form long homo-
132 polymeric repeats, while these sequences are absent in CPEB1 (**Figure 2**). These regions
133 have high scores for sequence simplicity and repetitiveness, and a high propensity to undergo
134 LLPS, as predicted by tools as such FuzDrop and ParSe [28].
135
136 Assessment of sequence similarity shows that there is a substantial difference between the
137 sequence of CPEB1 and its family members, CPEB2-4 (**Figure 2B-D**) [30, 31]. This suggests

that CPEB1 and CPEB2-4 may regulate distinct sets of RNA targets and operate through different mechanisms [32, 33]. Supporting this view, the regulatory pathways of CPEB1 and CPEB2-4 are also different, with CPEB2-4 being co-regulated by miR-26 and miR-92 [34, 35], while CPEB1 is regulated by miR-22 [36]. When over-expressed individually as GFP fusions in mammalian cells, all four vertebrate CPEBs form foci, although with significant differences in number, sphericity and fluorescence intensity. CPEB1 and CPEB2 foci are larger than those of CPEB3 or CPEB4, and CPEB1 foci are less fluorescent and significantly more abundant than foci of the other CPEBs. CPEB3 foci, while fewer in number and lower in volume, display the highest fluorescence intensity of all CPEBs and also showed the fastest recovery after photobleaching, suggesting dynamic molecular exchange. Overall, CPEB1 foci resemble RNP granules in which RNA is required for condensate formation, while CPEB2-4 can undergo LLPS in the absence of RNA [29]. In neurons, CPEB3 is present in RNP granules and P bodies, colocalising with Staufen-2, Pumilio and FMRP. A mutant form of CPEB3 (S240-242A) shows reduced co-localisation with P bodies and inhibition to the translation of a reporter construct [37], linking a key role of CPEB3 both in RNP granule formation and translation control.

The high sequence variability in the NTD of CPEBs (**Figure 2C**) gives rise to disparate regulatory mechanisms of the different family members. For example, phosphorylation of CPEB1 at Ser174 by Aurora kinase A promotes the transition of the protein from a translational repressor to an activator [38], with translational activation thereafter being controlled by degradation mediated by Cdc2, Cdk1 and Plk1 [23, 39]. CPEB4, by contrast, is activated via phosphorylation at multiple sites in its NTD by ERK2 and Cdk1 [40]. CPEB3 regulation is modulated by post-translational modifications such as mono-ubiquitination and SUMOylation [41, 42], the latter being linked to the localisation of protein to cytoplasmic granules, including TDP-43 [43-45]. Furthermore, SUMOylation has been linked to synaptic function and memory formation more generally [46, 47].

Functionally, all CPEBs can recruit the CCR4-NOT deadenylase to the CPEB-repressor complex. This recruitment is crucial for the regulation of mRNA stability and translation. However, only a fraction of transcripts regulated by CCR4-NOT deadenylase is common to all four CPEBs, indicating specificity. While all CPEBs recognise the canonical cytoplasmic polyadenylation element (CPE, $UUUUA_{(1–2)}U$), CPEB2-4 also recognise the "G variant" sequence (UUUUGU) [15, 29] allowing a broader range of mRNA regulation. Together, these findings highlight the commonality in the role of CPEBS in translational control but highlight that the different family members have different mRNA targets, different mechanisms of activation and repression.

3. CPEBs in neurons and memory

Further detailed characterisation of CPEB throughout the 1990s, predominantly by Richter and coworkers, led to the discovery of CPEB as a regulator of local protein synthesis in neurons [48]. This finding supported the broader concept that synaptic plasticity is underpinned by protein synthesis from dendritically localised mRNA, especially Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) [49]. In rodent brain, CPEB1 is localised at synapses, where it binds to the CPE in the 3'UTR of CaMKII mRNA, regulating its

183 polyadenylation and translation [48]. This regulatory mechanism is activated by NMDA
184 receptor stimulation [50, 51]. Indeed, appending the CPE-containing CaMKII 3'UTR to a GFP
185 reporter mRNA enhances dendritic trafficking of the reporter [52]. Furthermore, Ca^{2+} influx
186 leads to CaMKII activation, which in turn phosphorylates CPEB1 at Thr171, modulating its
187 activity, linking neuronal activation to CPEB1 activity in translational control [53].

189 *In vivo* studies of CPEB1 knockout (KO) mice show no significant alterations in baseline
190 behavioural characteristics. However, the KO mice exhibit impaired hippocampal memory
191 extinction, as demonstrated in a swim-maze task and a fear conditioning test [54] and deficits
192 in NMDA-dependent long-term potentiation (LTP) [55, 56]. It has been suggested that the role
193 of CPEB in NMDA-dependent LTP is dependent on its phosphorylation by CaMKII [57]. Similar
194 to CPEB1 KO mice, CPEB3 KO mice also show no changes in physical performance or
195 behaviour in their home cage, but they have enhanced short-term fear memory and long-term
196 spatial memory [58]. Brain homogenates from these KO mice show elevated levels of NMDA
197 receptor subunits (NR1, NR2A and NR2B), PSD-95 and the AMPA receptor subunit GluA1
198 [58], consistent with previous findings that CPEB3 represses GluA2 translation [32] (**Figure
199 1A**). The consequent increase in NMDAR-mediated Ca^{2+} influx leads to CaMKII
200 hyperactivation and abnormal LTD responses [59]. Recent findings also show that CPEB3
201 also limits the nuclear translocation of the transcription factor, STAT3, reducing the expression
202 of NMDAR subunits [60]. CPEB3 has also been implicated in visual imprinting in the chick
203 intermediate medial mesopallium (IMM), a brain region critical for linking visual input and
204 learning. In this learning paradigm, aggregated CPEB3 was shown to be upregulated in the
205 IMM after the training phase [61]. Moreover, higher levels of aggregated CPEB3 showed a
206 significant positive correlation with performance in the testing phase [62], suggesting a
207 functional role of CPEB3 self-assembly in memory consolidation. Similarly, increased
208 detergent-insoluble CPEB3 is seen after fear conditioning [63]. Conditional knockout of
209 CPEB3 in the mouse forebrain also leads to multiple memory impairments, including deficits
210 in associative memory, spatial memory and memory maintenance [63]. RNA
211 immunoprecipitation has identified learning and memory as one of the top gene ontology
212 categories for CPEB3 targets, including genes such as *Grm5*, *Kalrn* and *Nr3c1*. Furthermore,
213 CPEB3 KO mice show increased hippocampal glucocorticoid receptor signalling and reduced
214 expression of brain-derived neurotrophic factor (BDNF), further supporting its role in cognitive
215 performance and emotional regulation [64]. In contrast to the effects of CPEB3, knockout of
216 CPEB4 does not result in any memory deficits [65]. These findings highlight the distinct and
217 diverse roles of CPEBs in regulating various aspects of memory, including synaptic plasticity,
218 memory formation and maintenance.

220 Interestingly, there is a short, highly conserved intronic sequence in *CPEB3* which acts as a
221 ribozyme, regulating its own expression. A substitution of U to C (rs11186856) in this sequence
222 enhances self-cleavage of CPEB3 by two-fold, resulting in a concomitant decrease in CPEB3
223 protein expression [66]. Individuals with the homozygous *CC* genotype showed a deficit in
224 semantic memory performance, while the heterozygote genotype had no effect [67].
225 Consequently, antisense oligonucleotide targeting of the *Cpeb3* ribozyme, leads to increases
226 in *Cpeb3* mRNA. This results in increased CPEB3 protein, alongside increases in GluA1,
227 NR2B and PSD95 expression, leading to enhanced long-term memory [68]. Furthermore, this
228 CPEB3 variant has also been recently linked to memory in the context of foreign language

229 proficiency [69]. Collectively, this panoply of evidence points firmly to a role of CPEBs in
230 memory formation, although the underlying cellular and molecular mechanisms remain
231 complex and are not yet fully elucidated.
232
233 *CPEBs in invertebrates*
234 Much valuable information about learning and memory has been garnered through the use of
235 *Drosophila melanogaster* as a model system [70]. The *Drosophila* CPEB, Orb2, has been
236 proposed to promote memory via its isoforms (Orb2A and Orb2B), but also through regulating
237 genes involved in neuronal growth and synapse formation [71-74]. Accordingly, deletion of the
238 N-terminal Gln-rich region of Orb2 resulted in defects in long-term, but not short-term
239 memories [75], which is suggestive of a role for aggregation in memory-related functions [76].
240 This memory deficiency can be mitigated by expression of the lingerer protein (Lig), which can
241 form a complex with Orb2 [77]. The function of Orb2 in memory may involve oligomerisation
242 acting as a switch between its activating and repressive activities [78]. Aggregation of Orb2A
243 has been shown to be crucial for synapse-specific activation of dormant mRNA and long-term
244 memory stabilization [76, 78, 79]. It has been reported that Orb2 oligomerisation is potentiated
245 by Hsp40 family member Mrj and the DNA-J family chaperone JJJ2 [80, 81]. Subcellular
246 localisation of Orb2 is dependent on its 3'UTR. Interestingly, 3'UTR deletion did not result in
247 any change in soma expression of Orb2, but synaptic Orb2 was significantly reduced [82, 83].
248
249 Furthermore, Orb2A mRNA is present as a non-protein coding unspliced transcript, where
250 long-term memory formation is potentiated by pasilla-mediated Orb2A splicing to a protein
251 coding transcript [84]. It has been suggested that Orb2A is primarily involved in memory
252 acquisition, with Orb2B required for consolidation [85]. Orb2B may act as a conventional
253 CPEB, involved in mRNA transport and regulation, but Orb2A is required for the formation of
254 stable Orb2 complexes [79].
255
256 There have also been several interesting reports on the *Aplysia* CPEB (ApCPEB). This protein
257 has been shown to have prion-like properties [86, 87], has high stability [88] and can localize
258 to RNP granules [89]. A conformational switch in ApCPEB has been described, involving the
259 conversation of soluble α-helix-rich oligomers to β-sheet-rich fibrils [90]. Activation of 5-HT
260 receptors can downregulate miR-22, leading to increased expression of ApCPEB and
261 activation of target genes supporting long-term facilitation (LTF, *Aplysia* equivalent of LTP)
262 [36]. However, this is dependent on local protein synthesis as its inhibition blocks LTF [91].
263 ApCPEB co-operates with the granule protein Staufen to ensure correct localization of
264 syntaxin mRNA [92], a system which may also operate in mammalian cells [37].
265
266 *CPEB in neurodevelopmental disorders and neurogenesis*
267 Adult neurogenesis is a key process underpinning learning and memory, primarily through the
268 formation of new neurons from neural progenitor cells (NPCs) in the hippocampus [93]. Recent
269 work has revealed a key role for CPEB3 in modulating transcripts associated with
270 neurogenesis, such as *Nav2, Lcn2 and Cyld* [94]. CPEB3 regulates the alternative splicing of
271 pre-mRNA for these genes, thereby influencing the production of functionally distinct protein
272 isoforms. *Nav2* (neuron navigator 2) is essential for axonal guidance and brain development
273 [95], and has been identified as a genetic risk factor for Alzheimer's disease [96, 97]. *Lcn2,*

274 which encodes lipocalin-2, contributes to neurogenesis, stem cell proliferation and
275 neuroinflammation, and has been linked to performance in spatial memory tasks [98, 99]. *Cyld*
276 encodes a lysine 63 deubiquitinase involved in synaptic signalling and fear memory formation
277 [100]. Mutations of Cyld have been linked to memory loss in frontotemporal dementia [101].
278 Finally, CPEB3 regulates FosB, a transcription factor that is critically involved in hippocampal
279 neurogenesis [102, 103], further highlighting the importance of CPEB3 in the molecular
280 regulation of adult brain plasticity. Together the findings highlight importance of translational
281 control of neuronal function and a key role of CPEBs in this activity.
282
283 CPEB3 has been implicated in the neurodevelopmental disorder Fragile X Syndrome (FXS).
284 Here CPEB3 is reported to act as a downstream effector of Fragile X mental retardation protein
285 (FMRP) which is deficient in individuals with FXS. In *Fmr1* KO model mice (*Fmr1* encodes
286 FMRP), CPEB3 binds the *Gria2* promoter and enhances STAT5b-driven transcription,
287 resulting in elevated GluA2 expression and altered AMPAR function [104]. The nuclear import
288 of CPEB3 has also been shown to be linked to NMDAR activation [105]. The finding that the
289 *Fmr1* KO phenotype can be ameliorated by concomitant knockout of CPEB1 supports the
290 notion that these different CPEB family members have distinct roles in controlling translation
291 in neurons [106].
292
293 Recent work has also linked CPEB function to Down's syndrome and autism spectrum
294 disorder (ASD), both of which can be associated with learning and memory deficits [107, 108].
295 This is mediated, at least in part, via the modulation of Down's syndrome cell adhesion
296 molecule (DSCAM), which plays a critical role in neural development and synaptic connectivity
297 [108, 109]. DSCAM has been shown to associate with CPEB1 in the dendrites of
298 hippocampus, regulating local translation of DSCAM upon neuronal activation [110].
299 Dysregulation of DSCAM expression has not only been linked to the cognitive deficits
300 observed in Down's syndrome [111], but also to autism-like behaviours and impairments in
301 spatial memory in animal models [112, 113]. These findings suggest that CPEB-mediated
302 control of DSCAM expression may represent a convergent molecular mechanism underlying
303 shared cognitive phenotypes across these disorders.
304
305 It has been suggested that addictive behaviour is an example of perturbed learning and
306 memory pathways [114, 115]. Interestingly, cocaine administration in mice causes significant
307 increases in striatal CPEB1 and CPEB3 expression, while mice deficient in CPEB1 and
308 CPEB3 showed reduced addition behaviours, again linking CPEBs to learning and memory.
309 Cocaine administration also caused significant increases in the transcription factor ΔFosB,
310 which is closely associated with addiction. This increase was not seen in CPEB1/3 knockout
311 mice, suggesting that CPEB1/3 may regulate translation of FosB and its splice variant ΔFosB
312 [103]. In addition to well described roles in addiction, FosB is also involved in spatial memory
313 [116, 117] and learning [102], and targeting ΔFosB signalling has been shown to restore
314 memory in a model of Alzheimer's disease [118]. In addition, rodent with sub-chronic exposure
315 to aluminium was shown to cause learning and memory deficits, mediated by miR-353-5p
316 repression of CPEB3 [119]. Similarly, exposure to prenatal diesel exhaust particulates resulted
317 in reduced CPEB3 expression, alterations in NMDAR subunit expression and defects in
318 hippocampal learning and memory [120]. Hence, exposure to drugs and other potentially toxic
319 compounds that are associated with memory defects are united by a role of CPEB in

320   translational control.

321

322   Overall, these data highlight the central role of CPEBs in gene regulatory hubs that govern
323   brain development and memory. Further understanding the function of CPEBs could provide
324   further insights on both neurodevelopmental and neurodegenerative disorders. While these
325   novel findings may pave the way for novel therapeutic strategies, it is unlikely that direct
326   targeting of CPEB3 is a viable therapeutic option given its links to cancer, including
327   neuroblastoma [121].

328

329   **4. CPEB structure**

330   ***Domain organisation***

331   AlphaFold predicted structures of all CPEB paralogues reveal a predominantly disordered
332   NTD with some structured segments interspersed throughout [28]. NMR analysis of the NTD
333   of CPEB3 using^{13}C labelled protein confirmed these models showing the disordered nature of
334   the NTD, which lacks secondary structure across the 426-residue region (**Figure 3**). However,
335   transient alpha-helix formation, involving residues 1-10, 202-210, 222-234 and 238-246 are
336   observed, along with partially populated polyproline II helices (PPII) at residues 86-93 and
337   166-175, highlighting the potential for these regions to form structured elements [122]. Two
338   prion-like domains (PrLD) have been identified (PRD1: residues 1-217 and PRD2 residues
339   284-449 in mouse CPEB3). When expressed and purified, PRD1 was shown to be capable of
340   forming amyloid-like fibrils [123, 124], with the amyloid core region involving residues 101-
341   194, as identified by hydrogen-deuterium exchange (HDX-NMR) and limited proteolysis [124].
342   This supports the view that self-assembly into amyloid-like fibrils potentially could be involved
343   in the function of CPEB3, suggesting that CPEBs might belong to the growing class of
344   functional amyloids [125].

345

346   Across different animal classes, the NTD of CPEB3 homologues are variable amongst
347   different sequences, with similarity to human CPEB3 ranging from 94% in dolphin to 49% in
348   zebrafish (**Table 1**). In contrast, the C-terminal domain (CTD) (residues 442-698 in human
349   CPEB3 (**Figure 3**)) is highly conserved, with sequence similarity to human CPEB3 ranging
350   from 99% in fish to 100% in all other classes (**Table 1**). This suggests a strong evolutionary
351   pressure to conserve the RNA-binding CTD, a constraint not observed in the more variable
352   NTD.

353

354   Within the NTD of human CPEB3, residues Q164-T325 form an actin-binding domain (ABD).
355   This contains a chameleon sequence (R192-K220), which can adopt two different secondary
356   structure conformations: α-helix or β-hairpin. Binding of F-actin to the CPEB3 ABD triggers
357   aggregation by promoting β-hairpin formation in this chameleon sequence [126]. In addition,
358   SUMOylation of CPEB3, potentially at K220, potentially could inhibit the ABD-F-actin
359   interaction, modulating the conformational preferences of this region, affecting CPEB function
360   [42, 126, 127].

361

362   PrLDs, such as those found in CPEB3, are commonly associated with RNP assemblies [128],
363   many of which are structurally defined, but functionally ambiguous [129]. In cultured mouse
364   neurons, CPEB3 has been shown to colocalise with Staufen-2 and FMRP in neuronal RNP

365 granules, both of which are associated with memory formation [9, 37]. Several other RBPs,
366 including Gadd45α and the ELAV family, have also been implicated with memory formation
367 [130-132]. These finding suggest that self-assembly of CPEBs into higher order assemblies
368 may be important in its function, as we discuss below.
369

370 ***Functional amyloid and CPEB3***

371 Amyloid fibrils are composed of protofilaments which are linear stacks of monomeric protein
372 subunits arranged in β-sheet layers spaced approximately 4.7-4.9 Å apart [133, 134]. Although
373 pathological amyloid assemblies have been linked to neurodegenerative diseases, such as
374 Alzheimer's disease and Parkinson's disease [135], several examples of functional amyloids
375 exist. These can be reversible, are typically stimulus-dependent and serve disparate
376 physiological functions. Examples include amyloids in bacteria (CsgA and FapC, involved in
377 biofilm formation), in archaea (MspA, wherein amyloid forms the protective outer sheath) [136],
378 and in humans (storage of peptide hormones [137]; promoting cell migration (fibronectin)
379 [138], synthesis of melanin in melanosomes (Pmel17) [139], and in cell death caused by
380 necroptosis (RIPK1 and RIPK3)) [140].
381

382 *Drosophila* Orb2 has been shown to form amyloid fibrils in which the fibril core comprises just
383 31 residues from its 704 amino acid sequence. Structural solution of these fibrils, extracted
384 directly from the brains of *Drosophila*, using cryogenic electron microscopy (cryoEM) showed
385 that the amyloid fibril core adopts a cross-β structure stabilised by inter-digitated glutamine
386 residues that form a hydrophilic hairpin [141] (**Figure 1B**). The fibril core is separated from the
387 RNA-binding domains by long, flexible regions rich in glycine and serine residues, offering
388 high conformational flexibility to these regions in a fuzzy 'halo' that surrounds the fibril core
389 (see image in **Figure 1B**) [141]. Immunogold labelling has demonstrated a potential functional
390 switch from a translation repression complex in the monomeric state to a translation activation
391 complex in the fibrillar state, upon which target mRNA remains bound [141], strongly
392 suggesting that, at least for Orb2, protein-self-assembly is involved in its functional role in
393 translation control (**Figure 1B**).
394

395 In *Drosophila melanogaster*, two Orb2 proteins appear to work in tandem, with Orb2B being
396 far more highly expressed and also more resistant to aggregation. Orb2A and 2B have
397 common glutamine-rich and RNA-binding domains, but differ in their N-terminal sequences:
398 Orb2A has a short, unique sequence before its Gln/His-rich region, while Orb2B has a longer,
399 serine-rich region at its N-terminus [142]. Orb2A is thought to be essential for the formation of
400 Orb2 fibrils; despite 97.5% of the total monomeric subunits in ex vivo Orb2 fibrils being Orb2B
401 [141], fibril assembly does not occur in the absence of Orb2A. Hence, Orb2A is required to
402 initiate Orb2B fibril formation [76]. In addition, calmodulin binding to Orb2A can inhibit fibril
403 formation [143]. The Orb2A N-terminal domain is 153 residues shorter than Orb2B and in
404 isolation the N-terminal 88 residues of Orb2A rapidly assemble into amyloid fibrils, albeit with
405 a different structure and including different residues in the ordered core when compared with
406 the *ex vivo* fibril structure [142]. The N-terminal 20 residues on Orb2A have also been
407 demonstrated to bind lipid bilayers, perhaps regulating Orb2A (and therefore all Orb2) fibril
408 assembly [144]. In vertebrates, CPEB3 appears to work in isolation and so the mechanism for
409 its fibril assembly differs significantly from Orb2.

410

411 Proteins enriched in glutamine (or asparagine) residues have been shown to form reversible
412 amyloid-like inclusions in yeast that are required for the inheritance of phenotypic features,
413 and hence to have prion-like capabilities [145]. Aligned with these findings, a Gln/Asp-rich
414 region in ApCPEB was also found to enable the formation of insoluble, aggregated multimers,
415 which has been implied to support long term synaptic changes [86, 87, 91, 146-148]. This self-
416 sustaining biochemical reaction and continuous macromolecular synthesis would offer
417 potential solution to the paradox of how long-lasting synaptic modifications can persist despite
418 the continual turnover of synaptic proteins [11, 149]: just the kind of protein that Crick
419 suggested would be required for memory formation [11].

420

421 Based on the sequence similarity to these Gln/Asp-rich proteins in its NTD, CPEB3 has been
422 proposed to form functional amyloids, though direct *in vivo* evidence remains limited. *In vitro*,
423 PRD1 can form fibrils as shown by negative stain EM. These fibrils bind Congo Red and
424 Thioflavin T, and exhibit β-sheet rich circular dichroism spectra, classic features of amyloid
425 [123, 124, 134] Furthermore, detergent-insoluble CPEB3 has been isolated from chick brain
426 and cultured neurons [62, 63]. However, amyloid-like CPEB3 assemblies have not been
427 observed definitively *in vivo* or *ex vivo* following physiological stimuli. With respect to their
428 primary sequences, CPEB2 and CPEB3 have the lowest sequence complexity and highest
429 repetitiveness among the vertebrate paralogues, features known to facilitate both LLPS and
430 amyloid fibril formation [28]. Hence despite much excitement and interest, the functional role
431 of protein self-assembly in vertebrate CPEB function remains speculative, due to the lack of
432 structural information on the insoluble aggregates in a physiological context [4] (**Figure 1B**).

433

434 ***Glutamine-rich regions and a role of proline in CPEB3***

435 Protein sequences with high glutamine content have long been linked to aggregation
436 propensity [150]. These regions can be divided into two broad categories. Those that contain
437 poly-glutamine tracts such in huntingtin, members of the ataxin family, TATA-box binding
438 protein and the androgen receptor [151]. Generally, the poly-glutamine tract in these proteins
439 is expanded in length in disease. For example, in huntingtin (associated with Huntington's
440 disease), there are 6-35 glutamine residues in the poly-Gln tract in normal cells, and this is
441 expanded to 36-121 repeats in disease [152]. The second category includes proteins that
442 contain Gln-rich regions in their sequence. CPEB3 belongs to this second category of proteins.
443 Human CPEB3 contains a Gln-rich region between residues Gln13 and Gln27, which contains
444 10 glutamine residues in total, but no more than four consecutive glutamines [153].

445

446 Interestingly, several of these proteins have proline-rich regions adjacent to the poly-Gln tract
447 (e.g. ataxin 2, ataxin 7, androgen receptor, huntingtin). In the case of huntingtin, expansion of
448 the proline-rich region has been shown to increase protein solubility and mitigate the toxicity
449 associated with poly-Gln tract expansion, suggestive of a role of proline-rich region in
450 protecting the proteins from aggregation [154]. Notably, CPEB3 also contains Gln-rich regions
451 interspersed with proline residues (**Figure 3**) [153]. This pattern of proline-rich regions flanking
452 Gln-rich segments is also observed in other RNA-binding proteins such as TIA-1 (T-cell
453 Intracellular Antigen 1), TIAR (T-cell intracellular antigen 1-related protein), FUS (Fused in
454 Sarcoma protein) and SFPQ (Splicing Factor Proline/ Glutamine rich protein), all of which are

455 associated with the formation of dynamic cytosolic granules [155-157]. Mutation of the proline-
456 rich region in TIA-1 has been shown to promote fibril formation and reduces granule
457 disassembly [115], further supporting a functional role for the proline-rich segments in
458 modulating aggregation. In CPEB3, PPII helices that form in these protein-rich segments may
459 contribute to the formation of insoluble CPEB3 assemblies [122]. The proline content and
460 poly(proline) length in CPEB3 have increased over evolutionary time, particularly in primates
461 compared to cartilaginous fish *Chondrichthyes* [28], suggestive perhaps of specific roles of
462 the balance of proline-rich and glutamine-rich sequences in different organisms.
463
464 Overall, therefore, it is evident that the conserved, and unusual patterning of the amino acid
465 sequence in CPEBs are of functional significance, and that their sequences may facilitate
466 protein self-assembly into aggregates, reversible condensates and/or stable amyloid fibrils,
467 possibly involving binding of RNA via their RRMs. However, there is still much to learn about
468 whether, and precisely how, these sequence features and their biophysical properties
469 contribute to memory formation.
470
471 5. **Progress and extant questions**
472
473 The progress in the field of CPEB proteins, particularly CPEB3, in learning and memory can
474 be assessed by the robustness and quality of the data addressing three key questions:
475
476 (i) Does CPEB3 have a role in human memory or related functions?
477 (ii) What are the functional consequences of CPEB3 dysfunction?
478 (iii) What are the molecular mechanisms underpinning CPEB3 function?
479
480 Two papers have directly addressed the role of CPEB3 in human memory, by Vogler *et al.* and
481 Yerdenova *et al.* [67, 69]. These reports assessed the correlation of the rs11186856 SNP
482 (intronic CPEB3 variant) with performance in an episodic memory task or foreign language
483 proficiency, respectively. This SNP is located in the CPEB3 ribozyme and is associated with
484 enhanced self-cleavage. The data from Vogler *et al.* report a strong correlation between
485 CPEB3 and outcome in a memory assessment, but the population size was modest (total
486 cohort 333 individuals) and causation cannot be inferred [67]. Yerdenova *et al.* reported
487 CPEB3 as part of a cohort of six other genes and foreign language proficiency was self-
488 reported [69]. The only other *CPEB3* SNP (rs855708, intronic) linked with brain function is
489 significantly associated with a panel of psychiatric disorders. However, it is of note that this
490 was a genome-wide association study, hence *CPEB3* was one of many genes reported [158].
491
492 The only direct investigation of CPEB expression in human brain samples showed significant
493 increases in CPEB1 and decreases in CPEB4 in Huntington's disease brains, but with no
494 significant changes in CPEB2 or CPEB3 [159]. In addition, risk genes for ASD contain CPEs
495 and are bound by CPEB4, which is mis-spliced in ASD, leading to risk gene deadenylation
496 [160].
497
498 It may be possible some make some inference on the involvement of CPEB3 in memory
499 dysfunction via analysis of regulatory microRNA. It has been reported that CPEB3 is regulated
500 by miR-26, miR-92 [34], miR-351 [119] and miR-21 [161] in neuronal models and miR-20 in

non-neuronal cells [162]. Furthermore, miR-92 is upregulated in neuronal extracellular vesicles from individuals diagnosed with frontotemporal dementia [163] and in Alzheimer's disease (AD) brain [164] and plasma samples [165]. Interestingly, miR-92-mediated repression of CPEB3 was beneficial in a mouse model of experimental autoimmune encephalomyelitis [35]. miR-20 and miR-26 are also increased in AD brain and blood samples, respectively [166, 167], but regulation of CPEB3 by miR-20 has only been shown in cultured hepatocellular carcinoma cells [162].

Overall, robust evidence for the role of CPEBs in human memory is sparse, based predominantly on a single study [67], hence future work is needed. Data supporting the role of CPEBs in diseases of memory dysfunction are also still speculative at the current time, with evidence predominantly based on changes in regulatory microRNAs. The complexity here is illustrated by the reported increase in miR-26 in AD [167], which may be expected to repress *CPEB3*, but also targets *BDNF*, which has a central role in AD [168]. Therefore, inferring a causal role for CPEB3 from these data is challenging.

In terms of mechanism, supporting data can be separated into structural and functional. Structurally, although recombinant CPEB3 PRD1 has been shown to form fibrils and the structure solved, to high resolution using cryo-EM [123, 124], this has not yet been shown to be the case for the full-length protein. Similarly, although Orb2 fibrils have been extracted from *Drosophila* brain and their structure solved to near atomic resolution [141], this has not been reported for any vertebrate CPEBs. In addition, there has been no demonstration of CPEB3 amyloid formation in cultured cells as determined by either fluorescent amyloid-specific dyes or by their extraction and structural characterisation. The notion of CPEB3 inclusion formation *in vitro* and *in vivo* rests on the formation of microscopic puncta or detergent-insoluble species [62, 63]. So, while it is possible that CPEB3 forms amyloid fibrils, this remains to be demonstrated.

Given that CPEB3 contains canonical RNA-recognition motifs [169] and the presence of similar domains in a range of other RNA-binding proteins [93], it is highly likely that CPEB3 functional as an RNA-binding protein in human neurons. Furthermore, RNA immunoprecipitation experiments have demonstrated CPEB3 binding to a range of targets in cultured mouse cells and mouse brain [58, 94]. However, transcriptomic analysis of this kind has yet to be performed in human cells or tissue, hence the RNA targets of CPEB3 in humans remain unclear.

The existence of temporally-regulated RNA granules, including P bodies, is now well-established and these granules can be purified [170, 171]. RBPs have been linked to these granules, including as part of a cellular stress response [172, 173]. The question, therefore, is the extent to which it has been demonstrated that CPEB3 can be recruited to P-bodies or other granules. CPEB3 has been shown to colocalise with P-body [153] and neuronal RNP markers [37], albeit using over-expression in mouse or non-neuronal cells. So, while there is some good evidence for CPEB3 localisation to RNA granules, this remains to be demonstrated for endogenous CPEB3 in human neuronal cells. Similarly, these caveats also apply to the regulation of CPEB3 by SUMOylation and the ability of CPEB3 to regulate glutamate receptor subunits [32, 42, 174].

Delineating the precise role of CPEB3 in memory is challenging. Initially, it was reported that CPEB3 knockout mice displayed enhancement of both short-term fear conditioning and long-term spatial memory [58, 64]. Similarly, inhibition of miR-92 leads to CPEB3 activation in mouse hippocampus, which impaired fear conditioning [175]. Yet, CPEB3 knockout mice were also shown to have impaired hippocampal LTP and spatial memory [63]. Conversely, aluminium-induced long-term spatial memory deficits in rats were alleviated by miR-351 inhibition and recovery of CPEB3 expression [119]. In line with this, inhibition of the *Cpeb3* ribozyme increased CPEB3 expression and improved object location memory [68]. The ability of CPEB3 to form detergent-insoluble species has also been linked to learning processes, specifically filial imprinting in chicks and spatial memory in mice [61-63]. It is likely that these conflicting data on the precise role of CPEB3 in memory derive from two potential opposing functions of CPEB3. Chao *et al.* reported that CPEB3 was acting to repress key transcripts encoding GluA1, PSD95 and NMDA receptor subunits [58], which may have been due to sequestration of these transcripts in RNA granules [153]. However, Chen *et al.* reported the opposite effect, insofar as CPEB3 enhanced expression of these proteins, ascribing this to increased polyadenylation of the relevant mRNA transcripts [68].

It is likely that CPEB3 is able to repress mRNA translation via sequestration and activate via polyadenylation, based on unclear, but potentially highly dynamic, regulatory mechanisms. Published data support a role for CPEB3 in memory processes, but the precise mechanism for any such roles remains unclear. Future analysis of human tissue and biofluids alongside advanced human cell models, such as neurons derived from induced pluripotent stem cells, will be needed to further our understanding.

Of course, the most significant overarching question is how these data integrate with Crick's prediction from over forty years ago. Might CPEB3 represent a "macromolecule which is relatively immune from molecular turnover" [11]? CPEB3 certainly has a primary sequence consistent with a protein capable of forming aggregated assemblies and certain isolated domains can form fibrils in a cell-free system [123]. Learning-related tasks can induce the formation of detergent-insoluble CPEB3 species [63]. However, the cellular lifespan of these species is unclear i.e. the extent to which they are relatively immune from molecular turnover or are able to template their structure onto newly synthesised CPEB monomers via fibril seeding activity. A pulse chase experiment could provide such information, but the ability to generate insoluble aggregates of endogenous CPEB3 in cultured cells has not yet been demonstrated. Also unclear is how the process of aggregation and amyloid formation regulates the apparent switch between target transcript polyadenylation or sequestration, although there are some interesting findings in *Drosphila* as to how this might be achieved [78].

6. Concluding remarks

Memory encoding and retrieval remain an enigmatic phenomenon. This review presents key evidence supporting the role of CPEBs in maintaining long term memory. There is a wealth of data now that support a role of CPEB3 in regulating the translation of dormant mRNAs at synapses, including those encoding AMPA and NMDA receptor subunits. This regulation enhances synaptic trafficking and modulates synaptic strength, both of which are essential for

592 long term memory consolidation. Structurally, CPEB3 is well suited for this function. Its
593 disordered NTD, enriched in glutamine and proline residues, promotes reversible aggregation
594 and phase separation. These properties enable its localisation to ribonucleoprotein granules
595 and may support the formation of functional amyloids. The conserved CTD contains RNA
596 recognition motifs that are critical for binding target mRNAs. Together, these domains allow
597 CPEB3 to act as a molecular switch, linking transient synaptic signals to sustained protein
598 synthesis.
599
600 Despite these advances, many questions remain. It is not yet clear whether CPEB3
601 aggregation depends solely on NMDA receptor signalling, or if other stimuli, such as cellular
602 stress, can also trigger this process. The role of SUMOylation in relieving translational
603 repression, the formation and reversibility of amyloid-like structures *in vivo*, and the extent of
604 CPEB3 regulation beyond glutamatergic pathways are all active areas of investigation.
605 Additionally, the functional significance of proteolytic cleavage remains to be determined. A
606 more detailed understanding of the individual sequence regions and any potential binding
607 partners would be of great interest and addressing such key extant questions will drive this
608 exciting field forward in the future, so that the enigmatic question of how long terms memories
609 are made and retrieved at a molecular level may soon be better understood.
610

614

615 **Author contributions**

616 David A. Hicks: conceptualisation, visualisation, writing- original draft (lead), writing- review
617 and editing; Liam D. Aubrey: visualisation, writing- original draft (supporting), writing- review
618 and editing; Jessica C. F. Kwok: writing- review and editing; Sheena E. Radford: writing- review
619 and editing.
620

621 **Conflicts of interests**
622 The authors have no conflicts of interest to declare.
623

624 **Data availability statement**
625 Not applicable
626

References

1. Ortega-de San Luis, C. and T.J. Ryan, *Understanding the physical basis of memory: Molecular mechanisms of the engram.* J Biol Chem, 2022. **298**(5): p. 101866.

2. Merlo, S.A., et al., *Memory persistence: from fundamental mechanisms to translational opportunities.* Transl Psychiatry, 2024. **14**(1): p. 98.

3. Kupke, J. and A.M.M. Oliveira, *The molecular and cellular basis of memory engrams: Mechanisms of synaptic and systems consolidation.* Neurobiol Learn Mem, 2025. **219**: p. 108057.

4. Huang, Y.S., et al., *CPEB and translational control by cytoplasmic polyadenylation: impact on synaptic plasticity, learning, and memory.* Mol Psychiatry, 2023. **28**(7): p. 2728-2736.

5. Malinow, R., *AMPA receptor trafficking and long-term potentiation.* Philos Trans R Soc Lond B Biol Sci, 2003. **358**(1432): p. 707-14.

6. Matsuo, N., L. Reijmers, and M. Mayford, *Spine-type-specific recruitment of newly synthesized AMPA receptors with learning.* Science, 2008. **319**(5866): p. 1104-7.

7. Pereyra, M. and J.H. Medina, *AMPA Receptors: A Key Piece in the Puzzle of Memory Retrieval.* Front Hum Neurosci, 2021. **15**: p. 729051.

8. de Leon-Lopez, C.A.M., M. Carretero-Rey, and Z.U. Khan, *AMPA Receptors in Synaptic Plasticity, Memory Function, and Brain Diseases.* Cell Mol Neurobiol, 2025. **45**(1): p. 14.

9. Sudhakaran, I.P. and M. Ramaswami, *Long-term memory consolidation: The role of RNA-binding proteins with prion-like domains.* RNA Biol, 2017. **14**(5): p. 568-586.

10. Naskar, A., et al., *Phase separation and pathologic transitions of RNP condensates in neurons: implications for amyotrophic lateral sclerosis, frontotemporal dementia and other neurodegenerative disorders.* Front Mol Neurosci, 2023. **16**: p. 1242925.

11. Crick, F., *Memory and molecular turnover.* Nature, 1984. **312**(5990): p. 101.

12. Lev-Ram, V., et al., *Do Perineuronal Nets Stabilize the Engram of a Synaptic Circuit?* Cells, 2024. **13**(19).

13. Tompa, P. and P. Friedrich, *Prion proteins as memory molecules: an hypothesis.* Neuroscience, 1998. **86**(4): p. 1037-43.

14. Rosenthal, E.T., T.R. Tansey, and J.V. Ruderman, *Sequence-specific adenylations and deadenylations accompany changes in the translation of maternal messenger RNA after fertilization of Spisula oocytes.* J Mol Biol, 1983. **166**(3): p. 309-27.

15. Fernandez, M. and R. Mendez, *Cytoplasmic regulation of the poly(A) tail length as a potential therapeutic target.* RNA, 2025. **31**(3): p. 402-415.

16. McGrew, L.L. and J.D. Richter, *Translational control by cytoplasmic polyadenylation during Xenopus oocyte maturation: characterization of cis and trans elements and regulation by cyclin/MPF.* EMBO J, 1990. **9**(11): p. 3743-51.

17. Paris, J., et al., *Maturation-specific polyadenylation: in vitro activation by p34cdc2 and phosphorylation of a 58-kD CPE-binding protein.* Genes Dev, 1991. **5**(9): p. 1697-708.

18. Simon, R., J.P. Tassan, and J.D. Richter, *Translational control by poly(A) elongation during Xenopus development: differential repression and enhancement by a novel cytoplasmic polyadenylation element.* Genes Dev, 1992. **6**(12B): p. 2580-91.

19. Hake, L.E. and J.D. Richter, *CPEB is a specificity factor that mediates cytoplasmic polyadenylation during Xenopus oocyte maturation.* Cell, 1994. **79**(4): p. 617-27.

20. Hake, L.E., R. Mendez, and J.D. Richter, *Specificity of RNA binding by CPEB: requirement for RNA recognition motifs and a novel zinc finger.* Mol Cell Biol, 1998. **18**(2): p. 685-93.

21. Merkel, D.J., et al., *The C-terminal region of cytoplasmic polyadenylation element binding protein is a ZZ domain with potential for protein-protein interactions.* J Mol Biol, 2013. **425**(11): p. 2015-2026.

676 22. Reverte, C.G., M.D. Ahearn, and L.E. Hake, *CPEB degradation during Xenopus oocyte*
677   *maturation requires a PEST domain and the 26S proteasome*. Dev Biol, 2001. **231**(2): p.
678   447-58.
679 23. Mendez, R., D. Barnard, and J.D. Richter, *Differential mRNA translation and meiotic*
680   *progression require Cdc2-mediated CPEB destruction*. EMBO J, 2002. **21**(7): p. 1833-44.
681 24. Stebbins-Boaz, B., L.E. Hake, and J.D. Richter, *CPEB controls the cytoplasmic*
682   *polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte*
683   *maturation in Xenopus*. EMBO J, 1996. **15**(10): p. 2582-92.
684 25. Kurihara, Y., et al., *CPEB2, a novel putative translational regulator in mouse haploid germ*
685   *cells*. Biol Reprod, 2003. **69**(1): p. 261-8.
686 26. Theis, M., K. Si, and E.R. Kandel, *Two previously undescribed members of the mouse*
687   *CPEB family of genes and their inducible expression in the principal cell layers of the*
688   *hippocampus*. Proc Natl Acad Sci U S A, 2003. **100**(16): p. 9602-7.
689 27. Turimella, S.L., et al., *Characterization of cytoplasmic polyadenylation element binding*
690   *2 protein expression and its RNA binding activity*. Hippocampus, 2015. **25**(5): p. 630-42.
691 28. Vaglietti, S., et al., *Divergent evolution of low-complexity regions in the vertebrate CPEB*
692   *protein family*. Front Bioinform, 2025. **5**: p. 1491735.
693 29. Duran-Arque, B., et al., *Comparative analyses of vertebrate CPEB proteins define two*
694   *subfamilies with coordinated yet distinct functions in post-transcriptional gene*
695   *regulation*. Genome Biol, 2022. **23**(1): p. 192.
696 30. Rouhana, L., et al., *Cytoplasmic Polyadenylation Is an Ancestral Hallmark of Early*
697   *Development in Animals*. Mol Biol Evol, 2023. **40**(6).
698 31. Mendez, R. and J.D. Richter, *Translational control by CPEB: a means to the end*. Nat Rev
699   Mol Cell Biol, 2001. **2**(7): p. 521-9.
700 32. Huang, Y.S., et al., *CPEB3 and CPEB4 in neurons: analysis of RNA-binding specificity*
701   *and translational control of AMPA receptor GluR2 mRNA*. EMBO J, 2006. **25**(20): p. 4865-
702   76.
703 33. Lee, S.H., et al., *ApCPEB4, a non-prion domain containing homolog of ApCPEB, is*
704   *involved in the initiation of long-term facilitation*. Mol Brain, 2016. **9**(1): p. 91.
705 34. Morgan, M., A. Iaconcig, and A.F. Muro, *CPEB2, CPEB3 and CPEB4 are coordinately*
706   *regulated by miRNAs recognizing conserved binding sites in paralog positions of their 3'-*
707   *UTRs*. Nucleic Acids Res, 2010. **38**(21): p. 7698-710.
708 35. Winkler, I., et al., *MicroRNA-92a-CPEB3 axis protects neurons against inflammatory*
709   *neurodegeneration*. Sci Adv, 2023. **9**(47): p. eadi6855.
710 36. Fiumara, F., et al., *MicroRNA-22 Gates Long-Term Heterosynaptic Plasticity in Aplysia*
711   *through Presynaptic Regulation of CPEB and Downstream Targets*. Cell Rep, 2015.
712   **11**(12): p. 1866-75.
713 37. Ford, L., et al., *CPEB3 low-complexity motif regulates local protein synthesis via protein-*
714   *protein interactions in neuronal ribonucleoprotein granules*. Proc Natl Acad Sci U S A,
715   2023. **120**(6): p. e2114747120.
716 38. Sarkissian, M., R. Mendez, and J.D. Richter, *Progesterone and insulin stimulation of*
717   *CPEB-dependent polyadenylation is regulated by Aurora A and glycogen synthase*
718   *kinase-3*. Genes Dev, 2004. **18**(1): p. 48-61.
719 39. Setoyama, D., M. Yamashita, and N. Sagata, *Mechanism of degradation of CPEB during*
720   *Xenopus oocyte maturation*. Proc Natl Acad Sci U S A, 2007. **104**(46): p. 18001-6.
721 40. Guillen-Boixet, J., et al., *CPEB4 is regulated during cell cycle by ERK2/Cdk1-mediated*
722   *phosphorylation and its assembly into liquid-like droplets*. Elife, 2016. **5**.
723 41. Pavlopoulos, E., et al., *Neuralized1 activates CPEB3: a function for nonproteolytic*
724   *ubiquitin in synaptic plasticity and memory storage*. Cell, 2011. **147**(6): p. 1369-83.
725 42. Drisaldi, B., et al., *SUMOylation Is an Inhibitory Constraint that Regulates the Prion-like*
726   *Aggregation and Activity of CPEB3*. Cell Rep, 2015. **11**(11): p. 1694-702.

727   43.   Verde, E.M., et al., *SUMO2/3 conjugation of TDP-43 protects against aggregation*. Sci
728   Adv, 2025. **11**(8): p. eadq2475.
729   44.   Keiten-Schmitz, J., et al., *SUMO: Glue or Solvent for Phase-Separated
730   Ribonucleoprotein Complexes and Molecular Condensates?* Front Mol Biosci, 2021. **8**:
731   p. 673038.
732   45.   Yau, T.Y., et al., *SUMO Interacting Motifs: Structure and Function*. Cells, 2021. **10**(11).
733   46.   Schorova, L. and S. Martin, *Sumoylation in Synaptic Function and Dysfunction*. Front
734   Synaptic Neurosci, 2016. **8**: p. 9.
735   47.   Gustin, A., et al., *Protein SUMOylation is a sex-specific regulator of fear memory
736   formation in the amygdala*. Behav Brain Res, 2022. **430**: p. 113928.
737   48.   Wu, L., et al., *CPEB-mediated cytoplasmic polyadenylation and the regulation of
738   experience-dependent translation of alpha-CaMKII mRNA at synapses*. Neuron, 1998.
739   **21**(5): p. 1129-39.
740   49.   Ouyang, Y., et al., *Visualization of the distribution of autophosphorylated
741   calcium/calmodulin-dependent protein kinase II after tetanic stimulation in the CA1
742   area of the hippocampus*. J Neurosci, 1997. **17**(14): p. 5416-27.
743   50.   Wells, D.G., et al., *A role for the cytoplasmic polyadenylation element in NMDA receptor-
744   regulated mRNA translation in neurons*. J Neurosci, 2001. **21**(24): p. 9541-8.
745   51.   Huang, Y.S., et al., *N-methyl-D-aspartate receptor signaling results in Aurora kinase-
746   catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses*.
747   EMBO J, 2002. **21**(9): p. 2139-48.
748   52.   Huang, Y.S., et al., *Facilitation of dendritic mRNA transport by CPEB*. Genes Dev, 2003.
749   **17**(5): p. 638-53.
750   53.   Atkins, C.M., et al., *Cytoplasmic polyadenylation element binding protein-dependent
751   protein synthesis is regulated by calcium/calmodulin-dependent protein kinase II*. J
752   Neurosci, 2004. **24**(22): p. 5193-201.
753   54.   Berger-Sweeney, J., N.R. Zearfoss, and J.D. Richter, *Reduced extinction of hippocampal-
754   dependent memories in CPEB knockout mice*. Learn Mem, 2006. **13**(1): p. 4-7.
755   55.   Alarcon, J.M., et al., *Selective modulation of some forms of schaffer collateral-CA1
756   synaptic plasticity in mice with a disruption of the CPEB-1 gene*. Learn Mem, 2004. **11**(3):
757   p. 318-27.
758   56.   Zearfoss, N.R., et al., *A molecular circuit composed of CPEB-1 and c-Jun controls
759   growth hormone-mediated synaptic plasticity in the mouse hippocampus*. J Neurosci,
760   2008. **28**(34): p. 8502-9.
761   57.   Atkins, C.M., et al., *Bidirectional regulation of cytoplasmic polyadenylation element-
762   binding protein phosphorylation by Ca2+/calmodulin-dependent protein kinase II and
763   protein phosphatase 1 during hippocampal long-term potentiation*. J Neurosci, 2005.
764   **25**(23): p. 5604-10.
765   58.   Chao, H.W., et al., *Deletion of CPEB3 enhances hippocampus-dependent memory via
766   increasing expressions of PSD95 and NMDA receptors*. J Neurosci, 2013. **33**(43): p.
767   17008-22.
768   59.   Huang, W.H., et al., *Elevated activation of CaMKIIalpha in the CPEB3-knockout
769   hippocampus impairs a specific form of NMDAR-dependent synaptic depotentiation*.
770   Front Cell Neurosci, 2014. **8**: p. 367.
771   60.   You, Z., et al., *CPEB3 can regulate seizure susceptibility by inhibiting the transcriptional
772   activity of STAT3 on NMDARs expression*. Mol Med, 2025. **31**(1): p. 77.
773   61.   Margvelani, G., et al., *Micro-RNAs, their target proteins, predispositions and the memory
774   of filial imprinting*. Sci Rep, 2018. **8**(1): p. 17444.
775   62.   Chitadze, L., et al., *Filial imprinting in domestic chicks; cytoplasmic polyadenylation
776   element binding protein 3, predispositions and learning*. Neuroreport, 2023. **34**(3): p.
777   144-149.

778 63. Fioriti, L., et al., *The Persistence of Hippocampal-Based Memory Requires Protein*
779 *Synthesis Mediated by the Prion-like Protein CPEB3.* Neuron, 2015. **86**(6): p. 1433-48.
780 64. Lu, W.H., et al., *CPEB3-dowregulated Nr3c1 mRNA translation confers resilience to*
781 *developing posttraumatic stress disorder-like behavior in fear-conditioned mice.*
782 Neuropsychopharmacology, 2021. **46**(9): p. 1669-1679.
783 65. Tsai, L.Y., et al., *CPEB4 knockout mice exhibit normal hippocampus-related synaptic*
784 *plasticity and memory.* PLoS One, 2013. **8**(12): p. e84978.
785 66. Salehi-Ashtiani, K., et al., *A genomewide search for ribozymes reveals an HDV-like*
786 *sequence in the human CPEB3 gene.* Science, 2006. **313**(5794): p. 1788-92.
787 67. Vogler, C., et al., *CPEB3 is associated with human episodic memory.* Front Behav
788 Neurosci, 2009. **3**: p. 4.
789 68. Chen, C.C., et al., *Inhibition of Cpeb3 ribozyme elevates CPEB3 protein expression and*
790 *polyadenylation of its target mRNAs and enhances object location memory.* Elife, 2024.
791 **13**.
792 69. Yerdenova, M.B., et al., *Association Between Genetically Predicted Memory and Self-*
793 *Reported Foreign Language Proficiency.* Genes (Basel), 2025. **16**(5).
794 70. Davis, R.L., *Learning and memory using Drosophila melanogaster: a focus on advances*
795 *made in the fifth decade of research.* Genetics, 2023. **224**(4).
796 71. Mastushita-Sakai, T., et al., *Drosophila Orb2 targets genes involved in neuronal growth,*
797 *synapse formation, and protein turnover.* Proc Natl Acad Sci U S A, 2010. **107**(26): p.
798 11987-92.
799 72. Pai, T.P., et al., *Drosophila ORB protein in two mushroom body output neurons is*
800 *necessary for long-term memory formation.* Proc Natl Acad Sci U S A, 2013. **110**(19): p.
801 7898-903.
802 73. Sanguanini, M. and A. Cattaneo, *A continuous model of physiological prion aggregation*
803 *suggests a role for Orb2 in gating long-term synaptic information.* R Soc Open Sci, 2018.
804 **5**(12): p. 180336.
805 74. Stepien, B.K., et al., *RNA-binding profiles of Drosophila CPEB proteins Orb and Orb2.*
806 Proc Natl Acad Sci U S A, 2016. **113**(45): p. E7030-E7038.
807 75. Keleman, K., et al., *Function of the Drosophila CPEB protein Orb2 in long-term courtship*
808 *memory.* Nat Neurosci, 2007. **10**(12): p. 1587-93.
809 76. Majumdar, A., et al., *Critical role of amyloid-like oligomers of Drosophila Orb2 in the*
810 *persistence of memory.* Cell, 2012. **148**(3): p. 515-29.
811 77. Kimura, S., et al., *The Drosophila lingerer protein cooperates with Orb2 in long-term*
812 *memory formation.* J Neurogenet, 2015. **29**(1): p. 8-17.
813 78. Khan, M.R., et al., *Amyloidogenic Oligomerization Transforms Drosophila Orb2 from a*
814 *Translation Repressor to an Activator.* Cell, 2015. **163**(6): p. 1468-83.
815 79. Kruttner, S., et al., *Drosophila CPEB Orb2A mediates memory independent of Its RNA-*
816 *binding domain.* Neuron, 2012. **76**(2): p. 383-95.
817 80. Desai, M., et al., *Mrj is a chaperone of the Hsp40 family that regulates Orb2*
818 *oligomerization and long-term memory in Drosophila.* PLoS Biol, 2024. **22**(4): p.
819 e3002585.
820 81. Li, L., et al., *A Putative Biochemical Engram of Long-Term Memory.* Curr Biol, 2016.
821 **26**(23): p. 3143-3156.
822 82. Kozlov, E.N., et al., *3'UTR of mRNA Encoding CPEB Protein Orb2 Plays an Essential Role*
823 *in Intracellular Transport in Neurons.* Cells, 2023. **12**(13).
824 83. Kozlov, E.N., et al., *Long-Term Memory Formation in Drosophila Depends on the 3'UTR of*
825 *CPEB Gene orb2.* Cells, 2023. **12**(2).
826 84. Gill, J., et al., *Regulated Intron Removal Integrates Motivational State and Experience.*
827 Cell, 2017. **169**(5): p. 836-848 e15.

828 85.   Kruttner, S., et al., *Synaptic Orb2A Bridges Memory Acquisition and Late Memory*
829   *Consolidation in Drosophila.* Cell Rep, 2015. **11**(12): p. 1953-65.

830 86.   Si, K., S. Lindquist, and E.R. Kandel, *A neuronal isoform of the aplysia CPEB has prion-*
831   *like properties.* Cell, 2003. **115**(7): p. 879-91.

832 87.   Si, K., et al., *A neuronal isoform of CPEB regulates local protein synthesis and stabilizes*
833   *synapse-specific long-term facilitation in aplysia.* Cell, 2003. **115**(7): p. 893-904.

834 88.   Heinrich, S.U. and S. Lindquist, *Protein-only mechanism induces self-perpetuating*
835   *changes in the activity of neuronal Aplysia cytoplasmic polyadenylation element binding*
836   *protein (CPEB).* Proc Natl Acad Sci U S A, 2011. **108**(7): p. 2999-3004.

837 89.   Chae, Y.S., et al., *Neuronal RNA granule contains ApCPEB1, a novel cytoplasmic*
838   *polyadenylation element binding protein, in Aplysia sensory neuron.* Exp Mol Med, 2010.
839   **42**(1): p. 30-7.

840 90.   Raveendra, B.L., et al., *Characterization of prion-like conformational changes of the*
841   *neuronal isoform of Aplysia CPEB.* Nat Struct Mol Biol, 2013. **20**(4): p. 495-501.

842 91.   Miniaci, M.C., et al., *Sustained CPEB-dependent local protein synthesis is required to*
843   *stabilize synaptic growth for persistence of long-term facilitation in Aplysia.* Neuron,
844   2008. **59**(6): p. 1024-36.

845 92.   Liu, J., et al., *Two mRNA-binding proteins regulate the distribution of syntaxin mRNA in*
846   *Aplysia sensory neurons.* J Neurosci, 2006. **26**(19): p. 5204-14.

847 93.   Chan, J.N., et al., *RNA-binding protein signaling in adult neurogenesis.* Front Cell Dev
848   Biol, 2022. **10**: p. 982549.

849 94.   Qu, W., et al., *CPEB3 regulates neuron-specific alternative splicing and involves*
850   *neurogenesis gene expression.* Aging (Albany NY), 2020. **13**(2): p. 2330-2347.

851 95.   Accogli, A., et al., *Loss of Neuron Navigator 2 Impairs Brain and Cerebellar*
852   *Development.* Cerebellum, 2023. **22**(2): p. 206-222.

853 96.   Yan, J., et al., *Hippocampal transcriptome-guided genetic analysis of correlated*
854   *episodic memory phenotypes in Alzheimer's disease.* Front Genet, 2015. **6**: p. 117.

855 97.   Wang, K.S., et al., *Family-based association analysis of NAV2 gene with the risk and age*
856   *at onset of Alzheimer's disease.* J Neuroimmunol, 2017. **310**: p. 60-65.

857 98.   Ferreira, A.C., et al., *Lipocalin-2 is involved in emotional behaviors and cognitive*
858   *function.* Front Cell Neurosci, 2013. **7**: p. 122.

859 99.   Ferreira, A.C., et al., *Lipocalin-2 regulates adult neurogenesis and contextual*
860   *discriminative behaviours.* Mol Psychiatry, 2018. **23**(4): p. 1031-1039.

861 100.   Li, H., et al., *Glutamatergic CYLD deletion leads to aberrant excitatory activity in the*
862   *basolateral amygdala: association with enhanced cued fear expression.* Neural Regen
863   Res, 2025. **20**(11): p. 3259-3272.

864 101.   Tabuas-Pereira, M., et al., *CYLD variants in frontotemporal dementia associated with*
865   *severe memory impairment in a Portuguese cohort.* Brain, 2020. **143**(8): p. e67.

866 102.   Manning, C.E., et al., *Hippocampal Subgranular Zone FosB Expression Is Critical for*
867   *Neurogenesis and Learning.* Neuroscience, 2019. **406**: p. 225-233.

868 103.   Drisaldi, B., et al., *Cytoplasmic Polyadenylation Element Binding Proteins CPEB1 and*
869   *CPEB3 Regulate the Translation of FosB and Are Required for Maintaining Addiction-Like*
870   *Behaviors Induced by Cocaine.* Front Cell Neurosci, 2020. **14**: p. 207.

871 104.   Hwang, J.Y., et al., *CPEB3-dependent increase in GluA2 subunits impairs excitatory*
872   *transmission onto inhibitory interneurons in a mouse model of fragile X.* Cell Rep, 2022.
873   **39**(10): p. 110853.

874 105.   Chao, H.W., et al., *NMDAR signaling facilitates the IPO5-mediated nuclear import of*
875   *CPEB3.* Nucleic Acids Res, 2012. **40**(17): p. 8484-98.

876 106.   Udagawa, T., et al., *Genetic and acute CPEB1 depletion ameliorate fragile X*
877   *pathophysiology.* Nat Med, 2013. **19**(11): p. 1473-7.

878 107. Casanas, J.J., et al., *CPEB1 is overexpressed in neurons derived from Down syndrome*
879   *IPSCs and in the hippocampus of the mouse model Ts1Cje*. Mol Cell Neurosci, 2019. **95**:
880   p. 79-85.
881 108. Montesinos, M.L., *Local translation of the Down syndrome cell adhesion molecule*
882   *(DSCAM) mRNA in the vertebrate central nervous system*. J Neurogenet, 2017. **31**(4): p.
883   223-230.
884 109. Jain, S. and K. Welshhans, *Netrin-1 induces local translation of down syndrome cell*
885   *adhesion molecule in axonal growth cones*. Dev Neurobiol, 2016. **76**(7): p. 799-816.
886 110. Alves-Sampaio, A., J.A. Troca-Marin, and M.L. Montesinos, *NMDA-mediated regulation*
887   *of DSCAM dendritic local translation is lost in a mouse model of Down's syndrome*. J
888   Neurosci, 2010. **30**(40): p. 13537-48.
889 111. Emili, M., et al., *Reversal of neurodevelopmental impairment and cognitive*
890   *enhancement by pharmacological intervention with the polyphenol polydatin in a Down*
891   *syndrome model*. Neuropharmacology, 2024. **261**: p. 110170.
892 112. Chen, P., et al., *DSCAM Deficiency Leads to Premature Spine Maturation and Autism-like*
893   *Behaviors*. J Neurosci, 2022. **42**(4): p. 532-551.
894 113. Neff, R.C., et al., *Cognitive behavioral phenotyping of DSCAM heterozygosity as a model*
895   *for autism spectrum disorder*. Genes Brain Behav, 2024. **23**(5): p. e70002.
896 114. Thomas, M.J., P.W. Kalivas, and Y. Shaham, *Neuroplasticity in the mesolimbic dopamine*
897   *system and cocaine addiction*. Br J Pharmacol, 2008. **154**(2): p. 327-42.
898 115. Ding, X., et al., *Disease-associated mutations affect TIA1 phase separation and*
899   *aggregation in a proline-dependent manner*. Brain Res, 2021. **1768**: p. 147589.
900 116. Solecki, W., et al., *Role of fosB in behaviours related to morphine reward and spatial*
901   *memory*. Behav Brain Res, 2008. **190**(2): p. 212-7.
902 117. Lamothe-Molina, P.J., et al., *DeltaFosB accumulation in hippocampal granule cells*
903   *drives cFos pattern separation during spatial learning*. Nat Commun, 2022. **13**(1): p.
904   6376.
905 118. Corbett, B.F., et al., *DeltaFosB Regulates Gene Expression and Cognitive Dysfunction in*
906   *a Mouse Model of Alzheimer's Disease*. Cell Rep, 2017. **20**(2): p. 344-355.
907 119. Ji, J., et al., *miR-351-5p regulation of CPEB3 affecting aluminium-induced learning and*
908   *memory impairment in SD rats*. Environ Pollut, 2024. **362**: p. 124973.
909 120. Yu, S., et al., *Prenatal diesel exhaust exposure alters hippocampal synaptic plasticity in*
910   *offspring*. Aging (Albany NY), 2024. **16**(5): p. 4348-4362.
911 121. Yang, J., et al., *Integrated analysis of the functions and prognostic values of RNA-binding*
912   *proteins in neuroblastoma*. PLoS One, 2021. **16**(12): p. e0260876.
913 122. Ramirez de Mingo, D., et al., *Conformational dynamics in the disordered region of*
914   *human CPEB3 linked to memory consolidation*. BMC Biol, 2022. **20**(1): p. 129.
915 123. Flores, M.D., et al., *Structural insights into functional regulation of the human CPEB3*
916   *prion by an amyloid-forming segment*. Structure, 2025.
917 124. Reselammal, D.S., et al., *Mapping the Fibril Core of the Prion Subdomain of the*
918   *Mammalian CPEB3 that is Involved in Long Term Memory Retention*. J Mol Biol, 2021.
919   **433**(15): p. 167084.
920 125. Kozlov, E., et al., *The role of CPEB family proteins in the nervous system function in the*
921   *norm and pathology*. Cell Biosci, 2021. **11**(1): p. 64.
922 126. Gu, X., et al., *Exploring the F-actin/CPEB3 interaction and its possible role in the*
923   *molecular mechanism of long-term memory*. Proc Natl Acad Sci U S A, 2020. **117**(36): p.
924   22128-22134.
925 127. Gu, X., et al., *A structural dynamics model for how CPEB3 binding to SUMO2 can*
926   *regulate translational control in dendritic spines*. PLoS Comput Biol, 2022. **18**(11): p.
927   e1010657.

928   128.   Boncella, A.E., et al., *Composition-based prediction and rational manipulation of prion-*
929   *like domain recruitment to stress granules.* Proc Natl Acad Sci U S A, 2020. **117**(11): p.
930   5826-5835.

931   129.   Ripin, N. and R. Parker, *Formation, function, and pathology of RNP granules.* Cell, 2023.
932   **186**(22): p. 4737-4756.

933   130.   Di Liegro, C.M., et al., *Role of Post-Transcriptional Regulation in Learning and Memory in*
934   *Mammals.* Genes (Basel), 2024. **15**(3).

935   131.   Aparisi Rey, A., et al., *Gadd45alpha modulates aversive learning through post-*
936   *transcriptional regulation of memory-related mRNAs.* EMBO Rep, 2019. **20**(6).

937   132.   Mirisis, A.A. and T.J. Carew, *The ELAV family of RNA-binding proteins in synaptic*
938   *plasticity and long-term memory.* Neurobiol Learn Mem, 2019. **161**: p. 143-148.

939   133.   Taylor, A.I.P. and R.A. Staniforth, *General Principles Underpinning Amyloid Structure.*
940   Front Neurosci, 2022. **16**: p. 878869.

941   134.   Benson, M.D., et al., *Amyloid nomenclature 2018: recommendations by the*
942   *International Society of Amyloidosis (ISA) nomenclature committee.* Amyloid, 2018.
943   **25**(4): p. 215-219.

944   135.   Gkanatsiou, E., et al., *Amyloid pathology and synaptic loss in pathological aging.* J
945   Neurochem, 2021. **159**(2): p. 258-272.

946   136.   Otzen, D.E., et al., *In situ Sub-Cellular Identification of Functional Amyloids in Bacteria*
947   *and Archaea by Infrared Nanospectroscopy.* Small Methods, 2021. **5**(6): p. e2001002.

948   137.   Maji, S.K., et al., *Functional amyloids as natural storage of peptide hormones in pituitary*
949   *secretory granules.* Science, 2009. **325**(5938): p. 328-32.

950   138.   Bascetin, R., et al., *Amyloid-like aggregates formation by blood plasma fibronectin.* Int J
951   Biol Macromol, 2017. **97**: p. 733-743.

952   139.   Berson, J.F., et al., *Proprotein convertase cleavage liberates a fibrillogenic fragment of a*
953   *resident glycoprotein to initiate melanosome biogenesis.* J Cell Biol, 2003. **161**(3): p.
954   521-33.

955   140.   Buchanan, J.A., et al., *Functional Amyloids: Where Supramolecular Amyloid Assembly*
956   *Controls Biological Activity or Generates New Functionality.* J Mol Biol, 2023. **435**(11): p.
957   167919.

958   141.   Hervas, R., et al., *Cryo-EM structure of a neuronal functional amyloid implicated in*
959   *memory persistence in Drosophila.* Science, 2020. **367**(6483): p. 1230-1234.

960   142.   Cervantes, S.A., et al., *Identification and Structural Characterization of the N-terminal*
961   *Amyloid Core of Orb2 isoform A.* Sci Rep, 2016. **6**: p. 38265.

962   143.   Soria, M.A., S.A. Cervantes, and A.B. Siemer, *Calmodulin binds the N-terminus of the*
963   *functional amyloid Orb2A inhibiting fibril formation.* PLoS One, 2022. **17**(1): p. e0259872.

964   144.   Soria, M.A., et al., *The Functional Amyloid Orb2A Binds to Lipid Membranes.* Biophys J,
965   2017. **113**(1): p. 37-47.

966   145.   Tuite, M.F., *Yeast prions and their prion-forming domain.* Cell, 2000. **100**(3): p. 289-92.

967   146.   Si, K., et al., *Aplysia CPEB can form prion-like multimers in sensory neurons that*
968   *contribute to long-term facilitation.* Cell, 2010. **140**(3): p. 421-35.

969   147.   Liu, J. and J.H. Schwartz, *The cytoplasmic polyadenylation element binding protein and*
970   *polyadenylation of messenger RNA in Aplysia neurons.* Brain Res, 2003. **959**(1): p. 68-76.

971   148.   Rahn, E.J., M.C. Guzman-Karlsson, and J. David Sweatt, *Cellular, molecular, and*
972   *epigenetic mechanisms in non-associative conditioning: implications for pain and*
973   *memory.* Neurobiol Learn Mem, 2013. **105**: p. 133-50.

974   149.   Kandel, E.R., *The molecular biology of memory storage: a dialogue between genes and*
975   *synapses.* Science, 2001. **294**(5544): p. 1030-8.

976   150.   Shattuck, J.E., A.C. Waechter, and E.D. Ross, *The effects of glutamine/asparagine*
977   *content on aggregation and heterologous prion induction by yeast prion-like domains.*
978   Prion, 2017. **11**(4): p. 249-264.

979 151. Scherzinger, E., et al., *Huntingtin-encoded polyglutamine expansions form amyloid-like*
980   *protein aggregates in vitro and in vivo*. Cell, 1997. **90**(3): p. 549-58.
981 152. Bonsor, M., et al., *Polyglutamine disease proteins: Commonalities and differences in*
982   *interaction profiles and pathological effects*. Proteomics, 2024. **24**(12-13): p. e2300114.
983 153. Ford, L., et al., *CPEB3 inhibits translation of mRNA targets by localizing them to P*
984   *bodies*. Proc Natl Acad Sci U S A, 2019. **116**(36): p. 18078-18087.
985 154. Pigazzini, M.L., et al., *An Expanded Polyproline Domain Maintains Mutant Huntingtin*
986   *Soluble in vivo and During Aging*. Front Mol Neurosci, 2021. **14**: p. 721749.
987 155. Gilks, N., et al., *Stress granule assembly is mediated by prion-like aggregation of TIA-1*.
988   Mol Biol Cell, 2004. **15**(12): p. 5383-98.
989 156. Huang, Z., et al., *Cytoplasmic Aggregates of Splicing Factor Proline-Glutamine Rich*
990   *Disrupt Nucleocytoplasmic Transport and Induce Persistent Stress Granules*. J Cell Mol
991   Med, 2024. **28**(23): p. e70261.
992 157. Udan, M. and R.H. Baloh, *Implications of the prion-related Q/N domains in TDP-43 and*
993   *FUS*. Prion, 2011. **5**(1): p. 1-5.
994 158. Ding, H., et al., *Shared genetics of psychiatric disorders and type 2 diabetes:a large-*
995   *scale genome-wide cross-trait analysis*. J Psychiatr Res, 2023. **159**: p. 185-195.
996 159. Pico, S., et al., *CPEB alteration and aberrant transcriptome-polyadenylation lead to a*
997   *treatable SLC19A3 deficiency in Huntington's disease*. Sci Transl Med, 2021. **13**(613): p.
998   eabe7104.
999 160. Parras, A., et al., *Autism-like phenotype and risk gene mRNA deadenylation by CPEB4*
1000   *mis-splicing*. Nature, 2018. **560**(7719): p. 441-446.
1001 161. Wang, B., et al., *MicroRNA-21-5p Reduces Hypoxia/Reoxygenation-Induced Neuronal*
1002   *Cell Damage through Negative Regulation of CPEB3*. Anal Cell Pathol (Amst), 2021.
1003   **2021**: p. 5543212.
1004 162. Li, Z., et al., *MiR-20b-5p promotes hepatocellular carcinoma cell proliferation, migration*
1005   *and invasion by down-regulating CPEB3*. Ann Hepatol, 2021. **23**: p. 100345.
1006 163. Manzini, V., et al., *miR-92a-3p and miR-320a are Upregulated in Plasma Neuron-Derived*
1007   *Extracellular Vesicles of Patients with Frontotemporal Dementia*. Mol Neurobiol, 2025.
1008   **62**(2): p. 2573-2586.
1009 164. Gugliandolo, A., et al., *MicroRNAs Modulate the Pathogenesis of Alzheimer's Disease:*
1010   *An In Silico Analysis in the Human Brain*. Genes (Basel), 2020. **11**(9).
1011 165. Siedlecki-Wullich, D., et al., *Altered microRNAs related to synaptic function as potential*
1012   *plasma biomarkers for Alzheimer's disease*. Alzheimers Res Ther, 2019. **11**(1): p. 46.
1013 166. Wang, R., et al., *Human microRNA (miR-20b-5p) modulates Alzheimer's disease*
1014   *pathways and neuronal function, and a specific polymorphism close to the MIR20B gene*
1015   *influences Alzheimer's biomarkers*. Mol Psychiatry, 2022. **27**(2): p. 1256-1273.
1016 167. Tuna, A., et al., *miR-26b, miR-30e, and miR-206 Polymorphisms May Play a Role in*
1017   *BDNF-mediated Development of Alzheimer's-type Dementia*. Clin Psychopharmacol
1018   Neurosci, 2025. **23**(2): p. 193-201.
1019 168. Phillips, H.S., et al., *BDNF mRNA is decreased in the hippocampus of individuals with*
1020   *Alzheimer's disease*. Neuron, 1991. **7**(5): p. 695-702.
1021 169. Tsuda, K., et al., *Novel RNA recognition motif domain in the cytoplasmic*
1022   *polyadenylation element binding protein 3*. Proteins, 2014. **82**(10): p. 2879-86.
1023 170. Riggs, C.L., et al., *Mammalian stress granules and P bodies at a glance*. J Cell Sci, 2020.
1024   **133**(16).
1025 171. Munier Godebert, A., D. Weil, and A. Safieddine, *Fluorescence-activated particle sorting*
1026   *for condensate purification*. Nat Protoc, 2025.
1027 172. Molliex, A., et al., *Phase separation by low complexity domains promotes stress granule*
1028   *assembly and drives pathological fibrillization*. Cell, 2015. **163**(1): p. 123-33.

1029 173. Riback, J.A., et al., *Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily*
1030 *Tuned Response.* Cell, 2017. **168**(6): p. 1028-1040 e19.
1031 174. Stephan, J.S., et al., *The CPEB3 Protein Is a Functional Prion that Interacts with the Actin*
1032 *Cytoskeleton.* Cell Rep, 2015. **11**(11): p. 1772-85.
1033 175. Vetere, G., et al., *Selective inhibition of miR-92 in hippocampal neurons alters*
1034 *contextual fear memory.* Hippocampus, 2014. **24**(12): p. 1458-65.
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Figure Legends

Figure 1: CPEB3 trafficking and regulation of AMPA receptors and possible modes of action of CPEB3 in memory. (A) SUMOylation of CPEB3 and its association with target mRNA can lead to phase separation and localisation to P bodies, where translation of target mRNAs is repressed. **(1)** Activation of AMPA and NMDA receptors leads to **(2)** LTP. Increased cellular Ca^{2+} concentration leads to activation of calpain-2 and proteolysis of CPEB3 into its NTD and CTD halves. **(3)** LTP leads to deSUMOylation of CPEB3 and translocation out of P bodies. **(4)** Subsequent oligomerisation of CPEB3 and its mono-ubiquitination by Neurl1 leads to CPEB3 association with polysomes and translation of target mRNAs, including GluA2. **(5)** This results in increased AMPA subunit trafficking to the cell surface and membrane insertion of functional AMPA receptors. In addition, CPEB3 is able to modulate GluA2 indirectly through STAT5b. Created in BioRender. Hicks, D. (2025) https://BioRender.com/tbnros4. *(B)* Possible structures into which CPEB3 (represented in blue) might self-assemble. Oligomers (represented using blue beads), condensates (LLPS), aggregates (represented using blue beads) or amyloid-like fibrils (represented using beads (light blue representing the core and dark blue representing the "fuzzy coat") and also in detail using the PDB 6VPS model of Orb2 fibril structures (light blue) with additional "fuzzy coat" regions (dark blue)). Each structure may actively facilitate translation of target mRNAs or result in their release. Which, if any, or all, of these is involved in long term formation of memory remains to be resolved and is a key question for future research. Created in BioRender. Aubrey, L. (2025) https://BioRender.com/nl6c5uk.

Figure 2: Domain organisation in CPEB family members. (A) Domain organisation in CPEBs 1-4, showing NTD and CTD with RNA Recognition Motifs (RRM) and zinc finger (ZnF) sub-domains*. (B)* Percentage sequence identity (black) and similarity (blue) between CPEB family members. Similarity is defined as GAVLI/ FYW/ CM/ ST/ KRH/ DENQ/ P.

Figure 3: Domain organisation in human CPEB3. **Top**: the main functional domains of CPEB3. **Middle**: Residues 160-325 (the actin binding domain). Lower: regions with repetitive sequences (AAR (amino acid repeat) regions) and those able to form transient secondary structural elements of different types (2°).

Table Footnote

Table 1: Sequence similarity of CPEB3 between animal classes. Percentages represent sequence similarity to the human sequence of CPEB3 defined as GAVLI/ FYW/ CM/ ST/ KRH/ DENQ/ P**.** The border between NTD and CTD is defined by RRM1, independently of residue number; 1-441 and 442-698 refer to the human sequence only.

Figure 1

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1090 **Figure 2**
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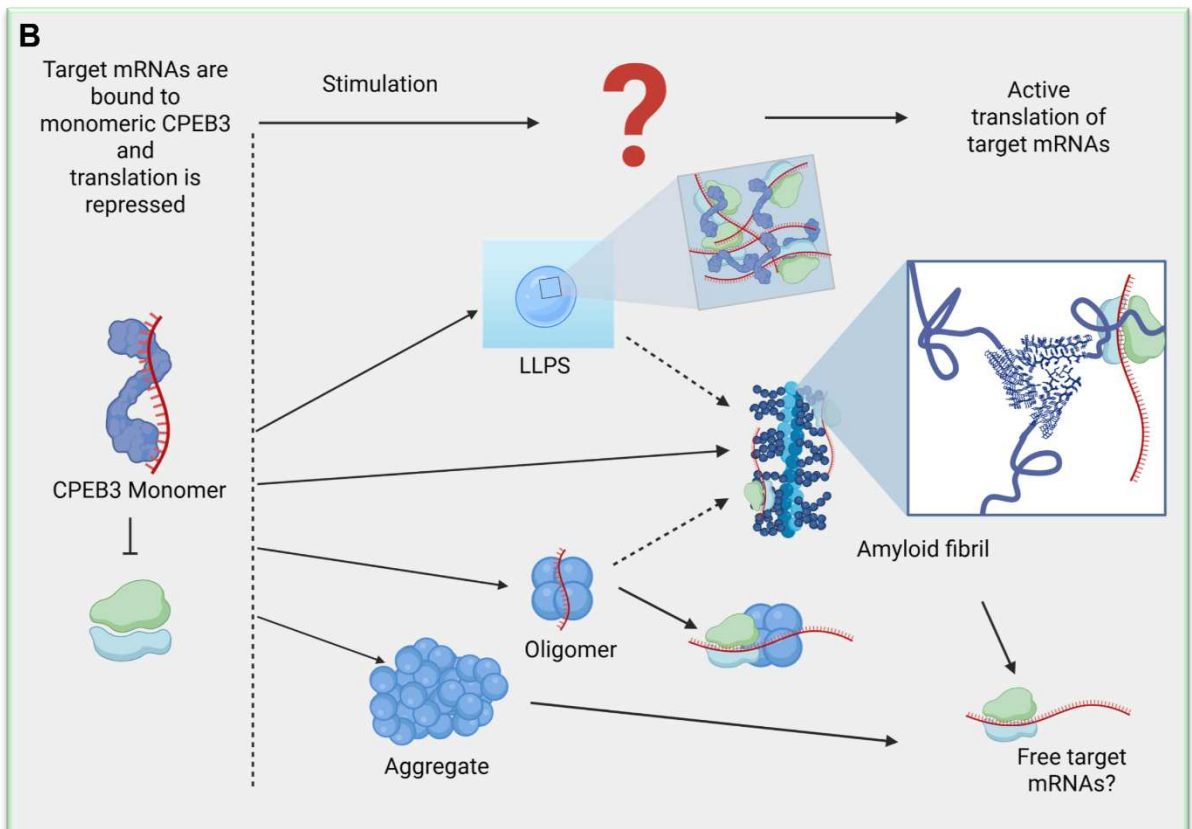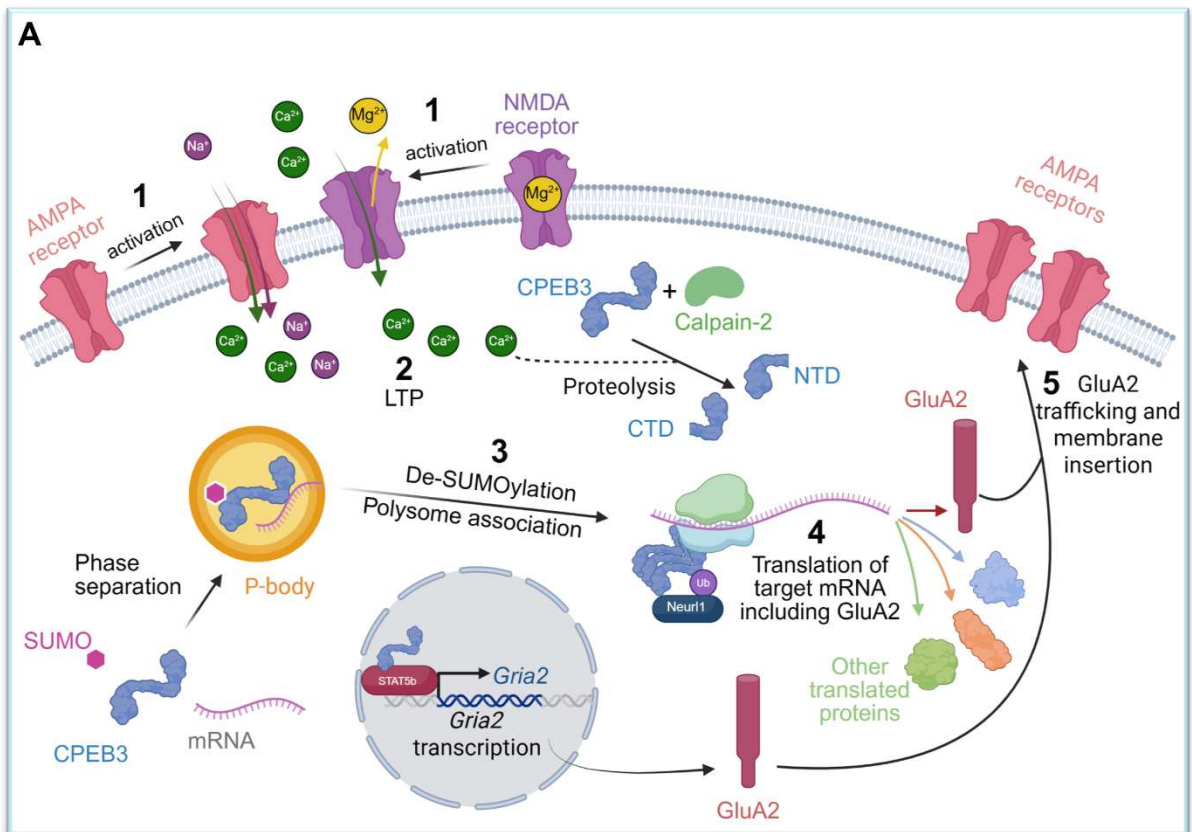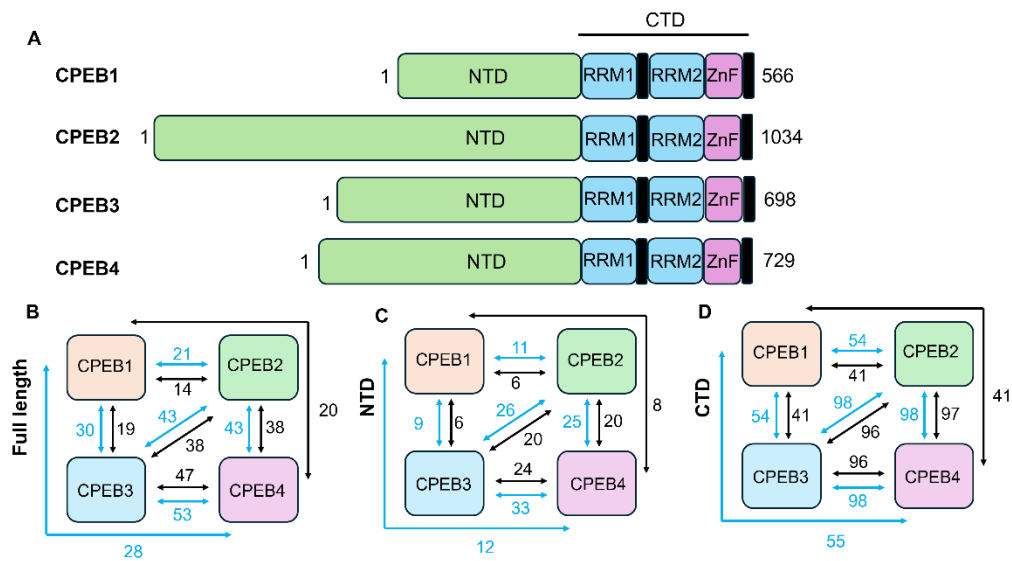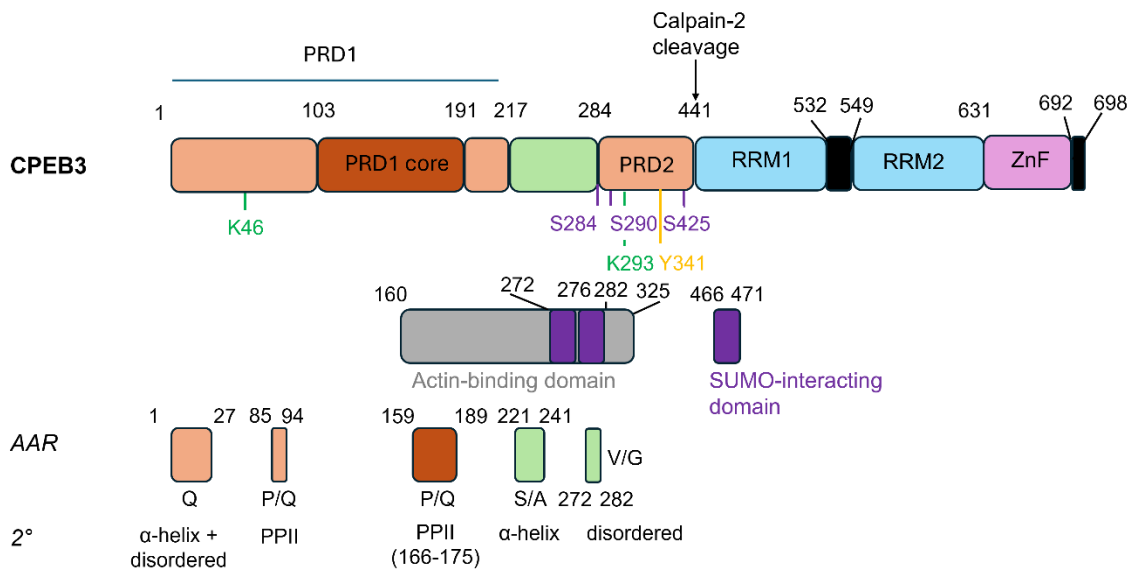
	Full length	NTD (1-441)	CTD (442-698)
Human	100	100	100
Mouse	95	92	100
Dolphin	96	94	100
Frog	75	62	100
Tortoise	85	76	100
Alligator	85	78	100
Chicken	85	77	100
Python	83	74	100
Lungfish	76	65	99
Zebrafish	66	49	99
Shark	76	63	99

1097
1098 **Table 1**