と，大きく影響されない成分に分かれる．前者はHomer，GKAP，Shankなどが含まれ，後者には膜表面受容体と結合するPSD-95などを含む．これらの分子群はおそらく，シナプス動態・形態制御とシグナリング制御の2つの事象に，それぞれ互いに相互作用を及ぼしながら関わっていると考えられている．各構成分子の機能異常は，シナプスの機能に多様で広範な障害を及ぼす．以下に，HomerおよびPSD-95の2つの分子を例として，構造と機能，およびその機能破綻による病態との関係について概説する．

5-1 Homerタンパク

Homerは，3つの遺伝子（Homer 1～3）からなるファミリータンパク質であり[9]，いずれもEVH 1ドメインとCoiled-coilドメインの2つのドメイン構造を有する（図3-2-5 a）．ここでは簡単にするため，これらのファミリーをまとめてHomerとして扱う．また，Homer遺伝子からはCoiled-coilドメインを欠損したアイソフォームも作られる．Homerはシナプス後肥厚部においてEVH 1およびCoiled-coilドメインを介して，多数のタンパク質と相互作用し，

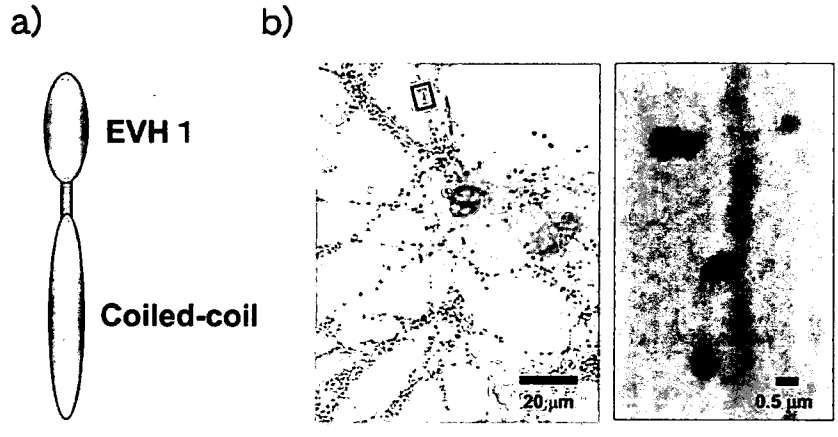


図3-2-5 Homerタンパク質の分子構造とシナプス集積

(a) Homerのドメイン構造．Homerはタンパク-タンパク相互作用モチーフであるEVH 1ドメインを持ち，さらに複合体形成に必要なCoiled-coilドメインをもつ．またCoiled-coilドメインを欠損するshort-form型も存在する

(b) Homerのシナプス局在．（左）海馬培養細胞の透過像に，抗Homer抗体による免疫染色像（黒色表示）を重ね合わせて示した．1つの培養神経細胞あたり数百以上の黒いスポットが観察される．（右）樹状突起の拡大図．左パネルのボックス部を拡大した．個々のHomerのスポットは，樹状突起の幹部から少し離れたスパインの頭部に見られる

これらのタンパク質の足場の構成成分として機能していると考えられている(図3-2-5 b). EVH 1ドメインと結合するタンパクには，代謝型グルタミン酸受容体mGluR1／5やIP3レセプター，TRP-Cチャネルなどが知られる．これらのタンパク質は，いずれもカルシウムシグナルと密接な関わりがあるものであることから，Homerタンパク質が細胞内のカルシウムシグナルの制御に重要な役割を果たしていることが示唆される．またEVH 1ドメインには，NMDA受容体複合体の構成成分Shankも結合するが，このShankはさらにCortactinというアクチン細胞骨格結合タンパク質と結合する．このタンパク質複合体により，Homerはアクチン細胞骨格との間接的な相互作用を持っていると考えられる．一方，Coiled-coilドメインは，Homerタンパク質同士の結合に必要であり，この部分を介してHomer-Homer複合体を形成することにより，EVH 1ドメイン結合タンパク質とともに巨大な複合体を形成すると考えられている．

Homerの生理的機能の解析は，主にノックアウトマウスを用いて進められてきた．Homerノックアウトマウスは，学習・記憶障害のほか，新規オブジェクトに対する関心の低下やプレパルス抑制の障害，また多動性を示すなど，多くの神経精神疾患様の症状を示す[9]．また，Homerノックアウトマウスでは，コカインやアンファタミンなどの薬剤に対する感受性が上昇しており，向精神薬中毒の分子メカニズムの解明の糸口として注目を集めている．さらにHomerを欠損させると，アルコールに対する行動レベルでの感受性が低下し，逆にHomerを強制的に発現させると，アルコールに対する感受性が上昇する．また興味深いことに，ヒトにおいてHomer1遺伝子の一塩基多型（SNP）が，ある群の統合失調症患者においては有意に認められたという報告もある[10]．これらの知見を統合すると，Homerは神経機能を正常に保つために必須であり，シナプスにおけるHomerの機能，局在，動態などの異常は，広範な神経精神疾患様症状を引き起こす原因となっている可能性を強く示唆している．

5-2 PSD-95タンパク質

PSD-95は，中枢神経系の興奮性シナプスのシナプス後肥厚部にもっとも豊富に存在する分子の1つである．PSD-95を中心的な足場として集積した複合

体には，シナプス可塑性やアクチン動態にも大きく影響を与えるNMDA型グルタミン酸受容体が含まれる．PSD-95は，3つのPDZドメイン，SH3ドメインならびにGKドメインの，5つのタンパク-タンパク結合ドメインを有し，それぞれを介したタンパク-タンパク相互作用により複合体形成に寄与すると考えられている（図3-2-6）．各ドメインは，特にきわめて相同性の高い3つのPDZドメインは，特異的なリガンドと独立に結合して，それぞれ特異性の異なるシグナルの橋渡しをするのだろうか？　このような問いに対する解を得るためには，PSD-95全長の分子構造を保持しつつ，特定PDZドメインの結合能のみ欠落している全長変異体を用いる工夫が必要である．

野中らは，PDZ1，2とPDZ3との結晶構造・NMR構造比較をもとに，結合活性を有する機能的なPDZドメインの数が0，1，2，3となるようなさまざまなPSD-95変異体を作成し，これらをマウス海馬の神経初代培養細胞に遺伝子

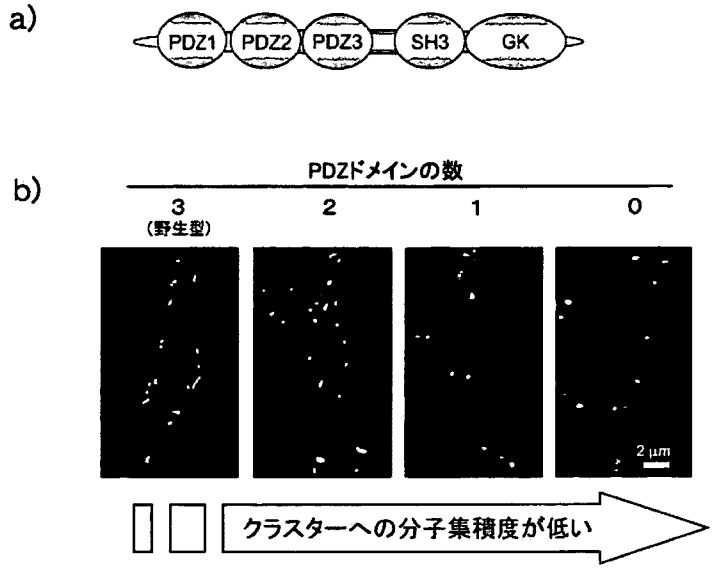


図3-2-6　PSD-95の分子構造とシナプス集積

(a) PSD-95のドメイン構造．タンパク-タンパク相互作用モチーフであるPDZドメインが3つ存在し，そのC末側には別のタンパク-タンパク相互作用モチーフであるSH3ドメインおよびGuanylate kinase様(GK) ドメインが存在する

(b) PDZ変異体を用いたPSD-95の機能解析．3つのPDZドメインに段階的に変異を加えることにより，徐々にPSD-95のシナプスへの集積度が低下し，また，スパインの長さが長くなるなどのシナプスの形態の異常が見られた（東京大学・野中美応博士のご厚意による）

導入して表現型解析を行った[11]．各PSD-95変異体はシナプスに集積したが，クラスター形成効率は，機能的PDZドメインの数が3，2，1，0と減るにつれて徐々に低下した．また，変異PSD-95を発現している神経細胞では，細長く伸展している幼弱な形状のスパインが数多く見られ，機能的PDZドメインが減るほどスパインが長くなる傾向が見られた．スパイン長の伸展は，前述した精神遅滞や，脆弱性X染色体症候群などの疾患で見られる長いスパインを思い起こさせる．これまでPSD-95の3つのPDZドメインは，リガンド特異性が異なることから，独立した機能を受け持つと考えられてきた．しかし上記の実験から，3つのPDZドメインは，高密度クラスター形成とスパイン形態成熟に対して相加的な作用を示すことが明らかになった．

PSD-95は，PDZ結合タンパクの1つであるSynGAPなどの細胞骨格制御分子との結合を通して，シグナル伝達をスパイン形態に反映させる機能を持つことが示唆されており[11]，また，ほかのPDZ結合タンパク質であるStargazinを介して，AMPA型グルタミン酸受容体のシナプス集積にも関与している．このようにAMPA型・NMDA型のグルタミン酸受容体や細胞骨格制御分子などの，多様かつシナプス後膜の中心的シグナリング分子群を結びつける核として機能するPSD-95は，シナプス伝達・シナプス可塑性に重要な役割を果たしていることが，ノックアウトマウスやRNA干渉によるノックダウン実験の結果から明らかにされている[12]．これまでのところ，PSD-95単独の欠損による神経精神症状は報告されていないが，これはPSD-95同様に，シナプス後肥厚部に数多く存在するSAP102やPSD-93などのPSD-95関連タンパクが，その機能を代償するためと考えられている．このような冗長性は，逆に，正常なシナプスにおけるPSD-95の役割の重要性を示唆している．興味深いことに，PSD-95結合タンパク質であるneuroliginは，統合失調症や自閉症の原因遺伝子の候補の1つとして最近とくに注目を集めている[13]．

6 ナノメディシンの新たな標的：シナプス機能制御・破綻修復から疾患の克服まで

本稿では，シナプスの両側にあるサブフェムトリットルのシグナル伝達空間において，シナプス伝達とそこから派生する細胞内シグナル伝達が正しく作動

することが，脳の高次機能発現に必須であることを述べてきた．紙面の関係上述べることができなかったが，神経細胞の発達段階，神経活動の頻度や持続時間，パターンによって，個々のシナプスで駆動されるシグナル伝達機構は異なることが明らかになってきている．特に，カルシウムシグナル，cAMPシグナル，脂質シグナルなどの，主要なセカンドメッセンジャーを介したシグナリングの時・空間的特異性，およびそれらシグナリングの相互作用の生理的意義は大きく，今後は，これらの系統的な解析が待たれるところである．最近の光技術の急速な進歩により，UVや多光子吸収による光融解を利用した単一シナプス反復刺激法が，培養ニューロン，脳スライスさらに*in vivo*においても可能になってきた（図3-2-7）．このような新しい方法を用いることにより，近い将来，シナプス内およびシナプス間シグナルの，非線形的相互作用のルールが解明されることが予感される．さらにこれらをより深く理解するためには，シナプス後肥厚部や神経突起先端の成長円錐のような，限局した容積を持つナノドメインにおける酵素反応や，タンパク質-タンパク質間，タンパク質-脂質二重膜間，タンパク質-細胞骨格間の相互作用などを網羅的に，かつ定量的に叙述可能な数学的なフレームワークの構築が急務であろう．

ヒトにおける遺伝性精神神経疾患の患者や，そのモデル動物である関連遺伝

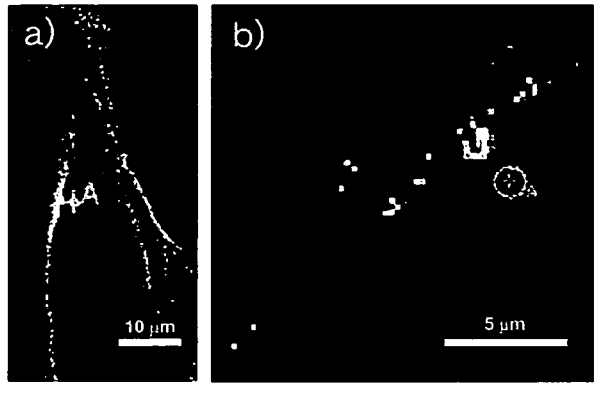


図3-2-7 UVレーザーフォトリシスによる光刺激

(a) ケイジドグルタミン酸を用いた光融解刺激．カルシウム指示薬を取り込ませた海馬培養細胞の樹状突起の一部にUVレーザーパルスを照射し，局所での細胞の応答を記録した．A点がレーザー照射位置を示し，白黒表示で局所のカルシウム上昇を示している

(b) レーザー強度をコントロールすることにより，さらに領域を限局させて単一シナプスの刺激が可能である

子改変マウスの脳などでは，異常なスパイン形態の増加や，突起形成，細胞移動による投射異常などが観察される．このような病態において，正常な脳高次機能を回復するためには，神経ネットワークがマクロ的およびミクロ的に正しく再形成され，神経細胞骨格修飾やシナプス形態制御を修復することが重要であろう．加えて，ナノドメイン空間におけるシグナル伝達共役の異常の克服が必要となる．シナプスシグナリングの定量的理解と，破綻メカニズムの理解をもとに，リン酸化酵素・脱リン酸化酵素・イオンチャンネルなどの機能モジュレーターの発見・開発や，遺伝子治療・ウィルス治療・光刺激法などの新しい技術を駆使することにより，遠くない将来，神経ネットワークの損傷・障害による脳高次機能低下からの回復法が確立されることを願ってやまない．

[執筆者プロフィール]

おくの ひろゆき

1990年東京大学理学部卒．大学院博士課程において東京大学医学部生理学教室にて大脳生理学を学び，東京大学医学部助手，ジョンズ・ホプキンス大学医学部博士研究員を経て，2003年より東京大学・大学院医学系研究科・尾藤研究室にて研究に従事．研究テーマは活動依存的シナプスシグナリングと長期神経可塑性について

ふじい はじめ

2004年京都大学大学院医学研究科修了，現在，東京大学・大学院医学系研究科（尾藤研究室）在学中，学術振興会特別研究員．蛍光分子イメージングを用いて，神経細胞間のシナプス伝達が細胞内シグナルへと変換される様子を明らかにしたい

びとう はるひこ

1990年東京大学医学部卒．同大学院にて学位取得後，スタンフォード大学医学系研究科博士研究員，京都大学医学部助手・講師を経て，2003年より東京大学・大学院医学系研究科・神経生化学にて教室主任を勤める．研究内容はhttp://www.neurochem.m.u-tokyo.ac.jpをご参照ください

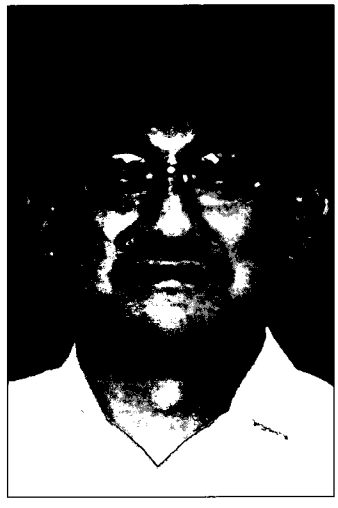
[参考文献]

1. Yuste, R. & Bonhoeffer, T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24 (2001) 1071-89
2. Arakawa, Y. et al. Control of axon elongation via an SDF-1alpha/Rho/mDia pathway in cultured cerebellar granule neurons. J Cell Biol 161 (2003) 381-91
3. Bito, H. et al. A critical role for a Rho-associated kinase, p160ROCK, in determining axon outgrowth in mammalian CNS neurons. Neuron 26 (2000) 431-41
4. Kennedy, M. B. Signal-processing machines at the postsynaptic density. Science 290 (2000) 750-4
5. Fiala, J. C., Spacek, J. & Harris, K. M. Dendritic spine pathology: cause or consequence of neurological disorders? Brain Res Brain Res Rev 39 (2002) 29-54
6. Sudhof, T. C. The synaptic vesicle cycle. Annu Rev Neurosci 27 (2004) 509-47
7. Kim, E. & Sheng, M. PDZ domain proteins of synapses. Nat Rev Neurosci 5 (2004) 771-81
8. Furuyashiki, T. et al. Citron, a Rho-target, interacts with PSD-95/SAP-90 at glutamatergic synapses in the thalamus. J Neurosci 19 (1999) 109-18
9. Szumlinski, K. K., Kalivas, P. W. & Worley, P. F. Homer proteins: implications for neuropsychiatric disorders. Curr Opin Neurobiol 16 (2006) 251-7
10. Norton, N. et al. Mutation screening of the Homer gene family and association analysis in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 120 (2003) 18-21
11. Nonaka, M., Doi, T., Fujiyoshi, Y., Takemoto-Kimura, S. & Bito, H. Essential contribution of the ligand-binding beta B/beta C loop of PDZ1 and PDZ2 in the regulation of postsynaptic clustering, scaffolding, and localization of postsynaptic density-95. J Neurosci 26 (2006) 763-74
12. Elias, G. M. & Nicoll, R. A. Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. Trends Cell Biol 17 (2007) 343-52
13. Craig, A. M. & Kang, Y. Neurexin-neuroligin signaling in synapse development. Curr Opin Neurobiol 17 (2007) 43-52
14. Harris, K. M. Structure, development, and plasticity of dendritic spines. Curr Opin Neurobiol 9 (1999) 343-8
15. Marin-Padilla, M. Structural abnormalities of the cerebral cortex in human chromosomal aberrations: a Golgi study. Brain Res 44 (1972) 625-9
16. Purpura, D. P. Dendritic spine "dysgenesis" and mental retardation. Science 186 (1974) 1126-8

CHAPTER 16

Activity-dependent Gene Regulation: How do Synapses Talk to the Nucleus and Fine-tune Neuronal Outputs?

Haruhiko Bito, Sayaka Takemoto-Kimura, Hiroyuki Okuno



Sayaka Takemoto-Kimura, and Hiroyuki Okuno are Assistant Professors working in close collaboration with Dr. Bito at the Department of Neurochemistry.

Dr. Haruhiko Bito is an Associate Professor at thes Department of Neurochemistry, The University of Tokyo Graduate School of Medicine. He graduated from The University of Tokyo, gaining an M.D., followed by a Ph.D. in Biochemistry and Molecular Neurobiology. Thereafter, he pursued a postdoctoral training at Stanford University. He was an Assistant Professor and Lecturer at Kyoto University School of Medicine before establishing his new laboratory in Tokyo.

MAJOR CONTRIBUTIONS

1. Bito H, Deisseroth K, Tsien RW. 1996. CREB phosphorylation and dephosphorylation: a Ca^{2+}- and stimulus duration-dependent switch for hippocampal gene expres- sion. *Cell*, 87:1203-1214.

2. Bito H, Furuyashiki T, Ishihara H, Shibasaki Y, Ohashi K, Mizuno K, Maekawa M, Ishizaki T, Narumiya S. 2000. A critical role for a Rho-associated kinase p160ROCK in determining axon outgrowth in mammalian CNS neurons. *Neuron*, 26:431-441.

3. Furuyashiki T, Arakawa Y, Takemoto-Kimura S, Bito H, Narumiya S. 2002. Multiple spatiotemporal modes of actin reorganization by NMDA receptors and voltage-gated Ca^{2+}-channels. *Proc Nat Acad Sci USA*, 99:14458-14463.

4. Takemoto-Kimura S, Terai H, Takamoto M, Ohmae S, Kikumura S, Furuyashiki T, Arakawa Y, Narumiya S, Bito H. 2003. Molecular cloning and characterization of CLICK-III/CaMKIγ, a novel membrane-anchored neuronal Ca^{2+}/calmodulin-dependent protein kinase (CaMK). *J Biol Chem*, 278: 18597-18605.

5. Nonaka M, Doi T, Fujiyoshi Y, Takemoto-Kimura S, Bito H. 2006. Essential contribution of the ligand-binding βB/β C loop of PDZ-1 and PDZ-2 in the regulation of postsynaptic clustering, scaffolding, and localization of PSD-95. *J Neurosci*, 26: 763-774.

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

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.

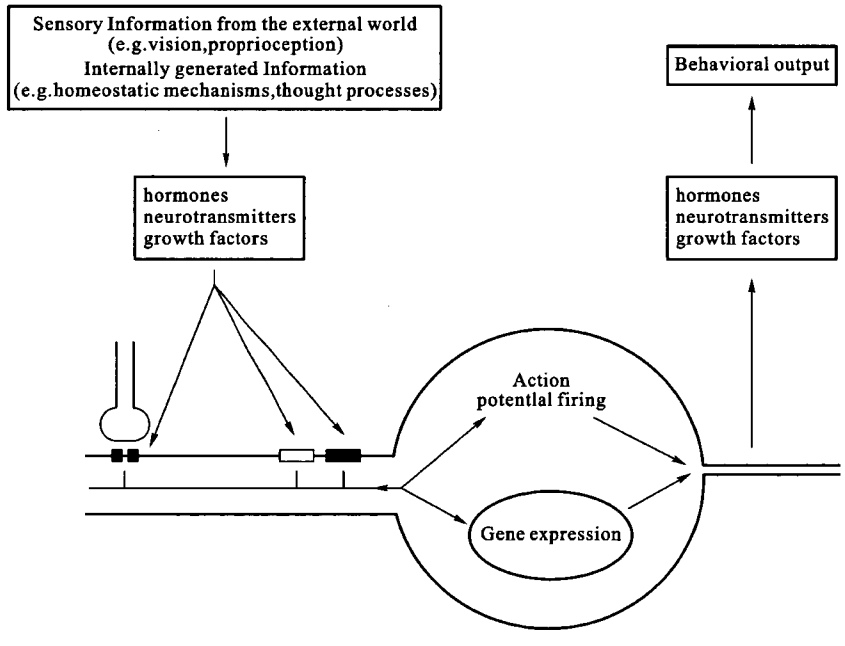


Fig.16.1 Activity-dependent gene expression critically tunes neuronal input-output function. Each neuron receives inputs from a high number of adjacent neurons via specialized contact domains called synapses. The number of synapses can sometimes reach over 10000, as in the case of hippocampal pyramidal 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.

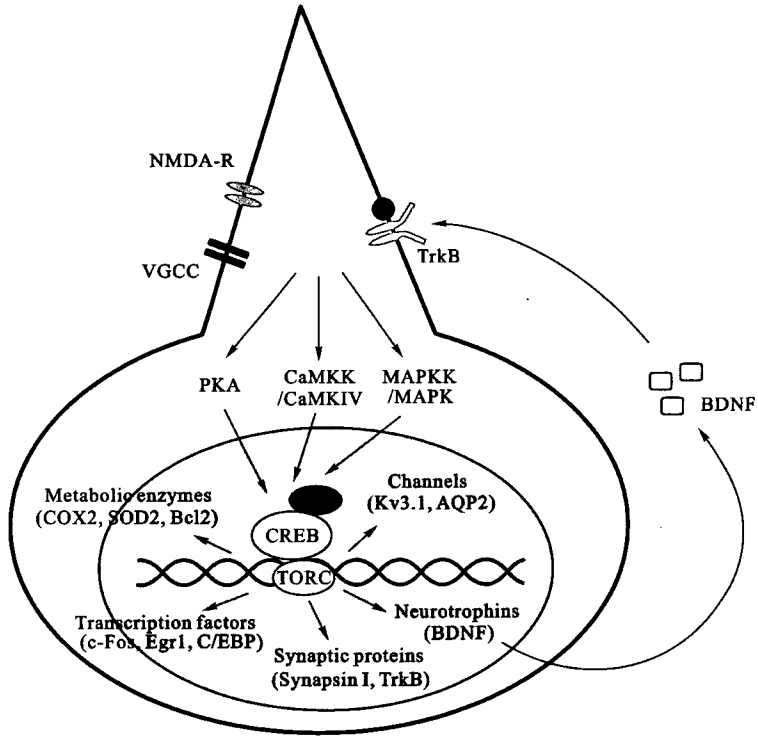


Fig.16.2 An example of signaling from synapse to nucleus: the case of CREB. CREB is a transcription factor that can be activated by many separate signaling routes that culminate in stimulating different nuclear CREB kinases. Three well described examples, the CaMKK/CaMKIV pathway, the PKA pathway and 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, Perl, 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.

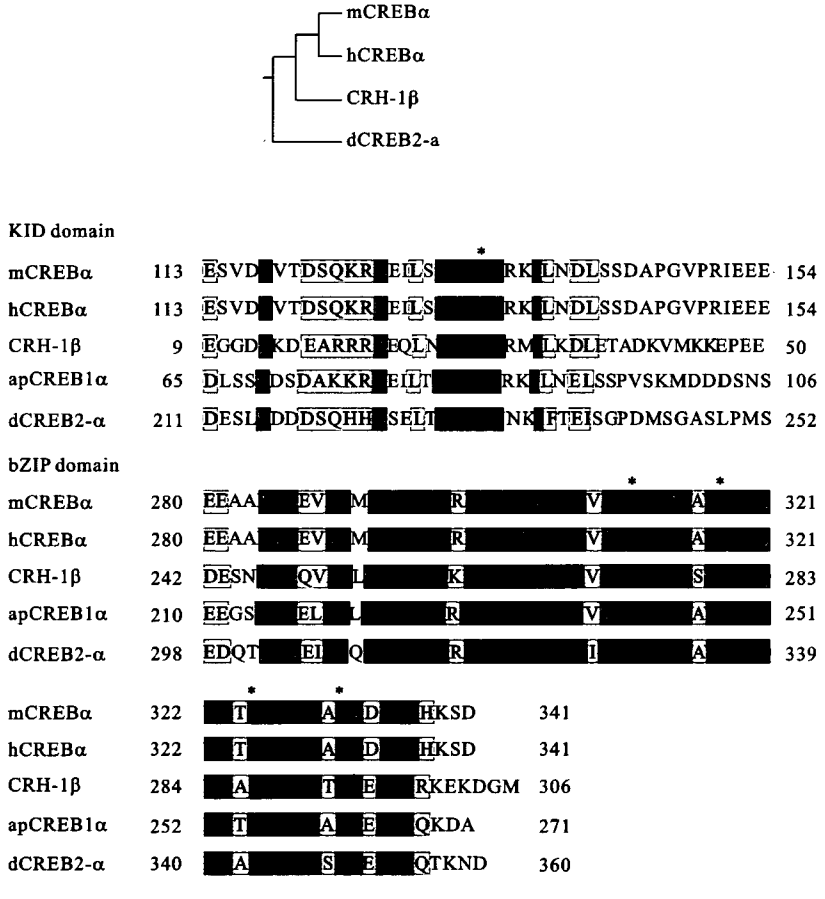


Fig.16.3 Conservation of CREB protein during evolution. The top panel shows a phylogenic tree analysis of the primary amino acid sequences of full-length CREB protein. It is likely that *D. melanogaster* CREB ortholog (dCREB2-a) has evolved separately from the mammalian CREB (mCREBα, hCREBα) gene, as 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.

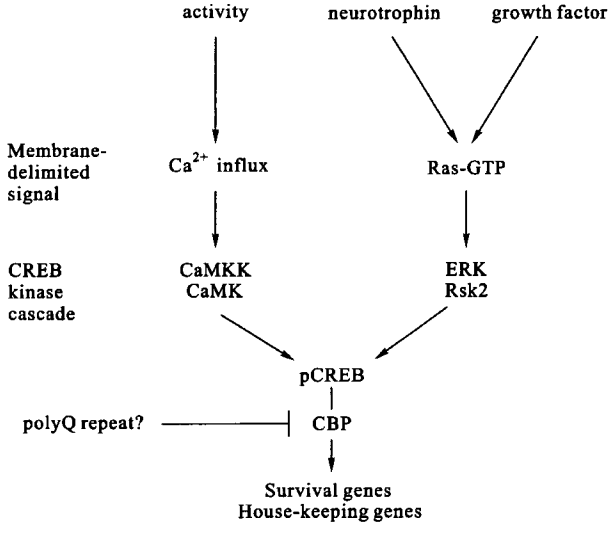


Fig.16.4 CREB/CBP complex is a biologically ritical node of signal convergence and its aberrance may underlie the molecular pathology of certain polyglutamine diseacses.Various kinds of neuronal signaling events implicated in neuronal activity or survival factors including neurotrophins or growth factors 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.

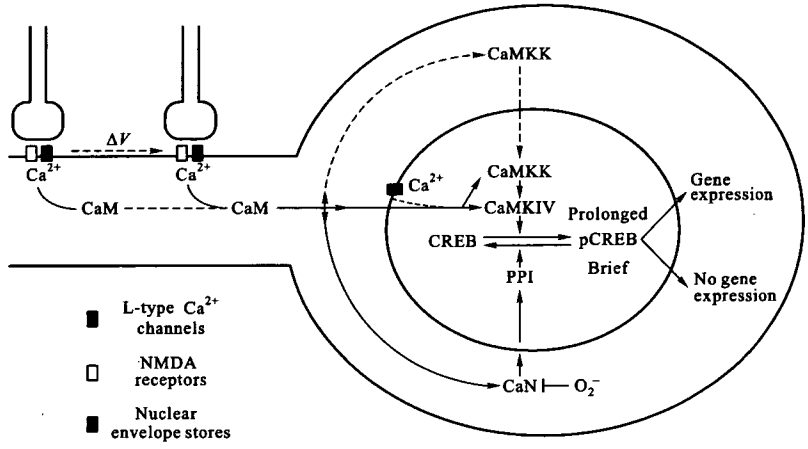


Fig.16.5 Complex control of nuclear phospho-CREB abundance by Ca^{2+}/CaM-mediated synapse-to-nucleus signaling. Patterned synaptic activity triggers robust Ca^{2+} mobilization at synaptic and perisynaptic locations via opening of NMDA-rece- ptors and voltage-dependent Ca^{2+} channels. Calmodulin (CaM) 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

posttraumatic stress disorder.

GENERAL CITATIONS

Bito H. 1998. The role of calcium in activity-dependent neuronal gene regulation. *Cell Calcium*, 23: 43-150.

Bito H, Takemoto-Kimura S.2003. Ca^{2+}/CREB/CB-P-dependent gene regulation: a shared mechanism critical in long-term synaptic plasticity and neuronal survival. *Cell Calcium*, 34:425-430.

Kandel ER. 2001. The molecular biology of memory storage: a dialogue between genes and synapses. *Science*, 294:1030-1038.

Martin SJ, Grimwood PD, Morris RG. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci*, 23:649-711.

Silva AJ, Kogan JH, Frankland PW, Kida S. 1998. CREB and memory. Annu Rev Neurosci, 21:127-148.

DISCOVERY CITATIONS

Barco A, Alarcon JM, Kandel ER. 2002. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell*, 108:689-703.

Bartsch D, Ghirardi M, Skehel PA, Karl KA, Herder SP, Chen M, *et al.*. 1995. Aplysia CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. *Cell*, 83:979-992.

Bito H, Deisseroth K, Tsien RW. 1996. CREB phosphorylation and dephosphorylation: a $Ca^{(2+)}$- and stimulus duration-dependent switch for hippocampal gene expression. *Cell*, 87:1203-1214.

Blendy JA, Kaestner KH, Schmid W, Gass P, Schutz G. 1996. Targeting of the CREB gene leads to up-regulation of a novel CREB mRNA isoform. *Embo J*, 15:1098-1106.

Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ. 1994. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell*, 79:59-68.

Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH. 1993. Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature*, 365:855-859.

Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, Hogenesch JB, *et al.*. 2003. TORCs: transducers of regulated CREB activity. *Mol Cell*, 12:413-423.

Dash PK, Hochner B, Kandel ER. 1990. Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. *Nature*, 345:718-721.

Deisseroth K, Heist EK, Tsien RW. 1998. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature*, 392:198-202.

Ferreri K, Gill G, Montminy M. 1994. The cAMP-reg- ulated transcription factor CREB interacts with a component of the TFIID complex. *Proc Natl Acad Sci USA*, 91:1210-1213.

Ginty DD, Kornhauser JM, Thompson MA, Bading H, Mayo KE, Takahashi JS, *et al.*. 1993. Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and a circadian clock. *Science*, 260:238-241.

Gonzalez GA, Montminy MR. 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell*, 59:675-680.

Hagiwara M, Alberts A, Brindle P, Meinkoth J, Feramisco J, Deng T, *et al.*. 1992. Transcriptional attenuation following cAMP induction requires PP-1-mediated dephosphorylation of CREB. *Cell*, 70:105-113.

Herzig S, Hedrick S, Morantte I, Koo SH, Galimi F, Montminy M. 2003. CREB controls hepatic lipid metabolism through nuclear hormone receptor PPAR-gamma. *Nature*, 426:190-193.

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, *et al.*. 2001. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature*, 413:179-183.

Impey S, McCorkle SR, Cha-Molstad H, Dwyer JM, Yochum GS, Boss JM, *et al.*. 2004. Defining the

CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell*, 119: 1041-1054.

Kida S, Josselyn SA, de Ortiz SP, Kogan JH, Chevere I, Masushige S, *et al.*. 2002. CREB required for the stability of new and reactivated fear memories. *Nat Neurosci*, 5:348-355.

Lonze BE, Riccio A, Cohen S, Ginty DD. 2002. Apoptosis, axonal growth defects, and degeneration of peripheral neurons in mice lacking CREB. *Neuron*, 34:371-385.

Mantamadiotis T, Lemberger T, Bleckmann SC, Kern H, Kretz O, Martin Villalba A, *et al.*. 2002. Disruption of CREB function in brain leads to neurodegeneration. *Nat Genet*, 31:47-54.

Mayr B, Montminy M. 2001. Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol*, 2:599-609.

Montminy MR, Bilezikjian LM. 1987. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature*, 328:175-178.

Ramanan N, Shen Y, Sarsfield S, Lemberger T, Schutz G, Linden DJ, *et al.*. 2005. SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. *Nat Neurosci*, 8:759-767.

Rudolph D, Tafuri A, Gass P, Hammerling GJ, Arnold B, Schutz G. 1998. Impaired fetal T cell development and perinatal lethality in mice lacking the cAMP response element binding protein. *Proc Natl Acad Sci USA*, 95:4481-4486.

Sée V, Boutiller AL, Bito H, Loeffler JP. 2001. Calcium-calmodulin dependent protein kinase type IV (CaMKIV) inhibits apoptosis induced by potassium deprivation in cerebellar granule cells. *FASEB* J, 15: 134-144.

Screaton RA, Conkright MD, Katoh Y, Best JL, Canettieri G, Jeffries S, *et al.*. 2004 The CREB coactivator TORC2 functions as a calcium-and cAMP-sensitive coincidence detector. *Cell*, 119:61-74.

Shaywitz AJ, Greenberg ME. 1999. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem*, 68:821-861.

Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, *et al.*. 1994. Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell*, 79:49-58.

Zhang X, Odom DT, Koo SH, Conkright MD, Canettieri G, Best J, *et al.*. 2005. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. *Proc Natl Acad Sci USA*, 102:4459-4464