Chapter 5: CREB — A Key Transcription Factor in the Nervous System

cAMP response element binding protein (CREB) is a complex transcription factor that integrates various cellular signals and outputs specific transcriptional programs. In response to extracellular stimuli, CREB is post-translationally modified, most notably by phosphorylation. This, in turn, alters CREB-dependent transcription and affects such processes as neuronal development and survival, drug addiction, and learning and memory. Here, we review the literature on CREB regulation and function in the nervous system.

Cyclic-AMP response element binding protein (CREB) is a key transcription factor that translates extracellular stimuli into specific gene programs[1-3]. CREB is expressed in all cells in the brain[3] and complete knockout of CREB in mice is lethal[4]. CREB is a member of a family of transcription factors that includes CREB, CREM, and ATF-1 and binds as a homo- or heterodimer to cyclic-AMP response elements (CREs) containing the DNA sequence TGACGTCA[1]. CREB consists of four domains: glutamine rich Q1 and Q2 domains, a central kinase inducible domain (KID) and a bZIP domain. The KID domain contains the majority of known CREB phosphorylation sites[2], including the major phosphorylation site at Ser133, and is necessary for phosphorylation-induced CREB activity. The bZIP domain is necessary for CREB to bind the DNA. The Q2 domain, primarily, and the Q1 domain, secondarily, are important for interactions with the basal transcriptional machinery and have been shown to mediate CREB transactivation independent on stimulus[5].

Post-Translational Modifications of CREB

Phosphorylation. The best-studied post-translational modification of CREB is phosphorylation of CREB at Ser133. Many different kinases, including CaMK, PKA, PCK, PKG, Rsk, and MEK, and many different stimuli, including growth factors, neurosignalling molecules, cytokines, and environmental stress[2] all phosphorylate CREB at Ser133. This phosphorylation event promotes the interaction between CREB and the coactivators CREB binding protein (CBP) and its paralogue p300, dramatically enhances CREB activity, and drives the expression on different CREB-mediated gene programs. Although phosphorylation at this site has been extensively studied and is often associated with CREB activation, it appears to be neither necessary[6] nor sufficient[2] for CREB activation.

CREB is phosphorylated at Ser142 by CaMKII[7] and this phosphorylation is modulated by a number of different stimuli, including formalin injection into the spinal cord, a model for peripheral noxious stimulation[8], circadian rhythm, light, and glutamate in the suprachiasmatic nucleus (SCN)[9], and calcium influx in cultured cortical neurons (which also leads to phosphorylation of Ser143)[10]. Ser142 phosphorylation is necessary for complete light-induced expression of *c-fos* and *mPer1* and for light-induced phase shifts of the circadian clock in the SCN[9]. Ser142Ala CREB, mutants show enhanced, while Ser142Ala / Ser143Ala double mutants show repressed, CREB activity in response to neuronal depolarization but no effect on CREB activity in response to increased cAMP levels compared to WT CREB[10]. Mechanistically, Ser142 phosphorylation has been shown to cause the dissociation of CREB dimers[7] as well as to block the interaction between CREB and the KIX domain of CBP[10].

Hypoxia induces hyperphosphorylation of CREB at sites other than Ser133[11], although the exact sites are unknown. Ionizing radiation, which causes DNA damage, promotes the phosphorylation of a cascade of CREB phosphorylation sites by ATM, CK1, and CK2 starting with Ser111 followed by Ser108, Ser114, Ser117, and Ser121, which finally blocks the interaction between CREB and CBP[12]. Alternatively, CREB undergoes cell-cycle-dependent phosphorylation at Ser108, Ser111, and Ser114, and CREB with Ser111 and Ser114 mutated to glutamic acid to mimic phosphorylation show enhanced transcription on non-induced CREB[13]. Furthermore, DNA damage leads to CREB phosphorylation at Ser271 by HIPK2, which enhances CREB transactivation by recruiting CBP[14]. Finally GSK-3 phosphorylates CREB at Ser129 subsequent to phosphorylation of Ser133, and this phosphorylation event is required for CREB transactivation by forskolin in PC12 cells[15].

Other Post-Translational Modifications. Along with phosphorylation, CREB is also modified by acetylation, glycosylation, ubiquitination, and SUMO-ylation. CREB is acetylated by CBP at Lys91, Lys96, and Lys136, and mutations that replace these lysines with alanines or arginines enhance both basal and PKA-induced CREB activity[16]. CREB is glycosylated *in vivo* within amino acids 259–261[17]. Finally, following onset of hypoxia, CREB is phosphorylated, ubiquitinated, and degraded[11], but after prolonged hypoxia, CREB is SUMO-ylated at Lys285 and Lys304, which stabilizes CREB, and in the case of Lys304 SUMO-ylation, may lead to increased nuclear localization[18]. Taken together, the abundance of post-translational modifications on CREB suggests that CREB is tightly controlled in response to stimuli and that, although Ser133 phosphorylation may

be a predominant post-translational modification for activating CREB, these other modifications may be important for the finely tuned modulation of CREB activity.

CREB-Interacting Proteins

CREB interacts directly with a variety of different proteins that either activate or repress CREB activity. Two of the most widely studied CREB-interacting proteins are CBP / p300 and transducer of regulated CREB activity (TORCs). CBP / p300 are transcriptional transactivators that interact with multiple transcription factors, including CREB. The interaction between CREB and CBP is modulated by phosphorylation of CREB at Ser133, which is necessary but not sufficient for CBP to bind CREB[19]. CBP / p300 promote the recruitment of RNA polymerase II through RNA helicase A and have histone acetyltransferase activity, which facilitates the opening of chromatin to allow access to the DNA[20].

TORCs are a recently discovered family of proteins that coactivate CREB transcription independent of CREB Ser133 phosphorylation[6,21] and have been shown to contribute to important physiological and disease processes such as gluconeogenesis[22] and diabetes[23]. TORCs bind to the bZIP domain of CREB, likely through ionic interactions[24], and have been proposed to enhance CREB transactivation by promoting the interaction between CREB and $TAF_{II}130$, a component of the basal transcriptional machinery[6]. TORCs are actively shuttled out of the nucleus, and thus, in untreated cells, TORCs are found predominantly although not exclusively in the cytoplasm[25]. TORCs are modified by phosphorylation and *O*-GlcNAc glycosylation. In response to low-energy signals such as low ATP levels or low glucose levels, AMPK in hepatic cells and

the hypothalamus and SIK in hepatic cells phosphorylate TORC2 at Ser171[22,26], and in response to low glucose in islet cells, MARK2 phosphorylates TORC2 at Ser275[27]. Both of these phosphorylation events recruit the 14-3-3 protein, which causes TORC to be sequestered in the cytoplasm. Alternatively, in response to high glucose, TORC2 is *O*-GlcNAc glycosylated at Ser70 and Ser171[23] in hepatic cells or dephosphorylated by calcineurin in islet cells[24,27], which blocks the interaction with 14-3-3, and allows TORC2 to accumulate in the nucleus and enhance CREB activity. In neurons, TORC1 and TORC2 translocate into the nucleus downstream of calcinuerin in response to neuronal depolarization[28], where they are required for activity-dependent gene expression of SIK, activity-dependent dendritic growth[29], stress sensitivity[30], and maintenance of L-LTP in the Schaffer collateral–CA1 pathway[28].

CREB can also interact with other proteins both to alter CREB activity as well as to alter the activity of the other protein. For example, CREB binds $TAF_{II}130/135$, which interacts with the Q2 domain of CREB and activates CREB[31], as well as YY-1, which inhibits CREB activity by bending the DNA around CREB and thus blocking the interaction between CREB and the basal transcriptional machinery[32]. Alternatively, CREB interacts with MeCP2, which modulates MeCP2 function such that it activates rather than represses genes[33], and CREB bridges p53 and CBP, thereby enhancing p53 transcriptional activation[34].

CREB Targets

The complement of confirmed and predicted CREB target genes is extensive and continues to grow. 1349 mouse and 1663 human putative CREB binding sites have been

identified[35] and 6302 CREB loci have been mapped from PC12 cells using a serial analysis of chromatin occupany (SACO) approach[36]. CREB has been shown to regulate the expression of many different classes of proteins, including proteins important in neurotransmitter release, cell structure, signal transduction, and metabolism[1]. Furthermore, microarray analysis of CREB knockdown in myeloid leukemia cells[37], CREB overexpression in the nucleus accumbens, and S133A CREB overexpession in the nucleus accumbens[38] identified many transcripts whose expression was modified in response to modified CREB activity.

Specificity of CREB Signal

CREB integrates a diversity of extracellular signals and translates them into unique gene programs. Yet how CREB differentiates each of these signals and transcribes the correct set of genes for the correct time period and for a given signal within a given cell type remains an open and pressing question in the field. For example, tyrosine hydroxylase, a CREB-dependent gene, is expressed in only specific cell types throughout the brain[39] and c-fos expression has a distinct time-course that returns to basal levels within an hour, independent of stimulation time, in 3T3 cells[40]. The post-translational modifications of CREB (discussed above), which can be induced or repressed in response to different stimuli, may account for some of these effects. Nevertheless other signals and events are also likely required to differentiate the set of targets transcribed following CREB activation.

The exact duration of CREB Ser133 phosphorylation may in part contribute to CREB transactivation. H_2O_2 induces transient (15 min) CREB phosphorylation without

CREB transactivation, whereas estradiol induces prolonged (5 hr) CREB phosphorylation and CREB activation[41]. Nevertheless the kinetics of CREB phosphorylation are not the sole mediator of CREB activity as forskolin, which activates PKA, and phorbol 12-tetradecanoate 13-acetate (TPA), which activates PKC; both phosphorylate CREB equally in NIH3T3 cells but only forskolin induces CREB activity[42], and, similarly, forskolin and TPA both produce comparable levels of CREB phosphorylation in PC12 cells, but only forskolin induces expression of the CREB target gene *Icer*[43]. Interestingly, in the latter case, only forskolin was found to promote the formation of a nuclear CREB–CBP complex although both forskolin and TPA promoted the formation of cytoplasmic CREB–CBP complexes[43].

Chromatin modification and accessibility may also contribute to stimuli-specific differences in CREB-mediated transcription. CREB forms a stable complex with HDAC1 and PP1, which is disrupted by forskolin[44]. Furthermore, treatment with Trichostatin A (TSA), an HDAC inhibitor, enhances the expression of a subset of CREB-mediated genes, including *c-fos* and *NUR77*, following forskolin treatment while blocking the activation of a different subset of CREB-mediated genes, including *ICER* and *NOR-1*[45]. Similarly, *BDNF*, a CREB-target gene, has exon-specific changes in chromatin modifications on its promoter following NMDA treatment[46]. Finally, CREB occupancy of different promoters, including the *somatostatin* promoter, differs depending on the cell type, possibly in response to different availability of the promoter for protein binding[47].

Finally, post-translational modifications of CREB coactivators or corepressors may specify which stimuli activate CREB transcription. CBP is phosphorylated[48],

methylated[49], SUMO-ylated[50], and glycosylated[51]. CBP Ser301 phosphorylation is induced by NMDA in hippocampal neurons and is required for full CREB transactivation[48]. Furthermore, methylation at Arg300 blocks the interaction between CREB and CBP and represses CREB transcription. Finally, CBP can be SUMO-ylated at Lys999, Lys1034, and Lys1057, which recruits the transcriptional corepressor Daxx and represses CBP transcriptional activity[50]. Thus, signals that modify the duration of CREB phosphorylation, the chromatin modifications around CREB target genes, and the activity of CREB coactivator and corepressor, in addition to activating CREB, may be important in differentiating specific stimuli and activating unique CREB-dependent gene programs.

Pathways that Activate CREB Following Neuronal Activity

Three major kinase pathways regulate CREB activity in neurons in response to neuronal activity — PKA, CaMK, and Ras/ERK. PKA is regulated by cAMP levels, which are themselves regulated by adenylate cyclase activity. Adenylate cyclase activity can be regulated downstream of G-protein-coupled receptors (GPCRs) for neurotransmitters as well as directly through Ca^{2+} flux[1,52]. Ca^{2+} flux also activates both MAPK and calmodulin. MAPK and calmodulin further active Rsk kinases and CaMK, respectively, which directly phosphorylate CREB[53]. Once phosphorylated, two phosphatases are able to directly dephosphorylate CREB, PP1 and PP2A[1]. Inhibition of calcineurin is also known to enhance CREB phosphorylation but calcineurin has yet to be shown to directly dephosphorylate CREB *in vivo*[54].

Functions of CREB in the Nervous System

CREB performs a number of different functions in the nervous system. Among the best studied include its role in neuronal development and survival, in drug addiction, and in learning and memory.

CREB and Neuronal Development and Survival. Inhibition of CREB family members *in vitro* and CREB knockout studies *in vivo* substantiate a critical role for CREB in neuronal survival[55-58]. *In vitro* transfection with a dominant-negative CREB construct blocks BDNF-mediated cell survival in cerebellar granular neurons[55] and NGF-mediated cell survival in sympathetic neurons[56]. Furthermore, overexpression of a constitutively-active CREB construct was sufficient to promote sympathetic neuron survival in the absence of NGF[56]. Finally, CREB null mice have a reduction in the size of the corpus callosum and the anterior commissures, as well as enhanced apoptosis of sensory and sympathetic neurons[57], and dentate gyrus neuronal survival following ischemic insult depends on CREB-dependent gene expression[58].

CREB has also been shown to contribute to neuronal development. Studies have shown that CREB, through the expression of Wnt-2, in hippocampal neurons[59] and CREB and TORC1 in cortical neurons[29,60] are necessary for activity-dependent dendrite growth both *in vitro*. Furthermore, CREB knockout studies indicate axonal growth defects in DRG neurons[57].

CREB and Drug Addiction. CREB activation, expression, and activity appear intricately linked to the process of drug addiction. Multiple regions of the brain demonstrate up-

regulation of the cyclic AMP pathway following opiate addiction[61]. In the locus coeruleus, morphine administration inhibits, whereas morphine withdrawal activates, CREB phosphorylation[62]. Alternatively morphine and cocaine administration induces CREB activity in the nucleus accumbens and striatum[63,64]. Furthermore, mice lacking CREB in the entire brain show decreased development of morphine dependence[65]. More specifically, overexpression of a dominant-negative form of CREB in the nucleus accumbens or the caudal ventral tegmental area (VTA) increases the rewarding effects of morphine and cocaine whereas overexpression of WT CREB decreases them[66-68]. Alternatively, overexpression of WT CREB in the rostral VTA makes cocaine more rewarding and overexpression of a dominant-negative form of CREB makes cocaine less rewarding[68].

CREB and Memory. CREB is a key transcription factor for regulating long-term memory across many different species. Long-term facilitation, a model of memory, can be blocked or induced in *Aplysia* by injecting CRE oligonucleotides or phosphorylated CREB, respectively, demonstrating that CREB is necessary and sufficient for this process[69]. Additionally, activation of long-term facilitation activates CREB-mediated transcription in *Aplysia*[70]. Induction of a dominant negative CREB transgene blocks while induction of a CREB activator isoform enhances long-term memory in *Drosophila*[71,72]. In mice, learning tasks are associated with an increase in CREB phosphorylation at Ser133[73,74]. Furthermore, injection of CREB antisense oligos into the hippocampus of WT CREB causes deficits in spatial memory[75,76]. Moreover, CREB αΔ knockout mice show a deficit in long-term but not short-term memory following

contextual and cued fear conditioning[76] and social transmission of food preferences[77] as well as a deficit in the Morris Water maze test, which could be caused by learning or memory impairment[76]. Although follow-up experiments have shown that these effects depend, in part, on the specific mouse strain used for the study and may require knockout of additional CREB isoforms[78,79], they nevertheless suggest a significant role for CREB in long term memory formation. Similarly, induction of a dominant-negative CREB blocks consolidation of long-term memory whereas overexpression of WT CREB enhances this consolidation[80,81]. Taken together, these studies demonstrate that CREB contributes to critical and complex functions in the brain.

Conclusions

CREB is a complex transcription factor that is activated by multiple, distinct signaling pathways in neurons and that is essential for many critical neuronal functions. Although CREB has been well studied, a number of open questions remain: How does CREB distinguish between the different pathways that induce phosphorylation at Ser133? Do other CREB post-translational modifications contribute, and if so, what are these other modifications, how are they regulated, and how do they affect CREB function? Finally, given a variety of inputs, how does CREB activate transcriptional programs specific for the context? Addressing these questions will provide in-depth information on the function of CREB in neurons. Moreover, insofar as CREB is a model for other transcription factors, additional data of CREB will more broadly inform our understanding of transcription.

References

1. Lonze, B.E. & Ginty, D.D. Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35, 605-23 (2002).
2. Johannessen, M., Delghandi, M.P. & Moens, U. What turns CREB on? *Cell Signal* 16, 1211-27 (2004).
3. Carlezon, W.A., Duman, R.S. & Nestler, E.J. The many faces of CREB. *Trends Neurosci.* 28, 436-45 (2005).
4. Rudolph, D. et al. Impaired fetal T cell development and perinatal lethality in mice lacking the cAMP response element binding protein. *Proc Natl Acad Sci USA* 95, 4481-6 (1998).
5. Shaywitz, A.J. & Greenberg, M.E. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* 68, 821-61 (1999).
6. Conkright, M.D. et al. TORCs: transducers of regulated CREB activity. *Mol Cell* 12, 413-23 (2003).
7. Wu, X. & McMurray, C.T. Calmodulin kinase II attenuation of gene transcription by preventing cAMP response element-binding protein (CREB) dimerization and binding of the CREB-binding protein. *J Biol Chem* 276, 1735-41 (2001).
8. Niederberger, E., Ehnert, C., Gao, W. & Coste, O. The impact of CREB and its phosphorylation at Ser142 on inflammatory nociception. *Biochem Biophys Res Commun* (2007).
9. Gau, D. et al. Phosphorylation of CREB Ser142 regulates light-induced phase shifts of the circadian clock. *Neuron* 34, 245-53 (2002).
10. Kornhauser, J.M. et al. CREB transcriptional activity in neurons is regulated by multiple, calcium-specific phosphorylation events. *Neuron* 34, 221-33 (2002).
11. Taylor, C.T., Furuta, G.T., Synnestvedt, K. & Colgan, S.P. Phosphorylation-dependent targeting of cAMP response element binding protein to the ubiquitin/proteasome pathway in hypoxia. *Proc Natl Acad Sci USA* 97, 12091-6 (2000).
12. Shanware, N.P., Trinh, A.T., Williams, L.M. & Tibbetts, R.S. Coregulated ataxia telangiectasia-mutated and casein kinase sites modulate cAMP-response element-binding protein-coactivator interactions in response to DNA damage. *J Biol Chem* 282, 6283-91 (2007).
13. Saeki, K., Yuo, A. & Takaku, F. Cell-cycle-regulated phosphorylation of cAMP response element-binding protein: identification of novel phosphorylation sites. *Biochem J* 338 (Pt 1), 49-54 (1999).
14. Sakamoto, K. et al. Regulation of Genotoxic Stress Response by HIPK2 through Phosphorylation of CREB at Serine 271. *Molecular biology of the cell* (2010).
15. Fiol, C.J. et al. A secondary phosphorylation of CREB341 at Ser129 is required for the cAMP-mediated control of gene expression. A role for glycogen synthase kinase-3 in the control of gene expression. *J Biol Chem* 269, 32187-93 (1994).
16. Lu, Q., Hutchins, A.E., Doyle, C.M., Lundblad, J.R. & Kwok, R.P.S. Acetylation of cAMP-responsive element-binding protein (CREB) by CREB-binding protein enhances CREB-dependent transcription. *J Biol Chem* 278, 15727-34 (2003).

17. Lamarre-Vincent, N. & Hsieh-Wilson, L.C. Dynamic glycosylation of the transcription factor CREB: a potential role in gene regulation. *J Am Chem Soc* 125, 6612-3 (2003).
18. Comerford, K.M. et al. Small ubiquitin-related modifier-1 modification mediates resolution of CREB-dependent responses to hypoxia. *Proc Natl Acad Sci USA* 100, 986-91 (2003).
19. Brindle, P., Nakajima, T. & Montminy, M. Multiple protein kinase A-regulated events are required for transcriptional induction by cAMP. *Proc Natl Acad Sci USA* 92, 10521-5 (1995).
20. Nakajima, T. et al. RNA helicase A mediates association of CBP with RNA polymerase II. *Cell* 90, 1107-12 (1997).
21. Iourgenko, V. et al. Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. *Proc Natl Acad Sci USA* 100, 12147-52 (2003).
22. Koo, S.-H. et al. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* 437, 1109-11 (2005).
23. Dentin, R., Hedrick, S., Xie, J., Yates, J. & Montminy, M. Hepatic glucose sensing via the CREB coactivator CRTC2. *Science* 319, 1402-5 (2008).
24. Screaton, R.A. et al. The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. *Cell* 119, 61-74 (2004).
25. Bittinger, M.A. et al. Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. *Curr Biol* 14, 2156-61 (2004).
26. Lerner, R.G., Depatie, C., Rutter, G.A., Screaton, R.A. & Balthasar, N. A role for the CREB co-activator CRTC2 in the hypothalamic mechanisms linking glucose sensing with gene regulation. *EMBO reports* 10, 1175-81 (2009).
27. Jansson, D. et al. Glucose controls CREB activity in islet cells via regulated phosphorylation of TORC2. *Proc Natl Acad Sci USA* 105, 10161-6 (2008).
28. Kovács, K.A. et al. TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. *Proc Natl Acad Sci USA* 104, 4700-5 (2007).
29. Li, S., Zhang, C., Takemori, H., Zhou, Y. & Xiong, Z.-Q. TORC1 regulates activity-dependent CREB-target gene transcription and dendritic growth of developing cortical neurons. *J Neurosci* 29, 2334-43 (2009).
30. Wang, B. et al. The insulin-regulated CREB coactivator TORC promotes stress resistance in Drosophila. *Cell Metab* 7, 434-44 (2008).
31. Tanese, N., Saluja, D., Vassallo, M.F., Chen, J.L. & Admon, A. Molecular cloning and analysis of two subunits of the human TFIID complex: hTAFII130 and hTAFII100. *Proc Natl Acad Sci USA* 93, 13611-6 (1996).
32. Zhou, Q., Gedrich, R.W. & Engel, D.A. Transcriptional repression of the c-fos gene by YY1 is mediated by a direct interaction with ATF/CREB. *J Virol* 69, 4323-30 (1995).
33. Chahrour, M. et al. MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* 320, 1224-9 (2008).

34. Giebler, H.A., Lemasson, I. & Nyborg, J.K. p53 recruitment of CREB binding protein mediated through phosphorylated CREB: a novel pathway of tumor suppressor regulation. *Mol Cell Biol* 20, 4849-58 (2000).
35. Conkright, M.D. et al. Genome-wide analysis of CREB target genes reveals a core promoter requirement for cAMP responsiveness. *Mol Cell* 11, 1101-8 (2003).
36. Impey, S. et al. Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell* 119, 1041-54 (2004).
37. Pellegrini, M. et al. Expression Profile of CREB knockdown in Myeloid Leukemia Cells. *BMC Cancer* 8, 264 (2008).
38. McClung, C.A. & Nestler, E.J. Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat Neurosci* 6, 1208-15 (2003).
39. Cambi, F., Fung, B. & Chikaraishi, D. 5' flanking DNA sequences direct cell-specific expression of rat tyrosine hydroxylase. *Journal of Neurochemistry* 53, 1656-9 (1989).
40. Greenberg, M.E. & Ziff, E.B. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature* 311, 433-8 (1984).
41. Kanda, N. & Watanabe, S. 17beta-estradiol inhibits oxidative stress-induced apoptosis in keratinocytes by promoting Bcl-2 expression. *J Invest Dermatol* 121, 1500-9 (2003).
42. Seternes, O.M., Johansen, B. & Moens, U. A dominant role for the Raf-MEK pathway in forskolin, 12-O-tetradecanoyl-phorbol acetate, and platelet-derived growth factor-induced CREB (cAMP-responsive element-binding protein) activation, uncoupled from serine 133 phosphorylation in NIH 3T3 cells. *Mol Endocrinol* 13, 1071-83 (1999).
43. Mayr, B.M., Canettieri, G. & Montminy, M.R. Distinct effects of cAMP and mitogenic signals on CREB-binding protein recruitment impart specificity to target gene activation via CREB. *Proc Natl Acad Sci USA* 98, 10936-41 (2001).
44. Canettieri, G. et al. Attenuation of a phosphorylation-dependent activator by an HDAC-PP1 complex. *Nat Struct Biol* 10, 175-81 (2003).
45. Fass, D.M., Butler, J.E.F. & Goodman, R.H. Deacetylase activity is required for cAMP activation of a subset of CREB target genes. *J Biol Chem* 278, 43014-9 (2003).
46. Tian, F. et al. Dynamic chromatin remodeling events in hippocampal neurons are associated with NMDA receptor-mediated activation of Bdnf gene promoter 1. *Journal of Neurochemistry* 109, 1375-88 (2009).
47. Cha-Molstad, H., Keller, D.M., Yochum, G.S., Impey, S. & Goodman, R.H. Cell-type-specific binding of the transcription factor CREB to the cAMP-response element. *Proc Natl Acad Sci USA* 101, 13572-7 (2004).
48. Impey, S. et al. Phosphorylation of CBP mediates transcriptional activation by neural activity and CaM kinase IV. *Neuron* 34, 235-44 (2002).
49. Xu, W. et al. A transcriptional switch mediated by cofactor methylation. *Science* 294, 2507-11 (2001).
50. Kuo, H.-Y. et al. SUMO modification negatively modulates the transcriptional activity of CREB-binding protein via the recruitment of Daxx. *Proc Natl Acad Sci USA* 102, 16973-8 (2005).

51. Tai, H.-C., Khidekel, N., Ficarro, S.B., Peters, E.C. & Hsieh-Wilson, L.C. Parallel identification of O-GlcNAc-modified proteins from cell lysates. *J Am Chem Soc* 126, 10500-1 (2004).
52. Poser, S. & Storm, D.R. Role of Ca2+-stimulated adenylyl cyclases in LTP and memory formation. *Int J Dev Neurosci* 19, 387-94 (2001).
53. West, A.E. et al. Calcium regulation of neuronal gene expression. *Proc Natl Acad Sci USA* 98, 11024-31 (2001).
54. Christie-Fougere, M.M., Darby-King, A., Harley, C.W. & Mclean, J.H. Calcineurin inhibition eliminates the normal inverted U curve, enhances acquisition and prolongs memory in a mammalian 3'-5'-cyclic AMP-dependent learning paradigm. *NSC* 158, 1277-83 (2009).
55. Bonni, A. et al. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* 286, 1358-62 (1999).
56. Riccio, A., Ahn, S., Davenport, C.M., Blendy, J.A. & Ginty, D.D. Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. *Science* 286, 2358-61 (1999).
57. Lonze, B.E., Riccio, A., Cohen, S. & Ginty, D.D. Apoptosis, axonal growth defects, and degeneration of peripheral neurons in mice lacking CREB. *Neuron* 34, 371-85 (2002).
58. Mabuchi, T. et al. Phosphorylation of cAMP response element-binding protein in hippocampal neurons as a protective response after exposure to glutamate in vitro and ischemia in vivo. *J Neurosci* 21, 9204-13 (2001).
59. Wayman, G.A. et al. Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron* 50, 897-909 (2006).
60. Redmond, L., Kashani, A.H. & Ghosh, A. Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. *Neuron* 34, 999-1010 (2002).
61. Nestler, E.J. Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* 2, 119-28 (2001).
62. Guitart, X., Thompson, M.A., Mirante, C.K., Greenberg, M.E. & Nestler, E.J. Regulation of cyclic AMP response element-binding protein (CREB) phosphorylation by acute and chronic morphine in the rat locus coeruleus. *Journal of Neurochemistry* 58, 1168-71 (1992).
63. Cole, R.L., Konradi, C., Douglass, J. & Hyman, S.E. Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* 14, 813-23 (1995).
64. Kano, T., Suzuki, Y., Shibuya, M., Kiuchi, K. & Hagiwara, M. Cocaine-induced CREB phosphorylation and c-Fos expression are suppressed in Parkinsonism model mice. *Neuroreport* 6, 2197-200 (1995).
65. Maldonado, R. et al. Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. *Science* 273, 657-9 (1996).
66. Carlezon, W.A. et al. Regulation of cocaine reward by CREB. *Science* 282, 2272-5 (1998).

67. Barrot, M. et al. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proc Natl Acad Sci USA* 99, 11435-40 (2002).
68. Olson, V.G. et al. Regulation of drug reward by cAMP response element-binding protein: evidence for two functionally distinct subregions of the ventral tegmental area. *J Neurosci* 25, 5553-62 (2005).
69. Kandel, E.R. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 294, 1030-8 (2001).
70. Kaang, B.K., Kandel, E.R. & Grant, S.G. Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. *Neuron* 10, 427-35 (1993).
71. Yin, J.C. et al. Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. *Cell* 79, 49-58 (1994).
72. Yin, J.C., Del Vecchio, M., Zhou, H. & Tully, T. CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in Drosophila. *Cell* 81, 107-15 (1995).
73. Viola, H. et al. Phosphorylated cAMP response element-binding protein as a molecular marker of memory processing in rat hippocampus: effect of novelty. *J Neurosci* 20, RC112 (2000).
74. Stanciu, M., Radulovic, J. & Spiess, J. Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: relationship to Fos production. *Brain Res Mol Brain Res* 94, 15-24 (2001).
75. Guzowski, J.F. & McGaugh, J.L. Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proc Natl Acad Sci USA* 94, 2693-8 (1997).
76. Bourtchuladze, R. et al. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* 79, 59-68 (1994).
77. Kogan, J.H. et al. Spaced training induces normal long-term memory in CREB mutant mice. *Curr Biol* 7, 1-11 (1997).
78. Gass, P. et al. Deficits in memory tasks of mice with CREB mutations depend on gene dosage. *Learn Mem* 5, 274-88 (1998).
79. Graves, L., Dalvi, A., Lucki, I., Blendy, J.A. & Abel, T. Behavioral analysis of CREB alphadelta mutation on a B6/129 F1 hybrid background. *Hippocampus* 12, 18-26 (2002).
80. Kida, S. et al. CREB required for the stability of new and reactivated fear memories. *Nat Neurosci* 5, 348-55 (2002).
81. Han, J.-H. et al. Neuronal competition and selection during memory formation. *Science* 316, 457-60 (2007).