CPEB: Cascades and Consolidation

The direct and indirect effects of cytoplasmic polyadenylation element binding protein and their implications for long-term potentiation

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Introduction

Although neurons consist of the same basic parts, memory is highly individualized. Therefore memory must be the cause of change and store in circuits (Martinez and Derrick, 1996; Hebb, 1949 as reviewed in Martinez and Derrick, 1996; Morris, 2003; Marr, 1971; McNaughton and Morris, 1987 reviewed in Martinez and Derrick, 1996; Carlson, 2012; Kandel, 2012). While some hunt for the locus of memory, many more, intrigued by patient HM's inability to generate new memories, search for how memories are made (Milner, 1970 as reviewed in Morris, 2003). Since the works of Hebb and attention drawn to the hippocampus by patient HM, long term memory formation has been postulated to be the works of long term potentiation (LTP) (Hebb, 1949 as cited in Mertinez and Derrick, 1996; Milner, 1970 as reviewed in Morris, 2003). Barnes (1979) demonstrated that LTP could not only increase stability of synapse, but that this stability is maintained over time. Although many credit memory as an emergent phenomenon of the neuron, it is one protein in particular, cytoplasmic polyadenylation element binding protein (CPEB), which acts indirectly and directly, producing the various changes so characteristic of LTP at the synapses.

Powering the Protein: from NMDA to Aurora A

Postsynaptically, the first step in signal transduction occurs at the NMDA receptor. By requiring not only glutamate as a ligand, but also glycine and Mg^{2+}'s dissociation by depolarization as prerequisites, NMDARs function as fantastic filters (Martinez and Derrick,

1996; Lynch, 2004). NMDAR's requirements for functionality provides a check system to assure LTP will only occur if it is worth the energy expense of synapse stabilization, which occurs by the influx of Ca^{2+}. Ca^{2+} influx demonstrates the efficiency of NMDARs for Ca^{2+}'s higher charge depolarizes neurons faster than sodium, and acts as a crucial second messenger (Carlson, 2012; Kandel, 2012, Bliss & Collingridge, 1993). When Ca^{2+} influx is blocked by D,L-AP5, an NMDA-antagonist, LTP is diminished in rodents (Morris et al., 1986). After eight trails of swimming to a platform while under the influence of D,L-AP5, on the ninth trail with the platform removed, these drugged rats swam without preference, demonstrating they made and retained no memory of where the platform was and should be (Morris et al., 1986). Therefore, if by blocking NMDARs one removes the expression of LTP, then NMDARs and the consequences of its activation - calcium influx - are integral to the phenomenon of LTP.

Calcium though may have a more prominent role than its keeper. Not only does Ca^{2+} enter the spines of dendrites, which concentrates calcium and amplifies its signal, but neurons will release their own internal store of Ca^{2+} to heighten this effect (Regehr & Tank, 1990; Müller & Connor 1991; Alford, Frenguelli, Collingridge, 1993). The necessity for calcium is highlighted demonstratedby the fact that Ca^{2+} chelator EGTA, thapsigarin and ryanodine, drugs that block Ca^{2+} (Tsien & Tsien as cited in Alford & Collingridge, 1993; Bao, Kandel, and Hawkins, 1997). As a result, they deplete Ca^{2+} from intracellular stores, and inhibit calcium-induced Ca^{2+} release all resulting in a common outcome: the inhibition of LTP (Tsien & Tsien as cited in Alford & Collingridge, 1993; Bao, Kandel, and Hawkins, 1997). If Ca^{2+} had no role in LTP, antagonists would not have altered the course of LTP after NMDAR stimulation.

Once inside the cell, calcium signals are quickly amplified by activation of protein kinases causing mass and rapid responses. One of these protein kinases is Aurora A[1], which phosphorylates CPEB (Haung et al., 2002; Plotnikova et al., 2010; Cao et al., 2005). CPEB, once activated, binds to CPE-containing-mRNA to elongate the mRNA's poly-A tail of mRNA which facilitates translation (Kandel, 2012; Keleman et al., 2007). To aid in CPEB's binding, it contains two RNA recognition motifs (RRMs) and two zinc fingers (Huang et al., 2006; Keleman et al., 2007). The form of CPEB's RRMs fits their function by giving CPEB a high affinity for CPEs (Huang et al., 2006; Keleman et al., 2007). Although the end effect may sound simplistic, like memory, the bind of CPEB to CPE and polyadenylation is complex and contains checkpoints.

The core player to CPEB to CPE binding, and thus mRNA translation, protein expression, synaptic change, and LTP, which likely supports long term memory, is Symplekin (eee appendix figure one). Symplekin is a scaffolding protein that anchors CPEB to cleavage and polyadenylation specificity factor CPSF (Huang et al., 2006; Kim & Richter, 2006 as reviewed in Richter, 2007; Barnard et al., 2004 as reviewed in Richter, 2007; Rouhanan et al., 2005 as reviewed in Richter, 2007; Rouhanan & Wickens, 2007 as reviewed in Richter, 2007). Also clustered onto Symplekin are the proteins; polyA ribonuclease PARN, a deadenylating enzyme, and Germ-Line Development Factor 2 Gld-2, a poly(A) polymerase (Radford et al., 2008; Andresson and Joan V.Ruderman, 1998; Barnard et al., 2004 as reviewed in Richter, 2007; Rouhanan et al., 2005 as reviewed in Richter, 2007; Rouhanan & Wickens, 2007 as reviewed in Richter, 2007). It may seem strange that CPEB, which is supposed to elongate the polyA tail of mRNA to induce translation, spends time intimately attached with PARN, a protein that does the

[1] Other proteins include APP and APLP1. They play a crucial role in anchoring the necessary components for activating CPEB. For more detail see Cao et al (2005).

exact opposite, deconstruct the polyA tail. This set up is a way for the cell to control expression of mRNA. PARN is more active than Gld-2, so although Gld-2 continuously stimulates the elongation of the polyA tail, PARN degrades it faster than it can be elongated(Radford et al., 2008; Huang et al., 2006; Barnard et al., 2004 as reviewed in Richter, 2007; Rouhanan et al., 2005 as reviewed in Richter, 2007; Rouhanan & Wickens, 2007 as reviewed in Richter, 2007;). So then, how do the tables turn so that Gld-2 can elongate the tail?

Phosphorylation cascades. Phosphorylation of CPEB by protein kinase Aurora A (also activated by a cascade triggered by NMDA receptors) results in the disassociation of PARN from the Symplekin scaffolding protein (Huang et al., 2002). Without PARN, Gld-2 continues to stimulate polyadenylation and the poly A tail is produced, aiding mRNA in undergoing translation (Kim & Richter, 2006 as reviewed in Richter, 2007; Radford et al., 2008). This mechanism, pCPEB and the disassociation of PARN provides another filter to the process of LTP in addition to the NMDAR. Yet, there is still another filter. Phosphorylation of CPEB by Aurora A increases interaction between CPEB and CPSF, as well as CPEB and Gld-2 (Huang et al 2006). CPEB and eIF4E are bound together by Maskin, which inhibits translation of CPE containing RNA by preventing the eIF4G initiation complex from forming (see appendix figure two). PABP aids in the dissociation of Maskin from eIF4E allowing translation(Huang et al., 2006; Radford et al., 2008; Jung, Lorenz & Richter, 2006). Similar to how PARN prevented polyadenylation and thus translation, Maskin prevents the binding of eIF4G which is needed for attaching the guanine cap of the mRNA to the small subunit of the ribosome, thus orienting it for translation. Without the orientation provided by eIF4G, the Symplekin complex will not attach to a ribosome and the protein will not be translated. Like the NMDAR and PARN - Gld-2

mechanism, the role Maskin plays in preventing eIF4E and EIF4G association protects the neuron from wasting energy on protein synthesis for a synapse that is not meant to be reinforced.

Once CPEB is phosphorylated and the polyA tail is produced, PABP can bind to both the poly A tail and eIF4E[2] aiding eIF4G in displacing Maskin, allowing for binding of the mRNA and the small ribosomal subunit for translation (Jung, Lorenz & Richter, 2006; Cao & Richter, 2002 as reviewed in Richter, 2007; Wakiyama et al., 2000 as reviewed in Richter, 2007; Richter & Sonenberg, 2005 as reviewed in Richter, 2007) Once CPEB has be bound and phosphorylated, PARN and Maskin disassociated, causing the mRNA strand to bind to a small ribosomal subunit, which attaches with the large ribosomal subunit becoming a functional ribosome, so that translation can begin.

This translation leads to protein expression of the many proteins that may strengthen the synapse in a variety of ways from building motor highways to bringing in more proteins or receptors, to actual increasing the strength of the synapse through the proteins that hold the synapse together. In addition CPEB affects many proteins by promoting their translation, such as alphaCaMKII, as well as interacting with dynein and kinesis that move mRNA into the dendrites for translation by CPEB (Richter, 2007; Wells et al., 2001). These proteins' actions and, more importantly, interactions produce not just the strengthening of the synapse, but the synapse's stabilization resulting in L-LTP, a time tested increase in the synapse's sensitivity.

Alpha-CaMKII and the indirect reach of CPEB[3]

[2] Although eIF4E's primary purpose it to aiding Maskin displacment, eIF4E is regulated by Neuroguidin. For futher discussion see Jung, Lorenz & Richter (2006)

[3] For other indirect effects of CPEB on LTP see Zearfoss et al. (2008) and Richter & Klann (2009), who describe CPEB facilitating translation of c-jun, a promoter of GH, which can stimulate LTP by itself.

Once alpha-CaMKII has been produced[4], it can either be stimulated by Ca^{2+} or it can autophosphorylate. This is thought to play a role in molecular memory, as if to say "we have LTPed here once before" (Lisman et al., 1988 as reviewed in Bliss and Collingridge, 1993). When alpha-CaMKII KO mice and mice heterozygous for the KO were bred with their NMDAR in tack, not only was the chance of LTP reduced, as demonstrated by spatial memory deficiency in Morris' water maze, but long term memory as measured by fear response was specifically inhibited (Frankland et al., 2001; Cho et al., 1998 as reviewed in Lynch, 2004; Martinez and Derrick, 1996; Lisman et al., 2002 as reviewed in Lynch, 2004.). Since NMDA is functional but lacks alpha-CaMKII, then alpha-CaMKII must be a crucial kinase triggered by Ca^{2+}-stimulated cascades. On the contrary, when autophosphorylation is blocked, LTP is severely impaired suggesting both functions of alpha-CaMKII are important to LTP (Lisman et al., 1998 as reviewed in Bliss and Collingridge, 1993).

Alpha-CaMKII's role in long term memory comes from its ability to alter neuron morphology. This protein kinase binds to alpha actinin, PSD95, the synaptic adhesion molecule densin-180, as well as microtubule associate protein 2 (MAP2) and Neurofilament L (Lynch, 2004). As these names (MAP2, alpha-actin, and Neurofilament L) suggest, phosphorylation of these proteins result in morphological changes in the cytoskeleton. Both actin and microtubules could be used to extend the reach of a dendrite, increase the size of the synapse, build motor protein pathways to deliver either vesicles or mitochondrion for energy, or cause dendrite spine splitting (Harris et al., 2003; Sorra et al., 1998). By extending the spine towards the synapse, the cleft's volume within decreases, thus increasing diffusion of the neurotransmitter from bouton to receptor, subsequently increasing communication rates. Furthermore, with less volume within

[4] It has been shown by Wells et al. (2001) that alpha-CaMKII is more often than not created by translation of already existing mRNA once NMDARs are stimulated, than by transcription.

the cleft, one not only shortens the distance of diffusion, but increases the concentration of neurotransmitters in the cleft (proportionally), which would, similar to saturation, facilitate more neurotransmitters to bind to receptors. These adaptations of increasing the surface area of the synapse proportionally either by synapse enlargement - allowing for more presysnaptically docked vesicles or postsynaptic receptors - or concentration of the cleft results in a direct relationship between bouton volume and synaptic strength as judged by the active zone site (Schikorski & Stevens, 1999). These postsynaptic phenomenon increase the likelihood of depolarization, LTP, and strengthen the synaptic connection.

Yet synaptic strengthening involves not just post-synaptic change, but synaptic and presynaptic modifications. Working back from the postsynaptic membrane to the synapse, Densin-180, physically helps hold the synapses together. Not all modifications are as direct or literal as Densin-180 in strengthening the synapse. Presynaptically alpha-CaMKII activates synapsin, synaptotgamin and synaptophysin, which work in conjunction to increase the amount of neurotransmitter release (Lynch, 2004). Mirroring the effects postsynaptically, the end result is a significantly heightened efficiency in neuron communication. Not only are synapses held tighter, reducing the volume for diffusion and increasing the proportion of neurotransmitter in the cleft, but alpha-CaMKII stimulates the increase of even more neurotransmitters. This compounded occurrence can not be understated. It has been shown, that alpha-CaMKII is such a key player in LTP, that if injected into the synapse can stimulate LTP without the need for NMDA activation (Markesbery, 1997 as reviewed in Lynch, 2004; Pettit et al., 1994 as cited in Lynch, 2004).

Further experiments support that alpha-CaMKII increases AMPA conductance as a result of recruitment of the AMPA receptor(Liao et al., 2001 as reviewed in Lynch, 2004; Shi et al.,

2001 as reviewed in Lynch, 2004; Shi et al., 1999 as reviewed in Lynch, 2004; Lüscher and Frerking, 2001). In reference to the recruitment of AMPA, the building of motor proteins, stimulated by alpha-CaMKII, allows for cells to use endo/exocytosis along with vesicle transporting to move receptors of one part of the cell to be localized at the synapse undergoing LTP (Isaac et al., 1995; Lynch, 2004; Lüscher and Frerking, 2001). Yet AMPA recruitment is only one branch of alpha-CaMKII's involvement with synapse sensitivity. By dephosphorylating Dynamin - a protein responsible for vesicle cleaving - and thereby activating it, alpha-CaMKII further facilitates its AMPA migration regime (Lüscher and Frerking, 2001; Reese et al., 2011).

AMPA's role in LTP is fundamental. If a synapse had no AMPARs before LTP induction, afterwards, AMPAR is abundantly found (Lüscher and Frerking, 2001; Isaac et al., 1995). Silent neurons - neurons without detectable or functioning AMPAR - therefore become active after LTP due to the addition of functioning AMPAR. It is this addition of AMPAR where there where none that gave this hypothesis its name, the Silent Synapse Theory (Isaac et al, 1995; Lüscher and Frerking, 2001). To prove this Silent Synapse Theory, Isaac et al. (1995) identified neurons that lacked AMPA receptors. By inducing LTP, they observed ESPCs increase over time as a result of AMPA receptors' contribution and that these recruited AMPA receptors remained present at the synapse throughout their experiment. Such supports that LTP strengthens synapse sensitivity as a direct result of the addition of more receptors, and that this increase remains over long durations of time due to the affixation of the receptors to the synapse by other proteins and cytoskeleton modification.

Implication of AMPA's spontaneous appearance is that these receptors are constantly moving through endocytosis and exocytosis (an observable phenomenon if AMPA receptors are tagged with GFP) (Lüscher and Frerking, 2001; Frey and Morris, 1997). If exocytosis is blocked,

then EPSPs decrease over time, while if endocytosis is blocked the opposite occurs (Lüscher and Frerking, 2001). What this means, is that LTP requires stimulus, and if that stimulus is insufficient (because of a lack of receptors, since they could not be transferred to the membrane through exocytosis) then LTP does not occur. Conversely, if a cell prepares for LTP but does not receive further stimulus, by prohibiting the removal of AMPA from these synapses, the synapse stays strengthened. Collectively, this evidence supports that AMPA receptor relocation is key to strengthening the synapse.

Therefore it becomes of great interest to understand how one might anchor these newly relocated receptors to the synapse. It is speculated that proteins of the cytoskeleton may serve this role, perhaps adhesions (Reese et al., 2011). Although the exact protein responsible for permanent anchorage is unknown, similar receptor anchorage has been seen in other LTP related receptors such as mGluR1 and cytoskeleton protein 4.1 (Lüscher and Frerking, 2001). Anchorage, although a most likely overlooked part of the process of LTP (in interest of following receptors, or phosphorylation cascades) are a finalizing component of LTP. Without the anchor, receptors would be relocated, and any other structural changes might as well have been for naught. While a larger synapse, with smaller cleft volume, and more neurotransmitter release may all be valuable in enhancing neuron communication, but if it falls upon deaf ears (a barren postsynaptic neuron) not much communication will occur.

Indirect reach of CPEB: motor highways for synaptic tagging

The proteins activated by Ca^{2+} induced cascades cause changes necessary for memory formation. Yet some of these proteins function with a different role; tagging. Tagging allows for faster recollection and maintenance of synaptic strength (Morris, 2003). Amnesiacs studied by

Warrington and Weizkrantz (1968) claimed that they were unable to form new memories, but those encoded from past key life events remained in tack. However to retrieve these memories, they had to be able to find them, explaining the cruciality of the synaptic tag. It was not that these amnesiacs were unable to form new memories, it is that synaptic potentiation only creates the potential of lasting changes, but does not commit to LTP without a tag. (Warrington and Weizkrantz, 1968; Redondo & Morris, 2011, Frey & Morris, 1997). In this sense, the Synaptic Tagging and Capture (STC) Hypothesis acts as yet another filter, where synaptic potentiation does not necessarily result in change, similar to how NMDAR requires both Glu and Mg^{2+} dissociation. Once a tag is set, plasticity related proteins (PRPs) are captured and their functions are expressed leading to increase in synaptic strength. By subdividing LTP, timing of the tag and the stimulus inducing PRP synthesis can alter LTP's effectiveness. The later the PRP stimulus arrives, the more the tag has degraded, and thus the time between these two events determine if stabilization occurs at all (Redondo & Morris, 2011).

Although STC Hypothesis offers much new insight, in light of recent work, it requires some modifications. PRP's (proteins) ignores the crucial role of mRNAs as plausible plasticity related products (Redondo & Morris, 2011). mRNA has a wide range of functions, from regulating gene expression to editing other strands of mRNA, and some are the precursors of proteins. For mRNA's to be translated and thereby be expressed, they need ribosomes. Ribosomes, like mitochondrion, are capable of traveling motor highways (similar to those established by alpha-CaMKII) and are useful as evidence in observing protein synthesis, since the act of transcription is observable (Harris et al 2003). Therefore they serve as a testament to LTP. To no surprise, they are found in abundance (27% increase compared to controls) in the spines of dendrites undergoing LTP, such as those observed by Schickorski & Stevens (1999)

(Steward and Schuman, 2001 as cited in Harris et al., 2003; Yuste & Bonhoeffer, 2001). Fazeli et al. (1993) monitored protein densities at synapses, and similarity found an increase in protein synthesis density after LTP. These proteins help strength the synapse in the multitude of ways already described. But how do these mRNAs and Ribosomes interact? As discussed towards the beginning of this paper, it is the work of proteins like CPEB, a PRP, that facilitates the translation of some of the most crucial kinases in LTP like alpha-CaMKII.

It is therefore no surprise that NMDA stimulation activates not only alpha-CaMKII, the agent that coordinates the proteins in causing the changes necessary for LTP, but also CPEB, the protein that fosters the agent. If the actor agent relationship between CPEB and alpha-CaMKII is as strong as suggested, then translational and polyadenylation factors should be sequestered together at the site of activation; the dendrites' synapses (Wu et al., 1998 as cited in Huang et al., 2002). Although Huang et al. (2002) observed that upon stimulation, Maskin and CPEB's colocalize at the dendrites, it was Bagni et al. (2000) who observed that this arrangement resulted in "translational competence" at the postsynaptic neuron's synapse (as cited in Huang et al., 2002). While translation could happen in the soma, the proteins upon completion would have to travel to the synapse, exposing them to potential degradation, and costing the cell time - as previous shown critical for STC - and ATP in transportation. Therefore proximity of all the proteins required for strengthening the synapses heightens cell efficiency without forfeiting flexibility, since many regulating factors (such as Maskin and PARN) are bound to a scaffolding protein Symplekin. It is through this arrangement the elegance of CPEB can be understood. Not only does CPEB facilitate translation of alpha-CaMKII whose wide reach on LTP have only been skimmed upon, but the activation of alpha-CaMKII establishes motor highways which

further facilitates CPEB by bringing more mRNA and ribosomes to be translated into proteins at the to-be-strengthen synapse.

Direct Action of CPEB[5]: prion formation

CPEB, although crucial for inducing changes required for LTM, may play a more direct role in LTP. Aplysia CPEB cultured in transgenetic yeast demonstrate similar behavior as yeast prions; spontaneous conformational change to another stable "active" state, which leads to amyloid genesis (Si K, Lindquist S, Kandel ER, 2003a, Papassotiropoulos et al., 2005; Levenson & Sweatt, 2004; Si et al., 2003b). Prion behavior is peculiar, because CPEB is relatively conserved amongst species, yet in humans, prions are known to generate neurodegenerative diseases. The same amyloid formation, which can mentally deteriorate man, can also stabilize his memory (Tompa and Friedrich, 1998).

Stabilization of memory is possible, because the prion nature of CPEB resembles that of yeast prions not PrPs. Unlike PrPs, Wickner (1994) hypothesized that yeast prions could be used to induce epigenetic changes in yeast, similar to histone methylation (as cited in Si, Lindquist, and Kandel 2003a). Why this behavior warrants the name prion, is that these proteins pass on their altered conformational folding in a dominant manner and as a result are self-perpetuating (Si, Lindquist, and Kandel, 2003a). CPEB's state (large/small aggregate or soluble protein) heritability was best demonstrated by Si, Lindquist and Kandel (2003a) who fuzed an active glucocorticoid receptor to CPEB in the yeast that resulted in a blue (active) white (inactive) color coding. Like yeast prions, these CPEB-GR colonies demonstrated, in an epigenetic fashion, heritability with seldom conversion. In other words, white colonies produced white, and blue

[5] For more direct action of CPEB see Chae et al. (2010) on ApCPEB1 and stress granules, or Wilczynska et. al (2005) on CPEB, dcp1 and stress granules

produced blue. These colonies were then tested and were show to have different resistant levels to proteases as well as level of activity, where colonies with the large aggregates of CPEB had higher resistance and activity. Projected into brain, these results demonstrate that the addition of aggregate CPEB to the synapse creates a resistant and self perpetuating, thus long term, change to the synapse by increasing translation of proteins. Therefore, it is CPEB's resistance to degradation and constant protein production that makes LTP L-LTP. If it where not for CPEB, many of the proteins translated could be degraded and the effects of LTP nullified, thus tagging the synapse with aggregated CPEB effectively defeats the rapidly fluctuating environment of the synapse and cell to over come the short lifespan of most cellular effects.

Si et al. (2010) suggests that polymerization of CPEB to itself, unlike prions, is determined by more than CPEB's Poly-Q section[6]. These excess structural properties needed for prion conversion are regulated by serotonin - a NT that can stimulate LTP by itself - adding yet another regulator of LTM (Si et al., 2003b; Si et al., 2010; Montarolo et al., 1986). Since it was Glutamate that originally triggered CPEB, serotonin indicates a shift from the initial stimulus, perhaps induced by rehearsal, which is significant given serotonins prominent effect in classical conditioning (Eliot et al., 1994; Bao, Kandel, and Hawkins, 1998; Si et al., 2003b).

While the explicit role of serotonin here is not fully understood, when used as stimulation, serotonin activate importins - proteins also activated upon depolarization in hippocampal neurons, LTP induction, or NMDA activation - which shuttle other proteins through nuclear pores (Thompson et al., 2004). Such is a reasonable conclusion given that for transcription, signals must be relayed to the nucleus (Thompson et al., 2004). Regardless of importins' cargo, Eliot et al. (1994) thoroughly demonstrated that serotonin and electrical stimulation paired together generate longer lasting LTP than either on its own, making serotonin

[6] a chain of glutamate within CPEB though responsible for prion formation

and Ca^{2+} a reasonable fit for each other and CPEB activation. Such can be demonstrated by N-actin and T(alpha)1-tubulin, motor highway proteins, whose mRNA becomes polyadenylated in response to serotonin, further supporting a tight-knit relationship between CPEB and promotion of proteins that bring mRNA to CPEB (Si et al., 2003b).

So how might an amyloid be useful in memory? One possibility is that a conglomeration of CPEB might increase the effectivity of CPEB and mRNA transcription (Heinrich & Lindquist, 2011; Si, Lindquist, and Kandel, 2003a). Increase the amount of proteins, increase the strength of the synapse, increase the lasting potential of the memory. Another thought is that once active, CPEB prions tag a synapse for long term maintenance. In other words, CPEB is another stamp of approval. Throughout the process of long term memory formation, there are numerous cascades (mGluR1, alpha-CaMKII, Ca2+), checkpoints (initial stimulus, tagging, repeated stimulus, anchoring of receptors, cytoskeletal rearrangement), and energy expediter (protein synthesis, vesicle transport, translation), but despite all of this, a neuron can undo it all. That is of course, unless there is some highly degradation resistant (can withstand SDS solubilization) marker signaling the neuron to not divii up and relocate resources (Alberti et al., 2009 as cited in Heinrich & Lindquist, 2011; Fowler et al., 2007 as cited in Heinrich & Lindquist, 2011). This is due to CPEB's prion's self perpetuating nature, and that, when activated, CPEB translates proteins, causing a self-sustaining and specific change at that synapse (Si, Lindquist, and Kandel, 2003a ; Si et al., 2003b; Tompa and Friedrich, 1998). In other words, prion state CPEB may be the welding and soldering that holds strengthen synapses together, and indeed aggregate CPEB does prove more resistant and active the CPEB in its non amyloid form (Si, Lindquist, and Kandel, 2003a).

Memories being stored in these synapses, CPEB suddenly takes on a much larger role than its size. If CPEB has such a foundational role in LTP, then its deletion should impair LTM. Surely enough, Keleman et al. (2007) demonstrated that fruit flies lacking Orb2 - the fruit-fly gene ortholog for CPEB - have impaired LTM. Further demonstration of CPEB's necessity is seen when homologous recombination mutants were bred. One mutation, missing the RRM and zinc finger motif, proved lethal, while the other - removal of the glutamine region - inhibited memory (Keleman et al., 2007). Using a mechanism well understood, Drosophilia courtship, conditions were produced to have males learn to suppress their advances on female by previous rejection of unreceptive mated females (Keleman et al., 2007). In the mutants, when exposed to a mated female (immobilized) they mated anyway. This lack of expression of previously learned suppression suggests the degradation of memory and the need for functional CPEB (Keleman et al., 2007).

Further demonstrations have involved CPEB KO mice, which displayed negatively affected LTP and extinction (Alarcon et al., 2004; Zearfross, Richter, Berger-Sweeny, 2006). Although specific outcomes of CPEB alteration can not be fully understood, these results, CPEB mutants leading to fatality and LTM impairment, further strengthen the cruciality of CPEB's role in LTP. Furthermore, the strength of CPEB is so potent that after 72 hours of sustained activation LTP can not hindered by emetine or TAT-AS oligonucleotide directed against ApCPEB (Miniaci et al., 2008). Therefore, it is the proteins produced as a result of CPEB that establish strengthened synapses, which not only aid in LTP, but the general viability of an organism, for without stabilized connections there can be no cognitive growth. The inextinguishable aspect of CPEB after 72 strongly reflects how protein synthesis is mandatory for L-LTP and its time-tested durability.

Preventing CPEB phosphorylation only strengthens the evidence of CPEB's role in LTP. Wells et al. (2001) utilized CPP, an NMDA antagonist to inhibit CPEB phosphorylation by Aurora A, which itself is activated downstream of NMDAR activation (Mendez et al. 2000). By testing experienced based activation of NMDARs in the visual cortex, they found that CPP, although indirectly, prevents the phosphorylation of CPEB resulting in a lack of translation of the alpha subunit of alphaCaMKII, whose role in LTP has been previously discussed. Huang et al. (2002) demonstrated that not only is NMDA required for Aurora A to phosphorylate CPEB, but that CPEB phosphorylation increases 7 fold as a result of Glu stimulation of NMDAR. Such supports the notion that CPEB is the focal point of LTP. The events of depolarization, glutamate release, and NMDAR stimulation all converge to the phosphorylation of CPEB, and as a result protein and amyloid genesis strengthen the synapse. CPEB's gravity in LTP proves itself in that most of the proteins and second messengers synthesized as a result of pCPEB come full circle and further facilitate CPEB's role in LTP[7]. However, it has yet to be shown directly that Aurora A phosphorylation of CPEB is integral for CPEB mediated translation and therefore LTP. Rather than testing this effect on LTP, most experiments test the effects of preventing CPEB phosphorylation on the development of early oocytes (Mendez et al., 2000; Katsu et al., 1999; Wong et al., 2011; Ota et al., 2011).

While unraveling how CPEB functions in oocyte development is crucial to understanding how it potentially functions in the brain, LTP can not be observed in these conditions. Therefore the extent of oocyte research on CPEB simply alludes that phosphorylation of CPEB is a requirement for translation, and inhibition of this results in deficits in development, or failure of protein expression (Wells et al., 2001; Mendez et al., 2000; Katsu et al., 1999; Wong et al., 2011;

[7] Consider alphaCaMKII, which is translated as a result of CPEB, and whose activation results in the construction of motor highways that deliver mRNA to CPEB for cytoplasmic adenylation induced translation.

Ota et al., 2011; Keleman et al., 2007; Zearfross, Richter, Berger-Sweeny. 2006; Alarcon et al., 2004; Sarkissian et al., 2004). However CPEB KO mice in conjunction with these experiments provide sufficient grounds to assert CPEB's necessity to the process of LTP (Keleman et al., 2007). Once the understanding of the cascades tied to CPEB and CPEB's nature is developed, a more direct approach can be taken to determine the effect of blocking CPEB's phosphorylation on LTP. Until then, as current research has demonstrated, CPEB is integral to an organism's survival and has various widespread affects on LTP, warranting human investigation to understanding this minuscule protein's role on the meta-phenomenon known as long-term memory.

Conclusion

Although there are many regulators and requirements strung to CPEB, when functioning it promotes the proteins dedicated to fortifying infrastructure so that synapses may be strengthened and memories made. Unlike the many proteins loyal to single tasks, CPEB's wide reaching indirect and direct actions helps explain how neurons, made of the same basic components, can store such complex and individualize memories. CPEB circular impact makes it not only practical, but elegant in that not only does it translate proteins, which activate congruently (alpha-CaMKII in response to NMDAR stimulation), but also the proteins these proteins activate (actin, t(alpha)1 tubulin, etc). While it is foolish to think that understanding of one protein can clarify the emergent phenomenon of memory, it has been shown that many of the drugs affecting more holistic systems (NMDAR, Serotonin or Ca^{2+}) actually disrupt memory through CPEB interference. It is therefore to be understood that while plasticity within a single neuron by no means explains memory in its many forms, CPEB explains the fortifying of a link

within a chain, which can withstand significant stretches of time. By assembling a chain with this understanding, CPEB and its indirect and direct affects of LTP provides a rational stronghold for the theory of LTM.

Appendix

Figures and figure legends from Richter (2007)

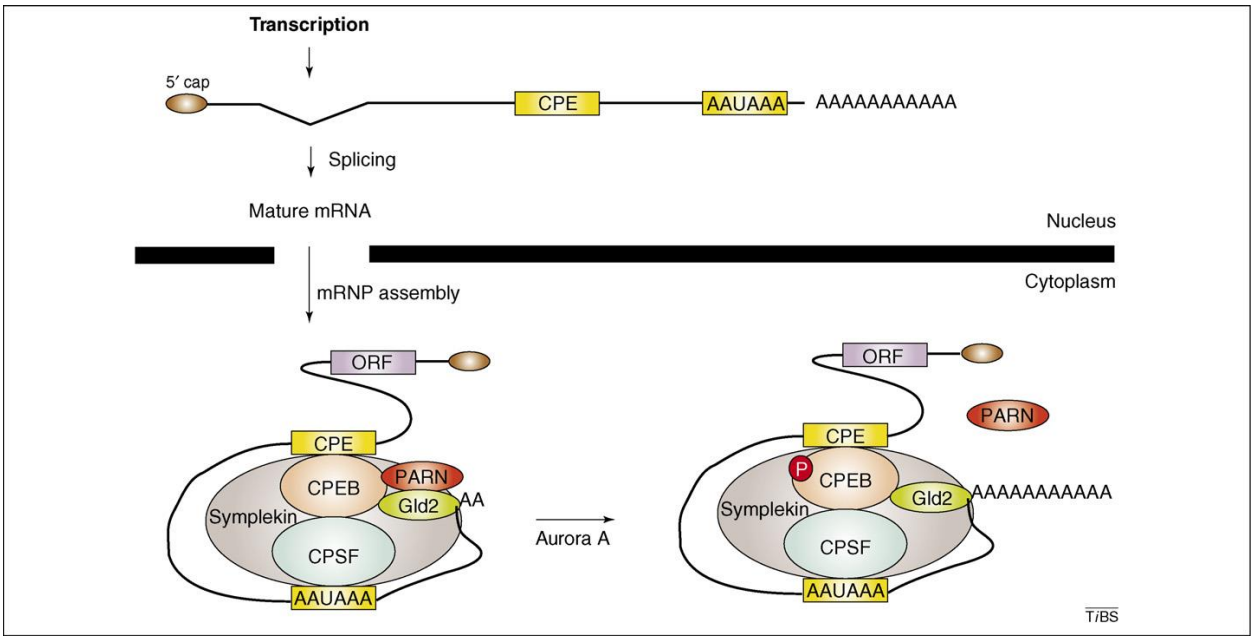

Figure 1. CPEB-regulated polyadenylation. In the nucleus, pre-RNAs containing or lacking a CPE acquire a long poly(A) tail. Following splicing and RNA export, the CPEB- containing RNAs assemble into a ribonucleoprotein (RNP) complex that is nucleated by CPEB. The other factors in this complex include: CPSF, which recognizes the AAUAAA polyadenylation hexanucleotide; PARN, a deadenylating enzyme; Gld2, a poly(A) polymerase; and symplekin, a scaffold protein. Although PARN and Gld2 are both active, PARN activity is more robust; it thus removes the poly(A) tail and keeps it short although Gld2 continues to catalyze polyadenylation. Upon the induction of oocyte maturation, the kinase Aurora A phosphorylates CPEB Ser174, which causes the expulsion of PARN from the RNP complex. Thus, by default, Gld2 elongates. Other factors such as Maskin, eIF4E and PABP have been omitted for clarity. Modified, with permission, from Ref. [8].

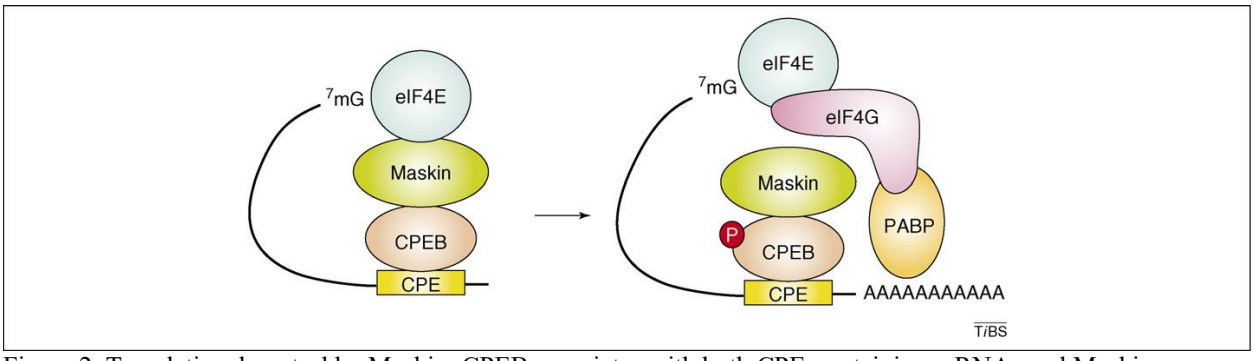

Figure 2. Translational control by Maskin. CPEB associates with both CPE-containing mRNAs and Maskin. Maskin, in turn, interacts with the cap (^{7}mG)-binding factor eIF4E. In this configuration, Maskin binding to eIF4E precludes eIF4G from binding eIF4E, thus, inhibiting translation. Following CPEB phosphorylation and polyadenylation (see Figure 1), PABP binds the newly elongated poly(A) tail; PABP also binds eIF4G and helps it displace Maskin from eIF4E. Because eIF4G is indirectly associated with the 40S ribosomal subunit (not shown), translation initiation proceeds. For clarity, other polyadenylation and translation factors are omitted.

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