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Review Article

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A Functional Role for CREB as a Positive Regulator of Memory Formation and LTP

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cAMP response element-binding protein (CREB), a transcription factor, has been shown to play a central role in memory formation, and its involvement in this process has been investigated using a wide range of animal models, from nematodes to higher animals. Various CREB mutant mice have been developed and investigated. Several types of mutant mice with loss of CREB function have impaired memory formation and long-term potentiation (LTP), suggesting that CREB plays essential roles in these processes. To characterize the roles of CREB in memory formation and LTP further, mutant mice displaying gain of CREB function have been generated and analyzed. Importantly, CREB-DIEDML mice and CREB-Y134F mice showed enhanced memory formation, whereas CREB-VP16 mice displayed a lowered threshold of long-lasting LTP (L-LTP) induction, strongly suggesting that CREB functions as a positive regulator of memory formation and LTP. In this review, I focus on the effects of the genetic activation of CREB in LTP and memory formation and summarize previous findings.

Key words: CREB, memory, LTP, LTM, STM, BDNF

ROLES OF GENE EXPRESSION IN MEMORY FORMATION AND LONG-TERM POTENTIATION

Short-term memory (STM) persists for several hours at most, while long-term memory (LTM) can last for up to a lifetime. The process underlying the formation of LTM is called memory consolidation [1]. A clear biochemical feature of memory consolidation at the cellular level, which occurs immediately after learning (an episode), is the induction of gene expression. This gene expression is considered to induce plastic changes in neurons, thereby allowing the long-term retention of memory. For example, experiments in rodents using a Pavlovian fear conditioning task and other tasks showed that when gene expression in the brain was inhibited immediately after conditioning (learning), STM (up to 2-4 h) was intact, but LTM (approximately 24 h) was disrupted [2, 3]. These findings indicate that memory consolidation is dependent on the activation of gene expression. In addition, long-term potentiation (LTP) is considered to be a cellular model that reflects one aspect of memory formation. Field recording analyses showed that long-lasting LTP (L-LTP) induced by tetanic stimulation of hippocampal CA1 neurons also requires gene expression; the inhibition of gene expression impairs L-LTP without affecting the induction or early phase of LTP [1, 3].

CREB, MEMORY FORMATION, AND LTP

CREB was cloned in 1988 as a transcription factor that binds to the cAMP-responsive element (CRE) [4]. Subsequent studies showed that CREB belongs to the CREB/ATF family together

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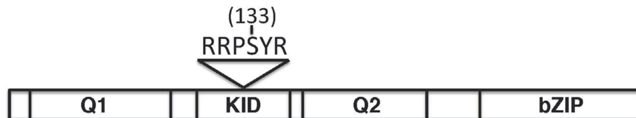

Fig. 1. Primary structure of CREB. Domain structure of CREB. RRPSYR represents aminoacid sequence around Serine 133. Q1 and Q2; glutamine rich regions. KID, kinase-inducible domain; bZIP, basic leucin zipper domain.

with ATF-1, cAMP-responsive element modulator (CREM), ATF2 and ATF3 and ATF4, which are highly homologous to CREB at the primary structure level [5]. The N-terminal region of CREB contains two glutamine-rich regions (Q-rich domains) and a region with serine residues (KID; kinase induceble domain), the latter of which is the target of various kinases and functions as a transcriptional regulatory domain (Fig. 1). In addition, the C-terminal region harbors a b-ZIP domain with a leucine zipper following a basic region and contributes to the formation of homo- and hetero-dimers and binding to CRE (Fig. 1).

CREB is located downstream of the signal transduction pathways of cAMP and Ca^{2+}. The activation of transcription by CREB depends on its phosphorylation at serine 133 (S133) [1, 5-8], and CREB is activated when S133 is phosphorylated mainly by protein kinase A (PKA) or Ca^{2+}/calmodulin-dependent kinase IV (CaMKIV). CREB can interact with CREB-binding protein (CBP), a transcriptional co-activator, only when S133 is phosphorylated, thereby inducing transcription [8]. In this way, the activation of transcription by CREB is strictly controlled by the phosphorylation of S133; therefore, S133 phosphorylation is widely used as a marker of transcriptional activation by CREB.

To clarify the roles of CREB in learning and memory formation, many mutant mice have been developed and their phenotypes have been investigated. Importantly, previous studies using mutant mice demonstrated that the genetic loss of CREB function impaired the formation of LTM without affecting STM [2, 9,10]. Furthermore, mutant mice with inhibited CREB activity also showed deficits in hippocampal L-LTP [9]. These findings indicate that CREB is required for memory consolidation and LTP, suggesting that CREB plays a central role in these processes. CREB target genes include c-fos, activity-regulated cytoskeleton-associated protein (Arc), and brain-derived neurotrophic factor (BDNF) [11-13]; CREB is believed to control memory consolidation and LTP by regulating the expression of these genes. Importantly, previous studies have shown that CREB plays critical roles in memory formation not only in rodents but also in *Aplysia* and *Drosophila* [1, 14, 15]

EFFECTS OF THE GENETIC ACTIVATION OF CREB ON LTP AND MEMORY FORMATION

The finding that the loss of CREB function blocks memory consolidation and LTP suggests that it functions as a positive regulator of these processes. To examine this hypothesis, three types of transgenic mice displaying gain of CREB function have been generated and investigated.

CREB-VP16 mice

VP16, a virus-derived protein, contains a transcription activation domain displaying significantly high transcription activity and has been used in abundant transcription studies as a model of transcription activation domain in eukaryotes. To investigate the gain-of-function of CREB, CREB-VP16, a chimeric transcription factor, was developed in which VP16 was fused with CREB. Transgenic mice expressing CREB-VP16 in the forebrain region were generated using the tetracycline system [16]. These mutant mice showed that CREB-VP16 is highly expressed in the hippocampal CA1 and dentate gyrus areas. However, even though these mice were expected to have improved memory formation, behavioral studies showed that they displayed abnormalities in spatial memory formation [17]. These observations are contradictory compared to the results of behavioral experiments using CREB-Y134F and CREB-DIEDML mice (see below). These findings are discussed in the next session.

Previous studies using field recordings have shown that LTP was induced in the hippocampal CA1 neurons of wild-type (WT) mice when 1 train of 100-Hz tetanic stimulation was applied for 1 s, although this LTP disappeared after approximately 2 h. On the other hand, when this tetanic stimulation was applied 4 times at 5-min intervals, L-LTP that lasted for over 3 h was induced [18]. Importantly, similarly with memory consolidation, L-LTP requires the induction of gene expression [3]. Interestingly, electrophysiological analyses showed that one train of tetanic stimulation is sufficient to induce L-LTP-like LTP in hippocampal CA1 neurons of CREB-VP16 mice [18]. Further studies indicated that the threshold of L-LTP induction was lower in CREB-VP16 mice than in WT mice [18].

As a next step, the mechanism underlying the reduction in the threshold of L-LTP induction observed in CREB-VP16 mice was investigated [18]. In WT hippocampal slices, even one train of tetanic stimulation was sufficient to induce L-LTP once L-LTP was induced in the other synapses of the same neuron by four trains of tetanic stimulation. This observation is thought to reflect the fact that the application of tetanic stimulation four times induces CREB-mediated gene expression; then,

the resulting gene products are transported to the synapses of the stimulated neuron, even to those in which L-LTP was not induced. Therefore, since the expression of CREB target genes is significantly enhanced in CREB-VP16 mice, one train of tetanic stimulation, as with four trains of tetanic stimulation, is thought to induce L-LTP. This hypothesis was supported by detailed analyses showing that the LTP induced in WT mice by one train of tetanic stimulation following four trains of tetanic stimulation has similar characteristics to that induced in CREB-VP16 mice by one train of tetanic stimulation. Furthermore, the increased expression of BDNF in CREB-VP16 mice under the basal condition, i.e., without any tetanic stimulation, has been shown to contbibute to the lowered threshold of L-LTP induction [18]. Consistently, four trains of tetanic stimulation failed to enhance LTP induced by one train of tetanic stimulation in CREB-VP16 mice. These observations suggested that synaptic capture of BDNF is sufficient for induction of L-LTP by a single tetanic stimulation and provided potential molecular mechanisms of synaptic tagging [19-21].

Y134F mice and DIEDML mice

CREB-Y134F (Y134F) contains a mutation in which tyrosine is changed to phenylalanine at position 134. This mutant protein displays increased affinity to PKA (a CREB kinase), thereby leading to the lowering of the threshold for CREB activation; this mutation makes it easier for the protein to be activated [22]. On the other hand, for CREB-DIEDML (DIEDML), six amino acids (RRPSYR, which include S133) are replaced with DIEDML (Fig. 1), the CBP-binding motif of sterol-responsive element binding protein (SREBP) [23]. Therefore, DIEDML interacts constitutively with CBP without phosphorylation by CREB kinases. Thus, Y134F and DIEDML function as dominant active mutants.

Transgenic mice were generated in which Y134F or DIEDML is expressed specifically in the forebrain region under the control of the αCaMKII promoter (Y134F and DIEDML mice) [24]. These mutant mice display higher expression levels of c-fos, a CREB-target gene, than WT mice, indicating that the expression of these dominant active mutants leads to the enhanced activation of CREB-mediated transcription.

Similarly with the results from the CREB-VP16 mice, electrophysiological analysis using field recordings in a transgenic line with a high level of Y134F expression (Line C) confirmed enhanced L-LTP in hippocampal CA1 neurons [24]. Further analysis using the patch clamp method showed enhanced spike-timing LTP in the hippocampal CA1 neurons of Y134F mice (Line C) [24]. Taken together with the observations from the CREB-VP16 mice, these results strongly suggest that CREB functions as a positive regulator of LTP, even though these studies were not performed under similar conditions to those used for the CREB-VP16 mice.

In contrast to the results from the CREB-V16 mice, behavioral studies showed that all of the transgenic Y134 and DIEDML lines displayed improved LTM at 24 h in social recognition and fear conditioning memory tasks [24]. Furthermore, improved LTM was also observed in a spatial memory task using the Morris water maze and a passive avoidance memory task [24].

In a contextual fear conditioning task, WT mice display impaired contextual discrimination at one month after conditioning. These mice display fear (freezing) responses when they are exposed to a box that is novel, but similar to the original box in which they had received an electrical foot shock. However, they did not display such a high level of freezing responses in the novel box compared to the original box at one day after conditioning. In contrast, Y134F mice (Line C), which highly express the CREB mutant, could discriminate between the novel and familiar boxes, even at one month after conditioning; mutant mice displayed significantly higher fear responses in the familiar context in which they received a foot shock compared to the novel context. These observations indicated that Y134F mice formed more accurate (stronger) memory than WT mice [24]. Thus, the behavioral experiments using Y134F and DIEDML mice showed that, in contrast to the results from CREB-VP16 mice, the activation of CREB significantly improved memory consolidation, indicating that CREB functions as a positive regulator of this process (Fig. 2).

In this section, I compare the results of behavioral analyses among CREB-VP16, Y134F, and DIEDML mice. In Y134F and DIEDML mice, CREB was activated only a few fold higher than in WT mice, whereas in CREB-VP16 mice, since VP16 contains a strong transcriptional activation domain [25], the activation level was 20- to 30-fold higher than in WT mice. Importantly, the

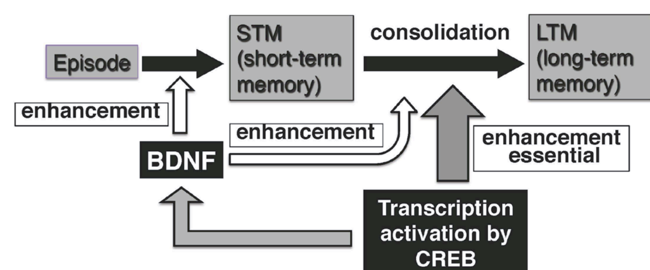

Fig. 2. Roles of CREB in the regulation of memory formation. CREB controls the expression of genes essential for memory consolidation. The strength of memory is determined by the level of transcriptional activation by CREB. Also, CREB indirectly controls STM by regulating the expression of BDNF, a CREB target gene, and further enhances memory consolidation using a positive feedback loop.

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dominant active CREB mutants were expressed simply under the control of the αCaMKII promoter in the Y134F and DIEDML mice. In contrast, in the CREB-VP16 mice, the expression of CREB-VP16 is amplified using the tetracycline system. Therefore, these comparisons suggest that the activation of CREB-mediated transcription was unusually high in the CREB-VP16 mice compared to the Y134F and DIEDML mice; Y134F and DIEDML mice display enhanced CREB-mediated transcription at a physiologically moderate level, while CREB-VP16 mice do not. Thus, although enhanced LTP was observed in the CREB-VP16 and Y134F /DIEDML mice, the levels of CREB activation seemed to exert a strong influence on memory; only Y134F /DIEDML mice, which show moderate CREB activation, display enhanced memory consolidation.

Interestingly, Y134F and DIEDML mice demonstrated improved STM from 30 min to 2 h as well as LTM [24]. Especially, DIEDML mice, with higher CREB activation levels than Y134F mice, displayed improved STM at 30 min. These observations strongly suggest that STM is improved in a dose-dependent manner by CREB activity [24]. Importantly, as STM is thought to be formed independently of new gene expression, it is suggested that CREB plays a regulatory role in STM, but this enhanced STM is not mediated by the transcriptional activation of CREB target genes immediately after training.

Expression analysis showed that Y134F and DIEDML transgenic lines with enhanced STM display increased levels of BDNF in the hippocampus; transgenic lines expressing higher levels of BDNF also exhibit enhanced STM [24]. Importantly, a microinfusion of BDNF or a BDNF inhibitor into the hippocampus of WT mice generated enhanced or impaired STM, respectively. Additionally, the infusion of a higher dose of the BDNF inhibitor into the hippocampus was required for the impairment of STM in DIEDML mice than in WT mice. These observations suggest that an increase in the expression levels of BDNF improves STM in Y134F and DIEDML mice [24]. From these findings, CREB is thought to play a regulatory role in STM through the regulation of BDNF expression (Fig. 2).

On the basis of the analysis of Y134F and DIEDML mice described above, it is suggested that CREB is a positive regulator of memory consolidation. In addition, it is suggested that CREB indirectly controls STM by regulating the expression levels of BDNF.

SUMMARY

Previous studies have shown that the loss of CREB function impairs memory consolidation and LTP. Conversely, recent studies using mouse genetics indicated that the gain of CREB function improves memory and LTP. Taken together, these findings clearly indicate that CREB positively regulates memory consolidation and LTP. Furthermore, CREB is suggested to play a regulatory role in STM though the activation of target gene expression such as BDNF. Thus, CREB plays essential and regulatory roles in STM and LTM.

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