

## CREB, plasticity and memory

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### 1. CREB AND TRANSCRIPTION

#### 1.1. THE MULTIGENE CREB FAMILY

cAMP Responsive Element Binding Protein (CREB) is a member of a family (CREB/ATF) of structurally related transcription factors that bind to promoter CRE sites. In mammals, at least three genes encode the CREB-like proteins, *CREB*, *CREM* (cAMP Response Element Modulator) and *ATF-1* (Activating Transcription Factor) (Hoeffler et al., 1988; Rehfuss et al., 1991; Foulkes et al., 1992). These three genes share a high degree of sequence homology and are well conserved throughout evolution. The CREB-like proteins share many structural and functional polymorphisms, and are expressed in a wide range of tissues and cell types.

#### 1.2. STRUCTURAL FEATURES

Transcription factors from the CREB family generally contain three key domains that mediate transcriptional activation, DNA binding and dimerization. The transcription activation domain is located on the N-terminal part of CREB and CREM and contains glutamine-rich domains (Q domain) that flank a cluster of phosphorylation sites (P-box, also referred to as the kinase-inducible domain (KID)) by which various kinases regulate the transactivational potential of CREB. DNA binding is mediated by a region rich in arginine and lysine residues (basic region), while dimerization is mediated by an adjacent leucine zipper domain (bZIP) (Kerppola and Curran, 1995). Most of the sequence homology amongst the different members of the CREB family of proteins is restricted to the bZIP region (Hai et al., 1989). However, not all of the CREB-like proteins contain these three key domains. For example, an isoform of the CREM gene, the Inducible cAMP Early Repressor (ICER), contains only the bZIP and the DNA binding domains (Molina et al., 1993).

#### 1.3. CREB/CREM ISOFORMS

The human and mouse CREB gene is composed of 11 exons (Hoeffler et al., 1990; Waeber et al., 1991; Cole et al., 1992). Three main transcriptional activators,  $\alpha$  (Gonzalez et al., 1989),  $\beta$  (Blendy et al., 1996) and  $\delta$  (Yamamoto et al., 1990) are generated from the CREB gene by alternative splicing. In addition to these transcriptional activators, the CREB family also includes repressors of transcription. For example, the CREM gene codes at least four

isoforms that repress CRE-dependent transcription: the CREM  $\alpha$ ,  $\beta$  and  $\gamma$  proteins as well as ICER (Foulkes et al., 1991; Molina et al., 1993). The diversity of CREB isoforms is produced by alternative splicing, start sites (e.g., CREB $\beta$ ) and even alternate promoters (e.g., ICER) (Molina et al., 1993).

In *Drosophila* and *Aplysia*, the CREB gene produces several isoforms by alternative splicing, some of which act as transcriptional activators and others as transcriptional repressors. Similar to the mammalian CREM gene, the *Drosophila* CREB gene expresses both transcriptional activators and repressors. The activator (dCREB2-a) contains the three key protein domains described above, while the repressor (dCREB2-b) contains a P-box but lacks the glutamine-rich region, which is required for the transactivation of CREB (Yin et al., 1994). The *Aplysia* CREB1 gene expresses three isoforms: ApCREB1a, ApCREB1b and ApCREB1c (Bartsch et al., 1995). ApCREB1a functions as a transcriptional activator, similar to mammalian CREB. ApCREB1b contains only the bZIP domain and lacks the N-terminal transcription activation domain of ApCREB1a, and functions as a repressor. ApCREB1c is a truncated protein that lacks a nuclear localization signal.

#### 1.4. DNA BINDING

The CRE site in the somatostatin promoter (5'-TGACGTCA-3') was the first CREB-binding site described (Montminy and Bilezikjian, 1987). These palindromic consensus CRE sequences are typically located 100 nucleotides upstream from the TATA box in the promoter regions of the target genes (Comb et al., 1986; Montminy et al., 1986; Short et al., 1986). CREB binds as a dimer to the CRE sites with an affinity of approximately 1 to 2 nM (Richards et al., 1996). Promoters may include more than one copy of the CRE sequence, and there may be considerable sequence variability between functional CRE sites. In fact, the exact nucleotide sequence of these CRE sites may affect CREB binding. Adding to this variability, and providing a further mechanism of transcriptional regulation, are the different transcriptional efficiencies of the various members of the CREB/ATF family (these vary by 10–20 fold).

There are two categories of factors that recognize CRE sites: those that form dimers with CREB and those that do not (Kerppola and Curran, 1995; Shaywitz and Greenberg, 1999). The first group of factors that are able to dimerize with CREB includes CREB, CREM and ATF-1. CREM and ATF-1 recognize, and bind to, CRE sites as homodimers or as heterodimers with CREB (Hai and Curran, 1991; Loriaux et al., 1994a), perhaps owing to a highly conserved leucine zipper region (Foulkes et al., 1991; Hoefler et al., 1991; Hurst et al., 1991). Factors that cannot dimerize with CREB include c-Jun, some of the other ATFs, such as ATF-4, and members of the CAAT/enhancer binding protein (C/EBP) gene family (Yun et al., 1990; Hai and Curran, 1991; Hummler et al., 1994). Heterodimerization may regulate CREB function by changing the affinity of these factors for the DNA sequences to which they normally bind. Moreover, this heterodimerization may cross-link distinct intracellular signaling systems, allowing a rich and complex regulation of gene function.

#### 1.5. TRANSCRIPTIONAL ACTIVATION

The transcriptional activity of CREB is regulated by phosphorylation of Ser133 in the P-box or KID of the protein (Gonzalez and Montminy, 1989). This domain includes several consensus phosphorylation sites for a variety of kinases, including protein kinase A (PKA), protein kinase C (PKC), casein kinases (CKII), calmodulin kinases (CaMK), glycogen synthase

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kinase-3 (GSK-3), p34<sup>cdc2</sup>, p70<sup>s6k</sup>, mitogen-activated p90 rsk, MAP kinase/extracellular signal-regulated kinase (ERK1/2), stress-regulated mitogen-activated protein kinase-2 and Akt/PKB (Montminy et al., 1986; Gonzalez and Montminy, 1989; Gonzalez et al., 1989; Yamamoto et al., 1990; Dash et al., 1991; Sheng et al., 1991; Fiol et al., 1994; Tan et al., 1996; Xing et al., 1996; Bullock and Habener, 1998; De Cesare et al., 1998; Deak et al., 1998) that may either increase or decrease the transcriptional activity of CREB (Brindle and Montminy, 1992; Sassone-Corsi, 1995). Thus, although CREB was initially identified as a mediator of the cAMP pathway, the KID domain of CREB may be phosphorylated by kinases in the calcium/calmodulin and/or growth factor pathways. An illustrative example of each of these distinct pathways that converge on CREB, namely cAMP, calcium/calmodulin and growth factors, will be discussed briefly.

Increases in the level of intracellular cAMP produced by forskolin administration (Deisseroth et al., 1998; Impey et al., 1998a; Sheng et al., 1990) or by activation of adenylyl cyclase by transmembrane receptors, such as the D<sub>1</sub> dopaminergic receptor (Liu and Graybiel, 1996), for example, activates PKA by dissociating the regulatory (R) from the catalytic (C) subunits. The C subunits of PKA passively translocate to the nucleus where they may phosphorylate CREB at Ser133 to induce transcription (Bacskai et al., 1993; Hagiwara et al., 1993).

A second pathway that leads to CREB activation is mediated by increases in intracellular calcium (Ca<sup>2+</sup>). Driven by the activation of synaptic *N*-methyl-D-aspartate receptors (NMDARs) and L-type Ca<sup>2+</sup> channels, the increase in intracellular Ca<sup>2+</sup> may lead to phosphorylation of CREB via the CaMK family of serine/threonine kinases. Increases in Ca<sup>2+</sup> in the nucleus do not seem to be sufficient for CREB phosphorylation in response to synaptic signals (Deisseroth et al., 1996). Instead, increased Ca<sup>2+</sup> levels located within 1–2 μm of the cell membrane seem to be crucial, suggesting that a calcium sensor (e.g., calmodulin (CaM)) associated with synaptic membranes may trigger a cascade of events leading to the activation of CREB in the nucleus (Deisseroth et al., 1996). Thus, increases in intracellular Ca<sup>2+</sup> may increase the concentration of Ca<sup>2+</sup>/CaM complex, which binds to, and activates, CaMKs that, in turn, phosphorylate CREB.

Phosphopeptide mapping indicates that CaMKI, CaMKII and CaMKIV all phosphorylate CREB at Ser133 *in vitro* (Dash et al., 1991; Sheng et al., 1991; Enslin et al., 1994; Matthews et al., 1994; Sun et al., 1994). However, CaMKII, in addition to phosphorylating CREB at Ser133, also phosphorylates CREB at Ser142, which inactivates the transcriptional activating properties of CREB by preventing dimerization but not DNA binding (Parker et al., 1998; Wu and McMurray, 2001). Furthermore, CaMKII-induced inhibition of CREB transcriptional activity overshadows the stimulatory effects of PKA (Gonzalez and Montminy, 1989) and CaMKIV (Sheng and Greenberg, 1990; Sun et al., 1996) on CREB.

CaMKIV may phosphorylate CREB at Ser133 following membrane depolarization in neuronal cells (Bito et al., 1996). Cotransfection of constitutively active CaMKIV drives CREB-dependent gene expression (Enslin et al., 1994; Matthews et al., 1994; Sun et al., 1994) while interfering with CaMKIV function inhibits membrane depolarization-induced Ser133 phosphorylation (Bito et al., 1996). Furthermore, CaMKIV-deficient mice show impairments in inducible CREB phosphorylation and decreases in the expression of c-Fos, an immediate-early gene that has CRE sequences in the promoter region (Ho et al., 2000). Therefore, it may be that the Ca<sup>2+</sup> signal generated at the synapse is conveyed to the nucleus by transport of calmodulin across the nuclear membrane and activation of CREB by CaMKs (Deisseroth et al., 1996).

However, a recent study suggests that CREB may be phosphorylated in the nucleus even when protein import into the nucleus is blocked with wheat-germ agglutinin (Hardingham et

al., 2001). This suggests that  $\text{Ca}^{2+}$  alone may carry the signal from dendrites to the nucleus. Therefore, waves of  $\text{Ca}^{2+}$  from the site of entry at the synapse may be propagated and amplified by intracellular stores of  $\text{Ca}^{2+}$  into the nucleus to activate CREB (Hardingham et al., 1997, 2001).

A third pathway by which CREB may be activated is via a cascade of kinase activity initiated by nerve growth factor (NGF). NGF stimulation activates NGF receptors (tyrosine kinase receptor, Trk receptors) that stimulate guanine-nucleotide exchange factors (GEF) to activate Ras, a small G protein. Activated Ras, in turn, stimulates the serine/threonine kinase, Raf, that triggers activation of MEK, and its targets, the ERK1/2 members of the MAPK family (Blenis et al., 1991). One downstream substrate of the Ras/ERK pathway is a 90 kDa ribosomal S-6 kinase-2 (RSK-2). Upon activation, both ERKs and RSKs translocate to the nucleus where they may phosphorylate CREB at Ser133 (Chen et al., 1992; Xing et al., 1996; Finkbeiner et al., 1997).

The functional importance of these different signaling cascades that culminate in the phosphorylation of CREB at Ser133 is not entirely clear. The complexity of the pathways upstream from CREB may allow for tight, fine-tuned regulation of CRE-mediated transcription. There is evidence for substantial cross-talk between the pathways that converge on CREB. For instance, CaMKIV produces a wave of CREB phosphorylation with a rapid onset and a rapid offset whereas the Ras-ERK-RSK2 pathway promotes a slow phase of CREB phosphorylation (Wu et al., 2001a). The distinct kinetic properties of the upstream pathways may allow CREB to compute information regarding the exact nature of synaptic stimuli. Perhaps this complexity allows for specific stimuli to be translated into specific patterns of gene expression.

Phosphorylation at Ser133 is essential for the activation of CREB (Yamamoto et al., 1990; Lee et al., 1993). Although CREB proteins bind to CRE sites as dimers, phosphorylation of both partners does not seem to be required for transcriptional activation. Nevertheless, dimers in which both partners are phosphorylated (at Ser133) are more active than hemiphosphorylated dimers (Loriaux et al., 1994b). Thus, the degree of phosphorylation may be another avenue to control CREB-dependent transcription.

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Phosphorylation of CREB at Ser133 does not have an appreciable impact on the structure of CREB (Richards et al., 1996), but promotes the phosphorylation-dependent interaction with the KIX domain of the CREB Binding Protein (CBP) or its close relative p300 (Chrivia et al., 1993). CBP and p300 are ubiquitously expressed coactivator proteins that may physically link phosphorylated CREB with the basal transcriptional complex (Kwok et al., 1994; Nakajima et al., 1997a,b). Overexpression of CBP enhances stimulus-induced transcription of a CRE-reporter gene, an effect that depends on the phosphorylation of CREB at Ser133 (Kwok et al., 1994). Conversely, inhibiting the function of CBP, or the formation of a CREB-CBP complex, blocks CREB-mediated transcription (Arias et al., 1994; Hu et al., 1999). CBP may facilitate transcription by recruiting RNA polymerase II to the transcription machinery through an interaction with RNA helicase A (Kwok et al., 1994; Swope et al., 1996; Nakajima et al., 1997a,b). In addition, both CBP and p300 may also possess intrinsic histone acetyltransferase (HAT) activity that may facilitate access of the basal transcription factors to the core promoter region by decondensing the chromatin (Bannister and Kouzarides, 1995, 1996; Ogryzko et al., 1996; Kouzarides, 1999).

Although binding of phosphorylated CREB to CBP is required for transcription, the recruitment of CBP does not appear to be sufficient for transcription. In order for transcription to be initiated, it seems that CBP, itself, must also be activated (Chawla et al., 1998; Hu et al., 1999). CBP may be phosphorylated via the  $\text{Ca}^{2+}$  signal transduction pathway, possibly

through a CaMKIV mechanism (Chrivia et al., 1993; Kwok et al., 1994). However, the ERK1/2 cascade does not seem to phosphorylate CBP as activation of the ERK1/2 pathway alone (by electrical activity in the presence of inhibitors of CaM kinases, selective stimulation of ERK1/2 with growth factors, or by genetic means) leads to CREB phosphorylation at Ser133 without appreciable induction of CREB-dependent transcription (Sheng et al., 1988; Bonni et al., 1995; Chawla et al., 1998; Hardingham et al., 1999; Hu et al., 1999). It seems, therefore, that CREB-mediated transcription requires two activation events (CREB and CBP).

CBP and p300 are global coactivators that interact with a number of other transcription factors, including AP-1, NF- $\kappa$ B, c-Fos, c-Jun, and transcriptional activator sites such as phorbol ester elements (TREs) and Serum-Responsive Elements (SREs) (Arias et al., 1994; Nordheim, 1994; Kamei et al., 1996). Thus, it may be that competition for the availability of CBP may also regulate the transcriptional responses mediated by CREB (Kamei et al., 1996).

Surprisingly, a recent report indicates that the activation of CREM is not always phosphorylation-dependent. ACT (activator of CREM in testis, found exclusively in the male germ cell) is a tissue-specific coactivator that interacts with CREM independent of its phosphorylation state (Fimia et al., 1999). This finding suggests that there may be another pathway in the regulation of CRE-mediated transcription, at least in this tissue.

## 1.6. TRANSCRIPTIONAL REPRESSION

Just as phosphorylation of Ser133 seems to be critical for activation of CREB, dephosphorylation of this residue is important for inactivation of CREB. As with all other phosphoproteins, therefore, the level of CREB phosphorylation at Ser133 reflects a balance between the oppositional actions of kinases and phosphatases, such as protein phosphatase 1 (PP-1 and PP-2) (Hagiwara et al., 1992). For example, dephosphorylation of CREB at Ser133 may be initiated by the activation of calcineurin (PP-2B) by the Ca<sup>2+</sup>-CaM pathway. Calcineurin may then activate the nuclear phosphatase PP-1 that goes on to dephosphorylate CREB (Bito et al., 1996).

In addition to dephosphorylation, the transcriptional activity of CREB may also be actively suppressed by transcriptional repressors. For example, the CREM  $\alpha$ ,  $\beta$  and  $\gamma$  repressors lack the glutamine-rich transactivating domains, but seem to bind CRE sites normally (Foulkes et al., 1991; Laoide et al., 1993). Phosphorylated CREB/CREM $\alpha$  complexes activate transcription, thus showing that repression by these factors is not mediated by heterodimerization with activators (Loriaux et al., 1994a). Instead, repressor dimers may compete with CREB activators for CRE sites. As the repressors are unable to interact with the basal transcription machinery (they lack Q-domains; but see above (Fimia et al., 1999)), the result may be a silencing of CRE-containing promoters.

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Furthermore, the 3' end of the CREM gene contains an alternative promoter that codes for ICER, a strong CREB repressor. The ICER promoter contains CRE sites, and, therefore, is induced by CREB/ATF activation (Molina et al., 1993). It is possible that activation of CREB results in the transcription of repressor isoforms, such as ICER. The accumulation of repressors could, in turn, lead to the eventual repression of CREB-dependent transcription. This regulatory feedback may have an impact on a number of biological functions, including plasticity.

Thus, the transcriptional activity of CREB seems to be determined by complex interactions between kinases and phosphatases and active transcriptional repression systems. These competing systems, furthermore, may allow for fine-tuning of CREB-mediated transcriptional responses.

## 2. PLASTICITY AND MEMORY

### 2.1. MEMORY AND PROTEIN SYNTHESIS

There is extensive evidence from a variety of animal model systems showing that protein synthesis during, or shortly after, training is essential for the formation of long-term memory (LTM) (Davis and Squire, 1984; Matthies, 1989). For example, systemic administration of the protein synthesis inhibitor anisomycin, before or immediately after training, blocks LTM (typically measured 24 h following training) but not short-term memory (STM; typically measured 30 min to 2 h following training) for conditioned fear (Abel et al., 1997; Bourtchouladze et al., 1998). A similar pattern of results is observed following infusion of anisomycin icv (Schafe et al., 1999) or directly into the amygdala (Gewirtz et al., 1998; Schafe and LeDoux, 2000), a neural structure known to be critical for the acquisition of fear conditioning (Davis, 1992; LeDoux, 1992; Fanselow and Kim, 1994; Fanselow and LeDoux, 1999).

Several lines of evidence show that CREB is one of the transcription factors regulating the synthesis of new proteins necessary for the formation of LTM. Insights into the biochemistry of CREB activity allow for the design of experiments to examine the mnemonic effects of both increases and decreases of CREB levels and function. The specificity and consistency of the results obtained by many laboratories using diverse model systems establish that CREB may be a key molecular player in the cellular events underlying memory formation.

### 2.2. MASSED AND SPACED TRAINING SCHEDULES AND MEMORY

In general, memory retention is stronger following training with multiple trials, than with one single trial (Bugelski, 1962; Cooper and Pantle, 1967; Zacks, 1969). Not only is the number of training trials an important determinant of memory retention but so too is the distribution of training trials over time. Spaced training (training in which the trials are presented with intervening rest intervals) is generally more effective than massed training (the same number of training trials presented with no or short intervening rest intervals) in producing strong LTM for a variety of memory tasks (Ewing et al., 1985; Rescorla, 1988). In species ranging from *Aplysia* (Carew et al., 1972; Pinsker et al., 1973; Frost et al., 1985; Cleary et al., 1998; Mauelshagen et al., 1998), *Drosophila* (Tully et al., 1994), *Chasmagnathus* crab (Freudenthal et al., 1998), mouse (Kogan et al., 1996), rat (Fanselow and Tighe, 1988; Barela, 1999; Josselyn et al., 2001) to human (Ebbinghaus, 1885), spaced training is needed to produce maximal LTM. Thus, spaced training is more likely to induce LTM than massed training. Extensive evidence from different species and behavioral tasks indicate that the levels of CREB play a key role in this trial-spacing effect.

## 3. MEMORY: THE ROLE OF CREB

One of the fundamental results that emerged from studies with protein synthesis inhibitors is that LTM, but not STM, requires protein synthesis following training. STM is thought to involve transient changes in synaptic strength, perhaps mediated by covalent modifications of preexisting proteins. By contrast, LTM requires both transcription and translation of genes, and is proposed to involve growth and restructuring of new synapses. CREB is thought to be one of the factors necessary for initiating the transcription of proteins required for LTM in a variety of species.

### 3.1. CREB AND ELECTROPHYSIOLOGICAL STUDIES OF LONG-TERM PLASTICITY IN *APLYSIA*

Tactile or electrical stimulation of the siphon of the marine mollusc *Aplysia* produces a defensive reflex in which the siphon and gill are withdrawn. If an electric shock is applied to the tail of *Aplysia* the subsequent reaction to siphon stimulation is increased, or sensitized. A single tail shock produces short-term sensitization of the withdrawal reflex that lasts several minutes, and does not require protein synthesis (Pinkser et al., 1970; Carew et al., 1971; Kandel et al., 1981). However, repeated intermittent (spaced) shocks to the tail produce long-term sensitization of the withdrawal reflex that lasts many hours and requires the synthesis of new proteins (Pinsker et al., 1973; Kandel and Schwartz, 1982; Frost et al., 1985; Goelet et al., 1986; Bailey et al., 1992; Cleary et al., 1998). Behavioral sensitization in *Aplysia* is mediated by facilitation of synaptic transmission between sensory (responding to the sensitizing stimulus) and motor neurons (mediating the withdrawal response) (Frost et al., 1985; Byrne, 1987). This monosynaptic connection is enhanced for a period of minutes following a single tail shock and for longer than 24 h following repeated spaced shocks (Walters et al., 1983; Buonomano and Byrne, 1990; Mercer et al., 1991; Cleary et al., 1998).

Long-term facilitation (LTF), a stable enhancement of synaptic function with properties that closely mirror behavioral long-term sensitization, may be observed in a co-culture preparation of sensory and motor neurons. One pulse of serotonin, a transmitter released by the sensitizing tail stimulus (Glanzman et al., 1989), produces short-term facilitation (STF) lasting only minutes, whereas five spaced pulses of serotonin produces LTF lasting longer than 24 h (Carew and Kandel, 1973; Walters et al., 1983; Montarolo et al., 1986; Rayport and Schacher, 1986; Sweatt and Kandel, 1989; Mercer et al., 1991; Emptage and Carew, 1993; Muehlshagen et al., 1996; Zhang et al., 1997). Similar to long-term behavioral sensitization, LTF is dependent on protein synthesis (Montarolo et al., 1986) whereas STF is not (Montarolo et al., 1986; Dash et al., 1990).

Thus, both STF and LTF may be triggered by serotonin, suggesting that this neurotransmitter may have distinct downstream actions that differentially mediate the formation of STF or LTF. Serotonin activates G protein receptors that are positively coupled to adenylyl cyclase. This increase in intracellular cAMP levels transiently activates cytoplasmic protein kinases, including PKA and the diacylglycerol-protein kinase C (DAG-PKC) system. This kinase activation, in turn, is thought to covalently modify (phosphorylate) a number of target proteins. One possibility is that this cascade culminates in the closing of  $K^+$  channels, prolonging the action potential and increasing the influx of  $Ca^{2+}$ , leading to the augmentation of transmitter release, and culminating in STF (Castellucci et al., 1980; Sugita et al., 1992; Byrne et al., 1993).

A similar mechanism, however, cannot account for LTF. Although intracellular cAMP levels rise dramatically shortly after serotonin treatment, this increase is not present 24 h later, at a time when LTF is observed (Bernier et al., 1982). Instead, it may be that initial increases in cAMP activate PKA and MAP kinase cascades, triggering CREB-dependent transcription of genes whose products are required for LTF (Bacskai et al., 1993; Martin et al., 1997).

The first study to suggest that CREB is required for memory formation or plasticity was performed in *Aplysia* cultured neurons (Dash et al., 1990). LTF, but not STF, was blocked by injection of oligonucleotides with CRE sequences into cultured sensory neurons (Dash et al., 1990). Presumably, the CRE-oligonucleotides trap the CREB proteins needed for the transcriptional activation of genes that ultimately mediate LTF (Kaang et al., 1993; Alberini et al., 1994). Moreover, a similar injection of a reporter gene driven by a CRE-containing

promoter shows that repeated pulses of serotonin that produce LTF also trigger CREB activation, while a single pulse of serotonin that does not produce LTF similarly does not trigger CREB activation (Kaang et al., 1993).

Cloning studies identified several CREB-like proteins in *Aplysia* (Bartsch et al., 1995). The *Aplysia* CREB1 gene encodes three proteins (ApCREB1a, ApCREB1b and ApCREB1c) by alternative splicing. CREB1a shares structural and functional homology with CREB transactivators in mammals. Injection of antibodies or antisense against CREB1a blocks the LTF normally induced by five pulses of serotonin. These treatments, however, have no effect on STF (Bartsch et al., 1995). Conversely, injection of phosphorylated recombinant ApCREB1a protein alone induces LTF and prevents further facilitation normally induced by additional pulses of serotonin (Bartsch et al., 1995). Thus, ApCREB1a seems both necessary and sufficient to induce LTF in cultured neurons (Bartsch et al., 1995).

On the other hand, ApCREB1b lacks a P-box and, therefore, cannot be phosphorylated by PKA, CaMK or PKC. Thus, ApCREB1b resembles mammalian ICER both structurally and functionally. CREB1b forms homodimers as well as heterodimers with CREB1a and represses CREB1a-mediated transactivation and LTF (Bartsch et al., 1995). Injection of antisense oligonucleotides specifically targeted to CREB1b lowers the threshold for producing LTF, such that a single pulse of serotonin (rather than five spaced pulses) now induces LTF. Thus, removing the inhibition presumably produced by CREB1b enhances formation of LTF (Bartsch et al., 1995).

*Aplysia* CREB1c is a cytoplasmic protein that does not contain a nuclear localization signal and is unable to bind DNA or form dimers. Injection of unphosphorylated CREB1c followed by a single pulse of serotonin enhances STF and induces LTF. Therefore, this cytoplasmic form of CREB may play an important role not only in the modulation of CREB-mediated transcription necessary for LTF but also in STF.

*Aplysia* CREB2 is structurally unrelated to *Aplysia* CREB1 but shares some homology with mouse ATF-4 (Hai et al., 1989). Pairing a single pulse of serotonin that normally induces STF (but not LTF) with antibodies against ApCREB2 produces LTF (Bartsch et al., 1995). Transfection studies in F9 cells show that ApCREB2 represses the function of ApCREB1 (a CREB activator). Thus, it may be that ApCREB2 inhibits LTF by forming heterodimers with ApCREB1, thereby masking the activation domains of ApCREB1 and inhibiting its function. However, other experiments also show that, under certain circumstances, ApCREB2 may function as an activator in F9 cells (Bartsch et al., 1995). Thus, when co-transfected with a PKA construct, ApCREB2 (the nominal repressor) and ApCREB1 (the nominal activator) produce similar increases in the levels of a LacZ reporter gene under the regulation of a promoter with five CRE sites. ATF-4, which shares homology with ApCREB2 (Hai et al., 1989; Bartsch et al., 1995), may also function as a transcriptional repressor (Karpinski et al., 1992) or an activator (Bartsch et al., 1995) depending on the conditions used. Thus, the precise mechanism underlying the effects that ApCREB2 exerts on LTF is unclear.

### 3.2. CREB AND MEMORY IN *DROSOPHILA*

Learning and memory in *Drosophila* may be assessed using a Pavlovian olfactory test in which flies learn to avoid a previously neutral odor that was paired with shock (conditioning stimulus +; CS+) in favor of another odor that was not paired with shock (CS-) in a T-maze (Tully, 1991). Several temporally distinct phases of memory are identified using this test, including STM and LTM. Similar to behavioral sensitization and facilitation in *Aplysia*, spaced training in *Drosophila* induces LTM that is dependent on protein synthesis (Tully et

al., 1994). Massed training, on the other hand, produces (protein synthesis independent) STM but no LTM (Tully et al., 1994). For example, 10 spaced training trials, but not 48 massed training trials conducted over the same span of time, produce robust LTM (Yin et al., 1995).

Memory has been studied using both forward and reverse genetics in *Drosophila* (Tully, 1991). Using a forward genetic approach, the progeny of flies that were treated with a mutagen were screened for learning and memory impairments. Two mutants identified by this screen were subsequently determined to have disruptions in  $\text{Ca}^{2+}$ /CaM-stimulated adenylate cyclase (*rutabaga*) and in cAMP-specific phosphodiesterase (*dunce*), both key enzymes in the regulation of intracellular levels of cAMP (Byers et al., 1981; Tully, 1991; Levin et al., 1992).

Using a reverse genetic approach, CREB function was disrupted in *Drosophila* by the transgenic expression of a CREB transcriptional repressor (Yin et al., 1994). The *Drosophila* dCREB2 gene encodes two isoforms, dCREB2a, a transcriptional activator, and dCREB2b, a transcriptional repressor. dCREB2b binds CRE sites, but lacks the two exons required for transcriptional activation, and represses CREB-mediated transcription in cell culture. To control the onset of CREB repression in the transgenic fly, dCREB2b was placed under the control of a promoter that is activated by temperature increases (heat-shock promoter). Inducing dCREB2b prior to spaced training in the olfactory task completely disrupts LTM without affecting STM. The finding that STM is intact indicates that the overexpression of this CREB repressor did not disrupt learning or the perceptual responses to the odors used, shock reactivity, or motor performance necessary for this task. Mutating two amino acids that disrupt the dimerization domain of dCREB2b results in normal LTM, thus showing the specificity of the inhibition produced by the dominant negative CREB protein (Yin et al., 1994).

Multiple spaced training is required to produce maximal LTM in the olfactory task in normal *Drosophila* while massed training produces strong STM but no LTM. However, massed training alone is sufficient to produce maximal LTM if a CREB activator (dCREB2a) is overexpressed in transgenic flies prior to training. Furthermore, overexpression of this CREB activator produces robust LTM following only one training trial (Yin et al., 1995). Transgenic flies overexpressing a mutant activator, where Ser231 (similar to Ser133 of the mammalian CREB gene) was replaced by an Ala, do not show LTM after one training trial, indicating that phosphorylation of CREB is required for the enhancement of LTM (Yin et al., 1995). Together, these results show the importance of CREB in LTM formation in *Drosophila* and, furthermore, suggest that CREB may be a limiting component of this process.

### 3.3. PKA, CREB AND MEMORY IN HONEYBEES

The findings of the role of CREB in memory using *Drosophila* are in agreement with similar results from an associative olfactory conditioning task in honeybees (*Apis mellifera*). In this task, the proboscis extension reflex is conditioned by pairing an odor (the CS) with a sucrose reward (the US) (Bitterman et al., 1983; Menzel and Müller, 1996). A single trial produces activation of PKA in the antennal lobes and memory for the association, both of which decay over several hours (Hammer and Menzel, 1995; Grunbaum and Müller, 1998). Multiple spaced training trials, on the other hand, produces prolonged activation of PKA in the antennal lobes and a prolonged memory (LTM that is protein-synthesis-dependent) (Müller, 1996, 2000; Grunbaum and Müller, 1998; Wüstenberg et al., 1998; Fiala et al., 1999; Menzel, 1999).

Injection of antisense against the catalytic subunit of PKA into the brain of a bee or systemic injection of the PKA antagonist, RpBrcAMP, blocks LTM, but not STM (Fiala et al., 1999; Müller, 2000). These results suggest that PKA activation at the time of training is critical to LTM formation. Moreover, artificially prolonging PKA activation in the antennal

lobes (by photorelease of cAMP) in combination with a single training trial is sufficient to induce LTM (Müller, 2000). Thus, similar to *Drosophila*, training that normally produces STM may produce LTM if CREB, or a pathway upstream of CREB, is artificially increased or activated.

### 3.4. CREB AND MEMORY IN SONG BIRDS

Young male zebra finch birds (*Taeniopygia guttata*) learn to sing from conspecifics during a song-sensitive period in development (Sakaguchi et al., 1999). This form of learning is sensitive to protein synthesis inhibitors (Chew et al., 1995) and triggers an increase in CREB phosphorylation in the Higher Vocal Center (HVC), a brain area that is critical for song learning (Sakaguchi et al., 1999). Neither exposure to songs from other species nor white noise increase the levels of phosphorylated CREB in the HVC, thus showing the specificity of this effect (Sakaguchi et al., 1999). Similar to mammalian CREB, zebra finch CREB (zCREB) contains a putative PKA phosphoacceptor site at Ser119 (homologous to Ser133 in mammals) and zCREB is readily phosphorylated at Ser119 by PKA in vitro. These results suggest that CREB activation may play a role in the production of long-lasting memory traces in the song system of zebra finches.

### 3.5. CREB AND MEMORY IN MAMMALS

The study of the role of CREB in mammalian memory began with the generation of a mouse in which the CREB gene was disrupted. A neomycin resistance (neo) gene was inserted into exon 2 of the CREB gene, which was thought to contain the translation initiation site for all CREB isoforms (Hummler et al., 1994). This neo insertion resulted in the loss of the two main CREB isoforms ( $\alpha$  and  $\delta$ ) in the CREB <sup>$\alpha\delta$ -</sup> mice (Hummler et al., 1994). However, the translation of a previously unknown CREB isoform (CREB $\beta$ ) starts from exon 4, and consequently the insertion of neo gene into exon 2 did not disrupt this isoform. Instead, the usually low expression level of CREB $\beta$ , is upregulated in the CREB <sup>$\alpha\delta$ -</sup> mutants (Blendy et al., 1996). The levels of CREM activator ( $\tau$ ) and repressor isoforms ( $\alpha$  and  $\beta$ ) are also increased in these mutants (Hummler et al., 1994). Despite this upregulation, the CREB <sup>$\alpha\delta$ -</sup> mutation decreases CREB-dependent transcription in these mutants (Hummler et al., 1994; Blendy et al., 1996).

#### 3.5.1. Fear conditioning

To determine whether the CREB <sup>$\alpha\delta$ -</sup> mutation affects memory, mutant mice and wild-type (WT) littermate control mice were tested in a fear conditioning paradigm (Bourtchuladze et al., 1994; Kogan et al., 1996). Fear conditioning is a form of Pavlovian learning in which animals learn to fear a stimulus (CS; typically a context or discrete cue such as a tone) that previously has been paired with an aversive stimulus such as footshock (US) (Frankland et al., 1998; Fanselow and LeDoux, 1999; LeDoux, 2000). LTM, but not STM, for fear conditioning depends on the synthesis of new proteins (Abel et al., 1997; Schafe et al., 1999). Similarly, the CREB <sup>$\alpha\delta$ -</sup> mutation disrupts LTM, but not STM, for both discrete cue and contextual fear conditioning.

A deficit in LTM is also found using a different paradigm to measure discrete cue fear conditioning, the fear-potentiated startle task (Falls et al., 2000). In the fear-potentiated startle paradigm, LTM is inferred from an increase in the amplitude of the acoustic startle response

of mice when the startle reflex is elicited in the presence of a light (CS) that was previously paired with footshock (US) (Davis, 1992; Josselyn et al., 2001). Protein synthesis within the basolateral complex of the amygdala is critical for LTM of fear-potentiated startle (Gewirtz et al., 1998). CREB<sup>αδ-</sup> mutants show LTM deficits in this task (Falls et al., 2000).

Interestingly, overexpression of a CREB repressor (Ser133Ala mutation) in the forebrain does not affect memory to the same extent as the CREB<sup>αδ-</sup> mutation (Rammes et al., 2000). In one of the transgenic lines studied, a LTM deficit was observed following cued fear conditioning, even though synaptic plasticity in the amygdala (as assessed by long-term potentiation (LTP)) appeared unaffected. It could be that the milder phenotype observed in these transgenic mice is due to a milder decrease in CREB function, and/or to upregulation or compensation by other transcription factors. In contrast, recent studies with a transgenic mouse expressing an inducible CREB repressor (Ser133Ala), show that the induction of the CREB repressor produces profound LTM deficits in both contextual and discrete cue fear conditioning (Josselyn, Kida and Silva, unpublished data).

Also important to note are the findings that CREB-mediated transcription is activated during fear-conditioning training that results in LTM. For example, transgenic mice with a β-galactosidase reporter construct under the regulation of a CRE-containing promoter (CRE-LacZ), show that fear-conditioning training induces CRE-mediated transcription in several brain regions. Contextual, but not discrete cue (tone), fear training induces LacZ expression in the hippocampus, while both forms of fear training induce LacZ expression in the amygdala (Impey et al., 1998b). Together, these findings suggest that although CREB seems to be critical for LTM, not all genetic lesions of CREB produce pronounced effects on LTM. Understanding why and how these different genetic manipulations of CREB affect memory will help to reveal the intricate relation between the regulation of CREB-mediated transcription and memory formation.

### 3.5.2. Social behavior

Besides a role in LTM for fear conditioning, CREB has also been implicated in other forms of memory, such as social memory. The response demands of the tasks used to assess social memory are different from those required by fear conditioning. Nevertheless, both social memory and fear conditioning memory may involve some overlapping brain regions (such as the hippocampus) (Winocur, 1990; Bunsey and Eichenbaum, 1995). In the social transmission of food preference task rodents develop a preference for foods recently smelled on the breath of other rodents (Galef and Wigmore, 1983; Strupp and Levitsky, 1984; Galef et al., 1988). CREB<sup>αδ-</sup> mutant mice show normal immediate memory but deficient LTM in this task (Kogan et al., 1996).

In another type of social memory, social recognition memory, the ability of rodents to remember conspecifics is evaluated (Thor and Holloway, 1982; Kogan et al., 2000). Social recognition is defined by a decrease in spontaneous investigation behaviors observed in a mouse re-exposed to a familiar conspecific. Lesions of the hippocampus disrupt longer-term social memory, but not memory assessed immediately following training (Kogan et al., 2000). Furthermore, protein synthesis inhibitors block LTM but not STM for social recognition in WT mice (Kogan et al., 2000). Similarly, CREB<sup>αδ-</sup> mice show intact STM but impaired LTM for social recognition (Kogan et al., 2000). Thus, in two types of social memory, CREB<sup>αδ-</sup> mice show LTM impairments but normal immediate or STM. The findings suggest that the CREB<sup>αδ-</sup> mutation specifically disrupts those processes required for the long-term retention of information, rather than its acquisition.

### 3.5.3. Spatial learning

In the hidden platform version of the Morris water maze (Morris, 1981), rodents learn to find a platform submerged in a pool of opaque water. In the visible platform version of this task, mice learn to find a marked escape platform. Hippocampal lesions disrupt performance on the hidden platform, but not the visible platform, version of the Morris water maze (Morris et al., 1982; Sutherland et al., 1982; Cho et al., 1999). CREB <sup>$\alpha\delta$ -</sup> mice show a profound impairment in spatial learning and/or memory in the hidden version of this task (Bourtchuladze et al., 1994; Kogan et al., 1996). In contrast, learning in the visible platform version of the water maze is intact in the CREB <sup>$\alpha\delta$ -</sup> mice, again showing the behavioral specificity of this mutation.

In agreement with these findings, injections directly into the dorsal hippocampus of antisense against CREB mRNA disrupt learning and/or memory of rats in the hidden platform version of the water maze (Guzowski and McGaugh, 1997). In contrast, injection of antisense 2 days following the completion of training does not affect subsequent performance in the water maze, indicating that decreasing CREB function does not disrupt expression of the memory. This finding is consistent with results from *Drosophila* and *Aplysia* suggesting that the critical period for CREB function in LTM is during, or shortly after, training.

Injection of CREB antisense oligonucleotides into the hippocampus first decreased the levels of  $\alpha$  and  $\delta$  CREB within 6 h of injection, but increased the levels of these isoforms 14 h later. This rebound effect may reflect mechanisms similar to those responsible for increases in the levels of CREB $\beta$  and of CREM isoforms in CREB <sup>$\alpha\delta$ -</sup> mutants. Surprisingly, training conducted at a time when CREB levels were lower (within 6 h of injection) or higher (within 20 h of injection) relative to normal levels produced similar deficits. It could be, therefore, that unlike Pavlovian conditioning in *Drosophila*, increased levels of CREB do not facilitate the storage of complex information, such as that accumulated in spatial tasks.

### 3.5.4. Conditioned taste aversion

CREB is also required for the development of a conditioned taste aversion (CTA). Pairing malaise or sickness (for example, induced by lithium chloride, LiCl) with exposure to a novel taste (for example, saccharin) may produce a strong and long-lasting aversion to the novel taste. Infusion of the protein synthesis inhibitor, anisomycin, directly into the amygdala during training blocks LTM for this aversion (Lamprecht and Dudai, 1996). Similar infusion of oligonucleotides against CREB into the amygdala decreases CTA memory measured 3–5 days, but not 2 h, following training (Lamprecht et al., 1997). Infusions of CREB sense into the amygdala or CREB antisense oligonucleotides into the basal ganglia do not disrupt LTM for the taste aversion. Furthermore, CREB antisense oligonucleotides produce no effect on memory retrieval if infused into the amygdala before the memory test, rather than during training. Interesting, the CREB <sup>$\alpha\delta$ -</sup> mice also show impaired LTM for CTA (Josselyn et al., 1999).

The finding that CREB in the amygdala is important for the development of CTA memory is supported by the observation of an increase in the levels of phosphorylated CREB (pCREB) in the lateral nucleus of the amygdala specifically following CTA training. Importantly, this CTA training does not induce increases in pCREB in other brain regions such as the insular or gustatory cortex. Furthermore, increases in pCREB are not observed following administration of either the CS (e.g., saccharin) or the US (e.g., LiCl) alone (Swank, 2000), attesting to the associative nature of the observed increase in pCREB. In addition, training in a similar appetitive task that leads to LTM also leads to increases in pCREB. For example, in the

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olfactory preference task an odor is paired with stroking in neonate rats. This pairing produces a reliable preference for the odor (tested 24 h later) and triggers an increase in pCREB in the olfactory bulb. Similar to the findings with CTA, presentation of the odor alone or stroking alone does not induce LTM for the olfactory preference and does not induce an increase in pCREB levels in the olfactory bulbs (McLean et al., 1999).

### 3.6. TRIAL SPACING EFFECT

Robust LTM for contextual fear conditioning may be produced by a single training trial in WT mice. Despite normal sensory perception, the same single trial produces only a transient (<60 min) memory in CREB<sup>αδ-</sup> mice (Bourtchuladze et al., 1994). Not even training that produces maximal LTM in WT mice (5 trials with 1 min intervals), compensates for the profound contextual fear amnesia of these mutants. However, two spaced trials with a 1-h intertrial interval (ITI), that in WT control mice does not produce higher levels of freezing than a single trial, nevertheless induces robust LTM in CREB<sup>αδ-</sup> mutants (Kogan et al., 1996). Similarly, a single 5-min interaction with another mouse is sufficient to trigger LTM for social transmission of food preference in normal mice, but not in the CREB<sup>αδ-</sup> mutants. In CREB<sup>αδ-</sup> mice, LTM for socially transmitted food preferences requires spaced training (two trials with a 1-h ITI) (Kogan et al., 1996).

Learning to find the hidden platform in the Morris water maze is gradual, and, to master the task mice must remember what is learned over days. As with the fear conditioning and social transmission of food preference tasks, increasing the ITI (more than 10 min and as long as 24 h) and doubling the amount of training (20 trials instead of 10) overcomes the LTM deficit of the CREB<sup>αδ-</sup> mutants in the water maze (Kogan et al., 1996). The finding that spaced training rescues the LTM deficits in CREB<sup>αδ-</sup> mutants in three different tasks indicates that the profound deficits in LTM observed following relatively more massed training may not be attributed to deficits in sensory, motor or motivational processes.

Together these results indicate that massed training induces STM, but less robust LTM in WT animals, and spaced training rescues the LTM deficit in CREB<sup>αδ-</sup> mutant mice. However, does the reverse hold true? Does increasing CREB levels rescue or facilitate LTM following massed training? A recent experiment addressed these questions. WT rats given massed fear conditioning training (4 CS (light)–US (shock) pairings with ITIs of 3, 5 or 10 s) show no or weak LTM, as measured by fear-potentiated startle, compared to rats given the same number of training trials presented in a spaced fashion (ITIs of 8 min) (Josselyn et al., 2001). However, increasing CREB levels specifically in the basolateral amygdala of these rats via viral vector-mediated gene transfer increases LTM following massed fear training. Thus, training that normally induces STM (but little LTM) produces robust LTM if CREB levels are increased in the amygdala. The enhancing effect of CREB overexpression on LTM formation depends on phosphorylation of the viral encoded CREB gene as similar overexpression of mCREB (in which Ala was substituted for Ser133) does not enhance LTM following massed training. Consistent with the key role that the amygdala occupies in fear conditioning, overexpression of CREB in regions surrounding the amygdala or directly into the caudate nucleus does not facilitate the formation of LTM following massed training. Furthermore, increasing CREB levels at the time of training (memory formation), rather than testing (retrieval), seems critical for the facilitatory effects of CREB on LTM.

There are striking parallels among the studies showing that CREB affects the training schedules required to produce LTM. First, multiple spaced applications of serotonin are needed to trigger LTF between *Aplysia* synapses, while a single application of serotonin

induces STF. However, a single application of serotonin given together with the CREB activator, ApCREB1a, produces LTF. Second, *Drosophila* given massed training for an olfactory avoidance task show no LTM. However, massed training, or even a single training trial, produces robust LTM if a CREB activator is overexpressed in flies before training. Third, rats given massed fear training show no or weak LTM as assessed by fear-potentiated startle. However, massed training induces robust LTM in rats if CREB is overexpressed in the basolateral amygdala. Finally, the LTM deficits for a variety of memory tasks observed in the CREB<sup>αδ-</sup> mutant mice (with decreased levels of CREB) are overcome with spaced training. Together, these results from a range of species and memory tests suggest that animals with relatively lower levels of activator CREB require longer rest intervals between trials to produce LTM whereas animals with higher levels of activator CREB may exhibit LTM following massed, as well as spaced, trials. Flies, mice and rats with presumably more activator CREB require less training than flies and mice with less activator CREB (Yin et al., 1994, 1995; Josselyn et al., 2001). Therefore, in species ranging from *Aplysia*, mice, rats and flies, manipulations of CREB function seem to directly affect the amount and distribution of training required for the induction of LTM. The ability of CREB to affect the schedules of training required for memory formation seems to be highly conserved across species.

A 10-min ITI is optimal for olfactory learning in WT *Drosophila* (Yin et al., 1995) and an 8-min ITI seems best for fear conditioning in WT rats using fear-potentiated startle (Josselyn et al., 2001). Nevertheless, CREB<sup>αδ-</sup> mutants trained with 1-h ITIs perform better than those trained with 10-min ITIs, suggesting that 10 min may be just below the threshold for optimal memory induction in these mutant mice (Kogan et al., 1996). The timing of these events is important because it may provide hints regarding the nature of underlying mechanisms. It may take 3–8 min for synaptic activation to trigger maximal CREB activation (phosphorylation) (Moore et al., 1996). After committing the general transcriptional machinery to genes with CRE promoters, it may take CREB a few more minutes before another round of transcription may be initiated (akin to a refractory period). Additionally, it is possible that the longer intervals result in optimal inactivation of phosphatases (Bito et al., 1996; Liu and Graybiel, 1996) that control the phosphorylation and activation of CREB transcription factors.

### 3.7. DISSECTING THE ROLE OF CREB IN MEMORY WITH AN INDUCIBLE, BRAIN-SPECIFIC TRANSGENE

The findings obtained using the CREB<sup>αδ-</sup> mice indicate that CREB function is required for LTM. However, it is important to test the role of CREB in memory with alternative tools, especially those that allow temporal control over CREB function. Thus, our laboratory developed a transgenic mouse expressing a brain-specific and inducible CREB repressor.

This inducible CREB repressor (CREB<sup>IR</sup>) mouse overexpresses the αCREB isoform with a mutation of Ser133 to Ala (αCREB<sup>S133A</sup>) that represses endogenous CREB function (Gonzalez and Montminy, 1989; Brindle and Montminy, 1992). The inducibility of the system is provided by fusing the mutant CREB to a ligand-binding domain (LBD) of a human estrogen receptor with a G521R mutation (LBD<sup>G521R</sup>), the activity of which is regulated not by estrogen but by the synthetic ligand, tamoxifen (TAM) (Danielian et al., 1993; Logie and Stewart, 1995; Feil et al., 1996). Therefore, in the absence of the inducer TAM, the LBD<sup>G521R</sup>-CREB<sup>S133A</sup> fusion protein is inactive (Feil et al., 1996). However, administration of TAM activates this inducible CREB repressor fusion protein, allowing it to compete with endogenous CREB and disrupt CRE-mediated transcription. The CREB<sup>IR</sup> construct represses CRE-mediated transcription of a CRE-luciferase reporter in a model system (COS cells) in a

TAM-dependent manner. In the transgenic mice, the inducible CREB repressor is under the control of the  $\alpha$ CaMKII promoter, which is active in excitatory neurons of forebrain areas including the hippocampus, amygdala, neocortex and striatum (Mayford et al., 1996). Thus, administration of the inducer TAM decreases in CREB function in forebrain areas.

Parametric studies using this transgenic mouse show that administration of the inducer, TAM, 6 or 12 h but not 30 min prior to contextual fear training produces a deficit in LTM measured 24 h following training. Importantly, similar administration of TAM to WT littermate mice, regardless of time of administration, produces no effect. These results suggest that the CREB<sup>IR</sup> system has good temporal and inducible control. Moreover, the finding that CREB<sup>IR</sup> mice administered TAM 24 h before training show no subsequent deficit in LTM suggests that the disruption of CREB function using this system is reversible within 24 h. STM for contextual fear conditioning is not disrupted in these transgenic mice administered TAM prior to training. Furthermore, acutely disrupting CREB function (by administering TAM to CREB<sup>IR</sup> mice) before a retrieval test produces no effect, indicating that CREB is not critically involved in the retrieval of fear memories.

#### 4. PKA, CREB AND LONG-TERM POTENTIATION

Long-term potentiation (LTP) is the most extensively studied candidate cellular plasticity mechanism thought to underlie memory (Bliss and Collingridge, 1993). LTP refers to a class of long-lasting enhancements in synaptic efficacy with properties expected of a memory mechanism (long-lasting, associative, reversible, etc.). A variety of different studies suggest that an LTP-like mechanism may be involved in memory formation (Barnes, 1995; Maren and Baudry, 1995; Mayford et al., 1996). LTP is not a single phenomenon; rather, there are various forms of LTP with distinct time courses and underlying biochemical mechanisms (Huang et al., 1996).

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The best studied LTP occurs between CA3 and CA1 pyramidal neurons of the hippocampus and is sensitive to blockers of CaMKs (Bliss and Collingridge, 1993; Chapman, 2001). One stimulus train typically produces LTP that dissipates within 1 to 2 h (early LTP or E-LTP), and is insensitive to inhibitors of protein synthesis (Frey et al., 1988, 1993; Huang and Kandel, 1994). Late-LTP (L-LTP) lasts much longer than E-LTP (>7 h), generally requires more stimulus trains to induce, and is blocked by protein synthesis inhibitors (Frey et al., 1993; Huang and Kandel, 1994).

In addition, L-LTP is blocked by pharmacological agents that inhibit PKA (Frey et al., 1993; Huang and Kandel, 1994; Nguyen et al., 1994; Woo et al., 2000). Moreover, transgenic mice expressing an inhibitory form of the regulatory subunit of PKA (R(AB)), which have significantly reduced levels of hippocampal PKA activity (approximately 40–50% of basal activity), show deficits in the late phase of L-LTP, even though synaptic transmission and the early phase of LTP are normal (Abel et al., 1997). These findings are consistent with those showing that the R(AB) mice, as well as systemic (Abel et al., 1997; Bourtchouladze et al., 1998), icv (Bourtchouladze et al., 1998; Schafe et al., 1999) or intra-amygdala (Ding et al., 1998; Schafe et al., 2000) administration of pharmacological agents that inhibit PKA to WT animals selectively impair LTM for fear conditioning. Together, these findings are consistent with a model that proposes that PKA is required for both L-LTP and LTM formation.

In contrast, administration of low levels of rolipram, a phosphodiesterase type IV inhibitor that increases cAMP levels, may selectively enhance the formation of LTP and LTM (Barad et al., 1998). In WT hippocampal slices E-LTP decays to baseline levels several hours after

tetanus. However, with the addition of rolipram, this stimulation protocol produces L-LTP that persists for several hours. Similarly, rolipram enhances LTM in contextual fear conditioning without affecting STM. Thus it could be that increasing cAMP, which may increase PKA activation and ultimately CREB phosphorylation, may facilitate both LTP and LTM.

Not only is PKA critically involved in L-LTP, but CREB may also play an important role. Hippocampal slices from the CA1 region of CREB<sup>αδ-</sup> mutant mice show normal E-LTP but impaired L-LTP (A.J.S., in prep). A single bout of high-frequency stimulation (100 Hz for 1 s with a 250-μs stimulus) induces a potentiation that lasts several hours in WT mice. In contrast, the same stimulus triggers a smaller potentiation that dissipates within 90 min in CREB<sup>αδ-</sup> mutants. Thirty minutes following this stimulation, however, slices from CREB<sup>αδ-</sup> mutants are potentiated, consistent with normal STM in these mice (Bourtchuladze et al., 1994). Thus, two genetic (CREB<sup>αδ-</sup> mutation and R(AB) transgenics) and several pharmacological manipulations that affect the PKA/CREB pathway produce comparable effects on LTP and LTM, suggesting that this second messenger pathway is required for L-LTP and LTM formation.

The hypothesis that CREB is important for L-LTP is also supported by the observation that the levels of pCREB increase in response to L-LTP-inducing synaptic stimuli in hippocampal slices (Matthies et al., 1997; Lu et al., 1999), amygdala slices (Huang et al., 2000), dissociated hippocampal neurons in vitro (Bitto et al., 1996) and in the hippocampus in vivo (Schulz et al., 1999; Davis et al., 2000). Increases in CRE-mediated gene expression are produced by L-LTP inducing stimuli in CRE-reporter mice (Impey et al., 1996). Importantly, the increases in pCREB levels are stimulus-specific. That is, increases in pCREB are not observed following stimuli that do not result in L-LTP (such as low-frequency stimulation of the perforant pathway) but are observed following stimuli that result in L-LTP (such as high-frequency stimulation of the perforant pathway) (Schulz et al., 1999).

Long-term depression (LTD), a use-dependent depression between synapses (Linden and Connor, 1995), may be observed in the cerebellum and is thought to mediate some forms of motor learning (Thompson, 1986). Together with LTP-like phenomena, LTD-like phenomena may modulate the storage capacity of neuronal networks, by fine-tuning synaptic weights (Malenka, 1994). Similar to LTP, a late-phase form of LTD is blocked by protein synthesis inhibitors (Ahn et al., 1999). Moreover, inhibition of CREB function with a dominant negative isoform (A-CREB) also inhibits L-LTD in cerebellar slices (Ahn et al., 1999).

In addition to PKA, CaM kinases are located upstream from CREB and may be critically involved synaptic plasticity such as LTP. Antisense oligonucleotides against the α and β splice variants of CaMKIV disrupt CREB phosphorylation and mutant mice with a targeted disruption of the CaMKIV gene show impaired CREB phosphorylation and CREB-dependent transcription of c-Fos (Ho et al., 2000). Furthermore, these mutant mice show impaired LTP (measured in hippocampal CA1 neurons) and LTD (measured in cerebellar neurons). Interestingly, these CaMKIV mutant mice show no LTM deficits, suggesting that other signaling pathways may compensate for the impairment of CaMKIV-dependent pathway in these mutant mice.

#### 4.1. MASSED VS. SPACED STIMULATION OF SYNAPSES AND LTP

Just as the interval between training trials is an important determinant of LTM in behavioral studies, the interval between tetani is important for the induction of L-LTP in hippocampal slices. Under certain conditions, 1-min intervals between periods of high-frequency stimulation (e.g., 100 Hz) produces unstable LTP that lasts only a few hours. The same tetani,

however, delivered at 10-min intervals is more likely to trigger a stable, longer-lasting LTP (>4 h) that requires protein synthesis (Huang et al., 1996). Results from our laboratory suggest that increasing the interval between tetani may overcome the L-LTP deficits observed in the CREB <sup>$\alpha\delta$</sup> - mutants (A.J.S. and J.K., in preparation). Thus, the loss of the  $\alpha$  and  $\delta$  CREB activators does not block either L-LTP or LTM under all conditions. Instead, the mutants require spaced training to show normal LTM (Kogan et al., 1996) and spaced tetanization to show normal L-LTP.

Consistent with the findings in hippocampal cultures (Deisseroth et al., 1996), a brief stimulus (100 Hz) fails to activate CREB-dependent transcription in hippocampal slices from the CRE-LacZ (reporter) mice, and fails to induce L-LTP. In contrast, multiple-spaced tetani (three tetanic trains given with a 5-min ITI), that induce protein-synthesis-dependent L-LTP, also trigger an increase in LacZ expression in the CRE-LacZ hippocampal slices (Impey et al., 1996). This increase is detectable 2 h after stimulation and reaches a peak within 4–6 h. Both types of LTP stimuli used (a single tetanus or three tetani) increase the levels of CREB phosphorylation, although only the repeated tetanic stimulation increases LacZ expression (Impey et al., 1996).

It is important to note that even though the levels of CREB may help determine how tetanic patterns affect LTP, CREB activation itself may not be the only biological sensor that reads out these patterns. A recent study shows that the pattern of electrophysiological stimulation affects the duration of a kinase upstream of CREB. Thus, spaced membrane depolarizations, but not a single prolonged stimulus, produces a persistent activation of the MAPK pathway (Wu et al., 2001b; but see also Fields et al., 1997). Importantly, these authors also show that MAPK activation triggers a slow-onset activation (phosphorylation) of CREB (Wu et al., 2001a). Thus, in a treatment that may be akin to spaced training in behavioral experiments, spaced electrophysiological ‘training’ may activate a kinase upstream from CREB.

#### 4.2. CREB AS A GAIN CONTROL DEVICE FOR MEMORY

Together, the data reviewed thus far suggest that CREB may have a specific computational role in memory formation. CREB may assist in ‘setting the height of the bar’ that must be attained in order for LTM and LTP formation to occur. High levels of CREB activity may allow neuronal circuits to acquire memory relatively quickly, while circuits with low CREB levels would acquire memory relatively slower. This gain control mechanism could be critical in determining the number and distribution of trials required to lay down memories in a given circuit.

It is important to note that CREB-like proteins function with many other transcription factors. There is evidence that the results obtained by manipulating CREB are also found in studies using other transcription factors (Alberini et al., 1994). Therefore, CREB may be simply one of several related gain control mechanisms that contribute to the regulation of memory acquisition in neural circuits.

### 5. CREB AND SYNAPTIC REMODELING

Behavioral long-term sensitization in *Aplysia* results not only in a stable increase in neurotransmitter release, but also in structural changes, such as the growth of new synapses. These structural changes include alterations in sensory neuron active zones and an increase in the number of presynaptic varicosities (Bailey and Chen, 1988). There are many parallels

between agents that induce (or block) LTF and those that induce (or block) these structural changes, a finding that confirms the relationship between synaptic function, structural changes and memory formation in *Aplysia* (Bailey and Chen, 1988). Intracellular injection of cAMP into the intact ganglion (Nazif et al., 1991) and repeated pulses of serotonin that induce LTF in culture (Glanzman et al., 1990; Bailey et al., 1992) also induce the growth of new synapses. A single pulse of serotonin paired with an injection of an antibody against a CREB repressor (ApCREB2) is sufficient to induce the growth of new synapses, just as it is sufficient to induce LTF (Bartsch et al., 1995). These results indicate that CREB-dependent proteins are involved in the growth of new synapses during LTM formation in *Aplysia*.

However, CREB-mediated transcription seems to be necessary but not sufficient for the growth of new synaptic connections. Experiments using a single sensory neuron composed of two branches that contact two spatially separated motor neurons shows that local application of serotonin onto a single synapse induces LTF that is branch-specific (Martin et al., 1997; Casadio et al., 1999). This branch-specific LTF requires local protein synthesis and CREB activation in the nucleus of the presynaptic neuron. Repeated application of serotonin onto the cell body of the sensory neuron induces a cell-wide transient LTF (not beyond 48 h) that is CREB-dependent, but is not accompanied by synaptic growth. A similar pattern (transient LTF and no synaptic growth) is produced by injection of phospho-CREB1 into the sensory neuron. In order for this transient LTF to become stable and for growth to appear, a single pulse of serotonin at either synapse is required. Thus, CREB-mediated transcription cooperatively induces synaptic plastic changes in concert with local stimulation by serotonin.

In addition to a proposed role for CREB in synaptic remodeling in *Aplysia*, there may also be a role for CREB in structural plasticity in mammals. A recurrent theme in the study of development and learning is that these two forms of plasticity may share some common mechanistic features (Hebb, 1949; Carew et al., 1998). Thus, CREB may be important to both to the formation of memory and to the synaptic restructuring that takes place during development.

### 5.1. DEVELOPMENTAL PLASTICITY OF VISUAL CORTEX

During a critical period in development, manipulating the input to the visual system may dramatically change neuronal connections in the visual cortex (Hubel and Wiesel, 1998). For example, depriving visual input from one eye during this critical period shifts the responses of cortical neurons towards the non-deprived eye (Wiesel and Hubel, 1963; Gordon et al., 1996). This process may require new protein synthesis, and perhaps CREB-dependent transcription.

Monocular deprivation during this critical period produces an increase in the transcription of a CRE-reporter (CRE-LacZ) construct in the visual cortex of transgenic mice (Pham et al., 1999). Specifically, the primary visual cortex (V1) receiving the input from the non-deprived eye shows an increase in CRE-LacZ expression compared to the area of primary visual cortex receiving input from the deprived eye. Importantly, both the shift in visual cortical responses and the increase in CRE-mediated transcription occur only during the critical period. Moreover, binocular deprivation, which does not induce cortical plasticity (Wiesel and Hubel, 1965; Gordon et al., 1996), similarly does not increase CRE-mediated transcription in the visual cortex of the transgenic mice. These results suggest that CREB is important in developmental visual cortical plasticity.

## 5.2. CREB AND PLASTICITY OF THE BARREL CORTEX

Unlike the visual cortex, plasticity in the somatosensory cortex does not seem to be restricted to a brief developmental critical period. Under certain conditions, there is a preferential re-allocation of cortical resources to areas representing the most-used sensory inputs. That is, there may be a use-dependent shift in cortical resources (for review see Buonomano and Merzenich, 1998). In rodents, experience-dependent plasticity has been extensively studied in the barrel cortex, a region of the somatosensory cortex that represents the long facial vibrissa. The vibrissa are topographically represented in barrel-like structures in the cortex. Cells within a barrel respond primarily to stimulation of their corresponding (principal) whisker but also show weaker responses to stimulation of whiskers neighboring the principal (Woolsey, 1967; Woolsey and Van der Loos, 1970; Welker, 1971).

The anatomical map of the barrels shows plasticity during a developmental critical period such that removal of the vibrissa follicles shortly after birth only leads to abnormal anatomical development of the barrels (Van der Loos and Woolsey, 1973). In contrast, the functional map of the barrel cortex exhibits plasticity outside the critical window, even in adulthood. Removing or even trimming some of the whiskers in adulthood, no longer alters the size or morphology, but does change the functional properties of the barrels (Hand, 1982; Fox, 1992). The receptive field properties of neurons in barrel-columns corresponding to the spared whisker typically expand in size and exhibit potentiation (Hand, 1982; Fox, 1992; Glazewski and Fox, 1996). A corresponding depression of cortical responses to the areas representing the deprived whiskers is observed. Interestingly, the CREB<sup>αδ-</sup> mutation, which disrupts LTP and LTM (see above), also disrupts this use-dependent plasticity in the barrel cortex (Glazewski et al., 1999). Similar to the results summarized above for the visual cortex, manipulations (whisker deprivation) that trigger plasticity in the barrel cortex also increase the expression of a transgenic CRE-LacZ reporter in the spared barrel (Barth et al., 2000). Importantly, manipulations that do not lead to plasticity, also do not produce increases in LacZ expression. Thus, normal undeprived animals and animals in which all the whiskers have been removed show low levels of LacZ expression and do not exhibit potentiated barrel responses (Barth et al., 2000). Therefore, CREB may be important in structural plasticity that takes place during development and the use-dependent functional plasticity that takes place during adulthood. These findings are in agreement with the idea that learning and development may share common mechanistic features.

## 6. TARGET GENES OF CREB

### 6.1. POSSIBLE DOWNSTREAM TARGETS OF CREB IN *APLYSIA*

The long-lasting plastic changes induced by CREB may be orchestrated by one or a choir of CREB target proteins. Multiple pulses of serotonin activate CREB in *Aplysia* and induce a set of genes, any one of which may regulate synaptic plasticity. Several synaptic plasticity candidate genes have been identified and will be briefly discussed. The CCAAT/enhancer binding protein, ApC/EBP is a candidate gene as it contains CRE sites in the promoter region, is rapidly induced by cAMP and exhibits properties consistent with an immediate-early gene. Low levels of ApC/EBP are present in unstimulated sensory neurons but higher levels are observed during the initial phase of LTF. Decreasing the expression of ApC/EBP selectively blocks the formation of LTF but not STF (Alberini et al., 1994). Thus, ApC/EBP may be a

transcriptional regulator downstream of CREB that induces the transcription of target genes necessary for LTF. Interestingly, inhibitory avoidance training in rats induces two homologs of ApC/EBP (C/EBP  $\beta$  and C/EBP  $\alpha$ ), suggesting that the function of ApC/EBP may be evolutionarily conserved (Taubenfeld et al., 2001).

Using a yeast two-hybrid screen with ApC/EBP as bait, another synaptic plasticity candidate gene was identified, the *Aplysia* Activating Factor, ApAF (Bartsch et al., 2000). Unlike ApC/EBP, ApAF is constitutively expressed in sensory neurons and is not up-regulated by serotonin application. ApAF shares homology with the mammalian PAR family of transcription factors, including TEF, E4BP4 and DBP. ApAF forms dimers with ApC/EBP and ApCREB2, but not with ApCREB1. PKA, CaMKII and PKC phosphorylate ApAF in vitro. Injection of recombinant ApAF into a co-cultured neuronal preparation converts the STF normally produced by one pulse of serotonin into LTF. However, unlike injection of ApCREB1a, injection of ApAF alone is not sufficient to induce LTF. Injection of antibodies against ApAF, or injection of a dominant negative form of ApAF, that contains only the bZIP domain, blocks the LTF produced by either five pulses of serotonin or injection of ApCREB1a itself (Bartsch et al., 2000). These manipulations, however, have no effect on STF. Thus, although ApAF does not directly interact with CREB1a, it may act downstream from, or somehow parallel to, CREB1a. It could be that ApAF heterodimerizes with ApCREB2, thereby removing the repression that ApCREB2 normally exerts on CRE-mediated transcription.

Another gene that is rapidly induced by LTF in *Aplysia* codes for ubiquitin C-terminal hydrolase (ApUch), an enzyme associated with proteasome-dependent proteolysis (Hegde et al., 1997). The ubiquitin-proteasome pathway degrades target proteins and ApUch enhances the degradation of substrates in proteasomes by removing ubiquitin. Injection of an antibody or antisense against ApUch blocks LTF, but not STF. Therefore, it seems that ubiquitin-dependent proteolysis is important for the induction of LTF, perhaps by removing or degrading inhibitory proteins. One possibility is that ubiquitin-mediated proteolysis cleaves the regulatory (R) subunit of PKA, freeing the catalytic (C) subunit, thereby producing a persistent activation of PKA (Bergold et al., 1992). Support for this comes from the finding that addition of C subunits of PKA may rescue the block of LTF produced by proteasome inhibitors. Thus, it could be that the activation of PKA C subunits (by ubiquitin-dependent degradation of R subunits of PKA) may be a key step in the phosphorylation of CREB necessary to produce LTF. Interestingly, a mutation of a related Angelman ubiquitin ligase in mice produces deficits in LTM for contextual fear conditioning and LTP (Jiang et al., 1998).

Another gene that may interact with CREB in mediating synaptic plasticity in *Aplysia* is *Aplysia* tolloid/BMP-1 (bone morphogenic protein)-like protein (ApTBL-1) (Liu et al., 1997). ApTBL-1 may function as a protease that activates growth factors such as transforming growth factor- $\beta$  (TGF- $\beta$ ). Inhibitors of TGF- $\beta$  block LTF produced by electrical stimulation, while treatment with TGF- $\beta$  induces LTF (Zhang et al., 1997). Hence, LTF may involve the expression of ApTBL-1 (CREB-dependent?) that activates TGF- $\beta$ , that, in turn, triggers a cascade of events that result in increased neurotransmitter release. This cascade may resemble that triggered by neurotrophins in hippocampal neurons (Kang and Schuman, 1996). It is interesting to note that TGF- $\beta$  triggers LTF without inducing long-term increases in neuronal excitability or STF (Zhang et al., 1997) thus confirming the previous finding that these two phases of sensitization are independent (Emptage and Carew, 1993; Mauelshagen et al., 1996).

## 6.2. POSSIBLE DOWNSTREAM TARGETS OF CREB IN MAMMALS

Many genes have been identified as potential target genes for CREB in mammals based on the presence of CRE sites within their 5'-flanking region. CREB-mediated plasticity may be ultimately mediated by any one, or a chorus, of these target genes. Although the precise downstream targets of CREB that are essential for mediating synaptic plasticity remain unknown, some studies have begun to evaluate the potential target genes. Two criteria should be met in order for a gene to be a potential mediator of CREB-induced plasticity. First, the gene should be regulated by CREB and second, the gene should be involved in synaptic plasticity.

One set of potential downstream targets of CREB is the immediate-early genes. The immediate-early genes, including Fos, Zif268 (also known as EGR1 and NGFI-A) and HZF-3 (also known as NURR1), contain CRE sites in their promoter regions and are transcriptionally regulated by CREB (Berkowitz et al., 1989; Changelian et al., 1989; Sakamoto et al., 1991; Saucedo-Cardenas et al., 1997). Furthermore, these genes are induced by neural stimulation and behavioral experience (Nikolaev et al., 1992; Robertson, 1992; Dragunow, 1996; see also Chapter VIII by Kaczmarek as well as Chapter XII by Leah and Wilce, this volume). The immediate-early genes are thought to function as nuclear third messengers that mediate a secondary cascade of transcription that leads to the delayed expression of effector proteins.

Fos is perhaps the most studied immediate-early gene in the central nervous system. Expression of the *c-fos* gene is increased by manipulations that also induce LTM or hippocampal LTP (Worley et al., 1991; Hess et al., 1995; Lanahan et al., 1997; Guzowski et al., 2001). Similar to CREB, antisense against Fos blocks LTM, but STM, in several behavioral tasks (Lamprecht and Dudai, 1996; Grimm et al., 1997; Morrow et al., 1999; Tolliver et al., 2000).

A second well-studied immediate-early gene that may be a downstream target mediating at least some of the synaptic plasticity effects of CREB is zif268. The levels of zif268 are upregulated following LTP-inducing stimulation (Cole et al., 1989; Abraham et al., 1991; Richardson et al., 1992). Furthermore, mutant mice lacking the zif268 gene show deficits in L-LTP and LTM for a variety of learning tasks (Jones et al., 2001). Of particular interest is the finding that, similar to the CREB<sup>αδ-</sup> mutant mice (Kogan et al., 1996), the spatial memory deficits of the Zif268 mutant mice are rescued by spaced training (Jones et al., 2001).

Less is known about the role of the immediate-early gene, HZF-3, in plasticity. HZF-3 encodes a transcription factor that is a member of the nuclear hormone receptor superfamily (Law et al., 1992; Mages et al., 1995; Peña de Ortiz and Jamieson, 1996). *hzf-3* mRNA is induced in the hippocampus during acquisition of a spatial discrimination task or induction of mossy fiber-CA3 LTP (Onton et al., 1996; Peña de Ortiz et al., 2000). Furthermore, antisense against *hzf-3* infused into CA1 or CA3 regions of the hippocampus impairs retention of a previously acquired spatial discrimination (Colón et al., 2000). Additional studies, perhaps using inducible transgenic or knockout approaches, are required to further assess the potential role of *hzf-3* in CREB-dependent plasticity.

Recent studies show that CREB mediates the Ca<sup>2+</sup>-dependent transcriptional activation of brain-derived neurotrophic factor (BDNF), a gene with CRE sites in its promoter region (Shieh et al., 1998; Tao et al., 1998). BDNF plays a critical role not only in neuronal development but also neuronal plasticity including synaptic transmission and LTP. Therefore, BDNF is a strong candidate for a CREB target that mediates some of the synaptic functions of CREB.

Additional candidates for CREB-regulated effector proteins await identification and char-

acterization. Studies combining genetically modified mice that are impaired in CREB function and gene profiling techniques such as microarrays may be useful techniques to this end.

## 7. CONCLUSION

The findings reviewed here represent a significant advance in our understanding of memory formation and plasticity. In a dizzying range of species, including *Aplysia*, *Drosophila*, song birds, honey bees, mice and rats, CREB-dependent transcription has been shown to be crucial for the formation of LTM. The studies reviewed above show that altering CREB function (with pharmacology, antibodies, antisense, viral vectors, targeted and inducible genetic mutations) effect LTM. The convergence of these results argues that CREB function is required for memory formation. This extensive body of data also suggests that the levels of active CREB are an important determinant of the amount and schedule of training required for the formation of LTM; in general increases in CREB obviate spaced training, while decreases in the levels of this transcription factor may be overcome by extended spaced training. Additionally, these studies show that CREB may have a universal impact on memory formation. Tests as diverse as olfactory conditioning in flies, fear conditioning, spatial memory, conditioned taste aversion, social recognition and social transmission of food preferences in rodents demonstrate the involvement of CREB in memory formation. Furthermore, the data reviewed here also indicate that CREB plays a key role in synaptic plasticity such as LTP and LTD. In addition, evidence was reviewed suggesting that CREB is important in structural and functional plasticity in the cortex. This finding adds credence to the suggestion that mechanisms important for development are recapitulated during learning. Overall, these findings suggest that CREB has a highly conserved role in various types of plasticity.

## 8. ABBREVIATIONS

ApAF	<i>Aplysia</i> activating factor
ApUch	<i>Aplysia</i> ubiquitin C-terminal hydrolase
ATF	activating transcription factor
BDNF	brain-derived neurotrophic factor
C/EBP	CAAT/enhancer binding protein
CaM	calmodulin
CaMK	calmodulin kinase
cAMP	cyclic adenosine monophosphate
CBP	CREB binding protein
CK	casein kinase
CRE	cAMP responsive elements
CREB	cAMP responsive element binding protein
CREB <sup>IR</sup>	CREB-inducible repressor
CREM	cAMP responsive element modulator
CS	conditioned stimulus
CTA	conditioned taste aversion
E-LTP	early LTP
ERK	extracellular signal-related kinase
GSK	glycogen synthase kinase

HAT	histone acetyltransferase
ICER	inducible cAMP early repressor
ITI	intertrial interval
KID	kinase-inducible domain
LiCl	lithium chloride
L-LTP	late LTP
LTD	long-term depression
LTF	long-term facilitation
LTM	long-term memory
LTP	long-term potentiation
NGF	nerve growth factor
NMDAR	<i>N</i> -methyl-D-aspartate receptor
PKA	protein kinase A
PKC	protein kinase C
PP	protein phosphatase
STF	short-term facilitation
STM	short-term memory
TAM	tamoxifen
TGF- $\beta$	transforming growth factor
Trk	tyrosine kinase
US	unconditioned stimulus
WT	wild type

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