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CREB: a message to remember

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Abstract. The prevailing hypothesis for the formation of long-term memory (LTM) is that introduction of a memory item alters the pattern of existing neuronal connectivity to form a neuronal network that will subserve the information for long-term storage. Modulation of synaptic efficacy is induced by changes in synaptic transmission within selected synapses or alteration in synaptic contacts. These changes are in turn supported by molecules that underlie transmission or synaptic remodeling. It is suggested that modulation of gene expression is needed for LTM formation to overcome the relative short lifetime of proteins in neurons (as compared with enduring memory) [1–3]. One of the most salient results consonant with this hypothesis is that the transcription factor cAMP response element binding (CREB) is involved in the formation of memory in organisms with diverse phylogenetic background from mollusks to mammals. CREB subserves the formation of memories of various types of tasks that utilize different brain structures. Circumstantial evidence is available suggesting that CREB regulates the transcription of genes that subserve LTM. The present review is focused on the CREB protein, its role in memory formation and considers mechanistic models pertaining to CREB action in modulating neuronal networks that underlie LTM.

Key words. CREB; learning; gene expression; synaptic plasticity.

Introduction

CREB is a member of a multigene family regulating calcium and cAMP-mediated gene expression

CREB is a central transcription factor that mediates cyclic AMP (cAMP) and calcium-dependent gene expression through the cAMP response element (CRE) [4, 5]. CRE, which consists of an 8-bp sequence (TGACGTCA), is typically located 100 nucleotides upstream from the TATA box [6–9] in promoters of numerous genes.

CREB is a member of a large family [CREB/ATF (activating transcription factor)] of structurally related proteins that bind to the CRE promoter. At least 10 additional genes are part of the CREB family. The number of factors is enlarged by the fact that most of these genes produce several isoforms by alternative splicing; for example, the CREB gene generates three main factors (α, β and δ isoforms [10–14]). In addition to transcriptional activators, the CREB family also includes transcriptional repressors. For instance, the cAMP response element modulator (CREM) includes at least four different repressors that block CRE-dependent transcription (CREM α, β, γ and the inducible cAMP early repressor (ICER) [15, 16].

Members of the CREB family contain the basic leucine zipper (bZIP) domain responsible for DNA binding and dimerization, the kinase-inducible domain (KID) expressing several consensus phosphorylation sites (e.g. PKA, CaMKIV) and the glutamine-rich domains Q1 and Q2 (transactivation domain) [17]. Most of the homology between members of the CREB family is restricted to the bZIP region. Not all the factors contain all the regions (e.g. CREM, ICER).

Intricate tiers of enzymes and factors control CREB activity

CREB can be activated seconds or minutes following external stimulation via an intracellular rise in cAMP or calcium (fig. 1A) [18, 19]. Following stimulation which leads to an increase in the cytosolic level of cAMP, the

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protein kinase A (PKA) catalytic and regulatory subunits rapidly dissociate, allowing passive translocation of the catalytic subunit into the nucleus. Translocation reaches a plateau 15–20 min following stimulation, which leads to serine-133 (Ser-133) phosphorylation in the consensus PKA phosphorylated site (RRPSY) on the CREB protein [19]. Ser-133 phosphorylation is considered to be a critical event that mediates the initiation of transcription, since mutation of Ser-133 to alanine abolishes transcription [20]. A rise in the intracellular calcium level activates CREB via Ca^{2+}/calmodulin-dependent kinase IV (CaMKIV) [21, 22]. The application of antisense CaMKIV oligonucleotide to hippocampal neurons blocks calcium-mediated phosphorylation of CREB on Ser-133 [22]. Activation of CREB by Ca^{2+} might be mediated by translocation of calmodulin from the cytoplasm into the nucleus [23]. Other candidates for calcium-regulated CREB kinases include p70 S6 kinase ($p70^{S6K}$), p90 ribosomal S6 kinase ($p90^{RSK}$) family members and cAMP-dependent PKA. These enzymes can phosphorylate CREB in vitro. Upon their activation, the kinases are localized in the nucleus.

The phosphorylation state of CREB is also regulated by phosphatases. It has been suggested that in hippocampal cells Ca^{2+}/CaM activates calcineurin, which in turn activates a nuclear phosphatase, most probably protein phosphatase-1 (PP-1), resulting in the dephosphorylation of pCREB [22]. In the striatum, the duration of

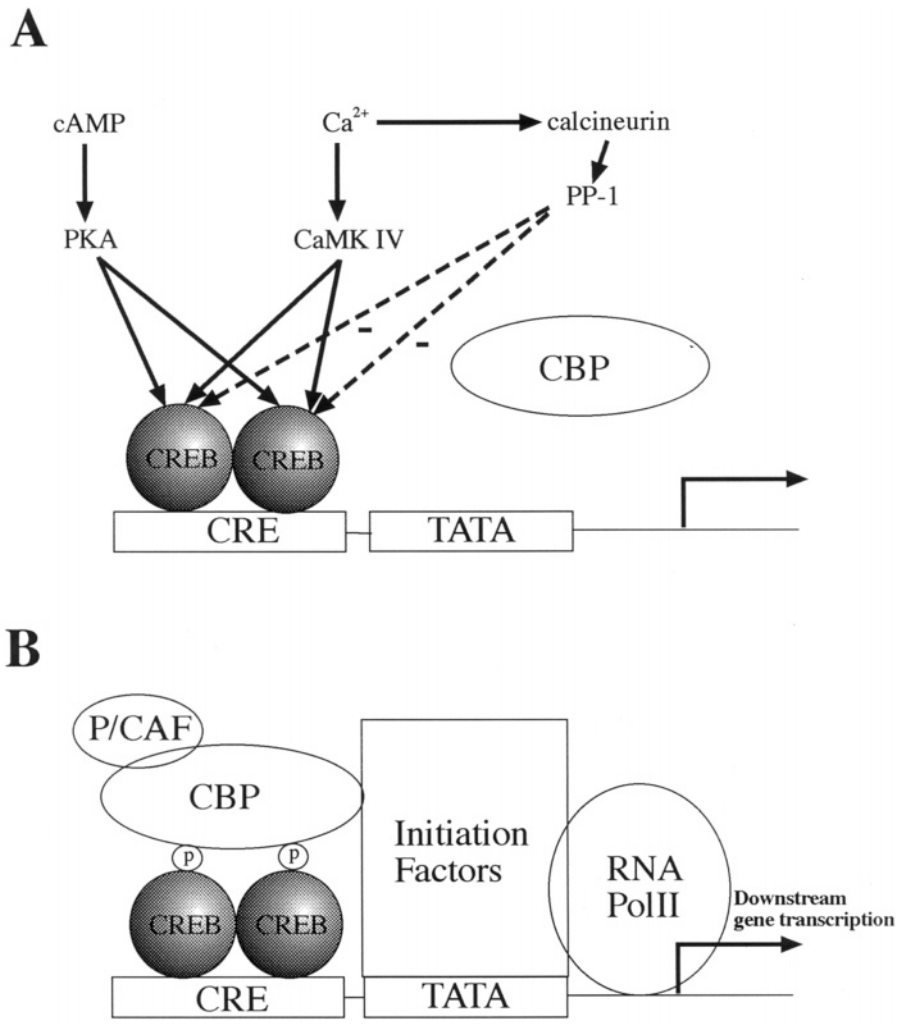

Figure 1. Regulation of gene expression by CREB. (*A*) Phosphorylation of CREB on Ser-133 is considered to be a critical step in CREB activation. CREB is phosphorylated on Ser-133 by CaMKIV or PKA following a rise in cellular calcium and cAMP, respectively. The phosphorylation of Ser-133 is also regulated by phosphatases such as PP-1. (*B*) Upon phosphorylation on Ser-133, CREB binds to the coactivator CBP. CBP interacts with the basal transcription factors and RNA polymerase II and initiates transcription. CBP and P/CAP exhibit histone acetyl transferase activity that may facilitate the assembly of the preinitiation complex.

Table 1. Summary of the involvement of CREB family members in the behavior of different organisms.

Organism	CREB family member	Paradigm	Brain area	Ref.
Aplysia	ApCREB1 ApCREB2	GSW sensitization	abdominal ganglion	39, 40
Drosophila	dCREB2-a dCREB2-b	olfactory learning	mushroom bodies	49, 50
Mouse	CREB α, δ CREB β (?)	– water maze – social transmission of food preference – fear conditioning	hippocampus amygdala	52, 53
Rat	CREB	– conditioned taste aversion – water maze	amygdala hippocampus	59 63

CREB phosphorylation initiated by D1/D5 dopamine receptor is prolonged by activation of the dopamine and adenosine 3′,5′-monophosphate-regulated phosphoprotein (DARPP-32), an inhibitor of PP-1 phosphatase [24].

In addition, CREB activity can be suppressed by transcription repressors [4]. For example, CREMs α, β and γ, which lack the Q domains and therefore are unable to interact with the transcription machinery, negatively regulate cAMP-dependent gene transcription by CRE. It has been shown that CREM-repressor forms exert their suppressive action either by competing with CREB activators for binding CRE sites or by complexing with the activators to generate nonfunctional heterodimers. Of note, CREB regulates the activation of repressors such as ICER and may thereby induce a negative feedback on its gene induction activity.

CREB recruits a complex molecular machinery to initiate transcription

The aforementioned observations indicate that CREB activity is carefully controlled by intricate tiers of enzymes and factors. But how does CREB regulate the transcription of genes? It was recently shown that when CREB is phosphorylated on Ser-133, it becomes bound to a transcriptional coactivator protein, CREB binding protein (CBP), or its cognate relative p300 (fig. 1B) [25, 26]. CBP and p300 are large molecules that contain multiple protein-protein interaction domains. CBP interacts in vitro with the basal factor TFIIB, and current biochemical evidence suggests that CBP may be a component of the RNA polymerase II holoenzyme complex. CREB-CBP complex can therefore recruit basal transcription and RNA polymerase II on the promoter site to initiate transcription [27]. In addition, CBP is a histone acetyl transferase (HAT) that binds to another protein, P/CAF (p300/CBP-associated protein), that also exhibits HAT activity [28, 29]. HAT catalyzes acetylation of lysine residues located within the amino terminal tails of histones. The acetylation neutralizes charges that mediate DNA-histone interactions. The CREB-CBP-P/CAF complex may therefore induce acetylation that loosens the histone-DNA interactions and consequently facilitates the assembly of the preinitiation complex on the promoter of immediate early genes (IEGs). Microinjection of CBP antiserum blocked induction of a CRE reporter plasmid, and overexpression of CBP was able to potentiate CREB activity in a phospho (Ser-133)-dependent manner [26]. Notwithstanding these findings, functional studies with CREB predict that its interaction with CBP and the consequent recruitment of RNA polymerase II is not sufficient per se for cAMP-dependent transcription. Other components such as TFIID are also needed for the initiation of transcription [30].

Several signaling pathways in addition to CRE utilize CBP. These pathways activate phorbol ester elements (TREs) and serum responsive elements (SREs), revealing that CBP is a multifunctional coactivator. The different signaling lanes may interfere and cross-couple with one another when the level of CBP is limited, and therefore serve as additional regulatory mechanisms for cAMP-induced gene expression via CREB [26].

Cumulatively, these observations show that CREB is a pivotal protein that mediates cAMP and calcium signaling pathways and induction of gene expression. Consequently, much attention is presently centered on the role of CREB in the modulation of gene expression that underlies LTM.

Involvement of CREB protein in learning and memory

In recent years vast evidence has been accrued on the central role of CREB protein in the formation of memory. It is fascinating to see how the role of CREB in the formation of memory was conserved during evolution. CREB supports memory in various organisms that perform different behavioral tasks, from simple reflexes in

mollusks to complex emotional behaviors in mammals (table 1).

CREB and memory in *Aplysia*

The first studies indicating that CREB is intimately involved in the formation of memory were performed in the marine snail *Aplysia californica*. The behavioral paradigm used was the sensitization of the gill and siphon-withdrawal reflex (GSW) which is a nonassociative form of learning [31]. In the GSW reflex, application of a tactile stimulus to the siphon leads to the withdrawal of the gill or the siphon [32, 33]. Tactile or electrical stimulation to the tail or head results in the sensitization of subsequent GSW response. The administration of a single stimulation causes short-term sensitization (minutes to hours) of the response, whereas repeated stimulation results in long-term sensitization (days to weeks). The GSW reflex is controlled by an array of sensory, motor and interneurons [32]. To simplify the study on the molecular and cellular mechanisms of the GSW sensitization, a reductive preparation was used. In this preparation learning can be mimicked by coculturing a single *Aplysia* sensory neuron with a single *Aplysia* motor neuron. A single application of serotonin (5-hydroxytryptamine; 5-HT), a transmitter that mediates sensitization, to the culture, results in the enhancement of transmitter release from the sensory neuron to the motor neuron which is independent of protein synthesis (short-term facilitation, STF). Five spaced applications of serotonin cause long-term enhancement (long-term facilitation, LTF) which is dependent on translation and transcription [34–36]. STF and LTF therefore parallel, at least in part, the sensitization behavior. It was found that sustained administration of cAMP (a second messenger of serotonin), to the media, leads to LTF [37]. In addition, a single application of 5-HT releases the catalytic subunit of PKA to the cytoplasm of the sensory neuron especially in the presynaptic terminals. Following multiple applications of 5-HT, the PKA catalytic subunit is translocated to the nucleus of the sensory neuron, where it phosphorylates CREB transcription factors that in turn activate gene expression [19]. The *Aplysia* CCAAT enhancer-binding protein (ApC/EBP), which is rapidly induced by cAMP and contains a CRE element in its promoter, is essential for the formation of LTF [38]. Dash et al. [39] extended these studies and showed that CRE has functional significance in the formation of LTF. They microinjected double-stranded CRE oligonucleotide into the sensory neuron. The CRE oligonucleotide impeded CREB binding to the endogenous CRE and selectively blocked LTF but not STF.

LTF formation requires, in addition to *Aplysia* CREB1 (ApCREB1) activation, the relief of *Aplysia* CREB2 (ApCREB2)-mediated repression of ApCREB1. ApCREB2 is constitutively expressed in *Aplysia* and contains consensus sites of phosphorylation for mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) [40]. ApCREB2 is homologous to human CREB2 and mouse ATF-4. Since CREB2 is a repressor of CREB-mediated gene expression, it has been suggested that ApCREB2 might serve as a repressor in *Aplysia*. Indeed, relief of ApCREB2 activity facilitated LTF in neuronal cell culture. As mentioned above, repeated application of 5-HT induces LTF, whereas a single application of 5-HT provokes STF. Microinjection of antibodies against ApCREB2 into the sensory neurons induced neuronal facilitation—namely, a single application of 5-HT becomes sufficient to induce LTF [40]. The relief of ApCREB2 repression in vivo might be regulated by covalent modification of ApCREB2. Indeed, the level in phosphorylation of ApCREB2 changes following repeated application of 5-HT, but further experiments are mandatory to clarify this issue. Thus, relief of ApCREB1, from ApCREB2, might be the limiting event in the initiation of CREB-mediated transcription that subserves LTF in *Aplysia*.

Odor memory in *Drosophila*

In the fly *Drosophila*, CREB and the molecules involved in its activation were shown to be implicated in learning [41, 42]. Using both forward and reverse genetics, mutant flies were tested for the acquisition and consolidation of an olfactory memory. The paradigm used was an olfactory-avoidance assay whereby flies are exposed to an odor and an electric shock simultaneously and subsequently exposed to a second odor without an electric shock. The flies are then tested in a two choice maze. One arm of the maze contains the conditioned odor and the other arm the nonconditioned odor. Migration toward the nonharmful odor indicated that the flies had memorized the harmful odor and thereby avoided it [41, 42].

Three mutant flies that showed impairment in learning were *dunce*, *rutabaga* and *amnesiac*. The *dunce* and *rutabaga* flies are defective in cAMP phosphodiesterase and Ca^{+2}/calmodulin-stimulated adenylyl cyclase [43, 44], and the *amnesiac* fly lacks a pituitary adenylyl cyclase activating peptide-like transmitter [45]. The finding that these mutated molecules cause learning deficiencies emphasized the key role of the cAMP pathway in learning. The central role of the cAMP pathway in olfactory learning in flies was further strengthened by the observation that the cAMP-dependent PKA is required for learning [46]. Transgenic flies that express inhibitors of PKA or additional PKA catalytic subunit (DCO) under the control of a heat shock-inducible promoter show attenuation in memory [47].

The role of the *Drosophila* CREB gene in long-term memory (LTM) was also investigated. To obtain LTM in flies, a modified olfactory learning paradigm was developed whereby the basic olfactory avoidance protocol (see above) is applied several times with a rest interval of 15 min between cycles [48]. To study the role of CREB in LTM, the CREB repressor isoform (dCREB2-b) was placed under the control of a promoter that can be activated by an increase in temperature. Thus, developmental complications are spared, since the gene is expressed only in the adult fly 3 h before training. When dCREB2-b was induced under the control of the heat shock promoter, prior to behavioral training, it specifically blocked the formation of LTM, without affecting immediate learning or anesthesia-resistant memory (ARM) [49]. ARM is induced like LTM but without the rest interval and is different from LTM in several ways: it lasts only 4 days (LTM remains at least 7 days) and is not affected by protein synthesis inhibitors. Conversely, induction of the active isoform of dCREB2 (dCREB2-a) led to enhancement of LTM where a full level of LTM was accomplished following only a single training session [50]. Thus, CREB may serve in *Drosophila* as a gate which permits the consolidation of meaningful learning events for LTM, namely an event that repeats for several time with interevent intervals. This restriction is bypassed by overexpression of dCREB 2-a. Relief of dCREB2-b repressor might be the limiting event for dCREB2-a activation, as observed with ApCREB2.

CREB subserves memory in mice

CREB is also involved in the formation of memory in mammals. Mutated CREB mice were created by insertion of a neomycin resistance (neo) gene into exon 2 [51]. This insertion causes the loss of two main CREB isoforms, α and δ, in the $CREB^{-\alpha\delta}$ mice and the increase of other CREB and CREM isoforms (e.g. CREBβ). The mice exhibited no gross developmental abnormalities either at the behavioral or neuroanatomical level [52].

To test the effect of $CREB^{-\alpha\delta}$ deletion on behavior, mice were tested for three hippocampal- and amygdala-dependent tasks [52, 53]. In the first amygdala-dependent task, the fear-conditioning paradigm [54, 55], $CREB^{-\alpha\delta}$ mice were trained to associate between a context (the chamber where they were placed) or a cue (specific tone) and a mild electrical foot shock. The mice received a discrete sound (conditioned stimulus, CS; for 30 s) together with a mild electrical foot shock (in the last 2 s of the CS; unconditioned stimulus, UCS). Memory was assessed at subsequent time points by measuring the duration of 'freezing' where mice are completely immobile, a behavior that signals fear response. In the contextual fear paradigm the mice were placed in the same cage, at different time points following the conditioning, and freezing was measured. In cued-fear conditioning the mice were placed in a novel context, and their response (freezing) to the sound (the same as the CS) was monitored. The mutant mice showed significantly fewer fear responses, compared with normal mice, when tested 1 h (context conditioning) or 24 h (in both tasks) but not 30 min following conditioning. These results indicate that $CREB^{-\alpha\delta}$ mutation does not interfere with the immediate association of the CS and the UCS but provokes disruption in memory between 30 to 60 min post-training. The response of mutated mice to the UCS alone was similar to the control animals, indicating that the animals were not less sensitive to shock.

The $CREB^{-\alpha\delta}$ knockout mice also exhibited impairment in the Morris water maze paradigm [52]. In this hippocampal-dependent task [56, 57], mice memorize the location of a submerged platform in a pool using spatial cues located outside the pool. The overall performance of the knockout mice was significantly lower than that of the control animals. The mutated mice were not different from controls in locating a visible platform, indicating that they were not impaired in their motor or visual faculties required to perform the water maze task.

In addition, $CREB^{-\alpha\delta}$ mutated mice were tested for the social transmission of food preference [53]. In this paradigm mice prefer food that they have smelled on the breath of another mouse. The memory is induced by interaction among the animals and lasts for days. Consistent with the results from the fear-conditioning task, the mutated mice showed normal performance of immediate memory but were deficient in LTM (24 h later).

Cumulatively, these results indicate that the $CREB^{-\alpha\delta}$ mutant mice exhibit deficits in performing learning paradigms involving different brain areas (hippocampus and amygdala) and various behavioral characteristics. However, it is not clear whether the deficits in learning are caused by the loss of the α and δ isoforms or by the increase in the CREM and/or CREBβ isoforms [51].

As mentioned above, one of the enzymes that activates CREB is the cAMP-dependent PKA. To test whether PKA activation is important for memory, transgenic mice that overexpress the R(AB) inhibitory subunit of PKA in forebrain areas were generated [58]. These mice showed low PKA activity in the hippocampus. The R (AB) transgenic mice exhibited deficits in spatial learning when tested in the Morris water maze task, and in long- but not short-term context-dependent fear conditioning.

Conditioned taste aversion learning in rats is mediated by CREB

CREB is essential for the formation of long-term conditioned taste aversion (CTA) memory in rats [59]. In CTA, ingestion of a novel taste stimulus (CS) is paired with internal malaise produced by administration of toxic substance (UCS). In this context, the animals memorize the taste and reject it when the CS is presented on a subsequent occasion (conditioned response, CR) [60].

To study the role of CREB in CTA, antisense oligonucleotide complementary to the CREB RNA sequence was microinjected into the central amygdaloid nucleus (CeA). Microinjection of the CREB antisense into the CeA reduced, 14 h later, the level of CREB protein (by 35%), which returned to basal level 24 h following microinjection [59]. CREB antisense did not affect the level of other proteins in the amygdala (e.g PKCγ and ATF-2). It also did not cause any neuroanatomical abnormalities in the amygdala and surrounding tissue. Bilateral intraamygdala CREB antisense injection 14 h before CTA markedly impaired CTA learning without affecting any sensory, motor or motivational faculties required to perform the task. CREB antisense impaired long-term (tested 72 h after training) but not short-term taste memory (tested 2–4 h following training) [59].

c-Fos protein in CeA was also shown to be involved in the formation of long-term CTA memory [61, 62]. Since *c-fos* transcription is regulated under certain conditions by CREB, one may argue that CREB in amygdala affects CTA memory formation via regulation of *c-fos* expression.

Another study that has utilized CREB antisense to elucidate the role of CREB protein in LTM was performed by Guzowski and McGaugh [63]. They showed that intrahippocampal infusion of antisense to CREB several hours prior to learning reduced the level of CREB protein in hippocampus and disrupted long- but not short-term spatial (water maze) memory.

Mechanisms of CREB-mediated modulation of synaptic strength

The aforementioned observations provide ample evidence that CREB protein is essential for the formation of LTM across disparate species and in various behavioral paradigms. However, the molecular and cellular mechanisms whereby CREB subserves LTM formation remain elusive. Several questions central to the understanding of these mechanisms may be addressed: What are the behavioral-induced neuronal inputs that activate CREB? How do CREB-regulated gene products affect modulation of specific synaptic connectivity? and How does modulation of these synapses subserve memory? In this context I will review recent studies that examined the specificity between stimulus and CREB activation and between CREB-regulated gene products and modulation of specific synapses using reductive preparation.

The spatiotemporal pattern of synaptic input can affect CREB activation and gene expression

Recent studies have indicated that CRE-mediated gene expression and the duration of CREB phosphorylation on Ser-133 correlate and depend on the nature of the incoming synaptic input [64]. For example, Bito et al. [22] showed that both short- (18 s) and long-term (180 s) electrical stimuli at 5 Hz in hippocampal neurons were able to induce Ser-133 phosphorylation of CREB. However, the extinction of CREB phosphorylation was significantly faster following the short stimulus compared with the long stimulus which induced a more stable pCREB. Interestingly, only the enduring stimuli producing the long-lasting CREB phosphorylation provoked an increase in the level of c-Fos and somatostatin (SS-14) proteins, which served as markers for CRE-mediated gene expression. Liu and Graybiel [24] found that activation of the striatal-organotypic culture with the D1/D5 receptor agonist SKF-81297 led to rapid (7 min) and widespread phosphorylation of CREB in the striatum. PCREB converged 30 min later to anatomical restricted areas within the striatum known as the striosomes. Treatment of the striatal culture with BAY K 8644, which prolonged open time of L-type voltage-sensitive Ca^{2+} channels, similarly caused rapid widespread phosphorylation of CREB in the striatum. However, in contrast to SKF-8197, 30 min following BAY K 8644 treatment, phosphorylated CREB was localized to the matrix surrounding the striosomes. Importantly, the sustained but not transient phosphorylation of CREB was correlated with c-Fos expression. In both studies the event that determines the duration of CREB phosphorylation was the ability of the stimuli to inhibit phosphatases, such as PP-1, thus preventing dephosphorylation of CREB.

It seems, therefore, that in some instances CREB activates IEG transcription only following neuronal stimuli that result in long-term CREB phosphorylation. Thus, the tenable hypothesis may be suggested that behavioral experiences that cause activation of CREB beyond a certain period of time (e.g. by repeated spaced stimulation) will be consolidated into LTM by way of modulation of gene expression. Consequently, CREB might serve as a limiting factor that gates information for long-term storage. It is noteworthy, however that in addition to the duration of Ser-133 phosphorylation of CREB alternative factors may play a 'gating role' in CRE-mediated gene expression in response to various stimuli [65]. As indicated previously [40], CREB repres-

sors may also restrain CREB activators. Relief of repression may allow CREB to activate gene transcription following only a single short stimulus.

In addition to the temporal properties of the stimulus, the activation of CREB could be influenced also by discrete spatial stimuli impinging on the postsynaptic neuron. It has been reported that diverse calcium pools influence the activation of CREB in distinctive ways. A number of studies indicate that nuclear, but not cytoplasmic Ca^{2+}, influx is essential for CREB- mediated transcription [66]; conversely, reports are available suggesting that calcium at the synapses induces CREB phosphorylation [67]. These observations afford interesting speculative views on CREB activation within a neuronal network. Thus, it could be implied—as suggested by Ginty [68]—that stimulation of receptors located at various distances from the nucleus might affect activation of transcription factors differently and as a consequence selectively modulate the expression of late response genes and long-term neuronal changes. For example, activation of close synapses affecting the level of calcium within the nucleus might activate CREB, whereas activation of distal synapses that provoke calcium increase within spines and cytoplasm might influence mostly SRE-mediated gene expression [66].

The aforementioned studies show that CREB activation may lead to gene expression. However, increase of CRE-regulated products per se is not sufficient to induce specific synaptic changes since it does not provide information on the location of the synapse where modulation is required. How, therefore, are CREB-regulated gene products recruited to specific synapses?

CREB-activated gene products are recruited by specific synapses

An attractive model, based on recent studies [69, 70], proposes that synapses can be selectively tagged to recruit proteins produced in the cytoplasm. In one of these studies Martin et al. [70] have shown local synaptic plasticity which is dependent on protein synthesis and CREB activation. The authors have used a modified *Aplysia* sensory-motor system by culturing a single bifurcated sensory neuron with two spatially separated follower motor neurons. They studied whether a single branch of the sensory neuron can undergo short- or long-term synaptic facilitation and elucidated molecular mechanisms underlying such facilitation. Toward these aims, perfusion microelectrodes were used to apply serotonin (5-HT) to the synapses made by only one branch of the sensory cell onto one motor neuron. Martin and colleagues found that a single application of 5-HT produced synapse-specific short-term facilitation (10 min), whereas repeated applications of 5-HT induced long-lasting (24 h) branch-specific facilitation. The synapses made on the contralateral motor neuron not exposed to 5-HT exhibited neither short-term nor long-term facilitation. Microinjection of antibodies raised against CREB into the sensory neuron 1 h before repeated application of serotonin blocked branch-specific LTF. In addition to the need for gene transcription in the nucleus mediated by CREB, local presynaptic protein synthesis was also essential. The investigators suggested that the consolidation of synapse-specific long-term plasticity requires two separate events: first, a retrograde messenger produced in the synapse and translocated to the nucleus to activate CREB-dependent gene expression, and second, recruitment of CREB-mediated gene products to the stimulated synapse but not to others made by the same neuron.

Another line of evidence [71] showed that CREB products can induce increase in synaptic transmission if these synapses have undergone specific molecular and structural changes. In this study the investigators have expressed the activator form of CREB (dCREB2-a) in mutated *Droshophila*, which expresses $\sim 50\%$ of wild-type Fasciclin II (Fas II) protein. Fas II is an adhesion molecule required for synapse remodeling and for sprouting of additional synaptic contact, events which are activity-dependent. In mutant flies there is an increase in the number of motor neuron boutons on muscle 6. The increase in synaptic boutons is not accompanied by an increase in synaptic strength. The induction of dCREB2-a resulted in a significant increase in quantal content and therefore synaptic strength in Fas II mutant but not in wild-type or mutant flies exhibiting reduction in synaptic boutons. These results suggest that the products of downstream genes regulated by CREB affect synaptic strength but only in sites of new synaptic growth where Fas II is reduced. It is therefore suggested that Fas II and CREB work in combination to control enhancement of synaptic strength.

How does CREB affect synaptic efficacy?

The strength of synaptic connectivity can be modulated by altering transmission or neuronal morphology (e.g. number of synaptic connections or spine structure; see Moser, this issue). Several lines of evidence are available showing that CREB affects growth of new synapses and synaptic transmission. For example, microinjection of ApCREB2 antibodies into the sensory neuron of *Aplysia* relieved the suppression of ApCREB1 and led to long-term facilitation of synaptic transmission after only a single pulse of 5-HT (see above [40]). Microinjection of ApCREB2 antibodies followed by a single pulse of 5-HT resulted in long-term changes in both strength of sensory-motor neuron connection (enhancement of

excitatory postsynaptic potential amplitude) and in the number of sensory neuron varicosities contacting the motor neurons. Murphy and Segal [72] have recently shown that an estradiol-induced increase in the density of dendritic spines in hippocampal neurons can be blocked by inhibiting PKA activity or reducing pCREB by means of antisense. In addition, induction of CREB activator in Fas II *Drosophila* mutants increased transmitter release [71].

One can therefore argue that CREB protein is involved in synaptic plasticity by way of affecting synaptic transmission efficacy and synaptic growth. As outlined previously, activation of CREB may result in induction of gene expression. The gene products are translocated from the nucleus to specific synapses. What is the nature of these products which are the building blocks that underlie CREB-mediated synaptic plasticity? CREB regulates the expression of genes which may serve as modulators of synaptic plasticity. The CRE downstream genes such as synapsin I, [4] which are part of the neurotrasmitter release machinery and involved in synaptic growth, are likely candidates to subserve synaptic plasticity. However, further studies are needed to elucidate the specific roles of such proteins in provoking and maintaining changes in synaptic strength. How these changes in turn subserve memory is a cardinal and central issue in the field of memory research.

Conclusions

Evidence has accrued showing the involvement of modulation of gene expression and protein synthesis in the formation of LTM [1–3, 73]. Several studies presented in this review suggest that the transcription factor CREB may mediate these events. Alteration of CREB expression and activity in disparate organisms including *Aplysia*, *Drosophila*, mouse and rat impaired LTM and LTF. In addition, relief from CREB repressors impeding CREB activity facilitates LTM and LTF formation. These results indicate that the regulation of CRE-mediated genes, leading to LTM, is probably controlled by the balanced activity of CREB activators and repressors. The elucidation of control mechanisms involved in the regulation of CREB activity, which leads to LTM, and insight into the mode(s) of action of CREB-regulated gene products in the modulation of specific synaptic efficacy in vivo are central issues for future research.

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