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16.原田倫世、藤本陽子、城戸康年、坂元君年、松崎素道、籔義貞、鈴木高史、笹原武史、中井裕、北潔 「ミトコンドリア」を持たない寄生原虫・クリプトスポリジウムにおける呼吸鎖の生化学的解析 第 82 回日本生化学会大会 平成 21 年 10 月

17.原田倫世、松崎素道、城戸康年、坂元君年、籔義貞、中井裕、北潔 *Cryptosporidium parvum* マイトソームの調製法の確立とシアン耐性酸化酵素（AOX）の解析 第 8 回感染症沖縄ワークショップ 平成 22 年 2 月

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H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得・申請

1.発明の名称：「MRI用プローブ」，発明者：**菊地和也**，水上進，滝川利佳，白川昌宏，出願番号：特願2007-68753，出願月日：2007年9月12日。

2. 発明の名称：タンパク質を蛍光標識する方法
出願番号：特願2008-273182
出願者：大阪大学
発明者：**菊地和也**，水上進，渡辺修司
出願年月：2008年10月

3. 発明の名称：タンパク質を蛍光標識する方法
出願人：大阪大学
発明者：**菊地和也**，堀雄一郎，上野秀樹
出願番号：特願 2009-056305
出願月日：2009 年 3 月 10 日

4. 発明の名称：タンパク質を二段階標識する方法，
出願人：大阪大学
発明者：**菊地和也**，堀雄一郎，上野秀樹
出願番号：特願2009-056306
出願月日：2009年3月10日

5. 発明の名称：タンパク質を蛍光標識する方法
出願番号：PCT／JP2010／054024
出願者：大阪大学
発明者：**菊地和也**，堀雄一郎，上野秀樹
出願日：2010年3月10日

2. 実用新案登録
特になし

3.その他
特になし

研究事業名：医療機器開発推進研究事業：ナノメディシン研究

研究課題名：「カルシウム恒常性破綻のナノイメージングに関する研究」

研究期間：平成19年度～平成21年度

研究組織：

研究代表者：尾藤晴彦　（東大・院医・准教授）
研究分担者：北潔　（東大・院医・教授）
研究分担者：菊地和也　（阪大・院工・教授）
研究分担者：奥野浩行　（東大・院医・助教）

研究目的：

カルシウム（Ca^{2+}）恒常性と細胞内Ca^{2+}動態の破綻は、生活習慣病・脳高次機能障害や骨粗鬆症など多くの病態において示唆される。本研究では、Ca^{2+}恒常性破綻のナノイメージングを可能にする融合的学際的研究を実施し、疾病時に起こると考えられるCa^{2+}シグナリングの様々なレベルでの破綻を疾患動物モデルにおいて計測する基盤技術を開発し、新たな光工学的技術開発に向けた産学連携の基礎を築く。

研究成果：

1.個体イメージングに適した赤色シフトFRET　Ca^{2+}センサーRed TNXLを開発した。

2.器官特異的プローブ導入をウィルスベクターにより実施。

3. Ca^{2+}感受性人工プロモーターによるルシフェラーゼ発光イメージングの開発。

4.新規酵素活性検出MRI造影プローブの開発改良。

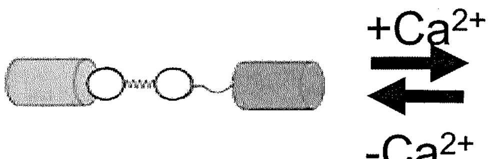
今後の期待される発展：
1. Ca^{2+}センサーの個体発現・オルガネラ発現技術の完成。
2. 動物個体カルシウム計測のための基礎・病態イメージング。
3. 国産機器メーカー等への可視化ノウハウ等の供与・共同研究開発。

分子ナノイメージングから疾患病態解明への融合的アプローチ

医・薬・化・ゲノム連携による成果

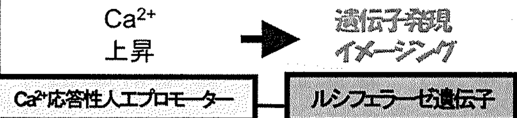
多様な生体レベルに対応したCa^{2+}シグナルプローブの開発

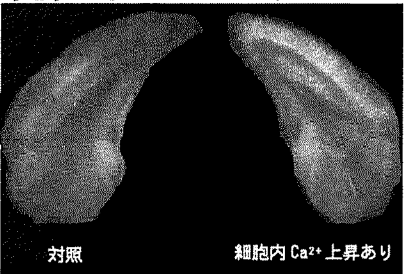
①Ca^{2+}FRET蛍光プローブ



(Takemoto-Kimura et al., **Neuron** 2007; Ageta-Ishihara et al. **J. Neurosci.** 2009)

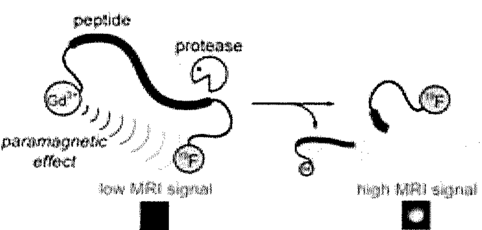
②Ca^{2+}感受性ルシフェラーゼ発光イメージング

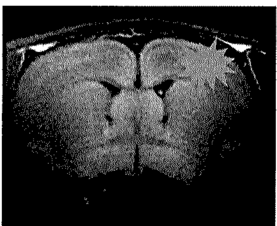




(Kawashima et al. **PNAS** 2009;
Kim et al. **Nature** 2010)

③MRI感受性Ca^{2+}応答酵素活性プローブ



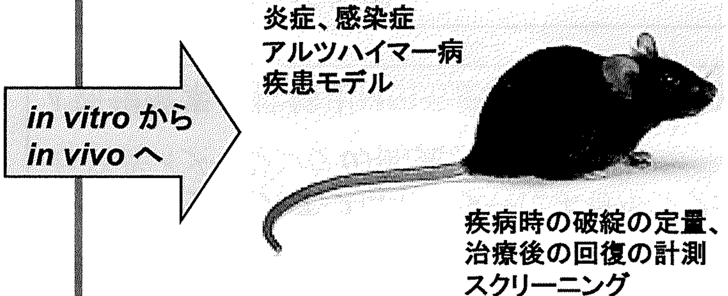


(Mizukami et al. **JACS** 2008;
「MRI用プローブ」発明者　菊地、他5名　特願2007-68753, 2007年)

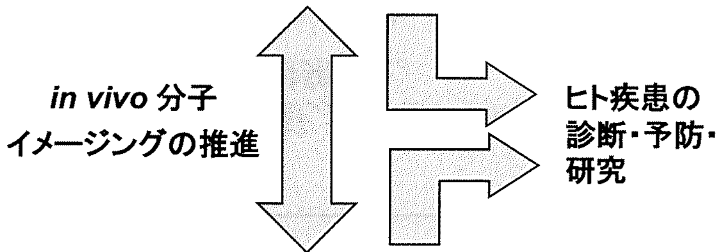
現在の到達目標と今後の展開

疾患動物モデル等における*in vivo* Ca^{2+}シグナル測定への応用

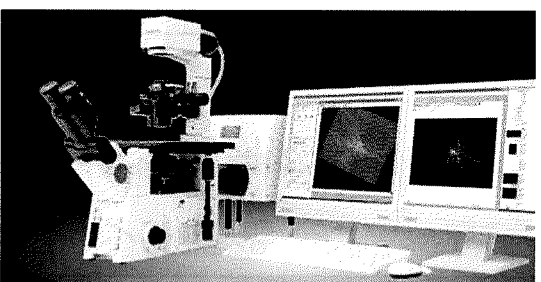
in vitro から
in vivo へ



疾病時の破綻の定量、
治療後の回復の計測
スクリーニング



技術仕様の
開示・提供



オリンパス（株）などの
国内光学機器メーカー

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Bito, H., Takemoto-Kimura, S., & **Okuno, H.**	Activity-dependent gene regulation: How do synapses talk to the nucleus and fine-tune neuronal outputs?	M. Zhuo	*Molecular Pain*	Springer	New York	2008	207-217
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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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竹本ー木村さやか、上田(石原)奈津実、布施俊光、上條諭志、**尾藤晴彦**.	神経疾患と細胞骨格	分子細胞治療	8	243-248	2009
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Kita, K., Shiomi K., and Õmura, S	Parasitology in Japan: Advances in drug discovery and biochemical studies	Trends in Parasitol	23	223-229	2007
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Activity-dependent Gene Regulation: How do Synapses Talk to the Nucleus and Fine-tune Neuronal Outputs?

CHAPTER 16

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SUMMARY

A large number of molecular mechanisms contribute to ensuring that the neuronal transcriptome can be adapted in function of the various kinds of external and internal events that the neuronal network is exposed to.

In recent years, activity-induced gene expression/ protein synthesis has received much attention as a potential mechanism likely to play a significant role in synaptic plasticity and long-term memory formation. The involved regulatory processes are intrinsically complex, and we still lack a detailed understanding of how specific neuronal nuclear factors are activated and modulated in concert to give rise to reliable and reproducible gene induction. In this chapter, we will consider the regulation of one of the most studied neuronal nuclear factor, the transcription factor CREB (Ca^{2+}/cAMP-response element-binding protein).
CREB structure is conserved from mollusk to rodents, and neuronal CREB mediates long-lasting forms of synaptic plasticity. Its activation was shown to be essential for higher brain functions such as learning and memory in many species. CREB usually resides in the nucleus, and is tightly bound to CRE loci, thus being ideally suited to rapidly convert cellular signaling into transcription. A large number of neuronal signaling pathways (e.g. Ca^{2+}/CaM/CaMKK/CaMKIV, cAMP/ PKA, Ras/MAPK, CaN/PP1) are employed and converge onto the regulation of the phosphorylation state of CREB Ser-133, consistent with its presumed importance in many adaptive biological processes, including long-term neuronal plasticity and survival. The amount of information storage available in the neuronal network will soon saturate quickly, however, without built-in mechanism for reversibility and regulated extinction/erasure of plasticity. Resolving all these problems will be of an immense clinical value when addressing cases involving aberrant persistence of pain sensation or posttraumatic stress disorder.

MAIN TOPICS

Stability and plasticity of a neuronal circuit: requirement for activity-dependent gene expression to sustain a long-term adaptive response in input-output relationship
Activity-regulated neuronal transcription factors: what are they?
CREB as a transcriptional regulator
Control of CREB activity by regulated phosphorylation at residue Ser-133

INTRODUCTION

In order to execute a higher cognitive task in response to external and internal stimuli, the brain needs to compute an output, based upon a barrage of input information that it receives from the outside world. As our brain is able to successfully compute a correct answer above par on a continuous basis, it has been speculated that there must a particular mechanism for online storage of data about the input-output relationship of the events that have received attention (and not been neglected) from our brain. Furthermore, it is also believed that "useful" information can be consolidated within a neuronal network, thereby perhaps allowing the brain to store experience as a memory and become smarter. Such external stimuli-dependent changes in the brain have been proposed to be acquired by using mechanisms of synaptic plasticity. According to the *synaptic plasticity and memory hypothesis*, as defined by Richard Morris and colleagues, "activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed". In recent years, both activity-induced gene expression/ protein synthesis and activity-induced changes in neuronal morphology have received much attention as potential mechanisms likely to play a significant role in synaptic plasticity and long-term memory formation. Experiments in hippocampal pyramidal excitatory neurons have shown that robust electrical activity can induce a large number of Ca^{2+}-dependent gene expression events. A crystal-clear picture of the molecular events following synaptic Ca^{2+} entry still remains missing, however, in part because the repertoire of activity-dependent transcription factors is not fully understood. Indeed, the mechanisms for their activation and their physiological significance ha7ve been elucidated for only a few of them, such as the Ca^{2+}/cAMP-response element-binding protein (CREB) or the nuclear factor of activated T-cells (NFAT). In this review, we shall overview some of the key signaling events by which the Ca^{2+}/CREB/CREB-binding

protein signaling system might critically control long-term adaptive responses such as long-term synaptic plasticity.

STABILITY AND PLASTICITY OF A NEURONAL CIRCUIT:REQUIREMENT FOR ACTIVITY-DEPENDENT GENE EXPRESSION TO SUSTAIN A LONG-TERM ADAPTIVERESPONSE IN INPUT-OUTPUT RELATIONSHIP

The central nervous system (CNS) is a complex organization consisting of 10-100 billion cells. Higher cognitive tasks and the principal information processing function of the CNS are thought to be supported in large part by the activity of excitable cells in the CNS, namely, the neurons. Each neuron receives inputs from a high number of adjacent neurons to which it physically connects to, via specialized contact domains called synapses. The number of synapses can sometimes reach over 10000, as in the case of hippocampal pyramidal neurons or cerebellar Purkinje cells. The major output axons are however relatively scarce, sometimes as few as one or two. Nonetheless, each axon can synapse onto many distinct dendritic arborizations via an array of *en passant* boutons, which can be triggered to release their neurotransmitters by the same action potential firing event with a high degree of synchrony. These features have led to the commonly believed notion that neurons are computing units that fires action potentials with accuracy and fidelity when it receives appropriate barrages of inputs within a neuronal cell ensemble.

The firing features of a neuron are very reliable, to the extent that a neuron can often be defined, classified or categorized based on its temporal pattern of firing (see chapters on excitatory and inhibitory transmissions). Similarly, the spatial input-output relationship of a neuron, or more commonly called hardwiring diagram, is also strictly reproducible, as demonstrated by many anatomical tracing experiments. Remarkably, a large number of these spatial and temporal characteristics seem to be conserved across species through evolution, as critical determinants of neuron types are encoded by tightly regulated sets of genetic programs.

Such a built-in stability of neuronal circuits is most likely to be at the foundation of the reliability and the reproducibility of sensory perception over tens of years in long-living animal species such as human. While common sensory events are usually chance events and mostly with neutral valence, certain sensory modality such as pain sensation has a special affective valence (usually negative in the case of pain), which is ethologically linked to specific patterns of emotional behavior (such as escape, cry etc.). Repeated exposure to physical stimuli that activate pain sensation may create an altered state of responsiveness (escape or coping) towards these stimuli. Similar alteration in response behavior was shown in many situations where exposure to specific and neutral sensory stimuli was associated with specific affective valence (such as in addictive state, during classical Pavlovian conditioning, or under spatial maze tasks with food reward or water escape as a reinforcer).

This experience-dependent adaptation of the behavioral output occurs entirely based on the specific patterns of the experienced incoming sensory stimuli. Many pioneering works suggest that such an adaptive responsiveness is most likely to be mediated, at least in part, by mechanisms of synaptic plasticity at the circuit level. Thus, activity-dependent synaptic plasticity (either long-term potentiation, LTP or long-term depression, LTD) is postulated to be induced at specific sets of synapses during association of sensory events with affective valence. Experimental evidence in favor of this hypothesis has been accumulated during hippocampal spatial learning and memory, fear memory, and pathological pain.

One major feature of activity-dependent synaptic plasticity is its perpetuation for sometimes more than days. The physical and molecular nature of such "memory trace" has been much debated, yet remains highly controversial. Among many hypotheses, one strong postulate that has survived intense scrutiny over dozens of years of research is the contribution of activity-induced up-regulation of novel gene products. This hypothesis posits that synaptic activity of the

kind that triggers synaptic plasticity (usually a strong burst of activity or prolonged and patterned stimuli) is necessary and sufficient to give rise to an increase in new protein synthesis, which in turn allows the potentiated/depressed synaptic efficacy states to be sustained over days, if necessary.

ACTIVITY-REGULATED NEURONAL TRANSCRIPTION FACTORS: WHAT ARE THEY?

One of the immediate challenges that face any attempt for the molecular dissection of activity-dependent neuronal gene expression is the accurate understanding of how synaptic activity can be possibly and tightly coupled to activation of specific nuclear transcriptional machinery. Early work suggested that mRNA transcription was a major site of regulation, as many gene transcripts were found to be induced upon receipt of strong depolarizing stimuli such as high K^+ depolarization. Screening of various induced genes suggested that a large number of genes up-regulated by neuronal activity contained *cis*-regulatory elements such as the cAMP-responsive element (CRE) or the serum-responsive element (SRE). Consistent with these findings, recent gene knockout experiments confirmed that the respective cognate transcription factor CREB (CRE-binding protein) and SRF (serum-responsive factor) are involved in activity-dependent gene activation in the CNS *in vivo*. Other *cis*-regulatory elements involved in neuronal activity-dependent gene expression include AP-1, NFAT, DREAM, CaRF, NFαB, and USF. Recent evidence indicates that regulation of chromatin remodeling is also implicated in these processes as well. Clearly, activity-dependent gene regulation is likely to be a complex orchestration of these various nuclear events, triggered and modulated by many independent yet interacting cytosolic and nuclear signaling pathways.

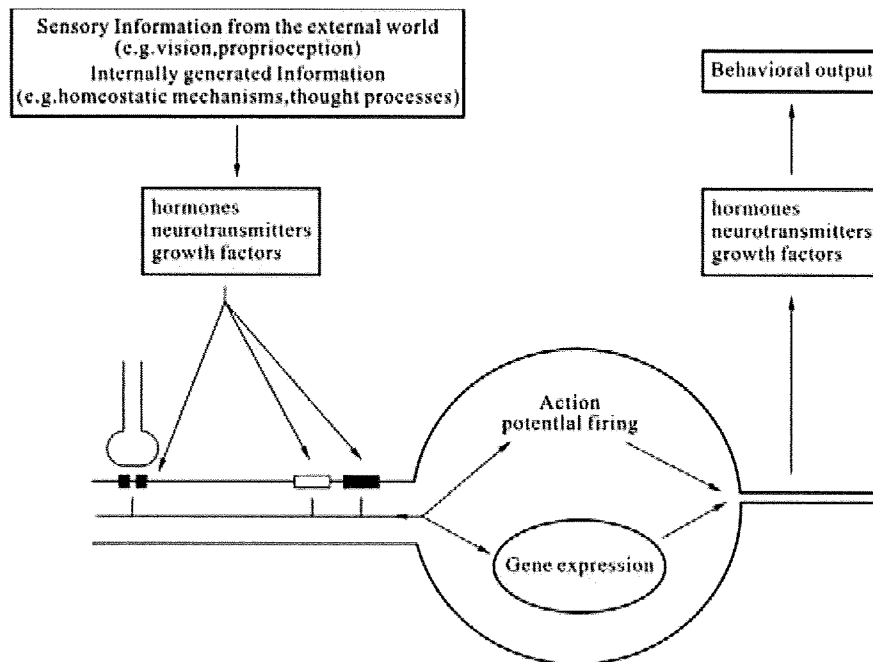


Fig.16.1 Activity-dependent gene expression critically tunes neuronal input-output function. Each neuron receives inputs from a high number of adjacent neurons via specialized contact domains called synapses. The number of synapses can sometimes reach over 10000, as in the case of hippocampal pyramidal neurons or cerebellar Purkinje cells. Each synapse is a source of EPSP that can be integrated into an axon potential in an all-or-none fashion when the summated potential exceeds the threshold to generate a sodium spike. Activity-dependent gene expression is triggered by a distinct kind of local (synapse)-to-global(nuclear)signaling with a different logic of summation.

What then are the specific sets of gene products up-regulated upon induction of synaptic plasticity? Are there common transcriptional regulatory elements and shared transcriptional machinery involved? How can synaptic activity traverse the space separating the synapses from the nucleus and yet not lose much of the specificity? We will address these few issues in the following sections.

BOX 16.1. An example of signaling from synapse to nucleus: the case of CREB.

Both gain-of-function and loss-of-function effects of CREB have been evaluated *in vivo* in various animal species including *Aplysia, Drosophila*, and mice and rats. The involvement of CREB on neuronal functions was first suggested in cultured neurons of *Aplysia*. The *Aplysia* neuronal culture, in which sensory neurons make synaptic contacts with motor neurons, shows stimulus-dependent enhancement in synaptic transmission efficiency (synaptic facilitation), which is a basis of behavioral sensitization to stimuli. Injection of CRE-containing oligonucleotide duplex into sensory neurons blocked long-term facilitation (LTF) without affecting short-term facilitation (STF). A series of following studies have demonstrated that CREB-induced gene expression is crucial for establishment and maintenance of LTF.

A direct relationship between CREB and memory has first been shown in *Drosophila*. Transgenic flies expressing a dominant negative dCREB-2 showed impairment of long-term olfactory memory, but not short-term memory. Conversely, transgenic flies expressing a dCREB-2 activator showed enhanced performance in olfactory memory task. The phenotype of this fly line, however, remains controversial, as the dCREB-2 activator transgene contained a mutation that induced a frame-shift and a premature stop in the coding region.

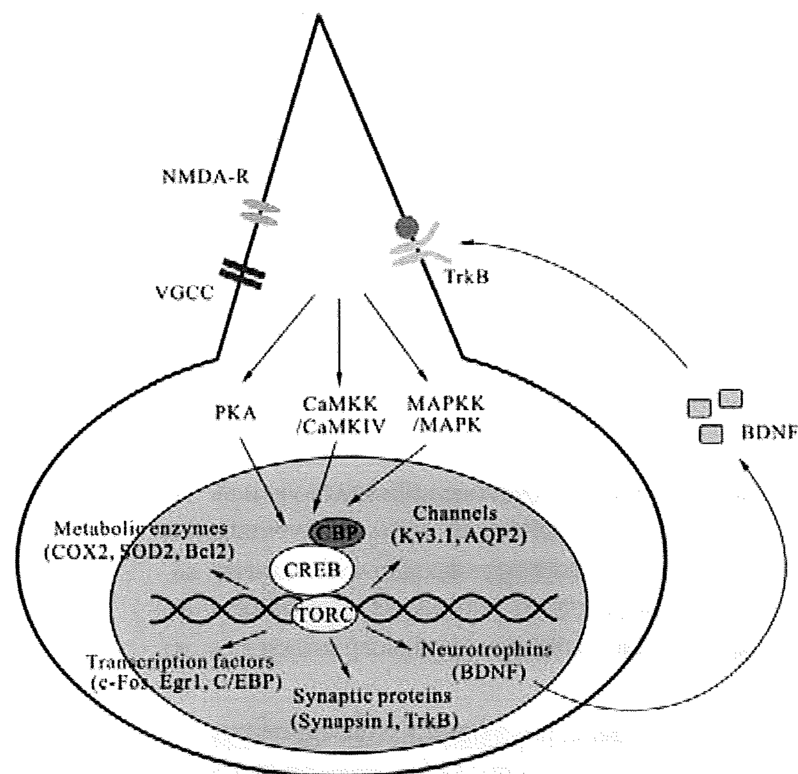


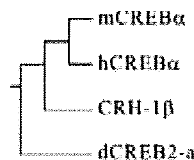
Fig.16.2 An example of signaling from synapse to nucleus: the case of CREB. CREB is a transcription factor that can be activated by many separate signaling routes that culminate in stimulating different nuclear CREB kinases. Three well described examples, the CaMKK/CaMKIV pathway, the PKA pathway and the MAPK pathway are illustrated. The activation kinetics and the resulting persistence of pCREB in the nucleus are thought to be distinctively regulated by the relative contribution of each one of these pathways, thereby accounting for the variety and diversity of the CREB-regu- lated transcriptome. Some of the CREB target genes such as BDNF could also participate in the late phase control of nuclear phospho-CREB amount. Such a positive feedback mechanism may critically determine the set of genes required for BDNF-dependent long-term plasticity and survival.

CREB AS A TRANSCRIPTIONAL REGULATOR

CREB is a member of the CREB/ATF family nuclear transcription factor, which includes CREB, activating transcription factors (ATFs) and cAMP-re- sponsive element modulator (CREM), and shares similar

CRE recognition characteristics with family members. CREB homodimerizes via the leucine zipper motif at the C-terminal, and binds to the cAMP-re- sponsive element (CRE), a specific palindromic DNA sequence, 5′-TGACGTCA-3′, often found in the 5′-upstream vicinity of transcription initiation sites of many neuronal genes. These include transcription factors (e.g., c-Fos, Egr-1, Per1, C/EBPα), cellular meta- bolic enzymes (e.g., cytochrome c, phosphorenolpyruvate carboxy kinase, cyclooxygenese-2, superoxide dismutase 2, bcl-2), growth factors and neuropeptides (e.g., somatostatin, enkephalin, brain-derived neurotrophic factor, insulin-like growth factor, fibroblast growth factor 6, vasopressin), and neuronal proteins (e.g., synapsin I, β1- and β2- adrenergic receptors, trkB). Furthermore, systematic genome-wide approaches, using comprehensive transcriptome and chromatin immunoprecipitation analyses, have confirmed the extreme complexity of CREB target gene profile, which seems to be highly cell-type and context-dependent.

CREB in a heterodimeric complex can also bind to a half-site CRE motif (5′-TGACG-3′). CREB homodimers usually reside in the nucleus, tightly bound to CRE loci, and are ideally suited to rapidly convert cellular signaling into transcription. Through regulation of CRE-dependent gene expression, CREB mediates cell growth, survival, death, proliferation and differentiation, in response to a variety of extracellular stimuli in different types of cells. CREB structure is conserved from mollusk to rodents, and neuronal CREB mediates long-lasting forms of synaptic plasticity and has been implicated in higher brain functions such as learning and memory in many species. Box 16.6 Gain-of-function and loss-of-function phenotypes of CREB in invertebrates.



KID domain

mCREBα	113	ESVDSVTDSQKRREILSRRPSYRKILNDLSSDAPGVPRIEEE	154
hCREBα	113	ESVDSVTDSQKRREILSRRPSYRKILNDLSSDAPGVPRIEEE	154
CRH-1β	9	EGGDSKDEARRRREQLNRRPSYRMILKDLETADKVMKKEPEE	50
apCREB1α	65	DLSSSDSDAKKRREILTRRPSYRKILNELSSPVSKMDDDSNS	106
dCREB2-α	211	DESLSDDDSQHHRSELTRRPSYNKIFTEISGPDMSGASLPMS	252

bZIP domain

mCREBα	280	EEAARKREVRLMKNREAARECRRKKKEYVKCLENRVAVLENQ	321
hCREBα	280	EEAARKREVRLMKNREAARECRRKKKEYVKCLENRVAVLENQ	321
CRH-1β	242	DESNRKRQVRLLKNREAAKECRRKKKEYVKCLENRVSVLENQ	283
apCREB1α	210	EEGSRKREFLRLLKNREAARECRRKKKEYVKCLENRVAVLENQ	251
dCREB2-α	298	EDQTRKREIRLQKNREAARECRRKKKEYIKCLENRVAVLENQ	339

mCREBα	322	NKTLIEELKALKDLYCHKSD	341
hCREBα	322	NKTLIEELKALKDLYCHKSD	341
CRH-1β	284	NKALIEELKTLKELYCRKEKDGM	306
apCREB1α	252	NKTLIEELKALKELYCQKDA	271
dCREB2-α	340	NKALIEELKSLKELYCQTKND	360

Fig.16.3 Conservation of CREB protein during evolution. The top panel shows a phylogenic tree analysis of the primary amino acid sequences of full-length CREB protein. It is likely that *D. melanogaster* CREB ortholog (dCREB2-a) has evolved separately from the mammalian CREB (mCREBα, hCREBα) gene, as the *C. elegans* full-length CREB protein (CRH-1β) has higher homology with its mammalian, rather than the fruitfly counterpart. The KID and the bZIP domains show overall a remarkable degree of conservation, as shown in the lower panels. Marked in red and green are identical and conserved amino acid residues, respectively. Ser-133 and the leucine residues forming a leucine zipper are shown with an asterisk.

CONTROL OF CREB ACTIVITY BY REGULATED PHOSPHORYLATION AT RESIDUE SER-133

CREB Ser-133 phosphorylation is a key switch that turns on CREB function. CREB is constitutively bound to CRE sites in the chromatin, regardless of cellular activity. However, several nuclear kinases have the potential to activate CREB via Ser-133 phosphorylation. When Ser-133 residue is phosphorylated, the kinase inducible domain (KID) which comprises this Ser-133 becomes a high-affinity binding site for the KID-interacting (KIX) domain of a general transcriptional co-activator, CREB-binding protein (CBP). Whether the high affinity of phosphorylated Ser-133 (pS133) site is sufficient in a native chromatin complex to trigger the docking and to promote the recruitment of a CBP-containing transcriptional pre-initiation complex at regions immediately adjacent and downstream to the CRE sequence remains unknown. The epigenetic state of the surrounding chromatin-DNA complexes, as well as the affinity between CREB and $TAF_{II}130$ in the TFIID complex and/or CREB-specific co-factors such as transducers of regulated CREB activity (TORCs) may also significantly contribute to this process.

In keeping with this complexity of regulation surrounding the interaction of pS133-CREB with the transcriptional pre-initiation complex, aberrance of this nuclear protein complex in the long-term appears to play a detrimental role in CNS function. Indeed, CREB/CBP-dependent transcription was shown to be repressed in several model systems of polyglutamine diseases, and this may be causative of the typical neurodegeneration associated with these diseases. Molecular or pharmacological manipulations that alleviated this transcriptional disruption significantly rescued neuronal cell death.

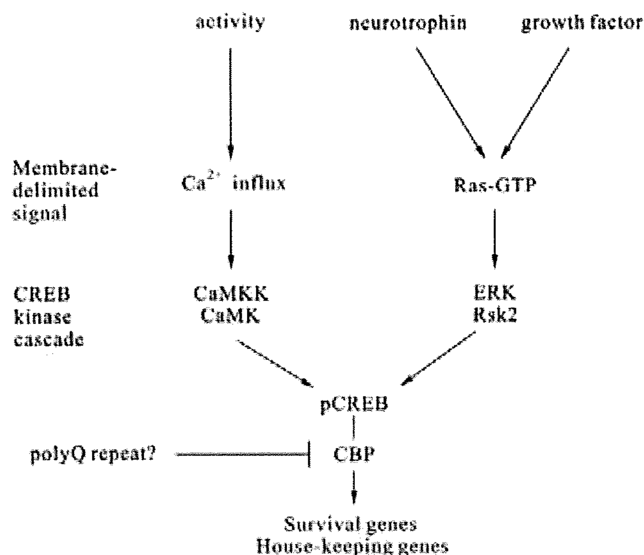


Fig.16.4 CREB/CBP complex is a biologically ritical node of signal convergence and its aberrance may underlie the molecular pathology of certain polyglutamine diseacses.Various kinds of neuronal signaling events implicated in neuronal activity or survival factors including neurotrophins or growth factors induce specific membrane-delimited signals that triggers and converge onto CREB phosphorylation. Phosphorylated CREB, by virtue of an increased affinity to CBP, a co-activator, can then turn on transcription of a battery of survival as well as house-keeping genes. In polyQ-diseases, the efficiency of pCREB-CBP interaction may be diminished, thus creating a deficit in the pool of CREB-dependent survival genes. This may create an increased susceptibility to insults, thereby augmenting the chance for neuronal death in the long run.

Although Ser133 phosphorylation of CREB was originally reported to be mediated by cAMP-dependent protein kinase (PKA), a large body of evidence now shows that the phosphorylation of this site is also

achieved by a variety of kinases including protein kinase C (PKC), Rsk1-3, Msk-1, MAPKAP-K2/3, Akt, and CaMKI, II and IV. The kinases that mainly phosphorylate Ser133 of CREB may depend on types of stimuli as well as types of cells. Studies *in vitro* in cultured neurons and *in vivo* in either knockout or transgenic mice have revealed that fast Ser-133 phosphorylation triggered immediately after strongly synaptic activity is predominantly mediated by a CaMKK/CaMKIV pathway. A temporally delayed phase of phosphorylation was shown to involve a MAPK pathway as well, though the specific isoform(s) and the relevant downstream CREB kinase(s) have not been firmly elucidated.

Compared to kinases, less information is available about phosphatases that dephosphorylate and thereby critically determine the decay of the nuclear amount of phospho-Ser133. Depending on the cell types, either Ser/Thr protein phosphatase 1 (PP-1) or PP-2A were shown to directly dephosphorylate CREB at Ser133 both *in vitro* and in culture cells, after phospho-CREB is unbound from CBP. Several other phosphatases such as calcineurin (PP2B), PTEN and protein tyrosine phosphatase 1B (PTP1B) were suggested to be indirectly involved in dephosphorylating pS133 by suppression of upstream CREB kinases or by activation of CREB phosphatases. Together, a huge amount of neuronal signaling pathways are employed and converge onto the regulation of the phosphorylation state of pS133, consistent with its presumed importance in many adaptive biological processes, including long-term neuronal plasticity and survival.

BOX 16. 2

Box 16.2 Gain-of-function and loss-of-function phenotypes of CREB in genetically engineered mice.

Although CREB is ubiquitously expressed in all tissues in the body, significance of CREB function has been most extensively investigated in the central and peripheral nervous systems as well as in some other systems including T-cell maturation. Also, several studies using mice expressing a dominant-negative CREB transgene have demonstrated CREB functions in glucose and lipid metabolism.

In mice, disruption of the exon 2 of the CREB gene, which contains the first ATG codon, resulted in abolishment of expression of major alternative spliced isoforms (CREB alpha/delta knockout), but at the same time, unexpected upregulation of another activator CREB isoform, CREB beta, and related protein CREM in a large number of organs. These CREB hypomorphic mutant mice showed no obvious developmental abnormality in the body, in a mixed 129 x BL6 background. There were also no obvious anatomical disorders in the brain. However, when the mice were assessed with hippocampal-dependent memory tasks, the mice showed impairments in formation of long-term memory, but not short-term memory, consistent with findings reported in invertebrates (see Box 16.1).

CREB null mutants that lack all functional activator CREB isoforms have been generated by disrupting the bZIP domain. In contrast to the CREB hypomorphic mice, the CREB null mutants were smaller than wild-type littermates and died immediately after birth from respiratory distress. The commissural fibers in the brain were dramatically reduced in the mutant mice. The mice also showed severe impairments in development of T-cells of the alpha/beta lineage, but not the gamma/delta lineage.

The CREB null-mutant mice also exhibited excess apoptosis and degeneration as well as impairment in axonal growth and projections in sensory neurons, suggesting that CREB plays a role in survival and growth of peripheral neurons. Conditional forebrain-specific CREB knockout mice with a CREM null background also showed extensive neuronal apoptosis during development and progressive neurodegeneration in adult brains.

A study with transgenic mice with an inducible and reversible CREB repressor showed that CREB is crucial *in vivo* for the consolidation of long-term conditioned fear memories, but not for encoding, storage, or retrieval of these memories. In the study, it has also shown that CREB is required for the stability of reactivated conditioned fear memories.

Transgenic mice with spatially restricted and temporally regulated expression of a constitutively active CREB (VP16-CREB) have been generated to assess CREB roles in hippocampal long-term potentiation (LTP), a possible cellular basis of learning and memory. In the transgenic mice, hippocampal CA1 neurons showed facilitation of a persistent late phase of LTP (L-LTP) elicited by weak stimuli, which usually produce only an early phase long-term potentiation (E-LTP) in wild type animals. The results indicate that elevated CRE-driven gene products by VP16-CREB may be sufficient for consolidation of LTP, and support the "synaptic tag and capture" hypothesis.

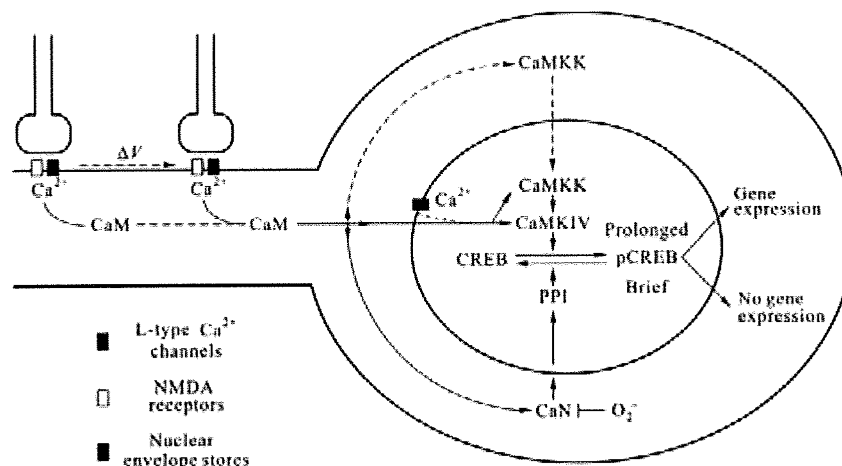


Fig.16.5 Complex control of nuclear phospho-CREB abundance by Ca^{2+}/CaM-mediated synapse-to-nucleus signaling. Patterned synaptic activity triggers robust Ca^{2+} mobilization at synaptic and perisynaptic locations via opening of NMDA-rece- ptors and voltage-dependent Ca^{2+} channels. Calmodulin (CaM) is particular enriched at and near synapses and synaptic Ca^{2+} entry dramatically augments the amount of activated (Ca^{2+}-bound) CaM. Ca^{2+}/CaM acts as a cofactor to various neuronal enzymes including CaM kinases, calcineurin, type I adenylate cyclase, GDP/GTP-exchange factors for small GTPases such as Ras and Rac. Thus, Ca^{2+}/CaM increase could in principle participate in activation of CaMK, Ras/MAPK, and cAMP/PKA pathways. In hippocampal CA1 pyramidal neurons and in anterior cingulate cortical neurons, rapid nuclear translocation of activated CaM was shown. This is believed to account, at least in part, for the predominant role of nuclear CaMKK/CaMKIV route in CREB phosphorylation in these cell-types. In parallel to this pathway, hippocampal neurons in which Ca^{2+} was strongly mobilized generate superoxide anions, which are able to inactivate calcineurin activity. This event seems to alleviate calcineurin-mediated regulation of protein phosphatase-1-dependent dephosphorylation of pS133, thereby contributing to the persistence of pS133 amount in the neuronal nuclei.

CONCLUSION AND FUTURE DIRECTIONS

Due to limitation in our current knowledge, we were able to review only a very small subset of the nucleus-to-synapse signaling critical for long-term adaptive regulation of neuronal input-output relationship. However, there is no doubt that a far larger number of molecular mechanisms contribute to ensuring that the neuronal transcriptome can be adapted in function of the various kinds of external and internal events that the neuronal network is exposed to.

One key issue that still escapes our studies concerns the spatial distribution of the up-regulated gene products within the activated neuron. Are they just widely distributed as resources with a higher availability, or are there further specific regulations to traffic them to special locations in the neuron, such as the sites of previous strong activity? Obtaining an insight to this question will provide a clue to better understand how plastic synapses (potentiated or depressed responses triggered by predetermined sets of stimuli/sensation) could possibly coexists with the normal synapses (unchanged responses at other synapses) within the same neurons. A critical test in the future will be to determine whether these up-regulated gene products are correlated in any way (temporal or spatial) with the distribution of persistent changes in individual synaptic weights within the relevant neuronal circuit.

Another predominant issue is how a stabilized synapse could possibly be reset back to baseline value by activity. Without such a dynamic bistability, that is, the existence of regulated extinction/erasure of plasticity, the amount of information storage will soon saturate quickly. However, how stabilization of plasticity and erasure can co-exist in concert remains totally unknown. Resolving this question would also be of immense clinical value to addressing clinical cases involving aberrant persistence of pain sensation or